Hematology 2020

AMERICAN SOCIETY of HEMATOLOGY
Education Program™
62nd ASH® Annual Meeting and Exposition
December 5–8, 2020

Hematology 2020

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*Articles Not Eligible for Continuing Medical Education Credit

Editors' Message

Welcome to the 62nd annual meeting of the American Society of Hematology. This year's congress is unique among ASH annual meetings—the first to be all-virtual and the first to be held in the midst of a raging pandemic.

And just as the annual meeting has had to adapt to the circumstances, so too has *Hematology 2020*. For the first time, this year's edition is electronic-only. Some of the chapters reference COVID-19 and its impact on hematologic disorders. Other new features include a new page design, visual abstracts, and shorter manuscripts with the aim of making *Hematology* more graphical.

In spite of these changes, the essence of *Hematology* remains the same. As always, you can count on high quality content spanning the breadth of malignant and nonmalignant hematology, based on a state-of-the-art Education Program prepared by this year's Education Co-Chairs, Dr. Sioban Keel and Dr. Christopher Flowers. Dr. Andrew Roberts will deliver the Ham-Wasserman Lecture on Therapeutic Development and Current Uses of BCL-2 Inhibition, and his article is also included.

This volume is the culmination of the efforts of many individuals and their selfless commitment to ASH, including the authors, reviewers, designer (Debra Naylor), and ASH staff (Michelle Lee, Kenneth April, and Brian Cannon).

In these difficult times, we hope that you find *Hematology* to be the valued resource that it has always strived to be: a means of helping you keep current with the torrid pace of progress in hematology by highlighting recent practice-changing clinical advances as well as putting cutting edge basic advances into clinical context. Happy reading, and please stay safe.


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The editors thank the session chairs and the reviewers who worked hard to ensure the reliability of the material presented in *Hematology 2020*:

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Continuing Medical Education Information

Hematology: the ASH Education Program, is an annual publication that provides practicing hematologists with invaluable information on the most important areas of clinical progress.

Hematology 2020 is a peer-reviewed collection of 92 articles written by the 2020 ASH Education Program speakers and the Ham-Wasserman Lecturer. The papers showcase groundbreaking advances and new concepts in 30 different fields. Every year, the periodical provides an updated and comprehensive review of each of the topics covered in the annual meeting education sessions.

Educational objectives

1. Employ the knowledge gained regarding the diagnosis and treatment of malignant and nonmalignant hematologic disorders to improve patient care.
2. Discuss the state-of-the-art therapeutics in hematology.
3. Analyze the potential contribution of novel, not-yet-approved modalities of therapy to current evidence-based management of malignant and non-malignant hematologic disorders.

Date of release

December 2020

Date of expiration

On December 31, 2021, the ability to earn Continuing Medical Education (CME) credit and American Board of Internal Medicine Maintenance of Certification Medical Knowledge points for this product expires. This is the last date for users to claim credit for this product. For questions about credit, please contact the ASH Education Department at cme@hematology.org or call toll-free 866-828-1231 within the United States only; 1-202-776-0544 internationally.

Accreditation and credit designation

The American Society of Hematology is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

The American Society of Hematology designates this enduring material for a maximum of 40 *AMA PRA Category 1 Credits™*. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

Physicians who participate in this CME activity but are not licensed in the United States are also eligible for *AMA Category 1 PRA Credit™*. To earn these credits, readers must pass two online tests (malignant and non-malignant) based on chapters from the book.

ABIM Maintenance of Certification

Successful completion of this CME activity enables the participant to earn up to 40 Medical Knowledge points in the American Board of Internal Medicine's (ABIM) Maintenance of Certification (MOC) program. Participants will earn MOC points equivalent to the amount of CME credits claimed for the activity. It is the CME activity provider's responsibility to submit participant completion information to ACCME for the purpose of granting ABIM MOC credit.

Claiming CME credits and ABIM points

The estimated time to complete this educational activity is 40 hours. To claim CME credit, users must complete an evaluation of the product and a test of medical knowledge, both of which are accessed through the ASH Academy (academy.hematology.org). There is a one-time processing fee for claiming CME/MOC credit. Users with scores of 80% or better on these self-assessment modules are eligible to claim credit for the activity.

To facilitate claiming of credit, the test for this product is divided into two subtests, one of which focuses on malignant hematology content with the other focusing on nonmalignant content. Successful completion of each test earns the user *20 AMA Category 1 PRA Credits™*. Users claim CME and/or ABIM MOC credit for each test individually. You can take one or both tests, depending on your CME and MOC needs.

Each test consists of 25 questions. The 25 questions can be answered in one sitting, or a user can save their progress and return to complete the test at a later time.

The Malignant Hematology Test covers information presented in the following sections:

- A Map for the Changing Landscape of CLL
- Acute Myeloid Leukemia—So Many Treatment Options; How Do You Decide?
- Aggressive Lymphomas: What Novel Approaches Are Ready for Prime Time?
- Caring for Patients with Acute Leukemia in Community Hospitals: Who, What, and When to Refer?

- Challenging Situations for Patients with Aggressive Lymphomas
- Handling Challenging Questions in the Management of CML
- Immunotherapy in Multiple Myeloma
- Improving Symptom Control for Children with Hematological Malignancies
- Indolent Lymphomas: Answers to Smoldering Questions
- Managing Toxicities of Targeted Therapies in CLL
- Monoclonal Gammopathies of Determined Significance
- Myelodysplastic Syndromes: What We Have and What We Want
- Myeloproliferative Disorders: Too Many Cells, Too Few Therapies—How Do We Choose?
- Pediatric Hematological Maligancies: CARs for Kids
- The Emerging Role of Targeted Therapies and Cell Therapy in Transplant
- Understanding How to Manipulate the Immune System in Immunotherapy for Lymphoma

The Nonmalignant Hematology Test covers information presented in the following sections:

- Advances in the Laboratory Assessment of Hemostatic and Thrombotic Disorders
- Beyond the Marrow: Major Nonhematologic Complications of Inherited Bone Marrow Failure Syndromes
- Chronic Transfusion Support: Challenging Cases
- Diagnostic and Prognostic Models in VTE Management: Ready for Prime Time?
- Genetic Testing for Heritable Hematologic Disorders 101
- Infection Risk, Immunization Recommendations, and Antimicrobial Prophylaxis Needs when Treating Nonmalignant Hematologic Disorders—Wash Your Hands and What Else?
- More Anxiety-Provoking Hematology Consults
- Out of Balance: Anemias Due to Disordered Iron Homeostasis
- Platelet Transfusions for Hematology/Oncology Patients: Taking a More Granular Look
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- What Hematologists Need to Know About Giving and Stopping Aspirin
- Yin and Yang of Autoimmunity and Immunodeficiencies in Hematology

Therapeutic development and current uses of BCL-2 inhibition

Andrew W. Roberts

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B-cell lymphoma 2 (BCL2) is a key protein regulator of apoptosis. It is variably highly expressed in many hematological malignancies, providing protection from cell death induced by oncogenic and external stresses. Venetoclax is the first selective BCL2 inhibitor, and the first of a new class of anticancer drug (BH3-mimetics) to be approved for routine clinical practice, currently in chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML). To help understand the potential and limitations of this therapy, this brief review will touch on the history of development of venetoclax, dissect its mechanism of action, and summarize critical evidence for its approved use in the management of patients with CLL and AML. It will also consider recent data on mechanisms of resistance and explore concepts pertinent to its future development based on key lessons learned to date.

LEARNING OBJECTIVES

- Understand how venetoclax inhibits BCL2 to trigger apoptosis of CLL and AML cells and other blood cancers and how resistance can develop
- Understand the results of pivotal trials in CLL and AML and how tailored venetoclax combinations may prove effective in other diseases

Introduction: the discovery of BCL2 and its function

B-cell lymphoma 2 (*BCL2*) was the name given to a gene of unknown function discovered as the anonymous partner of the immunoglobulin heavy chain locus in the typical translocation seen in follicular lymphoma: t(14;18).[1] How it was oncogenic remained a puzzle, until it was revealed that rather than promoting proliferation, the BCL2 protein acted to protect cells from apoptosis when overexpressed.[2-4] BCL2 was the first mammalian gene product associated with apoptosis, and recognition of its function led to an explosion of research into apoptosis and subsequently recognition that evasion of cell death is a hallmark of cancer. Avoidance of apoptosis is a prominent feature of many hematological malignancies.

We now recognize a large family of BCL2-related proteins, all operating by nonenzymatic protein:protein interactions to regulate the intrinsic or mitochondrial pathway to apoptosis, some protecting against apoptosis and some promoting it.[5] In the prosurvival subfamily, MCL1, BCLxL (BCL2L1), BCL2A1 and BCLB, like BCL2, inhibit the initiation of apoptosis. Through direct binding they hold in check the 2 key cell death effector proteins, BAX and BAK, which when activated congregate on the outer membrane of the mitochondria and create pores which permeabilize and depolarize the organelle, releasing cytochrome C and activating caspases that execute the destruction of cells in a manner we recognize as apoptosis.[5,6] BCL2 and other prosurvival proteins are naturally antagonized by the proapoptotic BH3-only protein subfamily comprising BIM, BID, NOXA, PUMA, BAD, HRK, BMF, and BIK. Although BIM, PUMA and BID can bind and neutralize the function of all prosurvival proteins, BAD only binds and inhibits BCL2, BCLxL and BCLW, and NOXA preferentially inhibits MCL1 and BCL2A1.[7]

The balance of activity between prosurvival proteins and BH3-only proapoptotic proteins determines whether a cell will live or undergo apoptosis, and collectively they serve to integrate the diverse extracellular and intracellular signals promoting either survival (eg, growth factors, nutrients) or death induced by stress (eg, oncogenic/proliferative, DNA damage, etc). This balance is deregulated in many hematological malignancies by altered expression of

BCL2 (or related proteins) or loss of BH3-only proteins or effector proteins.[6] The various genetic mechanisms by which these abnormalities can occur are summarized elsewhere,[8] but it is important to realize that high-level expression of prosurvival proteins can be an epigenetically regulated adaptive response to cellular stress. By way of general summary, malignant cells with upregulated BCL2 are being protected from undergoing apoptosis despite cellular stresses which would kill their normal counterpart cells. As described by Letai, these malignant cells are "primed for death,"[6] and in theory should be highly susceptible to loss of BCL2's protective function.

Targeting BCL2 with the BH3-mimetic, venetoclax

BH3-mimetics are a new class of anticancer drug that mimic the actions of BH3-only proteins in that they bind to prosurvival proteins like BCL2 in the same way (indeed the same groove) and inhibit BCL2's ability to bind BAX or BAK.[9] As BCL2 also exists bound to native BH3-only proteins, BH3-mimetics can also displace these endogenous activators of apoptosis. Venetoclax is a BCL2-selective BH3-mimetic and its addition to BCL2-overexpressing cancer cells in vitro potently triggers apoptosis[10] (Figure 1).

Extensive preclinical and in vivo patient data confirm that the principle mechanism by which venetoclax kills malignant blood cells is by induction of apoptosis.[10,11] Killing is absolutely dependent on BAX/BAK and for most susceptible cells is very rapid in onset,[12] with permeabilization of the mitochondria occurring within minutes and death within hours, including in patients.[10,11,13] In some less susceptible cells, mitochondrial permeabilization induced by venetoclax is insufficient to directly generate sufficient caspase activation for apoptosis, but disruption of mitochondrial energy production can prove lethal to vulnerable cells (eg, some AMLs)[14-16] and release of mitochondrial DNA can trigger an antiviral like cell death response in others.[17]

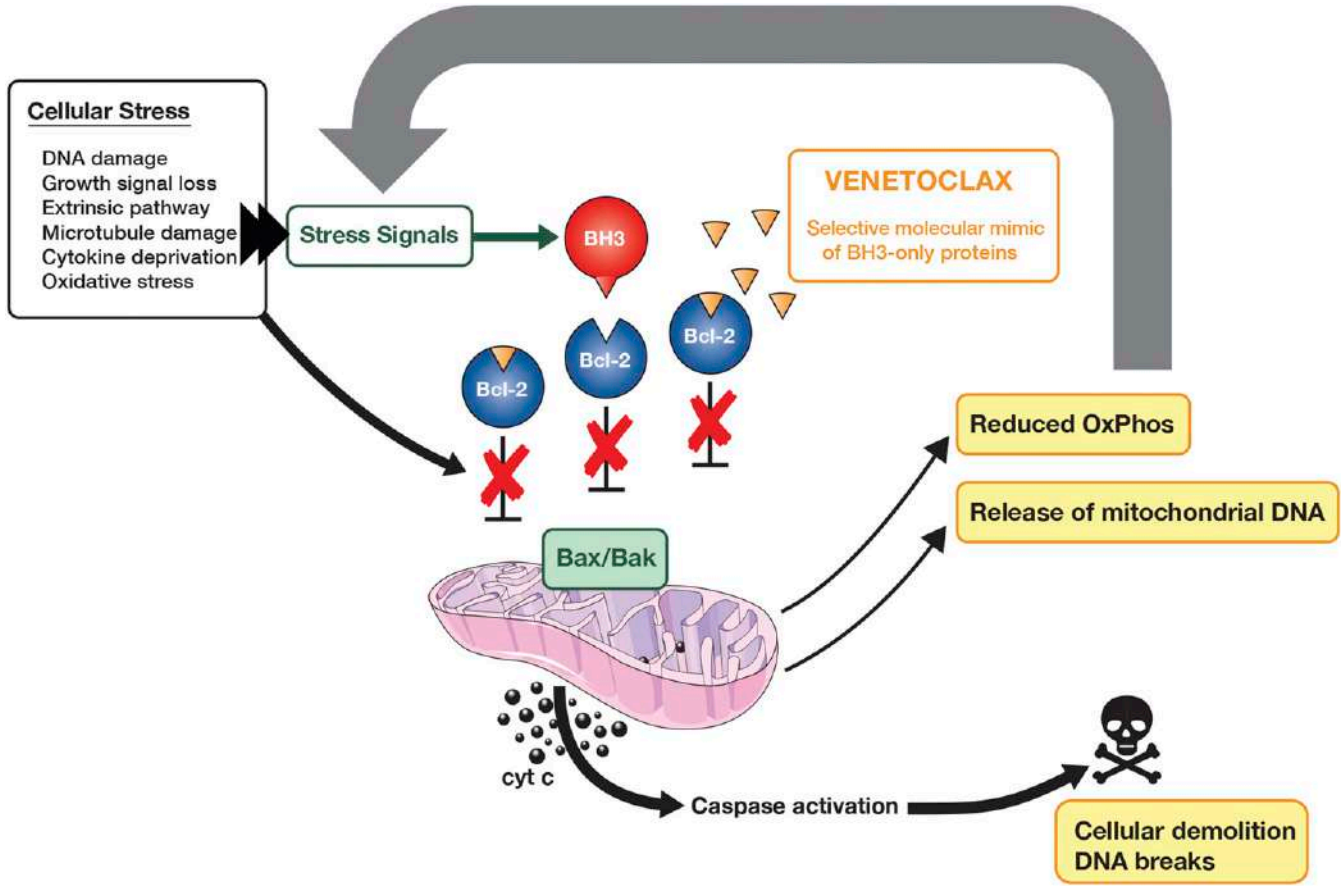

Figure 1. The mechanism of action of venetoclax, a BH3-mimetic that is a highly selective inhibitor of BCL2. The diagram illustrates in cartoon form how the small molecule venetoclax acts intracellularly in a BCL2-overexpressing leukemic cell to initiate apoptosis by mimicking the action of the endogenous antagonists of BCL2, the BH3-only proteins. Heightened expression of BCL2 protects leukemia cells from apoptosis by inhibiting activation of BAX and BAK, even when normally lethal cellular stresses induce prodeath BH3-only proteins such as BIM and NOXA. Venetoclax interacts with BCL2 selectively in the BH3-binding groove to directly and indirectly (via release of BIM) relieve repression of BAX/BAK which homodimerize or heterodimerize to permeabilize mitochondria,[10] unleashing apoptosis through release of cytochrome C and subsequent caspase activation, which demolishes cellular organelles and the nuclear structures.[5] Release of cytochrome C and caspase activation are generally considered the point of no return for cells. In some cells, apoptosis is not immediately fully established, but downstream effects such as disruption to oxidative phosphorylation[14,16] or release of mitochondrial DNA[17] amplify cellular stresses and complete commitment to apoptosis. Modified from Anderson et al[56] with permission.

Whether an individual cancer cell lives or dies after venetoclax therapy depends on the general dependence on BCL2 of that cell type (high for mature B lymphocytes, low for macrophages),[18] the cell-intrinsic oncogenic stresses (high for Myc-driven, lower for kinase-driven tumors),[19] the microenvironment in which the cell resides,[20] and the presence of other stressors (eg, additional therapy, such as DNA-damaging agents).[10] Consequently, different hematological malignancies display varying susceptibility to BCL2 inhibition in preclinical testing (reviewed elsewhere).[8]

The current sum of preclinical and clinical data suggests that as a highly specific therapy, we should think of venetoclax as an agent that directly hits the "bullseye" (the Achilles' heel of the cancer) only in instances where direct inhibition of BCL2 rapidly induces cell death in the majority of cells (Figure 2A). In other cell types, this drug also hits the "target," but in a different way, with direct inhibition of BCL2 immediately setting off a wave of secondary consequences that encompass the "bullseye" and cause cell death (Figure 2B). This may be via triggering secondary inhibition of other prosurvival proteins (eg, MCL1, BCLxL) through displacement of previously bound BIM from BCL2, or reduction in oxidative phosphorylation by permeabilized mitochondria in AML, etc. For some diseases, such as AML, there will be heterogeneity in how cell populations respond, reflecting how BCL2-dependent individual cells are, and this will vary between patients depending on the genetic and epigenetic makeup of the cancer. The more cells that are impacted in "hammer-strike" fashion rather than an "arrow to the bullseye" fashion, the more important concomitant therapy will be to achieve a high degree of cell killing.

Venetoclax and CLL

BCL2 is highly expressed in all CLL cells in all patients, and the great majority of CLL cells appear dependent on BCL2 for survival.[10,12,21] Consistent with this, venetoclax is effective as monotherapy in ~75% to 80% of patients with relapsed CLL, and complete remission (CR; including CR with incomplete recovery [CRi]) can be expected in 15% to 20% of patients[13,22] (Table 1). The rate of response, including CR, is independent of genetic subtypes, but the negative prognostic genetic markers del17p, TP53 mutations, and NOTCH1 mutations are associated with less durable responses in multivariable analyses.[23] Achieving CR and/or having undetectable minimal residual disease (uMRD; $<10^{-4}$) in the peripheral blood (PB; achieved in 30% of patients) or bone marrow (BM) is associated with prolonged remissions.[23,24] Venetoclax monotherapy is approved therapy for relapsed del17p CLL in many jurisdictions.

No randomized trials have been performed to determine whether addition of rituximab or other anti-CD20 antibodies increases response rate or durability of response. Nevertheless, rates of CR (51%) and uMRD (57% in BM) appeared higher in the first early phase combination trial with rituximab.[25] This combination trial also provided evidence that indefinite continuous daily venetoclax therapy was not required in CLL, with highly durable remissions continuing in patients who ceased in either CR or uMRD response.[26] These 2 characteristics led to the use of limited-duration combination venetoclax-anti-CD20 regimens in pivotal trials. Combination therapy with rituximab in the relapsed setting and with obinutuzumab in the frontline setting are associated with high rates of CR (27% and 50%, respectively) and PB uMRD (62% and 76%, respectively) at the end of combination therapy.[27,28]

In randomized trials (Table 2), venetoclax-rituximab proved superior to bendamustine-rituximab for patients with relapsed CLL in terms of efficacy (progression-free survival [PFS] and overall survival) and toxicity (febrile neutropenia)[27]; and venetoclax-obinutuzumab proved superior to chlorambucil-obinutuzumab for treatment-naive older patients with comorbidities in terms of

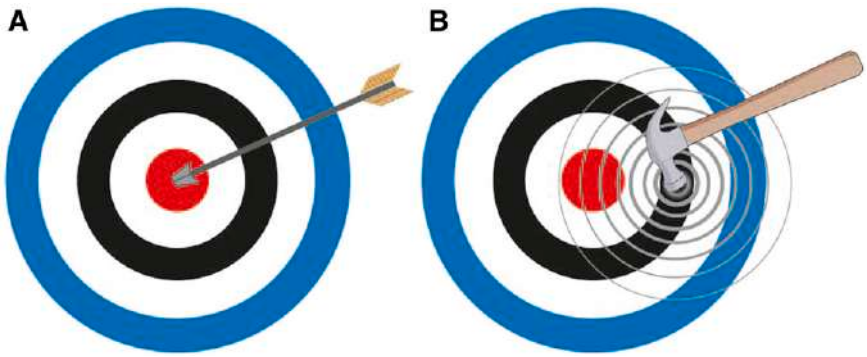

Figure 2. Schema for understanding variability in susceptibility of hematological malignancies to BCL2 inhibition. Venetoclax is a highly targeted therapy, binding almost exclusively to BCL2 when used in clinically achievable concentrations. (A) An illustration of the concept that venetoclax can hit the "bullseye" in malignancies such as CLL. In cells with invariably high expression of BCL2 and relatively minor expression of other prosurvival proteins, inhibition of BCL2 is sufficient to trigger apoptosis reliably in a high proportion of cells.[10,11] Such cells can be considered BCL2-dependent, and we recognize these diseases clinically through their high rate of response and complete response to venetoclax monotherapy.[13,24] (B) An illustration of the more common scenario, in which BCL2 is a target worth hitting, but inhibition of BCL2 directly is insufficient to "hit the bullseye." Cell killing by venetoclax is dependent on a wave of secondary events including secondary inhibition of other prosurvival proteins by displaced BH3-only proteins,[8,57,58] or mitochondrial depolarization[14,16] (see main text on this page). Such cells are not strictly BCL2-dependent. Additional stressors are likely to be required to maximize apoptosis and achieve high response rates in diseases in which the majority of cells are affected in this manner. Diseases such as AML and some lymphomas typically have a mix of malignant cells, some displaying susceptibility akin to panel A; others are vulnerable only through the secondary wave effect shown in panel B.

Table 1. Summary of indicative mature clinical trial data for venetoclax in hematological malignancies

Monotherapy					Combination			
	Response rate, %					Response rate, %		
Phase	Overall	CR*	Median PFS, mo	Partner drug(s)	Phase	Overall	CR*	Median PFS, mo†
CLL								
Relapsed/refractory								
1[13]	79	20	25	+ Rituximab	1b[25,26]	86	51	80% @ 2 y 56% @ 5 y
2[22,24]	79	16	27	+ Rituximab	3[27,59]	92	27	85% @ 2 y 71% @ 3 y
				+ Ibrutinib	2[29]	89	51	100% @ 1 y
First-line				+ Obinutuzumab + Ibrutinib	3[28] 2[30]	84.7 NR	49.5 74‡	88% @ 2 y 98% @ 1 y
AML								
Relapsed/refractory								
1b[31]	38	19	2§	+ Aza/Decitabine	2[35]	64	51	9‖
					2[36]	50	22.5	?
First-line								
(Elderly and/or unsuitable for standard induction)				+ Aza/Decitabine	1b/2[34]	62	60	11§
				+ Azacitidine	3[38]	3[38]	66 (61-72)	10 (8-12)¶
				+ Low-dose Ara-C	1b/2[33] 3[37]	64 NA	62 48 (39-56)	13.2 4.7¶
(Fit, unsuitable for standard induction)				+ Ara-C/Idarubicin	1b[41]		72	13.5‖
Lymphoma (relapsed)								
Follicular								
1[42]	38	14	11	+ Bendamustine/ Rituximab	1b[60]	75	38	NR @ 24 mo
Mantle cell								
1[42]	75	21	14	+ Ibrutinib	2[46,61]	75	71	29
Diffuse large B cell								
1[42]	18	12	1	+ Bendamustine/ Rituximab	1b[60]	41	14	4
Myeloma (relapsed)								
All								
1[43]	21	7	3#	+ Bortezomib/ Dexamethasone	1b[49] 3[50,62]	67 84	20 29	9.5 23
t(11;14)	40	14	7#	+ Bortezomib/ Dexamethasone	1b[49] 3[50,62]	78 95	NA 55	NA NR @ 29 mo

Initial phase 1 and phase 2 trials and all phase 3 trials formally reported to date for CLL and AML are included here, but the table is incomplete for recent combination phase 1b and phase 2 trials in myeloma, lymphoma, and other malignancies. The first early phase combination trials have been selected to provide the most simple indirect comparisons with monotherapy activity.
Ara-C, cytosine arabinoside; Aza/Decitabine, azacitidine or decitabine; NA, not reported; NR, not reached; PFS, progression-free survival.
*CR indicates complete response (and/or CR with incomplete count recovery) as assessed by investigators as best response during trial.
†Where median PFS not reached, estimate at specific time point is provided.
‡CR by intention to treat at time of reporting when many patients had not completed planned therapy.
§Duration of response.
‖Leukemia-free survival for CR achievers.
¶Event-free survival reported.
#Time to progression.

Table 2. Key results of randomized trials related to FDA-approved indications

Treatment	Durability of benefit			Overall survival			Toxicity			
	PFS/EFS*	HR	*P*		HR	*P*	Nausea, %	≥G3 febrile neutropenia, %	Pneumonia, %	Discontinued due to AE, %
CLL-relapsed[27,59]										
Ven-Ritux	71% @ 3 y	0.16	<.001	88% @ 3 y	0.50 (.3-.85)	.009	21	3.6	8.2	17
Ben-Ritux	15% @ 3 y			80% @ 3 y			34	9.6	8.0	16
CLL–first-line[28]										
Ven-Obin	88% @ 2 y	0.35	<.001	92% @ 2 y	1.24 (.64-2.4)	.52	19	17.5	4.7	22†
Chl-Obin	64% @ 2 y			93% @ 2 y			22	15.0	4.2	23†
AML first-line (including sAML pretreated with HMA)[37]										
Ven-LoDAC	4.7*	0.58	.002	8.4	0.70 (.50-.99)	.04	42	32	13	9
Pbo-LoDAC	2.0*			4.1			31	29	10	9
AML–first-line (no prior HMA)[38]										
Ven-Aza	9.8*		<.001	14.7	0.66 (.52-.85)	<.001	44	30	16	NR
Pbo-Aza	7.0*			9.6			35	10	22	NR

≥G3, grade 3 or higher; AE, adverse event; Aza, azacytidine; Ben, bendamustine; Chl, chlorambucil; EFS, event-free survival; FDA, US Food and Drug Administration; HMA, hypomethylating agent therapy; HR, hazard ratio; LoDAC, low-dose cytosine arabinoside; NR, not reported; Obin, obinutuzumab; Pbo, placebo; PFS, progression-free survival; Ritux, rituximab; sAML, secondary AML; Ven, venetoclax.
*EFS for AML.
†All cause discontinuation excluding PD.

PFS, with similar toxicity.[28] These 2 regimens are approved in many jurisdictions as a standard of care.

In ongoing trials, venetoclax is being combined with ibrutinib and other BTK inhibitors, with and without anti-CD20 monoclonal antibodies. Preliminary data with ibrutinib-venetoclax combinations indicate high rates of CR (51% to 74%; Table 1) and PB uMRD (53% in the relapsed[29] and 61% in the frontline settings[30]) after ~1 year of combined therapy, but efficacy and safety relative to currently approved regimens is unknown at this point.

A key unresolved question relates to the optimal duration of treatment with venetoclax, and whether this should be a fixed duration of therapy as currently approved or whether it should be informed by response assessment (ie, adaptive to depth and speed of response, with slow and incomplete responders receiving more prolonged therapy, and rapid responders less). Related to this is whether the use of time-limited therapy will reduce the emergence of resistant clones at progression, thereby enabling effective reuse of venetoclax-based regimens when progression occurs off therapy.

Venetoclax and AML

BCL2 is variably expressed in AML, and only a minority of patient samples show marked sensitivity in vitro.[31,32] Consistent with this, single agent activity is evident in patients. In the phase 1 monotherapy trial in relapsed disease a minority demonstrated major reductions in blasts, and only 19% achieved CR/CRi.[31] Even then the median duration of response was short (Table 1). These data reflect that inhibition of BCL2 in AML results in a "hammer-strike" effect, with BCL2 being a significant target, but not a bullseye. Consequently, combination therapy is essential. The first partners evaluated in trials were low-dose cytosine arabinoside (LoDAC)[33] and the hypomethylating agents azacytidine and decitabine[34] (Table 1). In relapsed AML, the CR/CRi rate appears higher with these combinations than with monotherapy,[35,36] but how much each drug is contributing and whether the antileukemic effects are subadditive, additive, or synergistic is unknown.

Not surprisingly, response rates in frontline therapy with the combinations are higher, with CR/CRi rates of 48% to 66% observed.[33,34] There is little evidence for benefit in patients with AML who do not achieve CR. These venetoclax-combination regimens received provisional US Food and Drug Administration (FDA) approval as frontline treatments of elderly or unfit patients on the basis of early phase trial data, and placebo-controlled phase 3 trial data have just been reported in 2020 (Table 2).

With extended follow-up, the addition of venetoclax to LoDAC in patients with primary or secondary AML (20% pretreated with hypomethylating agents) and aged >75 years or unfit for intensive induction therapy modestly improved overall survival and event-free survival (EFS; HR 0.7 and 0.58, respectively).[37] CR rates were substantially higher with the venetoclax combination across prognostic categories (cytogenetic risk groups, primary or secondary, prior hypomethylating therapy, selected driver mutations) and similar magnitudes of relative survival benefit seen for the whole population were suggested in an underpowered exploratory analysis.

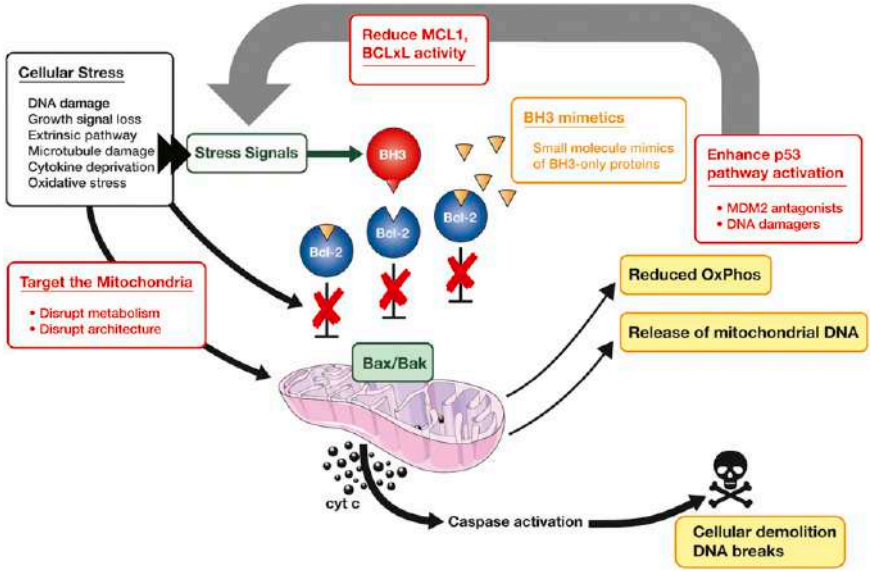

Figure 3. The anticancer effect of venetoclax theoretically can be enhanced through rational combination with other targeted therapies. This cartoon builds on the illustration of the mechanism of action of venetoclax in Figure 1 to highlight opportunities for enhancing apoptosis. A major avenue for amplifying the proapoptotic signal is to reduce the expression or activity of other pro-survival BCL2-like proteins (eg, MCL1 or BCLxL; red box, top center). This can be achieved directly by adding selective inhibitors of these proteins. Examples where this has been demonstrated preclinically[32,54,63] and are being explored in clinical trials include AML, ALL, and mantle cell lymphoma. Reduction in prosurvival protein function can also be achieved indirectly via induction of their natural antagonists (eg, NOXA which antagonizes MLC1 and BCL2A1) by enhancing activity of the TP53 pathway through DNA damage or inhibition of MDM2[64] (red box, right side). These strategies are being explored clinically in lymphomas and AML. Preclinical evidence further indicates that killing can be augmented through direct targeting of mitochondrial structures and functions, such as energy production (red box, left side). This has been demonstrated particularly, but not only, in AML.[15,16] A partial explanation of the enhanced efficacy of the azacitidine-venetoclax combination in AML includes disruption to energy metabolism.[65] The cartoon also depicts how combinatorial approaches can both amplify the proapoptotic effect upstream of BAX/BAK and also reduce the threshold for mitochondrial vulnerability to BAX/BAK activation. To maximize the therapeutic index for any of these combination approaches, each will need to be tailored to the specific vulnerabilities of individual diseases, and biomarkers may prove advantageous in this regard.

Addition of venetoclax to azacytidine in similar patients, but excluding those previously receiving hypomethylating agents for preceding myelodysplasia, also improved overall survival (HR, 0.66) and EFS.[38] In both trials, improvement in median overall survival was 4 to 5 months, with no plateau evident on survival curves and the majority of patients dying within 2 years. In both treatment-naive and relapsed settings, well-established prognostic factors appear to still be relevant with venetoclax-based therapy. Lowest survival is still seen in high-risk cytogenetic subgroups and where *TP53* mutations are detected, and more favorable outcomes in intermediate cytogenetic risk AML with either *NPM1* or *IDH1/2* mutations.[39,40] Mature follow-up and meta-analyses will be required to determine if any genetic marker is a true response-modifier that can be used to refine clinical decision-making.

A key question being addressed by several trials is whether venetoclax could have a role in treatment of patients fit for induction chemotherapy. Given that venetoclax induces selective killing of granulocytic progenitor cells in vitro and neutropenia in vivo, substantial additional bone marrow toxicity is anticipated, and scheduling issues are not yet resolved. Initial publications are expected during 2020, with an early trial indicating that venetoclax 600 mg per day for 14 days can be safely added to a 5+2 cytosine arabinoside/idarubicin regimen and achieve high CR rates in a mixed population of patients >60 years[41] (Table 1).

BCL2 inhibition in other hematological malignancies

Currently, venetoclax is being evaluated in >230 clinical trials in a wide range of hematological malignancies. Venetoclax has shown clinically meaningful single agent activity in selected lymphomas,[42] multiple myeloma,[43] blastic plasmacytoid dendritic cell neoplasm,[44] and T-cell prolymphocytic leukemia.[45] It is also being evaluated in myelodysplasia using AML-style combinations and in relapsed acute lymphoblastic leukemia. Table 1 summarizes some illustrative published results for lymphoma and myeloma.

Mantle cell lymphoma is susceptible to single-agent BCL2 inhibition, with a 75% response rate in the relapse/refractory setting and durable responses particularly in the 21% achieving CR.[42] Combination with ibrutinib appears additive at least, with PET-negative complete responses observed in >70% of patients, including 67% with uMRD, and in 50% of patients with *TP53*-aberrant disease.[46] At 30 months, 74% of responders remain relapse-free, and indefinite therapy is not necessarily required. Randomized trials are now comparing venetoclax-BTK inhibitor combinations with BTK inhibitor monotherapy.

Follicular lymphoma stands out as a disease with high and uniform expression of BCL2, yet only modest response rates with

venetoclax alone.[42] This paradox remains to be resolved. Experience with venetoclax monotherapy in DLBCL was sobering, and the limited responses could not be associated with any specific pattern of BCL2 expression.[42] In both these lymphoma types, combinations with DNA-damaging regimens and non–DNA-damaging regimens are being explored.

Multiple myeloma commonly expresses BCL2 at high, but variable, levels, as do normal plasma cells. However, responses to monotherapy are largely restricted to patients with the t(11;14) subclass,[43] as predicted preclinically,[47,48] where BCL2 expression is highest. In some non-t(11;14) myeloma with high *BCL2/BCL2L1* (BCL-xL) expression ratios, responses can also be seen. Response rates and CR rates are higher when venetoclax is used in combination.[49] However, the therapeutic index of the venetoclax-bortezomib-dexamethasone combination in unselected patients with myeloma is problematic. Preliminary presentations of the randomized trial indicate increased antimyeloma activity but excess toxicity in patients whose myeloma lacks t(11;14) or high *BCL2/BCL2L1* expression ratio.[50]

Lessons from clinical experience with venetoclax to date

As the first approved drug in this new class of anticancer therapy, experience with venetoclax has provided several key lessons that should help inform its ongoing development and that of future BH3-mimetics, for example, MCL1 inhibitors. First, because of its mechanism of action, venetoclax is a cytotoxic that kills vulnerable cells quickly,[10-12] with responses occur rapidly, typically with the first cycle.[13,31] Second, durable benefit is predominantly seen in patients achieving CR, as seen in CLL,[13,23] AML[31,33] and sensitive lymphomas.[42] Further in CLL, the most durable remissions are seen in patients who achieve MRD-negative remissions.[23,24] Third, to achieve maximal tumor reduction, combination therapy is necessary. To date, venetoclax has been shown to be tolerable when combined with many different classes of drugs.

Fourth, among sensitive tumors, secondary clinical resistance may occur due to genetic or epigenetic changes in apoptosis regulators or by the acquisition of constitutive growth factor signaling. Changes that affect regulators of the intrinsic pathway to apoptosis have emerged as important in several lymphoid malignancies. Mutations in *BCL2* that encode proteins that maintain prosurvival function but have reduced (up to 180-fold) binding to venetoclax are prominent as a cause of late CLL relapse in long term venetoclax-treated patients.[51] The most common is G101V, but several others have been described.[52,53] MCL1 overexpression related to focal amplifications on chromosome 1q are also seen,[16] as is upregulation of BCL-xL in CLL[51] and in mantle cell lymphoma.[54] Importantly, each of these changes can co-occur in independent clones in the same patient. Data to date on secondary resistance in AML indicate that outgrowth of FLT3-ITD or RAS-MAPK pathway mutant subclones is common.[39,40] Again, parallel emergence of clones with distinct mechanisms of resistance is observed in individual patients. Polyclonal heterogeneity is the norm for venetoclax-resistance, consistent with patterns now emerging for other highly targeted agents.[55]

Finally, more translational research is urgently needed. Validated biomarkers are required to better select patients for venetoclax-based therapy in diseases where targeting BCL2 is not an "arrow through the bullseye." Similarly, rigorous preclinical experiments are required to guide improvement in overall response rates and length of remissions in AML, lymphomas and myeloma. Figure 3 provides suggestions as to how adding targeted agents should amplify the apoptotic effect of BCL2 inhibition, based on recent insights into biology and mechanisms of resistance. It may be that we are just at the beginning of our understanding of how best to use BCL2 inhibitors like venetoclax.

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Conflict-of-interest disclosure

A.W.R. is an employee of the Walter and Eliza Hall Institute, which receives milestone and royalty payments related to venetoclax; receives a share of these royalties from the Institute; and has received research funding to his institutions from AbbVie, Janssen, and Servier for investigator-initiated clinical trials or laboratory research.

Off-label drug use

None disclosed.

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Approaches for relapsed CLL after chemotherapy-free frontline regimens

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Novel agents, including Bruton's tyrosine kinase inhibitors (BTKi; ibrutinib, acalabrutinib), venetoclax, and phosphatidylinositol 3-kinase inhibitors (PI3Ki; idelalisib, duvelisib), have fundamentally changed the chronic lymphocytic leukemia (CLL) treatment landscape, allowing for a chemotherapy-free paradigm for many. Randomized trials that demonstrated efficacy of these agents in the relapsed/refractory setting rarely included patients with prior novel agent exposure. Herein, we review available data, including single-arm prospective studies and retrospective cohorts, on outcomes for novel agent approaches after novel agent exposure. We examine data for subsequent treatment options in 3 specific scenarios: (1) progression of disease while receiving BTKi, (2) progression of disease after discontinuation of BTKi for intolerance, and (3) after treatment with venetoclax. Data are most robust for venetoclax-based regimens after progression on BTKi. For patients who experience progression of disease after discontinuation of BTKi for intolerance, venetoclax-based regimens and retreatment with BTKi (depending on severity of initial intolerance) are 2 data-driven options. After frontline venetoclax/obinutuzumab, subsequent treatment approaches depend on whether patients experience progression of disease during or after discontinuation of their fixed duration frontline regimen and whether venetoclax/obinutuzumab was discontinued for intolerance. After progression of disease while on venetoclax, we recommend BTKi as second-line therapy. For patients who experience progression after completion or premature discontinuation (because of intolerance) of fixed duration venetoclax/obinutuzumab, either BTKi or retreatment with venetoclax (with aggressive supportive care if prior intolerance) are reasonable considerations. Subsequent lines of therapy in these scenarios include PI3Ki and consideration of cellular therapies. Finally, clinical trial enrollment for interested patients in any line of therapy is recommended.

LEARNING OBJECTIVES

- Compare treatment strategies for patients with CLL requiring therapy after progression on BTK inhibitor or discontinuation for toxicity
- Understand data for efficacy of novel agents in the treatment of CLL after discontinuation of venetoclax.

Introduction

Based on promising results from trials examining chemotherapy-free regimens in the front-line setting, Bruton's tyrosine kinase inhibitors (BTKi) with or without anti-CD20 monoclonal antibodies and venetoclax/obinutuzumab are increasingly being used as first chronic lymphocytic leukemia (CLL)-directed therapy.[1-3] This paradigm shift in first-line treatment has altered the therapeutic landscape. As such, optimal sequencing of therapies within a chemotherapy-free paradigm has and will continue to become a pressing issue in the care of patients with relapsed/refractory (R/R) CLL.

Novel agents approved in the R/R setting include ibrutinib, acalabrutinib, idelalisib + rituximab, duvelisib, and venetoclax ± rituximab. Although these agents have demonstrated efficacy in R/R cohorts, the studies that led to their approvals largely examined patients who had received prior chemoimmunotherapy and rarely prior novel agents (Table 1).[4-10] As patients receiving novel agents often do well for extended periods of time, data regarding efficacy of novel agents in exclusively novel agent-treated patient populations is limited. Therefore, the sequences being explored are partially a consequence of the order in which agents were approved rather than intrinsic tumor biology. How readily data from chemoimmunotherapy exposed patient cohorts can be extrapolated to patients exclusively treated with novel agents remains to be seen, particularly given potential for differences in accumulated toxicity and resistance mechanisms.

Although many questions regarding the optimal sequence of novel agents in a chemotherapy-free treatment

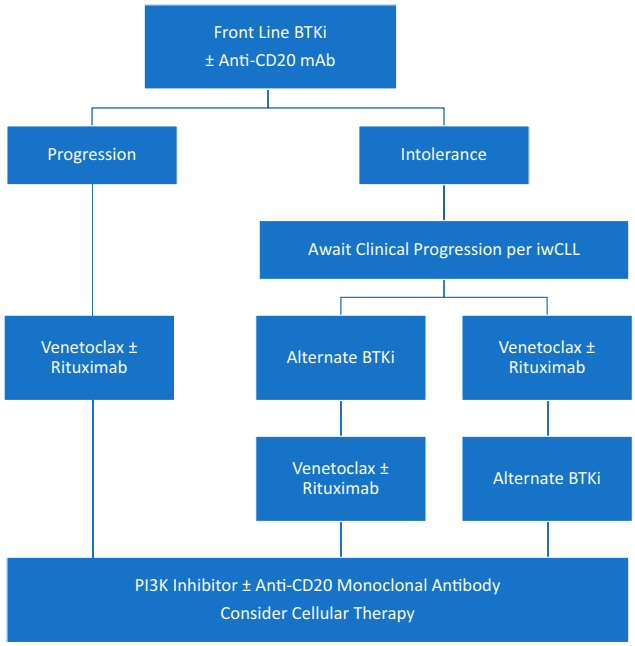

Figure 1. Proposed treatment algorithms after frontline BTK inhibitor for CLL.

sequence remain, we will review the available data regarding treatment approaches for patients based on first novel agent exposure and outline potential therapeutic pathways.

Clinical case 1

A 74-year-old man with CLL presents for follow-up. He was diagnosed at 70 years of age after presenting to his primary care physician for fatigue, at which time his complete blood count (CBC) showed lymphocytosis with a white blood cell count of 18.8 × 10^9/L, absolute B-lymphocyte count (ALC) of 11.6 × 10^9/L, hemoglobin of 9.4 g/dL, and platelet count of 89 × 10^9/L. He was noted to have firm, discrete, nontender, and freely mobile lymph nodes ranging from 1 to 4 cm (bidimensional) in the cervical, supraclavicular, and axillary chains and splenomegaly to 3 cm below the costal margin. Prognostic testing at the time of diagnosis showed unmutated immunoglobulin heavy chain variable region gene (IGVH), fluorescence in situ hybridization (FISH) with deletion of chromosome 11q [del(11q)], and no *TP53* mutation by next-generation sequencing. Given that he had both anemia and thrombocytopenia, he met criteria for therapy per the International Workshop on Chronic Lymphocytic Leukemia[11] and was started on ibrutinib 420 mg oral once daily.

He achieved partial remission (PR) with lymphocytosis after 6 months and PR after 15 months of ibrutinib therapy with reduced size of lymph nodes in all chains and normalization of hemoglobin and platelet count. At today's visit, he has been on ibrutinib 420 mg daily for 48 months and is tolerating treatment well. However, over the past 6 weeks, he has noted reappearance of lymph nodes in the cervical, axillary, and inguinal chains with development of fatigue. CBC today shows white count of 79.4 × 10^9/L, ALC of 73.6 × 10^9/L, hemoglobin of 9.8 g/dL, and platelet count of 80 × 10^9/L. Lactate dehydrogenase is within the normal range. There is no evidence of autoimmune hemolytic anemia.

Treating relapsed disease after progression on BTKi

For patients experiencing progression while on BTKi therapy, discontinuation of drug can lead to tumor flare, which can be difficult to control and life threatening.[12] As such, BTKi can be continued despite progression until the next therapy is ready to be administered. In some scenarios, a brief period of overlapping therapy or bridging to next therapy with steroids may also be warranted.[13] If CLL is behaving aggressively or Richter's transformation is suspected, positron emission tomography (PET)/computed tomography (CT) is recommended for initial evaluation. However, in the setting of progression on B-cell receptor inhibitor (BCRi), sensitivity and specificity of PET is diminished (71% and 50% for lesions with standardized uptake value ≥ 10, respectively), and biopsy is warranted if suspicious lesions are present.[14] Characterization of Richter's transformation through pathologic features is not impacted by prior chemoimmunotherapy vs novel agents.[15]

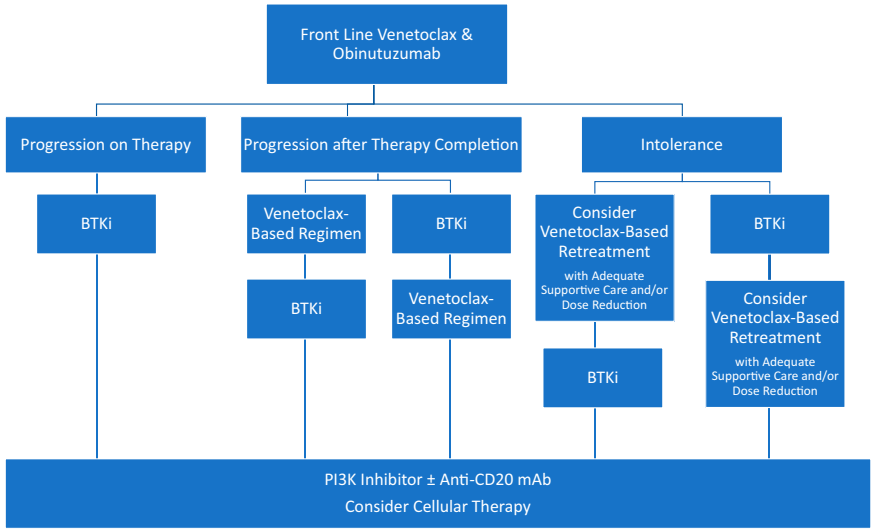

Figure 2. Proposed treatment algorithms after frontline venetoclax and obinutuzumab for CLL.

Table 1. Completed phase 3 clinical trials examining chemotherapy-free regimens vs standard of care in relapsed/refractory CLL

Novel agent	Control arm	Prior lines of therapy in novel agent arm, median (range)	Patients with prior novel agent exposure	Outcomes (novel agent vs control arm)
Ibrutinib[5]	Ofatumumab	3 (1-12)	Not reported	ORR: 91% for ibrutinib
				Median PFS: 44.1 vs 8.1 mo
				Median OS: 67.7 vs 65.1 mo
Acalabrutinib[6]	Investigator's choice: bendamustine or idelalisib/rituximab	1 (1-8)	Patients with prior BCRi or venetoclax were excluded	ORR: 81 vs 76%
				1-y PFS: 88% vs 68% vs 69%
Idelalisib/ rituximab[7]	Placebo/rituximab	3 (1-12)	None; prior BTK or PI3Ki as exclusion criteria	ORR: 81% vs 13%
				6-mo PFS: 93% vs 46%
				1-y OS: 92% vs 80%
Duvelisib[8]	Ofatumumab	2 (1-10)	None; prior BTK or PI3Ki as exclusion criteria	ORR: 74% vs 45%
				Median PFS: 13.3 vs 9.9 mo
Venetoclax/ rituximab[9,10]	Bendamustine	1 (1->3)	BCRi in 5 patients (2.6%)	ORR 92% vs 72%
				3-y PFS: 71% vs 15%
				3-y OS: 88% vs 80%

For all patients in whom therapy is anticipated in the R/R setting, assessment of clonal evolution through FISH and *TP53* mutational testing is indicated. Resistance mutations, including mutations in *BTK* and 1-phosphatidylinositol 4,5-bisphosphate phosphodiesterase γ-2 (*PLCγ2*) for patients on BTKi and *BCL2* for patients on venetoclax, have been described in patients progressing on targeted agents, although testing for these mutations is not routinely performed at this time,[16-21] likely because of limited availability. Furthermore, selection of the subsequent line of therapy is not affected by results of these tests based on currently approved agents, although understanding mutational profile may eventually inform treatment selection as we further understand efficacy of new agents in the setting of these mutations.

Although initial reports of progression on BTKi described a poor prognosis,[12,22] use of BTKi in earlier lines of therapy and development of additional classes of effective novel agents have significantly improved the outlook for patients experiencing progression of CLL while on BTKi.[23,24] Selection of agents after progression on frontline ibrutinib is informed by a series of prospective and retrospective real-world studies of patients treated with ibrutinib in the R/R setting and subsequently treated with novel agents (Table 2). Robust data on selection of therapy in a non–chemotherapy-exposed cohort is not available at this time.

With a median of 4 prior lines of therapy (range, 1-15), a phase 2 clinical trial examined efficacy of venetoclax as a continuous monotherapy in 91 patients who had been previously treated with ibrutinib and progressed before the start of venetoclax. Venetoclax monotherapy was associated with an overall response rate (ORR) of 65%. Regarding outcomes, 1-year progression-free survival (PFS) and overall survival (OS) were estimated to be 75% and 91%, respectively.[24] Venetoclax in combination with rituximab for a 2-year fixed duration was studied in the phase 3 MURANO study, which demonstrated the efficacy of this regimen in patients who had received 1 to 3 prior therapies with an ORR of 92% and 3-year PFS and OS of 71% and 88%, respectively. Although only 5 patients (2.6%) in this study had received prior BCRi, results of this study have been applied widely to patients progressing on ibrutinib in clinical practice, and therefore the combination of venetoclax and rituximab after progression on BTKi is also a reasonable choice.[9,10] In patients treated with venetoclax and rituximab, achieving deep responses with undetectable minimal residual disease (U-MRD) at the completion of therapy is associated with improved PFS.[9] The optimal approach for patients with residual detectable disease at the end of planned fixed-duration therapy requires further investigation.

A pooled analysis of 4 clinical trials examining venetoclax with or without rituximab in the R/R setting included 436 patients, of whom 149 were BCRi exposed (115 refractory, 34 nonrefractory). ORR for the entire cohort was 75% with a complete remission (CR) rate of 22% and median PFS of 30.2 months. Refractoriness to BCRi was associated with increased risk of failure to respond (odds ratio, 2.3; 95% confidence interval [CI], 1.4-3.7), failure to achieve CR (odds ratio, 4.8; 95% CI, 2.3-9.9), and relapse (hazard ratio, 1.9; 95% CI, 1.2-3.1). Although duration of response was shorter for BCRi-refractory patients independent of depth of response, many of these patients were heavily pretreated (median number of prior therapies, 3; range, 1-15) and had additionally received chemoimmunotherapy.[25]

A retrospective cohort study described 187 patients who had discontinued a BCRi (143 ibrutinib and 35 idelalisib), of whom 114 required subsequent therapy. This included 13 patients treated with venetoclax after discontinuation of either ibrutinib or idelalisib, in whom ORR was 76%. The study further describes that patients treated with idelalisib after ibrutinib discontinuation (n = 16) had an ORR of 28%.[26] In a cohort of 683 patients treated with BCRi (91% ibrutinib, 9% idelalisib) or venetoclax, 167 (24%) received a subsequent therapy after BCRi discontinuation. ORR to venetoclax after

Table 2. Available evidence for standard of care treatment strategies after BTKi discontinuation for CLL progression or intolerance

Subsequent therapy	Study design	Number of patients in group of interest	Clinical setting	Prior therapies, median (range)	ORR	Progression data on subsequent therapy	Survival data on subsequent therapy
Venetoclax	Prospective[24]	91	Progression on ibrutinib (n = 50), progression following ibrutinib discontinuation (n = 41) reasons for ibrutinib discontinuation: intolerance (n = 30), achievement of maximal benefit on ibrutinib (n = 6), completion of defined ibrutinib course (n = 3), unspecified (n = 2)	4 (1-15)	65% (63% in patients with prior ibrutinib intolerance, 54% for progression on ibrutinib)	1-y PFS: 75% median PFS: 24.7 mo	1-y OS: 91%
	Retrospective[26]	13	KI discontinuation (progression or intolerance)	Not reported	76%	Not reported	Not reported
	Retrospective[27]	Not reported	BCRi discontinuation (progression or intolerance)	Not reported	74%	24-mo PFS: 75%	Not reported
	Retrospective	115	Prior ibrutinib	3 (0-11)	69%	12-mo PFS: 68% For entire cohort of 141 venetoclax treated patients, prior BTKi was not associated with inferior PFS	12-mo OS: 88% For entire cohort of 141 venetoclax treated patients
	Retrospective[43]	62 post-BTKi alone, 10 post-BTKi and PI3Ki	BTKi discontinuation (progression or intolerance)	3 (1-15) post BTKi alone, 5 (3-15) post BTKi and PI3Ki	85% in post-BTKi alone, 80% in post BTKi and PI3Ki	1-y PFS 65% Estimated for entire cohort, prior exposure to BTKi was not significantly associated with inferior PFS	1-y OS 75% Median OS 61% Estimated for entire cohort, prior exposure to BTKi was not significantly associated with inferior OS
Acalabrutinib	Prospective[23]	33	Ibrutinib intolerance	4 (2-13)	76%	1-y PFS: 83%	Not reported
Idelalisib	Retrospective[26]	16	Ibrutinib discontinuation (progression or intolerance)	Not reported	28%	Not reported	Not reported
	Retrospective[27]	Not reported	Ibrutinib discontinuation (progression or intolerance)	Not reported	46%	Median PFS: 9 mo	Not reported
Umbralisib	Prospective[38]	44	BTKi intolerant	2 (1-7)	Not reported	Median PFS: 23.5 mo For entire cohort of 51 patients, including 7 with prior PI3Ki intolerance	Not reported

BCRi discontinuation (for progression or toxicity) was 74%, whereas ORR to idelalisib after ibrutinib discontinuation was 46%. Both of these approaches were associated with superior PFS compared with chemoimmunotherapy in this setting (median PFS, 5.1 months; $P < .001$; ORR, 50%).[27]

After progression on venetoclax, retrospective data suggest low response rates when retreating with BTKi if patients have previously experienced progression on a BTKi.[28] Additional US Food and Drug Administration (FDA)-approved novel agents for the treatment of R/R CLL are idelalisib with rituximab and duvelisib. These agents were approved based on studies that did not include BCRi- or venetoclax-treated patients.[7,8] Therefore, all data regarding their efficacy in novel agent exposed patients come from retrospective studies. In a phosphatidylinositol 3-kinase inhibitor (PI3Ki)-naïve but BTKi-exposed (intolerant or resistant) patient population who discontinued venetoclax, ORR to PI3Ki was 47% with short median PFS (6 months).[28] Based on current limited retrospective data, absence of prospective studies, and hypothesized overlapping mechanisms of resistance, responses to PI3Ki are not expected to be durable in patients who are double refractory to BTKi and venetoclax. Patients who are BTKi and venetoclax exposed are considered

Table 3. Available evidence for standard of care treatment strategies after venetoclax discontinuation for progression or intolerance

Subsequent therapy	Study design	Number of patients in group of interest	Clinical setting	Prior therapies, median (range)	ORR	Progression data on subsequent therapy	Survival data on subsequent therapy
Venetoclax or venetoclax/rituximab	Prospective[9]	14	Progression after fixed-duration venetoclax/rituximab (13 completed MURANO regimen, 1 discontinued early)	Not reported	55% (of evaluable patients)	Not reported	Not reported
Ibrutinib	Prospective[41]	8	Progression after fixed-duration venetoclax/rituximab	1 (1-4)	100%	4 on treatment, 3 with PD (median time on ibr 15 mo (3-48))	No deaths reported
	Retrospective[44]	27	Venetoclax discontinuation (18 with PD, 9 for other reasons)	2 (0-9)	56%	9 patients progressed on ibrutinib, time on ibrutinib 3-53 mo	Not reported
	Retrospective[27]	6	Progression on venetoclax	4 (1-7)	5/6 with PR	3 of 6 remain on therapy (6, 13, and 16 mo on therapy)	3 deaths (2 of toxicity, 1 due to progression)
BTKi	Retrospective[28]	44	Venetoclax discontinuation (progression, toxicity), BTK naïve	2 (0-8)	84%	Median PFS 32 mo	Not reported
	Retrospective[28]	30	Venetoclax discontinuation (progression, toxicity), BTK exposed (33% intolerant, 66% resistant)	4 (1-11)	53%	Median PFS 12 mo	Not reported
	Retrospective[42]	23	Venetoclax resistance, BTK naïve	4 (2-9)	91%	Median PFS 34 mo	
PI3Ki	Retrospective[28]	17	Venetoclax discontinuation (progression, toxicity), BTK exposed, PI3K naive	4 (1-6)	47%	Median PFS 5 mo	Not reported

high risk, and therefore, consideration of cellular therapy (allogeneic stem cell transplant or chimeric antigen receptor T [CAR-T] cell therapy) or enrollment in a clinical trial is appropriate for these patients.

Although data on allogeneic stem cell transplant in patients previously exposed to novel agents are limited,[29] current consensus guidelines recommend consideration of cellular immunotherapy (transplant or CAR-T) for patients considered high risk. This is defined as any patient with R/R CLL after chemoimmunotherapy, responding to BTKi or venetoclax with high risk features (TP53 aberration, complex karyotype, multiple lines) and low cellular immunotherapy risk; with nonresponse to first novel agent; or refractory to 2 novel agents.[30] In a cohort of 48 patients with CLL previously treated with a median of 2 (range, 1-9) lines of therapy before ibrutinib who subsequently underwent allogeneic stem cell transplant, 12-month PFS was 60% and OS was 72%. Compared with series of ibrutinib-naïve patients undergoing transplant, prior ibrutinib did not appear to adversely affect the safety or efficacy of transplant. Given that many patients with CLL have advanced age and comorbidities, CAR-T therapy, often associated with less morbidity and mortality, provides an appealing cellular immunotherapy option. Twenty-four patients with CLL resistant to ibrutinib subsequently received CD19-directed CAR-T cells and experienced an ORR of 71% and CR rate of 17%.[31] TRANSCEND CLL 004, an ongoing study, has reported an ORR of 87% with CR rate of 47% and U-MRD rate of 47% in 16 patients treated with the CD19-directed CAR-T lisocabtagene maraleucel in patients with R/R CLL previously exposed to BTKi.[32] Ibrutinib has further been studied as a tool for enhanced response to or persistence of CAR-T cells given its immune modulatory effects. Ibrutinib exposure appears to result in greater ex vivo T-cell expansion and higher ORR.[33] Despite the risks associated with cellular therapy, the potential for durable remission may outweigh risks for patients with high-risk disease and should be considered. Current studies are examining CD19-directed CAR-T cells in patients with failure of or incomplete response to ibrutinib and/or other novel agents (NCT03331198, NCT03960840, NCT03676504, NCT03085173) and CD19 CAR-T cells, CD19/CD20 CAR-T cells, CD19/CD20 or CD22, CD19/CD28 CAR-T cells, CD20 CAR-T cells, or γδT cells in patients with R/R CLL with or without prior novel agent exposure. Allogeneic cell sources are also being explored in patients with R/R CLL with or without prior novel agent exposure (NCT03881774, NCT03056339).

Agents with alternate mechanisms of action are currently under investigation with promising preliminary data. Two promising noncovalent reversible BTKi (LOXO-305 and ARQ 531) have reported data from small cohorts treated in early-phase clinical trials. The phase 1 clinical trial of LOXO-305 has reported outcomes of 9 patients with CLL, of whom 7 had received prior ibrutinib and 5 had prior PI3Ki. All CLL patients with available response assessments had documented response, including 1 with BTK C481S mutation.[34] Results from the phase 1 study of ARQ 531 demonstrated acceptable safety and evidence of

efficacy with PR in 7 of 26 (27%) patients who had CLL, of whom 85% had documented BTK C481S mutations.[35] Enrolling clinical trials are additionally examining other potential mechanisms for treating CLL, including inhibition of Syk, ATR, MALT1, STAT3, CDK, JAK1, MELK, PKC-B, XPO1, NEDD8-activating enzyme, and checkpoints; monoclonal antibodies targeting CD38, CD32-b, ROR1, FcγRIIB, Mcl-1, PSMB5, and B-cell activating factor; bispecific T-cell engagers; peptide vaccination; and combination therapies, among others.

Recognizing limitations of applying available data to patients who have received only 1 prior line of therapy, venetoclax ± rituximab after BTKi failure appears to produce higher response rates and improved outcomes than other standard of care options (PI3Ki, anti-CD20 monoclonal antibodies, or chemoimmunotherapy). For patients treated with a BTKi in the frontline setting, we recommend second-line treatment with venetoclax as a continuous therapy or venetoclax with rituximab as a 2-year fixed duration therapy as standard of care options, with selection between these regimens dependent on patient preference. Alternately, enrollment in clinical trials should be considered if available and of interest to the patient. Subsequent lines of therapy may include PI3K inhibitors (FDA approved but associated with lower response rates, shorter durations of response, significant risk of adverse effects, and limited data in patients previously exposed to novel agents), cellular therapies (depending on availability and patient age/comorbidities), and clinical trial enrollment (Figure 1).

Treating relapsed disease after intolerance to BTKi

Although there is no standard definition of intolerance, real-world evidence suggests that intolerance, rather than progression, is the most common reason for ibrutinib discontinuation.[36] With 6 years of follow-up for patients treated in the RESONATE trial, 16% had discontinued ibrutinib because of an adverse event, whereas retrospective series have suggested 20% discontinued for intolerance.[5,36] These data suggest that discontinuation of ibrutinib for adverse event is a commonly encountered clinical scenario.

For all patients in whom therapy for R/R CLL is being considered, it is first important to recognize that CLL may not require therapy immediately on progression. Instead, therapy should be initiated when CLL becomes symptomatic, including development of disease-related symptoms, anemia, thrombocytopenia, or massive or symptomatic splenomegaly, lymphadenopathy, or extranodal involvement.[11]

Patients with PR or CR may be able to discontinue therapy without immediate or significant disease progression, thus allowing for a treatment-free interval. For instance, among 354 patients who received ibrutinib/rituximab through the E1912 trial, 95 have discontinued ibrutinib (51% for adverse effects, 24% for progression, 25% for other reasons). For patients who had discontinued ibrutinib, a median of 23 months elapsed from discontinuation to disease progression.[37]

Once a patient requires treatment, providers must first determine their comfort with retreating patients with an alternate BTKi dependent on the reason for initial intolerance. Although some patients discontinue BTKi for persistently bothersome, but not life-threatening, adverse events, others have more substantial adverse events (ie, major bleeding, cardiac arrythmia) in which retreatment with an alternate BTKi is not deemed safe.

A study of 33 patients with ibrutinib intolerance examined the efficacy of acalabrutinib (a second-generation, highly specific BTKi), which results in an ORR of 76% with estimated 1- and 2-year PFS of 83% and 76%, respectively.[23] In this series, the overall discontinuation rate of acalabrutinib was 12%. For patients who experienced nonsevere toxicity with their initial BTKi, changing to an alternate BTKi is likely to produce response, and toxicity may not recur. For patients in whom BTKi associated toxicity was severe, venetoclax-based regimens are often a more appropriate second-line therapy.[24] Additionally, PI3Ki have been examined in BTKi intolerant patients. A phase 2 trial examined the safety and efficacy of the PI3Ki umbralisib in patients with BCRi intolerance (44 with ibrutinib intolerance, 7 with idelalisib intolerance) and demonstrated minimally overlapping toxicity profile (4 patients had recurrent toxicity, 1 required dose modification) and median PFS of 23.5 months.[38] Retrospective cohorts examining idelalisib after ibrutinib discontinuation (for intolerance or progression) have reported an ORR of 28% to 46%.[26,27] Thus, PI3Ki have data to support their use in this setting as well.

Clinical case 2

A 57-year-old man was diagnosed with CLL after presenting to his primary care physician with cervical lymphadenopathy and cough. A CBC showed a white blood cell count of 32.7 × 10^9/L, ALC of 31.1 × 10^9/L, hemoglobin of 11.0 g/dL, and platelet count of 125 × 10^9/L. Flow cytometric analysis of peripheral blood confirmed the diagnosis of CLL. Given that cough was a presenting symptom, a CT scan was obtained and demonstrated bulky hilar adenopathy, as well as bulky adenopathy throughout his abdomen and pelvis, up to 12 cm. IGVH was unmutated, FISH was without abnormalities, and next-generation sequencing revealed *TP53* mutation. Given the desire to pursue time-limited therapy, the decision was made to treat with a 1-year fixed-duration of venetoclax and obinutuzumab.[3] He was hospitalized for dose escalation per the venetoclax FDA package insert and tolerated therapy well without any complications.

At the end of 1 year of therapy, he had achieved CR with detectable MRD (0.45% of the peripheral blood). CBC at completion of therapy was consistent with CR, showing a white blood cell count of 6.4 × 10^9/L, ALC of 1.3 × 10^9/L, hemoglobin of 15.4 g/dL, and platelet count of 165 × 10^9/L. A CT scan showed resolution of all prior lymphadenopathy.

Twenty-eight months after completion of therapy, he returned to clinic with fatigue and night sweats. CBC showed development of anemia with hemoglobin of 9.6 g/dL. PET was performed and showed bulky adenopathy above and below the diaphragm, although no lesion had a standardized uptake value greater than 4.0. He presents to discuss therapeutic options.

Treating relapsed disease after progression on or after a venetoclax-based regimen

Venetoclax and obinutuzumab as a 1-year fixed duration combination regimen was FDA approved as a first-line regimen for CLL in 2019 based on findings from the CLL14 study.[3] Notably, this regimen appears to produce less durable remission for those with detectable MRD (vs U-MRD), and 6 of 14 early relapses after venetoclax discontinuation in CLL14 had aberration in *TP53*. Even with this in mind, the number of patients who have received 1 year of fixed duration therapy and subsequently progressed to the point of requiring therapy is low at this time. Thus, data

regarding efficacy of novel agents in this setting are limited, particularly in a population who had not previously received chemoimmunotherapy. To inform decision making about treatment in this case, data are extrapolated from data regarding patients who have progressed on venetoclax-based regimens in R/R settings.

The initial phase 1b study examining the safety of venetoclax/rituximab allowed for protocol-guided drug discontinuation for patients who achieved CR or U-MRD in the bone marrow compartment. This study included 49 patients, of whom 13 stopped therapy (2 with CR but detectable MRD, 11 with U-MRD). Both patients with detectable MRD experienced progression of disease after 24 months and subsequently responded to venetoclax retreatment.[39] Of the 194 patients treated with venetoclax-rituximab in the phase 3 MURANO study, data regarding response to subsequent therapy are available for 22 patients who have experienced progression of disease and required retreatment. Of 14 patients who received subsequent venetoclax-based therapy (8 with venetoclax/rituximab for 2-year fixed duration, 3 with venetoclax monotherapy, 2 with venetoclax/rituximab continuous therapy, and 1 with venetoclax/ibrutinib), the ORR was 55%.[40] Notably, the patients who have relapsed at this time had relatively short treatment-free intervals, likely because of the lack of deep response to venetoclax/rituximab or more aggressive disease biology. Retreatment with venetoclax may be more favorable for patients who derive more benefit (ie, deeper response or longer treatment-free interval) from their first course of venetoclax-based therapy. Of 8 patients who were subsequently treated with ibrutinib, the ORR was 100% in a heavily pretreated cohort.[41]

Retrospective studies have examined various therapeutic strategies after venetoclax discontinuation (Table 3). In a cohort of 326 patients who had discontinued venetoclax for any reason, 74 were subsequently treated with BTKi (44 BTKi naïve, 10 BTKi intolerant, 20 BTKi refractory). The ORR to BTKi in a BTKi naïve population was 84%, whereas BTKi-exposed patients had an ORR of 54%. Treatment with BTKi after venetoclax discontinuation was associated with a median PFS of 32 months in BTKi-naïve patients. For BTKi-exposed patients, the setting of prior BTKi failure (progression vs intolerance) significantly impacted PFS (median, 4 months vs not reached).[28] In a cohort of 23 patients treated with BTKi after venetoclax discontinuation in the setting of disease progression (median prior lines of therapy, 4; range, 2-9), ORR was 91%, median PFS was estimated to be 34 months, and median OS was 42 months.[42]

For patients who have progressed after frontline venetoclax and obinutuzumab, initial data suggest that BTKi is likely to produce high response rates and durable remissions. For patients who had deep responses and/or prolonged treatment-free intervals after fixed duration venetoclax therapy, retreatment with a venetoclax-based regimen is an appealing option, although additional data on the efficacy of this approach are needed. Subsequent lines of therapy may include PI3Ki, cellular immunotherapy, and treatment in a clinical trial as described previously (Figure 2).

Summary

We have examined the available data guiding treatment sequence decisions in a chemotherapy-free paradigm and noted gaps in the current literature. Decision making is largely extrapolated from studies that included heavily pretreated patients. As such, it is likely that response rates and outcomes will be improved when these agents are used in earlier lines of therapy. As CLL is a chronic disease in which we aim to sequence many therapies to extend survival, future clinical trials should include long-term follow-up to observe subsequent therapies and incorporate sequencing decisions in their design.

Several novel agent combination therapies are currently being studied (NCT03755947, NCT03836261, NCT03580928, NCT03824483). Although these regimens are designed to induce high rates of response and deep remissions with the goal of extending PFS, the optimal approach to therapy for patients who are treated with multiple novel agents simultaneously in the R/R setting remains entirely unexplored and will present an opportunity for active investigation in the future.

Review of this data further highlights the need for studies examining sequencing in a chemotherapy-free paradigm. Because these agents tend to be highly effective with extended periods of PFS, examining these sequences in retrospective, real-world studies is likely to provide data that will be difficult to capture in a prospective fashion.

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Conflict-of-interest disclosure

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Off-label drug use

Umbralisib is not FDA approved for the treatment of CLL. Data regarding investigational (non-FDA approved) agents are reviewed.

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Chemotherapy-free frontline therapy for CLL: is it worth it?

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The treatment of chronic lymphocytic leukemia (CLL) embodies one of the great success stories in translational research, with the development of therapies aimed at disrupting crucial pathways that allow for the survival and proliferation of the malignant clone. The arrival of targeted agents into our armamentarium, along with the advent of novel monoclonal antibodies that can achieve deeper remissions, has steered the field to a new treatment paradigm. Given the panoply of therapeutic options available, the question arises whether chemotherapy still has a role in the management of CLL. The novel targeted agents, which include the Bruton's tyrosine kinase inhibitors, ibrutinib and acalabrutinib, along with the B-cell lymphoma-2 inhibitor, venetoclax, are highly effective in achieving a response with improved remission duration and survival, particularly in high-risk patients. Despite this major progress, the new agents bring a unique set of toxicities unlike those associated with cytotoxic chemotherapy. There is a paucity of head-to-head comparisons among all of the novel agents, because their approval was based on randomization against traditional chemoimmunotherapeutic regimens. Parallel to the increase in the number of available targeted agents, there has been a significant improvement in quality of life and life expectancy of the patients with a CLL diagnosis over the last decade. Our review will examine whether "chemotherapy-free" frontline treatment approaches are worth the associated risks. Our goal is to help identify optimal treatment strategies tailored to the individual by reviewing available data on monotherapy vs combination strategies, depth of response, treatment duration, and potential toxicities.

LEARNING OBJECTIVES

- Review the current "chemotherapy-free" regimens available for the management of patients with treatment-naive chronic lymphocytic leukemia
- Review the prognostic markers that identify which patients may benefit from chemoimmunotherapy based approach vs the use of targeted agents
- Recognize criteria for selection of the optimal treatment strategy

Introduction

In 2020, the Surveillance, Epidemiology and End Result program database estimated 21 040 new cases of chronic lymphocytic leukemia (CLL) in the United States, with 4060 deaths attributed to this disease.[1] The natural history of CLL is variable, and outcomes are influenced by patient characteristics, clinical factors at the time of diagnosis, and the intrinsic biology of the tumor. Given the heterogeneity of the disease, there is no "one size fits all" recommendation. Until recently, systemic chemoimmunotherapy (CIT) had been considered the standard of care for frontline management. The CIT regimens of fludarabine/cyclophosphamide/rituximab (FCR) and bendamustine/rituximab (BR) had demonstrated excellent response rates, progression-free survival (PFS), and overall survival (OS) in the patients who could tolerate these regimens.[2,3] Nonetheless, significant myelosuppression and infectious complications made their use difficult to tolerate in elderly patients with comorbidities. Additionally, patients with a deletion 17p (del17p) or TP53 mutation (TP53mut) were considered ultrahigh risk,[4] because these patients progressed more rapidly and invariably relapsed shortly after CIT. The frontline treatment paradigm changed with the approval of the first-in-class Bruton's tyrosine kinase inhibitor (BTKi), ibrutinib, in patients with del17p.[5] This approval was a revolution in the treatment of del17p/TP53mut, achieving an OS never seen with prior therapies.[6-8] Shortly after, ibrutinib was approved for all patients and, later, other

targeted agents followed, including the second-generation BTKi, acalabrutinib, and the B-cell lymphoma-2 (BCL-2) inhibitor, venetoclax. All of these agents are now preferred regimens in the United States for the initial treatment of CLL, with or without del17p.[9]

In the era of multiple available targeted agents and CIT approaches, the following questions remain: Which patients benefit most from each regimen? Are the novel regimens worth their risk for potential complications? Is there a role for early intervention now that these novel agents are available? Are these drugs best used sequentially or in combinations?

Clinical case part 1

A 63-year-old man with a history of hypertension and hyperlipidemia presents for evaluation after an incidental finding of lymphocytosis (8000 lymphocytes per microliter) on routine complete blood count with normal hemoglobin and platelet count. On examination, he has mildly enlarged axillary and inguinal lymph nodes (~1.5-2 cm). No hepatosplenomegaly is appreciated on physical examination. He denies any constitutional symptoms. Flow cytometry reveals a $CD5^+$ $CD10^-$ $CD19^+$ $CD20^{dim}$ $CD23^+$ $CD200^+$ and κ-restricted monoclonal B cell population, confirming a diagnosis of CLL. Additional prognostic testing reveals unmutated immunoglobulin heavy chain (UM-IGHV) with a normal β-2 microglobulin level. Fluorescence in situ hybridization (FISH) testing is positive for trisomy 12. Next-generation sequencing does not reveal TP53mut.

Management of treatment-naive CLL in the era of targeted therapies

The role of prognostic factors

Risk stratification gives us the tools to deliver appropriately targeted care in a disease with a clinical presentation as varied as CLL. Outcomes are influenced by comorbidity burden, clinical factors, and the genetics of the tumor. Two staging criteria, the Rai[10] and Binet[11] systems, are widely used in clinical practice because of their simplicity (only a physical examination and a complete blood count are needed) and accuracy in predicting outcomes. High-risk group patients (Rai III/IV, Binet C) have a more aggressive clinical course associated with shorter survival. Further risk stratification is based on molecular and genomic studies, including IGHV mutation status[12] and cytogenetic analysis with FISH testing, including del17p,[13] del11q, trisomy 12, and del13q.[14] Complex karyotype, defined as ≥3 to 5 chromosomal abnormalities is associated with a worse prognosis.[15,16] Genomic mutations, particularly in TP53,[17,18] mark a more aggressive disease course. In 2016, the CLL International Prognostic Index (CLL-IPI) incorporated important genomic factors (IGHV, TP53 mutational state) with Rai/Binet stage, β-2 microglobulin level, and age to predict survival.[19] The validity of the CLL-IPI has been confirmed across several series, including mostly younger patients treated with CIT.[20] This novel prognostic index appears to be predictive of time to first therapy[20] in early-stage disease, thus identifying high-risk cohorts that may benefit from participation in early-intervention clinical trials. Nonetheless, the utility of the CLL-IPI to predict OS in patients starting targeted therapy remains uncertain.[21]

Timing and selection of therapeutic strategy

Consensus guidelines of the International Workshop on Chronic Lymphocytic Leukemia recommend initiating treatment only at the onset of constitutional symptoms (Table 1).[22] Data are lacking at this time to suggest a benefit of early intervention based on prior studies utilizing CIT at the time of diagnosis.[23,24] Trials studying the role of novel agents for patients with high-risk CLL are ongoing,[25-27] with a trial on ibrutinib against placebo presented in abstract form in 2019. The primary end point was event-free survival, defined as time from randomization until occurrence of active disease, new treatment, or death. At a median observation time of 31 months, event-free survival was 47.8 months in the placebo arm vs not reached in the ibrutinib arm (hazard ratio [HR], 0.25; 95% confidence interval [CI], 0.14-0.43; $P < .0001$). PFS was 14.8 months in the placebo arm vs not reached in the ibrutinib arm (HR, 0.18; 95% CI, 0.12-0.27). Time to next treatment was longer in the ibrutinib arm.[27] Until data from the full survival analysis are available, continued active surveillance of early-stage patients who have an increased risk for progression remains the standard of care.

Clinical case part 2

Based on the above review of clinical presentation and prognostic markers, our patient did not meet criteria for initiation of CLL-directed therapy at the time. Over the next 4 years, the patient's absolute lymphocyte count increases to 120 000 cells per microliter, and he develops symptomatic anemia with a hemoglobin of 9.8 g/dL and thrombocytopenia (platelets, 98 000 per microliter). He now has stage 3 chronic kidney disease (glomerular filtration rate, 50 mL/min per 1.73 m^2) attributed to hypertension. His Eastern Cooperative Oncology Group (ECOG) performance status is 0, and he remains active. He has palpable cervical (3×3cm) and axillary (3×4-cm) lymphadenopathy, and his spleen tip is palpable 3 cm below the costal margin. Repeat FISH testing reveals trisomy 12 without del17p, and next-generation sequencing is negative for TP53mut. He understands that he is in need of therapy and wishes to discuss available treatments.

Brief overview of chemotherapeutic approaches

Fludarabine-based chemotherapy regimens were standard of care for patients younger than 65 to 70 years of age based on the data from the CLL8 trial, which demonstrated an improvement in PFS for patients treated with FCR compared with fludarabine-cyclophosphamide.[2,28] Patients with mutated immunoglobulin heavy chain (M-IGHV) had the longest PFS, with the subset of patients achieving undetectable minimal residual disease (uMRD, defined as no evidence of CLL at a level of 1 in 10,000 cells) showing no evidence of disease relapse, even a decade posttreatment.[29] However, this regimen is generally too toxic for older patients and those with comorbidities. CLL10 compared FCR with BR in patients with CLL who were fit to receive CIT.[30] In the intention-to-treat population, BR was found to be inferior to FCR, with the exception of the subset of patients older than 65 years where the PFS was equivalent. Furthermore, FCR was associated with a higher rate of severe neutropenia and infectious complications, and this was more pronounced in patients above the age of 65 years. Based on these findings, BR was frequently used as a standard of care for patients over 65 years of age who were fit to receive intensive CIT. To determine optimal therapy for older unfit patients, CLL11 randomized patients who were not candidates for CIT to chlorambucil (Chl) monotherapy, chlorambucil-rituximab, or chlorambucil-obinutuzumab (ChlO).[31] ChlO demonstrated a superior overall response rate (ORR) of

Table 1. International Workshop on Chronic Lymphocytic Leukemia 2018 indications for therapy

Progressive marrow failure, as evidenced by development or worsening anemia < 10 g/dL or thrombocytopenia (<100 000 platelets per liter)
Massive or progressive symptomatic splenomegaly
Massive lymph nodes (>10 cm) or progressive symptomatic lymphadenopathy
Rapidly increasing lymphocytosis defined as an increase of 50% over a 2-mo period or a lymphocyte doubling time < 6 mo*
Autoimmune complications (anemia, thrombocytopenia) poorly responsive to corticosteroids
Symptomatic extranodal involvement
Constitutional symptoms
Unintentional weight loss > 10% within 6 months
Progressive fatigue
Temperature > 100.5°F for >2 weeks without another cause
Night sweats for >1 month without alternative etiology
ECOG PS > 2 if progressive/worsening

ECOG PS, Eastern Cooperative Oncology Group Performance Status.
*Absolute lymphocytosis alone is not an indication for treatment but can be used to determine disease pace; leukostasis rarely occurs in patients with CLL.

75.5% compared with ChlR (65.9%) and chlorambucil-rituximab (30%), with a median PFS of 29.2 months for ChlO.[32] Given the shorter PFS compared with historical data for FCR and BR, Chl-based combinations were traditionally reserved for patients who were unable to tolerate intensive CIT.

The new era of chemotherapy-free frontline treatment regimens: ibrutinib, acalabrutinib, venetoclax, and beyond

The success of the BTKi, ibrutinib, in the relapsed/refractory setting,[33] particularly for patients with high risk features, such as del17p/TP53mut, allowed for its quick approval as frontline treatment. This was followed by the RESONATE-2 trial, the frontline trial for patients over the age of 65 years who were unable to tolerate intensive regimens. Patients were randomized to receive ibrutinib vs Chl monotherapy. At 18.4 months of follow-up, median PFS for patients treated with ibrutinib was not reached compared with 18.9 months for patients treated with Chl, with a corresponding decrease in the risk of death of 84% in the ibrutinib group (HR, 0.16; $P = .001$).[5] Long-term follow-up (median, 60 months) demonstrated a sustained PFS and OS benefit for patients treated with ibrutinib compared with Chl (5-year PFS: 70% vs 12%; HR, 0.146; 5-year OS: 83% vs 68%; HR, 0.45).[34] Improvement in PFS was seen across subgroups, including del11q and UM-IGHV. There were more hematologic adverse events with Chl monotherapy and more nonhematologic adverse events with ibrutinib. Six percent of patients developed atrial fibrillation, and 4% of patients developed major hemorrhage. After 58 months of follow-up, the most common adverse events were diarrhea (50%), cough (36%), and fatigue (36%).[34] Rates of atrial fibrillation did not increase over time, but rates of hypertension remained consistent over 5 years. Ibrutinib yields long-term responses and is well tolerated for long durations of continuous therapy. Responses are improved in patients across all risk groups, including patients with historically poor prognostic factors, such as del11q and UM-IGHV.

Two pivotal trials confirmed the benefit of upfront treatment with ibrutinib in older patients (age ≥ 65-70 years) compared with chemotherapy. A047102, an Alliance-led National Clinical Trials Network study, compared ibrutinib with ibrutinib-rituximab (IR) and with BR in patients over the age of 65 years.[35] At a median follow-up of 38 months, ibrutinib and IR demonstrated superior PFS compared with BR (HR, 0.38 and 0.39, respectively). There was no difference in PFS between ibrutinib and IR, and 2-year PFS was 87% with ibrutinib, 88% with IR, and 78% with BR. PFS was improved in patients with del17p (median not reached in ibrutinib arms vs 7 months with BR). Zap70 methylation did not show a significant difference in PFS among the 3 cohorts. No differences in OS between the ibrutinib-containing arms and BR were reported, although patients initially treated with BR were allowed to crossover to ibrutinib upon confirmed disease progression. Nonhematologic toxicities were more common in the ibrutinib-containing arms. Atrial fibrillation occurred in 14% and 17% of patients treated with ibrutinib and IR, respectively. Other common side effects in the ibrutinib-containing arms are hypertension (~30% of patients), hematologic problems (anemia, neutropenia, thrombocytopenia), and infections. The rates of serious bleeding events were <5%. It is important to note that a numerically higher number of unexplained deaths occurred in the ibrutinib-containing arms. When compared against CIT, treatment with an ibrutinib-containing regimen improves PFS in older patients. Although ibrutinib is generally considered to be well tolerated, patients still require close monitoring for the potential development of toxicities, particularly cardiovascular toxicity.

The iLLUMINATE trial tested ibrutinib in combination with obinutuzumab (IO) in patients older than 65 years (or in patients younger than 65 years with comorbidities unsuitable for fludarabine-based CIT). Patients were randomized to IO or ChlO.[36] At a median follow-up of 31.3 months, PFS was not reached for patients treated with IO vs 19 months for patients treated with ChlO; 30-month PFS was 79% for IO and 31% for ChlO. ORR was higher for patients on IO vs ChlO (88% vs 73%), with more patients achieving a complete response (CR; 41% vs 16%). In patients with del17p, median PFS was not reached with IO compared with 11.3 months with ChlO. Similarly, patients with UM-IGHV had improved PFS (not reached vs 14.6 months), but there was no difference in PFS in patients with M-IGHV. Hematologic adverse

events were similar between the 2 treatments. Patients treated with IO had higher rates of hypertension (17%), atrial fibrillation (6%), upper respiratory tract infections (14%), and musculoskeletal toxicities (35%). These frontline ibrutinib trials demonstrate that ibrutinib (with or without an anti-CD20 antibody) improve PFS in older patients (or those unfit to receive CIT). Importantly, atrial fibrillation and hypertension remain 2 of the common toxicities that need close monitoring, particularly in an older patient population. In summary, ibrutinib improves the outcomes of elderly patients across prognostic groups compared with CIT regimens.

The ECOG 1912 trial sought to compare IR with FCR in younger patients (age < 70 years) fit to receive intensive CIT.[37] Patients were assigned 2:1 to IR (n = 354) or to FCR (n = 175). Notably, patients with del17p by FISH were excluded from the study, given the anticipated poor outcomes with FCR. The primary and secondary end points were PFS and OS, respectively. At a median follow-up of 34 months, PFS and OS favored ibrutinib-based therapy. Specifically, PFS was 89.4% (95% CI, 86-93) in the IR arm compared with 72.9% (95% CI, 65.3-81.3) in the FCR arm (HR, 0.35; $P < .001$). Although the number of deaths in both arms was limited, a statistically significant improvement in 3-year OS was also observed for IR vs FCR (98.8% vs 91.5%; HR for death, 0.17; 95% CI, 0.05-0.54; $P < .001$), with the majority of deaths in the FCR cohort attributed to CLL progression. On subset analysis, the PFS advantage observed with ibrutinib was statistically significant for UM-IGHV but did not reach statistical significance for M-IGHV. Higher rates of hematologic toxicity, including febrile neutropenia, occurred in the FCR group, and higher rates of hypertension, atrial fibrillation, and arthralgias were seen with IR. Updated results presented at the American Society of Hematology Annual Meeting in 2019 showed that, after a median follow-up of 48 months, IR continued to demonstrate superiority with regard to PFS and OS.[38] With extended follow-up, 73% of patients treated with IR remained on therapy, with the majority of treatment discontinuations attributed to drug toxicities rather than to progression. Patients who discontinued IR prior to progressive disease (PD) or death did not progress for a median of 23 months after the last dose of ibrutinib. Grade 3 and above treatment-related adverse events throughout the entirety of the study period were observed in 70% of IR-treated patients and 80% of FCR-treated patients (odds ratio, 0.56; 95% CI, 0.34-0.90; $P = .013$). Grade 3 or above events of interest, including hypertension and atrial fibrillation, occurred in 8.5% and 2.8% of IR-treated patients vs 1.9% and 0% of FCR-treated patients, respectively. There were higher rates of major bleeding with IR (1.1%) compared with FCR (0%). Lower rates of hematologic toxicities, including neutropenic fever, were seen in the IR group compared with FCR. Other common toxicities (all grades) with IR include upper respiratory tract infections (29%) and musculoskeletal toxicities (61%). IR demonstrates excellent clinical efficacy in younger patients, particularly those with UM-IGHV. Although the risk is smaller in younger patients, the commonly observed side effects of atrial fibrillation, hypertension, bleeding events, and infections remain a reason for drug discontinuation.

Notwithstanding the fact that ibrutinib is well tolerated overall, the toxic effects have been attributed, in part, to off-target inhibition of other kinases.[39] As such, several second-generation BTKi's with more selectivity have been developed in an attempt to improve the safety profile. Acalabrutinib, a second-generation covalent BTKi, was recently approved for frontline CLL treatment based on results from the ELEVATE-TN trial.[40] In this study, patients were randomized to receive acalabrutinib monotherapy, acalabrutinib-obinutuzumab (AO), or ChlO. At a median follow-up of 28.3 months, median PFS was not reached for AO vs ChlO (22.6 months), with a 90% reduction in relative risk of death or progression (HR, 0.10; 95% CI, 0.06-0.17; $P > .0001$). Similar PFS improvements were seen in patients treated with acalabrutinib (not reached) compared with ChlO (22.6 months), with an 80% risk reduction in death or progression (HR, 0.2; 95% CI, 0.13-0.30; $P < .0001$). Estimated 24-month PFS was 93% in the AO arm, 87% for acalabrutinib, and 47% for ChlO. Best ORR was 94% for AO, 86% for acalabrutinib, and 79% for ChlO. CRs were 13% for AO, 5% for ChlO, and 1% for acalabrutinib. AO demonstrated improved estimated 24-month PFS compared with ChlO across prespecified subgroups, including UM-IGHV (88% vs 76%), M-IGHV (96% vs 76%), and del17p (88% vs 22%). It is important to note that this trial was not powered to compare acalabrutinib vs AO, so the importance of adding an anti-CD20 antibody to upfront therapy remains unknown. Adverse events were equal across treatment arms, with headache (60-70%), diarrhea (62-69%), and upper respiratory tract infections (18-21%) being more common in the acalabrutinib-containing arms compared with ChlO. Neutropenia was more frequent with ChlO (45%) compared with AO (31.5%) and acalabrutinib (10.6%). With >2 years of follow-up, 79.3% of the patients in both acalabrutinib-containing arms remain on single-agent acalabrutinib, demonstrating the drug's activity and tolerability in treatment-naive patients, including patients with poor prognostic factors (eg, UM-IGHV and del17p).

Venetoclax, a novel orally bioavailable small molecule inhibitor for selective targeting of BCL-2, has proven efficacy and safety in CLL. The drug was initially approved by the US Food and Drug Administration (FDA) as continuous monotherapy for the treatment of patients with relapsed CLL with del17p.[41] Approval was then extended to all relapsed patients in combination with rituximab as a fixed-duration regimen[42]; more recently, it was approved for frontline therapy in combination with obinutuzumab based on the CLL14 trial.[43] CLL14 randomized patients with significant medical comorbidities to receive a fixed duration of 1 year of treatment with venetoclax-obinutuzumab (VenO) or 6 months of ChlO.[44] At a median follow-up of 28.1 months, 24-month PFS was 88.2% in the VenO group and 64.1% in the ChlO group (HR, 0.39; 95% CI, 0.22-0.44; $P < .0001$). At 39.6 months, recently updated data has demonstrated continued PFS benefit (not reached vs 35.6 months) for VenO and ChlO.[45] In patients with del17p/TP53mut, median PFS has not been reached for VenO, whereas it is 19.8 months for ChlO. For patients with UM-IGHV, median PFS was not reached for the VenO group, and it was 26.3 mo for ChlO. A PFS benefit was also seen for patients with M-IGHV with VenO (not reached) compared with ChlO (42.9 months).[45] Median OS was not reached in either arm, and no difference in OS was noted during this limited observation period. Three patients treated with VenO had laboratory evidence of tumor lysis syndrome (TLS) during treatment with obinutuzumab, but there were no TLS events reported during venetoclax ramp-up. Overall safety for hematologic and nonhematologic adverse events was similar in both groups, with neutropenia being the most common hematologic adverse event. Further follow-up is needed to determine the duration of remission and time to next treatment. Additionally, it remains unknown whether patients can be successfully rechallenged with venetoclax after disease relapse after therapy

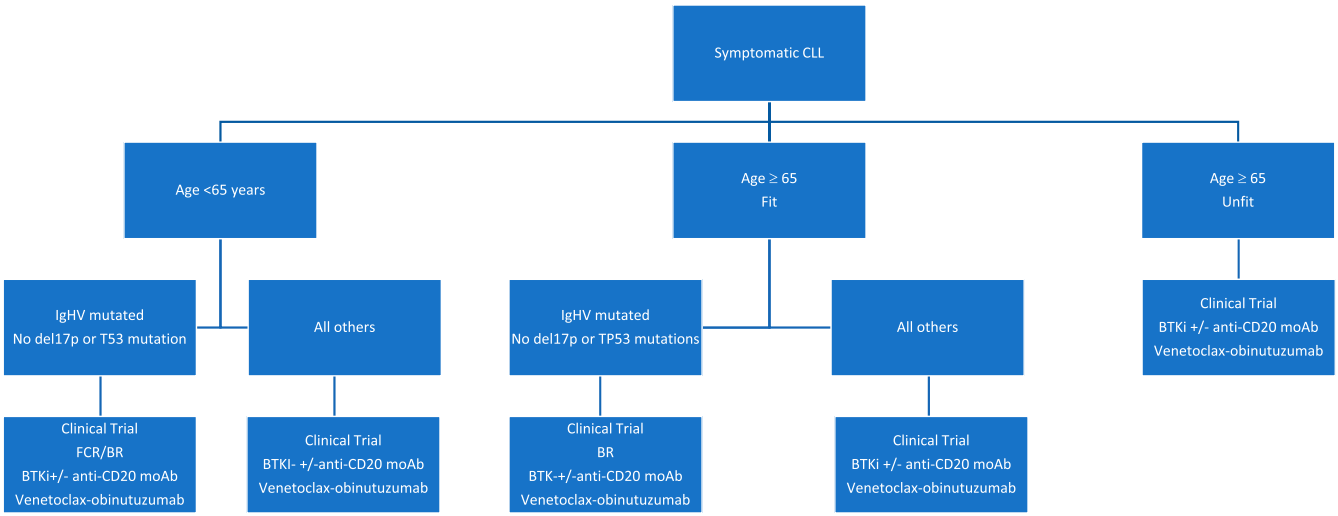

Figure 1. Proposed frontline treatment algorithm. moAb, monoclonal antibody.

discontinuation, although data from an early-phase clinical trial suggests that this is possible.[46] Similar to CIT regimens, this fixed-duration regimen offers the opportunity for planned treatment discontinuations with demonstrated improvement across prognostic groups, with a 3-year PFS of 82% (2 years posttreatment cessation).

Many combination strategies involving BCL-2 inhibitors and BTKi's, with or without anti-CD20 antibodies, are under study with the goal to induce deeper remissions with time-limited therapy. Ibrutinib-venetoclax has been studied in 2 trials. In a single-center phase 2 trial, the combination of ibrutinib-venetoclax was given for 12 cycles; it demonstrated an 88% CR or CR with incomplete count recovery (CRi) rate, with 61% of patients achieving uMRD.[47] Similar findings were noted in a multicenter phase 2 trial (CAPTIVATE) in which patients received ibrutinib-venetoclax for a total of 12 cycles. There were similar rates of MRD-negative responses in the peripheral blood and bone marrow (75% and 72%, respectively).[48] A single-center phase 2 study of the combination of ibrutinib, venetoclax, and obinutuzumab for frontline CLL treatment also demonstrated an ORR of 96%, with 63% of patients achieving uMRD.[49] Twenty-eight percent of patients achieved a CR with uMRD. Further studies looking at second-generation BTKi's are underway.[50] The triplet acalabrutinib-venetoclax-obinutuzumab showed deep responses, with 75% of patients achieving uMRD.[51] More mature data are needed to determine whether time-limited treatment with doublet or triplet therapy can improve long-term outcomes without increased toxicity burden.

Considerations determining upfront therapy

As discussed above, several factors are important when determining frontline treatment of CLL, including age, CLL prognostic factors, comorbidities, concomitant medications, and patient preferences on treatment duration. Data from phase 3 trials have demonstrated that CLL patients with high-risk features and UM-IGHV benefit from chemotherapy-free frontline regimens, and these options should be offered as the new standard of care.

Patients with M-IGHV CLL, in the absence of del17p or TP53mut, may still benefit from treatment with FCR or BR, and these

Table 2. Summary of significant nonhematologic adverse events on clinical trials with BTKi's

BTKi clinical trial	Arthralgias, %	Atrial fibrillation, %	Bleeding/hemorrhage, %	Hypertension, %	Infection, %
RESONATE-2: ibrutinib[34] (N = 136)	26	16	11	26	12*
A041202					
Ibrutinib[35] (n = 180)	1	17	2*	29*	20*
Ibruitnib-rituximab[35] (n = 181)	2	14	4*	34*	20*
iLLUMINATE: Ibrutinib-Obintuzumab[36] (N = 113)	22	12	NR	17	14*
ECOG E1912: Ibrutinib-rituximab[7] (N = 352)	4.8*	7.4	NR	18.8*	9.4†
ELEVATE-TN					
Acalabrutinib[40] (n = 179)	11.2	3.9	1.7‡	4.5	14†
Acalabrutinib-obinutuzumab[40] (n = 179)	9.5	3.4	2.2‡	7.3	20.8†

All data are percentages.
NR, not reported.
*Reported grade 3 or higher adverse event.
†Upper respiratory tract.
‡Grade 3 or any grade in central nervous system.

Table 3. Selected phase 3 trials for FDA-approved frontline regimens for unfit patients

Trial	Duration	ORR, %	PFS, %
RESONATE-2*: chlorambucil[33,34]	Continuous	37	12 (est. 60 mo)
RESONATE-2*: ibrutinib[33,34]	Continuous	92	70 (est. 60 mo)
A041202: IR[35]	Continuous ibrutinib; 6 cycles of rituximab	94	88 (est. 24 mo)
A041202: Ibrutinib[35]	Continuous	93	87 (est. 24 mo)
A041202: BR[35]	Up to 6 cycles	81	73 (est. 24 mo)
iLLUMINATE: IO[36]	Continuous	88	79 (est. 30 mo)
iLLUMINATE: ChlO[36]	Up to 6 cycles	73	31 (est. 30 mo)
CLL14: VenO[44]	Obinutuzumab 6 cycles, venetoclax 12 cycles	84.7	82 (est. 36 mo)
CLL14: ChlO[44]	Obinutuzumab 6 cycles, chlorambucil 12 cycles	71.2	50 (est. 36 mo)
ELEVATE-TN: acalabrutinib[40]	Continuous	86	87 (est. 24 mo)
ELEVATE-TN: AO[40]	Continuous	94	93 (est. 24 mo)
ELEVATE-TN: ChlO[40]	Up to 6 cycles	79	47 (est. 24 mo)

*Did not enroll patients with del17p.

regimens should be discussed as an option for this specific subgroup, taking into consideration their toxicity profiles and the patient's desire for time-limited therapy with the potential for long-term remission (Figure 1). Other frontline treatment options include a BTKi with or without anti-CD20 antibody or VenO (for patients who would prefer a fixed-duration regimen), because outcomes are excellent, and both regimens are well tolerated.

As a class, BTKi's are known for nonhematologic toxicities, particularly atrial fibrillation,[52] bleeding risk,[53] and hypertension[54] (Table 2). Ventricular arrhythmias and sudden cardiac deaths have also been reported.[35,37,55,56] It is important to consider the patient's age, cardiac risk factors (history of atrial fibrillation, hypertension on multiple medications), and risk for bleeding (concomitant anticoagulation, history of severe bleeding). The risks of potential side effects must be carefully weighed against the benefits of therapy on a case-by-case basis. Patients on BTKi's (in particular, ibrutinib) remain at risk for hypertension and quality of life toxicities, such as musculoskeletal toxicities, for the duration of treatment.[57] These toxicities may not be reversible upon discontinuation. For patients who are at high risk for cardiac complications, we involve a cardiologist who understands the potential risks of these agents. Infectious complications can occur, most commonly upper respiratory tract infections. Opportunistic infections have been reported, although they are more frequent in the relapsed/refractory setting.[58] Currently, there are no guidelines regarding infectious prophylaxis; thus, it should tailored to the patient's unique clinical presentation.

Despite its robust clinical efficacy, BTKi monotherapy seldom leads to CRs.[40,59] Hence, continuous therapy is recommended to prevent disease progression in the absence of unacceptable toxicity. In the E1912 trial, more than half of the patients discontinued ibrutinib use because of an adverse event.[38] Real-world evidence confirms this finding.[60,61] Nevertheless, patients have longer PFS than do patients who discontinue for progression of disease.[61] Second-generation BTKi's, which are designed to have fewer off-target effects, may be able to successfully overcome a toxicity after rechallenge. Most patients treated with acalabrutinib after ibrutinib discontinuation due to intolerance were able to be successfully treated without recurrence or an increase in the severity of the adverse events.[62,63] It is possible that the most common BTKi side effects are a class effect. There are several ongoing noninferiority phase 3 trials comparing second-generation BTKi's against ibrutinib that will help to answer this question.

In cases in which there is the potential for severe cardiovascular adverse events, our preference would be a venetoclax-based regimen. Although higher rates of neutropenia are observed, these respond to growth factor support. TLS remains the adverse event of highest concern when venetoclax is used, and frequent laboratory monitoring in real-time is required during the ramp-up period to recognize and treat acute laboratory changes at the earliest signs. For many patients, admission to

Table 4. Selected phase 3 trials for FDA-approved frontline regimens for fit patients

Trial	Duration	ORR, %	PFS
CLL8: FCR[29]	Up to 6 cycles	90	56.8 mo (est. 36-mo PFS, 65%)
CLL10: FCR[30]	Up to 6 cycles	95	55.2 mo (est. 36-mo PFS, 70%)
CLL10: BR[30]	Up to 6 cycles	96	41.7 mo (est. 36 mo PFS, 58%)
E1912*: IR[37]	Continuous ibrutinib, rituximab 6 cycles	96	Est. 36-mo PFS, 89%
E1912*: FCR[37]	Up to 6 cycles	81	Est. 36-mo PFS, 73%

est./Est., estimated.
*Did not enroll patients with del17p.

Table 5. Selected ongoing phase 2 clinical trials of frontline chemotherapy-free regimens

Regimen	Trial	Cycles
Venetoclax + ibrutinib[49]	CAPTIVATE trial; 150 patients. Primary end point: ibrutinib use in uMRD with assessment of disease-free survival at 1 y (n = 11). 55% CR; 100% uMRD in PB.	12, cont. ibrutinib based on MRD
Venetoclax + ibrutinib[48]	80 patients with high-risk features. Primary end point: CR/CRi. 92% CR/CRi at 12 mo.	24
Ibrutinib + venetoclax + obinutuzumab[50]	25 patients. Primary end point: MRD-negative CR at EOT. 52% CR/CRi; 58% uMRD.	14
	Phase 3: Alliance 041702 for elderly patients, ECOG 9161 for young fit patients	
Acalabrutinib + venetoclax + obinutuzumab[52]	72 patients. Primary end point: uMRD CR at EOT. 75% uMRD at EOT.	15-24, based on MRD
Zanubrutinib + venetoclax + obinutuzumab[52]	77 patients. Primary end point: establish rate of uMRD CR (time frame: 1 y). 68% uMRD at 8 mo.	Up to 12, based on MRD

cont, continuous; EOT, end of therapy; PB, peripheral blood.

the hospital is required for safe monitoring. Initial debulking therapy with obinutuzumab appears to decrease the rates of TLS and is a potential strategy to minimize the number of inpatient stays required.[44] The VenO regimen also offers the advantage of a time-limited treatment option. It is unknown whether there are patients with high-risk disease who may not benefit from this regimen. Combination strategies of BTKi with venetoclax appear to have higher rates of cytopenias than co either drug alone, and this should be factored into clinical decision making. This combination is not yet approved by the FDA, but the initial results seem very promising. Long-term follow-up for all of these trials is eagerly awaited.

The optimal treatment strategy for patients with del17p/TP53mut remains unknown. Venetoclax and BTKi regimens have both demonstrated improvement in outcomes compared with CIT. Mature data using continuous BTKi[8] or venetoclax[64] have demonstrated durable responses. In CLL14, the majority of progression events occurred in patients with del17p/TP53mut[44]; hence, it is unclear whether time-limited regimens would offer the same benefit to this cohort of patients. Ongoing clinical trials with time-limited therapies will answer this question in the future.

Currently, there are no available head-to-head data to determine which chemotherapy-free frontline regimen provides superior ORR, PFS, and/or OS. The CLL17 trial in Germany will compare ibrutinib, VenO, and ibrutinib-venetoclax to answer the question: What is the optimal chemotherapy-free frontline regimen? Results will not be available for years, but they will provide the answers to the important questions: Is treatment with BTKi or BCL-2i better in the frontline setting? Is the addition of an anti-CD20 antibody necessary? Many trials are underway to determine the best frontline therapeutic approaches. The phase 3 FLAIR trial in the United Kingdom is currently comparing FCR against ibrutinib monotherapy vs IR vs ibrutinib-venetoclax in the frontline setting. In the United States, phase 3 intergroup trials are underway for younger and older patients evaluating IO with or without venetoclax. These data will help to determine who may benefit most from monotherapy/doublet/triplet strategies. Triplet therapy appears to have higher rates of adverse events, although most patients are able to complete the intended duration of therapy. Combination strategies address continuous dosing by utilizing MRD-driven durations of treatment, but they come at the cost of higher incidences of hematologic adverse events upfront. As evidenced by all of these ongoing trials, frontline chemotherapy-free regimens have become the new standard of care. Further study of these combinations, particularly in older patients, will be needed to determine whether the side effect profile is acceptable for widespread use.

Clinical case part 3

Given his UM-IGHV status, our patient is not a candidate for CIT therapy. Potential treatment choices were discussed, including participation in a clinical trial, ibrutinib, acalabrutinib, or venetoclax with obinutuzumab because of his age, minimal comorbidities, and overall fitness. The patient chose the combination VenO, given its fixed-duration strategy. Staging computed tomography scans revealed a retroperitoneal lymph node conglomerate that measured 5.3 × 6.1 cm. Based on absolute lymphocyte count and tumor size, he is at high risk for TLS. He received the first cycle of obinutuzumab, which was only complicated by a grade 2 infusion reaction with test dose. He was admitted to the hospital for close TLS monitoring when venetoclax was started. After 3 cycles of therapy, his peripheral lymphadenopathy resolved, and his complete blood count showed improvement, specifically white blood cells (4400 per microliter), absolute lymphocyte count (1000 per microliter), absolute neutrophil count (1300 per microliter), hemoglobin (12.4 g/dL), and platelets (154 000 per microliter). Postcompletion of therapy, he is in a CR and remains under active surveillance.

Summary

Frontline treatment of CLL has dramatically evolved in the last few years with the rapid approvals of several novel targeted agents. Patients with high-risk genomic features have greatly benefited from this paradigm shift. CIT, once the backbone of treatment, has largely been replaced by targeted agents as a result of the PFS benefits seen across several phase 3 trials (Tables 3 and 4). To date, patients with M-IGHV without del17p or TP53mut remain the only group who may benefit (and potentially may achieve a functional "cure") with FCR or BR, although this may come at the expense of secondary malignancies, myelosuppression, and increased infection risk. Ongoing and upcoming studies are exploring regimens that can achieve better efficacy with deeper remissions to allow the patient a "treatment holiday" (Table 5). It is possible that achieving a deep response with a more intensive approach may be more beneficial to certain groups of patients (ie, fit with high-risk disease). Trial data comparing the efficacy of different chemotherapy-free

frontline regimens are important to develop a more tailored approach to treatment selection.

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J.M.R. has served as a consultant for Verastem, AstraZeneca, Pharmacyclics, and Abbvie/Genentech. J.C.B. has served as a consultant for Verastem, AstraZeneca, Pharmacyclics, and Abbvie/Genentech and received research support from Oncternal and Velosbio. She has received honoraria from Janssen.

Off-label drug use

The authors discuss off label drug use for ibrutinib, venetoclax, obinutuzumab, acalabrutinib and zanubrutinib.

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Standard treatment approaches for relapsed/refractory chronic lymphocytic leukemia after frontline chemoimmunotherapy

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Despite the effectiveness of chemoimmunotherapy (CIT), in most cases the clinical course of chronic lymphocytic leukemia (CLL) is characterized by consecutive episodes of disease progression and need for therapy. Treatment possibilities for patients with CLL in whom CIT fails whose disease progresses after initial CIT include pathway inhibitors (PIs) and, for selected patients, cellular therapy (ie, allogeneic stem cell transplant, chimeric antigen receptor T cells). PIs (ie, Bruton tyrosine kinase inhibitors, phosphatidylinositol 3-kinase inhibitors, and BCL2 inhibitors) are revolutionizing the treatment of CLL. PIs have proved to be more effective than CIT, both as upfront therapy and for relapsed/refractory disease, largely because they may overcome the negative impact of adverse biomarkers (eg, *TP53* aberrations, unmutated IGHV) on outcomes and because of their acceptable toxicity. In this article, the management of patients with relapsed/refractory CLL is discussed, with a particular emphasis on the role of PIs.

LEARNING OBJECTIVES

- Describe treatment strategies and the role of pathway inhibitors in patients with relapsed/refractory chronic lymphocytic leukemia
- Understand factors influencing therapeutic choices

Introduction

In the last few decades, the outcome of patients with chronic lymphocytic leukemia (CLL) has dramatically improved, and a fraction of patients may now expect to experience prolonged remission (>10 years). However, the cure of CLL is still elusive, and usually the course of the disease is punctuated by consecutive episodes of disease progression and need for therapy. Consequently, the overall survival (OS) of patients with CLL depends on the response to different treatments during the course of the disease.

Historically, treatment options for patients with relapsed/refractory (R/R) CLL were limited and treatment results unsatisfactory. This scenario has changed since the introduction of pathway inhibitors (PIs), including Bruton tyrosine kinase inhibitors (BTKis; ibrutinib, acalabrutinib), phosphatidylinositol 3-kinase inhibitors (PI3Kis; idelalisib, duvelisib), and time-limited therapy with venetoclax-based regimens. Selecting therapy for R/R CLL requires clinicians to take into consideration several patient, disease, prior therapy, and socioeconomic aspects (Figure 1).

Clinical case part I

A 60-year-old woman with relapsed CLL was referred to our center for evaluation. She had received frontline chemoimmunotherapy (CIT) with FCR (fludarabine, cyclophosphamide, and rituximab) for 6 cycles, and a complete response (CR) was achieved. Her laboratory test results immediately before starting fist treatment revealed the presence of poor prognostic variables, including del(11q), serum β_2-microglobulin 6 mg/dL, and unmutated IGHV genes.

Three years later, the patient presented with progressive lymphocytosis with an absolute lymphocyte count (ALC) of 50×10^9/L, hemoglobin (Hb) level of 110 g/L, and platelet count of 111×10^9/L. She was completely asymptomatic. Her physical examination revealed small lymph nodes of 2 to 3 cm that were palpable in all peripheral areas. Fluorescence in situ hybridization shows isolated del(11q) but no del(17p). No *TP53* mutations were present.

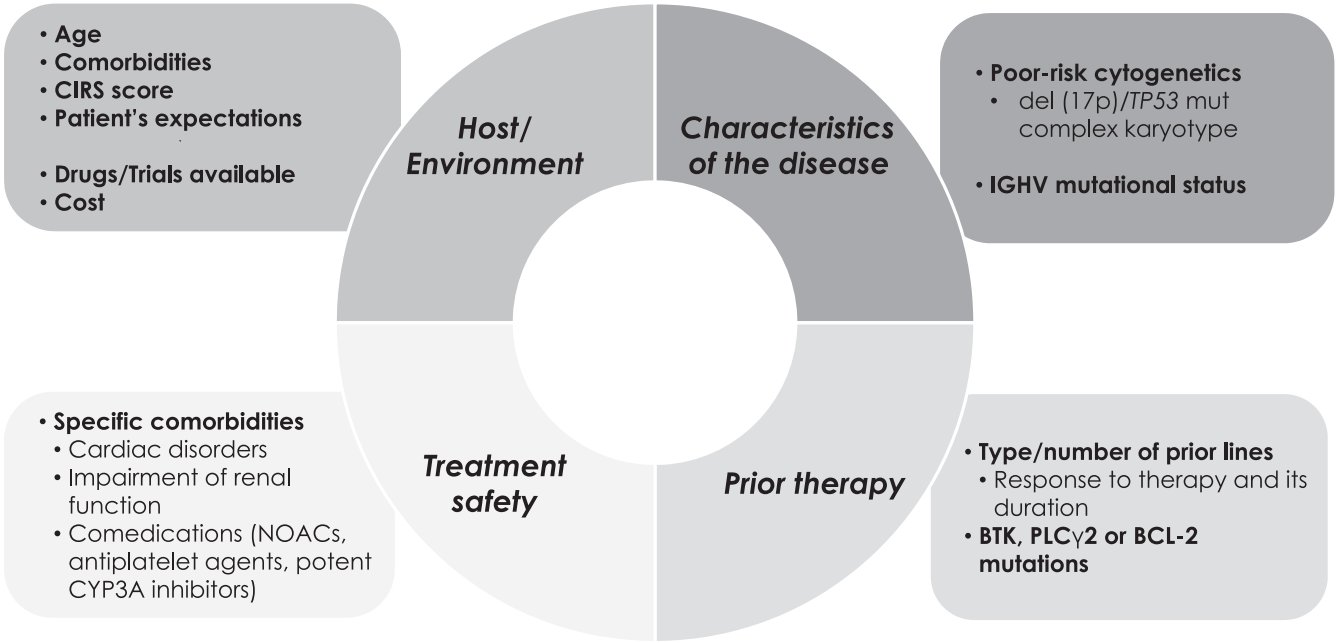

Figure 1. Patient-related, disease-related factors, and prior therapies need to be taken into consideration to select treatment modality.

Prognostic factors

Bulky disease, treatment refractoriness, extensive prior therapy, and adverse biomarkers (eg, *TP53* aberrations, unmutated IGHV) are poor prognostic factors. In a large retrospective study based on 2475 patients with R/R CLL treated in 6 PI trials, a prognostic model was used that consisted of 4 factors (1 point each for serum β_2-microglobulin >5 mg/dL, lactate dehydrogenase greater than the upper limit of normal, Hb <110 g/L for women or <120 g/L for men, and time from initiation of last therapy <24 months), separating patients into low (score 0-1), intermediate (score 2-3), and high (score 4) risk groups. The most important predictor is a short interval from treatment initiation to relapse.[1,2] An important caveat is that this model was generated in cohorts of patients treated with CIT, and treatment consisted of different PIs. Because prognostic factors may be treatment dependent, this is a limitation. Also, prognostic models for patients initially treated with PIs are needed.

Treatment options in R/R CLL

Treatment should be initiated only when International Workshop on Chronic Lymphocytic Leukemia criteria are met in the presence of signs or symptoms of disease activity, as in newly diagnosed patients.[3] *Relapsed*, *symptomatic*, and *refractory* (or *resistant*) disease should not be employed as synonymous terms. Disease relapse (ie, reappearance of the disease after a period of remission) is not necessarily an indication for therapy, because many patients in this situation are asymptomatic and can be followed up with no intervention for a long period of time. Likewise, refractory disease (ie, disease that does not respond to therapy) should not be confounded with disease progression (ie, symptomatic, relapsed disease). The main results of pivotal studies conducted in R/R CLL are summarized in Table 1.[4-11]

Clinical case part II

The patient was asymptomatic, and no therapy was provided. Nevertheless, 4 months later, she presented with extreme fatigue. Her blood test results revealed ALC 120 × 10⁹/L, Hb 96 g/L, and platelet count 60 × 10⁹/L. The result of a direct antiglobulin test was negative. The fluorescence in situ hybridization test was repeated and showed del(11q) but not del(17p). No *TP53* mutations were detected. Different treatment options were discussed with the patient.

BTKis

The significant clinical activity observed through the inhibition of Bruton tyrosine kinase (BTK), an important enzyme in the amplification of B-cell receptor signaling, represented a firm first step into the era of targeted therapies in CLL. BTKis such as ibrutinib and acalabrutinib disrupt B-cell receptor signaling and other circuits between CLL cells and the microenvironment. Ibrutinib was approved in 2014 as therapy for patients with CLL who had received at least one prior line of therapy, based on the initial results of the phase Ib/II PCYC-1102, which were further confirmed by the multicenter randomized RESONATE study in patients with previously treated CLL.[4,12] Long-term follow-up data of both trials demonstrated that ibrutinib administered as a single agent resulted in a significant benefit in R/R CLL, the estimated progression-free survival (PFS) and OS at 7 years being 34% and 55%, respectively, in the phase I-IIb study, and PFS and OS at 5 years of 40% and 60%, respectively, in the RESONATE study. The benefit of ibrutinib was observed across all risk groups, although patients with TP53 aberrations and complex karyotype had shorter PFS and OS.[5,13]

On the downside, ibrutinib is associated with side effects that, in some cases, can be severe. The most serious adverse events are bleeding and arrhythmias, mainly atrial flutter and atrial fibrillation; ventricular arrhythmias can also occur.[14,15] The

Table 1. Phase III trials comparing targeted therapies including BTKi, PI3Ki, and BCL2i vs anti-CD20 monoclonal antibodies or chemoimmunotherapy regimens in patients with R/R CLL

Treatment	Median age (y)	*TP53* aberrations	Prior lines of therapy, median (range)	3-y PFS	Neutropenia grade ≥3 AEs	Infection grade ≥3 AEs	Any nonhematologic grade ≥3 AEs*	Median follow-up
IBRU (vs ofatumumab)[4,5]	67	32% del(17p)	3 (1-12)	59%†	25%	30%	Hypertension (9%), AF (6%), major bleeding (4%)	59 mo (final analysis of the study)
IDELA-R (vs R)[6,7]	71	43% either del(17p) or *TP53* mutation	3 (1-12)	Median 19.4 mo	13%	53.6%	Diarrhea (16%), transaminitis (5%-9%), colitis (8%), pneumonitis (6%)	18 mo (final analysis of the study)
VEN-R (vs BR)[8,9]	65	26% del(17p), 25% *TP53* mutation	2 (1-4)	71.4%	58.8%	10%	TLS (2%), hyperglycemia (2%)	23.8 mo (last update up to 4 y)
A (vs IDELA-R or BR)[10]	67	16% del(17p), 24% *TP53* mutation	1 (1-8)	Median NR	17%	20%	AF (1%), hemorrhage (3%), hypertension (3%)	16 mo
DUV (vs ofatumumab)[11]	68	21% del(17p), 20% *TP53* mutation	3 (2-8)	Median 15.7 mo	23%	13%	Diarrhea (23%), colitis (11%), pneumonia (11%)	22 mo

A, acalabrutinib; AEs, adverse events; AF, atrial fibrillation; BR, bendamustine and rituximab; DUV, duvelisib; IBRU, ibrutinib; IDELA, idelalisib; NR, not reached; PFS, progression-free survival; R, rituximab; TLS, tumor lysis syndrome; VEN, venetoclax.
*Focused on diverse events of clinical interest.
†Median PFS 44.1 mo in the final analysis (6 years of follow-up).

most frequent toxicities, however, include arthralgias and pneumonia, and these are manageable. Other adverse events are diarrhea, hypertension, fatigue, cough, and skin lesions.[16] Ibrutinib must be discontinued in a significant proportion of patients (20%-50%). In a long-term analysis, only 28% of patients with R/R disease continued on ibrutinib beyond 5 years of initiating therapy; 33% discontinued treatment because of disease progression and 21% due to ibrutinib-associated toxicities, particularly frequent in older and heavily pretreated patients. This study showed that most adverse events tend to decrease over time, except hypertension, and that patients discontinue treatment mainly because of atrial fibrillation, diarrhea, infections, and bleeding events.[13] In a real-world scenario, treatment discontinuation was observed in 50% of patients with R/R disease.[17]

Second-generation BTKis are designed to improve upon first-generation agents such as ibrutinib by having less cardiotoxicity, fewer adverse events requiring treatment discontinuation, and fewer off-target effects. Acalabrutinib is a second-generation BTKi approved by the US Food and Drug Administration in 2019 for treatment of patients with CLL. In R/R CLL, acalabrutinib administered as a single agent showed a significant benefit in PFS compared with control arms (bendamustine with rituximab or idelalisib with rituximab). With a median follow-up of 22 months, the estimated 18-month PFS was 82% vs 48%; no differences in PFS were observed across risk groups.[10]

Although the follow-up is short, acalabrutinib seems as effective as ibrutinib, and its tolerance is likely better; atrial fibrillation, hypertension, and bleeding have been reported to be less frequent than with ibrutinib. Indeed, acalabrutinib is considered a therapeutic alternative in patients who do not tolerate ibrutinib.[18] The most frequent adverse event associated with acalabrutinib is headache, which can be easily managed. Other possible adverse events are neutropenia, diarrhea, cough, and upper tract respiratory infection. The acalabrutinib discontinuation rate has not been studied extensively. In one study, treatment discontinuation due to adverse events occurred in 16% of patients after a median follow-up of 22 months.[10] The results of a randomized phase III study, ACE-CL-006, comparing acalabrutinib with ibrutinib in previously treated patients with high-risk CLL, defined by the presence of del(11q) or del(17p), are eagerly awaited because they could be practice changing.

Another selective BTKi in advanced phases of clinical development is zanubrutinib. A phase I/II study reported a promising safety profile, with atrial fibrillation and major bleeding being infrequent. Ninety-five percent patients continued treatment after a median follow-up of 14 months, and only 2% discontinued therapy due to adverse events. Zanubrutinib at a dose of 160 mg twice daily achieved a steady inhibition of BTK. In the R/R setting, the overall response rate (ORR) was 93%, and the estimated PFS at 12 months was 100%.[19] A phase II study in patients with R/R CLL showed similar results; after a median follow-up of 15 months, the ORR was 84.6%, and the estimated PFS and OS at 12 months were 92.9% and 96%, respectively. Nine percent of patients discontinued zanubrutinib due to adverse events.[20] Updated results of the initial phase I/II study with a follow-up of 24 months showed a persistent benefit in PFS, regardless of the presence of del(17p).[21] A randomized phase III trial is comparing ibrutinib with zanubrutinib in patients with R/R CLL, but no data are yet available.

As mentioned above, a proportion of patients must discontinue treatment with BTKis due to disease progression. Although mechanisms of resistance are not fully understood, the acquisition of secondary mutations of BTK leading to impaired binding of the BTKi is the most common cause of resistance to BTKis. Acquired mutations in BTK involving Cys481 or phospholipase C-γ2 have been reported in >80% of patients with progressive disease treated with ibrutinib[22] and in 69% in a small cohort of patients treated with acalabrutinib.[23] Recently, a novel

mutation of BTK involving Leu528Trp has been found along with Cys481 mutations in patients who experienced progression under zanubrutinib therapy.[24] Furthermore, in patients showing resistance to BTKis, other non-BTK mutations (ie, TP53, SF3B1, and CARD11) have been described.[25] Several strategies aimed at overcoming resistance associated with mutations of BTK are in development, such as the use of reversible noncovalent BTKis, including LOXO-305 and ARQ-531.[26] Also, several combinations of BTKis with CIT and targeted therapies are under investigation. An important objective of these studies is to shift continuous therapy to time-limited therapy, to achieve deeper responses, and to prevent resistance to therapy.

PI3Kis

The selective inhibitor of phosphatidylinositol 3-kinase δ, idelalisib, is another treatment for patients with CLL who experience relapse after CIT. Idelalisib in combination with rituximab significantly improved PFS and OS compared with rituximab alone in a pivotal study including patients with R/R CLL and comorbidities.[6] In addition, a post hoc analysis suggested that the benefit of this combination extends to patients with complex karyotype and *TP53* aberrations.[27] However, immune-mediated adverse events (ie, hepatitis, colitis) and risk of infections can be a limiting factor, and because of this, idelalisib is generally reserved for later lines of therapy.[28]

Duvelisib is an oral dual phosphatidylinositol 3-kinase δ and γ inhibitor approved as treatment of patients with CLL who have received at least 2 prior lines of therapy, based on the results of the DUO trial. This study showed significant benefit in PFS in patients receiving duvelisib compared with the control arm; the median PFS was 13.3 months vs 9.9 months, respectively.[11] In addition, duvelisib showed a high response rate (77%) and a benefit in PFS in patients who experienced progression under the control arm (ofatumumab) and were subsequently treated with duvelisib in the DUO trial. However, duvelisib is associated with PI3Ki characteristic toxicity.[29]

Umbralisib is a second-generation PI3Ki with promising activity and moderate toxicity, especially hepatotoxicity and colitis. Combinations of umbralisib with novel anti-CD20 monoclonal antibodies, BTKis, or BCL2 inhibitors (BCL2is) are being investigated.[30-32]

BCL2is

Venetoclax is an oral selective inhibitor of the antiapoptotic BCL-2 protein that was first approved as monotherapy for patients with relapsed disease or with 17p deletion.[33] This agent was approved as a fixed-duration regimen in combination with rituximab for R/R CLL, based on the results of the MURANO trial.[8] This regimen consists of venetoclax for 2 years combined with rituximab for 6 months and is the first "time-limited therapy" in the era of targeted therapies. The last update of the MURANO trial with 4 years of follow-up demonstrated a consistent benefit in PFS and OS compared with bendamustine and rituximab in previously treated patients with CLL; the PFS at 4 years was 57.3% vs 4.6%. The progression rate after treatment discontinuation is 32% at 2 years. Patients with high-risk features, particularly those with *TP53* aberrations, are those more likely to experience progression.[9,34]

The combination venetoclax plus rituximab eradicates the disease in a high proportion of patients. Importantly, those patients with undetectable minimal residual disease (uMRD) at the end of therapy have a significantly better outcome than those in whom leukemic cells remain detectable, emphasizing the importance of minimal residual disease (MRD) as a treatment goal not only in patients treated with CIT but also in those receiving PIs.[35] Many trials are exploring combinations of PIs with uMRD as an endpoint and landmark to discontinue therapy. In patients with bulky disease previously treated with BTKis or having received >3 lines of therapy, treatment results are poorer.[34]

In early trials, tumor lysis syndrome (TLS) was observed in a not negligible proportion of patients.[36] Currently, a 5-week ramp-up dosage and adequate general prophylaxis have drastically limited TLS (<1% of patients), which, in most cases, is only a laboratory finding. In clinical practice, the risk for TLS (ie, subjects with bulky disease with any lymph node ≥10 cm or ≥5 cm with an ALC ≥25 × 10^9/L) can be considered as inconvenient, but the risks of TLS are counterbalanced by the high efficacy of venetoclax. The most frequent toxicity associated with venetoclax is neutropenia that needs to be managed with granulocyte colony-stimulating factor. Less frequent events are diarrhea, nausea, fatigue, anemia, and infections.[37] In real-world data, venetoclax therapy was discontinued in 29% of patients (20.5% due to adverse events and 53.8% due to disease progression). The most frequent adverse event leading to discontinuation was hematological toxicity.[38]

The main limitation of treatment with venetoclax is disease progression. Although there is little information on mechanisms of resistance to venetoclax, the acquisition of the Gly101Val point mutation in *BCL2* and other alternative *BCL2* mutations at clonal and subclonal levels interfere with the engagement of venetoclax in the binding site of BCL2.[39] However, these mutations are not found in all patients resistant to venetoclax, and other mechanisms, such as upregulation of antiapoptotic proteins such as MCL-1 and BCL-xL overexpression, might cause treatment resistance.[40,41]

CIT

CIT could still be considered a treatment option for patients experiencing late relapses (ie, >24 to 36 months) and in those initially treated with poorly effective regimens (eg, chlorambucil, rituximab). However, the short- and long-term toxicity, mainly hematologic and secondary malignancies associated with CIT, are of concern.[42] Because of this, CIT is gradually replaced by PIs. In patients with R/R CLL, CIT could still be an option when PIs are not available.

Clinical case part III

To sum up, this patient with CLL experienced relapse and had symptomatic disease after 3 years of receiving CIT. Of note, the patient did not have prior comorbidities except for hypercholesterolemia. Among the prognostic factors with which the patient presented was unmutated IGHV gene del(11q). After discussing the different therapeutic choices mentioned above, the patient started oral ibrutinib at a standard dose of 420 mg daily. The patient's tolerance of treatment was good; she only complained of fatigue that got worse during the first 2 months but progressively improved after 3 months of receiving ibrutinib. After 1 year of treatment, her blood test results revealed recovery of her Hb level and platelet count with an ALC of 6 × 10^9/L, consistent with partial remission.

Combination strategies and cellular therapies

The emergence of mutations driving treatment refractoriness to ibrutinib and venetoclax is a compelling argument to combine

different PIs and/or anti-CD20 monoclonal antibodies. Overall, combinations of PIs (ibrutinib and idelalisib) with anti-CD20 monoclonal antibodies and CIT regimens in R/R CLL have been shown to increase CR rates, including in patients with uMRD. However, the improvement of the quality of response has not always been followed by a significant benefit in PFS and OS. For example, the addition of rituximab to ibrutinib did not improve PFS and OS compared with ibrutinib alone.[43] In contrast, the addition of the novel anti-CD20 monoclonal antibody ublituximab to ibrutinib improved not only the quality of response but also PFS in patients with high-risk CLL [(del(17p), del(11q), and/or a *TP53* mutation] compared with single-agent ibrutinib.[44] Further studies with long follow-up are necessary to determine whether second-generation anti-CD20 monoclonal antibodies in combination with BTKi or PI3Ki agents provide a benefit as compared with single-agent therapy.

Another strategy consists of combining BTKis with BCL2is, based on the synergy found in preclinical models. Several trials are combining ibrutinib as a single agent during the first 2 or 3 cycles to mobilize CLL cells from microenvironment niches and reduce tumor burden, followed by the addition of venetoclax for a limited number of cycles. The main endpoint of most of these trials is the eradication of MRD to guide treatment discontinuation. A recent report has shown impressive results, with MRD negativity rates of 53% in peripheral blood and 36% in bone marrow in patients with R/R CLL after 12 months of combined therapy with ibrutinib and venetoclax. Although the follow-up is still short, only 1 of 53 patients experienced progression.[45] The same approach is being investigated with PI3Kis such as umbralisib or duvelisib with venetoclax with or without anti-CD20 monoclonal antibodies. The main results of all these trials are summarized in Tables 2 and 3.[45-52]

However, whether any additional benefit is derived from adding CIT or anti-CD20 monoclonal antibodies in the form of "doublets" and "triplets" to PIs administered as single agents remains unclear due to the lack of randomized clinical trials in the R/R setting. Treatment costs ("financial toxicity") and treatment alternatives once these regimens fail are of concern.

Another field that is moving forward is CD19-targeted, chimeric antigen receptor (CAR)-engineered T-cell immunotherapy. Early phase I /II clinical trials have shown encouraging results with durable responses in patients with R/R CLL who are resistant to ibrutinib and venetoclax. In line with this, the combination therapy of BTKi (ibrutinib) and CAR T cells is appealing. Preclinical studies suggest that ibrutinib may improve T-cell function and result in better CAR T-cell proliferation and antitumor effect. Results of a pilot study showed an ORR of 83%, with 61% cases with uMRD in bone marrow; the 1-year OS and PFS were 86% and 59%, respectively. Of interest, the concurrent administration of ibrutinib and CD19-targeted CAR T cells was well tolerated with lower severity of cytokine release syndrome than that observed with CD19-targeted CAR T cells without ibrutinib.[53-55]

Which therapeutic strategy should be used in patients experiencing relapse after CIT?

Whenever possible, patients should be included in clinical trials. In those patients not included in trials, choosing the optimal treatment strategy depends on several factors (Figure 1):

1. *Host factors*: Age, comorbidities, Cumulative Illness Rating Scale score,[56] and patient's expectations.
2. *Characteristics of the disease*: Bulky disease, extensive prior therapy (≥3 regimens), and a short PFS (<24 months) are high-risk features that predict poor outcome in patients initially treated with CIT. Other adverse factors that should be considered are *TP53* aberrations and complex karyotype. Patients with complex, high-risk genetic lesions should be included in clinical trials. In selected cases, T-cell therapy (allogeneic stem cell transplant, CAR T cells) can be an option.[57] Of note, disease relapse or progression may reveal Richter syndrome, whose therapy is not covered in this review.
3. *Treatment safety*: Specific comorbidities such as cardiac disorders, impairment of renal function or certain comedications (eg, oral anticoagulants, antiplatelet agents, potent CYP3A inhibitors) may interfere with PIs.
4. *Practical aspects*: Availability of drugs and their cost.
5. *Resistance to PI*: Presence of mutations associated with refractoriness to targeted therapies (ie, BTK, phospholipase C-γ2, or *BCL-2* mutations).[22,23,40]
6. *Goals of therapy*: The goals of therapy are prolonging survival and improving quality of life. Achieving response with uMRD is associated with longer PFS and OS. The importance of obtaining uMRD, however, depends on the target population and treatment objectives. For example, achieving uMRD is not critical in very old patients (>80 years), in whom disease palliation is the most reasonable objective. In contrast, in younger patients, it can be of paramount importance. In the allotransplant setting, achieving uMRD is important because results are better in patients in CR. In such cases, CIT and/or venetoclax plus rituximab, if not previously administered, are the

Table 2. PI3Ki combinations with venetoclax with or without anti-CD20 monoclonal antibodies in patients with R/R CLL

Trial phase	Treatment	No. of patients	TP53 aberrations	Schedule	Strategy	uMRD rate	Discontinuation rate	Median follow-up
Phase I/II[46]	Umbralisib-venetoclax-obinutuzumab	21	38%*	UMBRA cycle 1-12 + UBLI cycles 1-3 + VEN cycles 4-12	BM uMRD at cycle 12, discontinue all therapy BM MRD-positive at cycle 12, continue UMBRA monotherapy	4 patients with BM uMRD (19%)	4.7%	4.2 mo
Phase I[47]	Duvelisib-venetoclax	12	25% del(17p) and 42% TP53 mutation	DUV days 1-7, then DUV+VEN for 12 cycles	uMRD on 2 assessments, discontinue all therapy; resume VEN monotherapy at MRD recrudescence MRD-positive, continue VEN monotherapy	22% uMRD in BM and PB	25%	Reported with median number of cycles 6 (range, 1-9)

BM, bone marrow; c, cycles; DUV, duvelisib; PB, peripheral blood; UBLI, ublituximab; UMBRA, umbralisib; VEN, venetoclax.
*Also includes patients with del(11q).

Table 3. BTKis combined with venetoclax with or without anti-CD20 monoclonal antibodies in patients with R/R CLL

Trial phase	Treatment	No. of patients	TP53 aberrations	Schedule	Strategy	uMRD* rate	Discontinuation rate	Median follow-up
Phase II[48]	IBRU-VEN	80	30% del(17p) and *TP53* mutation	IBR for 3c, IBRU+ VEN 4-27c	MRD+ in BM could continue ibrutinib	67% uMRD in BM at 24 c	19%	22 mo
Phase II[45]	IBRU-VEN	53	22% del(17p)	IBRU 1-2c, IBRU+VEN after cycle 2	PB/BM uMRD at cycle 8: cease therapy after cycle 14; PB/BM uMRD between cycles 14 and 26: cease therapy after cycle 26; MRD-positive at cycle 26: continue IBR monotherapy	uMRD 53% in PB at 12 mo; uMRD 36% in BM at 12 mo	Not reported	21 mo
Phase II[49]	IBRU-VEN	51	18% del(17p) and TP53 mutation	IBRU 1-2 c, IBR + VEN 3-15 c	> PR and BM uMRD after cycle 15 randomized to observation or IBR monotherapy*; MRD recrudescence retreated with combination	uMRD 55% in PB; uMRD 39% in BM	16%	15 mo
Phase II[50]	IBRU-VEN	24	50% del(17p)/ 50% TP53 mutation	IBR+VEN for up to 2 y	uMRD CR on 2 assessments: discontinue all therapy or continue IBR monotherapy*; patients MRD-positive or less than CR: continue IBR monotherapy	67% uMRD in BM at 12 mo (in 15 evaluable patients)	12%	NA
Phase Ib[51]	IBRU-VEN-OBINU	12	8% del(17p)	OBINU 1-8 c + IBRU 2-14 c + VEN 3-14 c	After cycle 14, can continue IBR monotherapy	100% uMRD in PB/50% uMRD in BM at 14 c	Not reported	24 mo
Phase II[52]	IBRU-VEN-OBINU	25	Not reported	OBINU 1-8 c + IBRU 2-14 c + VEN 3-14 c	After cycle 14, can continue IBR monotherapy	100% uMRD in PB/50% uMRD in BM at 14 c	Not reported	18 mo

BM, bone marrow; c, cycles; IBR, ibrutinib; IBRU, ibrutinib; NA, not available; OBINU, obinutuzumab; PB, peripheral blood; PR, partial response; VEN, venetoclax.
*uMRD defined as < 1 CLL cell per 10000 leukocytes.

best options to cross the bridge.[57] Importantly, ibrutinib may be effective in patients experiencing progression after transplant.[58]

How should patients be managed during the coronavirus disease 2019 pandemic?

The coronavirus disease 2019 (COVID-19) pandemic is challenging the management of patients with cancer. CLL mainly affects older people with comorbidities. This fact, along with the severe immunosuppression inherent to the disease, suggests that in patients with CLL, severe acute respiratory syndrome coronavirus 2 infection could be more severe than in the general population. A detailed discussion of the management of patients with CLL with associated COVID-19 infection is beyond the scope of this review and has been addressed thoroughly by different organizations.[59,60] Nevertheless, in those patients with active CLL, treatment should not be withheld, because there are data showing that the outcome of patients with COVID-19 is better if their cancer is under control. In patients requiring intervention, BTKis may represent the preferred therapeutic option.[61]

Conclusions

The management of patients with R/R CLL is changing with the advent of PIs (BTKis, PI3Kis, and BCL2is). Continuous therapy with BTKis and fixed duration of venetoclax with anti-CD20 monoclonal antibodies have significantly improved the outcomes of patients with R/R CLL. However, most patients with CLL must discontinue PIs because of treatment-related toxicity, treatment failure, or disease progression . To overcome this, second-generation BTKis are being incorporated into CLL therapy, and newer combinations of PIs with other agents are being investigated. Achieving uMRD is increasingly employed as an endpoint and to define treatment duration. The inclusion of patients in clinical trials, large databases, and meta-analyses is critical to continue advancing CLL therapy.

Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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EVIDENCE-BASED MINIREVIEW

Treatment of relapsed chronic lymphocytic leukemia after venetoclax

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LEARNING OBJECTIVES

- Review the literature examining outcomes for CLL patients after progression on venetoclax-based treatment
- Discuss a patient-specific approach to therapy selection following CLL progression on venetoclax

Clinical case

Two years after his initial diagnosis, a 65-year-old man with chronic lymphocytic leukemia (CLL) developed progressive fatigue and anemia requiring initiation of therapy per International Workshop on Chronic Lymphocytic Leukemia (iwCLL) guidelines. Mutational profiling before treatment was initiated revealed mutated *IGHV*, del(13q), and normal karyotype. There was no del(17p) or *TP53* mutation. He was treated with 6 cycles of chemoimmunotherapy (fludarabine-cyclophosphamide-rituximab) and achieved complete remission (CR). However, 24 months after completion of therapy, he developed progressive lymphadenopathy that required initiation of second-line therapy. Repeat mutational profiling again revealed no evidence of *TP53* disruption. Venetoclax-rituximab was chosen for treatment, and the patient completed a total of 6 months of rituximab and 24 months of venetoclax per the MURANO study regimen. He initially had a CR, but 12 months after stopping venetoclax, he now has progressive lymphadenopathy and worsening anemia. What would be the next treatment that you would select for this patient?

Introduction

The BCL2 inhibitor venetoclax has shown deep and durable responses in treating CLL, including in traditionally high-risk patients such as those with del(17p) or complex karyotype.[1-7] On the basis of the results of the CLL14 and the MURANO trials, venetoclax in combination with an anti-CD20 antibody is now a standard-of-care treatment option in both the first-line and relapsed/refractory (R/R) settings.[2,3] Given its increasing use in clinical practice, it is expected that more patients will eventually require a subsequent therapy after discontinuing a venetoclax-based regimen given in earlier lines of therapy. Herein, we discuss reasons for progression on venetoclax, review the existing literature on clinical outcomes of treatment after progression on venetoclax, and highlight patient-specific considerations for selecting the next therapy.

Reasons for progression during or after venetoclax-based therapies and mechanisms of resistance

Data from clinical trials, registries, and real-world series demonstrate that most patients discontinue venetoclax because of progressive disease (PD) rather than because of adverse events.[7-10] Patients whose disease progresses on venetoclax have been shown to have traditionally high-risk features. A retrospective analysis of 445 patients treated with venetoclax on 4 early-phase clinical trials showed that patients who had received previous therapy with a Bruton tyrosine kinase inhibitor (BTKi), 3 or more previous lines of therapy, or had bulky lymphadenopathy were less likely to have a durable response to venetoclax-based therapy. In addition, del(17p), *TP53* mutations, *NOTCH1* mutations, and unmutated IGHV status predicted less durable responses to venetoclax.[11] A separate analysis of 67 patients on 4 early-phase venetoclax clinical trials showed that complex karyotype and fludarabine refractoriness were associated with progression on venetoclax.[8]

Minimal residual disease (MRD) status also seems to be an important predictor of progression for patients who have completed venetoclax-based therapy. At a median follow-up of 36 months for patients who completed 24 months of therapy with venetoclax-rituximab on the MURANO trial, patients with detectable MRD at the end of treatment were more likely to have PD than those with

undetectable MRD (uMRD). In addition, patients with a partial response (PR) to venetoclax-rituximab with MRD status at the end of treatment had an inferior progression-free survival (PFS) from 18 months onward from the end of combination treatment compared with patients with uMRD, suggesting that MRD status, regardless of iwCLL response, may best predict progression after cessation of venetoclax-based therapy.[4]

Early preclinical in vitro studies suggested that the development of a mutation to prevent venetoclax from binding to its target site on *BCL2* may serve as a mechanism of acquired resistance,[12,13] and a recurrent mutation (Gly101Val) in *BCL2* was recently identified in patients progressing on venetoclax.[14] In a study of 15 CLL patients with paired pre-venetoclax and progression samples, 7 patients had a Gly101Val mutation in *BCL2* at progression. Of note, *BCL2* Gly101Val was first detected after 19 to 42 months of receiving venetoclax therapy; variant allele frequencies were low at this time, and detection of the mutation preceded clinical progression by up to 25 months.[14] The authors demonstrated that Gly101Val decreases Bcl2 affinity for venetoclax by ~180-fold.[14] Given the late occurrence of *BCL2* mutations while receiving venetoclax therapy, it is unclear how relevant *BCL2* mutations will be for patients with progression after fixed-duration venetoclax regimens of 12 to 24 months. Another study examined CLL cells from patients who had persistent MRD after 1 year of treatment with venetoclax-rituximab; of 6 patients with persistent MRD and PD, a *BCL2* Gly101Val mutation was identified in 3 patients (50%). The variant allele frequencies of the identified *BCL2* mutations were less than 20%. In this study, CLL cells from patients with PD also had higher levels of ROR1 and BCL2 expression and greater cancer-stemness gene expression on transcriptome analysis.[15]

Other studies suggest additional factors that may contribute to venetoclax resistance. A recent study performed whole-exome sequencing on 8 specimens from a CLL patient that were collected before treatment with venetoclax and at the time of progression while receiving venetoclax and did not detect *BCL2* Gly101Val mutations. However, an increasing number of acquired copy number mutations or aneuploidy were identified at progression, which demonstrated that heterogeneous patterns of clonal evolution and increased genomic instability occur with exposure to venetoclax.[16] Another recent study also did not find *BCL2* Gly101Val mutations at progression in 6 CLL patients, but it demonstrated MCL-1 overexpression in venetoclax-resistant cells as well as increased oxidative phosphorylation. This suggests that metabolic reprogramming may also contribute to venetoclax resistance.[17]

Re-treatment with venetoclax

For patients with an initial response to venetoclax-based therapy who progress after completing therapy and have no identifiable acquired resistance mutation (ie, BCL2 Gly101Val), an unanswered clinical question is whether re-treatment with venetoclax should be considered. This question is particularly relevant because the MURANO and CLL14 studies used fixed-duration treatment regimens (continuous venetoclax therapy for 24 months in the MURANO study and 12 months of therapy in the CLL14 study).[2,3] At a median follow-up of 4.9 years from the original phase 1b study of venetoclax-rituximab, 18 patients stopped venetoclax treatment in deep response, and 4 patients (2 with MRD-positive CR, 2 with uMRD CR) had progressive disease after stopping venetoclax and were subsequently re-treated with venetoclax or venetoclax-rituximab. Of 3 patients who had a repeat response assessment, all achieved at least a PR and 2 had ongoing responses.[18] In 4-year follow-up data from the MURANO trial, 14 of 64 patients with PD in the venetoclax-rituximab arm received subsequent venetoclax or were re-treated with venetoclax-rituximab.[19] Six of 11 patients with evaluable responses had a response for an overall response rate (ORR) of 55%.[20] Notably, in follow-up data from the CLL14 trial, data on re-treatment with venetoclax are not available.[21] Early data suggests that re-treatment with venetoclax yields responses in select patients, but further study is needed to validate this approach. It is not yet known whether the activity of venetoclax re-treatment depends on the duration and/or depth of previous response to venetoclax, and future studies evaluating venetoclax re-treatment should take these factors into consideration.

Covalent BTKi's after venetoclax

Covalent BTKi's, including ibrutinib,[22-26] acalabrutinib,[27,28] and zanubrutinib,[29-31] have transformed the treatment of CLL in both the R/R and first-line settings. However, despite many studies demonstrating excellent efficacy of BTKi's in R/R settings, initial studies largely preceded clinical trials and the subsequent approvals of venetoclax. Therefore, data are limited on the efficacy of BTKi therapy after venetoclax-based treatment.

A recent retrospective study examined outcomes for ibrutinib-naïve patients who had been treated with ibrutinib after they progressed following treatment with venetoclax.[32] This study included 27 patients with a median of 2 therapies before venetoclax, including 1 patient treated with another BTKi. Notably, this was a high-risk population, with 12 (60%) of 20 patients with del(17p), 12 (50%) of 24 patients with complex karyotype, and 13 (86.7%) of 15 patients with unmutated *IGHV*. Of the 27 patients, 18 had discontinued venetoclax because of PD. The ORR to ibrutinib was 56.0% (PR, 13 of 25; CR, 1 of 25). Time to progression on ibrutinib ranged from 3 to 53 months, and the median duration of therapy was 18.3 months, suggesting that ibrutinib has clinical activity when used after venetoclax.

Another single-institution study examined the outcomes of 23 heavily pretreated patients who had progressed on venetoclax therapy and subsequently received a BTKi (ibrutinib or zanubrutinib).[33] Notably, these patients had received a median of 4 previous lines of therapy, with 91% having received previous fludarabine-cyclophosphamide-rituximab. The population was also high risk from a genetic perspective, with 76% of patients with *TP53* disruption and 68% with a complex karyotype. Of 23 patients treated with a BTKi, 20 patients had ORRs of 90%, with 15 PRs or PRs with lymphocytosis and 4 with a CR. Twelve patients had discontinued treatment with a BTKi (8 because of PD and 4 because of toxicity), and 11 patients continued to receive BTKi therapy at a median follow-up of 33 months. In subset analyses, previous CR or uMRD during venetoclax therapy (hazard ratio, 0.029) and ≥24 months during venetoclax therapy (hazard ratio, 0.044) were associated with longer PFS after initiation of a BTKi. Notably, 8 of 19 tested patients had a *BCL2* Gly101Val mutation; at a median follow-up of 33 months, the median PFS while receiving a BTKi had not been reached for these 8 patients. This small study suggests that a BTKi has clinical efficacy for patients with acquired resistance to venetoclax.

There are other small retrospective reports of using a BTKi after venetoclax. A pooled analysis of venetoclax-treated patients from early clinical trials reported that 6 of 8 patients with

progressive CLL received ibrutinib after venetoclax and 5 had a PR.[8] Another report of 11 patients showed that 10 of 11 patients achieved PRs when treated with ibrutinib after venetoclax.[34] In addition, an analysis of patients treated with venetoclax in clinical practice reported 23 patients receiving therapy after venetoclax, with 5 patients receiving ibrutinib and 1 with a PR, 2 with stable disease, and 2 with PD.[9]

Long-term follow-up from the MURANO study reported outcomes for 8 patients who were given ibrutinib after PD following treatment with venetoclax-rituximab.[35] All 8 patients had a response to ibrutinib (7 PR and 1 very good PR) and, at last follow-up, 4 patients continued to receive ibrutinib and 4 had discontinued treatment. The median duration of treatment with ibrutinib was 15 months.

To date, the largest cohort series is a retrospective international, multicenter study that reported treatment outcomes for patients requiring treatment after venetoclax discontinuation.[10] Notably, this cohort included both patients who were BTKi naïve and those with previous exposure to BTKis. The study included 326 patients who discontinued venetoclax, 188 (58%) of whom went on to subsequent treatment. BTKis were the most common therapy after venetoclax (74 of 188 patients: 44 BTKi-naïve, 30 with previous exposure to BTKis). Among BTKi-naïve patients, the estimated PFS for post-venetoclax BTKi treatment was 32 months compared with not reached in BTKi-intolerant patients and 4 months in BTKi-resistant patients (median follow-up, 7.7 months). These data suggest that BTKis after venetoclax are active and produce durable remissions, particularly in BTKi-naïve or -intolerant patients. Responses to BTKis were not durable after venetoclax for patients with known BTKi resistance. However, for patients with previous BTKi exposure who discontinued because of intolerance, a trial with an alternative BTKi is a feasible option.[36]

In summary, BTKis should be considered after venetoclax therapy for BTKi-naïve patients; however, prospective sequencing data are still needed. Alternative therapies should be considered for patients with previous exposure to BTKis and known failure or resistance.

Noncovalent BTKis after venetoclax

Resistance to ibrutinib, an irreversible, covalent BTKi, is mediated by an acquired cysteine-to-serine mutation in BTK.[37,38] Reversible, noncovalent BTKis, including GDC-0853,[39] LOXO-305,[40] ARQ 531,[41] and vecabrutinib,[42] may overcome BTKi resistance. Although trials of noncovalent BTKis are ongoing and in early phases, preliminary data suggest that these agents have clinical activity in heavily pretreated populations; however, the exact number of patients who have received previous treatment with venetoclax is not reported.[40-42] In the preliminary results from the phase 1 dose-escalation trial of LOXO-305, there was a reported response to the noncovalent BTK in an R/R CLL patient who had an acquired *BCL2* Gly101Val mutation after venetoclax therapy.[40]

Phosphatidylinsositol-3 kinase inhibitors after venetoclax

Existing data from small retrospective series suggest that phosphatidylinsositol-3 kinase inhibitors (PI3Kis) have limited activity after treatment with venetoclax, particularly in patients who have progressed on both BTKis and venetoclax (double progressors). In a recent retrospective series, 17 CLL patients received idelalisib or duvelisib after venetoclax. At a median follow-up of 5 months, there was an ORR of 46.9% with a median PFS of 5 months and a discontinuation rate of 78%, suggesting that PI3Ki therapy did not produce durable responses and was difficult to tolerate; all of these patients had previous exposure to BTKis.[10] Notably, the clinical trials leading to the approval of idelalisib and duvelisib did not include patients with previous exposure to venetoclax.[43,44]

Cellular therapy: allo-HSCT and CAR T-cell therapy

Allogeneic stem cell transplantation (allo-HSCT) remains the only potential curative therapy for CLL, although little is known about outcomes in the era of novel agents. A recent multicenter retrospective cohort study showed a PFS rate of 60% and an overall survival rate of 82% at 24 months for CLL patients undergoing allo-HSCT after being treated with 1 or more novel agents.[45] In the largest multicenter international case series of patients receiving treatment after venetoclax, 18 patients subsequently received anti-CD19–directed chimeric antigen receptor (CAR) T-cell therapy with an ORR of 66.6%, including 33.3% of patients with CRs; notably, all of these patients had previous exposure to BTKi's.[10] The phase 1/2 study of R/R CLL patients treated with the anti-CD19–directed CAR T-cell product lisocabtagene maraleucel (TRANSCEND-CLL-004) included 9 patients who had progression with BTKi therapy and for whom venetoclax had failed. Four of these patients had ongoing responses (3, PR; 1, CR/CR with incomplete hematologic recovery [CRi]) at the time of a presentation at the 2019 American Society of Hematology meeting.[46] In addition, a pilot study of 19 CLL patients treated with CD19-targeted CAR T cells with concurrent ibrutinib after ibrutinib therapy had failed included 11 patients with previous venetoclax treatment, 6 of whom had progression during treatment with venetoclax. Although outcomes of patients treated with venetoclax are not reported separately, the high 1-year PFS of 59% suggests that ibrutinib in combination with anti-CD19–directed CAR T-cell therapy could be a promising strategy in the future.[47] These data suggest that cellular therapy with either allo-HSCT or CAR T-cell therapy may have a role in select, fit patients with high-risk disease. Whether CAR T-cell therapy should precede allo-HSCT is not known.

Conclusion: considerations for selection of the next therapy

Our approach to therapy selection after venetoclax has been discontinued is summarized in Figure 1. Considering the reason for venetoclax discontinuation and taking an inventory of previous lines of CLL therapies are essential steps in selecting a therapy after venetoclax. For patients without previous exposure to BTKis, retrospective data support using a BTKi after venetoclax. Specifically, ibrutinib has the most data to support its use in this setting.

In the clinical case presented here, the patient is BTKi naive, and therefore we recommend initiation of ibrutinib. However, this patient also had an initial response to venetoclax and had a treatment-free interval of 12 months after completion of therapy. Re-treatment with venetoclax may be an option in such patients with an initial response and without known resistance to venetoclax. Before re-treatment, the duration of previous response to venetoclax should be considered, as well as the treatment-free interval after completion of therapy. Longer-term follow-up from clinical trials and prospective studies is required to investigate the efficacy of this approach. If fit patients achieve disease control with venetoclax retreatment or BTKis, allo-HSCT may be considered for select patients.

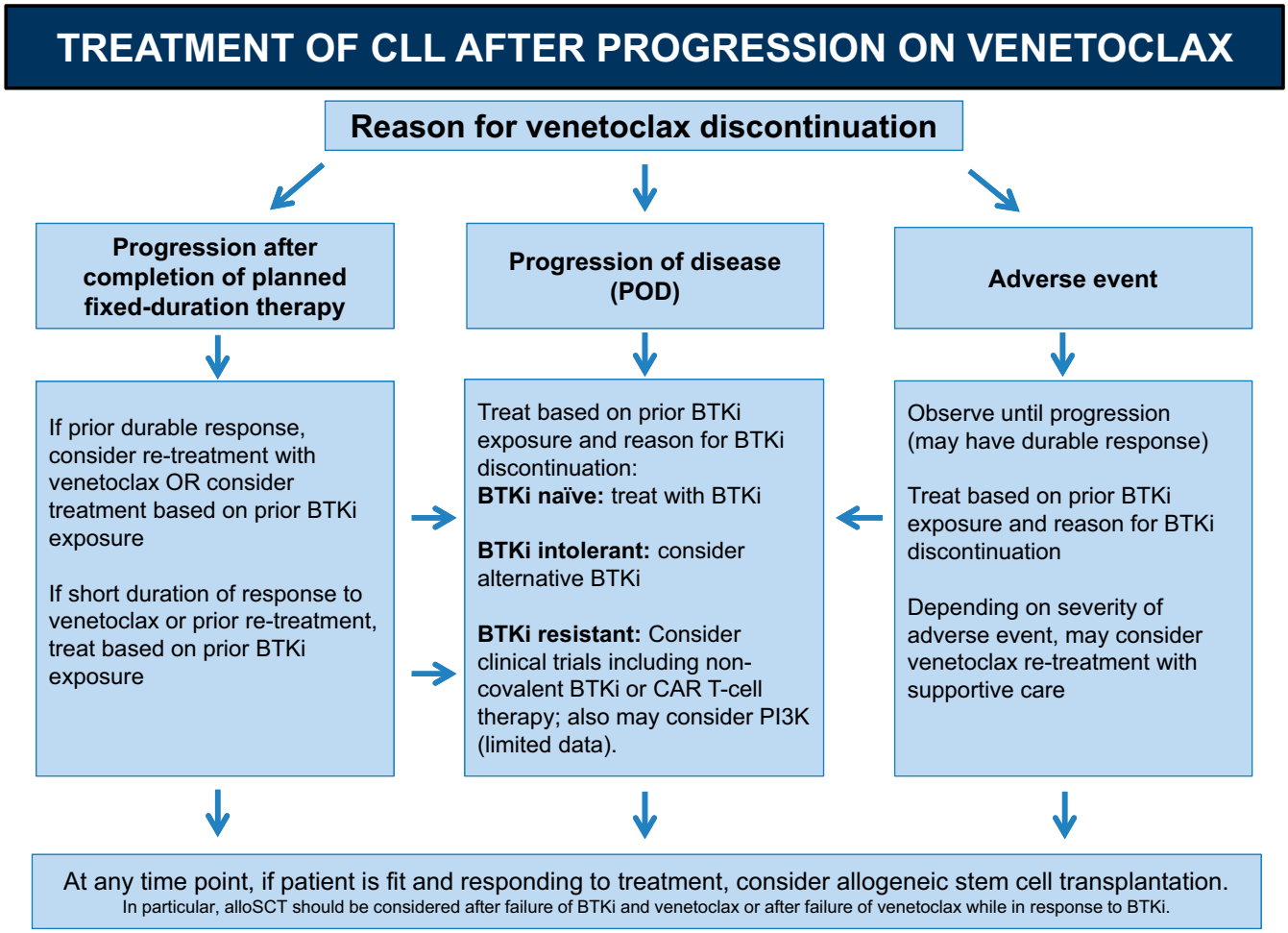

Figure 1. Algorithm for treatment of CLL after progression on venetoclax-based therapy.

For patients with previous exposure to a BTKi, the reason for discontinuation of the BTKi is important. For patients who have discontinued BTKi treatment because of intolerance rather than PD, it is reasonable to next try an alternate BTKi such as acalabrutinib. If the patient does not tolerate acalabrutinib or has no response to it, treatment on a clinical trial with a noncovalent BTKi (eg, LOXO-305 [NCT03740529], ARQ 531 [NCT03162536], or zanubrutinib [NCT04116437]) should be considered.

For patients who have developed PD while receiving a BTKi with or without a known acquired resistance mutation, allo-HCST may be considered in select fit patients. Investigational therapies that include CD19-directed CAR T-cell therapy or noncovalent BTKis are other options if they are available on clinical studies. There are few data to support the efficacy of PI3Kis after exposure to both BTKis and venetoclax in earlier lines of therapy, but PI3Ks should be considered if other options are not available.

In the currently available data on heavily pretreated patients, there is no evidence supporting chemotherapy or immunotherapy for PD after venetoclax progression. However, there are no data exploring the role of chemoimmunotherapy in the R/R setting in patients treated via chemotherapy-free pathways (ie, BTKi → venetoclax → PI3Ki). For IGHV-mutated patients, chemoimmunotherapy could be considered. The following are graded recommendations: (1) For patients with R/R CLL and PD during or after treatment with venetoclax, a BTKi should be chosen as the next therapy if the patient is BTKi naïve (evidence grade: moderate). (2) For patients with R/R CLL who responded to and have completed venetoclax-based therapy and then experience PD while not receiving therapy, re-treatment with venetoclax may be considered (evidence grade: low).

Conflict-of-interest disclosure

A.R.M. has served as a consultant for Celgene, Acerta, and Janssen; has served as a consultant for and received research funding from AbbVie, Loxo, Genentech, Pharmacyclics, AstraZeneca, Sunesis, and Johnson & Johnson; has received research funding from DTRM Biopharma and Gilead; and has served as a consultant for, received research funding from, is a DSMB member, and other for TG Therapeutics. M.C.T. declares no competing financial interests.

Off-label drug use

None disclosed.

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Does patient fitness play a role in determining first-line treatment of acute myeloid leukemia?

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The treatment choice for newly diagnosed patients with acute myeloid leukemia (AML) is no longer straightforward. Historically, patient fitness has been a major driver of the initial therapy decision based on the belief that intensive chemotherapy would be the optimal choice if a patient were "fit" enough to receive it. Tools based on chronological age, performance status, and comorbidities have been developed to help estimate patient fitness. With newer approved therapies that include nonintensive options such as IDH1 inhibition or less intensive options such as hypomethylating agent (HMA)- or low-dose cytarabine (LDAC)-based combinations with venetoclax, the choice of frontline AML therapy places more emphasis on disease-specific features, including cytogenetics and mutational profile. Moreover, newer treatments have higher response rates than what has been expected with older nonintensive options such as LDAC or HMA monotherapy. We present cases of three patients with AML with varying cytogenetic and molecular risks to demonstrate the important but changing role of patient fitness in the current era of expanding therapeutic options.

LEARNING OBJECTIVES

- Learn how to incorporate fitness and disease features in frontline AML treatment selection
- Know the tools available for estimating patient fitness and disease responsiveness

Introduction

The choice of therapy for newly diagnosed patients with acute myeloid leukemia (AML) is increasingly complicated. Until recently, therapeutic options have been limited to intensive induction chemotherapy with combination cytarabine and daunorubicin (eg, "7 + 3") for any "fit" patient or nonintensive strategies with hypomethylating agent (HMA) or low-dose cytarabine (LDAC) monotherapy. Various algorithms incorporate prognostic factors such as chronological age and comorbidities to estimate patient fitness and predict the likelihood of treatment success and treatment-related mortality.[1–4] The Ferrara et al consensus-based criteria (Table 1) also provide a practical framework for assessing fitness for intensive chemotherapy and have been used in recent phase 3 AML trials for this purpose.[5,6] However, these tools are not routinely used in clinical practice, and patient fitness remains a largely subjective determination for many clinicians. With the approval of newer AML treatment options, the role of patient fitness in treatment selection has only grown in complexity. Now, clinicians must determine not only whether a newly diagnosed patient with AML is fit to withstand intensive induction chemotherapy but also whether induction chemotherapy is the optimal option, given the patient's disease features and availability of newer, less intensive treatment options.

Frontline AML treatment landscape in the modern era

Current frontline intensive treatment options include cytarabine and daunorubicin-based induction chemotherapies such as conventional 7 + 3 and CPX-351. CPX-351 is a liposomal formulation of cytarabine and daunorubicin in a fixed molar ratio that is approved for older patients with newly diagnosed therapy-related AML or AML with myelodysplastic syndrome (MDS)-related changes. In the randomized phase 3 trial comparing CPX-351 to 7 + 3 for patients aged 60 to 75 years with newly diagnosed high-risk/secondary AML (defined as patients with a history of prior cytotoxic treatment, preceding MDS, or chronic myelomonocytic leukemia or having MDS-associated cytogenetic abnormalities), CPX-351 was associated with an improved overall remission rate (47.7% vs 33.3%; $P = .016$) and median overall survival (OS; 9.56 vs 5.95 months; hazard ratio [HR], 0.69; 95% confidence interval [CI], 0.52 to 0.90; $P = .003$).[7]

Current less intensive frontline options have expanded greatly and include HMA- and cytarabine-based combinations with venetoclax or glasdegib, and current frontline nonintensive options include the targeted IDH1 inhibitor ivosidenib (Figure 1 and Table 2). Venetoclax plus HMA (Ven/HMA) or LDAC (Ven/LDAC) and glasdegib plus LDAC combination therapies gained U.S. Food and Drug Administration

Table 1. Consensus criteria proposed by Ferrara et al for defining patients unfit for intensive chemotherapy

Fulfillment of at least one criterion suggests patient is unfit for intensive induction chemotherapy.
Advanced age (over 75 y)
Severe cardiac comorbidity
Severe pulmonary comorbidity
Severe renal comorbidity
Severe hepatic comorbidity
Active infection resistant to anti-infective therapy
Cognitive impairment
Low performance status (ECOG functional scale)
Any other comorbidity that the physician judges to be incompatible with chemotherapy

Adapted from Ferrara et al.[6]
ECOG, Eastern Cooperative Oncology Group.

(FDA) accelerated approval in 2018 for the treatment of patients with newly diagnosed AML age 75 years or older or of those who have serious cardiac, pulmonary, renal, or liver comorbidities that preclude intensive induction chemotherapy. These approvals were based on early-phase trials that demonstrated safety, tolerability, and robust composite response rates with each combination. Rates of complete remission (CR) and CR with incomplete count recovery (CRi) for Ven/HMA[8] and Ven/LDAC[9] were 67% and 62%, respectively. The subsequent phase 3 VIALE-A study confirmed that Ven plus azacitidine (Ven/Aza) compared with placebo plus azacitidine (Pbo/Aza) was associated with an improved CR + CRi rate (66.4% vs 28.3%, respectively) and median OS (14.7 vs 9.6 months; HR, 0.66; 95% CI, 0.52 to 0.85; $P < .001$).[5] In the concurrent phase 3 VIALE-C study, the planned primary analysis confirmed the improved composite response rate with Ven/LDAC compared with Pbo/LDAC (48% vs 13%), but there was only a trend toward improved median survival with Ven/LDAC (HR, 0.75; 95% CI, 0.52 to 1.07; $P = .11$).[10] A subsequent unplanned analysis with an additional 6 months of follow-up demonstrated a median OS of 8.4 months with Ven/LDAC compared with 4.1 months with Pbo/LDAC (HR, 0.70; 95% CI, 0.50 to 0.98; $P = .04$).[10] Of note, in the phase 1b/2 study, prior HMA exposure compared with those without was associated with worse outcome with Ven/LDAC (4.1 months; 95% CI, 2.9 to 10.1 months).[9] For the combination of LDAC + glasdegib, FDA approval was made after demonstration of an improved median OS of 8.8 months (80% CI, 6.9 to 9.9 months) compared with 4.9 months (80% CI, 3.5 to 6.0 months) with LDAC alone (HR, 0.51; 80% CI, 0.39 to 0.67, $P = .0004$).[11]

Importantly, clinical benefit with these newer, less intensive combination strategies have been observed across historically difficult-to-treat secondary AML and poor-risk cytogenetic and molecular subgroups commonly enriched in the older AML population.[12] In the phase 1b/2 trial evaluating Ven/LDAC in patients with previously untreated AML who were ineligible for intensive chemotherapy, CR + CRi rates of 35%, 63%, and 42% were seen in secondary AML and intermediate- and poor-risk cytogenetic groups, respectively.[9] In the VIALE-A study comparing Ven/Aza with Pbo/Aza, the CR + CRi rates were 74% vs 32% and 53% vs 23% in the intermediate- and poor-risk cytogenetic groups, respectively.[5] In addition, the CR + CRi rate for patients with *TP53* mutations was 55% with Ven/Aza compared with 0% for those who received Pbo/Aza.[5]

Notably, data in support of newer treatments have relied on composite CR data that incorporate rates of CRi or CR with partial hematologic recovery (CRh). Although these variations in CR are clinically meaningful when contrasted with rates of stable or progressive disease, they can obscure whether treatment has led to deep remission achievement vs less desirable outcomes such as treatment-related cytopenias. The impact of these treatments on minimal residual disease (MRD), which is highly prognostic for outcome,[13] is also just beginning to be understood. In the phase 1b study of Ven/HMA, 83 of 97 patients with CR/CRi had MRD data, and the median OS had not been reached for the 28 patients who achieved MRD $<10^{-3}$ or for the 55 patients who did not, though the median duration of response appears to favor those with MRD $<10^{-3}$ (not reached vs 11.3 months if MRD $\geq 10^{-3}$).[8] The rate of MRD $<10^{-3}$ is lower with Ven/LDAC. In the VIALE-A study, 6% of patients who received Ven/LDAC vs 1% of patients who received Pbo/LDAC had a flow cytometry–based MRD $<10^{-3}$.[10]

Overall, treatment selection in the modern era is increasingly influenced by disease-specific features such as cytogenetics or mutation profile and not solely by patient fitness. We present three patient cases to examine the current, changing role of patient fitness relative to other features in frontline AML treatment decision making using today's expanded therapeutic armamentarium (Figure 1).

Case descriptions

Case presentation 1

A 61-year-old man with obesity (body mass index, 33 kg/m^2), well-controlled type 2 diabetes mellitus (non–insulin dependent),

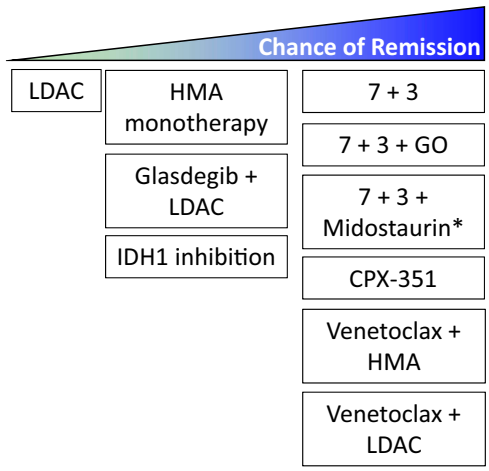

Figure 1. Spectrum of frontline AML induction therapies. Though most frontline regimens have not been compared directly, the chance of remission varies among frontline options. *The 7+3+midostaurin regimen (RATIFY trial[47]) was assessed in patients aged 18 to 59 years old with *FLT3*-mutated AML. The RATIFY trial did not show a difference in complete remission (CR) rate between 7+3+midostaurin and 7+3 alone using the strict protocol criteria of remission achievement within 60 days. However, when expanding the response definition to include CRs during protocol treatment and within 30 days after treatment discontinuation, the CR rate was significantly higher in patients randomized to the midostaurin arm (68% vs 61%; $P = .04$).

Table 2. Clinical outcomes, including response, survival, and notable toxicities in clinical trials of current frontline acute myeloid leukemia therapies

Phase	Patient population	Therapy arms	Sample size	CR	Other clinical parameters: duration of response, PFS, OS	Notable toxicities		Reference	Take-Home
						Grade ≥3 AEs	Frequency		
3	Age 17-60 y Treatment-naïve AML	Daunorubicin 45 mg/m² × 3 d + cytarabine 100 mg/m² × 7 d	318	57.3%*	OS 15.7 mo*	Cardiac	7.2% (ns)	Fernandez et al[47]	Daunorubicin 90 mg/m² improves upon 45 mg/m²
						F + N	34.9% (ns)		
						Death	4.5% (ns)		
		Daunorubicin 90 mg/m² × 3 d + cytarabine 100 mg/m² × 7 d	315	70.6%*	OS 23.7 mo*	Cardiac	7.9% (ns)		
						F + N	35.9% (ns)		
						Death	5.5% (ns)		
3	Age ≥60 y Treatment-naïve AML	Daunorubicin 45 mg/m² × 3 d + Cytarabine 200 mg/m² × 7 d	411	54%*	2-y EFS (age 60-65 y) 14%*	Infection	79%*	Löwenberg et al[48]	Daunorubicin 90 mg/m² improves upon 45 mg/m² but up to age 65 y
					2-y OS (age 60-65 y) 23%*	30-d mortality	12% (ns)		
		Daunorubicin 90 mg/m² × 3 d + cytarabine 200 mg/m² × 7 d	402	64%*	2-y EFS (age 60-65 y) 29%*	Infection	87%*		
					2-y OS (age 60-65 y) 38%*	30-d mortality	11% (ns)		
3	Age 16-72 y Treatment-naïve AML	Daunorubicin 60 mg/m² × 3 d + cytarabine 100 mg/m² × 7 d	602	75% (ns)	2-y OS 60% (ns)	60-d mortality	5%*	Burnett et al[49]	Daunorubicin 90 mg/m² is not superior to 60 mg/m²
					2-y RFS 48% (ns)				
		Daunorubicin 90 mg/m² × 3 d + cytarabine 100 mg/m² × 7 d	604	73% (ns)	2-y OS 59% (ns)	60-d mortality	10%*		
					2-y RFS 51% (ns)				
3	Age 18-59 y Treatment-naïve AML	Placebo + daunorubicin 60 mg/m² × 3 d + cytarabine 200 mg/m² × 7 d	354	53.5% (ns)	mOS 25.6 mo*	F + N	82% (ns)	Stone et al[50]	Midostaurin with induction chemotherapy is approved for newly diagnosed *FLT3*-mutant AML
					4-y OS 44.3% (ns)				
		Midostaurin + daunorubicin 60 mg/m² × 3 d + cytarabine 200 mg/m² × 7 d	335	58.8% (ns)	mOS 74.7 mo*	F + N	82% (ns)		
					4-y OS 51.4% (ns)				
3	Age 50-70 y Treatment-naïve AML, CD33 expression not required	Daunorubicin 60 mg/m² × 3 d + cytarabine 200 mg/m² × 7 d	139	72% (ns)	2-y EFS 17.1%*	TRM	8% (P = .051)	Castaigne et al[51]	GO + induction chemotherapy is approved for newly diagnosed CD33⁺ AML
					2-y OS 41.9%				
					2-y RFS 22.7%*				
		Daunorubicin 60 mg/m² × 3 d + cytarabine 200 mg/m² × 7 d + gemtuzumab ozogamicin (3 mg/m²)	139	73% (ns)	2-y EFS 40.8%*	TRM	2% (P = .051)		
					2-y OS 53.2%*				
					2-y RFS 50.3%*				

*Denotes statistical significance with $p < 0.05$. AE, adverse event; AML, acute myeloid leukemia; bid, twice daily; CR, complete remission; CRi, complete remission with incomplete count recovery; HMA, hypomethylating agent; ITT, intention to treat; LDAC, low-dose cytarabine; MDS, myelodysplastic syndrome; OS, overall survival; PFS, progression-free survival; t-AML, therapy-related acute myeloid leukemia. TLS, tumor lysis syndrome; sAML, secondary AML; R/R, relapsed/refractory; ns, not statistically significant with $p > 0.05$; F+N, fever and neutropenia; GO, gemtuzumab ozogamicin; mRFS, median relapse-free survival; mEFS, median event-free survival; mOS, median overall survival; EFS, event-free survival; CBF, core binding factor; BSC, best supportive care; CRp; CR without platelet recover.

Table 2. (Continued)

Phase	Patient population	Therapy arms	Sample size	CR	Other clinical parameters: duration of response, PFS, OS	Notable toxicities		Reference	Take-Home
						Grade ≥3 AEs	Frequency		
3	Age 60-75 y t-AML, s-AML, or de novo AML with MDS-related cytogenetic abnormalities	Daunorubicin 60 mg/m² × 3 d + cytarabine 100 mg/m² × 7 d	156	25.6%*	mOS 5.95*	F + N	70.9% (ns)	Lancet et al[7]	CPX-351 is approved for t-AML, s-AML, or de novo AML with MDS-related changes
					mEFS 1.31 mo*				
		CPX-351 100 U/m² (44 mg/m² daunorubicin and 100 mg/m² cytarabine)	153	37.3%*	mOS 9.56 mo*	F + N	68% (ns)		
					mEFS 2.53 mo*				
3	Primarily age >60 y	Hydroxyurea	99	1%*	OS odds ratio 0.61*	Cardiac	11% (ns)	Burnett et al[52]	Single-agent LDAC is a low-intensity option
		LDAC 20 mg bid	103	18%*		Cardiac	10% (ns)		
3	Age ≥65 y Treatment-naïve AML, >30% bone marrow blasts	Conventional care regimens (induction, LDAC, BSC only)	247	21.9% (ns)	mOS 6.5 mo*	F + N	30% (ns)	Dombret et al[53]	Single-agent azacitidine is a low-intensity option
					1-y OS 34.2%*				
					mEFS 4.8 mo (ns)				
					mRFS 10.5 mo (ns)				
		Azacitidine 75 mg/m²	241	19.5% (ns)	mOS 10.4 mo* (benefit driven by comparison of azacitidine with BSC)	F + N	28% (ns)		
					1-y OS 46.5%*				
					mEFS 6.7 mo (ns)				
					mRFS 9.3 mo (ns)				
3	Age ≥65 y Treatment-naïve AML with poor- or intermediate-risk cytogenetics	Treatment choice (supportive care, LDAC 20 mg/m²)	243	CR + CRp: 7.8%*	mOS (ITT) 5.0 mo*	F + N	22%	Kantarjian et al[54]	Single-agent decitabine is a low-intensity option
		Decitabine 20 mg/m²	242	CR + CRp: 17.8%*	mOS (ITT) 7.7 mo*	F + N	32%		
3	Age ≥75 y Treatment-naïve AML, ineligible for standard therapy	Placebo + azacitidine 75/ mg/m²	145	17.9%*	9.6 mo*	F + N	19%	DiNardo et al[5]	Led to accelerated approval for venetoclax + HMA for patients ineligible for intensive therapy
		Venetoclax 400mg + azacitidine 75 mg/m²	286	36.7%*	14.7 mo*	F + N	42%		
3	Age ≥ 18 y Treatment-naïve AML, ineligible for intensive chemotherapy	Placebo + LDAC 20 mg bid	68	7%*	mOS 4.1 mo*	F + N	29%	Wei et al [10]	Led to accelerated approval for venetoclax + LDAC for patients ineligible for intensive therapy
		Venetoclax 600 mg + LDAC 20 mg bid	143	27%*	mOS 8.4 mo*	F + N	32%		

*Denotes statistical significance with $p < 0.05$. AE, adverse event; AML, acute myeloid leukemia; bid, twice daily; CR, complete remission; CRi, complete remission with incomplete count recovery; HMA, hypomethylating agent; ITT, intention to treat; LDAC, low-dose cytarabine; MDS, myelodysplastic syndrome; OS, overall survival; PFS, progression-free survival; t-AML, therapy-related acute myeloid leukemia. TLS, tumor lysis syndrome; sAML, secondary AML; R/R, relapsed/refractory; ns, not statistically significant with $p > 0.05$; F+N, fever and neutropenia; GO, gemtuzumab ozogamicin; mRFS, median relapse-free survival; mEFS, median event-free survival; mOS, median overall survival; EFS, event-free survival; CBF, core binding factor; BSC, best supportive care; CRp; CR without platelet recover.

Table 2. (Continued)

Phase	Patient population	Therapy arms	Sample size	CR	Other clinical parameters: duration of response, PFS, OS	Notable toxicities		Reference	Take-Home
						Grade ≥3 AEs	Frequency		
2	Age ≥55 y Treatment-naïve AML, ineligible for standard therapy; high-risk MDS included	LDAC 20mg bid	44	2.3%*	mOS 4.9mo*	F + N	24.4%	Cortes et al[11]	Glasdegib + LDAC approved for patients ineligible for intensive therapy
		Glasdegib + LDAC 20mg bid	88	17.0%*	mOS 8.8mo*	F + N	35.7%		
1	Age 64-87 y *IDH1*-mutated, treatment-naïve AML, ineligible for standard therapy	Single-agent ivosidenib 500 mg	34	30.3%	mOS 12.6 mo	F + N	6%	Roboz et al[33]	Led to approval of ivosidenib for adults age ≥75 y with *IDH1*-mutant AML
					12-mo OS 51.1%	Differentiation syndrome	9%		
1/2	Age 58-87 y *IDH2*-mutant, treatment-naïve AML, ineligible for standard therapy	Single-agent enasidenib	39	18%	ORR 30.8%	TLS	8%	Pollyea et al[55]	Enasidenib as frontline therapy for *IDH2*-mutant AML has some benefit but is not yet approved
					mOS 11.3 mo	Differentiation syndrome	10%		
					mEFS 5.7 mo				
2	Age >60 y Newly diagnosed AML, newly diagnosed sAML, treated sAML, relapsed/refractory AML, high-risk MDS	Venetoclax 400 mg + 10 d of decitabine 20 mg/m^2	184	CR + CRi or marrow CR: 86% (newly-diagnosed AML)	mOS 18.1 mo (newly diagnosed AML)	F + N with infection	46%	Maiti et al[44]	Venetoclax with 10-d decitabine is an emerging treatment option
				67% (untreated sAML)	mOS 7.8 mo (untreated sAML)	F + N	28%		
				39% (treated sAML)	mOS 6.0 mo (treated sAML)				
				42% (R/R AML)	mOS 7.8 mo (R/R AML)				

*Denotes statistical significance with $p < 0.05$. AE, adverse event; AML, acute myeloid leukemia; bid, twice daily; CR, complete remission; CRi, complete remission with incomplete count recovery; HMA, hypomethylating agent; ITT, intention to treat; LDAC, low-dose cytarabine; MDS, myelodysplastic syndrome; OS, overall survival; PFS, progression-free survival; t-AML, therapy-related acute myeloid leukemia. TLS, tumor lysis syndrome; sAML, secondary AML; R/R, relapsed/refractory; ns, not statistically significant with $p > 0.05$; F+N, fever and neutropenia; GO, gemtuzumab ozogamicin; mRFS, median relapse-free survival; mEFS, median event-free survival; mOS, median overall survival; EFS, event-free survival; CBF, core binding factor; BSC, best supportive care; CRp; CR without platelet recover.

coronary artery disease (without anginal symptoms since coronary artery stenting 5 years ago), and a 40–pack-year smoking history presented to the emergency department with shortness of breath. He admitted to feeling unwell for the past 6 months. He had an Eastern Cooperative Oncology Group (ECOG) performance status of 1 and was living alone. He has an older sister who lives nearby. He was found to have a white blood cell (WBC) count of 10 000/μL, a platelet count of 7000/μL, and 80% peripheral blasts. A bone marrow biopsy confirmed a diagnosis of AML with MDS-related changes, del7q cytogenetics, and mutations in *SRSF2* (P95H; variant allele fraction [VAF], 29.8%), *ASXL1* (G642fs; VAF, 24.7%), and *SH2B3* (E208Q; VAF, 38.5%). His baseline echocardiogram revealed an ejection fraction of 55% with no evidence of heart failure. He had a normal chest radiograph. He was interested in treatment and bone marrow transplant but worried that his comorbidities may be limiting. How does fitness play a role in deciding between intensive and less intensive treatment?

This patient was ≥60 years old with multiple comorbidities, raising concerns about his fitness for intensive chemotherapy. There are no universally accepted criteria for assessing fitness. Chronologic age and performance status per the Karnofsky performance status scale or ECOG criteria are commonly used estimates of patient fitness, and both older age and poorer performance status (eg, ECOG ≥2) have been correlated with worse outcomes after intensive treatment.[14,15] More comprehensive tools include the Charlson Comorbidity Index and Hematopoietic Cell Transplantation Comorbidity Index (HCT-CI), which capture organ dysfunction and predict early death rates with induction chemotherapy or transplant.[3,4] AML-specific calculators such as the AML composite model (which integrates HCT-CI, cytogenetic risk, and age)[1] and a treatment-related mortality calculator (which integrates age, performance status, and select laboratory indices)[2] are also able to predict 2-year mortality with intensive therapy.[16] Geriatric assessment strategies may predict survival even more accurately by including dimensions such as cognitive impairment and objectively measured physical function.[17-19] Gait speed, for instance, is a marker of frailty that is prevalent among older patients with hematologic malignancies and is associated with increased chemotherapy-related toxicity, poor response to treatment, and mortality.[20,21] Despite these assorted tools, assessing a patient's fitness can remain a complex and subjective task for many clinicians, particularly when evaluating patients aged 55 to 75.

To assess the fitness of the patient presented in this case, our preference is to use the consensus-based, structured criteria proposed by Ferrara et al[5,6] (Table 1) that were recently adapted to determine patient eligibility in the phase 3 VIALE-A and VIALE-C trials. Using these criteria, we see that the patient has several comorbidities, but none clearly preclude his consideration for intensive induction therapy. Instead, we would rely more on his cytogenetics, mutational profile, and stated interests in a bone marrow transplant for our treatment decision. Given the patient's MDS-related cytogenetics and age between 60 and 75, CPX-351 would be recommmended.[7] In the phase 3 study comparing CPX-351 with 7 + 3 chemotherapy, the frequency of adverse events was comparable, although the duration of neutropenia and thrombocytopenia was longer with CPX-351 than with 7 + 3 (median time to absolute neutrophil count ≥500/μL, 35 vs 29 days; median time to platelet count ≥50 000/μL, 36.5 vs 29 days). Notably, prolonged neutropenia with CPX-351 was not associated with an increase in infection-related events or early mortality (5.9% and 10.6% at 30 days; $P = .149$; 13.7% and 21.2% at 60 days; $P = .097$).[7] In addition, for patients whom we intend to consolidate with a bone marrow transplant, CPX-351 is more likely than 7 + 3 to improve survival (HR, 0.46; 95% CI, 0.24 to 0.89; $P = .009$).[7]

If the patient had more significant comorbidities and were unfit for intensive induction chemotherapy, we would recommend the combination of Ven/Aza. In comparison with CPX-351, Ven/Aza has a high response rate among those with secondary AML (CR + CRi, 67%) and lower rates of febrile neutropenia,[5] though it should be noted that a head-to-head comparison with CPX-351 in a randomized trial has not been done. When compared with Pbo/Aza in the phase 3 VIALE-A study, Ven/Aza treatment resulted in increased rates of grade ≥3 thrombocytopenia (45% vs 38%, respectively), neutropenia (42% vs 29%, respectively), and febrile neutropenia (42% vs 19%, respectively), though 30-day mortality rates were similar between arms (7% vs 6%, respectively). Whether Ven/Aza compared with CPX-351 can improve survival for fit, elderly (age 60 to 75 years) patients who undergo consolidation with bone marrow transplant has not yet been assessed. In the VIALE-A trial, the OS benefit was significant in patients age ≥75 years receiving Ven/Aza compared with Pbo/Aza (HR, 0.54; 95% CI, 0.39 to 0.73). In contrast, a statistically significant OS benefit was not appreciated in patients <75 years of age (HR, 0.89; 95% CI, 0.59 to 1.33), though the study was not adequately powered to assess differences based on age.[5]

Treatment of older patients with AML will evolve further as we continue to tailor therapy on the basis of mutational profiles. The phase 3 trial comparing 7 + 3 with or without the e-selectin antagonist GMI-1271 (NCT03701308) is underway (Table 2), as is the Beat AML Master Trial that uses rapid genomic screening (<7 days) to assign therapy for patients 60 years of age and older.[22] Early results from Beat AML suggest that patients enrolled in the trial had longer OS than those who received standard therapy, but increases in OS were also appreciated in those who went on to receive alternative investigational therapy. There are also efforts to incorporate functional assays into treatment selection. Using 672 specimens from the Beat AML trial, large-scale integration of whole-exome sequencing, RNA sequencing, and in vitro drug sensitivity analyses has revealed drug sensitivities specific to previously unrecognized mutational combinations.[23] Incorporation of other functional tools, such as mitochondrial BH3 profiling assays,[24] into trial or treatment selection in the era of venetoclax-based therapies is also awaited.

Case presentation 2

A 74-year-old retired fireman was referred for a WBC count of 1000/μL with 40% peripheral blasts associated and 1 month of dyspnea on exertion. He had a history of well-controlled hypertension. Studies yielded a diagnosis of normal karyotype AML with mutations in *DNMT3A* (R882H, VAF, 41.5%), *NPM1* (W288fs; VAF, 44.6%), and *IDH1* (R132H; VAF, 43.3%). He had an ECOG performance status of 1. He was living with his supportive wife and was independent in all activities. He was eager to undergo treatment with the goal of prolonging his life, but he was not interested in bone marrow transplant. He refused to undergo any further isolation after struggling with the stay-at-home order during the coronavirus disease 2019 pandemic. He wished to spend as much quality time as possible with his four young grandchildren. He did not meet the Ferrara et al criteria for unfitness[6]. Which frontline treatment option is recommended?

This patient's case highlights treatment options for fit, elderly patients with newly diagnosed AML who are relatively chemosensitive. This patient had a favorable risk, non-core binding factor AML per European LeukemiaNet (ELN) criteria due to the normal karyotype, present *NPM1* mutation, and absence of an *FLT3* internal tandem duplication mutation with a high allelic ratio.[25] The favorable prognosis of *NPM1*-mutated AML is age dependent (median OS, 10.5 years vs 1.7 years in younger vs older patients,[26] respectively) and possibly diminished in the presence of select co-occurring poor-risk mutations.[27]

Treatment options of various intensities are available for patients with previously untreated *NPM1*-mutated AML. HMA monotherapy is a low-intensity option, but it confers a modest CR rate of 23% to 28% and a median OS ranging from 4.8 to 9.3 months.[28,29] Newer venetoclax-based combination therapy offers a higher response rate and more durable remission for this population (composite CR + CRi, >80%; 2-year OS, 71.8%).[30] Notably, our patient has a co-occurring *IDH1* mutation, and this can increase the relative chemosensitivity of *NPM1*-mutated AML.[31] In the phase 3 VIALE-A trial, patients with *IDH1/2*-mutated AML had a very high response rate when receiving Ven/Aza compared with Pbo/Aza (75% vs 11%, respectively), and those with *IDH1* mutations also had improved OS (HR, 0.28; 95% CI, 0.12 to 0.65).[5] Standard intensive induction chemotherapy with 7 + 3 is also reasonable for fit older patients with favorable-risk disease, but compared retrospectively with venetoclax-based combinations, the relative clinical benefit was less robust, with a CR rate of 56%, 1-year OS rate of 36%, and median OS of 10.8 months in patients older than 65.[29] Intensive induction chemotherapy also confers toxicities and risk of treatment-related mortality for patients >60 years old that are lessened with less intensive but active combinations such as Ven/Aza.

It should be noted that this patient technically does not meet the FDA indications for Ven/Aza: his age is <75 years, and he would not have been deemed unfit for intensive chemotherapy based on the adapted Ferrara et al criteria used by the phase 3 Ven/Aza VIALE-A study.[6] However, although the Ferrara et al criteria can be a useful tool, they lack prospective data assessing their role in identifying the optimal induction strategy (eg, 7 + 3 based vs Ven/Aza) in older patients with AML. Thus, our overall recommendation would be consideration of a clinical trial. In the absence of a trial, we would favor off-label Ven/Aza as his frontline therapy, given his previously discussed molecular profile and preference to reduce risk of prolonged hospitalization and to avoid bone marrow transplant. Grade 3 to 4 febrile neutropenia is seen in 43% of patients treated with Ven/Aza.[8] To reduce treatment-related toxicities such as neutropenic fever and infection that are seen with Ven/Aza, we recommend reviewing the management guidelines on antimicrobial, antifungal, and tumor lysis syndrome prophylaxis to reduce complications.[32] Notably, Ven/Aza shares a similar 30- and 60-day treatment-related mortality (TRM) rate with Pbo/Aza.

If this patient were less fit and were unlikely to tolerate myelosuppression or neutropenia-related infections, the small molecule inhibitor ivosidenib would be a reasonable option. Ivosidenib was originally approved by the FDA for relapsed or refractory AML and gained additional approval in 2019 for the treatment of patients with newly diagnosed *IDH1*-mutated AML who are ineligible for standard therapy based on age ≥75 or the presence of comorbidities. An expanded phase 1 trial in which 34 newly diagnosed *IDH1*-mutated patients with AML were treated with ivosidenib demonstrated a composite CR + CR with CRh rate of 42.4%.[33] The median duration of CR + CRh has not yet been reached, though the lower bound of the 95% CI was 4.6 months, and the median OS was 12.6 months (95% CI, 4.5 to 25.7).[33]

Even more treatment options may become available in the near future. Ivosidenib at the approved dose of 500 mg per day given continuously has been combined with standard dose azacitidine for up-front treatment in patients with IDH1-mutated AML who are ineligible for intensive chemotherapy based on investigator assessment.[34] Treatment has generally been tolerable, with common adverse events including thrombocytopenia, nausea, diarrhea, and anemia. Notably, combination ivosidenib plus azacitidine has an overall response rate of 78% (CR, 57%; CRi/CRp, 13%; morphologic leukemia-free state, 9%) with a median time to response of 1.8 months. There were also high rates of mutation clearance (79%; 11 of 14 patients with CR + CRh) and measurable residual disease negativity (83%; 10 of 12 patients with CR + CRh) with azacitidine plus ivosidenib.[35] Data from the phase 3 AGILE study comparing azacitidine plus ivosidenib vs azacitidine plus placebo are eagerly awaited to confirm these benefits of combination therapy. Finally, a phase 1b/2 study for IDH1-mutated myeloid malignancies is underway to assess the combination of ivosidenib and venetoclax with or without azacitidine to further deepen responses.[36]

Case presentation 3

A 64-year-old woman with a history of breast adenocarcinoma (definitively treated with surgical excision, local radiation, and 5 years of hormone therapy) and depression was referred to an oncology clinic after presenting to her primary care physician with petechiae. She had a WBC count of 4000/μL, hemoglobin of 8 g/dL, platelet count of 40 000/μL, and 30% circulating blasts. A bone marrow biopsy revealed monosomal complex karyotype (46,XY,del[5][q15q35],-12,-Y,-3,-7,-16[8]/43 to 44,idem,-Y,-3,del[7][q11.2],-16,-18,+22[2]/46,XY,del[5],del[9][p22][5]) and mutations in *TP53* (R248Q; VAF, 76.2%) and *U2AF1* (S34F; VAF, 11.3%). She had an ECOG performance status of 1. She was a recently retired nurse who lived with her partner. She was interested in remission-inducing therapy and transplant. She did not meet any criteria for unfitness according to Ferrara et al criteria.[6]

This patient has adverse-risk disease on the basis of ELN criteria, given her complex karyotype and presence of *TP53* mutation. These patients are not only less responsive to intensive chemotherapy but also frequently older, less fit with more comorbidities, and experience a higher risk of treatment-related adverse events and TRM. For patients ≥70 years of age, TRM can be 26% during the first month of conventional induction chemotherapy,[37] although TRM has been declining in newly diagnosed patients with AML who receive intensive induction regimens and may have less impact on outcomes than rates of disease resistance.[15,38] Still, CR + CRi rates are generally lower for adverse-risk disease after intensive chemotherapy than for more favorable risk groups, and the 10-year OS is ~10%.[39] Older patients are also more likely to have a complex karyotype, with 40% to 60% of these patients having a concurrent *TP53* mutation, for whom the median OS is 4 months with a 3-year OS <5%.[40] This patient also has therapy-related AML featuring *TP53* and a secondary-type lesion in *U2AF1*. The presence of secondary-type AML lesions defines a distinct disease subset associated with worse clinical outcomes, higher reinduction rates, and decreased event-free survival.[41]

Table 3. Ongoing investigational therapies for frontline acute myeloid leukemia

ClinicalTrials.gov identifier	Study	Phase	Target/agent	Key patient population
NCT03826992	CPX-351 + venetoclax	1	Venetoclax is a BCL-2 inhibitor.	Age <40 y relapsed/refractory
NCT03471260	Venetoclax + ivosidenib + azacitidine	1/2	Ivosidenib is an IDH1 inhibitor.	Age ≥18 y *IDH1* mutant, relapsed/refractory
NCT03248479	Magrolimab vs magrolimab + azacitidine	1	Magrolimab is an anti-CD47 mAb.	Age ≥18 y relapsed/refractory or treatment-naïve and unsuitable for intensive chemotherapy
NCT04086264	IMGN632 vs	1/2	IMGN632 is an anti-CD123 ADC.	Age ≥18 y CD123-positive relapsed/refractory or treatment-naïve
	IMGN632 + azacitidine vs			
	IMGN632 + venetoclax vs			
	IMGN632 + azacitidine + venetoclax			
NCT04150029	MGB453 + azacitidine + venetoclax	2	MGB453 is an anti-TIM-3 mAb.	Age ≥18 y treatment-naïve, unsuitable for intensive chemotherapy
NCT03701308	7 + 3 vs 7 + 3 + uproleselan (GMI-1271)	3	Uproleselan is an E-selectin inhibitor.	Age ≥60 y, eligible for induction
NCT03258931	Crenolanib + 7 + 3 vs midostaurin + 7 + 3	3	Crenolanib is an FLT3 inhibitor.	Age 18-60 y *FLT3*-ITD and/or D835 mutations, de novo AML
NCT04140487	Azacitidine, venetoclax, and gilteritinib	1/2	Gilteritinib is an FLT3 inhibitor.	Age ≥18 y relapsed/refractory or newly diagnosed *FLT-3*-mutated AML (for phase 2 portion)
NCT03709758	Venetoclax plus 7 + 3	1	Venetoclax is a BCL-2 inhibitor.	Age 18-60 y without *FLT3* mutation or inv16 or t(8;21)
NCT03113643	Azacitidine, venetoclax, and SL-401	1	SL-401 is a recombinant IL-3 genetically fused to truncated diphtheria toxin payload.	Age ≥18 y with CD123/IL3RA expression on myeloblasts

AML, acute myeloid leukemia; mAb, monoclonal antibody, ADC, antibody-drug conjugate; ITD, internal tandem duplication.

This patient is relatively fit for intensive therapy, but *TP53*-mutated AML is a notoriously chemoresistant disease subtype that makes intensive induction chemotherapy such as 7 + 3 less effective.[41] Although CPX-351 is an approved therapy for therapy-related AML, those with unfavorable cytogenetics did not benefit compared with conventional 7 + 3 (median OS, 6.6 vs 5.16 months, respectively; HR 0.73; 95% CI, 0.51 to 1.06).[7] Furthermore, no clear benefit was observed among those with mutations in *TP53* in either treatment arm (4.5 vs 5.1 months for CPX-351 and 7 + 3, respectively; HR, 1.19; 95% CI, 0.70 to 2.05).[42] Thus, we would not recommend CPX-351 for this patient.

Instead, we would prefer a clinical trial that is HMA-based (Table 3) or, in the absence of a clinical trial option, off-label use of Ven/HMA, with the caveat that in the phase 3 VIALE-A study, fit patients <75 years old, like the one in our vignette, would not have been included. Granted, for patients with *TP53*-mutated AML, Ven/HMA offers a median OS of 7.2 months,[8] which is not much longer than what has been observed with intensive chemotherapy, as described above. Durability of response remains a common issue for this poor-risk subset. However, Ven/HMA, which has a high CR + CRi rate for *TP53*-mutated AML (47%) compared with HMA alone, is much less toxic and has reduced TRM compared with 7 + 3, making it a more attractive regimen and bridge to bone marrow transplant. Another option is 10-day decitabine monotherapy, which results in a composite CR rate of 46% (including CR + CRi + marrow CR), robust though incomplete mutation clearance, and a median OS similar to that observed in intermediate-risk patients with AML who received the same regimen (median OS, 11.6 months).[43]

When considering Ven/HMA for the subset of patients with *TP53*-mutated AML, the choice of 5- vs 10-day decitabine warrants particular consideration. Early data from combination 10-day decitabine plus venetoclax show an impressive median OS of 18.1 months for those with newly diagnosed AML, but a median OS of only 7.8 months for patients with untreated secondary AML (n = 15).[44] However, encouraging early results show that all treatment-naïve patients treated with 10-day decitabine plus venetoclax and subsequently consolidated with bone marrow transplant were still alive at 1 year. Additional data are required to understand the optimal HMA dose in Ven/HMA-based therapies for the therapy-related TP53-mutated cohort.

Finally, two novel agents are under investigation with favorable early results for *TP53*-mutated AML, although the data are derived from small patient numbers (Table 3). Azacitidine plus the anti-CD47 antibody magrolimab showed a CR + CRi rate of 56% for the overall cohort and a CR + CRi rate of 75% for the *TP53*-mutated

cohort (n = 12) with a 6-month OS rate of 91%.[45] Azacitidine plus the small molecule "P53-refolding" agent APR-246 resulted in CR in 9 of 16 evaluable patients. Among the patients with CR, 7 of 9 had cytogenetic CR and 100% had TP53 mutation clearance (VAF cutoff was 2%).[46]

In summary, for the patient in our case vignette with very poor–risk disease, we would prefer a clinical trial if the patient is eligible and amenable. However, we recognize that a clinical trial may not be feasible on the basis of patient preference and location and that there may be strict eligibility criteria for early-phase studies. If a clinical trial is not an option, we would recommend a remission-inducing option such as Ven/HMA, which has a lower risk of TRM and higher chance of remission than current intensive chemotherapies such as CPX-351. We would also make a referral to our bone marrow transplant colleagues. If the patient's fitness level declines with disease progression, an early referral to our palliative care colleagues would be essential.

Conclusion

Frontline treatment options for AML have expanded substantially in the past few years. For several decades, treatment options have been dichotomous between intensive induction chemotherapy strategies and nonintensive options such as supportive care, and patient fitness primarily influenced the decision between these two options. Treatment options in the modern era now exist on a spectrum of treatment intensity and toxicity while having efficacy for more specific disease cytogenetics and mutational profiles (Figure 1). Outcomes are eagerly awaited from ongoing studies of novel targeted therapies such as CD47 and e-selectin inhibition and triplet therapy approaches combining the backbone of Ven/HMA with a targeted therapy (Table 3). Novel agents are also increasingly incorporated into up-front treatment strategies with the goal of deepening response. Crucially, prospective studies are still lacking that compare intensive regimens such as 7 + 3 or CPX-351 with Ven/Aza, which would help in deciding whether patients objectively fit for intensive chemotherapy might actually benefit more from less intensive therapeutic options that are now available. Tools such as the Ferrara et al[6] consensus criteria can be useful for determining unfitness for intensive induction chemotherapy, but, moving forward, functional and genomic biomarkers should be increasingly integrated to guide treatment selection, given the growing armamentarium of effective treatment options.

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Off-label drug use

None disclosed.

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How to approach shared decision making when determining consolidation, maintenance therapy, and transplantation in acute myeloid leukemia

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Until recently, treatment options for patients with acute myeloid leukemia (AML) were limited to cytotoxic chemotherapeutic agents that possessed little specificity for the cytogenetic and molecular mutations known to risk stratify patients with this disease. With the approval of multiple new therapies, not only have the agents that we treat patients with changed, but the way we talk about these options, decide on, and manage therapy has also been transformed. Given these complexities, it is important that we help patients make an informed decision by weighing the risk of relapse with patient wishes and desired quality of life. Shared decision making (SDM) is an approach to medical decision making for those situations in which most clinicians would agree that there is more than 1 correct choice for a patient. Here we review the principles of SDM and provide an overview of the 3-talk model and how it may be incorporated into the care of patients with AML.

LEARNING OBJECTIVES
- Review the principles of SDM and provide an overview of the 3-talk model of SDM
- Provide recommendations for implementing SDM processes when caring for patients with AML

Introduction

For those of us who care for patients with acute myeloid leukemia (AML), the approval of new therapies has had a significant impact not only on what treatments we have to offer, but also on how we discuss these options with patients and families. In addition to the complexity of explaining leukemogenesis and molecular mutations, we must also discuss differences in time to remission, length of treatment, need for hospitalization, and adverse effects. With experience we may refine our initial "AML talk" and be able to modify sections based on individual patient and disease characteristics. Although these conversations are well intentioned, they can be technically complex and one-sided as they tend toward delivering information and laying out options, risks, and benefits of therapy.[1] It is less likely that they begin by asking patients and families what information they want to know or how they prefer to receive this information. Similarly, an assessment of a patient's values and how they wish to participate in the decision-making process with their family may not be discussed at the time of diagnosis.[1]

For most patients, AML does not carry the familiarity of a more common solid tumor such as breast cancer, and it has come on with limited warning. This lack of understanding coupled with the suddenness of diagnosis has a significant impact on a patient's ability to process initial conversations when key decisions are being made.[1] Research has confirmed that many patients are so stressed that they may not be able to participate in the decision-making process in a meaningful way and make decisions quickly.[2,3] Patients may also feel as though they are making a choice about choosing to live or die, not realizing that there is more than 1 option for treatment. In addition to underestimating the number of treatments available, patients also tend to overestimate the possibility of cure, as well as the risk of intensive therapy.[1] One of the more extreme examples of impaired information processing becomes apparent when patients present for the first outpatient visit after induction chemotherapy and are unaware that there is a need for additional chemotherapy and possibly bone marrow transplantation.

Unfortunately, we may not become aware of how our patients are processing information until new decision points arise such as making choices for consolidation, whether or not to consider maintenance therapy, or assessing the role of bone marrow transplantation. How might we help patients process information so they can make knowledgeable decisions about their therapy? Shared decision making (SDM) is an approach to medical decision making for those situations in which most clinicians would agree that there is more than 1 correct choice for a patient.[4] During the SDM process, there is first a sharing of information between the physician and patient about existing options and the potential risks and benefits of each choice.[4] Although this sounds quite similar to our usual patient conversations, SDM differs in that patients are encouraged to consider their personal preferences and to make a decision based on what matters most to them after understanding pertinent benefits and harms. With this type of approach, patients feel better informed about their decisions and express satisfaction with the plan and process.[5] A systematic review of studies that measured SDM during a patient-clinician interaction and then evaluated the relationship of SDM with affective-cognitive, behavioral, and physiological and health outcomes confirmed this finding.[6] In 66% of the included studies, affective-cognitive outcomes, which include patient satisfaction, decisional conflict, and general perceptions about the interaction with the clinician, were statistically associated with SDM in a positive manner.[6] One of the initial models of SDM uses a framework with 3 sections: choice talk, option talk, and decision talk, which has evolved to team talk, option talk, and decision talk[4,7] (Figure 1; Tables 1-3). In both models, the physician notes that there are choices and describes them (choice/team talk), discusses these options in more detail (option talk), and then encourages patients to make a decision based on their preferences after understanding the risks and benefits (decision talk). Of note, while most physicians broadly support the inclusion of patient preference in decision making, commonly cited barriers to SDM include perceived time constraints, patient characteristics that suggest SDM cannot be successfully applied, and the notion that SDM does not have a role in certain clinical situations.[8]

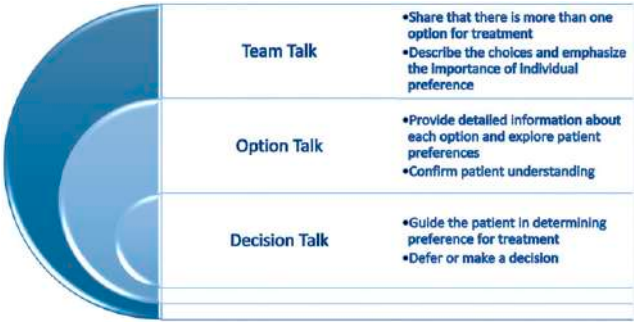

Figure 1. Three-talk model for shared decision making.[4,7] Illustration by Adrien Reidy, DPT.

Interestingly, data do not suggest a difference in the length of a clinic visit, but that the structure of the consultation did change after SDM was implemented, likely to accommodate the new interventions.[9] Regardless of level of training or years of experience, evidence suggests that training programs for physicians are essential to effectively implement SDM into regular practice.[10] In the following 3 cases we discuss how to incorporate SDM into decisions about consolidation, maintenance therapy, and transplantation for patients with AML.

Case 1

Patient 1 is a 67-year-old woman with a history of hypertension who presented to her physician for evaluation of increasing shortness of breath and was found to have a white blood cell count of 120 000. She is an active former kindergarten teacher and was otherwise asymptomatic. Her bone marrow biopsy confirmed a diagnosis of AML with normal cytogenetics and mutations in *DNMT3A* and *TET2*. She underwent leukapheresis and was started on 7+3 chemotherapy, which she tolerated well aside from anticipated cytopenias and febrile neutropenia. Her end-of-treatment bone marrow biopsy confirmed a morphologic complete remission (CR) with persistence of the *DNMT3A* mutation. She has been doing well at home and presents to the clinic for further recommendations for treatment.

Table 1. Team talk

Objective	Sample Questions/Statements
To explain the diagnosis of AML and that there is more than one option for treatment	I would like to talk more about your cancer and what the next steps are. How would you like to be involved in making this decision? We have several options for treatment and I'd like to talk to you about how each one is different. Before I share my opinion, would you like more details about your options?
To emphasize the importance of the patient's involvement, including the patient's support system if requested	I would like to work together with you and your family to come up with a decision for treatment. You do have a role in making this decision.
To review patient preferences	Each of the treatments can take you on a different path as you go through treatment. For us to make the best decision, we need to also understand what matters most to you. Do you any concerns about a prolonged hospitalization? Is receiving care close to home important to you? Are there certain side effects that you are most worried about? Do you have any financial concerns that we need to discuss? Should we consider how each treatment will impact your ability to spend time with family or care for dependents?

Adapted from MIDSOUTH PTN Practice Transformation Network.[25]

Table 2. Option talk

Objective	Sample Questions/Statements
Describe treatment options including risks and benefits and point out differences.	There are "X" treatment options for you. Let me list them before going into more detail. Not every treatment works for everyone, and the chances for side effects will be different for each person. These 2 options are different and will have a different impact on you and your family. Let me explain why.
Guide patients in considering their options, given their expressed preferences.	Based on what we've talked about, which treatment lines up best with what matters most to you? Given that you've expressed concern about being hospitalized for long periods of time, it would seem that "X" may not be a good option for you.
Check for understanding.	We've talked about a lot of things. Can you tell me what you remember from our discussion?
Consider providing written information.	Here is a summary of what we've discussed so that you can review this with your family.

Adapted from MIDSOUTH PTN Practice Transformation Network.[25]

This is the first time you are meeting her, and after reviewing her hospital course and bone marrow results, you assess her understanding of next steps for treatment. Despite the intentions of your colleagues, she left the hospital unaware of the need for additional chemotherapy and the probability of relapse. She is at her appointment with a friend today because she lives 2.5 hours away and "doesn't like to drive in the big city." She thought that this would be her final and only visit with you.

Without additional chemotherapy after achieving a morphologic CR, patients with AML will experience relapse of their disease.[11] Although most physicians, if not all, would agree with this statement, there are varied opinions as to which consolidation chemotherapy is the best for preventing relapse for patients older than age 60 years who are not proceeding to allogeneic stem cell transplantation (allo-SCT). Furthermore, we have yet to identify a regimen that prolongs survival in this patient subgroup. Potential options include a regimen similar to cytarabine and daunorubicin induction or assorted doses and schedules of cytarabine (1 to 1.5 g/m^2 once per day to twice per day on days 1 to 5 or on days 1, 3, and 5), possibly with an anthracycline or in combination with gemtuzumab ozogamicin.[12,13] HOVON97 was a phase 3 trial of patients age 60 years or older with AML or myelodysplastic syndrome (MDS) refractory anemia with excess blasts who had achieved a CR or CR with incomplete count recovery (CRi) after at least 2 cycles of cytotoxic chemotherapy who were then assigned to either observation or 12 cycles of azacitidine 50 mg/m^2 once per day for 5 days once every 4 weeks.[14] Despite gradual attrition in the azacitidine arm, therapy was well tolerated with few transfusion needs or adverse events. Importantly, disease-free survival was improved for those receiving azacitidine at the end of the 12 months (64% vs 42%; P = .04). There was no survival benefit in those receiving azacitidine: however, the trial was not powered to assess this. A more recent phase 3 trial, QUAZAR AML-001, is evaluating oral azacitidine (CC-486) vs placebo as maintenance therapy in patients age 55 years or older in first remission after intensive chemotherapy whether they received consolidation chemotherapy or not.[15] Early results have reported a survival benefit of 24.7 vs 14.8 months in those randomly assigned to azacitidine, regardless of whether they had received consolidation chemotherapy. Furthermore, relapse-free survival was also prolonged in these patients (10.2 to 4.8 months). Results of this study are pending; however, if they are confirmed, this would be the first agent to improve overall survival and relapse-free survival when given as therapy after remission in AML.

Case 1 (continued)

Recognizing that there are knowledge gaps, you take a step back and discuss with patient 1 how she has approached decision making during the course of her treatment (Table 4) and find out that she has made decisions on her own and that she prefers to think about how her choices impact her family. You also ask her how she prefers to receive information (ie, in small increments vs all at once) and whether she would like to have

Table 3. Decision talk

Objective	Sample questions
Guide the patient in determining a preference for treatment.	I now have a better understanding of what matters most to you, but just so I am sure, what I am hearing is —." You've mentioned that not being in the hospital for prolonged periods is important to you. The treatment option that is most likely to allow that to happen is —. You are worried about the side effects of nausea and emesis. Although these are possible with all regimens, we do have medications that can take care of them and prevent them from being an issue.
Defer or make a decision.	Are you ready to decide? Or do you need more time? Is there anyone else that you would like to talk to before you make a decision? Is there anything that you would like to ask me about that would help you make your decision?

Adapted from MIDSOUTH PTN Practice Transformation Network.[25]

Table 4. Preparing for a shared decision-making conversation

Objective	Sample Questions
To establish the patient's preference for amount of and delivery of information	How much information would you like to receive now? Do you prefer to receive the information in stages or all at once?
To determine how the patient wants to participate in the decision-making process	Do you prefer to make the decision on your own? Do you want family to be involved in the decision? Would you like me to make a recommendation?
To ascertain patient knowledge about the disease and treatment options	What do you already know about AML?
To clarify patient concerns, expectations, and long-term treatment goals	What do you worry about most now that you've been diagnosed with AML? Is there something that you worry might happen?

Adapted from Hashim.[24]

your help to guide her decisions, given that she has made all of her other decisions on her own. During this conversation you learn that she plays a significant role in her granddaughter's life because her own daughter is a single mother and that having to travel long distances for care will negatively impact her quality of life. This is not something that she is willing to compromise on. You then review the rationale for postremission therapy, emphasizing that there are several options for treatment, but you do so in the framework that she has set for you. She mentioned that she prefers to hear about "the long-term plan" and not just postremission therapy, so you discuss observation after treatment, detection of relapse, and salvage therapy. She asks for your help in deciding and you suggest proceeding with azacitidine on the basis of what you have understood her preferences to be. After considering her options she agrees, stating that this is the only choice that will allow her to receive care locally and hence spend more time with her family. She also agrees to telemedicine visits every 3 months so that you remain involved in her care and are able to monitor her progress.

Case 2

Patient 2 is a 73-year-old man with a history of diabetes mellitus and chronic kidney disease after he received a kidney transplant from a living relative 7 years before his diagnosis of AML. He was found to have new onset pancytopenia during routine follow-up with his nephrologist. A bone marrow biopsy confirmed cytogenetically normal AML with 3 separate *CEBPA* mutations. Although he is active and otherwise in good health, his baseline creatinine ranges from 2.7 to 3.5 mg/dL. Induction chemotherapy was initiated with decitabine 20 mg/m^2 per day for 10 days and he achieved a CR with incomplete neutrophil recovery after 2 cycles. He then started maintenance therapy with decitabine 20 mg/m^2 per day for 5 days once every 28 days. He experienced neutrophil recovery and has continued with treatment for the past 25 months uneventfully without hospitalization or delays in treatment. Today he presents for cycle 26 and would like to discuss whether to continue with maintenance therapy or to stop treatment.

Treatment with hypomethylating agents (HMAs), alone or in combination with small molecular inhibitors such as venetoclax, has become a new standard of care in AML patients age 60 years or older who are deemed unfit for intensive chemotherapy. Generally, patients begin therapy and receive between 2 and 4 cycles of HMA-based treatment in the hopes of achieving a remission or hematologic improvement that would suggest benefit for ongoing therapy.[12] For those patients for whom postremission therapy includes a bone marrow transplant, hypomethylating therapy is discontinued. For patients ineligible for transplantation, this becomes a chronic treatment until the time of relapse or the development of unacceptable toxicity. In a multicenter phase 1b study of patients age 65 years or older who are receiving venetoclax with either decitabine or azacitidine, the median duration of CR and CRi for those who received venetoclax 400 mg per day was 12.5 months.[16] In the phase 2 trial of single-agent decitabine in patients age 60 years or older, the CR rate was 47%, with a median disease-free survival of 46 weeks.[17] However, both studies include a range in duration of remission in which the upper limit was yet to be reached at the time of publication that may include patients such as the one mentioned here who has had a duration of response more than twice what was reported. In addition, many clinicians have "super responders" in their practice who have received chronic HMA therapy for several years.

There is little published data to guide cessation of HMA therapy in patients in a CR. Cabero et al[18] retrospectively reported on 16 patients with AML or higher-risk MDS who were treated on HMA-based trials or who were in CR when treatment ended either per protocol or by personal request. Eleven patients (69%) relapsed with a median time to relapse of 4 months (range, 2-68 months). Although the sample size was small, it seemed as though patients without high-risk cytogenetics who had received more than 12 cycles of therapy had a longer time to disease relapse. Minimal residual disease (MRD) monitoring and its ability to identify molecular, cytogenetic, or flow cytometric recurrence of leukemia is being incorporated into clinical trials and to some extent clinical practice to identify the depth of the first remission, inform postremission treatment strategies, and identify impending relapse.[19] Although published data about HMA "super responders" are limited, it is possible that evaluating MRD could be useful in cases such as this.

Case 2 (continued)

You discuss with patient 2 that data for guiding cessation of HMA therapy in patients who are in a CR are limited, and you mention and that he has 2 options for moving forward: continuing treatment without interruption or repeating a bone marrow biopsy to look for evidence of disease by morphology or MRD. If the bone marrow biopsy is negative, he could consider

discontinuing treatment in favor of active surveillance alone. Because you have been observing patient 2 for more than 2 years, you know that he has made all of his treatment decisions with input from his sons, enjoys being active in his church, and values his independence, but has said that he does not like having to come to the clinic every month for treatment. You ask him a few more questions and learn that what he values most is being independent and not having to rely on his wife for help because she has developed more health issues over the past year. You discuss that given his kidney disease, he is not a candidate for a bone marrow transplant, may not tolerate cytotoxic chemotherapy well, and is unlikely to be eligible for any clinical trials. For these reasons, his chances of achieving a second CR are more limited, and he may develop more treatment related side effects than he has had thus far. A decision that would offer him the best chance at maintaining his quality of life would be continuing with treatment. He agrees with this decision and the plan for therapy.

Case 3

Patient 3 is 52-year-old woman with a history of stage IIB breast cancer that was diagnosed and treated with chemotherapy 8 years before her diagnosis of AML. She was found to have new onset anemia after presenting for evaluation of shortness of breath. A bone marrow biopsy confirmed a therapy-related myeloid neoplasm, AML with MDS-related changes, and a complex karyotype with *TP53, RUNX1, DNMT3A* mutations. She received induction chemotherapy with liposomal daunorubicin-cytarabine. Her bone marrow biopsy on day 14 was consistent with persistent disease and she received a second induction with liposomal daunorubicin-cytarabine. She experienced complications in the hospital including a stay in the intensive care unit for neutropenic sepsis. Her counts slowly recovered and on day 55 of induction, she was discharged from the hospital. A bone marrow biopsy at the end of treatment confirmed both a morphologic and cytogenetic remission with disappearance of *TP53* and *RUNX1* mutations. She has been recovering at home and presents to the clinic with her partner to discuss further recommendations for treatment.

AML that arises after exposure to chemotherapy or radiation represents less than 10% of all AMLs but is more likely to be associated with high-risk features that portend a more aggressive clinical course with poor outcomes compared with de novo AML.[20] CPX-351 is a liposomal encapsulation of cytarabine and daunorubicin in a fixed 5:1 synergistic molar ratio that is now approved for patients with secondary AML or AML with MDS-related changes. In the randomized phase 3 study comparing CPX-351 to 7+3, patients enrolled on the experimental arm achieved higher remission rates (47.7% vs 33.3%; P = .016), and for those who underwent allo-SCT, there seemed to be a more favorable impact on survival.[21] Although only 19.6% of patients enrolled had therapy-related AML, CPX-351 is used in this patient population, but for those who are eligible and willing, post-remission consolidation with an allo-SCT is recommended.[21]

Case 3 (continued)

As you assess patient 3's performance status and recovery from induction, you learn that she has 3 children aged 12, 14, and 16 years. She also shares with you that this treatment had many more serious adverse effects than her breast cancer treatment and that her wife has been overwhelmed to the point that she is not able to contribute to discussions about additional treatment like she normally does. Patient 3 also expresses a lot of fear about additional treatment and an allo-SCT. Despite this, she is clear that her goal is to live as long as possible to be able to parent her young children and that she is willing to do anything that will help her to achieve that. You discuss the adverse risk of therapy-related AML and treatment with CPX-351 consolidation and allo-SCT within this framework, including the risk for toxicity and that SCT does not completely remove the possibility of relapse, particularly for *TP53*-mutated AML. She expressed understanding of these risks and decides that she wants to proceed with SCT when possible.

Conclusion

The principles of shared decision making are based on the belief that a patient's needs and desired outcomes should form the basis for all decisions. When using SDM, there is intentional engagement between the patient and his or her health care team as they navigate a diagnosis together.[22] SDM is not providing patients with a list of all possible treatments and asking them to decide. Patients want their health care providers involved in this process, and SDM allows us to integrate their values and preferences as we make decisions together. With the addition of new therapeutic options for patients with AML, consistent incorporation of SDM into our conversations may be needed now more than ever. To increase our understanding and confirm the impact of SDM on measureable outcomes in AML patients, additional studies of health care providers who use SDM techniques are needed, perhaps even in a randomized controlled study that includes usual practice as a comparator. One opportunity to consider might be the informed consent process for a clinical trial and a patient's understanding of adverse events, expectations of treatment outcomes, and the decision to participate.[23] Regardless, current data suggest that when SDM is used, patients are more likely to feel informed about their disease and satisfied with their decision.[5] Furthermore, health care teams are also more likely to feel as though they have done everything they can to honor a patient's wishes, which at the end of the day is one of the most important things that we can do.

Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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Management of toxicities associated with targeted therapies for acute myeloid leukemia: when to push through and when to stop

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The recent advent of myriad targeted therapies for acute myeloid leukemia (AML) has led to new hope for our patients but has also introduced new challenges in managing the disease. For clinicians, the ability to treat AML in the outpatient setting with novel agents of equal or greater efficacy than 7+3 has been transformative. Despite the enthusiasm, however, the reality is that many patients are still frail and remain at risk for treatment-related complications. Translating the results of clinical trials into improved outcomes for these individuals requires an understanding of how best to manage the adverse effects of these agents. Which patients benefit most and what to watch for? When to stop therapy? Using illustrative case presentations, this review details the unique toxicities associated with each of the approved mutation-specific and nonspecific targeted drugs for AML. The goal of this review is to help clinicians determine the risk:benefit ratio in decision making for individual patients with AML.

LEARNING OBJECTIVES

- Learn how to manage the most common toxicities of targeted therapies for AML to continue therapy and improve clinical outcomes
- Recognize unique and/or life-threatening adverse effects of targeted therapies that warrant rapid intervention and drug discontinuation

Introduction

Clinicians who treat patients with acute myeloid leukemia (AML) have for decades familiarized themselves with the risks of high-dose chemotherapy, specifically the need for prolonged hospitalization and the management of life-threatening cytopenia, sepsis, and organ failure. Over the years, innumerable prognostic models have been developed that purport to predict the "fitness" and appropriateness of individual patients to withstand 7+3 therapy.[1,2] On the basis of these models, older patients with newly diagnosed AML were often offered palliation over definitive chemotherapy.[3,4] Selecting appropriate patients capable of withstanding and benefiting from 7+3 therapy has remained one of the guiding tenets of AML therapy.[5]

The recent advent of myriad targeted therapies for AML therapy has led to new hope. For clinicians, the ability to treat AML in the outpatient setting with novel agents of equal or greater efficacy than 7+3 but with fewer toxicities in specific patient subsets has been transformative.[6,7] Despite the enthusiasm, however, the reality is that many patients are still frail and remain at risk for treatment-related complications. Translating the results of clinical trials into improved outcomes for these individuals requires an understanding of how best to manage the distinctive adverse effects of these agents to prolong and preserve quality of life.

This review article discusses the unique toxicities associated with currently approved targeted therapies for AML, including both mutation-specific and nonspecific agents. Each section uses specific patient scenarios to illustrate the decision making for individual patients and includes specific recommendations for when to proceed with therapy and when to stop.

Therapies for AML

FLT3 tyrosine kinase inhibitors

Midostaurin

Case 1. The patient is a 55-year-old woman with no significant medical history who presents with profound fatigue and easy bruising. Laboratory tests show a white blood cell count (WBC) of 170 000/μL, hemoglobin of 6 g/dL, and

platelets of 18 000/μL with many peripheral blasts. Bone marrow biopsy confirms AML with normal karyotype and FLT3-internal tandem duplication (FLT3-ITD) mutation. She starts 7+3 treatment followed by midostaurin on days 8 to 21. Her course is complicated by pneumonia, which requires broad-spectrum antibiotics. On day 12, she develops worsening nausea, vomiting, abdominal bloating, and diarrhea. She is started on around-the-clock antiemetics but is unable to take oral medication, including midostaurin, for 3 days. On the evening of the fourth day since beginning treatment, she develops neutropenic fever, and a computed tomography (CT) scan demonstrates new right-sided colitis. A stool test is positive for *Clostridium difficile* toxin. She begins therapy with oral vancomycin and gradually improves. Midostaurin is resumed, and she completes 14 days of drug therapy.

Currently, 2 tyrosine kinase inhibitors (TKIs) of mutant FLT3 kinase have achieved regulatory approval in the United States for therapy of *FLT3*-mutant AML: midostaurin in combination with induction and consolidation chemotherapy in the newly diagnosed setting and gilteritinib for relapsed/refractory (R/R) disease. Given that *FLT3* mutations are identified in 25% to 37% of newly diagnosed AML cases,[8] both agents have been welcome additions to the therapeutic armamentarium.

Midostaurin is a broad-spectrum multikinase inhibitor repurposed for its ability to block mutant *FLT3* signaling pathways. When added to 7+3 induction and cytarabine consolidation, midostaurin significantly improved overall survival (OS) and event-free survival in individuals age 18 to 60 years with newly diagnosed AML characterized by *FLT3*-ITD and/or tyrosine kinase domain mutations.[9] This agent was previously associated with significant gastrointestinal adverse effects leading to dose de-escalation from the originally planned dose of 100 mg twice per day continuously for 28 days to the currently recommended 50 mg twice per day for 14 days (days 8-21).[10] Schlenk et al[11] confirmed the event-free survival benefit of midostaurin added to first-line therapy in patients with *FLT3*-mutant AML up to age 70 years. Treatment in older individuals, however, was associated with cardiovascular (22%) and pulmonary events, primarily pneumonia. In addition, most did not complete the planned 12 months of midostaurin maintenance because of gastrointestinal toxicity and infections.

We adopt a 3-pronged approach to administer all 14 days of midostaurin therapy during induction and consolidation.[9] Antiemetics are given before each dose, and the medication is taken with food. Because nausea and vomiting are potentiated by the smell of the capsules, this is mitigated by airing out midostaurin capsules outside their blister pack for 10 to 15 minutes before administration. Once concomitant gastrointestinal disorders (i.e., *C difficile* infections) have been ruled out, around-the-clock use of antidiarrheal agents may be enacted. Once-per-week electrocardiograms (ECGs) should be performed to check QTc intervals with replacement of any concomitant QTc-prolonging medications, such as azoles and fluoroquinolones. Electrolyte supplementation is recommended to reduce the risk of arrhythmias in older patients. Life-threatening cardiac events and drug-induced interstitial pneumonitis should prompt discontinuation. At our institute, we routinely perform chest CT scans on all patients with newly diagnosed AML before therapy as a baseline study and to assess for asymptomatic fungal pneumonitis.[12] On the basis of data supporting the feasibility of continuous administration of midostaurin starting on day 8 until 48 hours before next chemotherapy,[11] we typically make up missed doses to ensure 14 days of midostaurin with each cycle (Table 1).

Gilteritinib

Case 1 (follow-up). The patient completes induction chemotherapy with achievement of complete remission (CR). Her brother is a full match, and plans are underway for her to proceed to allogeneic stem cell transplantation (allo-SCT). However, soon after completing consolidation cycle 1 consisting of high-dose cytarabine and midostaurin, she presents with new thrombocytopenia. Unfortunately, a repeat bone marrow biopsy reveals 75% myeloblasts with the same FLT3-ITD mutation. She begins treatment with gilteritinib 120 mg once per day. After 2 weeks, she has new elevations in liver enzymes, specifically aspartate aminotransferase (AST) 325 and alanine aminotransferase (ALT) 359 (greater than 7 times the upper limit of normal [ULN]). Gilteritinib is held for 1 week, and follow-up laboratory tests show improvement to AST 76 and ALT 140. She resumes drug treatment at a dose reduction of 80 mg per day and proceeds with plans for an allo-SCT.

Gilteritinib is a new-generation oral inhibitor with potent and specific inhibitory properties against FLT3 and AXL-1 kinases.[13] A randomized controlled phase 3 trial demonstrated higher overall response rates and significantly improved OS in patients with R/R *FLT3*-mutant AML who received gilteritinib vs conventional chemotherapy, including both high-intensity (mitoxantrone, etoposide, cytarabine) and low-intensity (azacitidine) regimens.[7] In our experience, patients, particularly those with low counts at baseline, experience significant cytopenias and symptoms after starting gilteritinib therapy. Our practice is to monitor them once per week in the ambulatory setting. Other adverse effects include elevated liver function tests, fever, myalgia/arthralgia, fatigue, mucositis, edema, rash, and diarrhea. Orthostatic hypotension is common and can be safely managed with intravenous fluids, adjustment of blood pressure medications, and addition of midodrine and/or fludrocortisone. ECGs should be performed before starting gilteritinib, on days 8 and 15 of cycle 1, at the start of cycles 2 and 3, and in additional cycles. Interruption of doses for QTc interval prolongation >500 ms is recommended. Increases in liver function tests, specifically transaminase (AST/ALT) elevations greater than 5× ULN, total bilirubin levels greater than 3× ULN, or evidence of pancreatitis (4%) should prompt temporary drug holds and resumption of the drug at a lower dose (80 mg once per day).

Gilteritinib is linked to 2 severe but rare complications. Differentiation syndrome (DS; 3%) may occur as early as 2 days and as late as 75 days after drug initiation and has been reported with several FLT3 inhibitors.[14] Symptoms include fever, shortness of breath, rapid weight gain, new pleural and/or pericardial effusions, heart failure, and hypotension. Laboratory tests typically show hyperleukocytosis with predominantly mature myeloid cells. Treatment consists of dexamethasone 10 mg intravenously or orally once every 12 hours for at least 3 days; if life-threatening complications and/or clinical deterioration occur despite 48 hours of steroid treatment, the drug should be held.[15] Posterior reversible encephalopathy syndrome is a rare neurologic complication reported in 1% of patients receiving gilteritinib. Symptoms of posterior reversible encephalopathy syndrome include seizure and acute changes in mental status. Diagnosis should be confirmed by magnetic resonance imaging, with permanent drug discontinuation. Because many patients experience gradual improvement in bone marrow blasts and responses over time, continuing therapy for 6 months in the absence of overt disease progression or

Table 1. FLT3 inhibitors: when to push through and when to stop

Drug name	Dose and frequency	Toxicity	When to push through	When to stop
Midostaurin[9,11]	50 mg orally twice per day on days 8-21 of 7+3 treatment and high-dose cytarabine consolidation	Pneumonitis, nausea/vomiting, diarrhea, fever, mucositis, infections, cardiac issues (in patients age 60 to 70 years)	Complete 14-day regimen during induction and consolidation	Unable to take oral medication because of nausea/vomiting or development of life-threatening cardiac issues; discontinue for possibly drug-related interstitial pneumonitis without infectious etiology or if there is evidence of R/R disease.
Gilteritinib[7]	120 mg orally once per day	Liver dysfunction, fever, PRES, DS, myalgia/arthralgia, fatigue, edema	First 6 cycles of therapy, mild to moderate renal impairment, mild DS responding to therapy	Severe DS with life-threatening complications or no improvement after 48 hours of steroid therapy; elevated liver function tests (AST and ALT >5× ULN, bilirubin >3× ULN). Restart at 80 mg; if pancreatitis is present, restart at 80 mg. QTcF >500 ms (1%), adjust medications and restart at 80-120 mg. Discontinue for PRES (1%) or if there is no response after 6 cycles.
Sunitinib[21]*	50 mg once per day for 4 weeks with 1 to 2 weeks off the drug	Edema, fatigue, oral ulcerations, decreased appetite, headache, gastrointestinal symptoms	Not recommended for AML therapy	Not recommended for AML therapy at this time.
Sorafenib[19,46]*	200-400 mg orally twice per day	Skin rash, fatigue, diarrhea, liver, myalgias, marrow hypoplasia, cytopenia	Off-label use for mutant *FLT3*-ITD AML in combination with other HMAs	Hold for excessive bleeding and severe cytopenias (consider resuming dose at 200 mg); hold or discontinue drug for severe persistent hypertension or cardiac ischemia or infarction; discontinue for severe skin reactions or if there is no response in 3 months.
Ponatinib[22]*	45 mg orally once per day	Pancreatitis, petechiae	Off label use	Hold drug for pancreatitis and resume at lower dose (30 mg); discontinue for life-threatening cardiac and peripheral vascular events.
Quizartinib[18]†	30-60 mg orally once per day	QTcF prolongation (dose related), DS	Per clinical trial only	Hold for significant QTC prolongation or development of cardiac arrhythmias (dose related) per clinical trial or for severe DS with life-threatening complications or no improvement after 48 hours of steroid treatment.
Crenolanib[17]†	100 mg orally three times per day plus induction/ consolidation or salvage	Nausea, vomiting, transaminitis, fluid retention, gastrointestinal bleeding	Per clinical trial only	Hold for gastrointestinal bleeding or toxicity and consider dose reduction (80 mg three times per day) per clinical trial.

PRES, posterior reversible encephalopathy syndrome; DS, differentiation syndrome; HMAs, hypomethylating agents (azacitidine, decitabine)
*Off-label use.
†Clinical trial use only.

intolerable toxicity is suggested. Patients for whom gilteritinib therapy fails should be strongly encouraged to pursue clinical trials.

Other FLT3 *TKIs*

Several other TKIs (reviewed in Daver et al[16]) have been evaluated for treating *FLT3*-mutant AML, including newer-generation inhibitors (crenolanib[17] and quizartinib[18]) and agents re-deployed in the off-label setting (i.e., sorafenib,[19,20] sunitinib,[21] and ponatinib).[22] Table 1 lists common toxicities observed with these agents and provides suggestions for management.

BCL-2 inhibition

Case 2

The patient is a 77-year-old man with a history of coronary artery disease, atrial fibrillation, and chronic obstructive pulmonary disease. He presents to his primary physician with worsening hypoxia. Laboratory tests show severe anemia (7.1 g/dL) with a WBC of 3200 cells per μL and platelet count of 78 000/μL. He is referred for workup of pancytopenia. Bone marrow evaluation confirms AML (29% blasts) with numerous cytogenetic abnormalities including del5q. Molecular profiling reveals NRAS, IDH1, and DNMT3A mutations. He is admitted and starts venetoclax and azacitidine. On day 7, the absolute neutrophil count (ANC) drops to 280 cells per microliter. He is started on posaconazole with a dose reduction of venetoclax to 100 mg once per day. On day 10, his platelet count decreases to <5000/μL. He receives once-per-day platelet transfusions with no interruption of venetoclax. Day 21 bone marrow evaluation demonstrates <5% blasts. Venetoclax is held. Granulocyte colony-stimulating factor (G-CSF) is administered. Several days later, counts improve (ANC, 1000 cells per μL, platelets 40 000/μL), and he is discharged home.

Addition of the oral B-cell lymphoma 2 (BCL-2) inhibitor, venetoclax, to hypomethylating agents (HMAs) or low-dose

Table 2. BCL-2, hedgehog, and CD33-directed therapies: when to push through and when to stop

Drug name	Dose and frequency	Toxicity	When to push through	When to stop drug
Venetoclax/HMAs (azacitidine or decitabine) or low-dose cytarabine[23,47]	400 mg orally once per day (azacitidine or decitabine) or 600 mg orally once per day (low-dose cytarabine)	Myelosuppression, tumor lysis, neutropenia, anemia, thrombocytopenia	First 2 cycles result in mild to moderate renal dysfunction, hepatotoxicity, cytopenias	Reduce dose by 50% for severe liver impairment (Child-Pugh score for cirrhosis). Hold if bone marrow evaluation on days 21 to 28 shows cytoreduction (<5% blasts). In responding patients, consider truncating duration of venetoclax therapy to 7 to 21 days in subsequent cycles; discontinue after 2 to 4 cycles if there is no response.
Glasdegib/low-dose cytarabine 20 mg subcutaneously twice per day × 10 days[34]	100 mg orally once per day	Fatigue, febrile neutropenia, dyspnea, anemia, dysgeusia, anorexia, QTc prolongation	Dysgeusia, fatigue, myalgias, cytopenias, QTc 480-500 ms during the first 6 cycles	Hold for QTc prolongation >500 ms and resume at 50 mg; discontinue for life-threatening cardiac arrythmias, or 30 days before donating blood, or for ANC <500 cells per μL and/or platelets <10 000/μL for >42 days in the absence of disease progression, or after 6 cycles of therapy if there is no response.
Gemtuzumab ozogamicin plus 7+3 therapy and consolidation (favorable- or intermediate-risk AML)[37,48]	3 mg/m^2 (maximum, 4.5 mg/m^2) intravenous infusion on days 1,4, and 7 of cycle 1 induction	Liver dysfunction, VOD/SOS, fever, myelosuppression, infusion reactions	Induction and consolidation for favorable- or intermediate-risk AML, mild or moderate hepatotoxicity, myelosuppression, or mild or moderate infusion reaction	Hold or discontinue gemtuzumab ozogamicin for severe hepatotoxicity or bleeding. Discontinue gemtuzumab ozogamicin for VOD/SOS or life-threatening anaphylaxis, or at least 2 months before planned allo-SCT.

VOD/SOS, veno-occlusive disease/ sinusoidal obstructive syndrome; HMAs, hypomethylating agents; alloSCT, allogeneic stem cell transplant

cytarabine has transformed the therapeutic landscape for older, unfit patients. These regimens deliver high response rates across diverse mutational subsets and median survival duration of more than 1 year.[23] Despite this, managing toxicity in these frail patients causes significant trepidation among clinicians. In a phase 1b trial, the most frequent severe adverse events of venetoclax with HMAs were febrile neutropenia (43%), leukopenia (31%), anemia (25%), thrombocytopenia (24%), neutropenia (17%), and pneumonia (13%). Infections of all grades occurred in three-fourths of patients (45% grade 3 to 4), with pneumonia (18%) being the most common. Deaths occurred primarily because of infections. No dose-limiting toxicities were detected with venetoclax up to 1200 mg once per day. Toxicities were similar between azacitidine and decitabine[23] (Table 2). Management of patients receiving venetoclax-based therapy varies. There are differences of opinion regarding the most appropriate setting for drug initiation (ie, outpatient vs inpatient), need for antifungal prophylaxis, timing of bone marrow evaluation, and duration and/or dosing of drugs in subsequent cycles.[24,25] A schema of how we administer venetoclax plus HMA/low-dose cytarabine is depicted in Figure 1.

Despite its designation as a low-intensity regimen, we consider this intermediate-intensity therapy, falling somewhere between 7+3 treatment and HMA/low-dose cytarabine monotherapy. Although it is not required, our practice is to admit all patients for cycle 1. Regardless of the clinical setting, it is essential that all unfit, elderly individuals who start this regimen receive careful monitoring with frequent ideally daily count checks and easy accessibility to blood products. For optimal efficacy, it is important to start venetoclax plus HMA/low-dose cytarabine concurrently, not sequentially (ie, starting HMA/low-dose cytarabine first and adding venetoclax later). Tumor lysis remains a primary concern. Hydroxyurea is administered before therapy is started if the WBC exceeds 25 000 cells per μL. For the first 3 to 5 days, all patients receive frequent laboratory checks, intravenous fluids, and allopurinol. Although gradual daily dose escalation of venetoclax is recommended (ie, 100 mg to 200 mg to 400 mg to 600 mg, if indicated), we have noted no clinically significant tumor lysis with full-dose venetoclax given in the inpatient setting.

Prolonged, often severe, myelosuppression (typically ANC <500 cells per μL or platelets <10 000 to 20 000/μL) associated with venetoclax and HMA/low-dose cytarabine therapy can last several days to weeks with or without clinical sequelae. Management depends on bone marrow response to therapy. Most individuals remain hospitalized during cycle 1 because of the requirements of once-per-day transfusions and/or complications of cytopenias such as neutropenic fever, infection, and bleeding. Dose reductions of venetoclax for concurrent azole use are standard: 70 to 100 mg with posaconazole or voriconazole (ie, strong CYP3A4 inhibitors) and 200 mg with isavuconazole (ie, moderate CYP3A4 inhibitors). Given the life-threatening nature of pulmonary infections in these patients, we perform baseline chest CT scans on all patients before starting therapy with a low threshold to initiate antibiotics and antifungals for any suspect initial or subsequent CT findings.[12]

To assess response, we perform bone marrow evaluation on cycle 1 day 21. If the bone marrow shows disease control (defined as <5% marrow blasts and/or marrow cellularity <10%), we hold venetoclax for 2 to 4 weeks until hematologic recovery (ANC >500

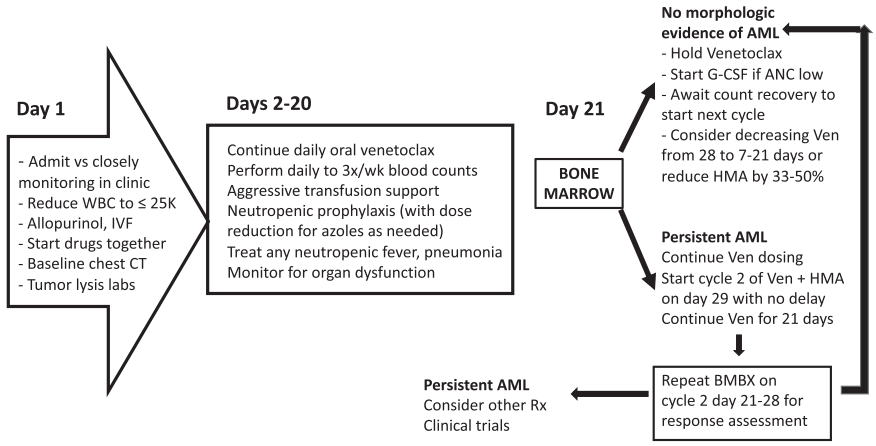

Figure 1. Management approach for venetoclax plus HMA/low-dose cytarabine. BMBX, bone marrow biopsy; IVF, intravenous fluid; Rx, prescription; Ven, venetoclax.

cells per μL and platelets >50 000/μL) before starting cycle 2 of therapy with both drugs. We often administer growth factor (G-CSF; granulocyte-macrophage colony-stimulating factor [GM-CSF]) in the interim to hasten neutrophil recovery. In contrast, patients with refractory AML without cytoreduction (>5% to 10% blasts and cellularity >10%) on day 21 bone marrow evaluation are continued on once-per-day venetoclax regardless of blood cell counts. Cycle 2 is initiated with both drugs on day 29 with venetoclax administered once per day until repeat bone marrow assessment on days 21 to 28 of cycle 2. Given the median time to response of 1 to 2 cycles for venetoclax-based regimens, the presence of persistent AML after cycle 2 should prompt physicians to consider alternative therapy.

In responding patients, both venetoclax and HMA/low-dose cytarabine should be administered indefinitely until intolerable toxicity or disease recurrence. In patients who maintain normal blood cell counts, no dose reduction or modification of treatment duration is recommended. Similar to others,[24,25] we have 2 approaches to treating patients with prolonged cytopenias and no evidence of bone marrow AML: (1) shorten venetoclax duration initially from 28 to 21 days and then to 7 to 14 days on successive cycles to minimize cytopenias without dose reduction, and (2) reduce HMA dose by 33% to 50% and/or extend the time between HMA cycles from 4 to 5 weeks. Additional bone marrow evaluations are performed to rule out relapsed AML as the cause of prolonged cytopenias that are not responding to these measures and routinely every 4 to 6 months to confirm continuing response. In practice, regimen adjustments are highly individualized and shaped by patient preference, logistics, and clinical nuance.

IDH1 and IDH2 inhibitors

Case 2 (follow-up)

The patient achieves a CR with incomplete count recovery with venetoclax and azacitidine. Unfortunately, after 12 months, routine bloodwork shows that he has increased peripheral blasts. Bone marrow biopsy reveals relapsed AML with NRAS, IDH1, and DNMT3A mutations. He elects to take ivosidenib. Three weeks into therapy, he presents to the clinic with new onset fever, hypoxia, weight gain of 10 pounds, and evidence of diffuse bilateral infiltrates on chest CT scan. Laboratory tests demonstrate a WBC of 21 000 cells with predominantly neutrophils and no blasts. A respiratory viral panel is negative. He is started on empiric azithromycin for possible infection and dexamethasone 10 mg orally twice per day. Five days later, he feels much better and continues uninterrupted treatment with ivosidenib.

Ivosidenib and enasidenib are first-in-class oral small molecule inhibitors of mutant isocitrate dehydrogenase-1 (*IDH1*) and -2 (*IDH2*), respectively.[26,27] Both agents work by decreasing abnormal production of the oncometabolite 2-hydroxyglutarate (2-HG), leading to differentiation of *IDH*-mutant myeloid blasts. Approximately 20% to 25% of patients on clinical trials develop hyperleukocytosis and clinical evidence of DS, a potentially lethal complication manifested by fluid retention, diffuse pulmonary infiltrates, hypoxia, and fever.[28] Onset is delayed with a median time of 19 to 20 days (range, 1-86 days) after treatment initiation. Fatal outcomes occur in 5% to 6% of patients.[29,30] Given the long half-life of these inhibitors, first-line treatment of DS is not to stop treatment with ivosidenib or enasidenib but to initiate dexamethasone 10 mg intravenously or orally twice per day for at least 3 days with hydroxyurea and/or leukapheresis for clinically relevant hyperleukocytosis. Inhibitors of IDH1 and IDH2 should be halted for life-threatening complications or lack of improvement after 48 hours, either temporarily or permanently depending on severity (Table 3).

Each inhibitor exhibits distinctive toxicities. More than one-third of patients receiving enasidenib develop asymptomatic increase of indirect bilirubin levels, which is generally self-limited and responds to dose reduction to 50 mg once per day. Almost 25% of patients treated with ivosidenib will have QTc prolongation >450 ms, with 10% developing a QTc interval >500 ms. ECG monitoring should be performed once per week for the first 3 weeks and then once per month for the duration of treatment. Ivosidenib can be resumed at a reduced dose of 250 mg once per day when QTc intervals return to within 30 ms of baseline or ≤480 ms. Another rare complication (incidence, 1%) of ivosidenib that requires permanent discontinuation is Guillain-Barré syndrome, which presents as progressive ascending muscle weakness, loss of reflexes, and sensory neuropathy leading to respiratory failure.[31]

It is important to recognize that both IDH inhibitors require long-term administration (at least 6 months) for optimal efficacy. In previous studies, the median time to CR with or without hematologic recovery was 2.8 months for ivosidenib and 1.9 months for enasidenib. Over time, many individuals experienced

Table 3. IDH inhibitors: when to push through and when to stop

Drug name	Dose and frequency	Toxicity	When to push through	When to stop
Ivosidenib[32]	500 mg orally once per day	DS, QTc prolongation, cytopenias, high WBC	First 6 months, mild to moderate renal and hepatic dysfunction, and mild DS	Hold for severe DS with cardiopulmonary compromise that requires emergent hospitalization with or without renal dysfunction, for QTc prolongation >480 ms (resume when <480 ms). Discontinue for life-threatening cardiac arrythmias, for Guillain-Barré syndrome, or if there is no response (hematologic recovery/blast clearance) after 6 months.
Enasidenib[33]	100 mg orally once per day	DS, elevated bilirubin, cytopenias, high WBC	First 6 cycles, mild to moderate renal impairment, mild DS, bilirubin <3× ULN	Hold for severe DS with cardiopulmonary compromise requiring emergent hospitalization with or without renal dysfunction, for bilirubin 3× ULN or more and reduce dose to 50 mg once per day. Discontinue if there is no response (hematologic recovery or blast clearance) after 6 months.

DS, differentiation syndrome; ULN, upper limit of normal; WBC, white blood cell count

decreased transfusion requirements and fewer clinical events despite incomplete hematopoietic recovery and persistent blasts.[32,33]

Sonic hedgehog inhibitor (glasdegib)

Case 3

The patient is a 75-year-old man with a history of chronic obstructive pulmonary disease and multiple myeloma after chemotherapy who develops pancytopenia with circulating blasts. Bone marrow biopsy reveals a therapy-related myelodysplastic syndrome with 15% blasts and complex karyotype. Despite several cycles of azacitidine, his disease progresses to AML. Treatment with glasdegib 100 mg orally once per day is started with low-dose cytarabine, and he achieves a partial response after 2 cycles. He presents to the clinic today with progressive weight loss and lack of a sense of taste. ECG demonstrates QTc prolongation to 490 ms (previously 440 ms). Glasdegib is reduced to 50 mg orally once per day. He is prescribed zinc lozenges. Posaconazole treatment is converted to treatment with micafungin, and levofloxacin treatment is changed to treatment with amoxicillin clavulanate.

Glasdegib is an oral sonic hedgehog inhibitor used in combination with low-dose cytarabine for older and/or unfit patients with AML. Glasdegib plus low-dose cytarabine significantly improved CR rates (17.0% vs 2.3%) and OS (8.8 vs 4.9 months; $P = .0004$) compared with low-dose cytarabine.[34] We consider this regimen in individuals at high risk for complications of venetoclax-based therapy (eg, infections, bleeding), specifically patients with several comorbidities and baseline pancytopenia, as well as in individuals who request a 100% outpatient regimen. Inhibition of hedgehog signaling in normal tissues causes adverse effects such as muscle spasms (20%), ageusia/dysgeusia (loss or alteration of the sense of taste, 24%), thinning or loss of hair (10%), and asthenia (fatigue). Other toxicities include anemia, hemorrhage, febrile neutropenia, edema, thrombocytopenia, nausea, dyspnea, constipation, and rash. Five percent of patients develop QTc prolongation >500 ms.[34] Management of glasdegib includes close monitoring of ECGs before and after initiation of therapy, antispasmodics, nutritional evaluation and supplementation, and anecdotal treatment with zinc lozenges to alleviate changes in sense of taste. Permanent discontinuation of glasdegib should be implemented for life-threatening cardiac arrythmias or severe neutropenia or thrombocytopenia lasting more than 42 days without overt bone marrow disease. Embryo-fetal toxicity remains a concern. It is recommended that patients do not donate blood products within 30 days of their last dose to avoid exposing pregnant women to the drug. Patients should receive 6 months of therapy for clinical response in the absence of progression (Table 2).

CD33 antibody-drug conjugate

Case 3 (follow-up)

The patient eventually develops worsening cytopenias, and a repeat bone marrow biopsy shows relapsed CD33+ AML (FLT3 and IDH wild-type). After discussion, he elects to start gemtuzumab ozogamicin monotherapy and receives the first dose in the clinic. He receives premedication with acetaminophen, diphenhydramine, and corticosteroid. After infusion, he is monitored every 10 to 15 minutes. Approximately 45 minutes after infusion, he develops fever, chills, and shaking rigors. His oxygen level drops to 85%. The nurse calls for additional orders. Additional doses of acetaminophen, diphenhydramine, and corticosteroid are given and he gradually improves. Vital signs return to normal, and he is discharged home 2 hours after infusion. One week after therapy, he has mild scleral icterus associated with acute increases in liver function tests: total bilirubin 4.5 mg/dL, AST 287, and AST 321. Hepatitis panel is negative. Right upper quadrant ultrasound and amylase/lipase levels are unremarkable. He is started on ursodiol (ursodeoxycholic acid) 3 times per day with gradual improvement and normalization of liver function tests.

Gemtuzumab ozogamicin is an antibody-drug conjugate composed of a monoclonal antibody directed against CD33 expressed on myeloid cells covalently linked to the DNA damaging agent calicheamicin. Gemtuzumab ozogamicin has been approved for treating patients with newly diagnosed CD33+ AML characterized by favorable-risk (specifically core binding factor) and intermediate-risk cytogenetics in combination with 7+3 induction and consolidation chemotherapy.[35-38] In the United States, gemtuzumab ozogamicin is also indicated as monotherapy for unfit patients with newly diagnosed AML and patients with R/R AML. In combination with chemotherapy, gemtuzumab ozogamicin is dosed on a hyperfractionated regimen of 3 mg/m^2 (maximum dose, 4.5 mg/m^2) per dose on days 1, 4, and 7. Adverse effects included veno-occlusive disease (VOD, also known as sinusoidal obstructive syndrome [SOS]), prolonged platelet recovery, severe hemorrhage, and infusion

Table 4. Toxicities of AML therapies by organ system

Toxicity	Responsible drug(s)	Description	Treatment of toxicity	When to stop drug
Hepatotoxicity	Gemtuzumab ozogamicin	VOD/SOS, elevated AST, ALT, bilirubin	Monitor liver function test results frequently, use ursodiol, avoid administration in patients with known hepatic dysfunction, use defibrotide for VOD/SOS	Hold or discontinue gemtuzumab ozogamicin for severe hepatotoxicity; discontinue for VOD/SOS and at least 2 months before planned allo-SCT.
	Enasidenib	Asymptomatic elevation of indirect bilirubin	Dose reduce to 50 mg once per day	No need to stop drug.
	Venetoclax	Elevated liver enzymes, bilirubin	Supportive care with no dose reduction	Reduce dose by 50% for severe liver impairment (Child-Pugh score for cirrhosis).
	Gilteritinib	Elevated liver function tests (transaminases, bilirubin), pancreatitis	Hold for elevated liver function tests (AST and ALT >5× ULN, bilirubin >3× ULN) and restart at 80 mg once per day	Hold for elevated liver function tests (AST and ALT >5× ULN, bilirubin >3× ULN) and restart at 80 mg once per day; hold drug for pancreatitis.
Nephrotoxicity	Venetoclax + chemotherapy	Tumor lysis syndrome	Allopurinol, intravenous hydration, tumor lysis laboratory tests, frequent monitoring	Severe renal dysfunction in setting of tumor lysis.
Embryo-fetal toxicity	Glasdegib	Embryo-fetal toxicity	Withhold therapy in all individuals suspected of being pregnant, contraceptives	Do not give to individuals who are pregnant or breastfeeding; do not give blood within 30 days of last dose.
Neurologic complications	Glasdegib	Fatigue (asthenia), ageusia/dysgeusia (loss or alteration of sense of taste), anorexia, myalgias	Symptomatic measures, nonsteroidal anti-inflammatory drugs, zinc lozenges, analgesics	Hold for significant debilitating weight loss.
	Gilteritinib	Posterior reversible encephalopathy syndrome	MRI scans, antiseizure medications	Permanently discontinue drug.
	Ivosidenib	Guillain-Barré syndrome	Lumbar puncture, MRI, ventilatory support	Permanently discontinue drug.
Cardiotoxicity	Glasdegib	QTc prolongation	Perform ECGs before, and 1 week after intitation of therapy, and monthly for 2 months, supplement electrolytes, adjust other medications	Hold for significant QTC prolongation or development of cardiac arrhythmias. Reduce dose to 50 mg once per day, and discontinue for life-threatening cardiac arrythmias.
	Midostaurin	QTc prolongation	ECGs once per week, electrolytes, substitute other medications	Hold for significant QTC prolongation or development of cardiac arrhythmias (dose related) per clinical trial.
	Gilteritinib	Orthostatic hypotension	Intravenous fluids, adjust blood pressure medications, add midodrine and/or fludrocortisone	Hold for severe symptomatic orthostasis.
	Gilteritinib	QTc prolongation	Electrolyte supplementation, baseline ECGs before cycle 1, on days 8 and 15, and start of cycles 2 and 3	Hold for QTc prolongation >500 ms.
	Ivosidenib	QTc prolongation	Electrolyte supplementation, adjust other medications, ECG at baseline and once per week for 3 weeks, then once per month during treatment	Hold for significant QTC prolongation >500 ms or development of cardiac arrhythmias; reduce dose to 250 mg once per day.
Gastrointestinal toxicity	Midostaurin 50 mg twice per day × 14 days	Nausea, vomiting, diarrhea, mucositis	Antiemetics before each dose, take with food, air each tablet outside of blister pack, rule out gastrointestinal infection, make up missed doses	Discontinue for life-threatening cardiac and peripheral vascular events.
	Glasdegib	Ageusia/dysgeusia (loss or alteration of sense of taste), anorexia	Symptomatic measures, nonsteroidal anti-inflammatory drugs, zinc lozenges, analgesics	Hold for significant debilitating weight loss.
Pulmonary toxicity	Gilteritinib	DS (2-75 days)	Dexamethasone intravenously or orally, oxygen, respiratory support, hydroxyurea, leukopheresis	Severe DS with life-threatening complications or no improvement after 48 hours of steroid treatment.

MRI, magnetic resonance imaging; DS, differentiation syndrome; VOD/SOS, veno-occlusive disease/sinusoidal obstructive syndrome.

Table 4. (Continued)

Toxicity	Responsible drug(s)	Description	Treatment of toxicity	When to stop drug
	Ivosidenib	DS (1-86 days)	Dexamethasone intravenously or orally, oxygen, respiratory support, hydroxyurea, leukopheresis	Severe DS with life-threatening complications or no improvement after 48 hours of steroid treatment.
	Enasidenib	DS (1-86 days)	Dexamethasone intravenously or orally, oxygen, respiratory support, hydroxyurea, leukopheresis	Severe DS with life-threatening complications or no improvement after 48 hours of steroid treatment.
	Midostaurin	Drug-related interstitial pneumonia	Consider baseline chest CT before therapy, and rule out infections	Discontinue drug.
	Venetoclax + chemotherapy	Respiratory infections, pneumonia,	Consider baseline chest CT, Infectious Disease consult, antibiotics, antifungals, fungal markers	Do not stop drug during cycle 1 until bone marrow evaluation is complete.
Hematologic toxicity	Venetoclax + chemotherapy	Prolonged myelosuppression	Daily transfusion support, neutropenic prophylaxis	Hold if cycle 1 days 21 to 28 bone marrow evaluation shows cytoreduction (<5% blasts), consider truncating duration of venetoclax therapy to 7-21 d in subsequent cycles.
	Glasdegib	Prolonged myelosuppression	Transfusion support, neutropenic prophylaxis	Discontinue for ANC <500 cells per μL and/or platelets <10 000/μL for >42 days in the absence of disease progression.
	Gemtuzumab ozogamicin	Prolonged thrombocytopenia with risk of severe hemorrhages	Transfusion support, monitor counts once per week with frequent platelet transfusions, and take precautions to avoid falls	Hold or discontinue gemtuzumab ozogamicin for severe bleeding.
Immunologic toxicity	Gemtuzumab ozogamicin	Infusion reaction before and 24 hours after dosing	Premedication (acetaminophen, diphenhydramine, corticosteroid) before each dose, frequent vital signs, clinical monitoring	Discontinue gemtuzumab ozogamicin for life-threatening anaphylaxis.

MRI, magnetic resonance imaging; DS, differentiation syndrome; VOD/SOS, veno-occlusive disease/sinusoidal obstructive syndrome.

reactions during and up to 24 hours after infusion. Premedications (ie, acetaminophen, diphenhydramine, and corticosteroid) should be given before each dose of gemtuzumab ozogamicin with frequent checks of vital signs and clinical monitoring before and immediately after infusion. Patients should be carefully monitored on at least a once-per-week basis to determine whether they need additional platelet transfusions to minimize bleeding risk as well as liver function tests. Gemtuzumab ozogamicin should be avoided in individuals with known hepatic dysfunction and for 2 months before allo-SCT. Any life-threatening anaphylaxis and VOD or SOS warrant permanent discontinuation of the drug (Table 3). Results of an expanded access trial in R/R AML confirmed the safety and feasibility of gemtuzumab ozogamicin administered alone or with chemotherapy in patients age 3 months or older. Hepatotoxicity was infrequent, with a VOD incidence of 0.5% to 1.1%[39] (Table 2).

Financial toxicities

Prohibitively high cost is common to all agents, also known as financial toxicity. Currently, the average wholesale price for 1 tablet of gilteritinib is estimated to be $315.00[40]; ivosidenib, $548.42[41]; enasidenib, $1081.19[42]; glasdegib, $710.85[43]; midostaurin, $192.18[44]; and venetoclax 100 mg, $122.92.[45] Therefore a 1-month supply would cost $7000 to $28 000. Addressing this issue requires dedicated clinical, pharmacy, social work, and case management staff. Given the aggressive clinical course of AML, timely insurance authorizations are needed. Philanthropic foundations, such as Patient Access Network Foundation, Cancercare, Good Days, and the Leukemia Lymphoma Society should also be pursued.

Conclusion

There are now myriad effective targeted agents for AML, an embarrassment of riches, after 4 decades of 7+3 treatment. Each of these agents has a distinctive mechanism of action and unique toxicity profile (summarized by organ toxicity in Table 4). Knowing when to push through and when to stop therapy is essential to striking the elusive balance between prolonging survival and preserving meaningful quality of life. It is our hope that these recommendations prove useful to both clinicians and patients who are navigating the difficult journey of AML therapy together.

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Conflict-of-interest disclosure

E.S.W. served on advisory boards and/or provided consulting services for AbbVie, Astellas, Daiichi Sankyo, Dava Oncology/Arog, Genentech, Jazz, Kite Pharmaceuticals, Kura Oncology, Macrogenics, Pfizer, PTC Therapeutics, and Stemline; served on independent data review committees for clinical trials for AbbVie, Genentech, and Rafael Pharmaceuticals; and served as a speaker for Stemline and Pfizer. J.B. declares no competing financial interests.

Off-label drug use

None disclosed.

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"TEG talk": expanding clinical roles for thromboelastography and rotational thromboelastometry

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Viscoelastic assays (VEAs) that include thromboelastography and rotational thromboelastometry add value to the investigation of coagulopathies and goal-directed management of bleeding by providing a complete picture of clot formation, strength, and lysis in whole blood that includes the contribution of platelets, fibrinogen, and coagulation factors. Conventional coagulation assays have several limitations, such as their lack of correlation with bleeding and hypercoagulability; their inability to reflect the contribution of platelets, factor XIII, and plasmin during clot formation and lysis; and their slow turnaround times. VEA-guided transfusion algorithms may reduce allogeneic blood exposure during and after cardiac surgery and in the emergency management of trauma-induced coagulopathy and hemorrhage. However, the popularity of VEAs for other indications is driven largely by extrapolation of evidence from cardiac surgery, by the drawbacks of conventional coagulation assays, and by institution-specific preferences. Robust diagnostic studies validating and standardizing diagnostic cutoffs for VEA parameters and randomized trials comparing VEA-guided algorithms with standard care on clinical outcomes are urgently needed. Lack of such studies represents the biggest barrier to defining the role and impact of VEA in clinical care.

LEARNING OBJECTIVES

- Describe the principle of viscoelastic testing and the technologies of thromboelastography and rotational thromboelastometry
- Appraise the evidence supporting viscoelastic assays in assessing coagulopathies and managing bleeding for various indications
- Recognize important considerations prior to implementing thromboelastography or rotational thromboelastometry at your institution

What are viscoelastic assays, and how does one interpret them?

Viscoelastic assays (VEAs) are global tests of coagulation performed on whole blood at the point of care. The thromboelastography (TEG) assay was first described in 1948 by Dr. Helmut Hartert at the University of Heidelberg, Germany. As blood clots, the fluid becomes less viscous and more elastic in nature. TEG and rotational thromboelastometry (ROTEM) are VEAs that assess clot formation, strength, and dissolution by measuring the effect of a continuously applied rotational force on whole blood that is transmitted to an electromechanical transduction system (TEG) or optical detection system (ROTEM), with results displayed as a graph. This allows the quantitative and qualitative measurement of the function of almost all components of clot formation and lysis, including platelets, other blood cellular components, fibrinogen, microvesicles, and soluble factors.

The TEG device has a pin suspended from a torsion wire immersed in a cup of whole blood (Figure 1A). The cup is held in a heating block and continually oscillates. Changes in viscoelastic clot strength are directly transmitted to the torsion wire and detected by an electromechanical transducer. The ROTEM device has a cup, which remains fixed in a heating block with whole blood while a pin suspended on a ball bearing mechanism oscillates (Figure 1B). The subsequent rotation of the pin is inversely related to the viscoelastic clot strength and is detected optically.

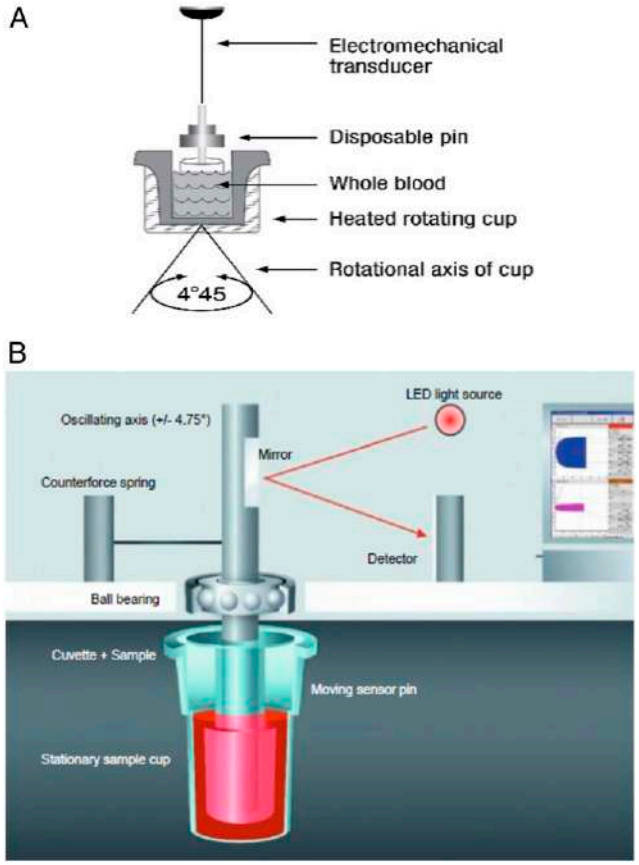

Figure 1. (A) Thromboelastography principle. (B) Rotational thromboelastometry principle.

The 2 common devices in use currently are the TEG 5000 (Haemonetics, Braintree, MA) and ROTEM Delta (Instrumentation Laboratory, Bedford, MA), shown in Figures 2A and 2B. More recent versions of these analyzers, the TEG 6s and the ROTEM Sigma, use cartridge-based systems with dry reagents designed to improve both usability at point of care by non–laboratory-trained personnel and interoperator reproducibility (Figures 2C and 2D). Although all TEG and ROTEM devices measure the same viscoelastic property (shear modulus), the newer ROTEM Sigma device is still based on the rotating pin described above, whereas the TEG 6s has changed to a microfluidic assay that measures clot resonance frequency.

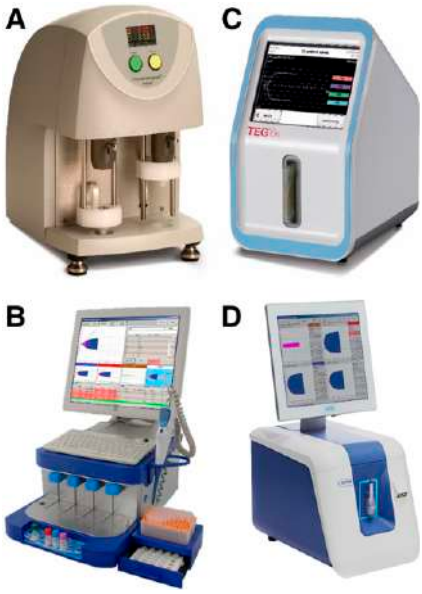

Figure 2. (A) TEG 5000. (B) ROTEM Delta. (C) TEG 6S. (D) ROTEM Sigma.

Although both TEG and ROTEM assess clot kinetics, strength, and lysis, the results are not interchangeable, owing to differences in both the operating characteristics and nomenclature of the parameters defined in the 2 systems. Figures 3A and 3B and Table 1 provide detailed descriptions of the parameters and their interpretation. A number of different assays can be performed on both TEG and ROTEM analyzers using various activators or inhibitors. In both systems, contact activation using ellagic acid or kaolin can provide information similar to the activated partial thromboplastin time. Tissue factor activation can provide information similar to the prothrombin time. Neutralization of heparin using lyophilized heparinase, blocking platelet contribution to clot formation by using platelet inhibitors, or inhibiting fibrinolysis with antifibrinolytics can help assess various aspects of coagulation. A comparison of the various assays available on the TEG 5000 and ROTEM Delta analyzers is summarized in Table 2. These assays can be customized for use in algorithms depending on the information needed to guide decision-making.

It is essential to note that diagnostic cutoffs or thresholds for the various parameters are assay, institution, or algorithm

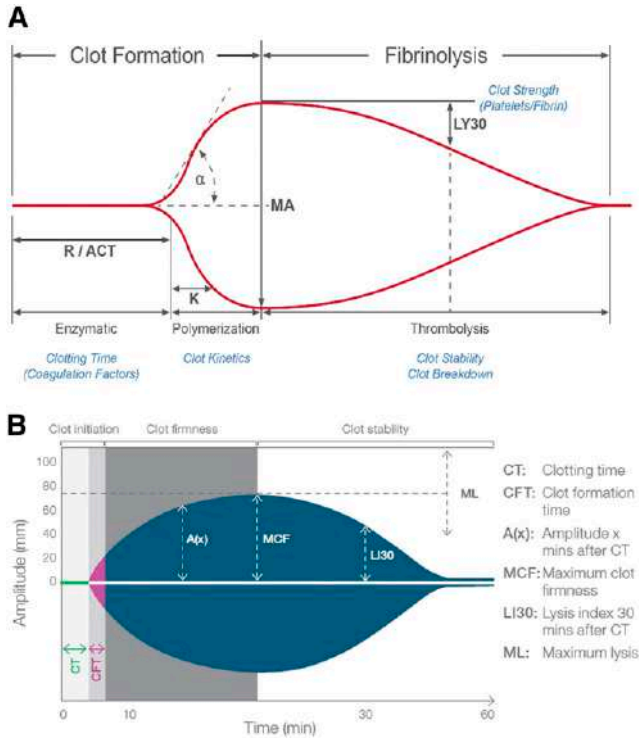

Figure 3. (A) Thromboelastography output demonstrating clot initiation, propagation, strength, and lysis. (B) Rotational thromboelastometry output demonstrating clot initiation, propagation, strength, and lysis.

Table 1. Comparison of TEG 5000 and ROTEM Delta parameters and their interpretation and physiological correlation to the phase of hemostasis

Parameters		Interpretation	Physiological correlation to phase of hemostasis
TEG 5000 (units)	ROTEM Delta (units)		
Reaction rate (R) (min)	Clotting time (CT) (s)	Time for the trace to reach an amplitude of 2 mm	Activation of coagulation, thrombin generation, time to initial clot formation, and influence of anticoagulants
Kinetics time (K) (min)	Clot formation time (CFT) (s)	Time for the clot amplitude to reach from 2 mm to 20 mm	Fibrin activation and polymerization (ie, speed of clot propagation)
Angle (α) (degrees)	Angle (α) (degrees)	Angle created by drawing a tangent line from the point of clot initiation (R or CT) to the slope of the developing curve	Fibrin activation and polymerization (ie, speed of clot propagation)
N/A	A10 (mm)	Amplitude reached 10 min after CT	Fibrinogen and platelet contribution to the strength of the clot
Maximum amplitude (MA) (mm)	Maximum clot firmness (MCF) (mm)	Peak amplitude or strength of the clot	Fibrinogen and platelet contribution to the strength of the clot
Lysis 30 (LY 30) (%)	Lysis index 30 (LI 30) (%)	TEG: Percentage reduction in the area under the TEG curve (assuming MA remains constant) that occurs 30 min after MA is reached ROTEM: Percentage clot remaining (compared with MCF) when amplitude is measured 30 min after CT is detected	Fibrinolysis
Lysis 60 (LY 60) (%)	N/A	Percentage reduction in the area under the TEG curve (assuming MA remains constant) that occurs 60 min after MA is reached	Fibrinolysis
N/A	Maximum lysis (ML) (%)	Degree of fibrinolysis relative to MCF achieved during the measurement (percentage clot firmness lost). It is not calculated at a fixed time.	Fibrinolysis

N/A, corresponding parameter not available on the analyzer; ROTEM, rotational thromboelastometry; TEG, thromboelastography.

specific and have not been standardized for many clinical indications. Evidence of correlation between VEA parameters and various conventional coagulation assays (CCAs) is also limited.

Case 1

A 75-year-old man underwent a complicated aortic and mitral valve replacement with tricuspid repair on cardiopulmonary bypass (CPB). Due to the extent of the surgery, the CPB run time was long (2.5 hours), which is generally associated with increased bleeding. He received a prophylactic loading dose of 2 g of tranexamic acid (TXA) intravenously over the course of 10 minutes at sternotomy, followed by an infusion of 16 mg/kg/hour and heparin 32 000 U during CPB. As he was being weaned off CPB, during the rewarming phase, he started to bleed severely. Protamine was administered at 0.7 mg/100 U of an initial heparin dose with normalization of the activated clotting time. The TEG assay performed on the TEG 6s analyzer (4 simultaneous channels) in the operating room (OR) showed the results in Figure 4.

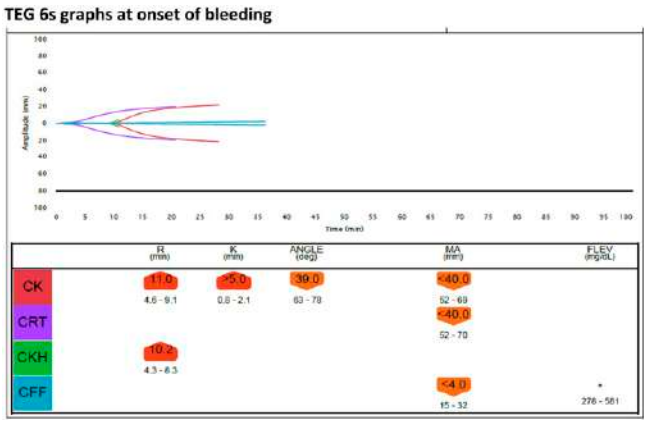

Figure 4. TEG 6s graphs at onset of bleeding.

TEG 6s graphs at onset of bleeding

The kaolin-activated TEG channel (CK) demonstrated a prolonged R time, indicating coagulation factor deficiency. The CK channel also showed a prolonged K time and reduced angle with reduced maximum amplitude (MA) on the functional fibrinogen (CFF) channel, indicating decreased fibrinogen contribution to clot formation. The MA on the CK channel was reduced, indicating decreased platelet function. There was no significant residual heparin effect, as evidenced by the minimal difference in R time between the kaolin sample in the CK channel and the kaolin sample with heparinase (CKH channel).

Is there a role for VEAs in the management of post–cardiac surgery bleeding?

Excessive bleeding after CPB occurs in approximately 20% of patients and is severe or massive in 10% to 15%, with up to 5% of patients requiring emergency surgical reexploration.[1] It accounts for almost 80% of blood transfusions after cardiac surgery and is

Table 2. Comparison of available assays on the TEG 5000 and ROTEM Delta analyzers

Assays		Interpretation/clinical indications
TEG 5000*	**ROTEM Delta†**	
Standard TEG (kaolin) Reagent contains kaolin activator.	INTEM Reagent contains ellagic acid activator.	These assays assess the contact activation pathway of coagulation and provide information similar to the activated partial thromboplastin time (APTT).
N/A	EXTEM Reagent contains tissue factor activator.	This assay assesses the tissue factor–initiated pathway of coagulation and provides information similar to the prothrombin time (PT).
Rapid TEG (rTEG) Reagent contains both tissue factor and kaolin as activators.	N/A	This assay assesses both the tissue factor–initiated and contact pathway–initiated coagulation and provides information similar to the PT and APTT.
Heparinase TEG (hTEG) Reagent contains kaolin activator and lyophilized heparinase for neutralizing unfractionated heparin.	HEPTEM Reagent contains ellagic acid activator and lyophilized heparinase for neutralizing unfractionated heparin.	These assays can assess heparin's effect when used in conjunction with the kaolin reagent (Standard TEG) and compared with the kaolin analysis on the TEG analyzer or when used in conjunction with the INTEM assay and compared with the INTEM analysis on the ROTEM analyzer.
Functional fibrinogen TEG (FLEV-TEG) Reagent contains tissue factor and abciximab, a GPIIb/IIIa inhibitor, to block the platelet contribution to clot formation.	FIBTEM Reagent contains tissue factor and cytochalasin D, an actin polymerization inhibitor, to block the platelet contribution to clot formation.	These assays can qualitatively assess the contribution of fibrinogen to clot strength independent of platelets when used in conjunction with the kaolin reagent (Standard TEG) and compared with the kaolin analysis on the TEG analyzer or when used in conjunction with the EXTEM assay and compared with the EXTEM analysis on the ROTEM analyzer.
N/A	APTEM Reagent contains aprotinin (fibrinolysis inhibitor).	This assay is designed to allow discrimination between fibrinolysis and platelet-mediated clot retraction.
TEG platelet mapping (TEGPM) Reagent contains ADP or arachidonic acid.	N/A	This assay is designed to assess platelet function and the effect of antiplatelet agents.
Native TEG Native whole blood sample analyzed after recalcification	NATEM Native whole blood sample analyzed after recalcification	These assays are impractical for clinical use, given their long reaction rate (R) on the TEG analyzer and clot formation time (CFT) on the ROTEM analyzer, respectively. They can be used to run custom hemostasis tests.

GP, glycoprotein; N/A, corresponding assay is not available on analyzer; ROTEM, rotational thromboelastometry; TEG, thromboelastography.
*TEG 6S analyzer: Standard TEG (kaolin) is CK channel; rapid TEG (rTEG) is CRT channel; heparinase TEG (hTEG) is CKH channel; and functional fibrinogen TEG (FLEV-TEG) is CFF channel.
†ROTEM Sigma analyzer: Assays have the same designation as the ROTEM Delta analyzer, except with a suffix C at the end (eg, EXTEM C).

an independent predictor of morbidity and mortality.[1-5] Bleeding after CPB is multifactorial and is related to surgical damage to blood vessels, technically complicated procedures, redo operations, and an acquired acute coagulopathy. Contributors to this coagulopathy include qualitative and quantitative platelet function defects; hemodilution causing reduction of procoagulants; use of intraoperative anticoagulants (heparin and potentially protamine); and activation of coagulation, inflammation, complement, and fibrinolysis.[1-7] Despite continuous efforts and clinical practice guidelines, blood product use after cardiac surgery has not shown significant declines over the past decade, remaining at ≥50% in high-risk patients.[1,2] Interventions to reduce bleeding and allogeneic blood exposure include preoperative hemoglobin optimization, heparin reversal after CPB, minimizing hemodilution, cell salvage, restrictive red blood cell (RBC) transfusion strategies, and monitoring of coagulation and platelet function to guide blood product replacement.[1,7]

Over the past 2 decades, 16 randomized controlled trials (RCTs) and many observational studies have assessed the role of VEA-guided transfusion algorithms after cardiac surgery, and 5 recent meta-analyses have analyzed these data.[8-12] In addition, an updated Cochrane systematic review and a health technology assessment (HTA) from the United Kingdom were published in 2015 and 2016, respectively, assessing the role of VEA in managing patients with bleeding. The majority of RCTs included in these 2 reviews were in adults undergoing elective CPB.[13,14] Six RCTs used TEG and 10 used ROTEM, comparing them to either CCA-guided algorithms or algorithms using clinician judgment. The majority of RCTs were small (between 22 and 224 patients), conducted in a single center, of poor methodological quality, and with significant heterogeneity and bias, thereby limiting the reproducibility of VEA parameters and the generalizability of their conclusions. One large, multicenter study by Karkouti et al[15] randomized 7402 patients at 12 Canadian hospitals in a stepped-wedge, clustered, pragmatic, randomized trial evaluating a combination of ROTEM and a point-of-care platelet function assay used in the OR in the context of a locally developed and validated transfusion algorithm.[16-18] In the preintervention phase of this trial, hospitals were instructed to continue their usual management of post–cardiac surgery

bleeding as per their institutional guidelines or standard of care.[15] The cumulative assessment of all these RCTs and meta-analyses in summary is that VEA-based management algorithms may be beneficial in reducing allogeneic transfusion of RBCs, frozen plasma, and platelets, as well as postoperative blood loss 12 and 24 hours after surgery, with a trend toward lowering mortality. Other clinical outcomes, such as surgical reexploration and intensive care unit (ICU) or hospital length of stay, are not significantly reduced. However, these conclusions are based primarily on poor-quality RCTs with a high risk of bias.[8-14] It is also possible that some of the benefit seen in these studies may be a result of VEA implementation often being paired with a renewed effort to standardize transfusion practice.

Case 1 follow-up

The initial patterns of TEG 6s graphs at the onset of bleeding indicated deficiency of coagulation factors, fibrinogen, and platelets. The following blood products were transfused: 4 U of frozen plasma, 4 g of fibrinogen concentrate, and 1 pool of platelets. TXA infusion was continued. Subsequent TEG 6s data at 80 minutes after transfusion are shown in Figure 5 and described below.

TEG 6s graphs after resuscitation

The postresuscitation TEG showed normal coagulation factors (CK R time, 7.2 minutes), fibrinogen (K time, 2 minutes; angle 63.1 degrees; CFF channel MA, 16.5 mm), and platelet function (CK MA, 57.0 mm). The bleeding slowed significantly, and the patient was transferred to the cardiovascular ICU in hemodynamically stable condition.

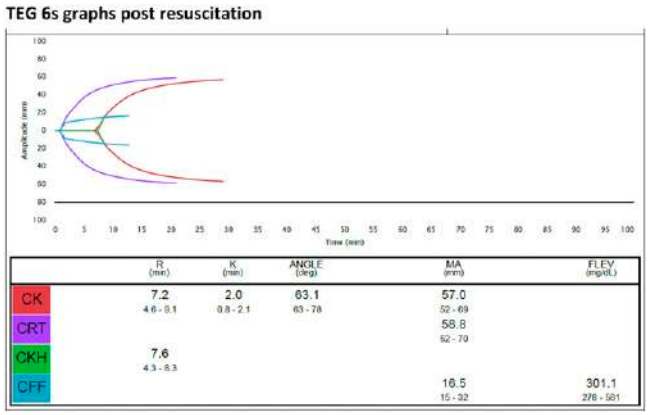

Figure 5. TEG 6s graphs postresuscitation.

Case 2

While jogging, a 48-year-old man was struck by a car traveling at 50 miles per hour. At the scene, he was alert and oriented but in severe pain in the lower abdomen, with no overt bleeding but with a systolic blood pressure of 85 mm Hg and a heart rate of 110 beats per minute. He was airlifted to a major trauma center. Initial resuscitation included IV fluids and a 1-g loading dose of TXA over the course of 10 minutes followed by 1 g of TXA infused over the course of 8 hours (per protocol). Trauma CT scans revealed a pelvic fracture with a large retroperitoneal hematoma. EXTEM and FIBTEM assays performed on a ROTEM Delta analyzer in the trauma bay showed the results in Figure 6.

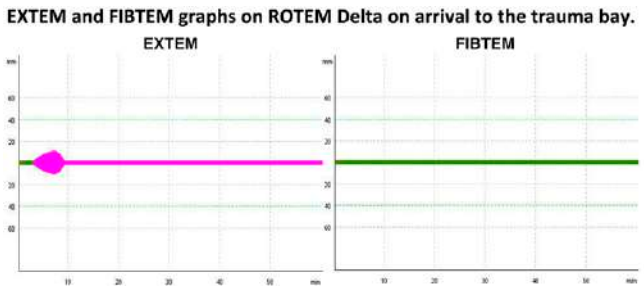

Figure 6. ROTEM Delta EXTEM and FIBTEM graphs on arrival at the trauma bay.

EXTEM MCF was 10 mm (reference interval [RI], 50 to 72 mm) with LI 30 at 0%. FIBTEM MCF showed no clot. This indicated severe coagulation factor and fibrinogen deficiency with severe hyperfibrinolysis. An initial complete blood count obtained from the laboratory within 15 minutes showed a hemoglobin level of 11.1 g/dL (RI, 12 to 15.5 g/dL) and a platelet count of 355 000 × 10^9/L (RI, 150 000 × 10^9 to 400 000 × 10^9/L).

Is there a role for VEA in the management of hemorrhage or coagulopathy in patients with major trauma?

Over the last 2 decades, more than 60 studies have evaluated either TEG or ROTEM assays in diagnosing early trauma coagulopathy and have assessed the role of VEA-guided transfusion algorithms in managing trauma-associated hemorrhage and in reducing mortality.[19-28] Both trauma-induced coagulopathy and hemorrhage are strong predictors of inpatient morbidity (massive transfusion, increase in complications, multiorgan failure, length of ICU stay, and perioperative cardiac arrest) and mortality.[19-21,29-31] Most of these studies are observational, single-center cohort studies; only one recent RCT has been published.[23] Reliable estimates of diagnostic accuracy of either TEG or ROTEM compared with CCA are not available, because very few studies have reported this information.[19,21] Almost half of the studies reported a trend toward improvement in blood product use and a mortality benefit when VEA-guided resuscitation algorithms were used.[19-28] Recently, Gonzalez et al[23] randomized 111 trauma patients who all met the criteria for activation of a massive transfusion protocol (MTP) to either a TEG-guided MTP algorithm or one guided by CCA, with a primary outcome of 28-day survival. The TEG-guided MTP algorithm significantly improved survival at 28 days compared with the CCA algorithm (36.4% deaths vs 19.6%; P = .049) and reduced deaths within the first 6 hours of arrival (7.1% in TEG group vs 21.8% in CCA group; P = .032). Patients randomized to TEG also received significantly fewer frozen plasma and platelet units than the CCA group. Although this is encouraging, large multicenter RCTs are needed to definitively determine the clinical effectiveness of VEAs in goal-directed resuscitation after major trauma.

One exception in which VEAs may uniquely contribute is in early hyperfibrinolysis due to hemorrhagic shock, seen in 2% to 5% of patients with major trauma but associated with up to 80% early mortality.[30,32-35] VEAs can detect hyperfibrinolysis early when CCAs cannot, thereby allowing specific interventions such as antifibrinolytic therapy to prevent death.[32-35] However, the lack of a reliable gold standard to diagnose hyperfibrinolysis

makes it difficult to assess if VEA identification of hyperfibrinolysis is sensitive or specific.

Although recent international guidelines have recommended that VEAs be used early in trauma resuscitation to assess coagulation status and guide transfusion of blood products and antifibrinolytics, experts were unable to recommend the exact thresholds to be used as transfusion triggers due to lack of data.[35,36] A recent large, prospective, multicenter study with 2287 patients from 6 European adult trauma centers attempted to systematically validate diagnostic cutoffs for both ROTEM and TEG parameters for the detection of coagulopathy, thrombocytopenia, hypofibrinogenemia, and hyperfibrinolysis and specific transfusion therapies based on these triggers.[24] Algorithms based on these VEA thresholds are now being compared with CCAs in an ongoing RCT and will require external validation in subsequent studies.

Case 2 follow-up

Initial EXTEM and FIBTEM graphs upon arrival indicated severe coagulation factor and fibrinogen deficiency with severe hyperfibrinolysis. On this basis, resuscitation was begun within 10 minutes with 8 U of frozen plasma and 4 g of fibrinogen concentrate. TXA infusion was continued. Eight units of RBC were administered. Coagulation laboratory results obtained 65 minutes after arrival confirmed the coagulopathy and hypofibrinogenemia: the patient's international normalized ratio (INR) was 2.9 (RI, 0.9 to 1.2), and his fibrinogen level was 0.29 g/L (RI, 1.7 to 4 g/L).

EXTEM and FIBTEM graphs at 4 hours after resuscitation (Figure 7)

The patient's EXTEM MCF corrected to 51 mm (RI, 50 to 72 mm), with LI 30 improving to 100%. His FIBTEM MCF was now normal at 11 mm (RI, 9 to 25 mm). His INR and fibrinogen were 1.35 (RI, 0.9 to 1.2) and 1.6 g/L (RI, 1.7 to 4 g/L), respectively, at the same time point. The patient was taken to the operating room for internal fixation of his pelvic fracture in stable hemodynamic condition.

Other clinical indications for VEAs

VEAs have been used in the management of bleeding in liver transplant (LT) surgery[37] and other noncardiac surgeries,[38] as well as in the assessment of the coagulopathy of end-stage liver disease (ESLD)[37,39] and sepsis.[40,41] In ESLD and LT surgery, the evidence base is limited to small, single-center, observational studies and only one RCT with 28 LT patients that compared a TEG-based algorithm with CCA.[37] These studies have shown inconsistent trends toward reduced bleeding or transfusions with the use of VEA. A recent meta-analysis of VEA in noncardiac surgical settings (trauma, burns, and ESLD and LT surgery) included only 4 small RCTs (a total of 229 participants) and concluded that data were insufficient to determine any reduction in transfusions in these settings.[38] Despite this, VEAs are used in the majority of LT surgeries, likely extrapolating the evidence from cardiac surgery studies. This is concerning because the coagulopathic states of ESLD and during LT surgery are markedly different from those seen in cardiac surgery.[37,39] Using VEAs for these indications must be accompanied by a clear understanding that VEA parameters suggesting coagulation factor, fibrinogen, or platelet deficiency or hyperfibrinolysis have not been validated in ESLD or during LT surgery and, if being used in the context of a locally validated VEA-based algorithm, should be used only to manage bleeding (not prophylactically).

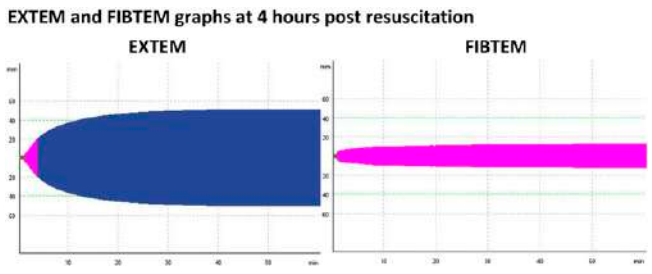

Figure 7. EXTEM and FIBTEM graphs at 4 hours postresuscitation.

In the evaluation of sepsis-induced coagulopathy (SIC), limited evidence suggests that, compared with CCAs, VEAs can assess both hypo- and hypercoagulability (associated with disseminated intravascular coagulation) and impaired fibrinolysis (associated with systemic inflammatory response syndrome).[40,41] The evidence thus far is limited to the characterization of SIC and early associations with clinical outcomes such as mortality.

VEAs have also been used in assessing hypercoagulability following surgery and its relationship to venous thromboembolism (VTE); characterization of malignancy-associated coagulopathy; and, most recently, the hypercoagulopathy seen in patients with severe coronavirus disease 2019.[42-44] Similar to studies in SIC, these studies are mostly observational and describe VEA parameters most indicative of a hypercoagulable state, the temporal course of this hypercoagulability, and associations with VTE. They are limited by varying definitions of VEA parameters that suggest hypercoagulability, varying populations of patients, and small sample sizes.[42,43] Several such studies have shown that hypercoagulable parameters in VEA testing are associated with increased thromboembolic complications and may be used to guide thromboprophylaxis.[42,43] Because CCAs cannot specifically identify hypercoagulable states or predict VTE, VEAs present an exciting potential opportunity for future research in this area.

VEAs have been evaluated extensively in pregnancy and puerperium in both the management of postpartum hemorrhage (PPH) and assessment of hypercoagulability. Normal reference ranges for both TEG and ROTEM have been developed for all trimesters of pregnancy, during labor, postpartum, and pre- and postoperatively for women undergoing cesarean delivery, and they correlate well with CCAs.[45] PPH is the commonest obstetrical scenario in which VEAs have been used; the ROTEM FIBTEM assay has been shown to correlate with fibrinogen, predict progression of PPH, and guide transfusion needs.[45,46] The Women's Health Scientific and Standardization Committee of the International Society on Thrombosis and Haemostasis has identified the lack of large RCTs comparing VEA-guided care with usual care with evaluation of important clinical outcomes as the biggest barrier to the adoption of VEAs in obstetrics.[45,46]

Evolving roles for VEAs include the management of hemophilia and the complex anticoagulation management of patients receiving extracorporeal membrane oxygenation (ECMO) and left ventricular assist devices. In hemophilia A, VEAs have been studied in determining clinical bleeding phenotype correlated with factor VIII (FVIII) levels, monitoring of routine FVIII prophylaxis, assessing the response to bypassing agents, and as a

means to determine the optimal dose of factor replacement in the perioperative period for patients undergoing surgery.[47] Although VEAs have been incorporated into institution-specific algorithms to assess the complexities of concurrent bleeding and thrombosis and to monitor anticoagulants in patients receiving ECMO and left ventricular assist devices, widespread use has been hampered by a lack of robust evidence.[48,49]

How do I implement VEAs at my institution?

VEAs are being used in clinical algorithms despite lack of evidence demonstrating benefit for many indications. This is driven largely by the inherent limitations associated with the CCAs, including their poor correlation with bleeding; their inability to reflect the contribution of platelets, factor XIII, and plasmin during coagulation; their lack of immediate turnaround times; and the need for rapid, serial monitoring for guiding blood products during severe hemorrhage. Given transport to the laboratory and centrifugation requirements, CCAs using citrated plasma will (at a minimum) have a turnaround time of 30 to 60 minutes. VEAs help to address this gap by providing coagulation information at the point of care within 10 to 20 minutes. However, until rigorous evidence establishing and externally validating diagnostic cutoffs for various parameters, their interoperator reproducibility, and their independent predictive value for clinical outcomes is available, the following considerations will help to ensure that VEAs are being used in a beneficial and safe manner. This requires close collaboration between clinicians and the laboratory.

1. Clinicians must clearly define their goals for introducing VEAs (eg, ensuring appropriate transfusion during MTPs to reduce allogeneic blood exposure).
2. Current practice in the area must be evaluated first to ensure that best transfusion practices are being adhered to.
3. If slow turnaround time of laboratory results is the major reason for needing to institute VEAs, assess if laboratory turnaround times for CCAs can be improved through institution of "stat" protocols that operationalize clinical, laboratory, and transport teams to achieve this goal.
4. Clinicians must develop a detailed clinical algorithm using the chosen VEA and must locally validate diagnostic thresholds for the specific clinical indication.[16-18]
5. The laboratory must be involved in decision-making about selection of both VEA devices and assays, their ideal location (laboratory vs point of care) based on device type and clinical goals, and assisting with the following implementation steps before the device can be put into clinical use:
   a. Evaluating and validating the selected VEA for accuracy and precision (ie, verifying manufacturer claims) and assessing interoperator reproducibility;
   b. Developing local reference ranges for various parameters or verifying manufacturer-provided reference ranges;
   c. Setting up a quality management program for the VEA, including the type and frequency of quality controls, enrolling in an external quality assessment program, training of individuals who will be performing the VEA (often nonlaboratory professionals), and assessing their competency at regular intervals;
   d. Developing and maintaining standard operating procedures;
   e. Assisting with managing inventory of reagents and consumables and service contracts with vendors, as well as troubleshooting device malfunctions and downtimes; and
   f. Assisting with reporting of VEA results in accordance with clinical needs and compliant with local laboratory accreditation standards.

Are VEAs cost-effective?

An HTA on VEAs conducted on behalf of the National Institute for Health and Care Excellence in the United Kingdom in 2015 concluded that VEAs are effective and cost-effective compared with CCAs in cardiac surgery, primarily on the basis of projected reduction in transfusions.[14] Depending on the assay combinations used, the costs may vary between TEG and ROTEM. For the trauma indication, both this HTA and a Canadian HTA conducted in 2017 concluded that VEAs may potentially be more cost-effective than CCAs in patients with trauma, given the larger volumes of blood transfusions than in cardiac surgery patients; however, the lack of high-quality evidence of clinical effectiveness limits the validity of their conclusions.[50] In the absence of robust cost-effectiveness data, institutions adopting VEAs must calculate annual costs of analyzer service contracts, test reagents, consumables, and quality control materials, as well as the costs of acquiring and maintaining a backup analyzer, training of personnel, and participation in an external quality assessment program, against the potential cost savings associated with reduced transfusions and associated complications.

Conclusions

VEAs have the potential to provide a complete picture of all elements of coagulation, clot strength, and lysis in assessing various coagulopathies. Although CCAs provide informative data on individual elements of coagulation, the presentation of clot formation and lysis visually and at the point of care have likely made VEAs an attractive alternative to CCAs.

Current evidence shows that using VEA-guided transfusion algorithms in cardiac surgery and major trauma may reduce allogeneic blood exposure and blood loss, which can translate to reduction in morbidity and mortality and can be cost-effective. There are also exciting opportunities to advance understanding of hypercoagulability and thrombosis prediction using VEAs, which is a gap currently not addressed by CCAs.

However, there is an urgent need for appropriately designed diagnostic studies correlating VEAs to CCAs, determining and externally validating diagnostic cutoffs for VEA parameters, and large multicenter RCTs establishing the independent contribution of VEA-guided algorithms in improving clinical outcomes compared with standard practice. Until such rigorous evidence is available, clinicians and institutions wishing to use TEG or ROTEM must do so appreciating the uncertainty regarding effectiveness, paying attention to monitoring the quality of results, and evaluating their benefits and costs locally.

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Conflict-of-interest disclosure

R. S. has no conflicts of interest to declare.

Off-label drug use

None disclosed.

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Improving interpretation of genetic testing for hereditary hemorrhagic, thrombotic, and platelet disorders

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The last 10 years have seen an explosion in the amount of data available through next-generation sequencing. These data are advancing quickly, and this pace makes it difficult for most practitioners to easily keep up with all of the new information. Complicating this understanding is sometimes conflicting information about variant pathogenicity or even about the role of some genes in the pathogenesis of disease. The more widespread clinical use of sequencing has expanded phenotypes, including the identification of mild phenotypes associated with previously serious disease, such as with some variants in *RUNX1*, *MYH9*, *ITG2A*, and others. Several organizations have taken up the task of cataloging and systematically evaluating genes and variants using a standardized approach and making the data publicly available so that others can benefit from their gene/variant curation. The efforts in testing for hereditary hemorrhagic, thrombotic, and platelet disorders have been led by the International Society on Thrombosis and Haemostasis Scientific Standardization Committee on Genomics in Thrombosis and Hemostasis, the American Society of Hematology, and the National Institutes of Health National Human Genome Research Institute Clinical Genome Resource. This article outlines current efforts to improve the interpretation of genetic testing and the role of standardizing and disseminating information. By assessing the strength of gene–disease associations, standardizing variant curation guidelines, sharing genomic data among expert members, and incorporating data from existing disease databases, the number of variants of uncertain significance will decrease, thereby improving the value of genetic testing as a diagnostic tool.

LEARNING OBJECTIVES

- Define the current resources available to clinicians, researchers, and patients to allow data sharing and standardization
- Identify the current ongoing efforts to improve and standardize interpretation and allow categorization of variants of uncertain significance
- Understand the role of the Clinical Genome Resource and ClinVar in standardizing and disseminating gene-specific variant curation results

Clinical case

A 14-year-old girl presented to a hematology clinic for evaluation of menorrhagia and long-standing thrombocytopenia. She had initially been noted to have thrombocytopenia at about 15 months of age, shortly after starting to walk, when she was presented to her primary care physician with bruising. At that time, she was also noted to have some petechiae. A complete blood count demonstrated normal counts, except for a platelet count of 55 × 10^9/L. Evaluation at that time provided a diagnosis of immune thrombocytopenia. She was treated with intravenous immunoglobulin and prednisolone, neither of which increased her platelet count above 50 to 60 × 10^9/L. She had bleeding symptoms of minor epistaxis as well as bruising and petechiae but was otherwise well, and no further attempts to alter platelet counts were made. At 14 years of age, she experienced menarche, which resulted in hemorrhage to a hemoglobin of 5.4 g/dL over 10 days with heavy menstrual bleeding. She was admitted, transfused with packed red blood cells, and treated again with intravenous immunoglobulin and prednisone as well as a platelet transfusion for a platelet count at that time of 37 × 10^9/L. Review of her peripheral smear demonstrated >50% large or giant platelets. She

was then transfused with platelets, and a 45-minute post-platelet count demonstrated an appropriate rise, with her platelet count 24 hours later still being >100 × 10^9/L. She was referred to hematology for further evaluation of her bleeding and thrombocytopenia. Evaluation at that time was concerning for inherited platelet disorder, despite lack of family history of thrombocytopenia or associated syndromes. A genetic evaluation demonstrated 2 variants in the *GP9* gene, inherited in *trans* with a maternally inherited known pathologic variant and de novo variant of uncertain significance (VUS).

Introduction

Rare diseases, defined as those affecting fewer than 1 in 2000 individuals in the general population, often have genetic causes, disproportionately affect children (50% to 75%), and tend to result in severe, multisystem disorders. Accurate genetic diagnosis of a rare disease enables access to disorder-specific support groups and provides information guiding management, therapy, and prognosis as well as determination of risk for family members.[1,2] This makes appropriate and accurate diagnosis important for many reasons. More recently, increased clinical sequencing has expanded phenotypes and revealed that some rare diseases are more common than originally thought and not always associated with severe phenotypes.[3] Among the platelet disorders with expanding phenotypes are *RUNX1*-related disorder,[4] Bernard-Soulier syndrome,[5] and others.[6] Even with Glanzmann thrombasthenia, some individuals are not diagnosed until later in life.[7]

Genetic testing started out with low-resolution cytogenetic testing, moving to microarray[8] and then the development of multiplexed single-gene sequencing in panels and then eventually high-throughput next-generation sequencing (NGS) of whole exomes and then whole genomes.[9-12] There are advantages of NGS testing, allowing massively parallel sequencing of millions to billions of genetic loci simultaneously, but this may come at the price of loss of resolution for some areas of the genome and inability to detect some types of copy number variation, which may have important implications, particularly in some types of disorders, such as those caused by small deletions (thrombocytopenia-absent radii syndrome),[13] or due to changes in genes with associated pseudogenes or to variations in copy number repeat changes (Quebec platelet syndrome).[14] One of the difficulties in NGS is the appropriate determination of which variants are clinically significant.[12,15] Sequencing so many genetic loci may provide thousands of single-nucleotide variants in a single individual, leaving the laboratory with the difficult task of assessing the clinical relevance of all of these genetic changes. Several groups have worked to develop tools and consensus to allow harmonization of clinical validity, pathogenicity, and clinical utility of genetic variants.[16]

Process of genetic test interpretation

When a patient is referred for genetic testing, several key elements should be in place to ensure accurate and timely testing as well as appropriate interpretation and return of results.

Test selection

The clinician ordering the test needs to determine the appropriate test to send on the basis of clinical phenotype and the suspected diagnosis. Various testing strategies exist and can be employed to arrive at a molecular diagnosis, including sequencing of targeted suspected genes, sequencing a panel of genes related to a specific disease or phenotype, or whole-exome or whole-genome sequencing, which is more agnostic to the diagnosis or potential genes involved.[17] Clinicians ordering genetic testing must be familiar with the available testing and be able to order the appropriate test for the disease category (Table 1). For example, patients with severe factor VIII deficiency rarely require exome sequencing to arrive at a molecular diagnosis, but instead targeted sequencing including examination for small deletions and duplications is appropriate in evaluating a patient with hemophilia due to factor VIII deficiency. In contrast, individuals with platelet function defects benefit from careful functional evaluation and, if appropriate, may benefit from broader panels or even exome sequencing to molecularly define the etiology of their disorder.

Many centers make available genetic counselors to aid in the appropriate selection of testing and subsequent consent and counseling of patients around the test and results (see below). The use of genetic counselors, if available, is recommended and provides an additional perspective both on the need for testing and on the potential risks/benefits of outcomes.[18] In addition, consultation with a disease expert may be helpful to determine the appropriate test and the best place to send the test among the various options to ensure timely results and aid with interpretation once those results are available.

Consent for testing

Once the appropriate genetic test has been identified, a clear conversation with the patient and, if appropriate, the patient's family should ensue with discussion of the risks and benefits of genetic testing. Particular ethical considerations may play a role in pediatric patients[19] who are more likely to carry genetic diagnoses but also may not be capable yet of participating in decision making about implications of results of genetic diagnoses. Full disclosure of not only the potential benefits of genetic testing but also the risks is critical to appropriate consent. Families who will be undergoing less targeted sequencing (panels or exome sequencing) need to understand risks of return of cancer predisposition, as well as incidental and secondary findings, and should be given the opportunity to "opt out" of especially some types of findings.[20] The consent and decisions about return of secondary findings should be documented in the medical record. The American College of Medical Genetics (ACMG) has published a list of secondary findings that are considered "medically actionable"[21] and recommends return of these results because these are results regarding which standard clinical interventions exist to prevent or mitigate the disease. Most participants in clinical studies have indicated a preference for return of secondary findings,[22-24] but these may not fully represent all patients (eg, those who would choose not to participate in research). Finally, regulations vary in some countries and should also be considered when providing consent because they may alter the availability of testing.

Interpretation and return of results

A patient's genetic data are analyzed, and each variant is assigned a classification based on the available literature and, today, information in public databases that helps to gather genetic testing information to inform the pathogenicity of findings. Variants may be "called" in 1 of 5 categories (Figure 1).

Table 1. Types of genetic testing and their advantages and disadvantages

Test type	Advantages	Disadvantages	Example of best use
Sanger sequencing (single gene)	• Less expensive	• Only provides information on one gene	Confirmation of known variant in affected family
	• Fast turnaround		
Gene panel	• More in-depth coverage of genes of interest	• Does not interrogate genes outside of panel (may miss novel associations)	Clinical scenario provides suspected diagnosis for confirmation
	• Custom designed to disease of interest	• Utility depends on frequency of panel update	
	• No secondary findings		
	• Generally faster turnaround		
Whole exome	• Agnostic and comprehensive	• Insensitive to deletions/duplications, depending on technique	Novel syndrome unclear diagnosis
	• Allows identification of novel variants	• Reading depth influences ability to find variants in some genes	
	• May be more readily available (not custom)	• Expensive	
Whole genome	• Allows sequencing of noncoding regions	• Expensive	Suspected genetic disease due to noncoding variant
	• Better than WES at detecting indels/duplications/inversions	• Lower read depth for some areas	
CGH array	• Detects large genomic changes	• Insensitive to single-nucleotide changes	Chromosomal deletions, loss

CGH, comparative genomic hybridization; WES, whole-exome sequencing.

Generally, variants that are benign or likely benign are not reported in genetic testing reports, because these are variants that have reliably been shown to be tolerated sequence variations. Definitive diagnostic results are returned with pathologic or likely pathologic variants identified in genes associated with the described phenotype. The result of a VUS needs to be carefully considered but does not mean the patient carries the associated diagnosis, and some VUSs have, since their initial description, been defined as benign. Return of a VUS may require consultation with genetic counselors, disease experts or additional testing and additional follow-up. If the results of testing are inconclusive, follow-up testing/analysis in 2 to 3 years may be advised because genetic testing is evolving with continued identification of additional genes and variants.

Finally, once the results of a genetic test are known, those results must be returned to the patient, which involves a complete discussion of the findings within the context of that patient's disease. Patients who carry single variants for autosomal recessive disorders generally do not have symptoms (although some genes are now being recognized as much more gene dose dependent than previously appreciated). Appropriate disclosure and discussion of the clinical significance of a VUS is one of the biggest challenges in genetic diagnosis, and several efforts are ongoing to help move variants out of this area of uncertainty.

Challenge of VUS

A VUS, generally a variant that has not previously been reported, cannot be conclusively assigned to either a benign or pathologic

Figure 1. Variant classifications. On the basis of American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines,[34] the variant classifications have been proposed along with a scoring system to standardize variant interpretation across clinical laboratories.

category on the basis of a single patient. Even if the variant has previously been reported, there may be conflicting information about how that variant may affect the gene product or even about the validity of the gene association.[25] Aggregation of patients with similar phenotypes/variants is a powerful tool to help sort VUSs into more helpful variant classifications.[26-28] In addition, experimental validation of the detrimental effect of a variant on protein function or expression can also help to define the role of a particular variant in the molecular pathogenesis of disease.[29]

Databases that collate and curate the vast amounts of information, making it available to other laboratories in interpreting results, allow more streamlined interpretation and better care for patients. Several databases have arisen over time; some are disease specific, collecting information about *RUNX1* variants, Glanzmann thrombasthenia–associated variants, and Bernard-Soulier syndrome–associated variants, whereas others are more general, collating information about any variant in any gene.

Development of public resources to assist in variant calling

There are multiple groups that have contributed to growing knowledge, especially within the genes responsible for inherited platelet disorders, including ThromboGenomics (http://thrombo.cambridgednadiagnosis.org.uk), the UK Genotyping and Phenotyping of Platelets group,[30] the Biomedical Research Centres/Units Inherited Diseases Genetic Evaluation Bleeding and Platelet Diseases consortium,[31] and groups in Spain[32] and Denmark.[33] These groups have pioneered the development of gene panels and NGS techniques for research and clinical purposes. Although a full discussion of the individual contributions of each of these groups, or even of the ways in which this field has advanced, is beyond the scope of this discussion, with their work came the explosion in information requiring curation and a need for a standardized approach to variant calling. As a direct result of international collaborations, several public resources have been developed over the past decade to help centralize information and provide open resources to scientists, clinicians, laboratories, and the public. Therefore, examining some of these resources and their roles and goals in the context of genetic testing may help in understanding how they can improve testing and serve as a model for comprehending the broader role of public resources in standardization.

ClinVar: a public database of the National Center for Biotechnology Information at the National Institutes of Health

ClinVar was launched in 2013[16] at the National Center for Biotechnology Information at the National Institutes of Health to provide a database of genetic variants that is centralized and open access to aid in interpretation of variants. One of the key missions of ClinVar was to gather the data generated from clinical genetic testing (variants and interpretations) and to add this together with other information about variants, providing a single source of information. The focus was for clinical laboratories to share variants, interpretations, and evidence for interpretations to improve variant interpretation overall. ClinVar then partnered with the Clinical Genome Resource (ClinGen) to create expert panels to provide the US Food and Drug Administration–approved database with consensus panel interpretation of all of the amassed data within ClinVar. ClinVar provides information within the database about whether variants have undergone any review and the level of that review (from single submission to aggregate interpretation to expert panel review). The level of evidence is provided and given a star rating from 0 to 4 stars, with 0 stars for a variant from a single submitter with criteria supporting an assertion and 4 stars representing evidence-based practice guidelines (eg, ACMG, Clinical Pharmacogenetics Implementation Consortium). The expert panel curations are given 3 stars, and there are multiple expert panels within ClinGen and recognized outside panels, including Pharmacogenomics Knowledgebase, Evidence-based Network for the Interpretation of Germline Mutant Alleles, and the panel for *CFTR2*.

ClinGen

ClinGen (https://clinicalgenome.org/), in contrast, was established to create evidence-based consensus for curating genes and variants, develop machine learning strategies, and disseminate collective knowledge and resources for unrestricted community use. ClinGen incorporates ClinVar and other resources (including extensive use of expert panels, some of which have been convened by the American Society of Hematology) to ask several questions (eg, Is the gene associated with disease? Is the variant causative? Is the information actionable?) about genes and variants and gather available knowledge to reach consensus.

ClinGen brings together physicians, clinical geneticists, laboratorians, research scientists, and biocurators to develop standards for variant and gene interpretation and dissemination of information. The work is split into several clinical domain working groups (CDWGs), and within these CDWGs, there are variant curation expert panels that specify the rules for variant curation (ie, How does one establish that a variant is pathologic?) and then curate genes/gene families as an expert panel. In addition, ClinGen has several gene–disease curation expert panels that curate the strength of association between a particular gene and phenotype/disease. ClinGen also has multiple other working groups/areas dedicated to improving variant interpretation guidelines, community curation, complex disease associations, somatic variability, and determining the actionability of secondary findings.

Hemostasis/Thrombosis CDWG: American Society of Hematology–ClinGen–ThromboGenomics partnership

In 2018, the Hemostasis/Thrombosis CDWG (https://www.clinicalgenome.org/working-groups/clinical-domain/hemostasis-thrombosis/) was established to improve the utility of genetic testing within this clinical domain. On the basis of prior experience of the ThromboGenomics group and the International Society on Thrombosis and Haemostasis Scientific Standardization Committee (ISTH-SSC) on Genomics in Thrombosis and Hemostasis, this international group of experts was organized to work in partnership to curate genes and variants within multiple domains of thrombosis and hemostasis. These curation results are publicly available on the ClinGen website (https://clinicalgenome.org/). The Hemostasis/Thrombosis CDWG within ClinGen has established several expert curation panels, as well as a gene–disease curation panel for this domain (Figure 2).

The expert panels within this CDWG have, over the past 2 years, curated 42 gene–disease associations and have established 5 variant curation expert panels whose variant curation rules are in various stages of approval by ClinGen. In partnership with ISTH-SSC, the gene curation panel was able to get a head

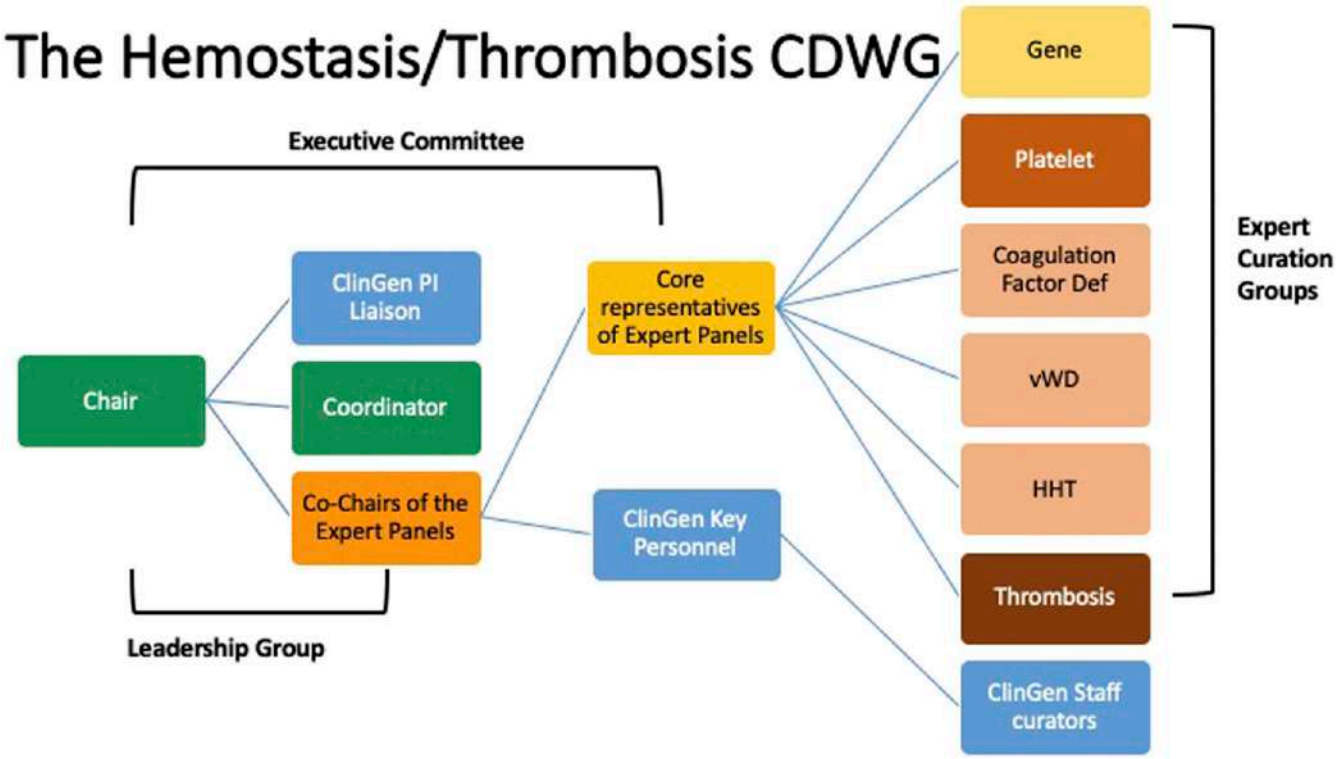

Figure 2. Structure of the Hemostasis/Thrombosis Clinical Domain Working Group.

start on platelet disorder gene curation by starting with the list of hemostasis genes that had been assigned a high likelihood of pathogenicity (https://www.isth.org/page/GinTh_GeneLists). They then harmonized the gene–disease associations of the 2 groups, providing a curated list of genes that had already been reviewed by the expert panel. This ongoing partnership benefits from the yearly platelet disorder gene review at the ISTH meeting and allows rapid incorporation of newly described genes into the list of associated genes.

Resolution of the case

The patient returned to the office to discuss the findings of her genetic testing. After discussion, her family opted for functional and flow cytometric evaluation to confirm the clinical diagnosis of Bernard-Soulier syndrome. Flow cytometry demonstrated markedly reduced expression of glycoprotein 1b/IX receptor on platelet surfaces, and platelet aggregation studies were limited due to thrombocytopenia but did demonstrate essentially normal aggregation to adenosine 5′-diphosphate and collagen and absent agglutination with ristocetin. The patient and family agreed to submission of the additional functional information to the laboratory to better define the VUS. Subsequently, the laboratory used this information, along with the clinical features and follow-up experimental evidence, for a submission to ClinVar through the online submission portal. Consent for submission was documented in the medical record along with the original consent for genetic testing.

The diagnosis of Bernard-Soulier syndrome markedly altered ongoing management, changing the discussions about the underlying pathophysiology of the disease, best available treatments, and risk of bleeding as well as conversations of about heritability and risks to any future siblings or offspring.

Future directions and ongoing needs

The development of these public databases, which have benefited from the work of prior public resources, has the potential to markedly improve genetic diagnoses by allowing the community to more quickly define the role of novel genes and variants in disease. Continued collaboration between researchers, clinical laboratories, and clinicians in sharing variants (regardless of the status) along with descriptive information allows development of more robust data and ultimately benefits the entire community.

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Conflict-of-interest disclosure

M.P.L. wrote the manuscript on behalf of the Hemostasis/Thrombosis Clinical Domain Working Group. M.P.L. has consulting relationships with Novartis, Dova Pharmaceuticals, Principia Biopharma, Octapharma, Sysmex, Shionogi, argenx, and Rigel. M.P.L. is a member of the Clinical Genome Resource Hemostasis/Thrombosis Clinical Domain Working Group, a co-chair of the Gene Curation Expert Panel, and a member of the platelet disorder variant curation expert panel and has received an honorarium for her participation. M.P.L. has received research funding from AstraZeneca, Novartis, Rigel, and Sysmex.

Off-label drug use

None disclosed.

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EVIDENCE-BASED MINIREVIEW

Laboratory surveillance of immune-mediated thrombotic thrombocytopenic purpura

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Clinical case

Three months after delivery of her first child, an otherwise healthy 30-year-old female presented with headaches and generalized malaise. For 3 days, she had experienced vomiting, diarrhea, easy bruising, and truncal petechiae. Laboratory tests revealed a platelet count of 11 × 10^9/L, a hemoglobin of 5.9 g/dL, numerous schistocytes on the peripheral blood smear, elevated lactate dehydrogenase and bilirubin levels, an undetectable haptoglobin, and a normal creatinine and prothrombin time (PLASMIC score[1] 7/7 points). The diagnosis of immune-mediated thrombotic thrombocytopenic purpura (iTTP) was confirmed by ADAMTS13 activity < 5% of normal in the presence of a strong functional ADAMTS13 inhibitor (>>2 Bethesda units/mL). Therapeutic plasma exchange (TPE) with replacement of plasma, glucocorticoids, and folic acid were initiated on the day of admission. Clinical response and a stable platelet count > 150 × 10^9/L were achieved with 5 sessions of TPE. Because of the rapid clinical response, treatment did not include rituximab, which is part of first-line treatment in many centers. After discharge on day 7, steroids were tapered over 4 weeks and discontinued. Among patients who reached remission from a first episode of iTTP, what types of outpatient clinical follow-up and laboratory testing are recommended to minimize the risk of relapse?

Introduction

Overall survival of patients presenting with their first episode of iTTP has improved significantly with the introduction of TPE in the early 1990s, by supplementing TPE with immune suppression in the 2000s, and, recently, with caplacizumab, an anti–von Willebrand factor nanobody that effectively inhibits the interaction between von Willebrand factor multimers and platelets but does not address the autoimmune response to ADAMTS13. At least half of survivors of an initial iTTP episode experience subsequently relapse(s) that are again associated with morbidity and mortality. In this minireview, we aim to examine recent literature on predictors of relapse in survivors of an initial iTTP episode, formulate a recommendation for patient follow-up, and discuss the possibility of minimizing the risk of relapse by preemptive treatment with rituximab.

Demographic risk factors of iTTP relapse

There are several nonmodifiable demographic risk factors for iTTP relapse. A recent multicenter cohort study that incorporated 124 patients from 6 major academic centers in Boston and Seattle identified the following clinical predictors associated with iTTP relapse: age younger than 25 years (hazard ratio [HR], 2.94; 95% confidence interval [CI], 1.2-7.2), non-O blood group (HR, 2.15; 95% CI, 1.06-4.39), and a prior episode of iTTP (HR, 2.97; 95% CI, 1.4-6.4).[2] In another study of 70 patients in Germany, male patients had a 1.5-fold increased risk for relapse compared with female survivors.[3]

Longitudinal measurement of ADAMTS13 activity as a predictor of iTTP relapse

In a natural history study from the Oklahoma TTP Registry, the investigators assessed the association between remission ADAMTS13 activity measurement and subsequent relapse in 67 iTTP patients who had ADAMTS13 activity determination(s) during follow-up.[4] In this study, ADAMTS13 activity < 10% at any point during remission was documented in 30% of patients. Eventual relapse occurred in 55% of patients within this group compared with only 4% of those who had persistent ADAMTS13 activity > 60% and 25% of those with fluctuating ADAMTS13 activity values > 10%. In patients with an ADAMTS13 activity < 10% during remission, the time to relapse ranged from 0.3 to 9.5 years. Thus, relapse was far from imminent for patients with low ADAMTS13 activity. In a subsequent retrospective cohort study from Italy, a similar association was reported between remission ADAMTS13 activity and relapse in 60 iTTP patients studied from 2002 to 2018.[5] ADAMTS13 activity < 20% and anti-ADAMTS13 antibody > 15 AU/mL at the time of remission, after 3 months, and at 6 months significantly correlated with iTTP relapse. Similar to the

Table 1. Key studies for preemptive rituximab strategy in iTTP management

Study	Population	Without preemptive rituximab	Preemptive rituximab treatment
Hie et al[7] Retrospective*	233 French iTTP patients with >1 y of follow-up (2000-2012). 48 had ADAMTS13 < 10% during follow-up; of these, 30 received preemptive rituximab, 375 mg/m^2 (1, 2, or 4 infusions).	TTP recurrence: 0.57 episodes per year (IQR, 0.46-0.70)	At 3 mo posttreatment: ADAMTS13 recovery‡ in 87%. No TTP recurrence episode per year (IQR, 0-0.81). Subsequent retreatment with preemptive rituximab necessary in 30%.
Jestin et al[8] Prospective*	92 French iTTP patients with >1 y of follow-up (2012-2017). 92 with ADAMTS13 < 10% during follow-up. 92 received preemptive rituximab 375-500 mg/m^2 (1-4 infusions).	TTP recurrence: 0.33 episodes per year (IQR, 0.23-0.66)	At 3 mo posttreatment: ADAMTS13 recovery‡ in 86%. No TTP recurrence episodes per year (IQR 0-1.32). Recurrence of ADAMTS13 < 10% in 45/79 (57%) patients. Subsequent retreatment with preemptive rituximab in 48% of patients.
Westwood et al[9] Retrospective†	45 British iTTP patients with 76 episodes of ADAMTS13 <= 15% (2005-2016) received preemptive rituximab: 375 mg/m^2 (n = 24; 4 infusions) or 500-mg fixed dose (n = 17; 4 infusions), 200-mg fixed dose (n = 19; 4 infusions), or various dose regimens (n = 16)	TTP recurrence incidence not reported	At 1 mo posttreatment, ADAMTS13 recovery‡ in 92%. 20/45 (44.4%) patients received ≥ 2 preemptive treatments with rituximab.

TTP, thrombotic thrombocytopenic purpura.
*Treatment trigger: ADAMTS13 < 10%.
†Treatment trigger: ADAMTS13 ≤ 15%. In 2 instances, treatment was initiated when ADAMTS13 activity was 16% and 17%.
‡ADAMTS13 recovery was reported differently in the 3 studies: In Hie et al,[7] median ADAMTS13 activity at 3 months was 46% (IQR, 30-68). Jestin et al[8] documented ADAMTS13 activity in 76 of 79 patients; it was normal in 56% (42/76) of patients and moderately decreased in 30% (23/76) of patients. In Westwood et al,[9] ADAMTS13 ≥ 30% in 70/76 (92%) episodes; complete remission as ADAMTS13 activity ≥ 60% in 60/76 (79%) episodes, and partial remission as ADAMTS13 activity in 30% to 59% in 10/76 (13%) episodes.

previous study, there was considerable heterogeneity in remission ADAMTS13 activity, and many patients with normal levels during follow-up experienced a relapse. There is emerging evidence that low-normal or reduced ADAMTS13 activity during remission is associated with an increased risk for ischemic stroke outside of iTTP relapse.[6] Based on the existing evidence, we believe that assessment of ADAMTS13 activity should be performed regularly for a minimum of 2 years after an acute iTTP episode; long-term or even lifelong assessment is preferable.

Preemptive rituximab strategy to prevent iTTP relapse

Over the past decade, preemptive rituximab therapy in iTTP patients with reappearing ADAMTS13 antibodies and severe ADAMTS13 deficiency in remission has shown promise (Table 1). In a retrospective study from France, preemptive rituximab use in 30 patients with ADAMTS13 < 10% led to an increase in ADAMTS13 activity (median, 46%) 3 months later, and the frequency of relapse decreased from 0.57 episodes per year (interquartile range [IQR], 0.46-0.7) to 0 episodes per year (IQR, 0-0.81; $P < .01$).[7] In a subsequent prospective study of the same group that was carried out between 2012 and 2017, 92 patients were treated with preemptive rituximab at doses of 375 to 500 mg/m^2 (1-4 infusions at the discretion of the treating physician) when ADAMTS13 remained at or dropped to <10% during remission. The median cumulative relapse incidence before and after treatment was 0.33 episodes per year (IQR, 0.23-0.66) and 0 episodes per year (IQR, 0-1.32; $P < .01$).[8] The optimal dose and schedule of preemptive rituximab have not been determined. In a retrospective study from the United Kingdom, the investigators compared different weekly doses,

Table 2. Recommendations for postremission laboratory surveillance and preemptive treatment of patients with iTTP

Timing of follow-up	ADAMTS13 activity and CBC	Preemptive rituximab
First 3 mo after stopping TPE	ADAMTS13 monthly; CBC every 1-2 wk until steroids have been discontinued and then monthly	Consider treatment when ADAMTS13 activity drops to 10-20% during follow-up in remission
3 to 24 mo	Every 3 mo	Optimal dose and schedule have not been determined; most studies have used the standard regimen for B-cell neoplasia (375 mg/m^2 per wk for 4 wk).
24 to 60 mo	Continue every 3 mo or extend interval to every 6-12 mo*	Associated favorable risk-benefit ratio.
61+ mo	No evidence, but recommend ongoing (yearly) surveillance*	

CBC, complete blood count.
*The patient's preference has to be taken into account; consider demographic risk factors of relapsing courses[2,3] in decision making.
†Other doses and <4 weekly infusions of rituximab may be effective in normalizing ADAMTS13 activity. Administration of 1 rituximab infusion (fixed or body weight–adapted dose), followed by re-measurement of ADAMTS13 activity after 1 month, is an alternative approach. However, according to Jestin et al,[8] the median time to first retreatment in patients receiving 4 rituximab infusions was 40 months (IQR, 18.1-57) compared with 18.9 months (IQR, 14.3-26) and 18.1 mo (IQR, 11.3-22) in those receiving 2 or 1 infusion(s), respectively ($P = 0.01$). Similarly, Westwood et al[9] documented a lower incidence rate for retreatment following preemptive rituximab in standard-dose regimens (0.17 per year) compared with reduced-dose regimens (0.38 per year) ($P = 0.039$).

administered for 4 weeks, of preemptive rituximab in 45 patients: 24 episodes were treated with 375 mg/m^2, 17 episodes were treated with a fixed dose of 500 mg, and 19 episodes were treated with a fixed dose of 200 mg.[9] There was no difference in the proportion of patients who had ADAMTS13 recovery, or subsequent relapse, however, the proportion of patients requiring retreatment was lower following standard-dose compared with reduced-dose regimens (Table 1).

Summary

Based on recent key publications, we have reviewed the clinical predictors and biomarkers of iTTP relapse after remission, as well as the utility of preemptive rituximab therapy when ADAMTS13 deficiency reappears (Table 2). To minimize the risk of overt clinical relapse and the associated risk for morbidity and mortality, we recommend regular measurement of ADAMTS13 activity, and preemptive rituximab treatment should be discussed with the patient as soon as remission ADAMTS13 activity drops to 10% to 20% (grade 1B). Although a standard-dose regimen of rituximab was used in most studies, a single dose (375 mg/m^2), with ADAMTS13 activity measured 4 weeks later, can be considered (grade 2C). After preemptive rituximab, resumption of follow-up with regular ADAMTS13 determination is advised. Routine medical care, cardiovascular risk reduction, and depression screening are beyond the scope of this minireview.

Conflict-of-interest disclosure

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Off-label drug use

None disclosed.

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Can we use epigenetics to prime chemoresistant lymphomas?

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Chemoresistance remains a challenging clinical problem in the treatment of many lymphoma patients. Epigenetic derangements have been implicated in both intrinsic and acquired chemoresistance. Mutations in epigenetic processes shift entire networks of signaling pathways. They influence tumor suppressors, the DNA-damage response, cell-cycle regulators, and apoptosis. Epigenetic alterations have also been implicated in contributing to immune evasion. Although increased DNA methylation at CpG sites is the most widely studied alteration, increased histone methylation and decreased histone acetylation have also been implicated in stem-like characteristics and highly aggressive disease states as demonstrated in both preclinical models of lymphoma and patient studies. These changes are nonrandom, occur in clusters, and are observed across many lymphoma subtypes. Although caution must be taken when combining epigenetic therapies with other antineoplastic agents, epigenetic therapies have rarely induced clinical meaningful responses as single agents. Epigenetic priming of chemotherapy, targeted therapies, and immunotherapies in lymphoma patients may create opportunities to overcome resistance.

LEARNING OBJECTIVES

- Understand general principles of chemoresistance in lymphoma: intrinsic and acquired
- Recognize the role of epigenetics in contributing to chemoresistance and evasion of antitumor immunity
- Gain knowledge regarding strategies to use epigenetic priming to overcome chemoresistance

Clinical case

In 2014, a 44-year-old woman presented with right flank and pelvic pain, 6 months of night sweats, and a 10-kilogram weight loss. Past medical history was significant for a bleeding gastric ulcer secondary to nonsteroidal anti-inflammatory drug use. Laboratory examination was notable for the following: hemoglobin, 7.0 g/dL; lactate dehydrogenase, 605 U/L; alkaline phosphatase, 209 U/L; γ glutamyl transferase, 16 U/L; albumin, 3.2 g/dL; and calcium, 10.6 mg/dL. Positron emission tomography/computed tomography scanning revealed hilar, para-aortic, mesenteric, and iliac adenopathy, and a renal mass, with involvement of bilateral psoas muscles and diffuse boney disease with standardized uptake values up to 21. Histologic evaluation of a psoas muscle biopsy revealed diffuse cellular infiltrate involving the skeletal muscle with large-sized cells positive for CD30, CD25, CD4, and CD5 and negative for anaplastic lymphoma kinase (ALK), consistent with ALK$^-$ anaplastic large cell lymphoma (ALCL). The patient was treated on the ECHELON-2 clinical trial and was randomized to receive either cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) or brentuximab plus cyclophosphamide, doxorubicin, and prednisone.[1] After 6 cycles, she attained a partial response and was consolidated with an autologous stem cell transplant. Posttransplant, she received 3000 cGy radiation for persistent disease in her humeral head. Six months later, the patient relapsed with cervical and pelvic lymphadenopathy and was treated on a clinical trial of combined epigenetic therapy with a novel antifolate, pralatrexate plus romidepsin.[2] Following 2 months of treatment, a positron emission tomography/computed tomography scan confirmed a complete response. The patient received therapy for a total of 1 year. As of June 2020, she remains free from disease and has remained healthy without any untoward sequalae.

Introduction

The recognition of drug resistance dates back to the study of antibiotic effectiveness. Luria and Delbrück uncovered the emergence of antibiotic resistance in 1943. Many of their discoveries have been applied to cancer chemotherapy resistance.[3] As Fox and Loeb have stated, "Cancers represent a microcosm of Darwinian evolution: tumor

Table 1. Epigenetic therapy plus chemotherapy

Drug	Disease type	NCT no.	Enrollment status	Response	Grade 3/4 toxicities
Vorinostat + R-DA-EPOCH vs R-DA-EPOCH	AIDS-related DLBCL	NCT01193842	Active, not recruiting; phase 1/2, N = 90	CR, 65% vs 77%; 1-y EFS, 75% vs 82% favoring R-EPOCH	Neutropenia, 44% vs 16%; thrombocytopenia, 27% vs 2%
Oral azacitidine + R-CHOP	DLBCL/FL/tFL	NCT02343536	Active, not recruiting; phase 1, N = 59	ORR, 95%; CR, 88%; 1-y PFS, 86%; 2-y PFS, 80%;	Neutropenia, 52%; febrile neutropenia, 25%; anemia, 17%
Vorinostat + R-CHOP	DLBCL	NCT00972478	Active, not recruiting; phase 1/2, N = 72	2-y PFS, 72%; OS, 86%	Febrile neutropenia, 38%; sepsis, 19%; neutropenia, 60%; anemia, 35%; thrombocytopenia, 35%
Vorinostat + R-CEP	DLBCL in elderly	NCT00667615	Completed; phase 1/2, N = 30	CR, 35%; median PFS, 9.2 mo	Neutropenia, 15%; thrombocytopenia, 12%; hyperglycemia, 11%
Azacitidine + R-CHOP	DLBCL	NCT01004991	Completed; phase 1/2, N = 12	CR, 92%	Neutropenia, 100%; febrile neutropenia, 25%
Tazemetostat + R-CHOP	DLBCL	NCT02889523	Completed; phase 1b, N = 17	Metabolic CR, 77%	Neutropenia, 47%; leukopenia, 29%; constipation, 24%
Decitabine + COP	DLBCL	NCT03494296	Recruiting		
Decitabine + R-DHAP	DLBCL	NCT03579082	Recruiting		
Oral azacitidine + R-ICE	DLBCL	NCT03450343	Recruiting		
Oral azacitidine + CHOP	PTCL	NCT03542266	Active, not recruiting		
Romidepsin + CHOP	PTCL	NCT01796002	Active, not recruiting; phase 1/2, N = 37	CR, 51%; ORR, 68%; 18-mo PFS, 77%	Neutropenia, 89%; thrombocytopenia, 78%; febrile neutropenia, 14%; pneumonia, 11%
Romidepsin + gemcitabine	PTCL	NCT01822886	Completed; phase 2, N = 20	CR, 15%; ORR, 30%; 2-y OS, 50%	Thrombocytopenia, 60%; neutropenia, 50%; anemia, 20%
Chidamide + CHOP	PTCL	NCT02809573	Completed; phase 1, N = 30	CR, 35%; 1-y PFS, 54%; 1-y OS, 100%	Leukopenia, 90%; neutropenia, 83%; lymphocytopenia, 40%
Romidepsin + GDP	PTCL	NCT01846390	Completed; phase 1, N = 20	ORR, 50%; median PFS, 2.3 mo; median OS, 7.16 mo	Thrombocytopenia, 55%; neutropenia, 30%; anemia, 30%
Romidepsin + ICE	PTCL	NCT01590732	Completed; phase 1, N = 18	CR, 13%; ORR, 93%; median PFS, 10 mo	Thrombocytopenia, 83%; anemia, 50%; neutropenia, 44%
Belinostat + CHOP	PTCL	NCT01839097	Completed; phase 1, N = 21	CR, 72%; ORR, 89%	Neutropenia, 26%; anemia, 22%
Vorinostat + CHOP	PTCL	NCT00787527	Completed; phase 1, N = 14	CR, 93%; 2-y PFS, 79%	Neutropenia, 76%
Romidepsin + CHOEP	PTCL	NCT02223208	Recruiting		
Decitabine + CHOP	PTCL	NCT035537	Not yet recruiting		
Panobinostat + ICE	HL	NCT0116936	Completed; phase 1, N = 29; phase 2, N = 24	Phase 1 CR, 72%; phase 2 CR, 82% vs 67%; no difference if FFS	Neutropenia, 55% vs 8%; thrombocytopenia, 100% vs 33%
Vorinostat + R-ICE	Relapsed B-cell NHL or frontline PTCL or MCL	NCT00601718	Completed; phase 1, N = 29	ORR, 70%	Febrile neutropenia, 28%; infection, 28%; gastrointestinal, 31%

ASCT, autologous stem cell transplant; CHOEP, prednisone, Oncovin (vincristine), cyclophosphamide, hydroxydoxorubicin, etoposide; COP, cyclophosphamide, Oncovin (vincristine), prednisone; CR, complete response; DHAP, dexamethasone, high-dose cytarabine platinum-based, chemotherapy; DLBCL, diffuse large B-cell lymphoma; EFS, event-free survival; FFS, failure-free survival; FL, follicular lymphoma; GDP, gemcitabine, dexamethasone, cisplatin; HL, Hodgkin lymphoma; ICE, ifosfamide, carboplatinum, etoposide; MCL, mantle cell lymphoma; NHL, non-Hodgkin lymphoma; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PTCL, peripheral T-cell lymphoma; R, rituximab; R-CEP, rituximab, prednisone, etoposide, cyclophosphamide; R-CHOP, rituximab, prednisone, Oncovin (vincristine), cyclophosphamide, hydroxydoxorubicin; R-DA-EPOCH, rituximab, dose-adjusted etoposide, prednisone, Oncovin (vincristine), cyclophosphamide, hydroxydoxorubicin; tFL, transformed follicular lymphoma.

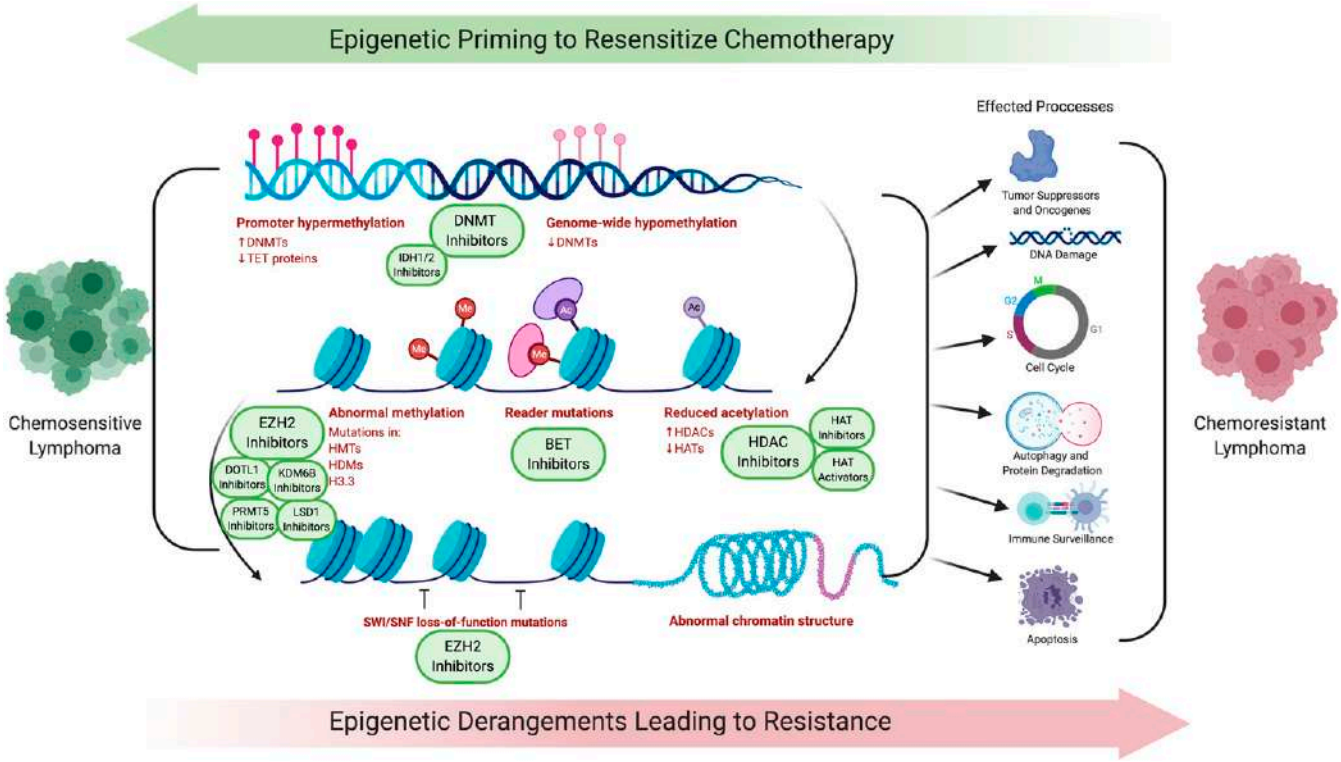

Figure 1. Epigenetic priming to resensitize chemotherapy. Derangements in multiple epigenetic processes lead to shifts in entire networks of signaling pathways, tumor suppressors, oncogenes, DNA-damage response, cell cycle, autophagy and protein degradation, and immune surveillance and apoptosis.

progression is a mutation-driven process that results from the adaptation of a heterogeneous cell population to different microenvironments through the preferential replication of the most suitable variants."[4]

In patients with lymphoma, chemotherapy resistance occurs by 2 main mechanisms. Intrinsic drug resistance is defined by resistance to treatment without any prior exposure to chemotherapy. It is associated with reduced drug transport, drug breakdown, altered expression or engagement of the target, or specific intrinsic biological properties of the lymphoma, such as alterations in cell-cycle regulators, and DNA-damage repair mechanisms.[5] Lymphomas harboring these attributes often have a dismal prognosis from the outset. One such example is the blastoid variant of mantle cell lymphoma characterized by cyclin D1 translocations and p53 mutations. Acquired drug resistance refers to the change in a susceptible tumor to one that has become resistant after repeated exposure to chemotherapy. This occurs via environmental factors that lead to genetic changes in the tumor, metabolic variations affecting the drug, modifications in the microenvironment and immune surveillance, and complex shifts of entire networks of signaling pathways. These events are often not related to specific chemotherapeutic drugs but rather are a result of intratumoral heterogeneity and random spontaneous events, which creates selective pressure for expansion of a clonal subpopulation. These mechanisms may occur alone, or more frequently together, which lowers the threshold for resistance to occur.[6]

Strategies to overcome chemotherapy resistance and expand the spectrum of activity of both traditional chemotherapy and novel agents have been in development[7] (Table 1). Epigenetic derangements have global effects, simultaneously influencing a greater set of pathways than direct genetic alterations of specific tumor-suppressor genes (Figure 1). Often, mutations in epigenetic processes do not occur in isolation, but rather simultaneously across multiple epigenetic controls, creating an opportunity to leverage epigenetic targeting as a means to overcome chemoresistance. This manuscript will build on the article "Harnessing lymphoma epigenetics to improve therapies" (see Green, in this book[8]). A better understanding of how targeting epigenetics have, and could be, used to overcome chemoresistance will hopefully spark innovation to improve outcomes for our patients with relapsed/refractory lymphoma.

Epigenetic factors have been implicated in both intrinsic and acquired drug resistance

Epigenetic plasticity allows for a diversity of survival following exposure to chemotherapy by cooperating with somatic mutations to generate resistant subpopulations of lymphoma.[9] Epigenetic polymorphisms, or epipolymorphisms demonstrate diversity both within and between lymphoma patient samples and has been found to evolve along with the development of a resistant clone.[10] The term epiallele refers to a specific DNA methylation pattern at a discrete genetic locus. Following chemotherapy, tumor cells undergo significant epiallele alteration and, as resistant clones are selected, epipolymorphism diversity narrows.

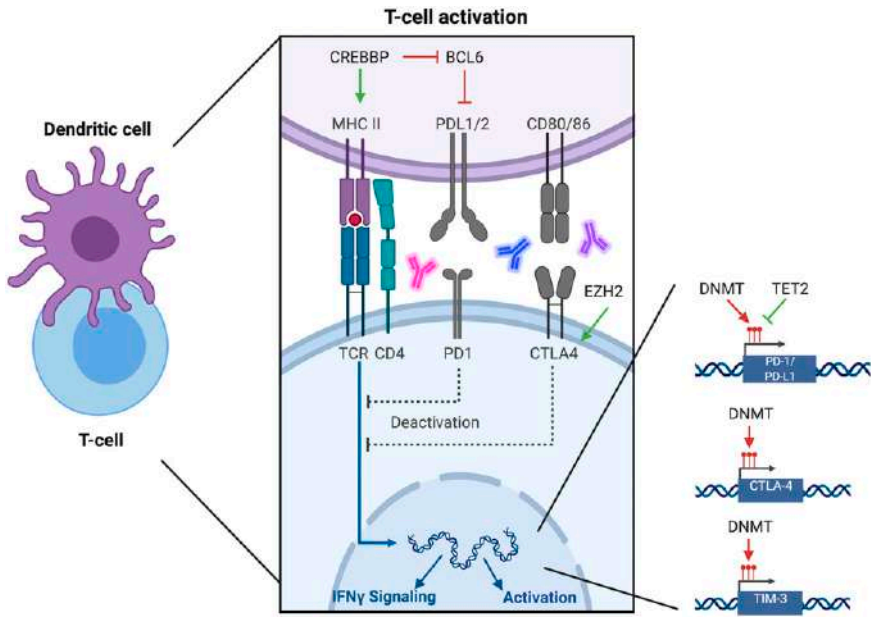

Figure 2. Intersection of epigenetics and immune surveillance. Regulation of immune surveillance is controlled in part by epigenetic operations. The histone acetyltransferase enzyme CREBBP activates expression of MHCII, which allows for engagement between the antigen-presenting cell and the T cell. CREBBP also abrogates BCL6, which in turn abrogates PD-L1 expression. These effects have been reversed with HDAC inhibitors. EZH2 is associated with increased CTLA4 activity therefore EZH2 inhibitors have combined favorably with CLTA4 inhibitors. Expression of PD-1, PD-L1, CTLA4, and TIM-3 is controlled by promotor region methylation; therefore, their expression may be modulated by DNMT inhibitors.

DNA methylation has been the most widely studied epigenetic process contributing to chemoresistance. To study this, researchers created a phylogenetic tree to relate the level of methylation of lymphoma subtypes follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) to patterning of normal B cells.[9] They found that the "farther" the methylation pattern was from normal B cells, the worse the survival. This held true even between FL grades. Furthermore, abnormal methylation patterns were not random, but were directed at the promoter regions of key regulatory factors such as *BCL6*, *EZH2*, and *MYC*. Building on these observations, investigators studied methylation heterogeneity between DLBCL patients who maintained a durable treatment response to those who relapsed.[11] They observed differentially hypermethylated regions enriched at promoter regions, specifically regulatory elements such as *CTCF*, a chromatin-binding factor that regulates the spread of DNA methylation through interactions with histone acetylases and deacetylases. In preclinical models of lymphoma,[12] utilizing a mafosfamide-resistant cell line, investigators demonstrated that methylation was quantified at cytosine guanine dinucleotide (CpG) sites to identify differential methylation between the parental and resistant cell lines. The authors found that gene regions functionally enriched for DNA binding, transcription factor activity, and cell differentiation were marked with increased methylation. Additionally, members of the histone methyltransferase polycomb-repressive complex 2 were strongly enriched over repressed genes in the resistant cell line and the histone demethylase *Uty* was downregulated, contributing to chromatin condensation and decreased gene expression. The investigators found that the expression profiles of the resistant cells were similar to that of progenitor B cells, recapitulating stem-like characteristics. Similarly, in a study of ALCL, investigators found significant increases in CpG island methylation in the aggressive chemorefractory ALK$^-$ subtype as compared with the chemosensitive ALK$^+$ subtype.[13] Furthermore, samples from relapsed ALCL patients demonstrated even greater hypermethylation. Interestingly, the investigators found similar hypermethylation patterns of resistant ALCL when compared with the profiles of DLBCL, suggesting that these patterns may not be random events but are common across lymphoma subtypes. The hypermethylation signature of the more aggressive lymphomas reverted back to that of ALK$^+$ ALCL following exposure to the DNA methyltransferase (DNMT) inhibitor 5-azacytidine. In a study designed to better understand the contribution of DNA methylation to chemoresistance and therapies to reverse these effects, investigators found that the SMAD1 member of the transforming growth factor β pathway is silenced via aberrant DNA methylation in chemoresistant DLBCL. Treatment with DNMT inhibitors reversed this phenomenon and sensitized cells to doxorubicin. These findings were translated to a clinical trial of azacitidine and rituximab plus CHOP chemotherapy in patients with DLCBL, which was well tolerated and led to a complete response in 11 of 12 patients.[14]

In addition to DNA methylation, epigenetic modification of histones has been recognized as an important contributor to chemoresistance. By directly leading to transcriptional silencing, enhancer of zeste homolog 2 (EZH2) and the polycomb-repressive complex 2 recruit DNMTs and DNA clusters over promoter regions known to be hypermethylated. In etoposide-resistant lymphoma cell lines, EZH2 expression has been linked to steering cells toward senescence rather than apoptosis. EZH2

Table 2. Epigenetic therapy plus immunotherapy

Drug	Target	Disease type/status	NCT no.	Enrollment status
Atezolizumab/tazemetostat	PD-L1/EZH2	DLBCL/FL	NCT02220842	Completed
Avelumab/utomilumab/azacitidine	PD-L1/IgG2 CD 127/4-1BB agonist/DNMT	DLBCL/HGBCL	NCT02951156	Completed
Vorinostat/pembrolizumab	HDAC/PD-1	DLBCL/FL/HL	NCT03150329	Recruiting
Romidepsin/5-azacitidine/pralatrexate/durvalumab	HDAC/DNMT/Anti-folate/PD-L1	PTCL	NCT03161223	Recruiting
Entinostat/pembrolizumab	HDAC/PD-1	HL/relapsed	NCT03179930	Recruiting
Decitabine/pralatrexate/pembrolizumab	DNMT/Anti-folate/PD-1	PTCL/CTCL relapsed	NCT03240211	Recruiting
Decitabine/SHR-1210	DNMT/PD-1	HL relapsed	NCT03250962	Recruiting
Romidepsin/pembrolizumab	HDAC/PD-1	T-cell lymphomas/relapsed	NCT03278782	Active not recruiting
Decitabine/SHR-1210/GVD	HDAC/PD-1/Chemo	PMBCL relapsed	NCT03346642	Recruiting
Decitabine/pembrolizumab/hypofractionated XRT	DNMT/PD-1/radiation	Lymphoma/CNS/solid tumor relapse	NCT03445858	Recruiting
CXD101/pembrolizumab	HDAC/PD-1	DLBCL/relapsed	NCT03873025	Not yet recruiting
Chidamide/decitabine/camrelizumab	HDAC/DNMT/PD-1	HL relapsed	NCT04233294	Recruiting
TAA-specific CTLs/azacitidine	TAA/DNMT	Lymphoma	NCT01333046	Recruiting

chemo, chemotherapy; CNS, central nervous system; CTCL, cutaneous T-cell lymphoma; CTL, cytotoxic T cell; GVD, gemcitabine, vinorelbine, doxorubicinl; HGBCL, high-grade B-cell lymphoma; HL, Hodgkin lymphoma; IgG2, immunoglobulin G2; NCT no., clinicaltrials.gov national clinical trial number; PMBCL, primary mediastinal B-cell lymphoma; TAA, tumor-associated antigen; XRT, radiotherapy.

inhibition induced sustained expression of p53 through modulation of MDM2-like p53 binding protein, while also upregulating p21 to overcome chemotherapy resistance.[15] In preclinical chemoresistance models, EZH2-mediated silencing of Schlafen 11 (SLFN11) led to impaired DNA-damage repair. Recent studies have identified SLFN11, an inhibitor of DNA replication, as a genomic determinant of response to agents such as doxorubicin, platinum, and methotrexate.[16] Treatment with EZH2 inhibitors restored SLFN11 expression and induced chemosensitivity in resistant models.[17] Disruptor of telomeric silencing 1-like (DOTL1), an H3k79 methyltransferase, promotes DNA repair through homologous recombination. DOTL1 inhibition prevents the recruitment of p53-binding protein 1 (53BP1) to sites of double-strand breaks. DOLTL1 inhibitors thus could sensitize cancer cells to DNA-damaging agents.[18,19] The DOTL1 inhibitor, pinometostat (EPZ-5676), is now in clinical trials. The histone lysine demethylase, KDM6B, was found to be correlated with reduced survival in DLBCL lymphoma patients receiving rituximab plus CHOP chemotherapy.[20] Following evaluation of 181 patients, the survival rate was 48% for high expressers vs 71% for low expressers. When the investigators treated lymphoma cell lines with the KDM6B inhibitor GSK-J4, they found marked increase in apoptosis when combined with chemotherapy, suggesting chemosensitization. The authors also found that GSK-J4 influenced B-cell receptor signaling and B-cell lymphoma 6 (BCL6) expression, suggesting that these drivers of disease may be modified with this treatment.

Histone acetylation has been known to affect a broad array of cellular mechanisms ranging from apoptosis, DNA-damage response, cell cycle, autophagy, protein degradation, and immune response. Through modulation of these pathways, therapeutic targeting of histone acetylation, mainly through histone deacetylase (HDAC) inhibition, has been a strategy for overcoming resistance, with the chemoresistant T-cell lymphomas as the model for HDAC-inhibitor activity. Three HDAC inhibitors are approved by the US Food and Drug Administration (FDA) for relapsed/refractory T-cell lymphoma: vorinostat, romidepsin, and belinostat; chidamide is approved in China. Circling back to our case, romidepsin has demonstrated an overall response rate (ORR) of 25% to 39%.[21,22] In the study published by Coiffier et al, there were 21 patients with ALK$^-$ ALCL, and although this disease entity is not marked by epigenetic derangements, 24% of those with ALCL achieved a response to therapy. Increased expression of p21 by HDAC inhibitors has been considered 1 potential mechanism of resensitizing chemoresistant lymphoma. This has been recognized across several lymphoma subtypes including DLBCL, mantle cell lymphoma, T-cell lymphoma, and Hodgkin lymphoma.[23] In a study of primary refractory DLBCL patient samples, vorinostat inhibited cell viability, induced p21 expression, and downregulated cyclin-dependent kinase 2, leading to resensitization to chemotherapy.[24] In rituximab-resistant DLBCL cell lines, entinostat led to decreased Bcl-XL levels and increased p21 and it potentiated the effects of cytarabine.[25] Similarly, in Hodgkin lymphoma cell lines, vorinostat led to induction of p21 and downregulation of cyclin D2, producing synergistic cytotoxicity with cisplatin.[26]

BCLs treated with anti-CD20 monoclonal antibodies are frequently associated with downregulation of CD20, which breeds resistance to rituximab. Epigenetic modulation of the CD20-coding gene, MS4A1, has been shown to contribute to loss of CD20. Treatment with the HDAC6-selective inhibitor, ACY-1215, has been demonstrated to induce CD20

Table 3. Combined epigenetic targeting agents with other small molecules

Drug	Disease type	NCT no.	Enrollment status	Response data	Grade 3/4 toxicity
Proteasome inhibitor: bortezomib					
Azacitidine	PTCL	01129180	Completed		
Vorinostat	Maintenance after ASCT	00992446	Completed		
Vorinostat	MCL and DLBCL	00703664	Completed; phase 2, N = 65	ORR, 10%; terminated early poor accrual	Thrombocytopenia, lymphopenia, diarrhea
Belinostat	HL and NHL	00348985	Completed; phase 1, N = 22		Anorexia, dehydration, fatigue
Panobinostat	PTCL	00901147	Completed; phase 2, N = 25	CR, 22%; ORR, 43%	Thrombocytopenia, neutropenia, diarrhea
Panobinostat	MCL	01504776	Completed		
Proteasome inhibitor: carfilzomib					
Romidepsin	PTCL	03141203	Active, not recruiting		
Belinostat	NHL	02142530	Completed		
Vorinostat	NHL	01276717	Completed; phase 1, N = 20	ORR, 5%	Toxicities led to dose reductions to dose level −1
Proteasome inhibitor: ixazomib					
Romidepsin	PTCL	03547700	Recruiting		
DNMT/HDAC: azacitidine					
Vorinostat	NK/TCL	00336063	Active, not recruiting		
Vorinostat	DLBCL	01120834	Completed; phase 1b, N = 18	ORR, 6.7%; 3-mo OS, 77%	Thromboembolism, diarrhea
Romidepsin	HL and NHL	01998035	Active, not recruiting; phase 1/2; phase 1, N = 33	ORR, 32%; in PTCL: ORR, 73%; CR, 55%	Thrombocytopenia, leukopenia, lung infection
Mocetinostat	HL and NHL	05433582	Terminated		
DNMT/HDAC: decitabine					
Vorinostat	HL and NHL	00275080	Completed; phase 1, N = 43	ORR, 0%	Neutropenia, thrombocytopenia
Chidamide	Relapse after CAR-T	04337606	Recruiting		
IMID: lenalidomide					
Azacitidine	FL/MZL	01121757	Terminated		
Panobinostat	HL	01460940	Completed; phase 1/2; N = 24	ORR, 17%; PFS, 3.8 mo	Neutropenia, thrombocytopenia
Romidepsin + carfilzomib	NHL	02341014	Active, not recruiting; phase 1b/2, N = 27	In TCL at MTD; CR, 36%; ORR, 45%	Neutropenia, thrombocytopenia
Tazemetostat + rituximab	FL	04224493	Recruiting		
PI3K: tenalisib					
Romidepsin	PTCL	00377000	Recruiting		
PI3K: duvelisib					

ADC, antibody drug conjugate; ALT, alanine aminotransferase; ASCT, autologous stem cell transplant; AST, aspartate transaminase; BET, bromodomain and extraterminal motif; CAR-T, chimeric antigen receptor T cell; CR, complete response; IMID, immune-modulatory drug; MCL, mantle cell lymphoma; MTD, maximum tolerated dose; MTOR, mammalian target of rapamycin; MZL, marginal zone lymphoma; NHL, non-Hodgkin lymphoma; NK, natural killer; OS, overall survival; PFS, progression-free survival; TCL, T-cell lymphoma. See Tables 1 and 2 for expansion of other abbreviations.

Table 3. (Continued)

Drug	Disease type	NCT no.	Enrollment status	Response data	Grade 3/4 toxicity
Romidepsin	PTCL	02783625	Recruiting; phase 1, N = 39	ORR, 51%; in PTCL: ORR, 55%; CR, 27%	Increased AST/ALT, neutropenia, hyponatremia
BET inhibitor: FT-1101					
Azacitidine	HL and NHL	02543879	Completed		
BET inhibitor: molibresib besylate					
Entinostat	HL and NHL	03925428	Not yet recruiting		
MTOR: everolimus					
Panobinostat	HL and NHL	00967044	Completed; phase 1, N = 30	ORR, 43%; CR, 15%	Thrombocytopenia, neutropenia, anemia
ADC CD30: brentuximab					
Romidepsin	CTCL	02616965	Recruiting		
Anti-folate: pralatrexate					
Romidepsin	NHL	01947140	Recruiting; phase 1/2; Ph 1, N = 29	ORR, 57%; in PTCL: ORR, 71%; CR, 17%	Anemia, mucositis, thrombocytopenia
Aurora A kinase inhibitor: alisertib					
Romidepsin	NHL	01897012	Completed; phase 1, N = 25	ORR, 28%; CR, 12%	Thrombocytopenia, anemia, infection
Retinoid: isotretinoin					
Entinostat	HL and NHL	00098891	Completed		
Sirtuin inhibitor: niacinamide					
Vorinostat	HL and NHL	00691210	Completed; phase 1, N = 28	ORR, 24%; SD, 57%	Thrombocytopenia, infection

ADC, antibody drug conjugate; ALT, alanine aminotransferase; ASCT, autologous stem cell transplant; AST, aspartate transaminase; BET, bromodomain and extraterminal motif; CAR-T, chimeric antigen receptor T cell; CR, complete response; IMID, immune-modulatory drug; MCL, mantle cell lymphoma; MTD, maximum tolerated dose; MTOR, mammalian target of rapamycin; MZL, marginal zone lymphoma; NHL, non-Hodgkin lymphoma; NK, natural killer; OS, overall survival; PFS, progression-free survival; TCL, T-cell lymphoma. See Tables 1 and 2 for expansion of other abbreviations.

expression and sensitize cells to rituximab.[27] This has also been demonstrated with entinostat and chidamide, suggesting a class effect.[25,28]

Epigenetics can also have effects on signaling pathways that are targetable with small molecules. One such example is targeting with the Bruton tyrosine kinase (BTK) inhibitor ibrutinib, in which resistance in mantle cell lymphoma has been recognized. Although mutations in the BTK-binding site are responsible for a subset of ibrutinib resistance, activation of alternative pathways leading to NF-κB signaling also play a role such as phosphatidylinositol 3-kinase (PI3K)-AKT activation.[29,30] HDAC inhibitors have been demonstrated to enhance activity of PI3K inhibitors through modulation of the JAK/STAT pathway. As such, the dual HDAC/PI3K inhibitor CUDC-907 has demonstrated activity in BTK-resistant mantle cell lymphoma and DLBCL preclinical models.

HDAC inhibitors have been implicated in inducing both mitochondrial and death receptor apoptotic pathways. Investigators studied a panel of etoposide-resistant lymphoma cell lines noted to have mutant BCL2.[31] Treatment with romidepsin led to acetylation and inactivation of heat shock protein 90, allowing for the upregulation of Bcl2-like protein 11. Although this effect is based on posttranslational rather than epigenetic modification, acetylation of heat shock protein 90 by HDAC inhibitors increased sensitivity to chemotherapy. Induction of Bcl2-like protein 11 has been noted across multiple lymphoma subtypes following exposure to HDAC inhibitors. As such, combination with BCL2 inhibitors such as venetoclax has demonstrated promising results.[32]

Posttranslational modifications induced by epigenetic drugs have the ability to influence many pathways known to contribute to chemoresistance. One such example is impaired p53 activity. Although there are many mechanisms responsible for reduced activity, acetylation of p53 activates the tumor suppressor and protects it from degradation.[33] Treatment with sirtuin inhibitors, class III HDAC inhibitors, can induce acetylation of p53. HDAC inhibitors have been known to influence the effects of master regulators and key oncogenes such as BCL6.[34,35] Our laboratory demonstrated synergy between romidepsin and niacinamide, a sirtuin inhibitor, through enforcing acetylation of the oncogene BCL6, abrogating its effects, and acetylation of p53 activating the tumor suppressor.[31] Romidepsin has been demonstrated to downregulate BCL6 and influence its target genes such as cyclin D2 and PRDM1. This in turn has led to synergistic targeting of MYC with the bromodomain and extraterminal domain (BET) inhibitor JQ1.

Intersection of epigenetics and immunotherapy

Immune evasion has been increasingly recognized as a mechanism of disease progression in lymphomas (Figure 2). Epigenetic regulation of immune surveillance in the microenvironment is an area of intense study (Table 2). Acquired chemoresistance has been linked to increased expression of programmed cell death 1 (PD-1) and programmed death ligand 1 (PD-L1), which have been demonstrated to be under epigenetic control.[36] De novo DNA methylation of promoter regions of Pdcd1 fix T cells in an exhausted state. Although this may be reversed with checkpoint blockade, when treatment is withdrawn, T cells revert to an exhausted state. Treatment with DNMT inhibitors has proven complimentary to checkpoint blockade, allowing for rejuvenation of the T-cell response.[37-39] Additionally, histone acetylation of both the PD-L1 and PD-1 promoter regions is implicated in PD-L1 expression. Accordingly, treatment with HDAC inhibitors has been demonstrated to increase expression in both.[40]Many of these studies have been performed in preclinical models of solid-organ malignancies or infection; however, their findings are now being explored in models of lymphoma and have rapidly been translated to clinical trials for relapsed/refractory lymphoma (Table 2).

Germinal center BCLs, DLBCL and FL, are not overtly responsive to checkpoint inhibition partly due to immune evasion. These entities are marked by activating mutations of *EZH2* and inactivating mutations in acetyltransferases *EP300* and *CREBBP*. EZH2 suppresses activity in T cells and regulates chemokines, leading to reduced antitumor immunity.[41] Investigators found that treatment with EZH2 inhibitor CPI-1205, reprogrammed T-cell antitumor immunity, suppressed regulatory T cells, and improved responses to anti-CTLA4 therapy. Derangements of both *EZH2* and acetyltransferases have been implicated in reduced major histocompatibility complex (MHC) expression and tumor-infiltrating lymphocytes. Treatment with EZH2 inhibitors leads to increased expression of the class II MHC transactivator enhancer, restoring MHCII expression. In addition, programmed cell death 1 and CTLA4 were increased following EZH2 inhibition.[42] *CREBBP* enhances MHCII expression and abrogates BCL6 inhibition of PD-L1. Mutations leading to CREB-binding protein (CREBBP) insufficiency have been linked to impaired B-cell to T-cell engagement and reduced T-cell expansion, activation, and antitumor immunity. Targeting this biology with HDAC3 inhibitors demonstrated a reversal of this phenomenon in preclinical models of DLBCL, resulting in synergistic cytotoxicity with PD-L1 inhibitors.[43] In a genomic analysis of microdissected Hodgkin Reed-Sternberg cells from 12 patients with primary refractory disease, 4 of 12 cases had mutations in *EP300* or *CREBBP* suggesting that this could play a role in reduced antitumor immunity.[44] This implies that priming with epigenetic modulators could potentiate checkpoint blockade in diseases marked by impaired immune surveillance (Table 2).

In peripheral T-cell lymphoma (PTCL), specifically those of follicular helper cell of origin such as angioimmunoblastic T-cell lymphoma, mutations in Tet methylcytosine dioxygenase 2 (TET2) are often observed.[45] TET2 is a dioxygenase that converts 5-methylcytosine to 5-carboxylcytosine. In addition, TET2 enzymatic activity is inactivated by the oncometabolite hydroxyglutarate generated by mutated isocitrate dehydrogenase 1/2 detected in up to 45% of angioimmunoblastic T-cell lymphoma patients.[46,47] Reduced TET2-mediated demethylation of PD-L1 promoter regions, chemokines, and the interferon γ pathway contribute to T-cell exhaustion.[48] These effects have been demonstrated to be reversed by treatment with DNMT inhibitors.[49,50] Adult T-cell leukemia/lymphoma is a highly aggressive disease associated with both intrinsic and acquired chemoresistance. Investigators surveyed adult T-cell leukemia/lymphoma patient samples and found that 25 of 58 had increased expression of T-cell immunoglobulin and mucin-domain containing 3 (TIM-3), which was associated with chemoresistance.[51] TIM-3 is expressed on immune cells such as macrophages, dendritic cells, natural killer cells, and T cells. Expression of TIM-3 regulates the immune response through downregulation of the interferon pathway leading to T-cell exhaustion. Expression of TIM-3 and its ligand, LGALS9, is known to be regulated by CpG island methylation within the promoter region, suggesting that expression could be regulated with DNMT inhibitors.[52]

Rewiring pathways driving chemotherapy resistance

There have been a multitude of studies evaluating combined epigenetic targeting for the treatment of lymphoma because aggressive lymphomas often have multiple epigenetic derangements and single-agent therapy has not proven clinically effective in many cases (Table 3). In preclinical studies of DLBCL, both vorinostat and decitabine induced cytotoxicity in chemoresistant cell lines and the combination was synergistic.[53] This has been demonstrated across other HDAC and DNMT inhibitor combinations.[54] The investigators translated these findings into a phase 1b study of relapsed refractory DLBCL patients and treated them with vorinostat and azacytidine; however, the study was closed early secondary to hematologic toxicity and disease progression. Interestingly, 7 of 18 patients went on to further treatment, 2 of whom achieved a complete response; 3 achieved clinical benefit from their subsequent therapies with responses ranging from 79 to 825 days. In a similar effort, investigators treated patients with relapsed DLCBL with the chidamide combined with decitabine then sequentially with rituximab plus gemcitabine and oxaliplatin.[55] All 13 patients enrolled achieved disease control with 3 achieving a complete remission. Not surprisingly, grade 3-4 hematologic toxicities were frequent, as were gastrointestinal side effects, mucositis, pyrexia, and liver abnormalities. Finding the appropriate dosing schedule and patient population to best benefit from combined epigenetic therapy will be necessary for successful application. For example, our team has demonstrated synergy between EZH2 and HDAC inhibitors only in cell lines with deranged EZH2 expression or activity, and not in those with normal EZH2 expression.[56] Our team evaluated the merits of the combination of oral azacitidine and romidepsin, for which the majority of responses were seen in T-cell lymphoma patients. Interestingly, among the 3 patients with germinal center–derived BCL, disease control was achieved in 1 patient with stable disease, 1 patient with a partial response, and 1 with a complete remission. Although the numbers with germinal center B-lymphomas are exceedingly small, this disease entity is marked by epigenetic derangements.[50]

Conclusion

Resistance to both chemotherapy and targeted small molecules continues to limit our ability to cure many with lymphoma. Epigenetic modulating drugs may be used in concert with other epigenetic targeting agents, or small molecules with the intention of rewiring pathways circumnavigating resistance. They may be used to enhance the effects of checkpoint inhibitors. Caution must be taken in efforts to combine these therapies as

toxicity could limit their usefulness. History has taught us that epigenetic drugs are unlikely to have clinical meaningful effects alone. Recognizing patterns of epigenetic derangements in specific disease entities could create an opportunity for precision medicine that will allow epigenetic therapy to be combined effectively. One of their greatest impacts may be as primers to chemotherapy or immunotherapy to overcome resistance.

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Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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Harnessing lymphoma epigenetics to improve therapies

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Affinity maturation and terminal differentiation of B cells via the germinal center reaction is a complex multistep process controlled by transcription factors that induce or suppress large dynamic transcriptional programs. This occurs via the recruitment of coactivator or corepressor complexes that epigenetically regulate gene expression by post-translationally modifying histones and/or remodeling chromatin structure. B-cell–intrinsic developmental programs both regulate and respond to interactions with other cells in the germinal center that provide survival and differentiation signals, such as T-follicular helper cells and follicular dendritic cells. Epigenetic and transcriptional programs that naturally occur during B-cell development are hijacked in B-cell lymphoma by genetic alterations that directly or indirectly change the function of transcription factors and/or chromatin-modifying genes. These in turn skew differentiation toward the tumor cell of origin and alter interactions between lymphoma B cells and other cells within the microenvironment. Understanding the mechanisms by which genetic alterations perturb epigenetic and transcriptional programs regulating B-cell development and immune interactions may identify opportunities to target these programs using epigenetic-modifying agents. Here, we discuss recently published studies centered on follicular lymphoma and diffuse large B-cell lymphoma within the context of prior knowledge, and we highlight how these insights have informed potential avenues for rational therapeutic interventions.

LEARNING OBJECTIVES

- Understand recent advances in understanding of the epigenetic etiology of follicular lymphoma and diffuse large B-cell lymphoma
- Describe how critical pathways can be targeted using epigenetic-modifying agents

Introduction

Translocations of the *MYC* and *BCL6* genes are long-standing examples of how genetic alterations in lymphoma can perturb important transcriptional programs. More recently, studies have revealed additional transcription factors, corepressor/coactivator complex components, histone-modifying enzymes, chromatin-remodeling complex components, and chromatin structural components that are targeted by genetic alterations in B-cell lymphoma. These alterations differ in frequency between histologies[1] and/or within transcriptionally or genetically defined subtypes,[2,3] function by perturbing epigenetic and transcriptional programs that control cellular pathways and cell fate decisions that are important for the tumor's cell of origin (reviewed elsewhere[4-6]), and are potentially targetable by an increasing number of epigenetic-modifying agents.[7] Therefore, understanding the epigenetic basis for lymphoma is an important challenge due to the high potential for clinical translation that could improve patient outcomes. Here, we discuss recently published (2018–2020) studies[8-14] and their translational implications.

EZH2: from H3K27me3 to immune synapse disruption

The *EZH2* gene encodes a lysine methyltransferase enzyme that catalyzes trimethylation of H3K27 (H3K27me3) as part of the polycomb repressor (PRC) 2 complex. EZH2 is highly expressed in germinal center B (GCB) cells[15-17] and normally functions to repress the expression of genes highly expressed in naïve B cells. Gene silencing also occurs in cooperation with BCL6 and BCOR[18] to temporarily repress the expression of transcription factors involved in plasma cell differentiation, such as PRDM1, IRF4, and XBP1,[19,20] and negative regulators of the cell cycle, such as CDKN1A/B.[19] Germinal center (GC)-specific conditional knockout (cKO) of *Ezh2* in transgenic mouse models prevents GC development,[19] but this can be rescued by co-deletion of the BCL6

target gene, *Cdkn1a*, highlighting an important role for Ezh2 in controlling GCB cell proliferation.[21]

Mutations of *EZH2* occur in 15% to 25% of follicular lymphoma (FL) and 5% to 10% of diffuse large B-cell lymphoma (DLBCL)[4] (25% to 45% of the C3/EZB subtype of DLBCL[2,3]; EZH2 mutation is a seed feature for the EZB subtype). These mutations most often encode a single–amino acid change at Y641 in the catalytic SET domain, causing a neomorphic change in activity that results in higher levels of H3K27me3.[22,23] Murine models using viral transduction or conditional knock-in showed that the expression of Ezh2-Y641 mutants results in GC hyperplasia after immunization and cooperates with Bcl2 overexpression to drive lymphomagenesis.[19,20] Recent studies have shown that this may result from deepening repression of canonical Ezh2 target genes within GCB cells, as well as spreading of the H3K27me3 mark to a large number of neighboring promoters[8] (de novo targets). This spreading may be restricted by 3-dimensional chromatin architecture, leading to gain of H3K27me3 within topologically associated domains and coordinated repression of neighboring tumor suppressor genes.[9] By performing targeted sequencing of 57 genes and immunohistochemistry for major histocompatibility complex (MHC) class I and class II on a cohort of DLBCL tumors, Ennishi et al[10] identified an association between *EZH2* mutations and dual loss of MHC class I and class II or single loss of MHC class I. GCB-like DLBCL tumors with loss of MHC expression also tended to have reduced frequencies of tumor-infiltrating CD4 and CD8 T cells,[10] but a subsequent comparison of DLBCL tumors by *EZH2* mutation status alone observed no significant differences between *EZH2* wild-type and mutant cases.[8]

Despite a prominent role for EZH2 in regulating BCL6 target gene expression within dark zone (DZ) GCB cells,[18,21] Béguelin et al[8] recently observed that *Ezh2-Y641F* conditional knock-in mice have an accumulation of light zone (LZ) GCB cells and no impediment to terminal differentiation. This was driven by Ezh2-mediated repression of genes involved in immune synapse formation with T-follicular helper (TFH) cells, resulting in a loss of interaction with TFH cells and reduced CD40/CD40LG signaling. This attenuated MYC expression and recycling to the DZ was associated with reduced somatic hypermutation and clonal diversity. Importantly, *Ezh2* mutant, but not *Ezh2* wild-type, GCB cells were able to maintain GCs in the absence of TFH interaction and CD40 signaling due to their increased association with follicular dendritic cells (FDCs). *Ezh2-Y641F* murine GCB cells were intermeshed with FDCs within the LZ, and human FL tumors with *EZH2* mutations more frequently had dense FDC networks within their follicles. Moreover, *Ezh2-Y641F* murine GCB cells were sensitive to FDC blockade. *EZH2* mutations may therefore function by uncoupling GCB cells from the normal process of clonal selection, allowing them to survive and proliferate within the LZ independent of their antigen affinity but dependent on survival signals from FDCs (Figure 1).

EZH2 mutant DLBCL is sensitive to catalytic inhibitors of EZH2, providing a rationale for targeting EZH2 in FL and DLBCL. Interim results of the phase 2 study of tazemetostat in relapsed/refractory FL were reported at the 2019 American Society of Hematology annual meeting[24] and showed a high response rate in *EZH2* mutant tumors. However, responses were also observed in *EZH2* wild-type cases. On the basis of the respective response rates and the frequency of *EZH2* mutations in FL, if an unselected patient population were treated with tazemetostat, the responders would be expected to consist of approximately equal proportions of *EZH2* wild-type and *EZH2* mutant cases. Thus, response to EZH2 inhibitors is dictated by more than just *EZH2* mutation status. Indeed, EZH2 function is critical for GCB cells, regardless of their mutation status,[19] so a subset of *EZH2* wild-type tumors may nonetheless be addicted to EZH2 activity. Furthermore, EZH2 suppresses the expression of genes involved in immune synapse formation, such as MHC classes I and II, the expression of which can be promoted on DLBCL cell lines and *Ezh2-Y641F* murine GCB cells by EZH2 inhibitors.[10] Therefore, EZH2 inhibition may also have an immune-potentiating effect. Tazemetostat recently received US Food and Drug Administration approval for treatment of patients with relapsed/refractory FL in whom 2 prior lines of therapy have failed if they have (1) an *EZH2* mutation or (2) no satisfactory alternative treatment options. Detailed analysis of the molecular and immunological bases for response/resistance to EZH2 inhibitors, and the redundant or compensatory role for EZH1[25] in this context, will be required to fully grasp the underlying mechanisms and

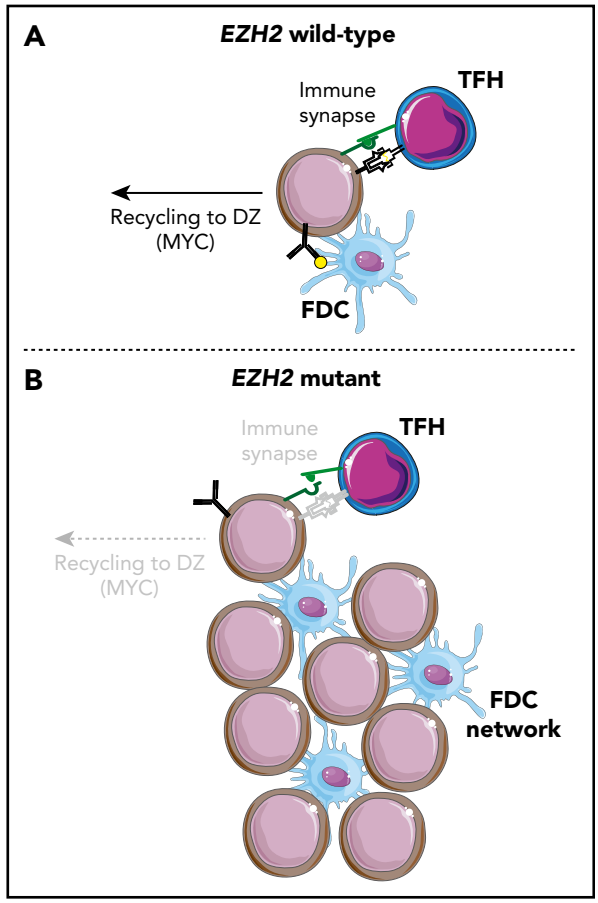

Figure 1. Loss of TFH immune synapse formation and gain of FDC interactions with *EZH2* mutations. (A) *EZH2* wild-type B cells undergo normal clonal selection by binding antigen on FDCs and presenting it on MHC class II. Those with the highest antigen affinity and presentation form an immune synapse with TFH cells, leading to CD40/CD40L signaling, which stimulates terminal differentiation or DZ recycling. (B) *EZH2* mutant B cells have reduced MHC expression and immune synapse formation with TFH cells that leads to decreased CD40/CD40L signaling and DZ recycling. However, these cells are able to proliferate and survive through interactions with an expanded network of FDCs.

inform potential combination strategies that may improve efficacy and durability.

CREBBP: not all mutations are created equal

The *CREBBP* gene encodes a lysine acetyltransferase (KAT) protein that activates gene expression through acetylation of histone H3 lysine 18 (H3K18Ac), histone H3 lysine 27 (H3K27Ac), and other residues. *CREBBP* is mutated in ~65% of FL and 10% to 15% of DLBCL[4,26] (53% of C3/EZB subtype[2,3]). The majority of mutations encode single–amino acid changes within the catalytic KAT domain that reduce acetyltransferase activity,[26] but nonsense/frameshift mutations also occur and are significantly more frequent in DLBCL than in FL.[27] *CREBBP* mutations are associated with poor outcome in FL,[28] but KAT domain mutations are associated with a worse progression-free survival than nonsense/frameshift mutations.[11] Murine studies using *Crebbp* knockout (KO)/knockdown found that *Crebbp* loss promotes B-cell lymphoma in cooperation with Bcl2 overexpression[27,29,30] and that regions of reduced histone acetylation associated with *Crebbp* loss were primarily located at enhancer elements bound by BCL6.[29] Consistent with this, cKO of *Crebbp* in GCB cells leads to reduced expression of genes that are expressed in LZ GCB cells, such as those involved in antigen presentation on MHC class II, B-cell receptor signaling, and interferon signaling.[12,29] This is consistent with observations in *CREBBP* mutant primary tumors, which showed a marked downregulation of MHC class II.[31] Importantly, a similar molecular phenotype was observed with cKO of *Tet2* in GCB cells due to hydroxymethylation loss that impaired enhancer acetylation,[32] suggesting that *TET2* mutations (a seed feature of the ST2 DLBCL subtype[3]) and *CREBBP* mutations may be alternative mechanisms for promoting a BCL6-associated DZ phenotype.

Key differences exist between the magnitudes of changes observed in mouse models using KO/knockdown versus primary tumors in which KAT domain mutations predominate[31] and when comparing patient outcomes by *CREBBP* mutational subtypes. Mondello et al[11] therefore investigated functional differences between *CREBBP* KAT domain mutations and nonsense/frameshift mutations. This was achieved using CRISPR/Cas9 gene editing to generate isogenic lymphoma cell lines with either wild-type *CREBBP*, *CREBBP-R1446C* mutation (a mutation hotspot in the KAT domain), or homozygous frameshift mutation (KO). The *R1446C* mutants had a more severe loss of H3K27Ac than KO mutants, which was linked with a greater reduction in expression of genes with a role in antigen presentation and interferon signaling. *CREBBP* KAT domain mutations may therefore have a dominant-repressive function which exceeds that of nonsense/frameshift mutations. Regions with reduced H3K27Ac were enriched for BCL6 target genes and were bound by both CREBBP and BCL6 in normal GCB cells. BCL6 suppresses gene expression by recruiting corepressors such as the SMRT–HDAC3 complex, suggesting that *CREBBP* mutation results in an imbalance in the antagonistic activity between CREBBP-mediated acetylation and BCL6/HDAC3-mediated deacetylation.[11,29] Consistent with this, HDAC3 inhibition promoted H3K27Ac and expression of genes that were reduced by *CREBBP* mutation. However, HDAC3 inhibition was capable of inhibiting the growth of both *CREBBP* wild-type and mutant DLBCL cell lines and patient-derived xenograft models via induction of the BCL6 target gene, *CDKN1A*. Furthermore, signatures of antigen presentation and interferon signaling were also induced by HDAC3 inhibition in both *CREBBP* wild-type and mutant cells. Therefore, *CREBBP* mutation determines the baseline level of antigen presentation

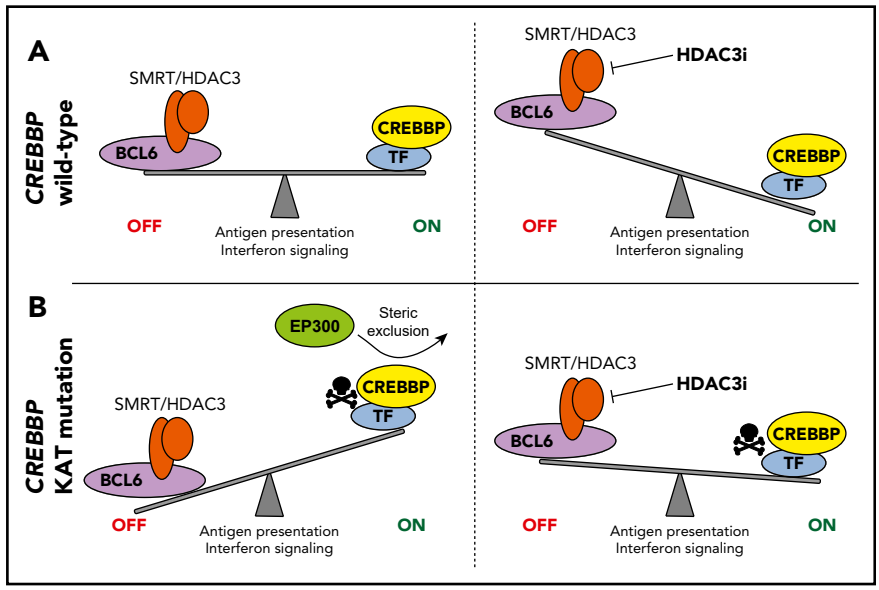

Figure 2. Control of antigen presentation and interferon signaling by CREBBP-mediated antagonism of BCL6/HDAC3. (A) In *CREBBP* wild-type GCB cells, BCL6 regulates the DZ signature by recruiting the corepressor complexes, including SMRT–HDAC3, to repress its target genes. These genes are reactivated in the LZ by CREBBP. Inhibition of HDAC3 in *CREBBP* wild-type B cells leads to increased expression of these genes, including those with a role in antigen presentation and interferon signaling, due to the conserved role of the CREBBP/BCL6–HDAC3 regulatory axis in wild-type cells. (B) KAT domain mutation of *CREBBP* inhibit its catalytic activity and leads to a dominant-repressive effect by preventing the participation of redundant acetyltransferases in transactivation complexes. This leads to loss of antagonism to BCL6-mediated gene repression and reduced expression of antigen presentation and interferon signaling genes. These genes can be restored in *CREBBP* mutant cells by using an HDAC3-selective inhibitor.

and interferon signaling rather than being a prerequisite for its inducibility by HDAC3 inhibition, because of the conserved role of the CREBBP/BCL6–HDAC3 regulatory axis in both contexts[11] (Figure 2). Notably, PD-L1 (*CD274*), a prominent interferon-inducible gene that blunts antitumor T-cell responses, was induced on tumor B cells by HDAC3 inhibition. This prompted investigation of the efficacy of combination HDAC3 inhibitor and PD-L1 blockade using a syngeneic Bcl6-driven lymphoma model. Treatment with HDAC3 inhibitor alone increased tumor-infiltrating Cd4 and Cd8 T cells to an extent similar to that of PD-L1 blockade alone, but their combination synergistically increased tumor-infiltrating T cells and reduced tumor B cells. This suggests that epigenetic modulation of immune response with HDAC3 inhibitors (or EZH2 inhibitors) may be best used in combination with PD1/PD-L1 blockade to prevent interferon-induced adaptive immune suppression.

The functional difference between *CREBBP-R1446C* and KO mutations is consistent with the catalytically inactive mutant CREBBP protein participating in transcriptional coactivating complexes and thereby preventing the participation of redundant acetyltransferases such as EP300.[11] A recent study by Meyer et al[12] showed, using *Crebbp*- and *Ep300*-cKO mice, that, despite having largely nonoverlapping functions with *Crebbp* in GCB cells, *Ep300* becomes critical for GCB cell proliferation in the absence of *Crebbp*. This was also confirmed in *CREBBP* mutant DLBCL cell lines using inducible Cas9 KO of *EP300* and through the use of CREBBP/EP300 inhibitors. Together, this suggests that paralogous lethality to *EP300* inhibition may be an alternative therapeutic approach for *CREBBP* mutant DLBCL.

C5/MCD subtype of DLBCL: getting in on the action

The C5/MCD genetic subtype of DLBCL is a subset of the activated B-cell (ABC)-like transcriptional subtype, with a propensity for extranodal sites of involvement. The defining genetic characteristics of the C5/MCD subtype are mutations of *MYD88* and *CD79B* that drive chronically active B-cell receptor signaling. DNA copy number gains of chromosome 18q are also found in 48% to 73% of the C5/MCD subtype of DLBCL and have been attributed to the *BCL2* oncogene. However, through the analysis of 1000 DLBCL tumors, Jain et al[13] recently identified the *TCF4* (also called *E2-2*) transcription factor gene as a more significant and frequent target of 18q gain[13]. The immunoglobulin heavy chain (IgM) and MYC were identified as important targets that have enhancers bound by TCF4, higher expression in primary ABC-like DLBCL tumors with *TCF4* copy number gains, and increased expression after tetracycline-inducible expression of TCF4 in cell lines. Genetic inhibition of TCF4 function was lethal to ABC-like DLBCL cell lines with *TCF4* DNA copy number gains, highlighting it as an attractive therapeutic target. Importantly, the *TCF4* gene is one of the most highly BRD4-loaded genes in DLBCL,[33] suggesting that BET inhibition may reduce its expression. Prior studies have also shown that ABC-like DLBCL is sensitive to BET inhibition,[34] but the underlying mechanism was not clear. Using a BET protein degrader, TCF4 expression was eliminated in DLBCL cell lines with *TCF4* copy gain, thereby reducing the expression of IgM and MYC and inducing cell death. MYC is also a direct target of BRD4 and can be directly reduced by BET inhibition.[35] However, both cell death and the expression of MYC and IgM were rescued by enforced expression of TCF4 during BET degrader treatment, showing that these phenotypes are at least in part a direct consequence of TCF4 reduction by the BET degrader. Therefore, DNA copy number gains of *TCF4* provide a direct mechanistic rationale for the use of BET inhibitors/degraders in the C5/MCD subtype of DLBCL (Figure 3).

The *TBL1XR1* gene encodes a core component of the SMRT–NCOR complex, and mutations of this gene are found in 20% to 35% of the C5/MCD subtype of DLBCL. The mutations primarily alter amino acids on the surface of the WD40 barrel structure that are predicted to have a role in protein–protein interactions. Venturutti et al[14] recently modeled these mutations using *Tbl1xr1*-cKO mice and mice with conditional knock-in of a *Tbl1xr1* D370Y mutant allele (*Tbl1xr1-D370Y*). Using a GCB-specific Cre allele, homozygous cKO and heterozygous *Tbl1xr1-D370Y* knock-ins were found to have significantly reduced frequencies of GCB cells and smaller GCs after immunization. This is in contrast to most murine lymphoma alleles, which increase or maintain the frequency of GCB cells. Furthermore, this phenotype was not evident with heterozygous cKO mice, suggesting a dominant-negative function for the *Tbl1xr1-D370Y* mutant allele. The *Tbl1xr1-D370Y* GCB cells had gene expression patterns reminiscent of human ABC-like DLBCL, with the upregulation of the ABC-like transcriptional signature and derepression of genes with BCL6-regulated enhancers and evidence of expansion of a pre–memory B-cell population. Further analysis revealed that reduced GCB cell

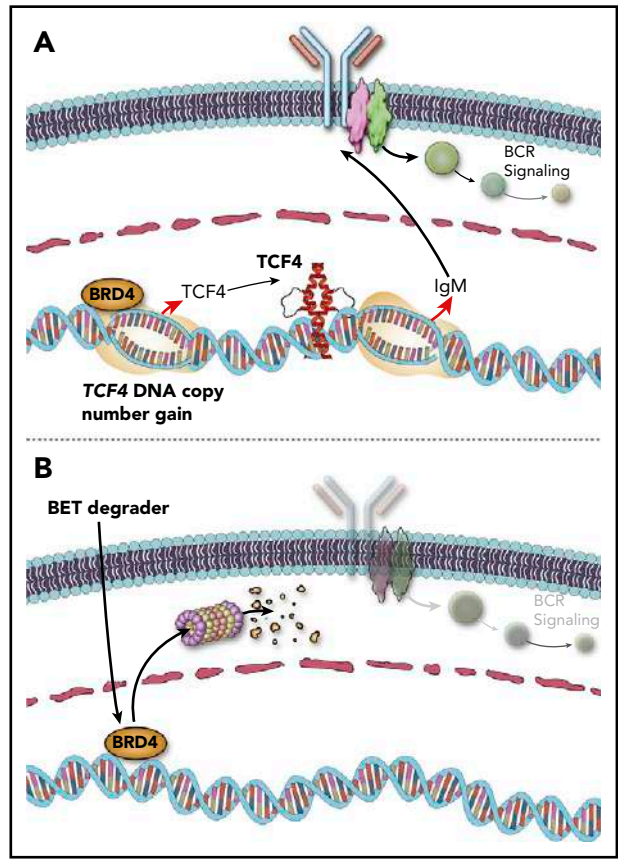

Figure 3. *TCF4* DNA copy number gains drive immunoglobulin expression and can be targeted by BET degraders. (A) DNA copy number gains of chromosome 18q increase the expression of the TCF4 (E2-2) transcription factor, which drives increased expression of IgM. (B) The *TCF4* gene is regulated by BRD4. BET protein degraders such as ARV-771 eliminate BRD4 protein and reduce the expression of *TCF4* and its target genes, including *IgM* and *MYC*.

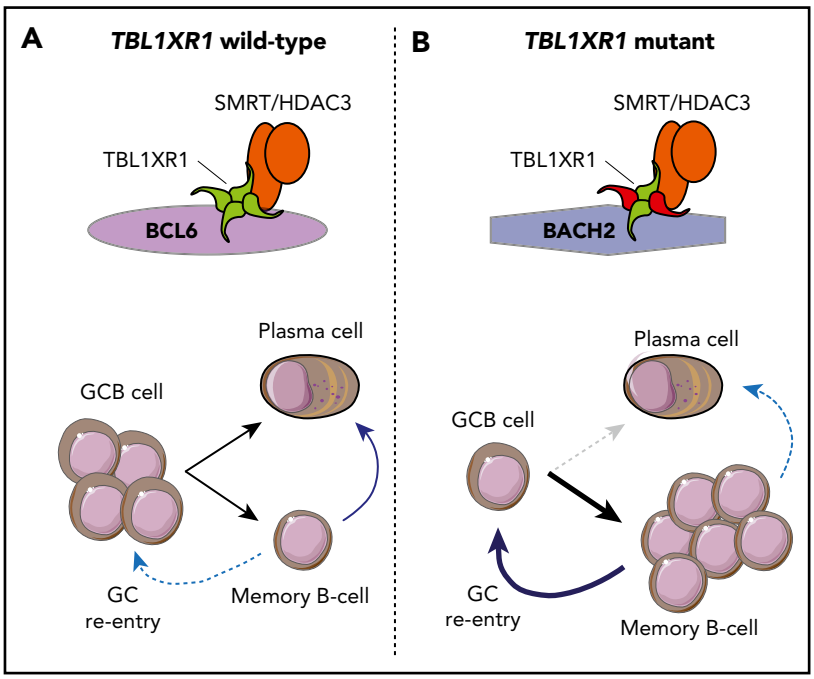

Figure 4. *TBL1XR1* mutation promotes a memory B-cell fate and germinal center reentry. (A) In *TBL1XR1* wild-type GCB cells, the SMRT–HDAC3 complex preferentially associates with BCL6 and facilitates expansion of GCB cells in the DZ of the germinal center. These can terminally differentiate via the LZ into either plasma cells or memory B cells. Upon antigen rechallenge (blue arrows), memory B cells become antibody-secreting plasma cells, and a small subset reenters germinal centers. (B) Mutant TBL1XR1 (red) acts as a dominant negative to increase association between the SMRT–HDAC3 complex and the BACH2 transcription factor and to decrease association with BCL6. This leads to reduced frequencies of GCB cells and promotes a memory B-cell fate. Upon antigen rechallenge (blue arrows), a reduced frequency of *TBL1XR1* mutant memory B cells become plasma cells, and an increased frequency reenter germinal center reactions and undergo additional somatic hypermutation.

frequencies in *Tbl1xr1* mutant mice were a consequence of cell cycle arrest of GCB cells and increased rates of GC exit into the memory B-cell compartment. The *Tbl1xr1* mutant memory B cells had a reduced frequency of class switching, leading to an IgM-positive bias, which persisted in the periphery over time, and had a significantly higher rate of GC reentry after antigenic re-challenge than wild-type B cells. When crossed with a *Bcl2* allele and serially challenged with antigen, *Tbl1xr1*-cKO mice develop premalignant lesions within extranodal sites, an increased rate of tumor development, and a more immunoblastic appearance compared with mice with the *Bcl2* allele alone. Mechanistically, the skew toward a memory B-cell phenotype resulted from a loss of interaction between mutant *TBL1XR1* and BCL6 and a concomitant gain of interaction with the BACH2 transcription factor. This resulted in a preferential association of the SMRT–HDAC3 complex with BACH2, an important regulator of memory B-cell development that recruits the SMRT–HDAC3 complex to silence genes involved in plasma cell differentiation, such as *PRDM1*[36] (Figure 4). The dependence of this process on the SMRT–HDAC3 complex strongly suggests that HDAC3-selective inhibitors may have a potential role in *TBL1XR1* mutant DLBCL.

Concluding remarks

There are still a large number of genetically altered transcriptional and epigenetic regulators that remain to be functionally explored, further details to be discovered regarding the function of previously investigated genes, and an additional level of complexity when considering combinations of genetic alterations that co-occur within the same tumor. Furthermore, epigenetic programs that are important for B-cell development and survival are controlled by epigenetic regulators, signaling pathways, and metabolic programs that are not directly targeted by genetic alterations but could nonetheless serve as therapeutic targets. Therefore, understanding how best to take the increasing yield of epigenetic-modifying agents[7,37] and rationally deploy them in a prioritized fashion will require a detailed, descriptive, and functional characterization of the epigenomic architecture of B-cell lymphoma in a manner similar to the approach taken for the coding genome.

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Conflict-of-interest disclosure

M.R.G. is a consultant for VeraStem Oncology and has stock ownership interest in KDAc Therapeutics.

Off-label drug use

None disclosed.

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Novel targets in aggressive lymphoma

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Targeting CD20 with the monoclonal antibody rituximab has improved survival in patients with aggressive B-cell lymphomas, the majority of which are cured with chemoimmunotherapy. Patients progressing through or relapsing after their treatment have a poor prognosis. Despite a number of promising novel agents with efficacy in relapsed disease, randomized trials building on the chemoimmunotherapy backbone have failed to show further survival benefit. Significant progress has been made in the last few years in relapsed or refractory disease with the emergence of therapies that harness the patient's immune system to fight disease. The approval of 2 chimeric antigen receptor T-cell products has provided potential for curative therapy, although challenges remain with toxicities and access. The approval of the antibody drug conjugate polatuzumab in combination with chemoimmunotherapy has offered survival benefit to patients who are not candidates for more aggressive approaches and has the potential to change the standard of care for initial management. Several targeted agents have proven effective, but the majority do not produce durable responses, requiring development in combination with other targeted or conventional therapies. Herein, promising targets in aggressive lymphoma with the greatest potential for improving outcomes in these patients are discussed. Novel therapies, their toxicities, and their potential role in initial or subsequent treatment are highlighted.

LEARNING OBJECTIVES

- Identify promising therapeutic targets in aggressive B-cell lymphomas and the different strategies to develop treatments directed at these targets
- Recognize emerging therapies and discuss results of the most promising clinical trials evaluating these therapies
- Understand further development of these therapies as single agents or in combination in the relapsed and frontline setting

Clinical case

A 55-year-old man presented with a 4-week history of left cervical lymphadenopathy (LN), drenching night sweats, and a 12-pound unintentional weight loss. Excisional LN biopsy confirmed an aggressive B-cell lymphoma. Biopsy showed diffuse infiltrate of medium to large atypical lymphocytes. Neoplastic cells were positive by immunohistochemistry for CD10, BCL6 (>70%), BCL2 (>90%), c-MYC (>90%), MUM1 (90%), and Ki67 ~70%. Fluorescence in situ hybridization was negative for rearrangements in *BCL2*, *BCL6*, and *MYC*. The lymphoma was classified as diffuse large B-cell lymphoma (DLBCL), not otherwise specified with a nongerminal center (non-GC) B-cell phenotype by Hans algorithm with coexpression of BCL2 and c-MYC, called double expressor lymphoma. Positron emission tomography/computed tomography (PET/CT) was remarkable for hypermetabolic lymphadenopathy above and below the diaphragm, with multiple hypermetabolic splenic and osseous lesions. He received 6 cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). His interim and end-of-therapy PET/CT were consistent with a complete metabolic response, Deauville 2. He noted enlarging right cervical LN just before 3-month follow-up, and biopsy confirmed relapsed disease. He enrolled in a clinical trial randomly assigning patients to standard of care (SOC) with salvage chemotherapy followed by autologous stem cell transplant (ASCT) versus an autologous anti-CD19 chimeric antigen receptor (CAR) T-cell therapy. He was randomly assigned to SOC and achieved a complete remission (CR) after 2 cycles of rituximab, ifosfamide, etoposide, and carboplatin. He received carmustine, etoposide, cytarabine, and melphalan conditioning and proceeded to ASCT with day +30 PET/CT showing complete metabolic response. Unfortunately, his day +100 PET/CT showed disease progression. He was initiated on ibrutinib bridging therapy, achieving a rapid CR, but he quickly progressed. He received his CAR-T cells without cytokine release syndrome (CRS) or neurotoxicity; however, his day +60 PET/CT showed progression. He

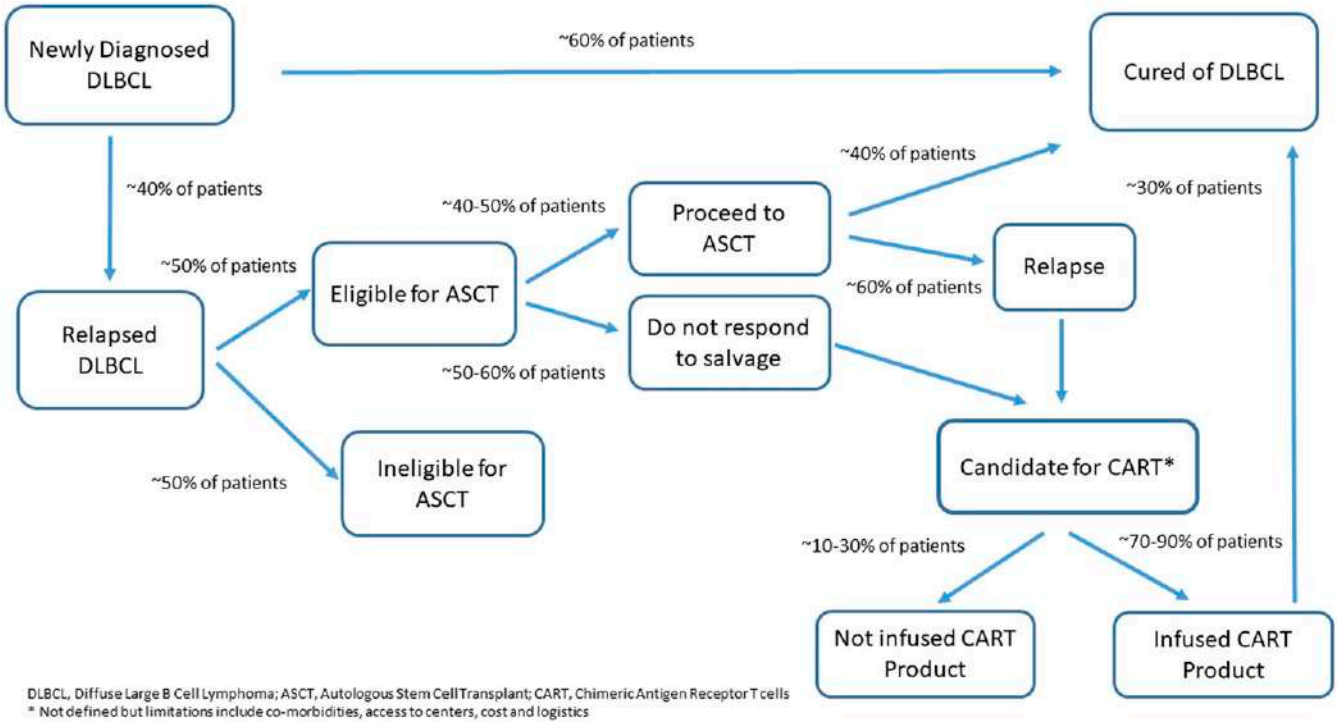

Figure 1. DLBCL. *Not defined, but limitations include comorbidities, access to centers, cost, and logistics.

enrolled in a clinical trial evaluating combination lenalidomide and nivolumab.

Introduction

The addition of the anti-CD20 antibody rituximab to CHOP results in a cure for ~60% of patients and, despite multiple trials, represents the last treatment breakthrough for untreated DLBCL.[1] Outcomes are driven by biological heterogeneity including distinct cell of origin (COO),[2] molecular clusters,[3,4] translocations of *MYC*, *BCL2*, or *BCL6* (high-grade B-cell lymphoma), and BCL2 and c-MYC protein overexpression without translocation.[5] For patients who relapse, there is curative potential with intensive treatment including ASCT, but this occurs in a minority of patients, with outcomes significantly worse for patients receiving previous rituximab-based therapy or progressing within 1 year of initial therapy.[6] CAR-T therapy targeting CD19 has shown promising results in relapsed or refractory (r/r) aggressive B-cell non-Hodgkin lymphoma (NHL), improving outcomes for patients not responding to salvage or relapsing after ASCT, where median overall survival (OS) is 6 months.[7] Two second-generation CAR-T therapies (axicabtagene ciloleucel and tisagenlecleucel) are approved, with overall response rates (ORRs) of 52% to 83% and 40% to 58% CR.[8,9] Despite impressive response rates, the majority of patients progress, and treatment is associated with significant toxicities including CRS and neurotoxicity (Figure 1). Accessibility to CAR-T centers, central manufacturing of cells, time to infusion, and financial burden remain challenges to broad access.

Improving frontline treatment for high-risk patients and identifying effective therapies for patients not candidates for or progressing after ASCT or CAR-T are of great importance. Herein, I highlight select recent and ongoing therapeutic approaches with promise to improve outcomes in r/r DLBCL.

Immunotherapy

Targeting CD19

The B-lymphocyte antigen C19 is expressed throughout B-cell development until terminal plasma cell differentiation, with high expression on most malignant B cells.[10] Expression is preserved throughout lymphoma treatment, making CD19 an ideal target.

Tafasitamab is a novel Fc-engineered, humanized, CD19 monoclonal antibody. A phase 2a trial of single-agent tafasitamab included 35 patients with r/r DLBCL with a 26% ORR. The median duration of response (DOR) for 9 responders was 20 months, including 5 patients with responses ≥12 months.[11] A phase 2 study evaluating tafasitamab and lenalidomide in 80 patients with r/r DLBCL considered ineligible for ASCT reported an impressive 60% ORR with 43% CR, median DOR 21.7 months with median follow-up 17.3 months, and median progression-free survival (PFS) 12.1 months.[12] Responses occurred irrespective of COO, with activity seen in patients with both GC and non-GC DLBCL and in patients with poor prognostic features including similar responses seen in patients with or without refractory disease. With an additional year of follow-up, the median DOR was 34.6 months, confirming durability of this immunologic combination.[13] The most frequent toxicities were hematologic toxicity, diarrhea, and fatigue. The US Food and Drug Administration (FDA) granted accelerated approval in late July 2019 for this combination in r/r DLBCL. Tafasitamab is being evaluated in a randomized phase 2/3 study in combination with bendamustine compared with bendamustine + rituximab (BR) in r/r DLBCL (NCT02763319) and in a frontline phase 1 study in combination with R-CHOP and R-CHOP + lenalidomide (NCT04134936).

Antibody–drug conjugates (ADCs) carry a cytotoxic payload directed against tumor-associated antigens in an effort to maximize efficacy while limiting off-target toxicity. Loncastuximab

Table 1. Select ongoing novel chimeric antigen receptor T-cell trials

Target	Phase	Identifier	Additional agents
CD19/CD20	1	NCT04215016 NCT04007029	
CD19/CD22	1	NCT03233854	
CD19/CD22	1/2	NCT03287817	Followed by PD-1 antibody pembrolizumab
CD19	1	NCT02706405	Followed by PD-1 antibody durvalumab
CD19	1/2	NCT04257578	BTK inhibitor acalabrutinib prior
CD20	1/2	NCT03277729	
CD22	1	NCT04088890	

BTK, Bruton's tyrosine kinase inhibitor; PD-1, programmed death 1.

tesirine (lonca) is a humanized anti-CD19 antibody conjugated to a pyrrolobenzodiazepine dimer. A phase 1 study included 61 patients with r/r DLBCL with a 49% ORR, 32% CR, and median DOR, PFS, and OS of 4.8, 2.9, and 10.9 months, respectively.[14] There was no maximum tolerated dose (MTD) in this trial; the most common toxicities were hematologic toxicity, fatigue, edema, liver test abnormalities, nausea, rash, and dyspnea, generally reversible and manageable with dosage delays or reductions. A phase 2 study of 145 patients with r/r aggressive B-NHL reported a 45.5% ORR and 20% CR, including activity in patients with refractory disease and a small number of patients with high grade B-cell lymphoma.[15] Lonca is being investigated in combination with ibrutinib (NCT03684694) and durvalumab (NCT03685344) with a randomized phase 3 trial of rituximab + lonca versus gemcitabine and oxaliplatin (NCT04384484) planned. A phase 2 study of the ADC coltuximab ravtansine, conjugated to a cytotoxic maytansinoid DM4, showed similar responses but is not being further developed in DLBCL.[16]

Bispecific T-cell engagers bring together T cells and tumor cells to trigger T-cell cytotoxicity and cytokine production when both binding sites are occupied. Blinatumomab, a bispecific T-cell engager targeting CD19 and CD3, was evaluated in a phase 1 study in r/r NHL with a 55% ORR in 14 patients with DLBCL.[17] A phase 2 study in 25 patients with r/r DLBCL demonstrated a 43% ORR, 19% CR, median DOR 11.6 months, and median PFS 3.7 months at a median follow-up of 15 months. Stepped-up dosing was required for tolerability, which limited activity for patients with aggressive tumors. Although serious neurologic toxicity occurred in the first 2 patients treated with flat-rate dosing at the target dosage, even with stepped-up dosing, 22% grade 3 neurologic toxicity occurred. Optimization of the dosing schedule and toxicity has limited development in DLBCL.

Adoptive cellular therapy using CAR-T cells has had a significant impact on our ability to treat r/r aggressive lymphomas. Although axicabtagene ciloleucel and tisagenlecleucel are highly active, a significant number of patients receiving these therapies progress, and their broad use has been limited by toxicity, with 13% to 22% grade ≥3 CRS and 12% to 28% grade ≥3 neurologic dysfunction.[8,9] A third product under priority review by the FDA, lisocabtagene maraleucel,[18] reported a 73% ORR and 55% CR with 1% grade ≥3 CRS and 15% grade ≥3 neurologic toxicity. The encouraging activity for high-risk patients has led to investigation of CAR-T earlier in the course of treatment, including at first relapse in both transplant-eligible and non–transplant-eligible patients. A plethora of ongoing studies are being conducted with CAR-T, including novel constructs and rational combinations (Table 1), with ongoing efforts aimed at improving durable remissions and decreasing toxicity. CAR T-cell exhaustion and immune evasion, CD19 antigen loss, and lack of persistence are all potential mechanisms of resistance. The development of CARs targeting multiple antigens, or bispecific CARs, are under investigation with both CD19/CD22 and CD19/CD20 bispecific CARs.[19,20] "Armored" CAR constructs have additional genetic modifications designed to secrete cytokines or express ligands that enhance or interact with endogenous immune cells.[21]

One of the challenges moving forward will be sequencing of CD19-directed therapies, because it remains to be seen whether efficacy of these agents will be hindered by previous use of others.

CD20 bispecific antibodies

Though initially promising, newer anti-CD20 monoclonal antibodies combined with CHOP have failed to show improvement over rituximab. Bispecific antibodies combine the specificity of two antibodies to simultaneously bind to different antigens, an antigen on the cancer cell and T-cell. Bispecific antibodies targeting CD20 and CD3 are in development, with mosunetuzumab and REGN1970 reporting promising activity. In a phase 1/2 study (NCT03677154), mosunetuzumab produced a 37.1% ORR with 19.4% CR in 124 patients with aggressive B-cell lymphoma with 1.1% grade ≥3 CRS and 3.7% neurotoxicity.[22] A phase 1 study of REGN1970 (NCT03888105) produced a 33.3% ORR and 17.8% CR, with no DLTs observed and 7.4% grade ≥3 CRS and no neurologic toxicity.[23] Both of these agents induced responses in patients progressing after CAR-T, which has been a difficult-to-treat population.

Immune checkpoint inhibition

Targeting programmed death 1 (PD-1) and programmed death-ligand 1 has proven disappointing in r/r DLBCL, with low ORRs to single-agent therapy.[24] Checkpoint inhibition of the innate immune system through antibodies to CD47 has shown promise. CD47 is the dominant macrophage checkpoint overexpressed on most cancers, acting as a "do not eat me" signal that enables macrophage immune evasion. Magrolimab is a humanized, anti-CD47 monoclonal antibody that induces macrophage phagocytosis of cancer cells by blocking the "do not eat me" signal. A phase 1b/2 study of magrolimab and rituximab (NCT02953509)

Table 2. Select ongoing clinical trials with novel combinations

Agent	Combination	Phase	Line of therapy	Identifier
Polatuzumab	Rituximab and lenalidomide	1	Relapsed	NCT02600897
Polatuzumab	Obinutuzumab, rituximab, venetoclax	1	Relapsed	NCT02611323
Polatuzumab	R-EPOCH	1	Frontline	NCT04231877
Polatuzumab	± RGemOx	3	Relapsed	NCT04182204
Polatuzumab	+ R-CHOP vs + R-CHP	3	Frontline	NCT04332822
Venetoclax	Obinutuzumab, lenalidomide, ibrutinib, prednisone	1	Relapsed	NCT03223610
Venetoclax	Rituximab and ibrutinib	1	Relapsed	NCT03136497
Venetoclax	Obinutuzumab and lenalidomide	1	Relapsed	NCT02992522
Venetoclax	Obinutuzumab	2	Relapsed	NCT02987400
Venetoclax	Rituximab and bortezomib	2	Relapsed	NCT02987400
Venetoclax	Rituximab and idasanutlin	1/2	Relapsed	NCT03135262
Venetoclax	RICE	1/2	Relapsed	NCT03064867
Selinexor	RICE	1	Relapsed	NCT02471911
Selinexor	RGDP or RDHAOx	1b	Relapsed	NCT02741388
Selinexor	Ibrutinib	1	Relapsed	NCT02303392
Selinexor	Venetoclax	1	Relapsed	NCT03955783
Selinexor	R-CHOP	1b	Frontline	NCT031478850

R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; R-CHP, rituximab, cyclophosphamide, doxorubicin, and prednisone; RDHAOx, rituximab dexamethasone, cytarabine, and oxaliplatin; R-EPOCH, rituximab, etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin; RGDP, rituximab, gemcitabine, dexamethasone, and cisplatin; RGemOx, rituximab, gemcitabine, and oxaliplatin; RICE, rituximab, ifosfamide, etoposide, and carboplatin.

included 59 r/r DLBCL with 36% ORR, 15% CR, and median DOR not reached (NR).[25] Therapy was well tolerated, with no MTD reached; the most common adverse events were on-target anemia and infusion reactions, with opportunities to safely incorporate this therapy into combination approaches.

Targeting CD79b

The ADC polatuzumab (Pola) targets CD79b, a component of the B-cell receptor (BCR). Pola was approved in combination with BR in r/r DLBCL based on a randomized phase 2 trial showing significant improvements in end-of-treatment ORR and CR compared with BR, with a median DOR, PFS, and OS of 12.6, 9.5, and 12.4 months, respectively.[26] Efficacy was seen across all risk groups, with activity independent of COO and patients benefiting regardless of refractory disease or number of previous lines of therapy. The addition of pola resulted in higher rates of grade 3-4 neutropenia without higher rates of infection and 44% grade 1-2 peripheral neuropathy with improvement or resolution in most. Other novel combinations are being investigated (Table 2). A phase 3 frontline trial of 875 patients evaluating R-CHOP versus R-CHOP + polatuzumab (NCT03274492) recently completed accrual, with potential to change the SOC for frontline treatment.

BCR signaling pathway

Targeting BCR signaling with oral kinase inhibitors has changed the treatment landscape in several B-cell lymphomas; however, these therapies have modest activity in limited subsets of patients with DLBCL. The highest single-agent activity is reported with ibrutinib, which is active and well tolerated in non-GC DLBCL but with short DOR.[27] Multiple combinations have been evaluated, the most promising being rituximab, ibrutinib, and lenalidomide (IR^2). A phase 1b trial of the combination reported an ORR of 65%, with 41% CR in 23 patients with non-GC DLBCL with no MTD reached.[28] The phase 2 enrolled 89 patients with non-GC DLBCL at 2 dose levels (n = 55 and n = 34, lenalidomide 20 mg and 25 mg).[29] The ORR was 47%, with 28% CR, with median DOR, PFS, and OS of 18, 5, and 14 months, respectively. Median DOR was NR in patients achieving CR, and median PFS and OS in responders were 21 months and NR, respectively. A single-arm phase 2 study of untreated patients evaluating 2 cycles of IR^2 followed by 6 cycles of IR^2 + CHOP/etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin (EPOCH) in non-GC DLBCL enrolled 60 high-risk patients.[30] The ORR and CR were 84.6% and 38.5%, respectively, after 2 cycles of IR^2, increasing to 98% and 92.3% after 6 additional cycles of IR^2 + chemotherapy. The 1-year PFS and OS were 92.5% and 96.5%, respectively. Longer follow-up is needed, but this trial is the first to show that "chemo-free" combinations are active in untreated DLBCL and may have a role in frontline therapy with or without combination chemotherapy.

Selective inhibitor of nuclear export

Selinexor is a first-in-class selective oral inhibitor of XPO1. Inhibition of XPO1 leads to nuclear accumulation and reactivation of tumor suppressor proteins such as p53 and p21 and reduction in oncoproteins including c-MYC, BCL2, and BCL6. A phase 1 study reported a 25.6% ORR in r/r DLBCL, with 4 patients achieving CR, 2 lasting ≥1 year. The most common gastrointestinal and constitutional adverse events (nausea, vomiting, anorexia, fatigue) were managed with antiemetics and appetite stimulators and

grade 3-4 hematologic toxicity with dose delays or reductions.[31] A multicenter phase 2 study in r/r DLBCL enrolled 129 patients with a 27.6% ORR, 10% CR, and median DOR of 9.2 months.[32] Activity was seen in both GC (ORR 34%, CR 14%) and non-GC (ORR 21%, CR 10%) subtypes, and responses were consistent across risk groups. Patients were required to have a 60-day washout from previous therapy, suggesting some selection for less proliferative or aggressive disease. The FDA has granted priority review for selinexor for r/r DLBCL. A number of ongoing studies are evaluating selinexor combinations (Table 2).

BCL2 inhibition

BCL2 is a pro-apoptotic protein overexpressed in ~30% of DLBCL.[33] BCL2 overexpression correlates with resistance to R-CHOP and is associated with overall worse prognosis, with dismal outcomes when c-MYC is concomitantly overexpressed (double-expresser [DE] or double-hit [DH] lymphomas).[34,35] Venetoclax (ven) is a highly selective BCL2 inhibitor with a potential role in overcoming resistance to chemotherapy. A phase 1 study including 34 patients with r/r DLBCL showed ven to be well tolerated, with an 18% ORR and 12% CR, with potential for rational combination therapy.[36] A phase 1b/2 study in combination with R-CHOP established the recommended phase 2 dose with no MTD reached.[37] Of the 8 patients with DE lymphoma, 7 achieved a CR. The phase 2 portion enrolled 211 patients, showing favorable PFS for patients with immunohistochemistry + BCL2 compared with historical controls.[38] A phase 1 study in aggressive B-cell lymphomas in combination with dose-adjusted rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin (R-EPOCH) reported a 93% ORR with 87% CR in DH lymphoma, with 10/13 patients with DH lymphoma maintaining CR at 11 months and 2/2 patients with DE lymphoma maintaining CR at 27 and 29 months.[39] An ongoing randomized frontline phase 2/3 trial (NCT03984448) is evaluating the addition of ven to R-EPOCH in patients with DH lymphoma and R-CHOP in patients with DE lymphoma to improve outcomes in these patients with a poor prognosis. Several combinations in relapsed disease are being evaluated (Table 2).

Clinical case

The patient in the clinical case presented with non-GC double expressor lymphoma, which is associated with inferior prognosis if treated with R-CHOP. An ongoing randomized phase 2/3 study is evaluating whether R-CHOP + ven is able to improve outcomes for these patients by overcoming chemotherapy resistance. Patients with primary refractory DLBCL are often resistant to further chemotherapy; the role of CAR-T in lieu of SOC salvage and ASCT (NCT03391466) is under investigation. Although he did achieve a CR to SOC and proceeded to ASCT, he relapsed shortly after, where median OS is <6 months. He responded to ibrutinib, which is active in non-GC DLBCL, but responses are not durable. He proceed to CAR-T without concerning toxicity; however, he progressed soon after, representing a population of patients where clinical trials are priority. He enrolled in a trial with nivolumab and lenalidomide, which have potential activity after chimeric antigen receptor T cell therapy. He is responding to treatment and has an allogeneic donor.

Concluding remarks

SOC for DLBCL has remained the same for nearly 20 years, and improving upon R-CHOP will probably require targeted therapy active across COOs or novel trial designs incorporating targeted therapies for select patients. Remarkable progress has been made in treating r/r aggressive lymphomas with the approval of CAR-T, and efforts to improve the efficacy, safety, and accessibility of these therapies is ongoing. Targeting CD19 with other immunotherapies is attractive, with additional agents showing promising responses and favorable toxicity profiles. A number of novel therapies are active but have limited single-agent activity, and durable responses will require combination therapy. Novel combinations targeting biologic drivers of disease are of high interest.

Conflict-of-interest disclosure

K.M. has received research funding from Pharmacyclics, BMS, Merck, and Novartis. K.M. has consulted for Pharmacyclics, Janssen, Morphosys, Celgene, Karyopharm, and Seattle Genetics.

Off-label drug use

None disclosed.

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Special pre- and posttransplant considerations in inherited bone marrow failure and hematopoietic malignancy predisposition syndromes

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Advances in the diagnosis and treatment of inherited bone marrow failure syndromes (IBMFS) have provided insight into the complexity of these diseases. The diseases are heterogeneous and characterized by developmental abnormalities, progressive marrow failure, and predisposition to cancer. A correct diagnosis allows for appropriate treatment, genetic counseling, and cancer surveillance. The common IBMFSs are Fanconi anemia, dyskeratosis congenita, and Diamond-Blackfan anemia. Hematopoietic cell transplantation (HCT) offers curative treatment of the hematologic complications of IBMFS. Because of the systemic nature of these diseases, transplant strategies are modified to decrease immediate and late toxicities. HCT from HLA-matched related or unrelated donors offers excellent survival for young patients in aplasia. Challenges include the treatment of adults with marrow aplasia, presentation with myeloid malignancy regardless of age, and early detection or treatment of cancer. In this article, I will describe our approach and evaluation of patients transplanted with IBMFS and review most frequent complications before and after transplant.

LEARNING OBJECTIVES

- Examine the effect of age, disease and disease status at transplant, prior treatments including chronic transfusion and other disease-specific complications on transplant outcomes
- An understanding of the consequences of transplant strategies as it pertains to the underlying disease and how these potentially add to the burden of morbidity and mortality associated with transplantation

Introduction

Although inherited bone marrow failure syndromes (IBMFS) are typically diagnosed in childhood or adolescence, an increasing number of patients may present to adult hematologists with atypical presentations. Careful consideration should be given to the patient's history and physical findings, and family history needs to be investigated to detect other relatives with IBMFS, hematologic malignancies, solid tumors, and pulmonary or hepatic complications.[1,2] Patients with IBMFS should undergo extensive clinical and laboratory evaluations before and after hematopoietic cell transplantation (HCT; Tables 1 and 2). A comprehensive review of systems involved in these syndromes was recently published by Alter in 2017.[1] Clinical manifestations are heterogenous and have variable penetrance within affected members of the same family, and screening of family members is essential to exclude them as potential donors.[1-4] (Figure 1) As IBMFS can impact organs other than the bone marrow, the care of these patients requires a multidisciplinary team. HCT is the treatment of choice for most patients with bone marrow failure and preferably with radiation-free reduced intensity conditioning (RIC) regimens.[5-7] Bone marrow is the preferred graft in T-replete transplants, because peripheral blood is associated with higher risk for graft-versus-host disease (GVHD) and second malignancy.[5,8,9]

Fanconi anemia

Clinical case 1

Patient 1 is a 14-year-old girl, and she presented with a history of mild anemia since the age of 5 treated with supportive care. Over the past months, her mother noticed progressive asthenia, petechiae, and oral bleeding. Physical examination was remarkable for short stature and 2 café au lait spots. Complete blood counts showed severe pancytopenia, and

she received red blood cell and platelet transfusions. A bone marrow aspirate and biopsy confirmed severe aplastic anemia. She was worked-up for Fanconi anemia (FA) at a tertiary referral center, and the diagnosis was confirmed with a positive diepoxybutane chromosome breakage test.

FA, predominantly an autosomal recessive disease, is characterized by genomic instability, progressive bone marrow failure, increased frequency of birth defects, and a striking predisposition for myeloid leukemias and head and neck squamous cell carcinoma (SCC).[3,6,9] HCT offers cure for the hematologic complications of FA and is indicated before onset of chronic transfusions, myelodysplasia (MDS), or acute myeloid leukemia (AML).[6,8,10] Recent studies have demonstrated an overall survival (OS) of 80% to 90% for young patients transplanted for aplasia from matched related (MRD) or matched unrelated donors (MUD).[6,11,12] For MRD transplantation, low-dose cyclophosphamide (CY) 20 to 40 mg/kg and fludarabine with or without antithymocyte globulin (ATG) is the most common regimen,[6,8,12] and irradiation is not necessary.[13] Our group has transplanted 91 FA patients in aplastic phase with MRD and CY 60 mg/kg with or without ATG; with a median follow-up of 7 years, 95% of patients were alive.[14] Outcomes after HCT for patients with FA transplanted in aplastic phase from MRD have improved dramatically over the last 30 years, and major long-term complications are now mainly related to endocrinologic problems and the development of cancer.[1,5,6,15]

Should we give androgens before HCT while waiting for an alternative donor?

We recommend androgens at the lowest possible dose (danazol, 2-5 mg/kg) while waiting for an unrelated donor. For patients in aplastic phase, androgen therapy (oxymetholone/danazol) leads to hematologic responses in up to 80% of patients.[16-18] Patients who respond to androgens may remain stable for years, enabling advances in donor selection and transplantation procedures.[6,19] In our daily practice, the decision to transplant a patient who has achieved a good hematologic response is never easy and usually depends on whether a well-matched alternative donor is available. Some patients or families may opt for continuing the use of androgens and delaying the transplant when only mismatched donors are available because of the higher risk related to these transplants. However, it is important to emphasize that androgens do not prevent the development of clonal evolution, the response may be transitory, patients may be older, and the donor may not be available when transplantation is needed. Side effects are also considerable, including dyslipidemia, abnormal liver function, liver adenomas, accelerated growth, and virilization, especially in young girls.[16-18] Regular assessment with ultrasonography, liver function, and lipid metabolism are necessary before transplant.[3,4,16-18]

What are the results and specific problems related to HCT in FA patients?

Patient 1 did not have a suitably matched related donor, and while awaiting an unrelated donor search, she received androgens and achieved a partial response. A matched unrelated donor was not found, and she developed signs of virilization and elevated liver enzymes. She was then considered for a mismatched unrelated donor transplantation or a haploidentical transplant.

The use of alternative donors has been historically associated with a lower survival because of high incidence of rejection and regimen-related toxicity, as well as GVHD.[6,8,10,11] Pivotal studies were able to identify changes that would enhance engraftment, decrease transplant-related mortality, and improve survival.[10] These factors included the incorporation of fludarabine in preparatory regimens, the use of in vivo or in vitro T-cell depletion, and better HLA typing and supportive care.[8,11,20] Young patients transplanted in aplastic phase from MUD receiving nonirradiation regimens have a survival that is now comparable to matched sibling donor (MSD) transplantation.[6,7,11]

For patients without a MRD or MUD, in vitro or in vivo T-cell depletion of grafts from a mismatched donor is an attractive option. In vitro T-cell depletion of the graft is effective in eliminating severe acute and chronic GVHD; however, this requires techniques that are not widely available.[10,11] Using haploidentical donors with the posttransplant cyclophosphamide platform is associated with a 2-year OS of 80% to 90%, but GVHD is higher, and longer follow-up is still needed to better identify late outcomes.[20,21] The use of cord blood transplantation has been associated with decreased rates of neutrophil engraftment and higher mortality, but using a fludarabine-based RIC regimen and selecting a unit with high cell dose and no more than one mismatch may help to overcome these complications.[10,22] In places where it is available and when feasible, families with known gene mutations have an option to go for in vitro fertilization with preimplantation genetic diagnosis to select unaffected HLA matched embryos and subsequent cord blood transplantation for their child with FA. However, this is very

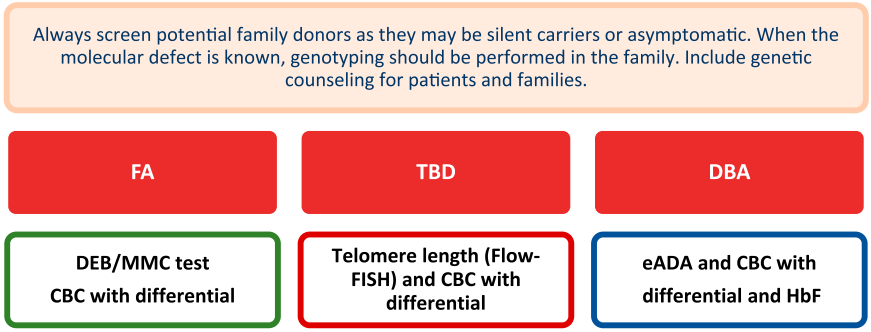

Figure 1. Screening of potential family donors. For a detailed discussion about diagnosis and genetic testing for IBMF and hematopoietic malignancy syndromes, the readers should refer to Furutani and Shimamura.[2] DEB, diepoxybutane chromosome breakage test; Flow-FISH, flow cytometry with fluorescent in situ hybridization. CBC, complete blood counts with reticulocytes; HbF, fetal hemoglobin.

expensive and can be physically and emotionally demanding for many families.[22,23]

Clinical case 1 continued

Because of the lack of complete response and unacceptable side effects related to androgens, patient 1 received a haploidentical HCT from her unaffected brother with fludarabine, ATG, and total body irradiation 200 cGy conditioning, followed by GVHD prophylaxis with posttransplant cyclophosphamide (total dose, 50 mg/kg), cyclosporine, and intravenous mycophenolate mofetil.[8] Posttransplant course was complicated by grade III mucositis, cytomegalovirus reactivation, hemorrhagic cystitis, and mild oral chronic GVHD. At the age of 21, she became pregnant and delivered a healthy baby.

Age is an important predictor for survival after HCT. In a study of 199 adults with FA, 46% had clonal disease, and the 3-year OS for the whole group was 36%.[24] Mortality secondary to transplant-associated complications was high (51%) and primarily caused by infections and GVHD. In the subset transplanted after 2000 with MRD, 3-year survival was 84%. Second malignancy remains an important complication, and transition from pediatric to adult care is challenging with many patients lost to follow-up in adulthood.[24]

Treatment of patients with MDS and AML can be very challenging.[6,10,24,25] As expected, those transplanted in remission have a better survival, but the risk of giving pretransplant chemotherapy is high and may lead to excessive toxicity and prolonged aplasia. A sequential approach using a FLAG regimen followed by RIC seems promising, but the numbers are still small to draw any conclusions.[25] Recently, the European Society for Blood and Marrow Transplantation group reviewed the data on 74 patients, most transplanted with MDS (n = 35) or had leukemia (n = 35). The 5-year OS was 42% with a relapse rate of 40% at 5 years.[26]

Long-term follow-up is essential to detect complications related to the disease or treatment. Endocrine abnormalities are the most frequent problems and may include growth hormone deficiency, thyroid dysfunction, dyslipidemia, hypogonadism, glucose intolerance, insulin resistance, or diabetes.[5,15,27] All males are infertile, and although fertility is low in females, successful pregnancies have been reported as described in our clinical case.[5,15] FA patients continue to be at risk for developing

Table 1. Pretransplant evaluation of patients with IBMFS

• Complete family and patient's history with careful evaluation of physical findings according to disease type.		
• Multidisciplinary team: Visual, hearing, endocrine, nutritional, and neuropsychologic evaluation in all patients. Oral examination performed by a dentist. Detailed skin examination in FA and TBD. Other evaluations as needed (such as gastrointeistinal endoscopy or nasolaryngoscopy screening)		
• Hematologic evaluation: disease phase (single or multilineage cytopenias, MDS, AML). CBC, fetal hemoglobin, eADA (Diamond-Blackfan anemia), α-fetoprotein, bone marrow aspirate, biopsy, cytogenetics and flow cytometry. FISH for chromosomes 3 and 7 in FA.		
• Previous transfusions (iron overload): Check number of transfusions, chelation history and ferritin levels. Consider T2* MRI to determine liver/heart iron overload. Check for alloimmunization and presence of DSA.		
• Prior use of androgens: describe type, duration and dose. Check for signs of virilization, growth problems, and liver dysfunction. Check abdominal ultrasound, liver function, lipid metabolism, and bone age.		
• Prior use of steroids: describe type, duration, and dose. Check for signs of Cushing's syndrome; hyperglycemia, hypertension, metabolic syndrome, avascular necrosis, and adrenal insufficiency.		
• For adults: special attention to genitourinary and gynecologic issues. Address fertility and options available for cryopreservation before HCT. Aggressive cancer screening is very important in this population.		
• Lifestyle evaluation: Recommend complete abstinence from smoking and alcohol especially for FA and TBD, good oral hygiene, sunscreen use, healthy diet, and exercise.		
Disease-specific pretransplant evaluation		
FA	DC	DBA
Address congenital abnormalities (head, heart, skeletal, genitourinary, and gastrointestinal).	Address congenital abnormalities. Brain MRI is indicated in HH and Revesz and/or development delay	Address congenital abnormalities
Brain MRI is indicated for children with multiple birth defects (pituitary gland evaluation)	Immune abnormalities are common in early-onset cases.	Prolonged use of steroids may cause pathologic fractures, avascular necrosis, cataracts, growth retardation, hypertension, and diabetes
	Avascular necrosis of the hips	
Attention to endocrine problems such as short stature, thyroid function, glucose, gonadal function, and lipid metabolism	Attention to PFTs and SpO_2 before HCT. Chest CT scan when indicated.	Iron overload is the major complication leading to heart and liver hemosiderosis, insulin-dependent diabetes mellitus, hypothyroidism, and delayed puberty
Postpuberty: Fertility evaluation	Check for signs of pulmonary and liver fibrosis and arteriovenous malformations in the lung, liver, and gastrointestinal tract, especially in adult patients	

These are disease-specific evaluations. All patients with IBMFS should undergo other regular pretransplant evaluation according to each BMT center. A more complete description of system involved in patients with IBMFS can be found in Alter.[1] AML, acute myeloid leukemia; BMT, bone marrow transplantation; CBC, complete blood counts with differential and reticulocytes; CT, computed tomography; DSA, donor-specific antibodies; eADA, erythrocyte adenosine deaminase; FISH, fluorescent in situ hybridization; HH, Hoyeraal-Hreidarsson syndrome; MDS, myelodysplastic syndrome; MRI, magnetic resonance imaging; SpO_2, saturation of oxygen by pulse oximetry.

cancer after transplant, especially head and neck SCC and the use irradiation-containing regimens and/or GVHD may increase the incidence of this complication. Lifelong aggressive surveillance for early detection of cancer is recommended because the treatment options in FA are limited, and complications related to chemotherapy and radiotherapy are frequent.[1,5,9,15,28,29]

Telomere biology disorders

Clinical case 2

Patient 2 is an 11-year-old boy presenting with development delay and progressive bone marrow failure who was eventually diagnosed with dyskeratosis congenita (*TINF2* gene mutation). He received a MUD transplant at age 13 with cyclophosphamide, fludarabine, ATG conditioning, and GVHD prophylaxis with cyclosporine and methotrexate. Three years later, he presented with progressive dyspnea. Physical examination was remarkable for multiple dental caries, cyanosis, digital clubbing, mild splenomegaly, and oxygen saturation of 87% in room air. High-resolution chest computed tomography scan showed no signs of parenchymal lung disease. However, intrapulmonary shunting was detected by contrast-enhanced echocardiography with agitated saline, suggesting hepatopulmonary syndrome (HPS). The patient was started on androgens (danazol) and supplemental oxygen without clinical response. He subsequently presented with seizures, fever, and a brain abscess attributed to a bacterial embolus consequent to right-to-left shunting and died despite appropriate management.

During the past decades, the discovery of germline mutations in 14 genes involved in the telomere maintenance has led to the definition of a group of disorders called telomere biology disorders (TBDs).[4] There is excessive telomere attrition, with very short telomeres, less than the first percentile for age measured in leukocytes subsets. Dyskeratosis congenita (DC) represents the prototype of TBD and is characterized by bone marrow failure and the classical triad of reticular skin pigmentation, oral leukoplakia, and nail dystrophy. Most patients develop cytopenia at least in 1 lineage by the age of 40.[4] The most severe variants present in childhood and include Hoyeraal-Hreidarsson syndrome (cerebellar hypoplasia, microcephaly, developmental delay, immunodeficiency) and Revesz syndrome (bilateral exudative retinopathy).[4] The TBD patient may also present with liver cirrhosis and pulmonary fibrosis and is at a lifetime higher risk for MDS, AML, and solid tumors.[3,4,9] Recent studies have focused on a group of vascular abnormalities including pulmonary arteriovenous malformations and gastrointestinal telangiectasias that are a cause of substantial morbidity and mortality.[1,4,30]

Treatment with androgens may improve the marrow function in most patients, and the use of danazol is preferred because it has fewer virilizing symptoms. Although side effects are similar to the ones described for FA, patients with DC may be more susceptible to liver complications, and the risk of splenic peliosis and splenic rupture is increased when growth factors are used simultaneously.[4,17,31]

What are the results and specific problems related to HCT in TBD patients?

HCT remains the only curative treatment of the hematologic complications, but survival is limited by a high incidence of both early and long-term complications.[1,4,5,7,32] Understanding the wide spectrum of clinical manifestations (pulmonary, gastrointestinal, liver, hematologic, neurologic, ophthalmic, and immunologic abnormalities) is important to systematically evaluate each patient before and after transplant (Tables 1 and 2).[1,4,15,30]

In a recent study analyzing the outcome of 94 patients, most were transplanted after 2000 (84%) and from MUD (49%). All patients received a RIC regimen, and the 5- and 10-year OS was 59% and 30%, respectively.[32] Consistent with another report, most early deaths and graft failure occurred after mismatched donor transplantations.[33] Late mortality was attributed mainly to liver and pulmonary fibrosis and other vascular complications, most likely related to the underlying disease.[32,33] To address the benefit of less toxic preparatory regimens, a prospective multi-institutional HCT clinical trial for patients with TBD is currently testing whether a radiation and alkylator-free regimen can lead to successful and sustained engraftment and fewer long-term complications (clinicaltrials.gov #NCT01659606). Our HCT center has transplanted 28 patients with TBD in aplastic phase over a 20-year period.[34] Most received a radiation-free RIC regimen and bone marrow from MUD (46%). With a median follow-up of 6 years, the 5-year OS was 53%. However, only 2 of 8 patients transplanted from mismatched donors are alive. We observed an unusually high frequency of vascular complications including severe hepatic VOD (n = 1), recurrent gastrointestinal bleeding (n = 1; Revesz syndrome), and HPS (n = 5). Only 1 patient with HPS is alive, and none had abnormal pulmonary function tests (PFTs) before transplant.[34] Patients with HPS may also develop liver dysfunction, but the diagnosis usually requires a liver biopsy to detect intrahepatic arteriovenous malformations and regenerative nodular hyperplasia.[1,4,30] In a recent report, patients with abnormal baseline PFT had a higher risk for significant pulmonary disease. In that study, the median time to pulmonary complications after transplant was 4.7 years,[35] very similar to what we observed in our cohort (5 years).[34] PFT and an evaluation to detect vascular abnormalities should be performed regularly before and after HCT, and with HPS, a complete hepatic evaluation.[1,4,35] Currently there is no effective treatment of pulmonary fibrosis, HPS, and liver cirrhosis other than solid organ transplantation.[4,35] Because these complications impact survival after HCT, a multidisciplinary team approach is essential for early detection of liver and lung problems. The risk of cancer is also high, and the most common types are head and neck and anogenital SCC; life-long screening is recommended.[4,9,15]

Can we do anything to prevent disease progression after transplant?

One prospective trial in patients with TBD demonstrated telomere elongation and no significant decrease in lung function during danazol administration, suggesting that the use of danazol could slow down the progression of pulmonary fibrosis.[31] Although these results are provocative, there is no confirmed role for the use of androgens in preventing disease progression in nonhematopoietic tissues.[4] Other approaches to prevent disease complications in the future may include the use of small molecules that specifically restore telomere maintenance in patient-derived stem cells, exploring the use of WNT pathway agonists such as lithium, and the use of small peptides derived from dyskerin that have a role in decreasing oxidative stress, DNA damage, and cell senescence.[4,36,37]

Diamond-Blackfan anemia

Clinical case 3

Patient 3 is a 4-year-old boy with a confirmed diagnosis of Diamond-Blackfan anemia (DBA) and was referred for an MSD

Table 2. Posttransplant evaluation of patients with IBMFS

• General recommendations: Maintain healthy diet, regular exercise, good oral hygiene, and sunscreen use. • Recommend complete abstinence from alcohol and smoking (including vaping). • Ensure HPV vaccination for all patients. • Posttransplant evaluation is a multidisciplinary teamwork. Consider organizing a LTFU clinic in collaboration with other specialists (endocrinologists, dentists, head and neck oncologists, gynecologists, and others). • Consider working together with family associations and organizing regional/national family meetings as this may help patients, increase disease awareness, and improve research • Attention to neurocognitive issues, especially in patients with development delays • Psychologic evaluation and psychologic support • Address visual and hearing problems as they may impact the learning process and decrease academic achievements and quality of life • Annual liver, kidney, and gastrointestinal evaluation. Cardiac and pulmonary evaluation every other year, except for DC patients (see below) • Annual endocrine evaluation: growth assessment, glucose, lipid metabolism. Assess gonadal function and bone mineral density • For postpubertal female patients: annual gynecologic evaluation. Discuss fertility options. • For male patients: gonadal function and spermogram • Iron overload: Check ferritin levels within 6 mo to 1 y of transplantation. Consider T2* MRI to determine liver iron overload. Phlebotomy is the first choice of treatment; second is desferasirox • GVHD increases the risk of cancer after HCT for all patients with IBMFS. Treatment of GVHD with steroids may be associated with metabolic syndrome, diabetes, avascular necrosis, and adrenal insufficiency. • Aggressive cancer surveillance. Cancer risk increases as patients get older and in the presence of GVHD • Dermatologic evaluation: skin cancer screening every 6-12 mo • Oral examination performed by a dentist every 6-12 mo. Encourage monthly oral self-examination		
Disease-specific posttransplant complications		
FA	DC	DBA
Endocrinologic problems are very frequent after HCT, including thyroid dysfunction, fertility, hypogonadism, and growth hormone (GH) deficiency. Short stature can be treated with GH after 6 mo of HCT, if GH deficiency is confirmed.[27]	Pulmonary and liver complications are the major problems after HCT Perform annual PFTs and check SpO_2 and signs of pulmonary and/or liver fibrosis and arteriovenous malformations (lung, liver, and gastrointestinal tract) after HCT [30,35]	Iron overload is the major complication after HCT, and LTFU problems include diabetes, delayed puberty, and hypothyroidism. Other problems include those related to chronic steroid use and fertility issues.[38,39]
Cancer risk[9]		
Skin SCC and basal cell carcinoma Head and neck and anorectal and vulvar SCC Esophagus, breast, and brain cancer	Skin SCC and basal cell carcinoma Head and neck anorectal SCC Esophagus, stomach, and lung cancer	Colorectal carcinoma Osteogenic sarcoma

These are disease-specific recommendations. All patients with IBMFS should undergo other regular posttransplant evaluation according to published guidelines.[1,15] LTFU, long-term follow-up; SpO_2, oxygen saturation by pulse oximetry.

transplantation. The donor was his healthy unaffected 8-year-old brother with normal blood counts. Patient 3 was treated irregularly with high-dose steroids since he was 8 months old and needed monthly red blood cell transfusions with inadequate chelation. Physical examination was remarkable for signs of Cushing syndrome, growth failure, and thumb abnormalities. He received a myeloablative conditioning (MAC) regimen with busulfan, fludarabine, ATG, and GVHD prophylaxis with cyclosporine and methotrexate. The transplant was uneventful, and the patient is alive 5 years after transplant. Major long-term sequelae include growth failure and iron overload.

DBA represents a group of disorders with a defective ribosome biogenesis and is characterized by anemia with reticulocytopenia, congenital abnormalities, and an increased predisposition to cancer. In the classical form, patients present in the first year of life with a macrocytic anemia and a normocellular marrow with selective paucity of red cell precursors. Congenital abnormalities are frequent, and nearly half of the patients have craniofacial, skeletal, heart, and kidney abnormalities.[38]

The standard of care for DBA includes corticosteroids and chronic transfusions with adequate iron chelation. Although 60% to 80% of patients respond to an initial course of corticosteroids, less than 50% can be maintained with doses that are low enough to avoid long-term toxicity, and fewer than 20% achieve spontaneous remission.[39] It is recommended to avoid steroids in the first year of life because of its deleterious effects on linear growth, and infants should be treated with regular blood transfusions. Chelation should be initiated when patients receive approximately 200-mL/kg red blood cell transfusions.[39,40]

HCT is the only curative option for the hematologic manifestations of DBA and is recommended for patients who fail to respond to corticosteroids and/or need chronic transfusion. We and others consider treatment failure as needing corticosteroids (dose >0.3 mg/kg per day) to maintain hemoglobin >8 g/dL.[7,39] Although not frequent, HCT may also be indicated for hematologic malignancy or aplastic anemia.[7,40] It is essential to screen potential family donors because they may be silent carriers or asymptomatic.[38,39] In our clinical case 3, the patient's genotype was known, and it was easy to screen the donor. However, when this is not available, donors should have a complete blood count, reticulocyte count, and fetal hemoglobin.

Table 3. HCT for other hematopoietic malignancy predisposition syndromes

Disease and reference	No. pts, median age	Disease phase	Prep regimen	Donor type stem cell source	GVHD	TRM	OS	Follow-up	Comments
CAMT[46]	63 pts 7 y (0.5-17)	NR	MAC 82%	MRD or MUD > 80% BM (n = 31); PB (n = 21); UCB (n = 11)	A-GVHD: II-IV 13%. Extensive C-GVHD: 6.3%	13% at 3 y	5-y OS: 76%	1,7 y (0.1-14)	No difference in OS according to donor or stem cell source
GATA2[47]	15 pts 15 y (5-20)	MDS (n = 12) AL (n = 3)	73% CY + TBI	MRD or MUD 53% BM (n = 11) and UCB (n = 4)	A-GVHD: II- IV: 33% Extensive C-GVHD 26%	20% at 1 y	5-y OS: 65% 5-y DFs:51	5,0 y (1-8.5)	Neurological toxicities and thrombotic events more frequent after *GATA2* transplants
MECOM[49]	10 pts 0.7 y (0.2-13)	Progressive BMF	NR	MRD or MUD: 9 pts BM (n = 9) and UCB (n = 1)	NR	3 pts died	7 pts alive	NR	Sensorineural deafness, kidney, heart, and skeletal defects
MECOM[48]	6 pts 1.3 y (0.5-3)	Progressive BMF	NR	NR	NR	2 deaths from cardiac problems	4 pts alive	10 mo	4 pts are alive with no major complications after HCT
ERCC6L2[48]	3 pts 13; 14; 22 y	BMF (n = 2) MDS (n = 1)	NR	NR	NR	1 death EBV lymphoma	2 pts alive	1 and 14 y	2 pts are alive with no major complications after HCT
SAMD9 *SAMD9L*[48]	*SAMD9*: 4 pts *SAMD9L*: 6 pts 3 y (1.5-33)	BMF (n = 3) MDS (n = 7)	NR	NR	NR	2 deaths	8 pts alive	4 y (0.1-7)	*SAMD9*: 3 pts alive and well *SAMD9L*: 5 pts alive, one with neurological complication
SAMD9 *SAMD9L*[50]	*SAMD9*: 6 pts *SAMD9L*: 6 pts 2.8 y (1.2-12)	MDS (n = 10) Other (n = 2)	MAC (n = 9) RIC (n = 3)	MRD or MUD (n = 9) Haplo (n = 1) UCBT (n = 2) BM (n = 10) UCB (n = 2)	A- GVHD: II-III 2 pts Mild C-GVHD: 2 pts	2 deaths AML (n = 1) DAH (n = 1)	10 pts alive	3.1 y (0.1-14)	*SAMD9*: all alive with developmental delays. MIRAGE syndrome (n = 4): short stature, adrenal insufficiency. *SAMD9L*: 4 pts alive. No neurologic manifestations thus far.

A-GVHD, acute GVHD; AL, acute leukemias; BM, bone marrow; BMF, bone marrow failures; CAMT, congenital amegakaryocytic thrombocytopenia; C-GVHD, chronic GVHD; CY, cyclophosphamide; DAH, diffuse alveolar hemorrhage; NR, not reported; pts, patients; TBI, total body irradiation; UCB, unrelated cord blood.

Although erythrocyte adenosine deaminase activity is elevated in most patients, the test is available only in a few countries around the world.[38]

What are the results and specific problems related to HCT in DBA patients?

Many patients present with complications related to the use of corticosteroids, Cushing syndrome (diabetes, hypertension, central adiposity), and chronic transfusion: iron overload (liver and heart), alloimmunization, and anti-HLA antibodies. Recent studies report excellent OS (90%-100%) after MRD HCT for patients <10 years of age.[40,41] Current guidelines recommend a busulfan or treosulfan with fludarabine and serotherapy (MAC regimen). Engraftment rates are high, and severe acute and chronic GvHD rates are low.[7] A MUD HCT is also associated with similar results.[40] However, mismatched unrelated donor HCT leads to high graft failure and GVHD. In a recent report from Brazil (n = 44), the 5-year OS after MSD HCT was 80%, for MUD was 73%, and for mismatched unrelated donors was 29%.[42] Common causes of death included graft failure and infections. As with other IBMFS, iron overload from chronic transfusions adds to the burden of morbidity post-HCT and requires intermittent phlebotomy. Aggressive iron chelation before and after transplant is necessary to reduce cardiac iron overload

because deaths from cardiac causes have occurred as late as 5 to 7 years after HCT.[1,15] Patients without venous access may tolerate oral iron chelators such as desferasirox.[1,15,17,39] The use of a MAC regimen may lead to infertility, and fertility preservation (when available) should be discussed upfront. DBA is a cancer predisposition syndrome, and the most common neoplasias include colon and genitourinary cancers, osteogenic sarcoma, and myeloid malignancies. Guidelines for long-term follow-up in patients with IBMFS have been recently updated.[1,15,39]

Other IBMFS with hematopoietic malignancy predisposition syndromes.

Severe congenital neutropenia is characterized by recurrent life-threatening bacterial infections, chronic neutropenia from birth, and predisposition to hematologic malignancies. Treatment with granulocyte colony-stimulating factor is the standard of care, and HCT is recommended for patients who are not responsive to high doses of daily granulocyte colony-stimulating factor or in the presence of clonal evolution. Young patients transplanted from matched related or unrelated donors with myeloablative preparatory regimens have the best outcomes.[7,43] Shwachman-Diamond syndrome is a recessive disorder characterized by bone marrow failure, exocrine pancreatic insufficiency, and skeletal abnormalities. Malignant transformation is frequent, ranging between 5% and 25%. The indications for transplant are worsening cytopenias and transformation into MDS and AML.[7] Recent studies from Europe and the United States showed a 5-year overall survival of 70% for patients transplanted in marrow failure and a dismal outcome for those transplanted with MDS or AML.[44,45]

Recent advances in genomic evaluation with next-generation sequencing can identify many suspected IBMFS with atypical presentations that remain undiagnosed after an initial workup.[2,3] Improving genetic diagnosis comes with a price and may delay referral to transplantation because these techniques are not routinely available worldwide. The balance between the urgency to proceed to transplantation and the need to confirm the diagnosis in a patient with suspected IBMFS to ensure that a healthy related donor is selected is still a matter of debate, especially in developing countries. Until we establish international partnerships, a great number of patients will remain underdiagnosed, and prognosis after transplantation will not be adequately established. Diseases such as congenital amegakaryocytic thrombocytopenia, *GATA2* deficiency, *MECOM* syndrome, *CTLA4* haploinsufficiency, and *SAMD9* are being increasingly diagnosed because of precision genomics, and transplant outcomes for these rare indications are shown in Table 3.[46-50]

Conclusions

Modifying well-known risk factors has resulted in substantial reduction in early mortality after HCT, thus extending survival into adulthood. An adequate selection and evaluation of patients before transplant, use of bone marrow from well-matched donors, and avoiding irradiation-containing preparatory regimens are universal recommendations for these patients. Screening of family members is essential to exclude affected individuals as potential donors. For all IBMFS HCT will not correct non-hematologic manifestations related to these diseases. The decision to proceed to transplant is complex, and all aspects of the transplant process should be considered, including but not limited to the phase of the disease, safety and quality of blood transfusions, pretransplant comorbidities, availability of techniques to manipulate the graft, and a capacity for adequate posttransplant care that includes long-term follow-up. IBMFS are rare, and international collaborative efforts are needed to advance the care of these patients.

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Off-label drug use

None disclosed.

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Extrahematopoietic manifestations of the short telomere syndromes

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The short telomere syndromes encompass a spectrum of clinical manifestations that present from infancy to late adulthood. They are caused by mutations in telomerase and other telomere maintenance genes and have a predominantly degenerative phenotype characterized by organ failure across multiple systems. They are collectively one of the most common inherited bone marrow failure syndromes; however, their most prevalent presentations are extrahematopoietic. This review focuses on these common nonhematologic complications, including pulmonary fibrosis, liver pathology, and immunodeficiency. The short telomere syndrome diagnosis informs clinical care, especially in guiding diagnostic evaluations as well as in the solid organ transplant setting. Early recognition allows an individualized approach to screening and management. This review illustrates a myriad of extrahematopoietic presentations of short telomere syndromes and how they impact clinical decisions.

LEARNING OBJECTIVES

- Describe the common presentations of extrahematopoietic short telomere disease
- Identify implications for diagnostic and management decisions

Introduction

Telomere length is genetically determined with a discrete normal range.[1,2] Germline mutations in telomerase and other telomere maintenance genes also accelerate the telomere shortening that normally occurs with aging and result in a spectrum of premature aging syndromes marked by degenerative disease. There is also a specific cancer risk that includes most commonly the marrow-derived malignancies, myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), with squamous cell cancers being more rare.[3,4] Studies in mice have shown that telomerase itself is not essential and that the short telomere length, which is inherited with genetic anticipation, is the primary determinant of phenotype severity.[5,6] Dyskeratosis congenita, characterized by a triad of mucocutaneous manifestations, including reticular skin pigmentation abnormalities, nail dystrophy, and mucosal leukoplakia, was the first recognized telomere disease in humans.[7,8] Bone marrow failure is a common complication, with a significant percentage of patients with classic dyskeratosis congenita developing at least a single-lineage cytopenia by the age of 30 years,[9,10] and often evolving into variable degrees of aplastic anemia.[11] Other rarer variants of infant-onset disease have recently been reviewed elsewhere.[12] Adult-onset disease is marked by pulmonary fibrosis; given the known prevalence of this lung disease and the percentage of patients with germline telomere defects, it is estimated to account for ≥90% of presentations.[13]

The pediatric and adult telomere phenotypes of bone marrow failure and pulmonary fibrosis have recently been found to share germline defects in telomere maintenance.[14,15] For this review, we refer to them as the "short telomere syndromes" to both indicate their underlying molecular defect and signify the frequent co-occurring syndromic manifestations.[2] This term also distinguishes this spectrum from the hypothesized group of "long telomere syndromes" that are thought to manifest as a familial clustering of cancer.[16,17] This review focuses on the nonhematologic features of the short telomere syndromes, which can precede the onset of clinically apparent hematologic disease, and their significance for patient care. The case-based approach highlights how the diagnosis of a short telomere syndrome impacts clinical management with a focus on the most common extrahematopoietic complications, including pulmonary fibrosis, liver pathology, and immunodeficiency.

Case 1

A 51-year-old woman presented to clinic with neutropenia 12 months after lung transplant for the diagnosis of idiopathic pulmonary fibrosis (IPF) (Figure 1A). She reported having a brother who was recently diagnosed with IPF. Her laboratory evaluation was remarkable for a white blood cell count of 2.0 × 10^3 cells/μL with an absolute neutrophil count of 800 cells/μL, an absolute lymphocyte tcount of 600 cells/μL, and a hemoglobin concentration of 11.3 g/dL. Her antirejection medication, mycophenolic acid, was discontinued without improvement in her neutrophil count (Figure 1B). Consultation with hematology did not reveal reversible or infectious causes, and bone marrow evaluation showed a hypocellular bone marrow for age. The co-occurrence of IPF with marrow hypoplasia prompted an evaluation for a telomere disorder. The patient's telomere length was found to be below the first age-adjusted percentile, and genetic testing identified a pathogenic mutation in the telomerase reverse transcriptase gene, *TERT* (Figure 1C). Because of progressive neutropenia, tacrolimus dosing was also reduced to target lower trough levels. She soon recovered and was able to discontinue granulocyte colony-stimulating factor support (Figure 1B). She continued to have excellent allograft function with no evidence of rejection at 36 months after transplant.

Genetics of the short telomere syndromes

Mutations in *TERT* are the most common cause of the short telomere syndromes, accounting for at least 40% of the cases.[3,4] Thirteen other genes have been linked to the short telomere syndromes. These genes are part of the telomerase complex itself, have roles in telomerase RNA biogenesis, telomerase recruitment or telomere protection, or are involved in other telomere maintenance functions. A contemporary list of genes and their roles in telomere length maintenance has recently been reviewed.[18] Approximately 20% to 30% of patients with classic short telomere phenotypes do not have an identifiable genetic cause of their disease,[3,4,19] and, in these cases, the diagnosis is made clinically on the basis of personal and family history along with measurement of telomere length and/or telomerase RNA levels.[20]

Age-dependent interpretation of telomere length

Interpretation of telomere length measurement is age dependent and must take the clinical and, when possible, genetic findings into account. There is not a single threshold applicable to all patients with short telomere syndrome across the age spectrum (Figure 2). Children and adolescents with symptomatic short telomere disease usually fall below the first percentile.[21] However, young, often asymptomatic mutation carriers can have telomere lengths between the 1st and 10th percentiles and are at risk for later-onset manifestations (Figure 2).[1,22] In those over age 40, telomere length has less diagnostic specificity, and in adults with symptomatic short telomere syndromes of this age, the telomere length can overlap with the lowest decile of the normal population. Furthermore, due to the early mortality of younger patients with short telomeres, nearly all patients over age 60 have telomere lengths above the first percentile.[1] In these cases, clinical recognition of the diagnosis based on personal and family history is fundamental along with genetic testing. Other nontelomere inherited bone marrow failure syndromes have also been associated with very short leukocyte telomere lengths.[1,23] The biology and natural history of these disorders are distinct from those of the short telomere syndromes, stressing the importance of clinical interpretation of telomere length studies (Figure 2). Finally, lineage-specific telomere length shortening can be seen in some acquired disease states. For example, some patients with autoimmunity have lymphocyte-specific shortening, whereas some patients with MDS acquire granulocyte-restricted shortening.[4,24] These observations highlight the importance of measuring telomere length in specific lineages by flow cytometry and fluorescence in situ hybridization (flowFISH).

Pulmonary fibrosis is the most common manifestation of the short telomere syndromes

Due to the overall higher prevalence of pulmonary fibrosis in the population, affecting ≥100 000 individuals in the United States alone, and the high prevalence of short telomere gene mutations in those patients, lung disease is the most common manifestation of the short telomere syndromes.[13] As in case 1, short telomere syndromes are the most commonly identified genetic cause for familial forms of IPF, accounting for up to 30% to 35% of familial cases.[15,20,25-31] In addition to IPF, short telomeres can lead to a broad spectrum of lung disease pathologies that share a progressive course.[32] These include nonspecific interstitial pneumonitis, bronchiolitis obliterans organizing pneumonitis, chronic

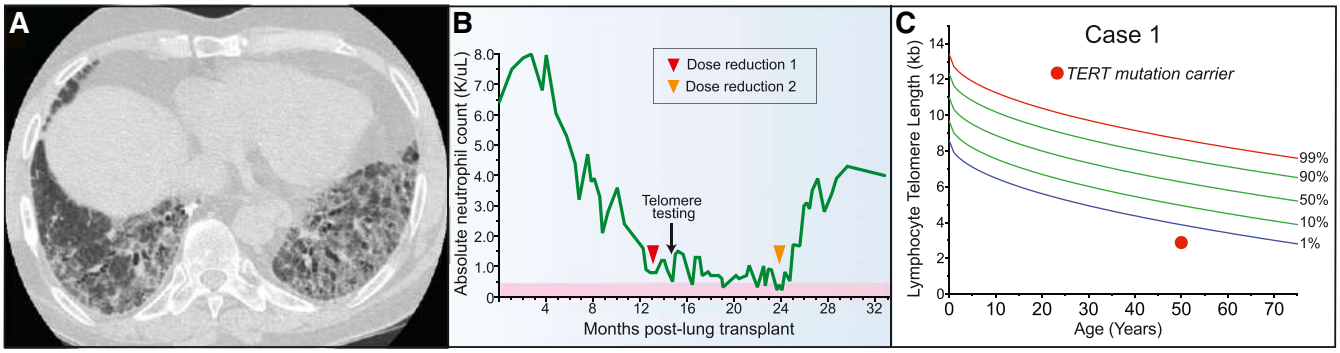

Figure 1. Pulmonary fibrosis is the most common short telomere manifestation. (A) Typical appearance of telomere-related IPF characterized on CT by honeycombing in the lung bases. (B) Schematic showing progressive neutropenia after lung transplant that improved after immunosuppressant regimen was attenuated. (C) Lymphocyte telomere length by flow cytometry and fluorescence in situ hybridization (flowFISH) in representative case.

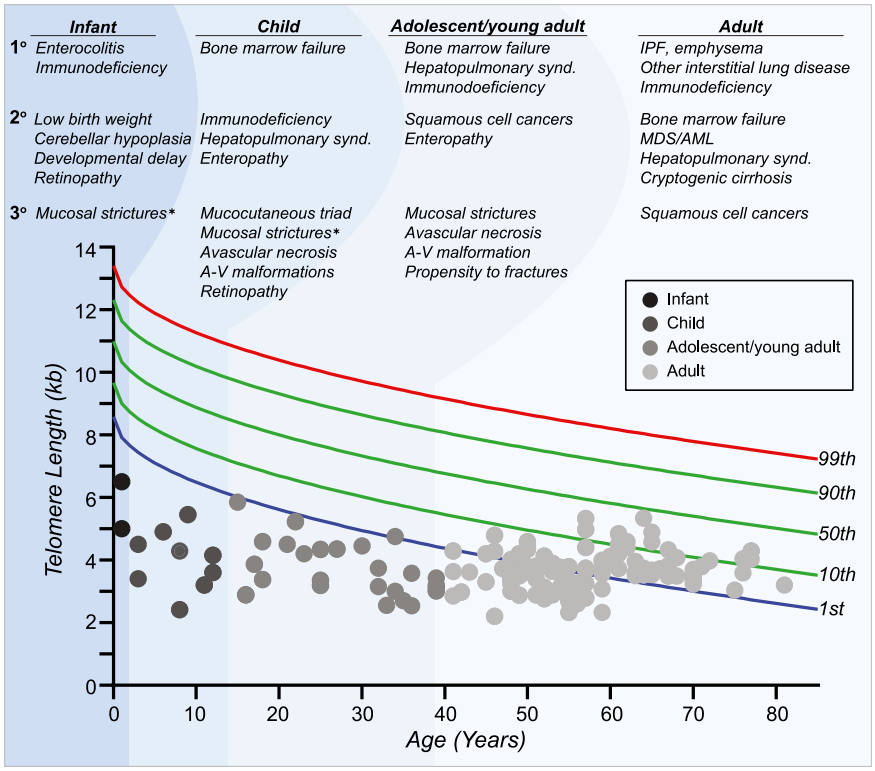

Figure 2. Age-dependent manifestations of the short telomere syndromes. A schematic telogram demonstrating the typical ranges of telomere length shortening by age of symptomatic disease onset. The threshold for clinically relevant telomere shortening is age and context dependent. Highlighted on top are the predominant manifestations in each of four age groups ordered by prevalence (primary, secondary, and tertiary). Telomere length displays a normal distribution in the population that is defined by the percentile lines labeled on the right. The telomere length is representative here of both total lymphocyte and granulocyte telomere lengths. Each dot represents one patient adapted from symptomatic patients included in Schratz et al.[4] *"Mucosal strictures" is a general term that encompasses mucosal defects affecting multiple systems, including lacrimal duct stenosis, esophageal stenosis/strictures/webs, and urethral stenosis/stricture/phimosis. A-V, arteriovenous; synd., syndrome.

hypersensitivity pneumonitis, pleuroparenchymal fibroelastosis, and emphysema.[15,31-34] Studies in mice have linked telomere dysfunction to alveolar stem cell senescence and a resultant epithelial proliferative defect that, with additional injury such as cigarette smoke or pulmonary toxic exposures, is thought to provoke lung remodeling and progressive respiratory disease.[35,36] Furthermore, studies in mice and humans suggest the primary short telomere–mediated lung phenotype in some individuals may be influenced by sex differences as well as environmental effects.[27,34] For example, within families with short telomere syndromes, pulmonary fibrosis predominates in never smokers, whereas smokers, especially females, are at risk for developing young-onset emphysema alone or in combination with pulmonary fibrosis.[34]

IPF, characterized radiographically by traction bronchiectasis and reticular honeycombing preferentially affecting the bases and periphery,[37] is the most common lung phenotype, accounting for 65% to 70% of lung disease presentations in adults with telomerase mutations.[33] Dyspnea and cough are the most common presenting symptoms of pulmonary fibrosis in the short telomere syndromes (Table 1). The average age of onset is in the sixth decade, and symptomatic disease is rare prior to the third decade.[27] Younger-onset lung disease can be precipitated by toxic medications previously used in the setting of ablative hematopoietic stem cell transplant.[38-40] Pulmonary fibrosis also precedes or co-occurs with telomere-mediated MDS/AML, complicating their treatment, and lung disease is the predominant contributor to mortality in these adult patients with MDS/AML.[4] In the short telomere syndromes, the diagnosis of pulmonary fibrosis can be made on the basis of clinical assessment and radiographic studies alone. Molecular and genetic testing for telomere abnormalities has also been suggested as an adjunct to the diagnostic evaluation of IPF due to the high morbidity and risk of mortality with open lung biopsy and the fact that the genetic diagnosis is predictive of the clinical course.[32,33]

Hematologic manifestations complicate lung transplant for telomere-mediated lung disease

There are currently no medical treatments that have been shown to reverse the extrahematopoietic diseases in the short telomere syndromes and the standard of care for end stage disease is organ transplantation.[32] IPF is the leading indication for lung transplant in North America underscoring a particular relevance for the short telomere syndromes.[41] The case presented highlights the vulnerable bone marrow reserves in short telomere syndrome patients with pulmonary disease that may be unmasked in the post-transplant setting.[42] Telomerase mutation carriers have higher rates of cytopenias, as well as opportunistic infections, particularly cytomegalovirus, and possibly renal

Table 1. Age-dependent presentations of short telomere extrahematopoietic disease

Age group	Immunodeficiency		Liver disease		Lung disease	
	Symptoms	Finding	Symptoms	Finding	Symptoms	Finding
Infant	Bloody diarrhea Failure to thrive Enterocolitis Sepsis	Intestinal mucosal atrophy Crypt apoptosis ↓ $CD19^+$ B cells ↓ IgA	Rare	Rare	Absent	Absent
Adolescent/ young adult*	Opportunistic infections (eg, herpes zoster reactivation, primary CMV with retinitis/ encephalitis; *P jirovecii* pneumonia; *Candida* esophagitis)	↓ $CD4^+$ T cells ↓ IgM ↓ T-cell repertoire	Splenomegaly Portal HTN Clubbing Dyspnea/hypoxemia Variceal bleeding	Nodular regenerative hyperplasia A-V malformations HPS Patchy fibrosis	Usually absent†	Usually absent†
Adult	SCC of the skin CMV viremia and disease after lung transplant *P jirovecii* pneumonia	↓ $CD4^+$ T cells ↓ T-cell repertoire ↓ T-cell proliferation	Splenomegaly Portal HTN Clubbing Dyspnea/hypoxemia Atrophic nodular liver Variceal bleeding	Nodular regenerative hyperplasia HPS Overt fibrosis/cirrhosis	Dyspnea/cough Basilar crackles Restrictive PFT findings	IPF-emphysema‡ Other interstitial lung disease

A-V, arteriovenous; CMV, cytomegalovirus; HPS, hepatopulmonary syndrome; HTN, hypertension; IgA, immunoglobulin A; IgM, immunoglobulin M; PFT, pulmonary function test; SCC, squamous cell cancer.
*These extrahematopoietic manifestations can also present in childhood. In this age group, however, hematologic manifestations are the primary feature (see Figure 2).
†Pediatric and young adult onset of pulmonary fibrosis has been precipitated historically by the use of ablative regimens in the bone marrow transplant setting, especially those regimens that include busulfan.
‡This nomenclature reflects the unique spectrum of lung disease phenotypes seen in the short telomere syndromes in which pulmonary fibrosis can occur alone (most commonly) or in combination with emphysema, particularly in female smokers.

insufficiency.[42,43] They invariably require dose reduction of the standard anti-rejection regimen, particularly antimetabolite medications.[42] Establishing the diagnosis of an underlying short telomere syndrome *prior* to lung transplant is critical so post-transplant morbidities can be anticipated and possibly averted.[42] Apart from attenuation of immunosuppression and use of less myelosuppressive antimicrobial prophylaxis, the primary treatment modalities for post-lung transplant cytopenias are supportive, including transfusions and granulocyte colony-stimulating factor for severe neutropenia. The benefit of the androgen danazol in this setting has not been studied and its use is controversial due to uncertainties regarding its safety profile in adults[44] and concerns it may promote clonal selection in these patients at risk for MDS/AML.[22] Management of cytopenias in this setting can be complicated and may require consultation with an experienced center. Going forward, as more patients with telomere-mediated lung disease are identified during the pre-transplant assessment, an inter-disciplinary team that includes hematologists and infectious disease specialists will be important for managing these patients.

Case 2

A previously healthy 57-year-old man presented to his physician for evaluation of unilateral vision loss. His family history was positive for acute leukemia in two siblings. His dilated fundus examination showed opacified lesions tracking centrifugally with the retinal vessels in the right eye with areas of necrosis and hemorrhage (Figure 3A). This was suspicious for cytomegalovirus (CMV) retinitis, and laboratory evaluation demonstrated lymphopenia with an absolute lymphocyte count of 800 cells/μL, absolute CD4 count of 339/μL (normal for age, 500 to 1400/μL), and macrocytic anemia (hemoglobin, 11 g/dL; mean corpuscular volume, 101 fL) but an otherwise intact complete blood count. The result of the patient's serum CMV polymerase chain reaction assay was positive at 79 000 IU/mL. The result of his HIV antibody test was negative. Treatment of CMV retinitis was initiated with intravitreal foscarnet and systemic ganciclovir. During follow-up care, computed tomography (CT) of the chest, performed for complaints of chronic exertional dyspnea, revealed interstitial lung disease (Figure 3B). These findings prompted an evaluation for a telomere disorder, which demonstrated telomere length below the first age-adjusted percentile (Figure 3C). Genetic evaluation showed no mutations in the known genes. The patient's hypoxia progressed, and he developed acute-on-chronic respiratory failure. Evaluation identified *Pneumocystis jirovecii* pneumonia as the cause (Figure 3B). He died 4 months after the diagnosis of his CMV retinitis despite maximum supportive care.

Short telomere–mediated T-cell immunodeficiency underlies propensity for CMV infection

CMV is a double-stranded DNA virus in the Herpesviridae family that causes opportunistic infections in individuals with T-cell immunodeficiency.[45] Patients with short telomere syndromes have been found to have a specific risk for herpes virus infections, with mortality from CMV infection reported in children and adults[46,47] (Table 1). In adults, CMV infection manifests often in immunosuppressed patients with short telomeres after lung transplant.[43] Lung transplant recipients with a history of IPF and telomere lengths shorter than the age-adjusted 10th percentile, regardless of germline mutation status, have been reported to

have a 5-fold increased risk of relapsing CMV viremia and potentially fatal CMV end-organ disease.[42,43] This predilection for CMV disease is due to both global and CMV-specific T-cell immunodeficiency.[43,47] Management of CMV in lung transplant recipients with short telomeres is complicated by drug resistance, as well as cytopenias related to myelosuppressive antiviral medications, such as older-generation medications valganciclovir and ganciclovir. Given these considerations, prevention is paramount, and our practice is to avoid high-risk CMV mismatch (seronegative recipient/seropositive donor) when possible, attenuate immunosuppression, and use CMV prophylaxis after transplant. Consultation with infectious disease specialists is helpful in identifying nonmyelosuppressive state-of-the-art strategies to prevent and manage CMV complications in at-risk patients.

Immunodeficiency as a presenting complication

As in the patient in case 2, outside of the lung transplant setting, opportunistic infection with absent or mild bone marrow failure can be the first presenting feature of an inherited short telomere syndrome.[47,48] Children with a rare and severe form of short telomere syndromes including Hoyeraal-Hreidarsson syndrome,[49,50] present in infancy with noninfectious enterocolitis due to a primarily B-cell immunodeficiency that is also compounded by telomere-related defects in the intestinal epithelium.[51] Outside of this early childhood period, opportunistic infections due to the T-cell immunodeficiency predominate and, in addition to CMV end-organ disease, may also present as herpes zoster reactivation, *Candida* esophagitis, and *P. jirovecii* pneumonia, for example (Table 1).[47,52,53] Due to short telomere-induced apoptosis and secondary depletion of T-cell precursors, children and adults with short telomere syndromes have low T-cell counts in addition to profound functional defects that include depleted naive T-cell pools and a restricted T-cell repertoire.[47] Laboratory evaluation of lymphocyte subsets and immunoglobulin levels at diagnosis may be helpful to identify those at highest risk of infection and for consideration for stem cell transplant if the patient is eligible or antimicrobial prophylaxis when the patient is not. T-cell immunodeficiency is an underrecognized indication for stem cell transplant in the absence of bone marrow failure in some patients.[47] Recognizing those with a short telomere–mediated immune defect is critical because these individuals require attenuated transplant protocols.[54] Patients with unrecognized short telomere syndromes who receive immunosuppression for "idiopathic" disease often develop severe opportunistic infections, underscoring the importance of a high index of suspicion for the diagnosis.

Case 3

A 62-year-old-man presented to clinic with dyspnea; he had been followed for a history of moderate thrombocytopenia unresponsive to immunosuppression. He had been diagnosed with liver cirrhosis 7 years prior and reported going gray at age 23. His family history was negative for lung or hematologic disease. His physical examination showed an age-appropriate man who was otherwise well but had digital clubbing (Figure 4A). Review of recent imaging showed a nodular liver contour, splenomegaly with evidence of varices (Figure 4B), and incidentally detected lower lobe predominant subpleural reticular opacities and traction bronchiectasis. The constellation of bone marrow failure, liver disease, and early signs of pulmonary fibrosis suggested a short telomere syndrome. The patient's telomere length fell between the 1st and 10th percentile for age, and genetic testing identified a heterozygous frameshift mutation in *RTEL1* (Figure 4C). Pulmonary function tests showed a markedly decreased carbon monoxide diffusion capacity with only a mild restrictive ventilatory defect. Agitated saline echocardiography showed delayed right-to-left shunting indicative of intrapulmonary shunting and supportive of the diagnosis of hepatopulmonary syndrome (HPS). The patient's immunosuppression was withheld, given the diagnosis of telomere-mediated bone marrow failure, and he is being monitored by a multidisciplinary team for progression of his lung, liver, and marrow disease.

Liver and lung disease co-occur with marrow failure in patients with telomere maintenance defects

Hepatic pathologies have been reported in ~10% of patients with short telomere syndromes,[55] and the triad of bone marrow

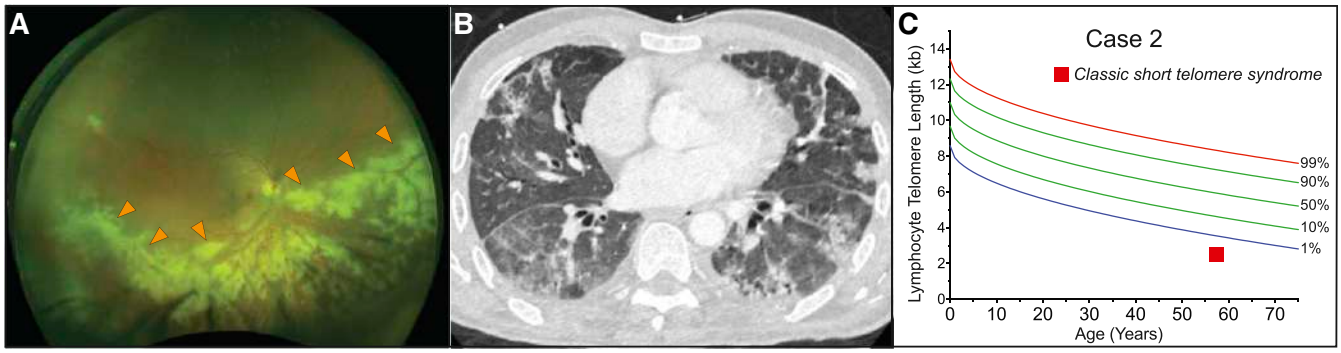

Figure 3. Infectious complications can precede onset of bone marrow failure in the short telomere syndromes. (A) Wide-field fundus photograph of CMV retinitis with intraretinal whitening (orange arrowheads) and necrosis along the retinal vasculature and progressing centrifugally. Image generously provided by Dr. Yonwook Kim of the Boston University Medical Center Department of Ophthalmology. (B) Chest CT image showing pleura-based patchy ground-glass opacities and interlobular septal thickening in a patient who developed *P. jirovecii* pneumonia in the background of interstitial lung disease and CMV retinitis that was ultimately fatal. (C) Lymphocyte telomere length by flowFISH in a patient with telomere-mediated primary T-cell immunodeficiency manifesting in fatal opportunistic infections.

failure, liver disease, and pulmonary fibrosis has been proposed to be defining for adult-onset disease.[14] Early reports identified cryptogenic cirrhosis in patients with dyskeratosis congenita and IPF.[55,56] Mouse studies suggested short telomeres decreased regenerative capacity and increased susceptibility to liver injury, resulting in hepatic steatosis and fibrosis with repeated insults.[57] Recent studies in patients have also linked the short telomere defect to noncirrhotic portal hypertension.[58] This liver disease predominantly manifests as HPS and is characterized by arterial hypoxemia with evidence of intrapulmonary vascular dilatation and secondary arteriovenous connections, both in the lung and in the abdomen.[58] As in case 3, the diagnosis of HPS is suspected in patients with short telomere syndrome with progressive dyspnea and a low carbon monoxide diffusion capacity on pulmonary function studies out of proportion to the extent of parenchymal lung disease and spirometry defects. Carbon monoxide diffusion capacity is also a useful, noninvasive method to monitor for disease progression, but it may be difficult to interpret in patients with lung parenchymal disease. Digital clubbing is generally identifiable by physical examination. Mild to moderate splenomegaly may be evident, and hypersplenism can be a significant contributor to thrombocytopenia and neutropenia; this pathology should also be considered in the differential diagnosis for delayed reconstitution or progressive cytopenias after allogeneic stem cell transplant for telomere-mediated marrow failure or immunodeficiency. The diagnosis of HPS can be made by transthoracic agitated saline echocardiogram. The stigmata of underlying liver disease may be subtle, and the diagnosis requires a high index of suspicion. With progression, the liver contour can become increasingly nodular with compensatory hypertrophy of the caudate lobe, which can appear similar to cirrhotic liver disease, but cirrhosis in young patients may not be detected. Histopathologic findings are often nonspecific and may be interpreted as nodular regenerative hyperplasia, among other pathologies.[55,58] In contrast to typical cirrhotic liver disease, liver synthetic function is generally well preserved. Patients with short telomere syndrome with HPS share a progressive natural history that has been well documented with complications due to severe hypoxia and portal hypertension, including gastrointestinal bleeding. The median time to death or liver transplant is 6 years from the onset of dyspnea symptoms, with a range of 4 to 10 years.[58] Liver transplant reverses the hypoxic defect in patients with telomere-mediated HPS; transplant recipients, however, remain at risk for progression of other short telomere manifestations, including pulmonary fibrosis.[58]

Clinical and diagnostic screening for extrahematopoietic manifestations

The physical examination with an informed index of suspicion for these age-dependent extrahematopoietic complications should drive the care of patients with short telomere syndromes. Because of the age-dependent natural history of the telomere disorders, there is no one-size-fits-all approach to screening and surveillance. As shown in Table 2, in the Johns Hopkins Telomere Center, we assess patient symptoms and rely on the physical examination to guide testing targeted at the complications highlighted in Table 1. We also factor in telomere length to anticipate the onset of symptoms and to individualize a program for surveillance following the data from large patient series.[1,4] We are judicious about diagnostic testing for lung and liver disease in the absence of symptoms, given the high risk of morbidity of interventions, the risk of radiation exposure, and the low yield in unselected age groups. Because the risk of parenchymal pulmonary disease increases with age but is rare prior to age 40, we discuss the risks and benefits of a high-resolution computed tomography in asymptomatic adults older than 40. The advantage of early detection in this group is to facilitate early referral to pulmonary clinics because some cases of pulmonary fibrosis in patients with short telomeres are known to have a rapidly progressive course. Early treatment, however, has not been shown to change the disease course, and the general natural history of short telomere syndromes is slowly progressive.

Summary

A subset of patients with short telomere syndromes can be clinically identified on the basis of personal or family history of hematologic, lung, and liver disease. In other cases, a high index of suspicion in certain settings, such as idiopathic disease of the bone marrow, immune system, lung, or liver, may be necessary to prompt testing. As consideration for an inherited syndrome is now standard for aplastic anemia management, this diagnosis should also be considered in some patients with extrahematopoietic presentations, including IPF, especially in those undergoing lung

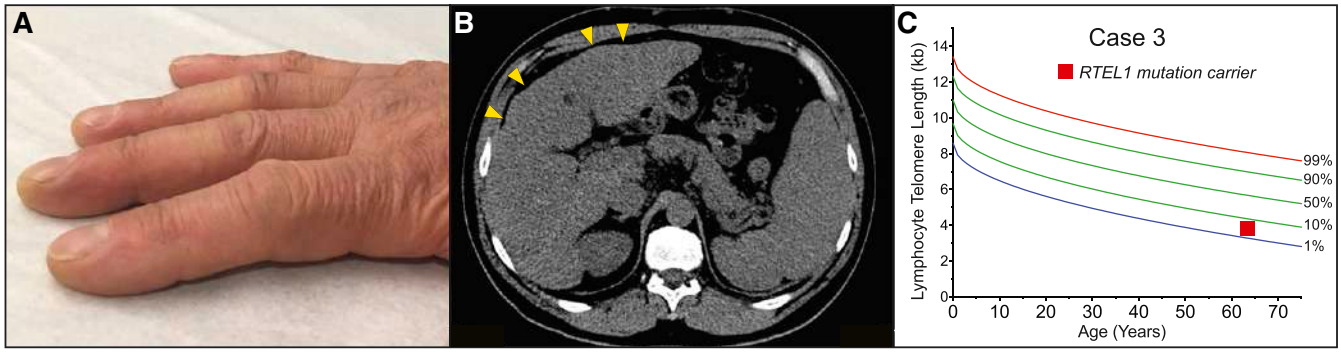

Figure 4. Clubbing and dyspnea can be a presenting feature of telomere-mediated hepatopulmonary syndrome. (A) Image of digital clubbing typically seen. (B) Abdominal CT image showing liver with nodular edges (yellow arrowheads) and secondary splenomegaly due to portal hypertension. (C) The lymphocyte telomere length by flowFISH in older patients with short telomere syndrome presenting over age 60 overlaps with the lower decile of the normal range.

Table 2. Framework for diagnosis and management of common extrahematopoietic complications of the short telomere syndromes

Immunodeficiency
At short telomere syndrome diagnosis, consider the following: • Lymphocyte subsets • Immunoglobulin levels (infants and where otherwise indicated clinically) Management of severe immunodeficiency: • Refer to center with experience in allogeneic stem cell transplant in patients with short telomere syndromes • Consultation with infectious disease specialist when indicated
Hepatopulmonary syndrome
At short telomere syndrome diagnosis: • Regular (1-2 y) history and physical examination to assess for symptoms and check for digital clubbing Diagnostic evaluation of dyspnea: • Agitated saline echocardiogram • Pulmonary function test with DLCO and assess for discordance with spirometry Management: • Refer to experienced liver transplant center
Telomere-related lung disease
At short telomere syndrome diagnosis: • Educate and counsel patients to seek attention for symptoms of dyspnea/cough • Discuss risks and benefits of high-resolution CT of the chest in asymptomatic individuals aged >40 y • Counsel on avoidance of environmental toxins (smoking) and oxygen (iatrogenic during elective procedures) Management: • Refer to pulmonologist with experience with telomere disorders • Avoid immunosuppression as therapeutic strategy • Evaluate for lung transplant at experienced center when patient is eligible

DLCO, carbon monoxide diffusion capacity.

transplant evaluations, those with primary immunodeficiencies, and patients with liver disease, particularly those with HPS. The diagnosis of a short telomere syndrome informs additional screening and diagnostic evaluations and has critical implications for management. As attenuated approaches to stem cell transplant for aplastic anemia have improved short-term outcomes, efforts are evolving for developing similar approaches to lung and liver transplantation.

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Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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Optimizing management of acute leukemia in community centers and when to refer

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Treatment of acute leukemia has been delivered predominantly in academic and larger leukemia treatment centers with the infrastructure and staff needed to manage patients receiving complex therapeutic regimens and supportive care. However, in recent years, several oral agents and less-myelosuppressive regimens were approved, making it possible for these patients to receive therapy in smaller community hospitals and oncology office practices. In this review, we discuss the optimum community setting, type of patient who can be treated, agents that can be applied, and an appropriate clinical circumstance in which a referral to a tertiary center should be made.

LEARNING OBJECTIVES
- Manage acute leukemia in the community setting
- Understand the barriers to treatment of leukemia outside of academic centers

Introduction

The incidence of leukemia in adults in the United States is 6.9 per 100 000 per year (from 1973 through 2014), and the lifetime risk of developing leukemia is 1.5%.[1] Acute myelogenous leukemia (AML) is the most common type of acute leukemia in adults and accounts for 80% of all leukemias.[2] The goal in younger patients with no comorbid conditions is a curative approach using intensive chemotherapy with or without targeted agents. This approach might be followed by bone marrow transplant on the basis of risk stratification and donor availability. For older patients and young patients with comorbid conditions, the use of curative intensive therapy is precluded, and the expectations are palliative, with an approach intended to prolong and maintain a reasonable quality of life.

For decades, the available agents for intensive induction have been "7 + 3" (anthracycline and infusional cytarabine).[3] This treatment was most frequently within the purview of academic teaching hospitals and larger community hospitals with programs to treat patients with acute leukemia. Alternatively, if the patient was incapable of tolerating intensive chemotherapy, other available options were hydroxyurea or low-dose cytarabine (LDAC).[4]

In the last 10 years, several new agents have been approved for AML, for both oral and parenteral use, offering additional options for older patients and making therapy of AML feasible in the community setting for most patients. Some of the newer agents are hypomethylating agents (HMAs), targeted agents such as FLT3 inhibitors, isocitrate dehydrogenase (IDH) inhibitors, hedgehog inhibitors, gemtuzumab ozogamicin (GO), and venetoclax.[5-13] Smaller community health care facilities and office-based private practices are increasingly treating patients with AML with some of the recently approved novel agents.

Because this is a recent paradigm, there is inadequate published literature on treating acute leukemia in the community. Hence, several of the topics discussed in this article and the recommendations suggested are derived from our own experience in developing and supporting a hematologic malignancy network in our catchment area (Table 1).

Our experience in engaging our community

For almost 25 years, our team has worked at developing a network of community hospitals and office-based practices in a catchment area comprising a population of 3.5 million. The catchment area is a patient referral base for the Georgia Cancer Center at Augusta University (Augusta, GA). Subsequently, we used this network to implement a clinical trial in the management of acute promyelocytic leukemia (APL). The cure rate and long-term survival for APL in clinical trials is >90%, although this is not true in the general population.[14-17] The induction mortality or early deaths (EDs) in APL is ~30%, and the long-term survival of all patients

Table 1. Our recommendations for considering a referral to an academic center

Reasons for referral	Our suggestions for considering a referral
Diagnostic challenges	Diagnosis is challenging, and pathology requests a second opinion.
	Consider referral/expert discussion before palliative treatment, even in elderly patients.
Treatment challenges	Presentation is complex and requires supportive care that is not available at the facility.
	Requires immediate therapy, but potential delays in diagnosis prevent start of therapy
Consider on the basis of subtype of leukemia	Acute promyelocytic leukemia
	Older adults
	Adolescents and young adults with acute lymphoblastic leukemia
	Consider referral for secondary leukemias
	Refractory after one induction
	Relapsed leukemia
	HCT is a consideration.

with this diagnosis is in the 65% range.[18-20] We conducted a study by developing a network of leukemia treatment centers in Georgia, South Carolina, and neighboring states. The study design provided a simplified 2-page treatment algorithm that emphasized quick diagnosis, prompt initiation of therapy, and proactive and aggressive management of the major causes of death during induction. APL expert support was available 24 hours per day, 7 days per week, to the treating physician very early in the diagnosis and was maintained until the completion of induction. As a result, patients were treated in local community hospitals by local oncologists rather than being transferred to a tertiary center. An aggressive outreach effort was made before initiating the trial by visiting most of the leukemia treatment centers to make our community partners aware of the availability of this program and educate treating physicians about ED in APL. A total of 120 patients were enrolled with no exclusion criteria at 5 large leukemia centers (n = 54 [45%]) and 18 community hospitals (n = 66). There were 12 EDs, one of which was in a Jehovah's Witness who declined transfusions and one in a patient who enrolled 12 days after diagnosis while already in multiorgan failure. The incidence of EDs was 10 (8.5%) of 118. With a median follow-up of 320 days, overall survival was 87%. This prospective trial showed that a simplified treatment algorithm along with support from experts and comanagement with treating physicians in the community decreased induction mortality and improved survival.[21] Although there was no control arm in this study and historical data were used for comparison, it is our impression that the process used and the academic–community partnership resulted in an improved outcome. This pilot study subsequently paved the way for activation of the ECOG-ACRIN 9131 trial to decrease induction mortality in APL using the same methodology. The trial is currently accruing patients nationally. Our experience and study demonstrate the possibility of improved outcomes with academic–community partnerships.

From the above study and experience, we learned the following to enhance community–academic partnership and collaboration:

- Initially, the community physicians did not think EDs were a problem in APL, but with time, support for the trial became stronger.
- It helps for referring physicians to have direct and easy access to academic physicians, such as through direct office and cell phone numbers.
- Physically visiting the community practices and developing a relationship with the physicians and staff are very effective.
- Participating in state and regional meetings attended by community physicians results in strengthening the collaboration.
- Meticulous communication and articulating the postdischarge plans in a written note and transmitting them to the referral practice create dependability.
- Hospital diversion and nonavailability of beds at the academic center can be a frustrating problem for community oncologists.
- Having the necessary support staff in an academic center to carry out the above functions is important.

General treatment of leukemias

Treatment centers

Before we proceed to how we manage patients in a community setting, it would be worthwhile to define what constitutes a community leukemia-treating center. Some data are available on where leukemia patients are treated in the United States. The majority of patients are treated in university teaching hospitals and larger leukemia-treating community hospitals. In a study by Zeidan et al[22] of 6442 patients with AML treated with induction chemotherapy, 95.6% of patients were treated in urban areas, 59.5% in teaching hospitals, and 55.5% in large hospitals with >500 beds. For instance, in the state of Georgia, with a population of 10.5 million, there are 134 hospitals, 20 of which treat patients with acute leukemia. Two of the 20 hospitals are university teaching hospitals, and the others are large community hospitals.[23] The large hospitals are of varying complexity, with one of them being a referral hospital that has a large leukemia service as well as a Foundation for the Accreditation of Cellular Therapy–accredited hematopoietic cell therapy (HCT) program. This might be true in other states as well. Some of the newer, less intense drugs can be administered in smaller community non–leukemia-treating hospitals as well as in private offices. As such, in general, there are 3 types of health care institutions that could provide care to patients with acute leukemia: (1) university teaching hospitals, (2) larger community hospitals, and (3) smaller community hospitals and private offices.

Do community centers want to treat patients with AML?

Obviously, community hospitals, private practices, and physicians would like to provide the whole spectrum of cancer care

and keep their patients in the community. Leukemias account for <3% of all cancers, and treating them requires familiarity with treating the disease and inpatient units with trained and dedicated nursing staff and oncology pharmacists. Blood bank support and availability of blood products are key to successful management. Treating these complicated patients adds a certain level of complexity to the hospital and practice site. The physicians may want to continue treating hematologic malignancies in order to maintain their leukemia treatment skills. An AML-specific quality-of-life questionnaire administered to 82 patients undergoing active treatment revealed that family support, friends, and community were the most important patient support systems.[24] As such, the added advantage is preventing inconvenience and travel hardship for patients and their families and thus would be attractive to both parties. Another reason for providing care to this patient population is a financial incentive and would be to the advantage of the smaller hospitals, private practices, and providers.

Workup

Guidelines for workup of patients with AML are available, and a comprehensive evaluation should be performed at diagnosis to make the best treatment decisions.[25,26] In our setting, if leukemia is a strong consideration, we suggest that the referring physician not perform the bone marrow biopsy but transfer the patient so that the procedure can be carried out in an academic hospital. This would also give the academic center an opportunity to conduct a comprehensive evaluation to help guide therapy and eliminate undue discomfort in the patient and duplication of the testing. Also, it offers the opportunity to screen and enroll the patient in a clinical trial if available or appropriate.

Fit and unfit patients

The treatment of AML is obviously going to be different in young, fit patients with no comorbid conditions. Similarly, older patients (aged >60 to 65 years) who are fit with no comorbid conditions may be treated like the previous group, aiming for cure and considered for stem cell transplant if applicable. Younger patients with severe comorbid conditions who are deemed unfit and older patients who are perceived to be ineligible for intensive treatment will be treated in a more palliative mode. Such patients can be treated in the community setting to allow patients to be closer to home and loved ones.

Treatment of AML in young and older fit patients

Treatment of young and older fit patients depends, for the most part, on the patient's goals and where the diagnosis is made. If the patient is diagnosed at an academic teaching hospital, management will be provided most likely at the diagnosing center. This would include the whole array of services, such as state-of-the-art diagnosis, induction, subsequent therapies as appropriate, and possibly enrollment in a clinical trial.

If patients are treated in community leukemia-treating centers, the programs are very capable of giving induction and consolidation, and some may also have access to cooperative group and industry-sponsored trials. But these centers, for the most part, do not treat many patients in any given year and generally seek advice in managing them. The centers tend to contact an academic center before initiating therapy to discuss what might be the ideal induction and consolidation regimens, as well as to seek advice should the patient have residual disease on a day 14 bone marrow biopsy or at the time of count recovery. In addition, they tend to refer patients to an academic center for evaluation for HCT, most probably at the end of leukemia induction, and for clinical trials in the event of failure of conventional treatment.

If the patient is diagnosed in a non–leukemia-treating center or private practice office, more likely than not the patient will be referred to an academic center. Given the distance and the constraints it places, these patients will receive their induction and consolidation most likely in the academic center. Due to the inconvenience distance causes to the patient and the family, we administer the consolidation at our facility but have the recovery laboratory tests, blood transfusions, and supportive care done at the local facility. There is a practical disadvantage to this approach should the patient develop a complication such as neutropenic fever or sepsis during the recovery time. The smaller hospitals may not have the infrastructure needed to manage complications, and the time it takes to get these patients to a larger center could be deleterious and compromise patient outcome. However, there is published data that supports this approach and reduces the burden of patient travel without compromising outcome.[27]

In the case of resistant disease, one option would be to coordinate with the non–leukemia-treating facility for the administration of an HMA along with targeted oral agents such as FLT3 inhibitors, IDH inhibitors, and venetoclax when appropriate.[28,29] However, it would help to discuss the toxicities associated with these drugs, such as cytopenias and differentiation syndrome, and also to discuss appropriate doses.[30]

In essence, for a fit patient, the options are treatment at an academic center or a community leukemia treatment center. Close collaboration between an academic center and community leukemia treatment center is essential. This facilitates enrollment of patients in clinical trials and management of resistant disease effectively and efficiently, and it helps in making the transition to HCT seamlessly if that is required.

Elderly and unfit patients

On the basis of available data, acute leukemia is seen in 1.3 per 100 000 people younger than age 65 years and in 12.2 per 100 000 older than 65 years of age.[31] The median age at diagnosis of AML is 66 years, and the incidence increases with age, with over half of the patients diagnosed at age 65 years or older. The prognosis in patients older than 65 is poor, with a median survival of 3 months. Further breakdown shows that the survival is slightly higher for patients aged 66 to 75 years (6 months) and is 2.5 months for patients aged 76 to 89 years. Merely 5% of patients older than 65 years of age are alive 5 years after their initial diagnosis.[1,32] Given these data, only half of Americans aged >65 years with newly diagnosed AML, and only 10% to 20% aged >80 years, receive AML-specific therapy.[33,34]

Older patients also have comorbid conditions that make them both older and unfit, and the general perception is that they are unlikely candidates for intensive therapy. Patients with comorbid conditions tend to be taking multiple medications (polypharmacy) that can cause drug–drug interactions, poor tolerance of chemotherapy, and a negative impact on overall survival.[35] However, data from the Swedish Acute Leukemia Registry showed that a certain subset of patients would benefit from intensive therapy at diagnosis. Similar results were also found in a population-based study in the United States.[33,36]

Because there is evidence to suggest that some of these patients may benefit from intensive treatment, it is reasonable to see patients aged <80 years at least once in an academic center to make a determination about whether such therapy would be appropriate. Applying one of the available tools for complete assessment of comorbidities, such as the Hematopoietic Cell Transplantation–Comorbidity Index or a geriatric assessment tool, would be reasonable and would provide a more precise prediction of induction mortality.[37,38] In addition, the academic center has the opportunity to perform a thorough pathologic evaluation to include molecular markers and next-generation sequencing before making a therapeutic decision. In patients with a lower white blood cell count, waiting for the cytogenetic and molecular results is not detrimental before initiating any form of therapy and offers valuable information that may influence treatment options and prognosis, so it should be encouraged for all patients. Those with very proliferative disease may be managed transitorily with hydroxyurea and aggressive supportive care while awaiting test results.[39] In older patients with a high comorbidity index, treatment-related mortality from intensive induction would be unacceptably high. Patients with secondary leukemia and high-risk cytogenetics are less likely to respond to intensive therapy. Hence, patients with a high risk of induction mortality and patients unlikely to respond would be the most likely groups to receive treatment in a community hospital or a private practice office. Still, support from an academic center would be valuable because treatment options such as glasdegib + LDAC or venetoclax plus either HMAs or LDAC may offer a survival benefit. Because of the intricacies of these regimens, optimal management is required to optimize benefit and minimize risks. Guiding the continuation of therapy in patients with glasdegib + LDAC that may require up to 6 cycles to demonstrate its best outcome, or deciding when to interrupt therapy with venetoclax or to start the next cycle, or when to adjust the dose of venetoclax may benefit from discussion with academic colleagues with significant experience with these regimens. In general, single-agent and combination low-intensity treatments would be considered for these patients.

Extreme elderly patients >80 years of age

This group, given their advanced age and comorbid conditions, tends to do poorly, with a median survival in the 2- to 3-month range. However, a carefully selected group of octogenarians may benefit from therapy.[40] Academic centers are frequently asked to render an expert second opinion, more so upon the insistence of family members, so that all the available options can be considered. These patients should be evaluated carefully, and the patient and the family should be engaged in an honest discussion regarding the prognosis, outcome, and, more important, the commitment required to undergo such therapy, as well as the real expectations with new therapies available for patients unfit for standard therapy. Patients and their families, for the most part, have not been through such circumstances previously, and this is uncharted territory. The visit could be used as an opportunity to educate the patient and family regarding the treatment, benefits, toxicity, and, more important, the need for multiple visits to the office and admissions to the hospital. Such honest engagement from an expert sets the expectations and has the potential to make it easier for the oncologist in the community.

Community options available for older unfit patients

LDAC

The Medical Research Council's AML-14 trial compared LDAC with the best supportive care for patients with untreated AML. This study showed that patients who received LDAC had a response rate of 18% vs 1% for best supportive care, leading to improved survival.[41] It should be mentioned that the group which had a response had a survival advantage. This continues to be the standard of care and could be applied to patients with minimal toxicity in the community. The possibility of self-administration may be attractive to some patients.

HMAs

Since their approval in 2004 and 2008, azacitidine and decitabine, respectively, have found increasing application in the treatment of unfit patients with AML. It is fair to say that the availability of these agents over the last 10 to 15 years has set the stage for a gradual transition of leukemia care in the community, and the recent approval of the oral combination of decitabine and cedazuridine may only strengthen this. In the AZA-AML-001 trial, older patients with AML with >30% leukemia cells in the bone marrow were randomized to receive either azacitidine or conventional care regimens. Median overall survival in the azacitidine arm was 10.4 months compared with 6.5 months in the conventional care arm. Similarly, the DACO-016 study compared the efficacy and safety of decitabine with best supportive care or LDAC in older patients ineligible for intensive chemotherapy. This study also showed an improvement in the median overall survival (7.7 months vs 5 months).[5,6] This is by far the most commonly applied treatment in older unfit patients in the community. Patients with AML with certain mutations, such as DNMT3A, TE T2, and TP53, are more likely to respond to the HMAs, and pointing this out to practitioners in the community may be valuable. Importantly, the clinical benefit of HMAs may take 4 to 6 cycles to be observed.

FLT3 inhibitors

The currently approved drugs for AML are midostaurin and gilteritinib.[7,8] In younger patients, the addition of midostaurin to chemotherapy improved survival compared with chemotherapy alone. The benefits of combining this agent with azacitidine and decitabine are currently being studied.[42] Gilteritinib is also approved for relapsed refractory AML and is a very potent inhibitor of FLT3 internal tandem duplication and FLT3 tyrosine kinase domain. Both of these are oral agents and can be safely administered in community- and office-based practices, but careful attention to possible risks such as tumor lysis syndrome and liver toxicity, as well as adequate prophylaxis and management of infectious complications, is required. There are several other agents in this class being tested that could be available options in the future.

IDH inhibitors

Both the IDH1 and IDH2 inhibitors are now approved for treatment of AML.[9,10] Enasidenib and ivosidenib are currently being studied in combination with chemotherapy and other agents in AML. The key to use of these agents is to test patients for the expression of these markers. Early recognition of differentiation syndrome is required. It is also important to understand the need for continuation of therapy to obtain the greatest benefit even for patients with only stable disease.

GO

The CD33 antibody has somewhat of a checkered history. GO is the CD33 antibody most studied. Pooled data from several studies showed that it produced a survival advantage, and it was approved by the US Food and Drug Administration in the United States in 2000. However, it was withdrawn from the market in 2010 after one randomized study of GO showed that the toxicity was excessive and survival inferior compared with standard chemotherapy alone. The drug was reinstated in 2017 when subsequent control trials of GO combined with chemotherapy showed a benefit in event-free survival and overall survival.[43] Gemtuzumab has been studied in combination with chemotherapy and HMAs, but administration of the drug requires close monitoring, given its tendency to cause both cytopenias and veno-occlusive disease. Practitioners in the community should be cautioned regarding its toxicity, and support should be offered in managing the appropriate doses.

Venetoclax

This BCL-2 inhibitor was found to be active in combination with other drugs in treating AML. When combined with an HMA or LDAC, high rate of responses have made them standard treatment for patients unfit for standard chemotherapy. The use of this option causes severe myelotoxicity and requires proper supportive care, including antimicrobial prophylaxis, optimal management of dosing and scheduling, and management of drug–drug interactions, with close communication with someone who has significant expertise that would be valuable if the patient is being treated in the community.[44]

Participation in clinical trials

This is an area where collaboration between community practices and academic centers could be of greatest benefit. Patients should be presented with options for clinical trials in most instances because the outcome with standard therapy, despite recent advances, remains poor. Studies conducted in rural and community settings have shown that accrual in clinical trials is <5%. Some of the reasons for poor accrual in the community include unavailability of trials, ineligibility, age, comorbid conditions, physician preference, distance, and financial considerations.[45,46] Academic centers have the potential to fill this need and also have the potential for biobanking, which should be encouraged whenever possible. This can be done at various stages of treatment to include pretreated samples and samples obtained in remission and at the time of relapse and resistance. Biobanking offers the opportunity to study the samples and understand response and resistance mechanisms. Additional areas of trial recruitment would include upfront trials for newly diagnosed patients, trials for resistant disease, and trials during and after bone marrow transplant. These can be made possible by encouraging community oncology programs to consider joining national community oncology research programs. This allows them to enroll patients in the cooperative group trial programs.

Commitment from academic centers

In order to optimize management of AML in the community, there has to be collaboration in several areas between the community practices and academic oncologists. It would be valuable to conduct periodic symposia to update the community regarding available resources at the academic center. These would serve as a platform for the academic providers, such as physicians, nurses, pharmacists, and advanced practice providers, to meet with their counterparts in the community. Academic centers not only should function as a resource in clinical care but also should show leadership in other areas, such as nursing, when new therapies become available. Being a resource to pharmacists in the community and exchanging information as well as periodic visits will benefit both groups. Administrative collaboration to discuss topics such as coding, billing, and collection is constructive and advantageous to both entities.

For the practitioner in the community, easy access to an academic leukemia expert is of paramount importance. It is common knowledge that providers in an academic center are often called on a Friday afternoon or on long holiday weekends with questions regarding sick patients who have to be sent to a tertiary center. A core group of experts from the academic center should be available as a resource to answer questions and be of assistance. With the coronavirus disease 2019 pandemic and the challenges it poses, along with the widespread use of telemedicine, quick consultation could possibly become easier. A process to transfer acutely ill patients and to execute this quickly should be present. In many of the smaller hospitals, however, little can be done for the patient with acute leukemia. Hospital diversion and unavailability of beds are stressors to community oncologists and cause undue pressure from patients and family members. This requires a commitment from the academic center to provide that support and to earn the trust of the referring physicians and community doctors. It also has to be emphasized that academic leukemia practices cannot survive without the support of the community practices. This partnership is crucial and benefits both parties equally. To fulfill the academic mission of patient care, research, and education, this concept has to be appreciated and not ignored or forgotten. In the end, the patients and their families benefit from this arrangement.

Conflict-of-interest disclosure

A.P.J. declares no conflicts of interest. J.E.C. consults with or has an advisory role with Amphivena Therapeutics, Astellas Pharma, Bio-Path Holdings Inc., BiolineRx, Bristol-Myers Squibb, Daiichi Sankyo, Jazz Pharmaceuticals, Novartis, Pfizer,Takeda, and received research funding from Astellas Pharma (Inst), Bristol-Myers Squibb (Inst), Daiichi Sankyo (Inst), Immunogen (Inst), Jazz Pharmaceuticals (Inst), Merus (Inst), Novartis (Inst), Pfizer (Inst), Sun Pharma (Inst), Takeda (Inst), Tolero Pharmaceuticals (Inst), and Trovagene (Inst). V.K.K. has provided consultancy or in an advisory board role for Novartis and Pfizer.

Off-label drug use

None disclosed.

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Practice patterns and outcomes for adults with acute myeloid leukemia receiving care in community vs academic settings

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Consistent with observations in other disease settings, retrospective studies have indicated that treatment outcomes for adults with acute myeloid leukemia (AML) are better in higher- vs lower-volume hospitals and academic vs nonacademic centers, with greatest benefits noted in acute promyelocytic leukemia. Younger age, more frequent receipt of chemotherapy and hematopoietic cell transplantation, and differences in comorbidities and socioeconomic factors may partially account for these differences. With new therapeutic options including oral small molecule inhibitors and parenteral drugs suitable for outpatient administration, there is increasing interest from patients and physicians in treating AML in the community setting and avoiding referral to academic centers. This may be particularly true for older adults, for whom treatment rates in the community have historically been low, and for those with comorbidities, because treatment benefits are estimated to be low, and thus travel to academic centers is perceived as especially burdensome. How the volume-outcome relationship is affected by the shift of the treatment landscape in AML over the last few years is unknown. Additionally, improvements in supportive care (transfusion support, broad-spectrum oral antimicrobials), resulting in gradually decreasing early death rates over time, and the growing focus on the impact of AML therapy on quality of life and treatment cost concerns further fuel the larger trend toward an increasing proportion of care delivered in the outpatient setting. Here, we examine whether the current shift of administering chemotherapy and supportive care to the outpatient setting can be translated to the community setting without compromising patient outcomes.

LEARNING OBJECTIVES

- Understand the impact of center type on outcomes in patients with AML
- Recognize characteristics of academic centers that may account for differences in outcomes compared with community settings
- Consider how emerging diagnostic and monitoring techniques, together with the availability of new drugs, will affect care delivery to AML patients in the future

Introduction

Until recently, treatment options for acute myeloid leukemia (AML) were relatively limited, and decision making followed an algorithm in place for almost 50 years.[1,2] For medically fit patients, cure was assumed possible with intensive chemotherapy and possibly allogeneic hematopoietic cell transplantation (HCT). Because of transfusion needs and risks of disease/treatment-related complications, patients receiving intensive therapies typically remained in the hospital, either at academic centers or nonacademic facilities, until resolution of cytopenias. In contrast, if the patient was judged medically unfit, cure was considered rare, prompting either nonintensive chemotherapy, most commonly low-dose cytarabine or single-agent azacitidine or decitabine, or an approach purely focused on supportive care.

Since 2017, the US Food and Drug Administration has approved 8 new drugs for AML in the United States.[3] With these, treatment options have substantially increased, and the line dividing intensive and less-intensive therapies has become less clear. With oral small-molecule inhibitors and parenteral drugs suitable for outpatient administration included, and helped by improvements in supportive care with resulting declines in early death rates, interest is mounting from patients

and physicians in treating AML in the community. This may be especially true for older adults and/or those with comorbid illnesses, because expectations for treatment benefits may be low; hence, travel to and treatment at academic centers be perceived as particularly burdensome. Treatment rates for such individuals in the community have historically been low. This trend is further fueled by an increasing interest in the impact of AML therapy on quality of life (QOL) and growing concerns over costs. Here, we review the evidence for and against the need to treat AML at academic centers and examine whether the current shift of transitioning therapy and supportive care to the outpatient setting can be translated to the community without compromising patient outcomes.

Volume–outcome relationship in AML

For many medical conditions and surgical procedures, both in oncologic and nononcologic settings, numerous studies and meta-analyses have shown a strong correlation between increasing patient volumes and better outcomes.[4,5] A study reported several years ago[6] suggested AML is no exception to this. A more recent analysis of patterns of care and clinical outcomes with conventional induction chemotherapy (IC) across diverse practice settings supports this conclusion by showing AML patients treated in high-IC–volume hospitals were less likely to die or be discharged to hospice than those treated at low-IC–volume hospitals.[7]

Rather than examining patient volume in the strict sense, however, most studies (all retrospective) have compared academic with community centers, with individual studies differing in how to define academic centers (patient volume vs designation as comprehensive cancer center by the National Cancer Institute or academic center by the Commission on Cancer), complicating comparisons and data interpretation. As one example, data from >60 000 AML patients treated in the United States between 2003 and 2011 suggested lower early death risks and better 1- and 5-year overall survival at academic centers.[8] The benefit was greatest for patients with acute promyelocytic leukemia (APL), consistent with previous observations.[9,10] However, although many studies have suggested better outcomes at higher-volume or academic institutions, findings have not been entirely uniform. In the European Organization for Research and Treatment/Gruppo Italiano Malattie Ematologiche dell' Adulto AML 8A study, for instance, which included patients treated at both transplant centers and referring centers (analogous to community centers), early death rates were higher and initial remission rates lower at referring centers, but remission rates after the second induction course and 6-year overall survival were similar.[11]

There are many reasons why AML patients may do better with nontransplant therapies at academic (or higher-volume) centers (Table 1). For one, patients treated at academic centers, especially when older, are more likely to receive chemotherapy than their counterparts in the community.[12,13] Furthermore, physicians, other medical providers, and supportive and ancillary staff (eg, physical therapists and nutritionists) at academic centers may have more experience managing disease/treatment-related complications of AML and have greater access to multidisciplinary teams and subspecialists dedicated to managing challenging complications The observation of lower early mortality when treated at academic centers[8] supports the notion of better supportive care playing a pivotal role in survival differences, with one study showing half the risk of early death at National Cancer Institute–designated cancer centers

Table 1. Potential reasons for improved outcomes at academic centers

Diagnostics	Improved access to molecular testing More rapid return of molecular testing results
Therapy	Older/comorbid patients more likely to receive antileukemia therapy Faster access to lifesaving drugs (eg, ATRA) Improved access to allogeneic transplant More clinical trial options
Complications	More resources for management of complications Multidisciplinary team with expertise Ancillary support staff Consulting specialists Early diagnosis of and expertise in managing complications (eg, differentiation syndrome, fungal infections) → decreased early death Standard operating policies (eg, antimicrobial prophylaxis, line care)

ATRA, all *trans* retinoic acid; DIC, disseminated intravascular coagulation.

compared with private hospitals with lower rates of renal failure, respiratory failure, and cardiac arrest[14]. Similarly, another study found patients treated at higher-volume hospitals were more likely to undergo bone marrow assessment and receive prophylactic antimicrobials than those treated at lower-volume hospitals.[7] Whether better access to clinical trials at academic centers translates into better outcomes (trial effect) remains controversial[13,15-17]; restrictive eligibility criteria for trial participation may bias such analyses.[18]

Perhaps unsurprisingly, the center effect in AML extends to survival outcomes with allogeneic stem cell transplantation (HCT), which are closely linked to overall AML outcomes.[19] Whether such benefits extend to other post-HCT composite endpoints such as graft-versus-host disease–free, relapse-free survival is, although plausible, currently unknown. Underlying reasons may include that patients treated at academic centers more likely undergo allografting, possibly because of earlier HLA typing and donor identification, easier care coordination facilitating the transplant workup and reducing the time to transplant, and access to a broader range of transplant protocols. Moreover, expansion of eligibility for allogeneic HCT (eg, to include larger numbers of older adults) combined with the fact that older patients are more likely to receive antileukemia therapy may contribute to improved overall outcomes at academic centers.

Attempts to decipher whether outcomes are better at higher-volume/academic centers are complicated by the increasing heterogeneity within academic settings as private oncology practices are bought by academic health systems, because this academic affiliation does not necessarily come along with the expertise or supportive care capabilities that long-standing academic centers provide. There are also likely unaccounted-for differences in the characteristics of patients treated at different sites. These confounders could be addressed by multivariate analyses accounting for prognostic covariates and site of treatment, but such models have limited prognostic ability, indicating many important prognostic factors remain unknown. Only randomization between treatment at

academic or community centers can account for such latent variables.

Are there distinct patient subsets that derive particular benefit from treatment at higher-volume or academic centers?

Certain subsets of leukemia patients have unique needs and challenges and may particularly benefit from the treatment environment offered at high-volume centers. As mentioned above, this is particularly true for patients with APL when treatment is initiated at academic centers.[9] This may be partially reflected in the substantially lower early death rates observed in the context of clinical trials (which are largely conducted at academic centers) compared with those seen in the general APL population,[20] although selection bias may play a role as well. Earlier diagnosis, faster availability and initiation of ATRA,[21] and improved diagnosis and management of complications (eg, disseminated intravascular coagulation, differentiation syndrome) may be contributing factors. Another distinct subset of patients particularly benefiting from higher-volume/academic centers are adolescents and young adults (AYA). As one example, one study evaluating outcomes of AML patients 18 to 39 years of age reported improved survival specifically in those with good-risk cytogenetics and those with APL when treated at academic centers, with the latter having half the risk of early death compared with patients treated at community centers.[10] Similarly, available evidence suggests that AYA with acute lymphoblastic leukemia (ALL) likewise benefit from treatment at academic sites, even after controlling for sociodemographic features.[22] Conceivably, this observation is closely linked to better outcomes of AYA with ALL following pediatric-inspired treatment regimens, which, because of their complexity, are more likely to be given at academic sites.[23] Increased enrollment on clinical trials of AYA with ALL at academic sites may also be contributing. Although data are lacking, it is likely that the same features that contribute to better outcomes in AYA with ALL and AML in general (better access to diagnostic testing and complex supportive care) may also apply to older adults with ALL given the complexity of the treatment regimens, many of which are now administered in the outpatient setting.

Several studies have evaluated whether improved outcomes seen at academic centers extend to older adults, for whom travel to these centers might be more burdensome and overall treatment outcomes worse. Prospective and registry data suggest that such patients do benefit from both intensive and less-intensive chemotherapy compared with no therapy.[24-26] However, intensive induction strategies are very rarely used in the community setting. In fact, most older patients with AML do not receive any type of AML-directed therapy, as indicated by data from a large retrospective Surveillance, Epidemiology, and End Results Medicare study showing only 40% of adults >65 years of age received anti-AML therapy within 3 months of diagnosis.[27] Results were similar in an analysis of US community oncology practice data,[12] with another Surveillance, Epidemiology, and End Results study showing >50% of AML patients >65 years of age received no anti-AML therapy even 9 years after azanucleosides (eg, azacitidine and decitabine) became available. Patients living in large metropolitan areas (with easier access to academic centers) were more likely to receive treatment, as were patients with a previous diagnosis of a solid or hematologic malignancy despite reduced performance status, possibly because of already having established specialist care.[28] Finally, for those who do receive azanucleoside therapy, published dose schedules are often not adhered to in the community (partially related to limited weekend infusion hours), and only a minority of patients surveyed in a recent population-based study in the United States received the recommended ≥4 cycles of therapy, potentially limiting the efficacy of these agents.[29]

Transition to outpatient delivery of intensive AML-directed therapy and supportive care

Historically, intensive therapy for AML has been delivered in the hospital in both academic and community settings because of the need for frequent transfusions and the likely occurrence of treatment/disease-related complications. However more recently, with improvements in supportive care such as introduction of broad-spectrum oral antifungals, ready availability of high-quality blood products, approval of new antileukemia drugs, and increasing focus on QOL[30] and treatment-associated costs, there has been a shift in care patterns with increasing efforts to administer chemotherapy and supportive care in the outpatient setting.

With availability of new drugs such as CPX-351 that have limited immediate toxicities and relatively convenient dosing schedules, there are now intensive treatment options that can be administered in the outpatient clinic even to older patients, both in the community and academic settings. Difficulties in recovering inpatient costs provide an additional incentive, prompting many centers to shift to outpatient administration of CPX-351,[31] with pilot data suggesting patients may remain outpatient after therapy,[32] although some centers currently routinely admit patients to the hospital for monitoring once CPX-351 is administered. Likewise, although treatment with venetoclax in combination with either low-dose cytarabine or an azanucleoside can be given in the outpatient setting, validated guidelines on how best to administer such therapies are currently missing, and many institutions admit patients routinely at the beginning for close monitoring of potential treatment-related complications (tumor lysis syndrome). Nonetheless, even conventional intensive induction and postremission therapy can be safely delivered in the outpatient setting in many patients.[33,34] However, given the complexities surrounding drug administration schedules and management of postchemotherapy care, a multidisciplinary team including social workers, nurses, pharmacists, and providers with expertise in the care of AML (largely available in academic centers only) is critical for successfully implementing this approach.

Despite this interest in administering newer therapeutics in the outpatient setting, most patients are still hospitalized for prolonged periods of time after intensive induction chemotherapy because of disease/treatment-related cytopenias, transfusion needs, and management of related complications.[35] This may, however, not be necessary for many patients. Based on multiple small studies suggesting feasibility of outpatient management after conventional induction chemotherapy,[36] we conducted 2 prospective clinical trials at our institution evaluating an early hospital discharge (EHD) strategy within 3 days after completion of intensive induction chemotherapy.[37,38] These studies supported the notion that early transition to outpatient care is feasible, safe, and associated with reduced care costs, which has since become standard of care at our

institution, logistics permitting. We recently evaluated our experience with this approach in the 4-year period since we completed our trials, with the application of the EHD strategy to a much broader patient base than was captured in the prospective trials, with confirmation of safety and reduced medical resource use.[39] Of note, we found no significant differences in care needs for patients undergoing initial induction treatment and those receiving postremission therapy.[40] This suggests that an EHD care strategy after induction therapy may be practically (and safely) implemented at many institutions that already have the infrastructure available enabling them to manage patients in the outpatient setting after standard postremission chemotherapy. At our institution, infrastructure available to support outpatient management of AML patients during the time of prolonged pancytopenia includes 24-hour phone access for patients to a provider familiar with outpatient management of AML, an infusion center with extended daily hours (including weekends and holidays) for transfusion needs, and the ability to rapidly evaluate and initiate treatment of neutropenic fever in the outpatient clinic before hospital transfer. These features (summarized in Table 2) are more likely present at academic than community cancer centers.

Unlike induction therapy, follow-up care after postremission chemotherapy has already shifted to the outpatient setting at both academic and community centers,[35] with multiple studies demonstrating feasibility, safety, and cost-effectiveness. For some patients, receiving outpatient care at the center where the chemotherapy was administered may pose logistic challenges. Here, a shared care model may address this barrier by allowing patients to receive their supportive care after postremission therapy at community centers closer to their homes.[41] Finally, the COVID-19 pandemic has forced rapid improvements in technology supporting telehealth, along with reimbursement for this service, potentially allowing academic sites to oversee some aspects of patient care with less travel for patients. How the rapid increase in access to telehealth will play out for the care of leukemia patients over the next few years remains unknown.

What about other major drivers for the shift from inpatient to outpatient care: QOL and health care costs? Studies in patients with hematologic malignancies undergoing autologous or allogeneic HCT have shown hospitalization is associated with reductions in QOL and increased depression.[42] The same has been found in AML.[30] Although there are no data comparing QOL of AML patients treated at academic vs community centers, more time spent in the outpatient setting (and closer to home) for both treatment and follow-up care may lead to gains in overall QOL. The same might apply to care costs, which remain dominated by inpatient charges.[43] The shift to more outpatient care and potentially more community-based care that newer medications facilitate may ultimately offset at least part of their high costs.

Will treatment at a higher-volume/academic center remain important with new lower-intensity and/or oral drugs?

Many of the new drugs in AML are molecularly targeted agents that are given orally and can be used at various stages along the AML treatment path. Whether there is a measurable benefit when such agents are given at a higher-volume/academic center vs the community setting is unclear. Undoubtedly, the availability of effective oral drugs (eg, venetoclax and oral decitabine/azacitidine in the AML pipeline) used alone or along with parenteral lower-intensity therapeutics (eg, azanucleosides) will increase the proportion of patients treated at community centers, including older and less-fit individuals. Thus far, the risk/benefits of delivering lower-intensity therapies at academic vs community centers are unknown (an important research agenda item for the near future), especially because these therapies can still lead to prolonged cytopenias and toxicities; thus, the issue of which centers provide better supportive care will likely remain relevant. In what way a center's access to molecular testing influences treatment outcomes is also unknown. For some of the newer agents (eg, inhibitors of mutated IDH1/IDH2/FLT3), anti-AML efficacy is primarily seen in patients carrying the corresponding mutations in their leukemia cells. Appropriate use of these therapeutics therefore requires access to timely molecular testing. One could therefore argue better testing availability at academic institutions may ultimately translate into better outcomes for patients getting care at such centers. However, although some data suggest molecular testing is more widely available at academic centers,[44] there is no clear evidence yet linking this availability to improved outcomes. It also remains uncertain whether targeted therapy in general will yield longer survival than nonspecific clinical trials.[45] Finally, many of these new drugs are costly to patients (particularly the oral drugs that come with high copays) that many, especially older individuals, cannot afford. With their resources, academic centers may be better positioned to help them find financial support from foundations and pharmaceutical companies to obtain these therapies.

Table 2. Infrastructure required to support delivery of supportive care after intensive chemotherapy in the outpatient setting

Inpatient management	Nursing education on treatment roadmap, expected complications CVC education and training Clear written discharge instructions with contact information for nonurgent and emergent situations Clear communication with outpatient team
Outpatient management	SOP for CVC care, antimicrobial prophylaxis, transfusion thresholds, management of neutropenic fever 24-h phone access to experienced provider in AML for emergencies Regular care team available for 3 times per week visits and nonscheduled evaluation of symptoms Infusion center with extended daily and weekend/holiday hours for frequent monitoring and transfusion Blood bank with large transfusion capability and rapid delivery of blood products to clinic setting Ability to rapidly evaluate and initiate treatment of neutropenic fever in clinic (eg, antimicrobial cocktail available for rapid administration before hospital transfer) Multidisciplinary expertise (infectious disease, pulmonary) in management of AML and therapy complications Ancillary support staff with expertise in AML management: nursing, social worker, pharmacists, physical therapists, nutritionists

CVC, central venous catheter; SOP, standard operating policy.

Conclusion and future perspective

Increasing access to new drugs has shifted the care of AML patients from academic to community settings. Thus far, this change in care pattern remains unsupported by data, especially for older and less fit patients who historically have not received antileukemia therapy in the community. Some of the potential advantages that academic centers provide (access to more rapid molecular testing, a broader range of clinical trials and allogeneic HCT, greater disease expertise, and availability of multidisciplinary teams for supportive care) have to be weighed against potential advantages of community settings (eg, less disrupted life, better family support, and QOL). With the rapidly changing treatment landscape in AML, the pros and cons of academic vs community setting treatment will need to be revisited constantly, ideally via randomized trial, as challenging as this would be. In the absence of strong data arguing for/against a particular care scenario, a shared academic-community care approach may currently best serve the interests of many patients.

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Conflict-of interest disclosure

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Off-label drug use

None disclosed.

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Updates in infection risk and management in acute leukemia

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Patients with hematologic malignancies are at increased risk of infection, with associated morbidity and mortality. Patients with acute myeloid leukemia (AML) have qualitative and quantitative deficits in granulocytes predisposing to bacterial and fungal infections. Acute lymphoblastic leukemia results in qualitative deficits in lymphocytes, resulting in hypogammaglobulinemia and reduced cell-mediated immunity predisposing to certain bacterial and viral as well as fungal infections. Chemotherapeutic regimens often compound these deficits, result in prolonged periods of severe neutropenia, and disrupt mucosal barriers, further elevating infection risk. Despite advances in antimicrobial therapies and prophylaxis, acute leukemia patients with disease- and treatment-related immunosuppression remain at risk for life-threatening infection, including with resistant organisms, antimicrobial-related adverse events, and higher treatment costs. Additionally, our knowledge of infection risk and drug-drug interactions with new immune-targeted cancer therapeutics is evolving. Here, we review 3 areas in which standard practice is evolving as challenges arise and new experience is gained, including antibiotic use in febrile neutropenia, fungal prophylaxis, and use of targeted therapies.

LEARNING OBJECTIVES

- Review the management of febrile neutropenia including early de-escalation of broad-spectrum antibiotics
- Review important issues in prophylaxis of fungal infections in patients with acute leukemia
- Review infection risk and management considerations with some targeted therapies for treatment of acute leukemia

Introduction

Neutrophils are a critical component of the innate immune system. Qualitative deficits from the underlying malignancy compounded by periods of neutropenia from chemotherapeutic agents are major risk factors for development of bacterial and fungal infections in patients with acute leukemia. The degree and duration of neutropenia correlate with infection risk, particularly for invasive fungal infections. Antimicrobial prophylaxis is used to reduce the risk of life-threatening bacterial and fungal infections, particularly in patients with disruption of the gut mucosa. During treatment, most patients experience long-term antimicrobial exposure, which can lead to adverse effects, drug-drug interactions, added costs, altered gut microbiome, and increased risk for infection with multidrug-resistant organisms requiring shifts in management strategies. Additionally, the chemotherapeutic field is changing as well, with increasing use of immune-targeted therapies for treatment of acute leukemia. These therapeutics act on many different targets and with a theoretical consequent risk of infection, though it is often difficult to ascertain true infectious risk given confounding risk from underlying disease state and prior immunosuppressive therapies. As experience is gained with targeted therapies, there is growing evidence for an association with some agents and susceptibility to infection, whereas for others, clear correlation is lacking. Identifying the best practice for prevention and management of infectious complications during treatment of acute leukemia in this changing landscape creates a clinical challenge requiring the collaboration of specialists in infectious diseases, hematology, and pharmacy.

Clinical case

A 56-year-old man with no past medical history presented to his primary physician with 2 months of progressive fatigue. Laboratory tests revealed a "very high white count" and he was referred to the emergency department. His

white blood cell count was 83.5 × 10^3/μL of blood with 65% blasts, his hemoglobin was 8.4 g/dL, and his platelet count was 35 × 10^9/L. A bone marrow biopsy revealed a hypercellular marrow (>95% cellularity) with 50% myeloid blasts with normal cytogenetics. He received 7+3 induction with cytarabine and daunorubicin, with levofloxacin, posaconazole, and acyclovir as antimicrobial prophylaxis. His course was complicated by febrile neutropenia (FN), treated with empiric cefepime. His fever resolved and no infectious agent was identified after 72 hours; cefepime was de-escalated to levofloxacin until neutropenia resolved. He achieved complete remission and underwent postremission chemotherapy.

Antibiotics for FN and their de-escalation

FN occurs in >80% of patients undergoing chemotherapy for acute leukemia. Despite improved diagnostic abilities in the last decade, an infectious etiology is identified in <50% of episodes.[1,2] Empiric broad-spectrum antibiotic (BSA) therapy is universally recommended in patients with FN.[3-5] Many studies have evaluated the best treatment regimens, and a number of consensus practice guidelines with stratified antibiotic recommendations are available. However, there is no consensus on duration of empiric treatment when patients clinically improve and no infectious etiology is identified.[3,6-8] Based on prior guidance, practice has typically been to continue antibiotics until resolution of both symptoms and neutropenia[4]; we now recognize increasingly that in patients with prolonged neutropenia, this leads to long-term antibiotic exposure, which can be associated with antibiotic-related adverse events, selection for multidrug-resistant organisms, and alteration of the microbiome. The concept of earlier de-escalation or discontinuation of BSAs is not new. A number of small studies indicate that de-escalation after 72 hours is safe in clinically stable patients with no infection identified, regardless of ongoing neutropenia and in some cases ongoing fever.[9-14] In recent years, early de-escalation has gained more traction. Guidelines from the European Conference on Infections in Leukemia advocate de-escalation after 48 hours if no infection is identified, regardless of anticipated duration of neutropenia.[3] Since then, there have been additional attempts to assess the safety and feasibility of this approach. Studies have varied in methodology, but all have concluded that de-escalation to prophylaxis in patients with resolution of fever and no documented infection after 48 to 72 hours does not lead to a significant increase in subsequent bacterial infections, clinical decompensation, or in-hospital mortality.[12,15-19] National Comprehensive Cancer Network (NCCN) guidelines now also suggest that clinically stable patients with persistent neutropenia without fever can be evaluated for discontinuation or de-escalation of BSAs to prophylaxis in some settings.[5]

Fungal prophylaxis

Invasive fungal infections (IFIs) are a major cause of morbidity and mortality in patients with acute leukemia. Patients with acute myeloid leukemia (AML) in particular are at increased risk of IFIs due to profound and prolonged duration of neutropenia, as well as the use of purine analogs in treatment.[20] Azoles are the most common agents used for prevention and treatment of fungal infections during chemotherapy. Fluconazole, a first-generation triazole, is commonly used due to low cost and toxicity, but emergence of resistant *Candida* species and lack of activity against molds are limitations. Fluconazole is effective in decreasing *Candida* infection in transplantation and in patients with graft-versus-host disease, but studies have not shown benefit in preventing invasive mold infections.[20-22] Voriconazole is a second-generation triazole that has activity against some opportunistic molds and is a first-line agent for treatment of invasive aspergillosis. Voriconazole has not been approved for use as primary prophylaxis, with studies showing a non–statistically significant trend toward decreased IFI incidence compared with fluconazole.[23] Voriconazole has excellent bioavailability, although use is complicated by toxicities and drug-drug interactions.[21]

Posaconazole is another second-generation, extended-spectrum triazole with activity against *Candida* and *Aspergillus* spp, as well as other invasive molds including *Fusarium* and *Mucorales*. In several studies, including a multicenter randomized trial, prophylaxis with posaconazole in neutropenic patients with AML or myelodysplastic syndrome receiving induction chemotherapy significantly reduced the rate of IFIs (2% vs 8%; $P < .001$) and showed survival benefit ($P = .04$) when compared with fluconazole and itraconazole.[24] Posaconazole is now recommended for primary fungal prophylaxis in patients with AML undergoing induction chemotherapy.[5,25,26] The newer formulation of posaconazole with the extended-release tablet allows for better bioavailability compared with the oral suspension.[27-29] Breakthrough IFIs have occurred, particularly at lower serum levels, thus therapeutic drug monitoring (TDM) may be warranted in obese patients or in those with concern for poor absorption.[30] A trough level after 5 to 7 days of therapy with a goal concentration of >0.7 μg/mL is recommended. Although to a lesser degree than voriconazole, posaconazole is a potent cytochrome P450 3A (CYP3A) inhibitor that can complicate its use with other CYP3A substrates, resulting in increased bioavailability of many drugs leading to toxicity.[31] Isavuconazole is a newer second-generation azole approved for treatment of both invasive aspergillosis and invasive mucormycosis. It is available in oral and IV formulations, has a broad spectrum of activity, and has a more favorable adverse effect profile and less significant drug-drug interactions compared with other triazoles. It is not routinely used for prophylaxis, although 1 study demonstrated safety and tolerability for use in high-risk patients.[32] Unlike other triazoles, isavuconazole does not induce prolongation of the QTc interval, but rather a dose-dependent shortening of unclear clinical significance, and thus may be an alternative option in high-risk patients limited by toxicity or baseline QTc prolongation.[33]

Although mold-active antifungal prophylaxis has become standard of care during neutropenic periods of most AML treatment regimens, there is no similar standardized recommendation during acute lymphoblastic leukemia (ALL) treatment. Patients with ALL are at intermediate to high risk, with the rate of IFIs ranging between 3% and 12%,[21] with higher rates in patients with longer duration of neutropenia, absence of antifungal prophylaxis, and relapsed disease. The strong inhibition of CYP3A4 by mold-active azoles can lead to significant toxicity when administered with several chemotherapy classes, particularly vinca alkaloids and alkylating agents, and targeted therapies such as tyrosine kinase (TK) inhibitors, which are mainstays in the chemotherapy regimens for ALL. In many instances, the azole would need to be discontinued prior to the chemotherapy initiation and not restarted until the chemotherapy agent has been discontinued and eliminated. In high-risk patients, the alternative use of an echinocandin or liposomal amphotericin may be warranted.[3,5]

Clinical case (continued)

At follow-up 9 months later, the patient's laboratory tests showed pancytopenia. A bone marrow biopsy showed a 60% cellular marrow with 80% myeloid blasts. The next-generation sequencing hematologic malignancy panel showed NPM1-W290Sfs*10 (variant allele frequency, 25%) and IDH2-R140Q (variant allele frequency, 43%). He was started on enasidenib and Infectious Diseases was consulted to determine antimicrobial prophylaxis.

Infection risks in targeted therapies

Biological-targeted therapies are those designed to act on a therapeutic target considered important in the pathogenic process of the disease. In recent years, an increasing repertoire of agents has changed the landscape of therapeutics for acute leukemias. As more experience is gained with these targeted drugs, there is growing evidence for an increased association between some agents and susceptibility to infection, whereas for others, clear correlation with infectious risk is lacking. Complicating the picture, many agents are being used for a wide array of disease processes, often in combinations and in the setting of numerous prior chemotherapy regimens and relapse making it difficult to delineate association with infectious risk (Table 1).

Therapeutics for AML

Ivosidenib and enasidenib are small-molecule inhibitors of mutant isocitrate dehydrogenase 1 (IDH1) and IDH2, respectively, and may be used to treat relapsed or refractory AML. Thus far, small studies to assess efficacy and safety have not demonstrated a clear increased risk for infection. This class is a substrate of CYP3A4 and when used in combination with strong inhibitors such as posaconazole, serum concentrations of the drug may be increased. Current recommendation is to avoid use of azoles when possible, but when use is required to reduce the ivosidenib dose.

Table 1. Infection risk, drug interaction and prophylactic considerations associated with the use of therapeutic agents for acute leukemia

Chemotherapy/Biological	Use	Infection risk	Interaction	Recommendations
Vinca alkaloids	ALL	Regimen related	Inhibits CYP3A4	Avoid with azole
Alkylating agents	ALL	Regimen related	CYP3A4/2C	Avoid with azole
BCR-ABL TK inhibitors (imatinib, dasatinib, nilotinib, bosutinib, ponatinib)	Ph+ ALL	Modest risk: bacterial infections, CMV, PJP, HBV reactivation	CYP3A4 inhibitor	No clear benefit from routine prophylaxis Screen for HBV infection Avoid with azole Monitor QTc
Anti-CD19 bispecific T-cell engager (blinatumomab)	ALL	HSV, VZV, CMV, PJP, PML, fungal per NCCN		Consider ACV and PJP prophylaxis Screen for HBV
Anti-CD22 antibody drug conjugate (inotuzumab)	ALL	Risk similar to anti-CD20		No clear benefit from routine prophylaxis Screen for HBV infection High risk for VOD
CD19 CAR-T (tisagenlecleucel)	ALL	Increased risk for IFI, PJP, prolonged IgG hypogammaglobulinemia in long-term; distinguish infection from CRS		Acyclovir viral prophylaxis PJP prophylaxis Screen for chronic HBV Consider levofloxacin and fluconazole prophylaxis Consider anti-mold azole if high-dose steroids or prolonged neutropenia
BCL-2 inhibitor (venetoclax)	AML	Possible increased risk of fungal infections in absence of antifungal prophylaxis	CYP3A4	Avoid with azole If azole is indicated dose reduce venetoclax (>50%)
IDH1/2 inhibitor (ivosidenib, enasidenib)	AML	No clear increased risk of infection; distinguish infection from differentiation syndrome		Avoid with azole Monitor QTc
Hedgehog pathway inhibitor (glasdegib)	AML	No data	CYP3A4	Avoid with azole Monitor QTc
Anti-CD33 antibody drug conjugate (gemtuzumab)	AML	Prolonged myelosuppression		Monitor QTc High risk for VOD
FLT3-TK inhibitor (midostaurin and gilteritinib)	AML	No significant increased risk of fungal infection	CYP3A4	Monitor QTc Monitor for midostaurin toxicity and use posaconazole TDM

ACV, acyclovir; BCL-2, B-cell lymphoma 2; CAR-T, chimeric antigen receptor T cell; CMV, cytomegalovirus; CRS, cytokine release syndrome; HBV, hepatitis B virus; HSV, herpes simplex virus; IDH, isocitrate dehydrogenase; IgG, immunoglobulin G; Ph+, Philadelphia chromosome–positive; PJP, *Pneumocystis jirovecii* pneumonia; PML, progressive multifocal leukoencephalopathy; TK, tyrosine kinase; VOD, veno-occlusive disease; VZV, varicella zoster virus.

Glasdegib is a selective inhibitor of the Hedgehog signaling pathway with primary use in patients who are not candidates for intensive chemotherapy. There has been no additional infection risk associated with the use of this agent to date. When used in combination with strong CYP3A4 inhibitors, the serum concentration of glasdegib may be increased. Avoiding this combination if possible is recommended, but, if used, it should be monitored for prolongation of the QTc interval and other potential toxicities of glasdegib.

Gemtuzumab ozogamicin is an antibody drug conjugate targeting CD33 on the surface or normal and leukemic myeloid cells and blasts, which leads to profound and prolonged neutropenia and thrombocytopenia. To date, demonstrated rates of infection are comparable to other regimens causing neutropenia. Standard prophylactic strategies for patients with AML and neutropenia are recommended.[34]

FMS-like TK 3 (FLT3) inhibitors, including midostaurin and gilteritinib, have emerged as treatment options to improve survival in patients with FLT3 duplication mutations. Studies have not shown an increased risk of IFIs when used with induction or consolidation chemotherapy. These agents are metabolized by CYP3A4, and thus concomitant use with strong inhibitors can be challenging. Patients requiring azole therapy should be monitored closely for potential midostaurin-related toxicity, and TDM is recommended.[35,36]

Venetoclax is a B-cell lymphoma 2 inhibitor that can be used in combination for patients unsuitable for intensive chemotherapy. No clear increased risk of infection has been identified.[37] However, venetoclax can induce severe and prolonged marrow suppression. When used in combination with CYP3A4 inhibitors, significant dose reduction of venetoclax is required (up to 75% dose reduction with strong CYP3A4 inhibitors such as posaconazole).[38]

Therapeutics for ALL

BCR-ABL TK inhibitors, including imatinib, dasatinib, nilotinib, bosutinib, and ponatinib, are used for Philadelphia chromosome–positive ALL. These may be associated with myelotoxicity, increasing risk for bacterial and fungal infection. There is some inhibition of $CD4^+$ and $CD8^+$ T-cell proliferation, which impairs cytomegalovirus (CMV)- and Epstein-Barr virus–specific $CD8^+$ T-cell responses and proliferation. TK inhibitors significantly impair B-cell responses leading to less robust response to vaccinations. In studies evaluating the incidence of infectious complications, there appears to be a modest increase in the risk of infection, more so with dasatinib, notably CMV and hepatitis B virus (HBV) reactivation. Screening for chronic HBV infection is recommended prior to therapy. To date, there are no data to suggest a clear benefit in the routine use of anti-infective prophylaxis.[5,37]

CD-19 targeted agents, including blinatumomab, are designed to direct CD3-expressing cytotoxic T cells to CD19-expressing B cells resulting in B-cell depletion and reduction in immunoglobulin G levels and hypogammaglobulinemia. There may be some inhibition in B-cell–dependent T-cell activation similar to anti-CD20 agents. Thus far, CD19-targeted agents have not demonstrated a significant increased risk of infection compared with conventional chemotherapy for relapsed or refractory ALL, but the risk of herpesvirus reactivation (herpes simplex virus and varicella zoster virus) and *Pneumocystis jirovecii* pneumonia [PJP] warrants prophylactic acyclovir and PJP prophylaxis. Some centers also give antifungal prophy as well. Patients should be screened for HBV prior to initiation of therapy and monitored or treated accordingly.[39]

Inotuzumab is a CD22-directed antibody-drug conjugate that targets the CD22 antigen that is expressed on mature B cells and most B-cell blasts. Risk of infection is similar to those treated with anti-CD20 monoclonal antibodies such as rituximab. In early studies, there has been no increased incidence of infection demonstrated when compared with standard chemotherapy. There is no expected benefit from universal prophylaxis, although patients should be screened for HBV infection prior to initiation and managed accordingly.[34]

Chimeric antigen receptor (CAR)-engineered T cells are engineered to express a receptor recognizing a target protein on cancer cells for B-cell malignancies. Infections are common in CAR T-cell therapy but may be a result of the underlying malignancy, persisting depression in cell-mediated immunity, and prolonged myelosuppression from prior therapies, exacerbated by the need for corticosteroids and tocilizumab for management of cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome. Early infections after CAR T-cell therapy are more often bacterial, but risk for IFIs increases with prolonged neutropenia, and management would be per standard of care for these scenarios.[40] Acyclovir for viral prophylaxis and fluconazole are recommended, with mold-active azoles to be considered depending on clinical scenario, duration of neutropenia, and need for high-dose steroids.[5,40]

As new antileukemic chemotherapy- or immune-based therapeutics are introduced into the armamentarium, vigilance in determining associated infection risk needs to be delineated so that appropriate caution and prophylaxis are considered. Many initial studies are performed in patients with relapsed or refractory disease, making it difficult to determine the additional infectious risk attributable to these agents vs associated with the underlying disease process and/or prior or concomitant immunosuppressive therapies. In addition, this list of agents is not exhaustive, and new agents enter the pipeline every year. Each patient should be managed based on a comprehensive risk assessment including disease status and prior and current therapies to ensure best management, and Infectious Diseases consultation and pharmacy involvement are strongly encouraged.

Conflict-of-interest disclosure

R.T. serves on a Merck advisory board. The remaining authors declare no competing financial interests.

Off-label drug use

None disclosed.

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Approaches to aggressive B-cell lymphomas in less fit patients

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Treating unfit patients with aggressive B-cell lymphoma poses the dilemma of balancing potential cure while minimizing toxicity because of frailty and comorbidities. Age greater than 80 years and common comorbidities such as cardiovascular disease and poorly controlled diabetes mellitus often preclude the use of full-dose anthracyclines and steroids, the backbones of standard regimens for aggressive B-cell lymphomas. Assessing patient fitness remains subjective, with no consensus on best practice or how to integrate assessment tools into decision making. Incorporation of prephase steroids for all unfit patients may markedly improve performance status with consideration of standard dose therapy, especially in patients less than age 80. Although randomized studies are lacking, current data suggest patients age ≥ 80 years are considered unfit a priori and should receive dose-reduced anthracycline regimens or anthracycline-free regimens. Severe toxicity is highest after the first cycle of chemotherapy. Dose reductions for cycle 1 in unfit patients with plans to escalate as tolerated is often an effective strategy. Unfit patients often benefit from comanagement with gerontologists, cardio-oncologists, and endocrinologists depending on age and the nature of comorbidities. Palliative therapy for patients with newly diagnosed aggressive B-cell lymphoma results in median survivals of less than 3 months, and in general, should only be considered in patients with untreatable comorbidities such as advanced dementia or refractory metastatic solid tumors. Incorporating new, potentially less toxic agents such as novel antibodies, antibody–drug conjugates, and bispecific antibodies into first-line therapy is an exciting future direction with potential for substantial benefit in less fit patients.

LEARNING OBJECTIVES

- Compare the benefit of maintaining dose intensity in unfit patients with DLBCL aged <80 and ≥80
- Describe the outcomes with anthracycline-free regimens for unfit patients with DLBCL

Clinical case

An 84-year-old woman with a history of diabetes mellitus (DM), chronic kidney disease, hypertension, atrial fibrillation, and diastolic dysfunction with preserved left ventricular ejection fraction (74%) presented with epigastric pain, night sweats, early satiety, and a 5-lb weight loss. Computed tomography scan revealed an 8.6-cm liver mass, and a biopsy was consistent with diffuse large B-cell lymphoma (DLBCL), germinal center B-cell (GCB) phenotype, with no evidence of *MYC* rearrangement. International prognostic index (IPI) was 4, performance status (PS) was 2, lactic dehydrogenase level was 415 U/L, hemoglobin level was 9.7 g/dL, creatinine level was 1.48 mg/dL, and brain natriuretic peptide level was 2700 pg/mL. Before her diagnosis, the patient was the full-time caregiver for her husband, who has Alzheimer disease. The patient and her family were considering palliative treatment options. Would you offer potentially curative therapy? If so, what are the chemotherapy options and what information can you provide the patient regarding prognosis, possible complications, and treatment-related mortality (TRM)?

Introduction

Patients with aggressive B-cell lymphoma who are unfit represent a unique challenge, framed by the common dilemma of whether to administer intensive therapy with the potential for cure or to de-escalate therapy, thereby reducing toxicity.[1] The aging population has led to a substantial increase in the number of older patients with DLBCL, with 40% greater than 70 years of age, which is a group for whom frailty and comorbidities limit options.[2] Age greater than 80 and common comorbidities such as cardiovascular disease and DM often preclude the use of the standard R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone), with prednisone, vincristine, and

doxorubicin each posing special risks to vulnerable patients.[3] Although many comorbidities may be manageable during chemotherapy, especially with the support of endocrinologists, cardio-oncologists, and gerontologists, others such as advanced dementia or concurrent metastatic solid tumor may prohibit curative intervention for lymphoma. Guidelines for best practices for unfit patients continue to rely on single arm phase 2 studies, as well as retrospective and population-based data. The European Society for Medical Oncology recently released recommendations for the clinical management of elderly patients with aggressive lymphoma that provide general guidance applicable to less fit patients.[4] Decisions about whether to treat unfit patients with an anthracycline-based vs anthracycline-free regimen, and when to dose reduce, are complex and driven by concerns that comorbidities, impaired marrow function, poor PS, and impaired nutritional status will contribute to more frequent treatment-related complications.[5] Clinical trials often exclude the oldest and least fit patients, and no prospective randomized studies have addressed the appropriate regimen for this population. Additional challenges include the complexity and often labor-intensive nature of formal comprehensive assessments needed to categorize fitness accurately, as well as the lack of data to support use of these objective tools in medical decision making. This article will summarize treatment options for unfit patients with aggressive B-cell lymphoma including the use of prephase steroids and other supportive care measures, review data on the effect of dose intensity in older and less fit patients, and discuss strategies for choosing a regimen that optimizes efficacy while minimizing toxicity.

Assessment of patient fitness

Despite several proposed tools to assess patient's baseline status as fit, unfit, or frail, there is no uniform consensus on the optimal tool, how to integrate tools into decision making, and the impact of frailty assessments on patient outcomes. Traditionally, comprehensive geriatric assessments are time-consuming and often require consultation with a geriatrician. This may be unrealistic for many patients with aggressive lymphomas given the need to start treatment expeditiously and the complexity of obtaining expedited referrals. The International Society of Geriatric Oncology task force reviewed several screening tools to assess fitness and identified the G8 tool, which includes only 8 questions and age, as one of the simplest and most predictive assessments.[6,7] The Charlson comorbidity index, a weighted index that takes into account the number and seriousness (scale of 0-5) of comorbid diseases, is commonly used in assessing the extent and severity of comorbidities.[8] More recently, the Fondazione Italiana Linfomi group defined and validated a new Elderly Prognostic Index integrating geriatric and clinical assessment in 1353 patients age ≥ 65 years with DLBCL using a simplified Comprehensive Geriatric Assessment (sCGA) together with age to classify patients as fit, unfit, or frail (Table 1).[9] sCGA incorporates the activities of daily living (ADL) score (1 point for bathing, dressing, toileting, transferring, feeding, and continence), instrumental activities of daily living (IADL) score (1 point for ability to use the phone, shop, prepare food, keep house, do laundry, travel on public transportation, handle money, and take own medication), and Comorbidity Index Rating Scale.[1,9,10] Multivariate analysis confirmed the 3 sCGA groups, IPI, and hemoglobin < 12 g/dL were independent prognostic factors. Based on these independent factors, the Elderly Prognostic Index defined 3 groups of patients with a 3-year overall survival (OS) of 87% in the low-risk (0-1) group, 69% in the intermediate-risk group (2-4), and 42% in the high-risk group (5-7). Importantly, this model categorizes all patients age ≥ 80 as unfit or frail. Efforts are ongoing to develop an even simpler but meaningful geriatric assessment tool, sometimes referred to as a frailty vital sign, such as gait speed or grip strength that can be easily incorporated into routine physical examination.[11,12]

Table 1. Categorization of fitness and integration of age and sCGA to define prognostic groups by the Fondazione Italiana Linfomi

	Fit	Unfit	Frail
ADL	6	5	≤4
IADL	8	7-6	≤5
CIRS-G	0 of score 3-4 <5 of score 2	0 of score 3-4 5-8 of score 2	≥1 of score 3-4 >8 of score 2
Age	—	≥80 fit	≥80 unfit

	Fit	Unfit		Frail	
Age		<80	≥80	<80	≥80
sCGA group	1		2		3

CIRS-G, comorbidity index rating scale for geriatrics.
Reprinted from Spina et al[9] with permission from the American Society of Hematology.

Use of even these simplified tools is uncommon in routine practice, because of time constraints and lack of data on how to incorporate the results into decision making. In a prospective trial of 100 patients age ≥ 70 years with DLBCL, Spina et al[1] prescribed modulated chemotherapy based on the sCGA. Patients scoring less than 5 on either the ADL or iADL scale had a 50% dose reduction and those scoring 5 on the ADL scale or 5 to 6 on the iADL scale had a 25% dose reduction, with excellent outcomes and low toxicity rates reported. Despite the lack of randomized or confirmatory trials, these guidelines represent a reasonable quantitative approach to dosing for unfit patients. Quantifying ADL and IADL scores, as well as a mini-mental status examination score as standard practice, both at baseline and intermittently on therapy, would potentially allow objective guidance for dose escalation or de-escalation during subsequent cycles.

Prephase treatment

Treatment-related deaths in older patients undergoing chemotherapy for DLBCL occur most frequently after cycle 1. In a retrospective study of 530 veterans age 80 and older treated for DLBCL, there was an 18% TRM, with 67% (32 of 48) of deaths associated with the first cycle of chemotherapy; most of these were related to infection.[13] PS at diagnosis was the most significant predictor of TRM: 27% in patients with a PS of 2 to 4 vs 8% in patients with a PS of 0 to 1. Prephase treatment was first introduced by the German High-Grade NHL Study Group in the NHL-B2 trial when a high rate of infection and death after the first cycle of CHOP chemotherapy was noted in older patients on the study.[14] A trial amendment required a single injection of 1 mg vincristine and 5 to 7 days of prednisone, 100 mg daily, before

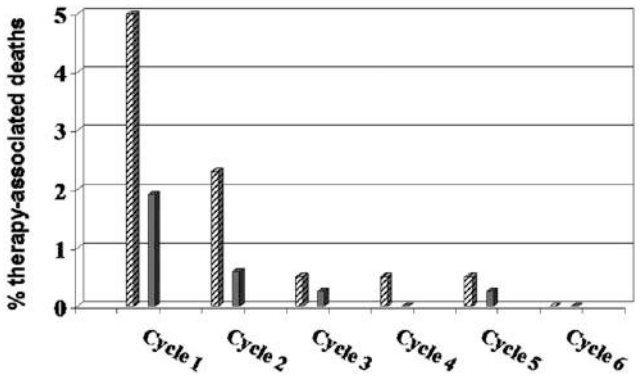

Figure 1. Therapy-associated deaths in the NHL-B2 trial of CHOP in DLBCL before and after the introduction of prephase treatment. Before (▨) and after (▩) the introduction of prephase treatment. Reprinted with permission. Reprinted from Pfreundschuh[14] with permission of the American Society of Hematology.

initiating CHOP. As shown in Figure 1, introduction of prephase treatment significantly decreased the incidence of TRM in the first 2 cycles.[15] Although not quantified, Pfreundschuh[15] also describes a lower incidence of tumor lysis after the addition of prephase treatment. In subsequent studies, the German High-Grade NHL Study Group eliminated vincristine and prescribed only prephase prednisone. Peyrade et al[16] incorporated vincristine 1 mg on day 7 and prednisone 60 mg on days −7 to −4 as prephase treatment in the ofatumumab miniCHOP study for patients age ≥ 80 with DLBCL, favoring a lower dose of steroids because of the increased risk of mania, psychosis, and hyperglycemia in patients over age 80. In this study, 5% of patients died on treatment, but none were treatment related, compared with the prior rituximab plus reduced-dose doxorubicin, cyclophosphamide, vincristine, and prednisone (R-miniCHOP) study, in which 27 of 150 (18%) of patients died on treatment, with 8% (12 of 150) due to treatment-related toxicity, mostly during cycles 1 and 2.[16,17] Other small series, not limited to older patients, have confirmed an improvement in PS and decrease in first cycle admissions with prephase treatment.[18,19] In patients with gastrointestinal involvement by aggressive lymphoma, prephase steroids may also decrease the risk of perforation and improve outcomes.[20] Despite the lack of randomized trials, there seems little downside to prescribing 5 to 7 days of prednisone, 60 to 100 mg/day for all unfit or older patients before initiating chemotherapy. Delaying decisions regarding dose reductions until re-evaluation of PS after prephase steroids may allow a subset of previously unfit patients to transition to standard dosing.

Anthracycline-based chemotherapy

For patients without cardiac contraindications, anthracycline-containing regimens remain the standard of care.[21] Lin et al[21] identified 9 retrospective cohort studies and 2 SEER-database derived analyses comparing chemotherapy regimens with and without anthracyclines in more than 11 000 elderly patients with DLBCL. With the limitations of inherent selection bias and the lack of objective assessment of fitness, collectively, these studies support an association between use of anthracycline-containing regimens and improved OS (3-year OS, 63% vs 44%) with acceptable toxicities.[21] Several other retrospective studies have tried to evaluate outcomes with and without anthracyclines based on measures of fitness. In a retrospective analysis of 135 patients with DLBCL ages 60 to 84, 53 (38%) were classified as unfit using CGA criteria.[22] Among patients treated with curative intent, 1-year progression free survival (PFS) was 83.7% for fit vs 66.5% for unfit patients (P = .011), with unfit patients having higher IPI scores. Outcomes for patients treated with palliative intent were dismal.

In an attempt to improve the tolerability of anthracycline-based regimens in the oldest patients, a large single-arm phase 2 study tested R-miniCHOP in 149 patients over age 80 with previously untreated DLBCL.[17] R-CHOP dose modifications included doxorubicin 25 mg/m^2, cyclophosphamide 400 mg/m^2, vincristine 1 mg, and prednisone 40 mg/m^2, but no prophylactic growth factors. The 2-year OS was 59% and 2-year PFS was 47%, with 12 (8%) treatment-related deaths (Figure 2). An IADL score less than 4 was predictive of outcome in univariate but not multivariate analysis. A follow-up study by the same group explored ofatumumab-miniCHOP in a similar patient group and

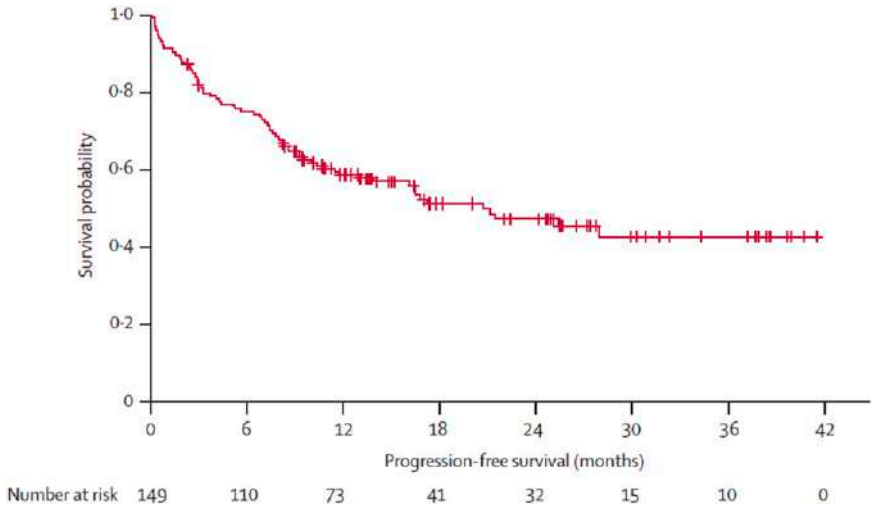

Figure 2. PFS in patients older than 80 years treated with R-miniCHOP (n = 150). Reprinted from Peyrade[17] with permission of Elsevier.

reported a 2-year OS of 64.7% and PFS of 57.2% with no treatment-related deaths.[16] In addition to adding prephase steroids, this study incorporated documentation of a simplified 4-question IADL, Buzby nutrition index, and Charlson Comorbidity score; however, the only factor predictive of outcome was the IPI score. These regimens have not been tested in patients age less than 80.

Impact of dose intensity based on age and fitness

Several retrospective series have attempted to address the impact of dose intensity in older, unfit, and frail patients with DLBCL. In a prospective study incorporating the CGA tool in 173 consecutive newly diagnosed patients age ≥ 70 treated during a 1-year period, curative treatment defined as >70% of the full-dose intent to treat resulted in improved outcomes in the 16% of patients categorized as unfit with a 2-year OS of 75% vs 45%, (P = .32). This was not the case in frail patients.[10] In an Asian population of 192 patients greater than age 60 with DLBCL, a PS of 2, age ≥ 75 years, and doxorubicin and cyclophosphamide relative dose intensity < 60% were all independent prognostic factors for survival.[23]

In 690 consecutive patients with age > 70 years with newly diagnosed DLBCL treated between 2009 and 2018 across 8 UK centers, the intended dose intensity <80% vs ≥80% in patients with age 70 to 79 years was highly predictive of PFS and OS (P < .001) but had no effect on outcomes in patients 80 or older (P = .88; Figure 3).[5,24] Comorbidities were associated with worse OS, but not lymphoma-specific survival. Similar results were reported in another retrospective study of 142 patients treated at a single medical center, with a marked reduction in OS for patients with age <80 years receiving <90% of the recommended dose intensity of doxorubicin (P = .005) or cyclophosphamide (P = .03), but not in patients with age ≥ 80 years after controlling for IPI and albumin.[25]

In a population based Danish cohort study of 1011 patients with age ≥ 75 years with DLBCL, the importance of dose intensity was age dependent.[2] Patients with ages 75 to 79, with or without comorbidities, were better served by standard treatment with R-CHOP than low-intensity (LI) treatment without anthracyclines. In patients with ages 80 to 84 years with no comorbidities, standard treatment also resulted in better PFS and OS than LI treatment. However, after adjusting for baseline characteristics, patients with age ≥ 80 years with comorbidities or patients with age ≥ 85 years regardless of fitness did not benefit from standard treatment over LI treatment. In all 3 age groups, palliative treatment resulted in a median OS of 0.2 years.

In another population-based study, in 530 veterans age 80 and older with newly diagnosed DLBCL treated between 1998 and 2008, only 285 received systemic treatment, including 193 with an anthracycline-based regimen.[13] Dose intensity ≥ 85% was associated with worse outcomes compared with dose intensity < 85%. Particularly striking was a first cycle mortality of 11% in patients given full-dose doxorubicin compared with 2% in those not treated with full dose. At 1 year, 59% of those treated at full dose intensity were alive compared with 70% treated with dose intensity < 85%; however, by 2 years, the OS rates were 53% vs 48%, perhaps suggesting higher relapse rates with lower dose intensity. After controlling for other variables, anthracycline use was not associated with OS. The 40% of patients who received no treatment had a median OS of 1.9 months.

If an anthracycline-based regimen is selected, a multidisciplinary approach including a cardiologist should be considered for all unfit and older patients, with cardiovascular profiling and risk stratification before treatment if feasible.[26] Following cardiac biomarker measurements (troponin and brain natriuretic peptide) on treatment may ameliorate cardiotoxicity by allowing earlier intervention.[26]

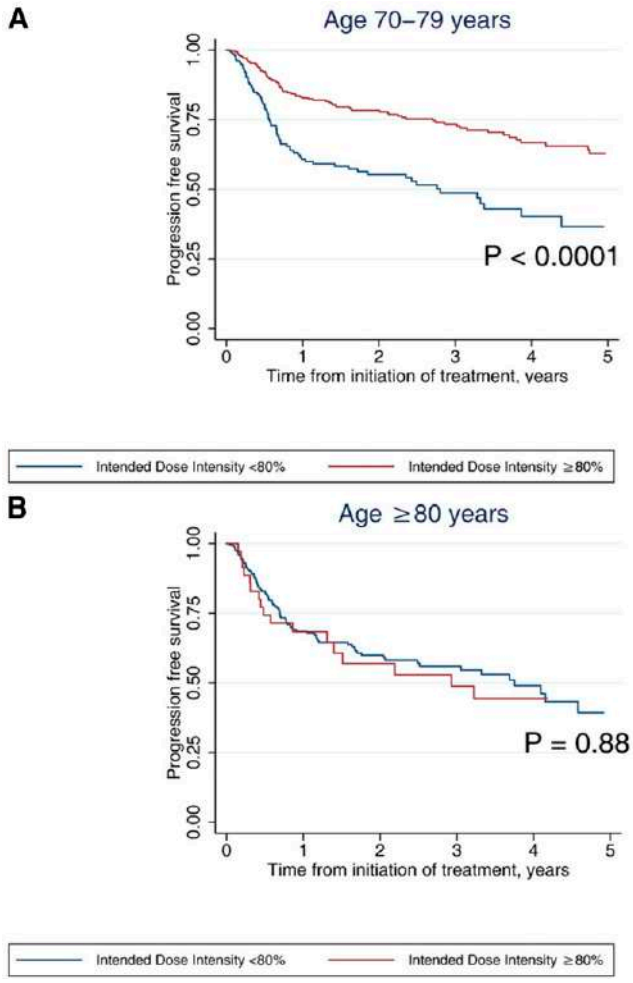

Figure 3. Impact of intended and relative dose intensity of R-CHOP in patients ≥ 70 years with DLBCL in a representative, consecutive cohort across 8 UK centers (2009-2018) treated with curative intent. (A) OS and (B) PFS by age and intended dose intensity. Reprinted from Eyre[24] with permission of the Journal of Internal Medicine.

Anthracycline-free regimens

Although regimens that do not include anthracyclines are considered most commonly for patients with cardiovascular comorbidities, a group of patients commonly excluded from trials, this approach may also be appropriate for patients greater than 80 years of age who are unfit. Anthracycline-free regimens have been associated with a higher risk of death in patients with age 75 to 79 regardless of comorbidities and in fit patients with age 80 to 84.[2] However, in patients with ages 80 to 84 years who were unfit, or patients with age 85 years or older regardless of comorbidities, outcomes without anthracyclines were not inferior to standard treatment.[1]

When anthracyclines are contraindicated, one approach is to simply omit the doxorubicin and administer R-CVP (rituximab,

cyclophosphamide, vincristine, and prednisone). In multiple retrospective series of patients ≥ 80 years of age, results with R-CVP are generally inferior to R-CHOP, in large part likely because of selection bias.[27-29] Replacing doxorubicin with an alternative active, but less toxic agent, such as etoposide or gemcitabine, is also an option. A phase 2 multicenter trial of R-GCVP (rituximab, cyclophosphamide, vincristine, gemcitabine, and prednisolone), accrued 61 patients (median age, 76.5 years) with newly diagnosed DLBCL and cardiac comorbidities.[30] Overall response rate was 61.3%, 2-year PFS was 49.8%, and 2-year OS was 55.8% with no significant difference in outcomes for patients with left ventricular ejection fraction >50% vs ≤50% (Figure 4A).[30] Common grade ≥3 toxicities included hematologic (55%), infection (27%), and cardiac (16%), with 3 fatal cardiac events on treatment. In the R-CEOP (rituximab, cyclophosphamide, etoposide, vincristine, and prednisone) regimen, doxorubicin is replaced by etoposide (50 mg/m^2 intravenously on day 1 and 100 mg/m^2 orally on days 2 and 3).[31,32]

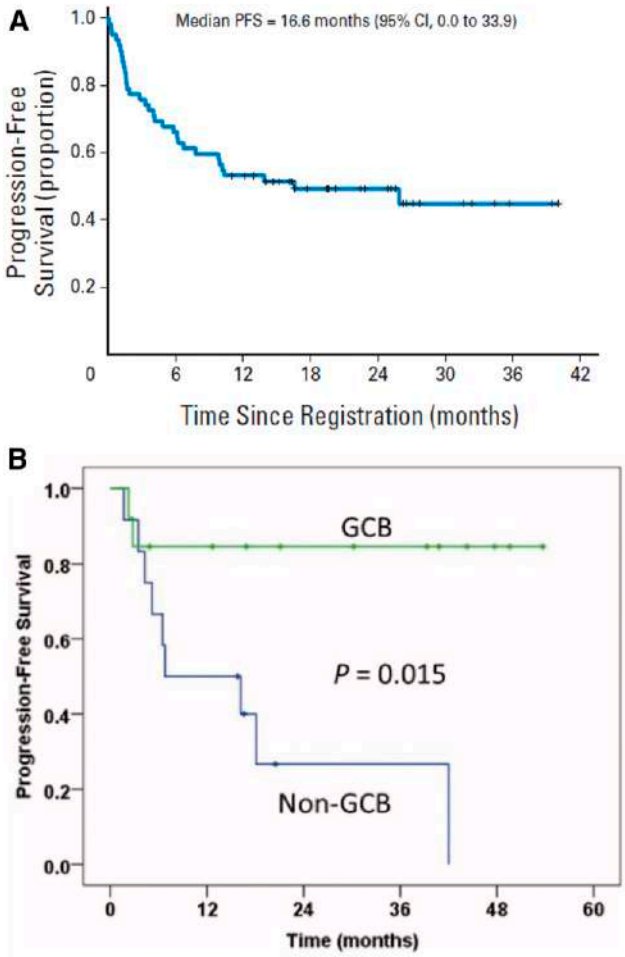

Figure 4. PFS for R-GCVP and R-CEOP in patients not eligible for R-CHOP. (A) PFS in patients (n = 63) considered unfit for anthracycline-containing chemoimmunotherapy because of cardiac comorbidity treated with R-GCVP. Reprinted from Fields[30] with permission of the American Society of Clinical Oncology. (B) Median PFS from a single institution report of R-CEOP in patients with DLBCL (n = 26) separated into GCB and non-GCB phenotype. Reprinted from Rashidi[32] with permission of Taylor & Francis.

One retrospective study of 81 patients (median age, 73 years) ineligible for R-CHOP had a 5-year time to progression of 57% after treatment with R-CEOP.[31] A single institution report of R-CEOP in 26 patients with a median age of 83 years reported 2-year event-free survival and OS rates of 49% and 59%, respectively.[32] An unexpected finding in this small series was a 2-year PFS of 32% in the 13 patients with a non-GCB phenotype compared with 80% in the 12 patients with GCB phenotype (Figure 4B).

Other non–anthracycline-based approaches tested in the front-line setting for unfit patients are regimens that are currently used in the DLBCL salvage setting. A phase 2 study of R-GemOx (rituximab, gemcitabine, and oxaliplatin) was conducted in 60 patients with previously untreated DLBCL age 70 or older, or age 60 to 69 with PS 2.[33] At a median follow-up of 45 months, the 3-year PFS and OS were 49% and 65%, respectively. In multivariate analysis, only IPI score was a poor prognostic factor, with 2-year OS of 38% in patients with IPI 3 to 5 compared with 85% for those with 0 to 2 (hazard ratio, 3.7). Febrile neutropenia occurred in 8% of patients with no TRM. A randomized trial is ongoing in China comparing R-miniCHOP to R-GemOx in the frontline setting (NCT02767674).

Tolerability and effectiveness of the bendamustine rituximab (BR) regimen in indolent lymphoma, stimulated evaluation of this regimen, initially in relapsed DLBCL but more recently as first line in older, less fit patients. In a phase 2 trial of 49 patients greater than 70 years of age with DLBCL and significant comorbidities (unfit and frail), the 2-year overall response rate was 62%, with 53% complete responses, but a disappointing 2-year PFS of 38%.[34] In a retrospective comparison of BR vs R-CHOP in 140 patients ≥ 75 years of age, or >65 years of age with PS ≥ 2, BR was associated with marked inferior median OS (16.3 vs 75.4 mo, P = .006).[35] BR patients were older and had higher-risk disease but no difference in comorbidities or PS. In the subset of patients with Charlson comorbidity index ≥ 6, there was no difference in outcomes between BR and R-CHOP. Although the BR regimen is well tolerated and has activity in DLBCL, the duration of remission in several reports appears significantly shorter that other regimens in this setting.[36,37] Table 2 summarizes outcomes with the anthracycline- and non–anthracycline-based regimens discussed above in unfit and older patients.

Supportive care measures

Supportive care measures are essential in older patients undergoing aggressive chemotherapy. In addition to specific recommendations described below, there should be consideration of more frequent follow-up of older patients, especially during the first 2 cycles of treatment. For example, assessing patients weekly with blood counts and the need for hydration or calorie supplementation may allow earlier intervention and avoid life-threatening complications. For patients with DM, engaging the primary physician or an endocrinologist to follow blood sugar control closely during treatment may lessen the risk of severe hyperglycemia related to prednisone.

Prophylactic antibiotics

As described above, increased rates of infection were observed in a number of R-CHOP–based trials in elderly patients. The German NHL study group noted an increased risk of grade 3 and 4 infections in the DENSE-R-CHOP14 trial, which enrolled patients 61 to 80 years of age.[38] After the first 20 patients, acyclovir (daily) and cotrimoxazole (twice a week) were added to the regimen,

Table 2. Clinical trials with elderly/unfit patients with DLBCL

	Phase	Patients, n	Median age, y	Patients age ≥ 80, %	ORR, %	CR, %	PFS, %	OS, %	TRM, %	Grade 3-4 F/N, %,	Reference
R-miniCHOP	II	150	83	100	73	62	47 (2-y PFS)	59 (2 y)	8	6	17
Ofa-miniCHOP	II	120	83	100	68	56	57 (2-y PFS)	65 (2 y)	0	21	16
R-GCVP	II	61	76	26	61	29	50 (2-y PFS)	56 (2 y)	7	0	30
R-CEOP	Retro	81	73	NR	NR	NR	57 (5-y PFS)	49 (5 y)	4	NR	31
	Retro	26	83	65	75	58	49 (2-y PFS)	59 (2 y)	4	19	32
R-Benda	II	45	81	NR	62	53	38 (2-y PFS)	51 (2 y)	0	2	34
R-GemOx	II	60	75	27	75	47	49 (3-y PFS)	65 (3 y)	0	5	33

CR, complete response; F/N, febrile neutropenia; NR, not reported; ORR, overall response rate; Retro, retrospective study.

and the serious infection rate dropped from 35% to 18%. This rate was also substantially lower than the 28% reported in a similar patient population treated with standard R-CHOP-14, leading the German NHL study group to recommend this prophylaxis in all older patients receiving an R-CHOP regimen.[15,38] Although no consensus guidelines exist for antibiotic prophylaxis in less fit patients with aggressive lymphoma, it is reasonable to consider acyclovir and cotrimoxazole.

Myeloid and erythroid growth factors

Prophylactic myeloid growth factors should be considered for all patients 80 years or older and those with significant comorbidities receiving cytotoxic chemotherapy for aggressive lymphomas, regardless of the regimen. Growth factors were not administered prophylactically in the R-miniCHOP regimen, and the incidence of febrile neutropenia was only 7%; however, 3 of 149 (2%) of patients died of neutropenic sepsis during cycle 1.[17] The American Society of Clinical Oncology guidelines recommend the use of myeloid growth factors for regimens with a ≥20% incidence of febrile neutropenia; however, data used to develop these guidelines would have included few patients age 80 and older and few unfit or frail patients.[39] I favor erring on the side of administering growth factors, particularly for cycle 1, in this patient population, even if administering a less intensive regimen.

Current guidelines do not recommend erythropoietin for patients with curable cancers because of concerns of increased thromboembolic complications and increased risk of progression in certain malignancies. A large randomized trial of darbopoeitin vs placebo in older patients with DLBCL receiving R-CHOP showed no detrimental effect of darbopoeitin on PFS or OS.[40] A large meta-analysis also suggested the relative safety of these agents in lymphoma.[41] Importantly, many centers limit transfusions to patients with hemoglobin less than 7 or 8 g/dL, which may lead to more symptoms in patients over age 80 or those with significant comorbidities. Even with less intense regimens such as ofatumumab-miniCHOP, 15% of patients required transfusions.[16] Based on individual patient circumstances, erythropoietin could be considered in the very elderly or unfit who are experiencing significant treatment-related anemia.

Vitamin D supplementation

Although difficult to prove causality, several studies have shown worse outcomes for a number of cancers in patients with low vitamin D levels. A retrospective evaluation of pretreatment serum samples from 359 patients 61 to 80 years of age with DLBCL treated on the RICOVER-60 trial, showed that 54% were vitamin D deficient (<10 ng/mL) and 46% were insufficient (10-30 ng/mL).[42] In contrast to the United States, there is no vitamin D fortification of milk in Germany, perhaps accounting in part for the remarkably high incidence of vitamin D deficiency in this patient population. In a multivariate analysis, both event-free survival and OS were significantly worse in patients with vitamin D levels ≤8 ng/mL compared with those with levels >8 ng/mL. Interestingly, there was no difference in patients who did not receive rituximab, supporting the hypothesis that vitamin D enhances rituximab-mediated cellular cytotoxicity.[43] Vitamin D supplementation to maintain levels >30 ng/mL is recommended in this particularly vulnerable population.

Conclusions and future directions

Evidence-based guidance for treating less fit patients with aggressive B-cell lymphoma is limited. Available data suggest that patients ≥ 80 years of age and those with significant limitations of ADLs and IADLs do not benefit from full-dose chemotherapy and should be treated with R-miniCHOP or an anthracycline-free regimen. Prephase steroids should be considered for all unfit and older patients, and decisions regarding treatment should be reassessed based on PS after prephase. In unfit patients, I start with a 25% to 50% dose reduction for cycle 1, and in those younger than 80 years of age, I attempt to escalate to at least 75% of standard dose with subsequent cycles if tolerated.

Returning to the earlier case presentation, I recommended R-CEOP with prophylactic pegfilgrastim for this 84-year-old woman with high-risk, GCB phenotype DLBCL after describing the very poor survival with palliative approaches and an approximately 40% to 50% chance of cure with a 5% to 10% TRM using chemotherapy. Although I did not administer prephase steroids, in retrospect, I should have. After cycle 1, she was hospitalized for 2 weeks because of volume overload with marked lower extremity edema, hyperglycemia requiring initiation of neutral protamine Hagedorn and sliding scale insulin per endocrinology consult, and a gastrointestinal bleed. The edema and hyperglycemia continued to be problematic throughout treatment; however, she was able to complete 6 cycles at full dose. Although end-of-treatment positron emission tomography-computed tomography was interpreted as a partial response, she

remains in remission 21 months after completion of treatment, living independently and caring for her disabled husband.

As new therapies emerge for aggressive B-cell lymphomas, especially targeted approaches, patients who are unfit and those >80 years of age are also likely to benefit because many of these agents, such as anti-CD19 antibodies, antibody drug conjugates, bispecific antibodies, and liposomal formulations have less toxicity than conventional chemotherapy. Combining new, less toxic agents with modified chemotherapy regimens either concurrently or as maintenance may lead to better outcomes. Trials designed specifically for the oldest and less fit patients are likely to have the greatest impact in improving treatment of this subgroup. Ongoing and recently completed trials such as R-miniCHOP vs R-miniCHOP/lenalidomide, R-miniCHOP vs R-miniCHOP/azacytadine, R-miniCHOP vs R-miniCHP/polatuzumab vedotin, single agent mosunutuzumab, R-lenalidomide, and R-lenalidomide plus a Bruton tyrosine kinase inhibitor may provide much needed evidence-based guidance on how to approach these challenging patients.

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Strategies for introducing palliative care in the management of relapsed or refractory aggressive lymphomas

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Recent advances in treatment of patients with aggressive lymphomas ranging from chimeric antigen receptor T-cell therapy to combination of antibody–drug conjugates with chemotherapy have improved survival outcomes. Despite these significant advances, patients with relapsed or refractory disease experience high physical and psychological symptom burden, and a substantial proportion still die of their lymphoma. In addition, end-of-life care outcomes are suboptimal with high rates of intensive end-of-life health care use and low rates of timely hospice enrollment. Integrating palliative care concurrently with disease-directed care for this patient population has strong potential to improve their symptom burden, quality of life, and end-of-life care. Multiple factors, including heightened prognostic uncertainty in the setting of relapsed/refractory disease, pose challenges to timely provision of palliative care. This article reviews benefits of primary and specialty palliative care for patients with relapsed/refractory aggressive lymphomas and barriers to such care. It also highlights strategies for effectively integrating palliative care for patients with relapsed/refractory aggressive lymphomas.

LEARNING OBJECTIVES

- Identify barriers to optimal palliative care for patients with relapsed/refractory aggressive lymphomas
- Learn strategies to improve timing and conduct of goals-of-care discussions for patients with aggressive lymphomas
- Identify patients with aggressive lymphomas who may benefit from specialty palliative care

Clinical case

A 66-year-old man presented with bilateral axillary lymphadenopathy, unintentional weight loss, and night sweats. An excisional axillary lymph node biopsy revealed a diagnosis of diffuse large B-cell lymphoma (DLBCL), not otherwise specified. Positron emission tomography (PET) showed enlarged fluorodeoxyglucose-avid lymphadenopathy above and below the diaphragm, as well as liver involvement. The result of his bone marrow biopsy was positive for disease involvement, consistent with stage IV disease. His lactate dehydrogenase level was elevated. He received six cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone and attained a complete remission. Four months after treatment, he developed fatigue, night sweats, and recurrent axillary lymphadenopathy, which prompted his hematologic oncologist to order a PET scan. This revealed lymphadenopathy above and below the diaphragm along with multiple bony lesions. Biopsy of an enlarged lymph node confirmed relapsed DLBCL. He began second-line chemotherapy with rituximab, ifosfamide, carboplatin, and etoposide. After two cycles, his repeat PET scan demonstrated refractory lymphoma. The patient and his wife met with his hematologic oncologist to discuss next steps in management. The patient reported fatigue and severe bone pain not relieved by the opioid analgesic regimen prescribed by his hematologic oncologist. He also stated, "I am very disappointed that the chemotherapy did not work, but I have learned of a treatment on the internet called chimeric antigen receptor T-cell therapy. Many people describe it as a magic bullet for lymphoma; I hope this will cure my lymphoma."

Introduction

Over 26 000 people are diagnosed with aggressive B- and T-cell lymphomas each year in the United States.[1,2] Despite advances in therapy, a substantial proportion of these patients develop relapsed/refractory disease. These patients

have significant palliative care needs, including high physical and psychological symptom burden and impaired quality of life (QOL).[3-5] In addition, many harbor misunderstandings regarding their prognosis,[6-8] hindering their ability to engage in informed decision making regarding their care and end-of-life (EOL) preferences. Integrating high-quality palliative care is thus essential for this population; yet, existing evidence suggests suboptimal palliative care uptake. In this review, we discuss the benefits of primary and specialty palliative care for patients with relapsed/refractory aggressive lymphomas and barriers to the delivery of such care. We also review approaches to optimize palliative care for this patient population.

Benefits of palliative care

Palliative care is an approach that improves QOL of patients facing life-threatening illness through the prevention and relief of suffering by early identification and impeccable treatment of pain and other physical, psychosocial, and spiritual problems.[9] It includes primary palliative care, such as goals-of-care discussions and basic management of physical (eg, fatigue, pain, neuropathy) and psychological symptoms (eg, anxiety and depression), which can be provided by hematologic oncologists. It also includes specialty palliative care, which is provided by palliative care specialists, and focuses on more complex symptom management, management of psychological distress, and complicated goals-of-care discussions (Figure 1).[10] Several studies have shown that palliative care improves QOL of patients and increases the likelihood of patients receiving care that is aligned with their goals.[11-13] Accordingly, early integration of palliative care concurrent with routine cancer-directed care is recommended.[14,15]

Goals-of-care discussions represent an important archetype of primary palliative care that can be provided by hematologic oncologists. These discussions entail eliciting patients' goals, values, and preferences regarding their treatment and EOL care options. These discussions are ideally conducted in the context of prognostic information to promote informed decision making. Patients who engage in goals-of-care discussions with their hematologic oncologists are more likely to receive care that is consistent with their preferences, to receive specialty palliative care, and to enroll in hospice.[16-18] They are also less likely to experience intensive EOL health care use (eg, multiple hospital admissions, hospital death),[19] with corresponding improvement in QOL, reduced risk of complicated grief for bereaved caregivers, and lower health care costs.[16]

Patients with aggressive lymphomas have substantial palliative care needs, including fatigue, dyspnea, depression, and pain, which typically worsen near the EOL.[3-5] Therefore, in addition to basic symptom management by hematologic oncologists, they stand to benefit from the support of specialty palliative care even when receiving curative intent therapy. In the first randomized controlled trial of specialty palliative care that focused solely on patients with hematologic malignancies (28% of whom had lymphoma), integrating specialty palliative care with hematopoietic stem cell transplant (HSCT) care significantly improved QOL and reduced symptom burden 2 weeks after transplant compared with routine transplant care.[12] Moreover, although the intervention was limited to the transplant admission, patients had sustained improvement in depression symptoms at 6-month follow-up compared with the standard care arm.[20] This study established the benefit of specialty palliative care for patients with lymphoma and illustrates that specialty palliative care can be combined with potentially curative therapy.

Barriers to optimal palliative care for patients with relapsed or refractory lymphoma

Despite increasing recognition of the benefits of palliative care, uptake of primary and specialty palliative care among patients with aggressive lymphoma is low.[19,21-23] With respect to goals-of-care discussions, 56% of hematologic oncologists in a national survey reported that EOL discussions typically occur "too late," and many would wait until death is clearly imminent to initiate discussions regarding resuscitation or hospice preferences.[22] In a study of patients who died of aggressive lymphomas and other hematologic malignancies, the median time from the first documented goals-of-care discussion to death was only 15 days.[19] In addition, symptom management in the primary palliative care

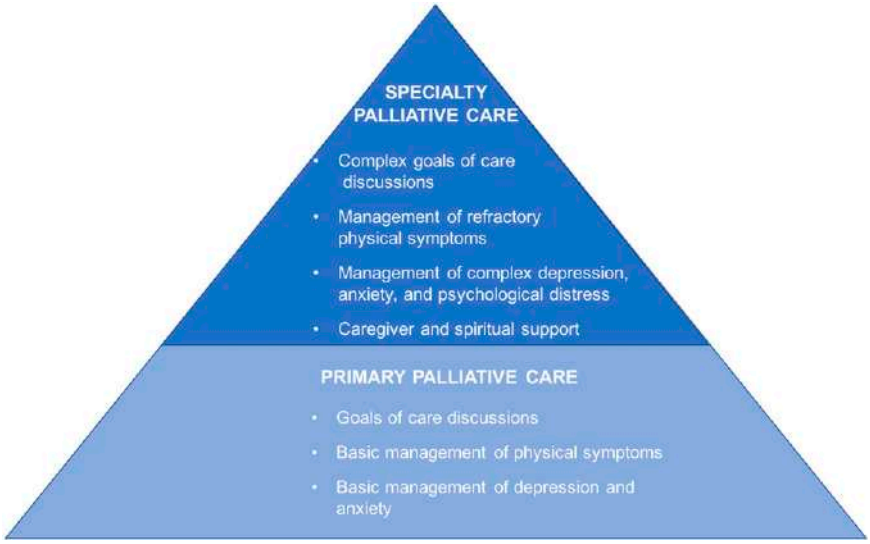

Figure 1. Primary and specialty palliative care.

setting is impeded by lack of systematic screening. Similar to primary palliative care, rates of specialty palliative care consultation are low, ranging from 33% to 40%,[21,24] with a median time between first consultation and death of 12 days,[21] and most occur in the inpatient setting.[23] Such low and untimely referrals preclude patients from fully benefiting from the longitudinal support that specialty palliative care provides. Limited integration of primary and specialty palliative care for patients with aggressive lymphoma contributes to prognostic discordance between patients and hematologic oncologists and impairment in QOL.[7,8]

Among several barriers to palliative care (Table 1), one that is amplified for patients with relapsed/refractory aggressive lymphomas is high prognostic uncertainty, given the potential of cure in relapsed settings and recent treatment advances.[25,26] For example, although the SCHOLAR-1 trial results were sobering with complete response and 24-month overall survival of 7% and 20%, respectively, for patients with refractory DLBCL,[27] complete response ranges from 40% to 58% with chimeric antigen receptor (CAR) T-cell therapy,[25,26] and the estimated 24-month overall survival is 50.5% for axicabtagene ciloleucel.[28] Although CAR T-cell therapy is a success story, a substantial proportion of patients will not experience a durable response, and the trajectory of decline in these patients is often rapid. The likelihood of durable response to treatments for relapsed/refractory aggressive T-cell lymphomas is typically even more dismal than that for B-cell lymphomas, and predictors of sustained response are unclear, highlighting the need for early palliative care.[29] Conducting nuanced goals-of-care conversations that effectively balance the potential promise of intensive disease-directed treatment in the relapsed/refractory setting with the risk of morbidity and mortality is difficult and may contribute to clinicians avoiding these discussions until death is clearly imminent.

With the backdrop of prognostic uncertainty, the effect of other barriers to palliative care (eg, clinician concern about taking away hope, not knowing the right thing to say to patients, misperceptions about specialty palliative care, unrealistic patient and clinician expectations) is compounded.[30-32] For example, prognostic uncertainty coupled with the misperception that specialty palliative care is synonymous with EOL care and needed only when no lymphoma-directed therapy is available[31,32] likely further delays integration of specialty palliative care. Systemic factors, such as limited access to specialty palliative care in some oncology settings and difficulty integrating specialty palliative and oncologic appointments in the same visit, also pose barriers to integration. Despite existing challenges, palliative care remains essential for patients with aggressive lymphomas; accordingly, effective strategies to ensure that patients receive high-quality primary and specialty palliative care are urgently needed.

Table 1. Barriers to primary and specialty palliative care

Disease-related barriers
High prognostic uncertainty
Rapid decline at the end of life
Physician-related barriers
Misperception that palliative care is synonymous with end-of-life care
Unrealistic physician expectations
Not knowing the right thing to say
Concern that the term "palliative care" will decrease patients' hope
Patient-related barriers
Misperceptions about palliative care
Unrealistic patient expectations
System-related barriers
Lack of universal and systematic symptom screening
Lack of universal and standardized training in primary palliative care
Limited access to specialty palliative care in some clinical settings
Difficulty integrating palliative and oncologic appointment schedules for patients

Optimizing goals-of-care discussions for patients with aggressive lymphomas

Patients with aggressive lymphomas desire to have goals-of-care conversations with their hematologic oncologists but seldom feel empowered to bring up these discussions.[6,33] In a study of patients with relapsed/refractory aggressive lymphomas, although 44.4% of patients had thought of their care preferences should they become critically ill, only 11.1% reported that they had the opportunity to discuss those preferences with their hematologic oncologists.[6] Hematologic oncologists thus need to be intentional in allotting time to engage in these discussions. It has been shown that when hematologic oncologists initiate goals-of-care discussions (compared with other clinicians), patients are less likely to die in hospitals and are more likely to enroll in hospice more than 3 days before death.[19] This underscores the powerful role that hematologic oncologists play in goals-of-care discussions and patient decision making. To optimize goals-of-care discussions, it is necessary to identify "when" and "how" to conduct these discussions.

When should goals-of-care discussion occur?

National guidelines recommend that goals-of-care discussions should occur early in the disease course for patients with life-limiting illness.[14] In addition, they should be revisited during the disease course because patients' preferences may evolve.[18] Although early engagement in these discussions is essential, this is difficult to operationalize in real time for patients with aggressive lymphomas. Therefore, practical triggers to conduct these conversations are critical (Table 2). Key transition points at which prognosis changes during the disease course, such as refractory disease or relapsed disease, can be used as triggers for initiating and revisiting goals-of-care discussions. These transition points were identified by lymphoma clinicians in a focus group as important signposts for engaging in goals-of-care discussions.[34] Lymphoma clinicians also identified other triggers for goals-of-care discussions, such as significant worsening of performance status and organ insufficiency, even in the absence of relapse. It is also critical to engage in these discussions with patients who are considering highly intensive therapies such as HSCT or CAR T-cell therapy. Another trigger with high utility is the surprise question, "Would you be surprised if this patient died in the next year?"[35,36] Indeed, this question has been shown to have a positive predictive value of 68.3% among patients with lymphoma and other hematologic malignancies.[36]

How should goals-of-care discussions be conducted?

Communication skills training and guides can equip hematologic oncologists to effectively conduct goals-of-care discussions. Examples of trainings and tools for goals-of-care discussions

Table 2. Triggers for goals-of-care discussions with patients with lymphoma

Relapsed disease
Refractory disease
Worsening performance status
Organ insufficiency
Planned hematopoietic stem cell transplant
Planned chimeric antigen receptor T-cell therapy
Surprise question (You would not be surprised if the patient died in the next 1 y.)

include the VITALTalk courses,[37] the Serious Illness Conversation Guide,[38] and the REMAP (reframe, expect emotion, map out patient values, align with values, and propose a plan) framework (Table 3).[39] These resources share the following best practices for conducting goals-of-care discussions. First, hematologic oncologists should explore the patient's understanding of their illness trajectory at the start of the conversation. This will help to appropriately frame the rest of the conversation on the basis of the patient's level of understanding of their illness and prognosis. Next, hematologic oncologists should provide information regarding their patient's illness and discuss prognosis (what is known of it) tailored to the degree of information the patient desires. This aspect of the conversation often elicits emotions from patients. It is important to acknowledge such emotions; one way to do so is by making reflective statements (eg, "I know this is really difficult news").

After taking the time to respond to emotions, hematologic oncologists can then explore their patient's values and goals in the context of their lymphoma. This part of the conversation may start with a question such as, "What is most important to you, given where things are with your lymphoma?" To have a comprehensive grasp of the patient's goals and values, clinicians will need to elicit the concerns, fears, and trade-offs the patient is willing to make to prolong life. It is important to summarize the goals and values of the patient, repeating them back to him or her to confirm that one's understanding is accurate. Next, if the patient is open to a recommendation, the hematologic oncologist should propose a plan that has the best chance of achieving the patient's goals on the basis of their expressed values and the available medical treatments that might feasibly help. Throughout the conversation, it is vital to affirm commitment to the patient so that he or she does not feel abandoned. It is also critical to engage the patient's loved ones (as preferred by the patient) in these conversations. Finally, documentation in the medical record is essential so that the patient's care preferences are known and honored across care transitions.

Optimizing specialty palliative care integration for patients with aggressive lymphomas

To improve rates and timing of specialty palliative care for patients with relapsed/refractory aggressive lymphomas, it is necessary to conceptualize specialty palliative care as an additional layer of support that is beneficial even when patients are receiving disease-directed care, similar to how other specialties (eg, infectious disease, cardiology) are consulted when there is a need for their expertise during cancer-directed care. With this understanding of specialty palliative care, consultation should be considered for patients with palliative care needs such as high symptom burden (physical or psychological), difficulty coping with illness, complex family dynamics, or complicated goals-of-care discussions (Table 4).[12] Indeed, in a large consensus study of criteria for specialty palliative care referral, there was a greater emphasis on needs-based triggers than on time-/prognosis-based triggers.[40] Specialty palliative care consultation should also be considered for patients undergoing highly intensive therapy with significant morbidity such as HSCT, given high-quality data demonstrating significant and long-lasting benefits of concurrent specialty palliative care in this setting.[12,20] Despite the shortcomings of relying solely on prognosis in the context of prognostic uncertainty to prompt specialty palliative care consultation, the surprise question can be used as a trigger because it is a practical way to identify patients with unmet palliative care needs.[36] Importantly, close collaboration between specialty palliative care teams and hematologic oncologists is vital for seamless and coordinated care provision to patients with relapsed/refractory lymphoma.

Clinical case revisited

Although the hematologic oncologist had previously engaged in goals-of-care discussions with the patient earlier in the disease course, she realized that refractory disease represented a key transition point at which to revisit goals-of-care discussions. The hematologic oncologist thus assessed the patient's understanding of his illness, reviewed prognostic information, and elicited the patient's values and goals. The patient and his wife expressed intense disappointment and sadness at the refractory nature of his

Table 3. Description of the REMAP[39] framework for goals-of-care discussions

REMAP step	Task
Reframe	Assess the patient's understanding of their lymphoma trajectory and, if needed, provide new information. Place the details of the patient's illness in a bigger context, and explain the need to initiate or revisit goals of care.
Expect emotion	Acknowledge the patient's emotion so that he or she can feel heard and supported.
Map out patient values	Explore what matters most to the patient in the context of their lymphoma, their concerns about the future, trade-offs they are willing to make, and their goals.
Align with values	Verbally reflect what is heard from the patient to ensure clear understanding of the patient's values. During this process, one may find that additional clarification of values is needed.
Propose a plan	With permission from the patient, recommend a plan that has the best chance of maximizing the likelihood of meeting the patient's goals using a combination of the patient's values and your knowledge of feasible medical treatments.

REMAP, reframe, expect emotion, map out patient values, align with values, and propose a plan.

Table 4. Triggers for specialty palliative care consultation for patients with lymphoma

High or refractory symptom burden
Psychological distress
Difficulty coping with illness
Misperceptions about illness understanding despite goals-of-care discussions
Complex goals-of-care discussions
Complicated family dynamics
Planned hematopoietic stem cell transplant
Recurrent unplanned hospital admissions
Surprise question (You would not be surprised if the patient died in the next 1 y.)

lymphoma, which the hematologic oncologist acknowledged. The patient noted that one of his goals was to be alive for his youngest daughter's wedding in 6 months and that he would be willing to undergo intensive treatment despite side effects and hospitalizations to try to attain this goal. He also longed for relief from his refractory bony pain. The hematologic oncologist reviewed benefits and risks of CAR T-cell therapy as a potential management option. She also discussed specialty palliative care with the patient for additional management of refractory pain and coping. The patient established care with the specialty palliative care team and decided to pursue CAR T-cell therapy. The palliative care specialist formulated a pain management plan, which led to improvement of bony pain; she also collaborated closely with the patient's oncologist during CAR T-cell admission and subsequent outpatient follow-up to provide additional support to manage the unmet palliative care needs of the patient and his family.

Conclusions

Despite recent treatment advances, patients with relapsed/refractory aggressive B- and T-cell lymphomas experience high symptom burden and many still die of their disease. Timely integration of both primary and specialty palliative care has strong potential to improve QOL for this population; yet, these are underused because of several barriers that are compounded in the context of high prognostic uncertainty. Practical triggers to prompt timely goals-of-care discussions and specialty palliative care consultations, as well as use of communication tools and training, are promising ways to overcome barriers to palliative care integration. Palliative care research for patients with hematologic malignancies has burgeoned in the past few years,[12,41,42] and the time is ripe to better characterize the palliative care needs of patients with lymphoma and develop effective strategies to integrate palliative care for this population.

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Strategies for aggressive T-cell lymphoma: divide and conquer

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The aggressive peripheral T-cell lymphomas (PTCLs) are a heterogenous group of uncommon lymphomas of mature T lymphocytes dominated by 3 subtypes: systemic anaplastic large-cell lymphoma, both anaplastic lymphoma kinase positive and negative; nodal PTCL with T-follicular helper phenotype; and PTCL, not otherwise specified. Although the accurate diagnosis of T-cell lymphoma and the subtyping of these lymphomas may be challenging, there is growing evidence that knowledge of the subtype of disease can aid in prognostication and in the selection of optimal treatments, in both the front-line and the relapsed or refractory setting. This report focuses on the 3 most common subtypes of aggressive PTCL, to learn how current knowledge may dictate choices of therapy and consultative referrals and inform rational targets and correlative studies in the development of future clinical trials. Finally, I note that clinical-pathologic correlation, especially in cases of T-cell lymphomas that may present with an extranodal component, is essential in the accurate diagnosis and subsequent treatment of our patients.

LEARNING OBJECTIVES

- Understand the pressing need for accurate diagnosis and subtyping of peripheral T-cell lymphomas
- Use the subtype of peripheral T-cell lymphoma to choose optimal upfront and subsequent therapies in an evidence-based manner

Much like their more common B-cell counterparts, T-cell lymphomas (TCLs) are a heterogenous group that comprise both indolent and more aggressive entities. In the 2016 SEER database, peripheral TCLs (PTCLs), those that originate from mature T lymphocytes, represent 5% of all non-Hodgkin lymphomas diagnosed in the United States.[1]The most common of these are mycosis fungoides/Sezary syndrome, representing a generally more indolent subtype of PTCL and 3 generally more aggressive subtypes of PTCL that are the focus of this report: PTCL-not otherwise specified (PTCL-NOS); nodal PTCL with T-follicular helper (TFH) phenotype; and anaplastic large-cell lymphoma (ALCL). The aggressive PTCLs comprise not only these 3 entities but an additional 9 lymphomatous entities, all of which may be treated with multiagent regimens designed with curative intent[2-4] (Table 1). Although the outcome of aggressive TCLs is far more dismal than that of their B-cell counterparts and indolent TCLs,[1] subtyping of PTCLs has been increasingly used to help select optimum therapy and improve outcomes (Table 2).

Clinical case

The patient was a 36-year-old male smoker who noted right axillary lymphadenopathy. He was diagnosed with classic Hodgkin lymphoma by means of a node biopsy and was to have outpatient follow-up. Two months later, he presented to another institution with dysphagia and respiratory difficulty requiring intubation. A left cervical node biopsy was performed and was thought to represent classic Hodgkin lymphoma; the patient received doxorubicin, vinblastine, and dacarbazine. Computed tomographic scanning showed widespread adenopathy above and below the diaphragm. Initial laboratory test results were remarkable for elevated lactate dehydrogenase. A week later he had not improved, and a second-opinion pathology consultation was obtained. At that time, the diagnosis was changed to anaplastic large cell lymphoma (ALCL), anaplastic lymphoma kinase negative (ACK$^-$). Morphologic and immunophenotypic evaluation of the cervical lymph node revealed an extensive sinusoidal infiltrate of large pleomorphic cells, with occasional

Table 1. 2016 WHO classification of mature, aggressive TCLs

PTCL-NOS
Nodal lymphomas of TFH-cell origin, including AITL
ALCL, ALK$^+$ and ALK$^-$
ATLL: acute and lymphomatous
Extranodal T-NK-cell lymphoma, nasal type
Intestinal TCL: EATL and monomorphic epitheliotropic intestinal TCL
Hepatosplenic TCL
Primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic TCL
Breast implant–associated ALCL: extended disease beyond the capsule (stages II- IV)

ATLL, adult T-cell leukemia/lymphoma; EATL, enteropathy-associated TCL; MEITL, monomorphic epitheliotropic intestinal TCL.

crescentic hallmarks cells that stained positively for CD45, CD2, CD43, MUM1, c-Myc, CD30, CD15, EMA, and granzyme B and demonstrated a markedly increased proliferation rate (90%). Fluorescence in situ hybridization for DUSP22 and TP63 were negative. The patient received an additional dose of doxorubicin, cyclophosphamide, and prednisone (CHP) with growth factors and experienced rapid improvement with extubation. He received brentuximab vedotin (BV) and CHP (BV+CHP) for an additional 6 cycles and achieved a complete remission followed by high-dose chemotherapy with peripheral stem cell rescue. At this writing, he had been in complete remission for 1 year.

The perils of TCL diagnosis and classification

The diagnosis and classification of TCLs continue to present a challenge to both the diagnostic pathologist and the clinician, because the entities are uncommon; there is a lack of reliable markers of clonality; specific genetic alterations have not been identified for most entities; and there is a need, especially in lymphomas that involve extranodal sites, to integrate clinical features with the available pathologic information.[8] In 2002, in a landmark study, the Non-Hodgkin's Classification Project found that PTCL could be diagnosed reliably by an experienced hematopathologist but that immunophenotyping was absolutely necessary. [4] Indeed, a recent prospective study that included both academic and community practices showed that the diagnostic workup for PTCL in the United States continues to vary widely and often lacks important phenotypic information to fully characterize the lymphoma.[9] The North American PTCL Study Group then undertook a project, suggesting that the use of a clearly defined algorithm as an approach to tissue diagnosis may render a diagnostic accuracy of greater than 90% in T-natural killer (T-NK) lymphomas[10] (Figure 1). Consideration of the necessity for accurate diagnosis to inform prognosis and treatment has no doubt led to the current recommendation of the National Comprehensive Cancer Center Network (NCCN) that a "review of all slides with at least one paraffin block representative of the tumor should be done by a hematopathologist with expertise in the diagnosis of PTCL."[11] The NCCN also proposed as essential a tentative panel of immunophenotypic tests; however, the performance of these have not been prospectively evaluated.[11]

ALCL: defining optimal induction therapy and beyond

Systemic ALCL is perhaps the best example of a subtype of TCL where a diagnostic antigen, CD 30, can also function as an effective target for treatment. In ALCL, all tumor cells are strongly positive for CD30. CD30 is most strongly present at the cell membrane and in the Golgi region, although diffuse cytoplasmic positivity is also common. This diagnostic feature of ALCL has been exploited in the search for new treatments of ALCL in the form of an antibody-drug conjugate, BV, a drug consisting of a chimeric anti-CD30 monoclonal antibody linked to monomethyl auristatin E, a disruptor of microtubules.

First studied in the relapsed or refractory setting in a phase 2 trial, BV was found to have an 86% response rate with 57% complete responses (CRs) and a duration of response of 12.6 months[12]

After the publication of that study, the ECHELON-2 trial, a 452-patient global, randomized, double-blind, double dummy, phase 3 study comparing BV+CHP with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) in the upfront treatment of

Table 2. Initial treatment considerations for subtypes of aggressive PTCL

Subtype	Suggested initial therapy
PTCL-NOS	Consider BV+CHP for CD30$^+$ histology; CHOEP,[5] CHOP, dose-adjusted (DA)-EPOCH[6]
Nodal lymphomas of TFH cell origin (including AITL)	Consider BV+CHP for CD30$^+$ cases; CHOEP, CHOP, DA-EPOCH
ALCL, ALK$^+$ and ALK$^-$	BV+CHP
ATLL: acute and lymphomatous	Dose-adjusted EPOCH, BV+CHP for CD30$^+$ cases
Extranodal T-NK lymphoma, nasal type	Nasal stages I and II include radiation; stage IV, extranasal: combination chemotherapy (asparaginase-based)
Intestinal TCL, EATL, and MEITL	Consider the Newcastle regimen[7]
Hepatosplenic TCL	Ifosfamide, carboplatin, etoposide (ICE)
Primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic TCL	Consider combination chemotherapy
Breast implant–associated ALCL: extended disease beyond the capsule	BV±CHP, CHOEP, CHOP, (DA)-EPOCH

ATLL, adult T-cell leukemia/lymphoma; CHOEP, cyclophosphamide, hydroxydaunorubicin, vincristine (Oncovin), etoposide, prednisone; (DA) EPOCH, (dose-adjusted) etoposide, prednisone, vincristine (Oncovin), cyclophosphamide, hydroxydaunorubicin.

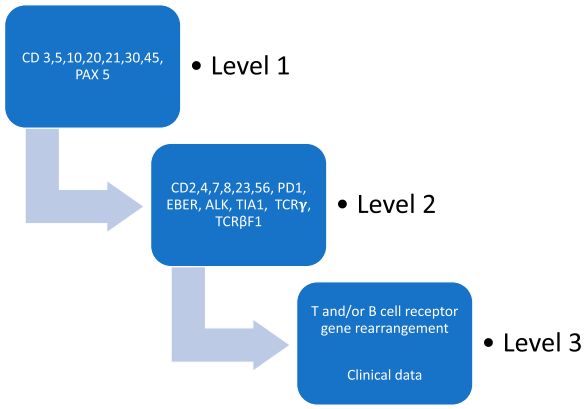

Figure 1. Algorithmic approach to diagnosis of PTCL after evaluation of basic demographics. Schematic of the case review algorithm. Adapted from the North American PTCL Study Group Project.[10]

patients diagnosed with PTCL, in which immunohistochemistry showed expression of CD30 in at least 10% of malignant cells, was published.[13] In that trial, patients were stratified according to the International Prognostic Index (IPI) and histologic subtype. By design, the study was enriched for patients diagnosed with ALCL. Seventy percent of the patients with intent to treat had ALCL, with 22% of patients having anaplastic lymphoma kinase (ALK)–positive ALCL with ≥2 IPI. The patients received either 6 or 8 cycles of chemotherapy, predetermined by the investigator at each site; 19% of the patients received more than 6 cycles. With blinded independent review, the patients treated with BV+CHP had a statistically significant improvement in median progression-free survival (PFS), from 20.8 to 48.2 months, compared with those who received CHOP. Treatment with BV+CHP also reduced the risk of death by 34%, compared with CHOP. The study was not powered to perform subtype analysis. ECHELON-2 is the first randomized study of PTCL to show an overall survival benefit of a novel therapy combined with a standard backbone with no increase in toxicity.[13]

A post hoc exploratory analysis of the data looked at the effect of consolidative high-dose therapy with stem cell rescue (autologous bone marrow transplantation [ABMT]) in those patients with ALK$^-$ systemic ALCL and those with histologic results showing other than systemic ALCL. In the investigational arm, 67% of patients with ALK$^-$ systemic ALCL attained CR, with 36% of those undergoing ABMT. Fifty-nine percent of patients with histologic diagnosis other than systemic ALCL also attained CR, and 29% of those underwent ABMT. Patients treated with ABMT tended to be younger (median age, 50) and from non-Asian countries. Although the sample size was small, PFS estimates favored the use of ABMT.[14]

In the relapsed or refractory setting, the presence of a t(2;5) translocation involving the ALK and the nucleophosmin genes, which result in the expression of a novel fusion protein and overexpression of ALK in ALK$^+$ ALCL, led to the study and effective use of crizotinib.[15]

The use of consolidative stem-cell transplant in the front-line treatment of PTCLs

Phase 2[16,17] and 3 studies[14] have suggested higher rates of PFS in patients with PTCL who receive consolidative stem-cell therapy in the first CR, and this procedure has become standard practice at many institutions for patients with high-risk disease or histologic findings (Table 3). In most of these instances, the expected 5-year overall survival is significantly <50%, with the exception of late-stage breast implant–associated ALCL, which may be as low as 60%.[18-21] In a prospective US cohort registry of patients with newly diagnosed PTCL, the Comprehensive Oncology Measures for PTCL Treatment (COMPLETE),[23] of the 119 patients with nodal PTCL (ALK$^-$ ALCL, angioimmunoblastic TCL [AITL], and PTCL-NOS), who achieved complete remission after induction therapy, 36 patients underwent ABMT and 83 did not. Among the patients who underwent ABMT, a significantly higher number had advanced disease and high IPI, although there were no significant differences in demographics. With a median follow-up of 2.8 years, the median overall survival was not reached in the group receiving ABMT, whereas the group that did not undergo ABMT had a median overall survival of 57.6 months. Although this difference did not reach statistical significance (P = .06), in a multivariate analysis, autologous stem cell transplantation was independently associated with improved survival. The role of upfront ABMT as consolidation of first complete remission in the absence of validation in a prospective, randomized trial continues to be controversial.

AITL and other nodal lymphomas of TFH cell origin: selection of treatment in the relapsed or refractory setting and of rational targets for investigation

Since the recognition of TFH cells as a unique subset of T-helper (Th) cells with a characteristic phenotype, a subset of PTCLs has been noted to have a TFH phenotype. The most well studied of these is AITL. In addition, up to 40% of cases of PTCL-NOS[24] have been found to share some of the clinical and pathologic features, the TFH phenotype, and some of the characteristic mutations of AITL. For this reason, in the 2016 revision of the World Health Organization classification of mature T neoplasms (an umbrella term), nodal lymphomas of TFH-cell origin, was introduced. For this designation, the malignant cell should express at least 2 or 3 TFH-related antigens including PD1, CD10, BCL6, CXCL 13, ICOS, SAP, and CCCR5. Recurrent genetic abnormalities associated with the TFH phenotype include the TET2, IDH2, DNMT3A, RHOA, and CD28 mutations, as well as gene fusions such as ITK-SYK or CTLA4-CD28.[2,3]

The recognition of this unique phenotype is advantageous in the classification of PTCL, enabling more specific diagnosis of some cases that previously would have been designated as PTCL-NOS, but identifying the function of the TFH cell in providing T-cell support to B lymphocytes may inform some of the clinical features of these conditions, such as the autoimmune phenomena and serologies, polyclonal hypergammaglobulinemia, and associated B-cell lymphomas.

Although the TFH phenotype has not yet been used to determine an induction therapy that would be most efficacious in these lymphomas, it has informed the selection of newer single agents that can be used for relapsed or refractory disease. Earlier uses of immunomodulatory drugs in treating AITL, such as cyclosporin and others, may serve as examples. Some of the elements of the TFH phenotype and its associated genetic

Table 3. Consider first-line consolidation and referral to stem cell transplantation program

PTCL-NOS
ALCL ALK$^+$ with IPI >2
ALCL ALK$^-$ with negative DUSP22 rearrangement or TP63 rearrangement
Nodal PTCL of TFH origin (including AITL)
ATLL, acute or lymphomatous
Extranodal T-NK lymphoma, nasal type, stage IV or extranasal
Intestinal TCL (EATL, MEITL)
Hepatosplenic TCL
Primary cutaneous CD8$^+$ aggressive epidermotropic cytotoxic TCL
Breast implant–associated ALCL: disease extended beyond the capsule

EATL, enteropathy-associated TCL; MEITL, monomorphic epitheliotropic intestinal TCL.

mutations are, at this time, also serving as targets for novel agents in the clinical trial arena or may do so in the future.

Mutations of IDH2, TET2, DNMT3A, and RHOA, frequently seen in AITL as well as in PTCLs, particularly those with a TFH phenotype, involve genes that encode epigenetic modifiers. Histone deacetylase inhibitors (HDACis) are agents that act in part to increase acetylation of the histones associated with DNA, effecting their condensation within the nucleus of the cell. It is this modification of histones by acetylation that plays a key role in epigenetic regulation of gene expression. Both romidepsin, a class 1 selective HDAC, and belinostat, a class 1 to 4 HDACi, have been approved by the U.S. Food and Drug Administration (FDA) for the treatment of relapsed and refractory aggressive PTCLs. Notably, the registration trials of these drugs showed that patients with AITL can have sustained responses with these agents, compared with other FDA-approved agents (Table 4).[25-27]

In AITL and in some cases of PTCL-NOS, CD30 overexpression has been noted in both the malignant T-cell compartment and in the B immunoblasts found in the microenvironment.[35] These B immunoblasts may be positive or negative for Epstein Barr virus–encoded small RNA. This feature of AITL qualified subjects with a diagnosis of AITL for enrollment in the ECHELON-2 trial.[13] Although 54 subjects with the diagnosis of AITL were randomized in the trial, they represented only 12% of the study population and cannot be analyzed separately. Treatment with BV+CHP represents a reasonable front-line therapy for patients with AITL, in which ≥10% of the malignant cells express CD30. In relapsed or refractory disease, a planned subset analysis of a study in which BV was administered to patients with CD30$^+$ PTCLs included 13 subjects with AITL who were found to have an overall response rate of 54% with 38% CR.[28]

In the clinical trial arena, multiple agents are already available that could target the specific antigens in a TFH phenotype, such as ICOS (MEDI-570 in NCT02520791), IDH2 (AG-221), CXCL13, and IL21. The finding of Epstein Barr virus–positive B cells in 80% to 95% of cases of AILT[3] would also commend trials with antiviral and HDACi combinations (www.clinical trials.gov, #NCT03397706). CD30 expression could also support the investigation of anti-CD30 chimeric antigen receptor T cells (#NCT04008394).

PTCL-NOS: future directions

Finally, PTCL-NOS is the heterogenous form of nodal and extranodal PTCL that does not correspond to any of the other specifically defined PTCL entities. Within this group, gene expression and microRNA profiling studies have delineated 2 biologic and prognostic subgroups: those with an increase in GATA-3 and those with an increase in TBX-21 expression.[2,3,22,36] Some cases within the latter group may show cytotoxic differentiation. Each group also has been found to have specific genetic mutations. The genetic abnormalities in the GATA-3 group are more complex and include loss or mutation of tumor-suppressor genes targeting the p53 and Pi3 kinase pathways and gains in STAT-3 and myc.[37] GATA-3 expression in PTCL-NOS is associated with an especially poor PFS and overall survival.[36,38] The TBX-21 group is enriched for genetic mutations of genes regulating DNA methylation.[38] TBX-21 and GATA-3 are transcription factors that regulate gene expression profiles in Th cells directing the Th cell into Th1- and Th2-cell–differentiating pathways, respectively. Immunohistochemical markers have been used in place of gene expression profiling and may have prognostic and therapeutic significance. Such observations provide biologic rationale for investigation of novel therapies for PTCLs, such as Pi3 kinase inhibitors, hypomethylating agents, and JAK/STAT pathway inhibitors, and may in future help us direct these therapies to the patients who may benefit most.

First appearances deceive many: the aggressive PTCL imposters

As the case history reported herein demonstrates, the accurate diagnosis of PTCLs and subtyping of these lymphomas is crucial to both prognostication and to enabling clinicians to make rational treatment decisions to benefit patients. Making these decisions may at times require collaboration with an experienced hematopathologist. It also may require the input of the clinician who is at the bedside or in the office, especially in cases with extranodal involvement, in which the provision of clinical data to the pathologist is vital. The most salient examples of the need for collaboration are in ALK$^-$ ALCL, which may appear indistinguishable or difficult to distinguish under a microscope from primary cutaneous ALCL and other subtypes of CD30$^+$ T- or B-cell lymphomas and classic Hodgkin lymphoma (as in our case), and large-cell transformation of mycosis fungoides. In both cases, the treatment may range from observation alone, to local radiation, to single noncytotoxic agents and single

Table 4. Response rates of FDA-approved agents for relapsed and refractory aggressive PTCLs

Agent	ORR PTCL-NOS (%)	ORR AITL (%)	ORR ALCL (%)
Romidepsin[25]	29	30	24
Belinostat[26]	23	54	15
Pralatrexate[27]	32	8	29
BV[12,28]	33	54	86
Crizotinib[15]	—	—	88 (ALK$^+$ only)
PD-1 inhibition*	—	—	—

ORR, overall response rate.
*Although PD-1 inhibitors, such as nivolumab and pembrolizumab, have been useful in the treatment of relapsed and refractory extranodal T-NK–cell lymphoma, nasal type,[29-31] their use in ATLL and other PTCLs has been reported to be associated with hyperprogression.[32-34]

cytotoxic agents. The use of the term "large cell" does not always connote aggressive PTCL.

Conclusions

The aggressive PTCLs are a heterogenous group of uncommon lymphomas of mature T lymphocytes dominated by 3 subtypes: systemic ALCL, both ALK$^+$ and ALK$^-$; nodal PTCL with TFH phenotype; and PTCL-NOS. Although the accurate diagnosis of TCL and the subtyping of these lymphomas may be challenging, there is growing evidence that knowledge of the subtype of disease can aid in prognostication and in selection of optimal treatments, both in the front-line and in relapsed or refractory disease. The focus of this article has been 3 most common subtypes of aggressive PTCL as examples of how current knowledge may dictate choices in therapy and consultative referrals and inform rational targets and correlative studies in the development of future clinical trials. Finally, note that clinic-pathologic correlation, especially in the case of TCLs that may present with an extranodal component, is essential in the accurate diagnosis and subsequent treatment of these patients.

Conflict-of-interest disclosure

Consultant to Kyowa Kirin, Acro Biotech, and Seattle Genetics.

Off-label drug use

Crizotinib for ALK-positive ALCL.

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Challenges in chronic transfusion for patients with thalassemia

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The introduction of regular red cell transfusions 60 years ago transformed β-thalassemia major from a fatal childhood illness into a chronic disorder. Further advances in the prevention of transfusion-transmitted infections and management of iron overload have allowed survival and quality of life to approach normal. However, transfusion therapy for some other thalassemia syndromes continues to challenge clinical decision-making. Nearly one-half of the patients with E ß thalassemia are transfusion-dependent, yet the criteria for initiating transfusions or hemoglobin targets are not well defined. Patients with thalassemia intermedia who begin transfusions as adults are at very high risk for developing red cell alloimmunization and serious hemolytic transfusion reactions. In the growing number of survivors of Bart hydrops fetalis, the approach to transfusion therapy and iron chelation is rapidly evolving. A collaboration between hematology and transfusion medicine specialists will be essential to improving patient care and developing evidence-based guidelines.

LEARNING OBJECTIVES

- Recognize the heterogeneity of transfusion-dependent thalassemia and its impact on management
- Understand barriers to the effective use of red cell transfusions in thalassemia intermedia, hemoglobin E beta thalassemia, and alpha thalassemia major.

In the past decade, the classification of patients into transfusion-dependent thalassemia (TDT) and non-transfusion-dependent thalassemia (NTDT) was widely adopted. These terms were beneficial in planning the management of iron overload or choosing stem cell transplant or other curative therapy based upon a patient's transfusion status. However, this approach can conceal the tremendous heterogeneity of TDT, a phenotypic group that encompasses β-thalassemia major, severe β-thalassemia intermedia, hemoglobin (Hb) E (HbE) β thalassemia, and certain α-thalassemia syndromes. Among these, β-thalassemia major is the largest category and is usually associated with the presence of 2 severe β-globin mutations.[1] These infants become symptomatic from anemia within the first year and regular transfusions are instituted before 2 years of age.[2] The natural history of β-thalassemia major has been the best characterized among various entities constituting TDT, and, consequently, the transfusion guidelines recommended by various groups for β-thalassemia major are largely similar.[1,3-8]

Heterogeneity of TDT

The guidelines developed for β-thalassemia major may not be appropriate in managing the other thalassemia syndromes that require regular transfusions. The severity of β thalassemia is determined by the imbalance between the α and non-α globin chains. A reduced or complete absence of β-globin synthesis leads to accumulation of excess α-globin chains that are toxic to the erythroid precursors.[9,10] The surplus of α-globin chains can be mitigated when 1 or both β-thalassemia alleles are mild (β^{+} or β^{++}), there is concurrent deletion of α-globin genes, or elevated synthesis of γ-globin persists.[9] Elevated γ-globin synthesis sometimes arises from hereditary persistence of fetal Hb, but milder increases are more often from quantitative trait loci in *Xmn1-HBG2*, *HMIP*, and *BCL11A*.[11] Various forms of β thalassemia may also differ in the total endogenous Hb (F, A, or E) or the oxygen-affinity characteristics (A and E compared with F)[12] that modify the adaptation to anemia.[13] Individuals with severe forms of α thalassemia, in contrast, produce nonfunctional Hb (Hb Bart or HbH), which causes underestimation of the true severity of anemia.[14] In β-thalassemia intermedia and HbE β thalassemia, the decision to commence regular transfusions is influenced not only by the severity of symptoms, but also on medical judgement. The latter is a subjective assessment of whether long-term prognosis would be

better by accepting symptomatic anemia without transfusions instead of transfusion dependence and the associated potential complications. The recognition that patients with NTDT have worse quality of life than those with TDT and are at risk for severe complications has led to the extension of chronic transfusions to a larger proportion of patients than in the past.[15-18]

The role of regular transfusions in HbE β thalassemia

HbE β thalassemia is caused by compound heterozygosity for the E mutation (HBB:c.79G>A) and a β-thalassemia mutation.[19] The prevalence of HbE β thalassemia follows the distribution of the E mutation, which reaches very high frequencies in southeast Asia, southern China, and south Asia. Immigration from Asia to the west has increased the awareness of this syndrome and its distinctive natural history compared with β-thalassemia syndromes (caused by 2 β-thalassemia mutations).[20] The severity of HbE β thalassemia ranges from a mild, asymptomatic anemia to the development of transfusion dependence from early life.[19] The E mutation activates a cryptic splice site that reduces synthesis of β^E messenger RNA.[21] The variable decrease in β^E output is 1 of the factors underlying the variable disease phenotype, even though HbE is a functional Hb. The severity of β mutation (β^+ instead of β^0), coinheritance of α-thalassemia trait, and genetic traits that increase γ-globin synthesis reduce the severity of HbE β thalassemia.[19] Why patients may have dissimilar physiological response to nearly identical Hb levels, and why erythropoietin response to anemia declines with age, is incompletely understood.[22] One characteristic that differentiates HbE β thalassemia from β thalassemia (intermedia or major) is the different functional properties of HbE and HbF. Patients with HbE β thalassemia compensate by rightward shift in the oxygen affinity, which is not seen in β thalassemia where the HbF is the predominant Hb.[12] Although clinical symptoms increase progressively with severity of anemia, it may not be possible to predict the likelihood of transfusion dependence based on Hb concentration alone.

Case 1

The patient was diagnosed with HbE β^0 thalassemia based on results of newborn screening. She was asymptomatic in early childhood with no limitation of physical activity, mild facial skeletal changes, and normal growth. Her Hb concentration was maintained between 6.7 and 7.1 g/dL without any blood transfusion. At 7 years of age, the spleen started to enlarge from 3 to 6.5 cm along with a decline in height velocity. She started regular blood transfusion at 10 years of age that led to resumption of normal growth and a decrease in spleen size to 2 cm (Figure 1).

Case 2

The patient was diagnosed with E β^0 thalassemia at 3 years and started regular transfusions with iron chelation. He was splenectomized at 12 years due to higher blood requirements and splenomegaly, following which Hb was spontaneously maintained between 6.9 to 7.2 g/dL. He was transfused intermittently 1 to 2 times per year when Hb dropped below 7 g/dL. In his early 20s, he developed dyspnea and continuous oxygen requirement from severe pulmonary arterial hypertension and heart failure. He had severe iron overload with liver iron concentration of 38 mg/g and cardiac magnetic resonance imaging T2* of 4.9 ms. Heart failure and pulmonary hypertension improved with supportive management, regular transfusions, and iron chelation (Figure 2).

Discussion

As expected for β-globin disorders, newborns with HbE β thalassemia do not develop anemia until the synthesis of HbF declines significantly over the first 6 months of life. Some infants display the classic symptoms observed in β-thalassemia major, including failure to thrive, hepatosplenomegaly, pallor, and fatigue.[23] More often, the symptoms are mild and escape attention until an incidental viral infection or a routine blood test reveals anemia. In California, where universal newborn screening is practiced for thalassemia, observations suggest that normal growth and development persist up to 1 year and beyond in many, but not all, infants with HbE β thalassemia. When the diagnosis is made later during life due to pallor, anemia, facial skeletal changes, growth failure, hepatosplenomegaly, or jaundice, the age at presentation is an important marker of the severity of disease.[24]

Decisions on appropriate timing to either initiate or discontinue chronic transfusion therapy, although difficult, are of principal importance to the management of HbE β thalassemia.[22] Patients with baseline Hb <6 g/dL should be placed on transfusions even when asymptomatic. Conversely, it is unlikely that patients with baseline Hb >8 g/dL would benefit from transfusions. Finally, those with Hb between 6 and 8 g/dL should be evaluated according to the proposed guidelines (Table 1). Our practice is to evaluate children with HbE β thalassemia in the clinic every 3 months for careful assessment of growth, splenomegaly, facial skeletal changes, and Hb level. Electronic medical records are useful to review trends over time and to store photographs for assessment of bony changes.

In our practice, splenectomy is discouraged as a strategy to avoid the need for transfusions because the effect on Hb may be short-term and it does not address the underlying severe pathophysiology.[22,25] On the contrary, splenectomy increases the risk of infections and a number of serious long-term complications such as thromboembolism, pulmonary hypertension, and iron-induced endocrinopathies.[25] Other avenues to improve anemia include hydroxyurea, which produces an ~1 g/dL rise in total Hb in 50% of patients,[26-28] however, the response in HbE β

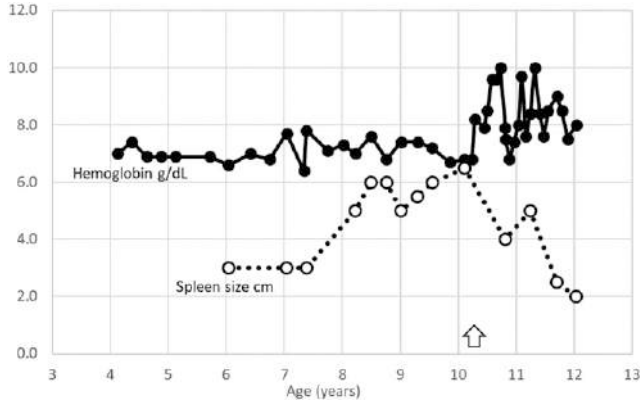

Figure 1. Case 1 depiction. In a patient with HbE β thalassemia (case 1), progressive increase in spleen size and reduced growth velocity led to start of regular transfusions (arrow). Transfusions led to reduction in splenomegaly over the following 2 years.

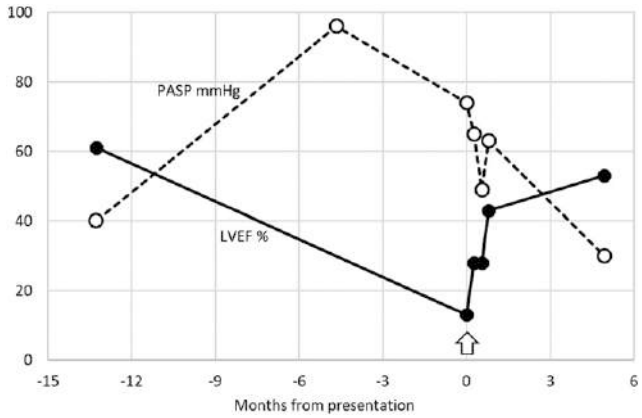

Figure 2. Case 2 depiction. In a transfusion-dependent patient with HbE β thalassemia (case 2), splenectomy was followed by discontinuation of regular transfusions. Fifteen years later, severe pulmonary arterial hypertension and congestive heart failure developed. These pathological changes were reversed by resumption of transfusions (arrow) and supportive care. LVEF %, left ventricular ejection fraction (percent); PASP, pulmonary artery systolic pressure.

thalassemia is variable and insufficient to eliminate the need for transfusions.[18] Luspatercept is an approved therapy to reduce the transfusion requirements in patients with TDT including HbE β thalassemia.[29] Results of a phase 2, open-label study of luspatercept in NTDT showed ≥1.5 g/dL improvement in Hb in 14 of 31 subjects,[30] but the pivotal trial has not yet been done. Mitapivat, a pyruvate kinase activator in the red cells, is also being evaluated in NTDT.[31]

Case 2 highlights the underrecognition of iron overload in patients with NTDT.[18,32] Patients who receive only intermittent transfusions can develop marked iron overload over time. Gastrointestinal absorption of iron is increased in nontransfused patients due to hepcidin insufficiency induced by ineffective erythropoiesis and elevated erythroferrone.[33-35] Screening for iron overload is recommended in all patients with NTDT irrespective of transfusion history. Although magnetic resonance imaging for assessment of liver iron concentration is more accurate,[36] we consider serum ferritin to be useful as a screening test for iron overload. Serum ferritin values underestimate the liver iron overload in NTDT compared with TDT, therefore, LIC should be measured when ferritin exceeds 300 ng/mL. Iron chelation is indicated if LIC is >5 mg/g liver weight[36] with deferasirox at a starting dose of 3.5 to 7 mg/kg per day (5-10 mg/kg if the dispersible tablet is used).[37] We evaluate liver iron concentration every 6 months during therapy and stop chelation therapy when LIC <3 mg/g is achieved.

Red cell alloimmunization in patients starting transfusions as adults

The development of antibodies to red cell antigens is a significant threat to the long-term success of transfusion therapy.[38-43] In the United States, the proportion of patients with thalassemia with red cell alloimmunization is 17% to 22%, only slightly lower than sickle cell disease (19% to 31%).[38,44,45] Older age at initiation of transfusions and splenectomy are identified as major risk factors for developing alloimmunization in thalassemia.[38,41,46] The specificity of antibody and the risk of alloimmunization is influenced by the disparity in red cell antigens among different ethnic groups.[47] In countries where thalassemia predominantly affects immigrant communities, the greater degree of donor-recipient red cell antigen mismatch can elevate the risk of alloimmunization, as shown in Table 2.[39,44,46,48-50] In particular, the low frequency of Kell and c antigens in patients of Asian background facilitates development of alloimmunization.[39,46] One-half of the patients with 1 alloantibody will develop further antibodies against 1 or more additional antigens.[44] Transfusions can become progressively more difficult and it may become nearly impossible to find matched units for certain patients. The most frequent antibodies are against Rh and Kell groups,[41,44] which implies that universal phenotypic matching for these antigens can be a cost-effective method to prevent development of most red cell antibodies in thalassemia.[41,46]

Case 3

A 50-year-old woman with β-thalassemia intermedia who had undergone splenectomy recently changed hospitals. She was diagnosed at the age of 30 years but was only transfused during pregnancy. Due to worsening fatigue, regular transfusions were recommended by her new hematology team. A red cell phenotype was checked, and an antibody screen was found to be negative. She was given 2 red cell units matched to c, E, and Kell antigens. Later that night, she developed chills and fever. Her Hb levels were 7.1 and 9.0 g/dL, respectively, before and after the transfusion. Laboratory testing showed that her lactate dehydrogenase was normal, bilirubin rose from 1.2 to 1.7 mg/dL, haptoglobin was low, and urine contained trace Hb. Two weeks later, the antibody screen was positive and anti-Fyb was identified. Historical antibody data obtained from the previous

Table 1. Indications to begin chronic transfusion therapy in HbE β thalassemia

Indications
Hb <6 g/dL at baseline
Hb 6-8 g/dL accompanied by symptoms
• Growth
– Infants (<2 y): failure to gain weight for 3 mo
– Children: Height velocity <3 cm/y
– Older children: Delay in puberty: >12 y in females, >13 y in males, endocrine evaluation
• Skeletal facial changes: subjective, discuss with patient and family
• Splenomegaly: Spleen >6 cm, or enlargement >1 cm/y after 2 y of age
• Extramedullary hematopoiesis: symptomatic or moderate to large masses
• Cerebrovascular: overt stroke, silent infarcts, arterial narrowing, moyamoya
• Venous thromboembolism
• Pulmonary hypertension
• Osteoporotic fracture
• Quality of life in adults: decline in capacity to work or perform usual activities

Table 2. RBC antigen frequencies and prevalence of alloantibodies

Antigen	White, %	Black, %	Chinese, %	Asian Indian, %	Lal et al, 2018,[39] n = 314 pts	Singer et al, 2000,[46] n = 64 pts	Thompson et al, 2011,[38] n = 697 pts
D	85	92	99	94	1		4
C	68	27	93	87	1		11
C	80	96	47	58	4	2	7
E	29	22	39	20	8	4	22
E	98	98	96	98	2		7
K	9	2	0	3.5	15	6	21
K	99.8	100	100	100			
Fy^a	66	10	99	87	2		2*
Fy^b	83	23	9.2	58	1		
Jk^a	77	92	73	81	4		9†
Jk^b	74	49	76	68	2	1	
M	78	74	79.7	89	1	1	
N	72	75	67.4	65	1		
S	55	31	8.7	55	3		3
S	89	93	100	89			

RBC antigen frequencies among ethnic groups[48-50] and prevalence of significant alloantibodies among patients with TDT in the western region of the United States,[39,46] and North America and United Kingdom.[38]
pts, patients.
*Fy^a or Fy^b.
†Jk^a or Jk^b.

blood bank showed anti-c, anti-E, anti-Kell, and anti-Fyb antibodies had been previously identified 15 years ago. The patient was successfully maintained on regular transfusions with extended phenotypically matched red blood cells. Anti-Fyb could no longer be identified after 4 years, although her antibody screen was intermittently positive due to development of anti-Cw antibody (Figure 3).

Discussion

Although considered a much greater risk in sickle cell disease,[51] delayed hemolytic transfusion reactions (DHTRs) are also observed in thalassemia.[52] Hemolytic transfusion reactions have been reported due to anti-E, anti-Jk^b, anti-Jk^a, anti-c, anti-S, anti-Kell, and anti-f.[39] More than 25% of older children and adults with thalassemia will develop an alloantibody following 1 or more transfusions when red cell matching is limited to ABO/D only.[41,44,46] In the absence of further antigenic exposure, one-third of alloantibodies become undetectable within the first year of follow up. Anti-Jka antibodies are very evanescent, falling below the limit of detection within the first month of initial detection, whereas the anti-Kell and anti-E antibodies are undetectable in >50% at 6 months.[53,54] Between 20% and 25% of individuals of Chinese and Asian Indian ethnicity are Jka^-, 60% to 80% are E^-, and virtually all are $Kell^-$.[48,55] An additional concern is c antigen which is negative in 40% to 50% of Asian patients, but the evanescence rate for anti-c antibody is lower (25%) compared with the other alloantibodies.[39]

Sensitized patients who receive a later transfusion, based on a negative antibody screen, rapidly increase antibody titer with reexposure that develops into a DHTR of variable severity.[51] DHTRs are less likely in regularly transfused patients where antibody screening is performed every few weeks and newly developed antibodies are unlikely to become undetectable before the next transfusion.[56] However, low-titer antibodies may be missed, or antibodies may develop while donor red cells are still circulating in significant amounts, leading to hemolysis. Intermittently transfused patients, on the other hand, are at high risk for antibody evanescence between transfusion episodes. The number of such patients with thalassemia, transfused 1 to 6 times per year, is small in the United States but significant in regions where blood availability is limited. Infrequent transfusions may be recommended during an infection, surgery, or

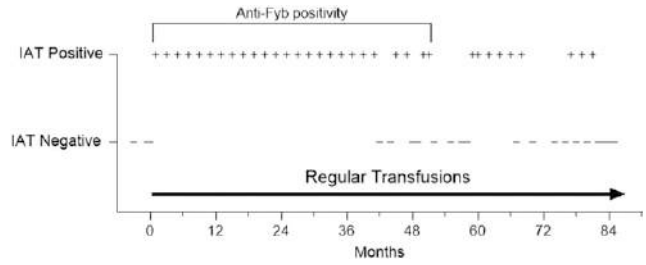

Figure 3. Case 3 depiction. In a patient with β-thalassemia intermedia with prior exposure to blood and negative antibody screen (case 3), the transfusion of red cell units matched only to Rh and Kell antigens led to the reemergence of anti-Fyb antibody and delayed hemolytic transfusion reaction. Regular transfusions were possible with extended phenotypic matching of red cell units. Anti-Fyb was no longer detectable after 4 years, though antibody screen remained positive intermittently due to other alloantibodies. IAT, indirect antiglobulin test.

pregnancy.[25] Transfusions during pregnancy are associated with a very high risk of alloimmunization, but these antibodies decrease in titer when transfusions are discontinued after childbirth.[57] Patient transfer between hospitals poses a persistent challenge to transfusion safety.[58] The lack of comprehensive antibody history can easily lead to the transfusion of red cells to which the patient is previously sensitized but now has a negative antibody screen. Although attempts have been made to provide patients with transfusion cards that list their phenotype and red cell antibodies, their use remains erratic. The absence of a centralized database for multiply transfused patients remains a serious shortcoming of the current practice of chronic transfusion therapy.[59]

Postnatal management of Bart hydrops fetalis: ATM

The deletion of all 4 α-globin genes (−/−, homozygous α^0 thalassemia, or α-thalassemia major [ATM]) causes severe fetal anemia.[14,60] As fetal viability depends upon the preservation of embryonic ζ genes (ζ-/ζ-, or −/ζ-), the most frequent α^0 deletion associated with ATM is the southeast Asian deletion ($-^{SEA}$).[14] The major Hb species in ATM is Hb Bart (γ_4), formed by self-association of γ chains into tetramers in the absence of α chains. Because Hb Bart is ineffective in transporting oxygen, most pregnancies end in fetal demise following a variable period of hydrops (Bart hydrops fetalis).[60] Intrauterine transfusions (IUTs) are essential for the fetus to reach viability with acceptable neonatal outcome.[61,62] All infants with ATM are transfusion-dependent from birth and require recognition of the nonfunctional Hb fractions for correct management.[62,63]

Case 4

A child was born to parents who were carriers of the $-^{SEA}$ deletion and had previously experienced a hydrops-associated stillbirth. During this pregnancy, fetal hydrops was detected at 20 weeks of gestation, and managed by IUT performed on 6 occasions. The baby, born at 37 weeks weighing 3.0 kg, was stable and underwent a red blood cell exchange transfusion at 48 hours. Following discharge, he started regular red cell transfusions every 4 weeks. The average pretransfusion Hb was 9.7 g/dL with Hb Bart and HbH accounting for 20% to 36% of the total value. Starting at 8 months, the transfusion regimen was changed to maintain HbH <20%, which corresponded to HbA >9.0 g/dL and total Hb of ~11 g/dL in the pretransfusion blood sample. Growth proceeded at the normal pace, and developmental assessment at 3 years showed age-appropriate attainment of milestones (Figure 4).

Discussion

The management of ATM is evolving with experience gained from data in international registries[61] and publication of case series.[62-65] In the absence of existing consensus guidelines, our institutional practices are provided in this discussion to fill gaps in published literature. Questions about the optimal management of pregnancies affected by ATM are being evaluated in an ongoing clinical trial (NCT02986698, fetus.ucsf.edu).

The hematological management of ATM commences as soon as the diagnosis is suspected during pregnancy. In that small proportion of cases in which both parents are known carriers of the α^0-thalassemia trait, the diagnosis should be established expeditiously with DNA testing from chorionic villus biopsy instead of ultrasound surveillance for fetal changes suggestive of hydrops. In most cases, however, the first indication is the detection of hydrops on ultrasound during the second trimester. In such cases, a presumptive diagnosis of ATM is appropriate when severe fetal anemia is observed by Doppler ultrasonography,[66] and the pregnant woman is not alloimmunized to RhD or other red cell antigens but has microcytosis and hypochromia. Hematologists have a critical role to play in confirmation of the diagnosis, nondirective counseling of the family, and prenatal management of the fetus.

When the decision is made to continue the pregnancy, the first IUT should be initiated as soon as possible. The strategy for conducting IUT in ATM is derived from consensus guidelines from the Society for Maternal-Fetal Medicine.[67] The decision to proceed with fetal blood sampling and IUT should be based on the detection of severe fetal anemia (defined as elevated peak systolic velocity in the fetal middle cerebral artery on Doppler ultrasonography[68]) irrespective of the presence of hydrops.[67] Because Hb Bart, which constitutes nearly all the Hb in ATM, does not participate in oxygen transport, fetal hypoxia is disproportionate to any given Hb level.[69] The goals of complete correction of anemia or suppression of fetal erythropoiesis must be balanced against the risk of acute cardiovascular alterations and hyperviscosity with IUT.[67] Fetal anemia develops early in ATM with a mean Hb of 6.8 g/dL at 18 weeks,[69] which implies that there could be a role for intraperitoneal IUT prior to 18 weeks of gestation when intravascular IUT using the umbilical vein becomes feasible.

Resolution of hydrops is expected with an adequate IUT regimen. Specific to the diagnosis of ATM is the recommendation to measure Hb Bart in addition to total Hb in the pretransfusion sample. Following the initial gradual correction of anemia, the target hematocrit following transfusion after 24 weeks of gestation should be chosen at the higher end of the recommended range (40% to 50%).[67] An HbA level >10 g/dL and Hb Bart <20% in the pretransfusion fetal blood sample is expected with

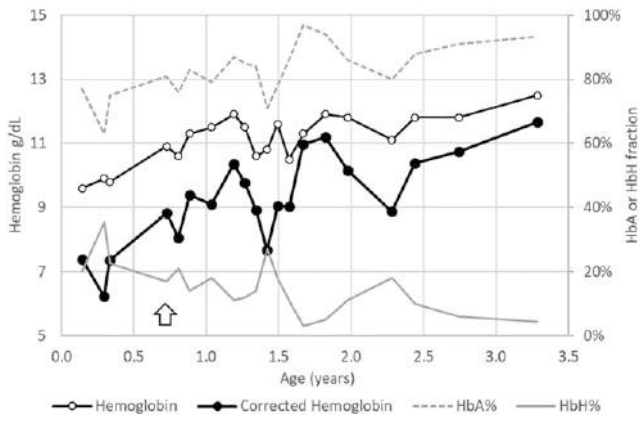

Figure 4. Case 4 depiction. A newborn with α-thalassemia major ($-^{SEA}/-^{SEA}$) was initially transfused with a goal of maintaining total Hb of 9 to 10 g/dL in the pretransfusion period (case 4). This was associated with effective functional Hb (total Hb − sum of HbH and Hb Bart) between 6 and 7 g/dL. Transfusion regimen was modified (arrow) to maintain HbA 9 to 10 g/dL, which was achieved with total Hb of ~11 g/dL in the pretransfusion blood sample.

these goals. The delivery is planned at 37 to 38 weeks with the last IUT no later than 35 weeks of gestation.[67]

Perinatal events in ATM following suboptimal fetal management are characterized by a high incidence of preterm birth, intrauterine growth retardation, cesarean delivery, birth trauma, and difficult resuscitation. Newborns exhibit respiratory distress, pulmonary hypertension, organomegaly, effusions, and hyperbilirubinemia.[61,62] Some may have congenital anomalies affecting the genitourinary system. Anemia is profound and further distinguished by a high proportion of nonfunctional Hb Bart if >2 to 3 weeks have elapsed from the last intrauterine transfusion. An urgent simple transfusion with 5 to 10 mL/kg of high hematocrit red cell unit is usually given. When the proportion of Hb Bart is very high, an exchange transfusion will rapidly improve tissue oxygenation. The goal during the first few weeks is to maintain total Hb >12 g/dL and Hb Bart <20% while the need for critical care continues.

Following stabilization, infants are in a transition period up to 6 months with intensive transfusion support under close monitoring. This period is marked by the switch from Hb Bart to HbH, resolution of hepatosplenomegaly and cardiomegaly, improvement in thrombocytopenia and transaminitis, and the establishment of consistent weight gain. Transfusions aim to maintain nadir total Hb >12 g/dL with the total nonfunctional Hb (Hb Bart plus HbH) <20%. The interval between transfusions is initially 2 weeks, but gradually lengthened to 3 weeks. Red cell antigens should be determined by genetic testing to provide antigen-matched blood. At the end of 6 months, infants transition to a chronic transfusion protocol in which pretransfusion HbA is >9.0 g/dL and transfusion frequency is 3 to 4 weeks. Following the absolute HbA level instead of total Hb is important, otherwise children with ATM are at risk for undertransfusion.[63] Infusion centers lacking access to rapid Hb electrophoresis or high-performance liquid chromatography can aim to maintain the pretransfusion total Hb at 10.5 to 11 g/dL and reticulocyte count <500 000/μL.[63] Splenectomy is not recommended in the management of ATM. Transfusional iron overload is observed early within a few months after birth, but the assessment and management of iron in ATM is not well defined. Because of the concerns over hepatic inflammation and renal immaturity, chelation is postponed until 12 months of age.

Summary

The management of TDT should be adapted to the heterogeneity conferred by various genotypes. Although a universal transfusion protocol for TDT is unfeasible, common principles underlie the long-term goals of transfusion therapy for individuals with thalassemia. With improvement in life expectancy, decisions about initiation and intensity of transfusion support in TDT should be guided by long-term natural history studies that span the various life stages.

Conflict-of interest disclosure

A.L. provided consultancy services to Chiesi USA and received research funding from Bluebird bio, Insight Magnetics, La Jolla Pharmaceutical Company, Novartis, Protagonist Therapeutics, and Terumo Corporation.

Off-label drug use

None disclosed.

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Outpatient transfusions for myelodysplastic syndromes

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Patients with myelodysplastic syndromes (MDS) often need extended periods of red blood cell or platelet transfusion support, with the goal to manage symptoms of anemia and thrombocytopenia, respectively, and improve quality of life. Many questions about the optimal approach to transfusion management in MDS, especially in the outpatient setting, remain unanswered, including hemoglobin and platelet thresholds for transfusion. Restrictive transfusion approaches are often practised, but whether these are appropriate for outpatients with MDS, who are often older and may be frail, is not known. Current schedules for transfusion-dependent patients are burdensome, necessitating frequent visits to hospitals for sample collection and blood administration. Questions of optimal schedule and dosage are being explored in clinical trials, including the recently completed REDDS study. Patient-reported outcomes and functional assessments are increasingly being incorporated into research in this area so that we can better understand and improve transfusion support for patients with MDS.

LEARNING OBJECTIVES

- Understand the goals of outpatient transfusions for patients with myelodysplastic syndromes (MDS)
- Review current evidence supporting optimal management of outpatient transfusions for patients with MDS
- Identify priority areas for future research in transfusion supportive care for MDS

Introduction

Anemia and thrombocytopenia in patients with myelodysplastic syndromes

Anemia is essentially universal in patients with myelodysplastic syndromes (MDS), and symptoms and signs of anemia, such as fatigue and dyspnea, are common presenting features.[1-4] Palpitations, headache, anxiety, insomnia, pain, and weakness are also described by patients, and these contribute to the well-documented findings that anemia is also associated with poorer health-related quality of life (QoL) in MDS.[1,4]

Both the presence and degree of anemia are important in MDS, and these have been incorporated into prognostic scores.[5,6] Malcovati et al[5] demonstrated that hemoglobin <90 g/L in men and <80 g/L in women was independently associated with worse overall survival and with both nonleukemic and cardiac causes of death. Oliva et al[7,8] reported that degree of anemia correlated with cardiac hypertrophy and remodeling, which may help explain the worse cardiac outcomes for anemic patients, although undoubtedly other factors contribute, including cardiac dysfunction from disease-related iron dysregulation and transfusion-related iron overload.[7-9] These data do not tell us whether we can modify these outcomes by applying a different transfusion policy.

Thrombocytopenia is also very common in MDS, with up to two-thirds of patients having platelet counts <100 × 10^9/L at diagnosis; 5% to 20% of patients have severe thrombocytopenia depending on the definition used (<20 or <10 × 10^9/L).[1,10,11] Thrombocytopenia in MDS is commonly multifactorial; platelet dysfunction is also common but often not recognized.[10] Presence and degree of thrombocytopenia are both associated with worse prognosis in terms of survival and progression to acute leukemia. In day-to-day management, the primary concern related to thrombocytopenia is risk of bleeding. Minor bleeding is common, and bleeding is variably reported as the cause of death in 5% to 24% of patients with MDS in different studies.[11]

In the context of these cytopenias, 50% to 90% of patients with MDS will need red blood cell (RBC) transfusions, and many become RBC transfusion dependent; 30% to 50% of patients will need ≥1 platelet transfusion.[1-3,12] With data indicating a high but variable burden of transfusion dependency in this patient population, this review

addresses recent research findings to help clinicians answer the questions: How do I decide whether a transfusion is needed for my patient, and if it is, how is it best delivered, in accordance with the principles of patient blood management? Key considerations include the following:

- The role of transfusion among the currently available therapeutic options for MDS
- The aims of, and optimal processes for delivering and monitoring, transfusion supportive care with a focus on patient QoL

Where does transfusion fit among the currently available therapeutic options for MDS?

Therapeutic approaches for MDS include those directed at ameliorating the underlying bone marrow disease or managing the resulting cytopenias. These options include growth factors such as erythropoiesis-stimulating agents (ESAs) or granulocyte colony-stimulating factor; hypomethylating agents such as azacitidine; immunosuppression or immunomodulation (eg, lenalidomide); chemotherapy; and allogeneic hematopoietic stem cell transplantation, the only current curative option.[1,9] Many novel agents, including molecularly targeted therapies, are in trials or coming into clinical practice. Recently, luspatercept has been shown to minimize anemia and RBC transfusion requirements, with 38% of luspatercept-treated lower-risk patients with MDS and ring sideroblasts becoming transfusion independent for ≥8 of the first 24 weeks on study, compared with 13% in the placebo arm, and with up to one-third of responders remaining transfusion independent for ≤48 weeks.[13] Starting disease-modifying therapy (DMT) earlier can be associated with better clinical outcomes, but these therapies are not yet widely used in routine practice, mostly because of problems with cost or tolerability.[14]

As a result, many patients with MDS, especially those with lower-risk disease, are managed with supportive care alone, including transfusion, often for months to years.[1-3,15-18] Therefore, even as new therapies emerge into practice, transfusion is likely to continue to be a central part of MDS management for the foreseeable future.

What are the aims of transfusion supportive care in MDS?

The main goals of transfusion supportive care are outlined in Table 1. RBC transfusions are given primarily to prevent serious complications of anemia, both acute and chronic, including heart failure and myocardial infarction.[1,15-17,19] Other reasons for transfusion are to manage broader consequences of bone marrow failure, including fatigue and other symptoms related to anemia, and to prevent and manage of bleeding related to thrombocytopenia, aiming to improve patient QoL. However, data on the optimal timing to start transfusions in MDS are lacking, and transfusion is usually introduced on the basis of symptoms and falling blood counts.

Various definitions of RBC transfusion dependency in MDS have been proposed, recently reviewed by Germing et al.[1] These include requirements for 2 RBCs per month, RBC transfusion three or more times in a year, and other variations which include minimum numbers of RBCs or admissions per defined time period – with these definitions being variably applicable either in the routine clinical management setting, in analysis of administrative datasets, or for determining entry or response criteria for clinical trials.[2,18,20] Transfusion intensity can be further described as low (3 to 7 RBCs per 16 weeks) or high (≥8 RBC per 16 weeks), and patients can be described either as not being transfusion dependent or as having low, medium, or high transfusion burden or dependency. These definitions can be helpful for research purposes but are probably less useful in day-to-day practice, except for the purpose of prognostication.

Management of transfusion dependency in MDS is a real conundrum: Transfusions are given with the aim of relieving symptoms, and indeed they can do so, but they provide only transient benefits related to the circulating lifespan of the transfused cells. They also carry the risks of both transfusion-related adverse effects and MDS-associated consequences: If you have MDS, being anemic or thrombocytopenic makes things worse for you, but being RBC or platelet transfusion dependent has itself been shown to be associated with worse prognosis, regardless of when it develops and even at low dose density.[1,20] This association may relate in part to the underlying disease (the worse the ineffective hemopoiesis, the worse the consequences and the worse the prognosis) but also to the transfusion and its sequelae.

What is optimal transfusion support in MDS? Is it the same for all patients?

The principles of patient blood management (PBM), such as those described by the International Society of Blood Transfusion[21] as "an evidence-based, multidisciplinary approach aimed at optimising the care of patients . . . put(ting) the patient at the heart of decisions made around blood transfusion, promoting appropriate use of blood and blood components and the timely

Table 1. Goals of RBC and platelet transfusion in MDS

Transfusion type	Goal of transfusion	Measured by	Desired outcomes
Red cell transfusion	• Improve acute and chronic symptoms of anemia (fatigue, dyspnea, chest pain, palpitations, effects on cognitive function) • Minimize major complications of (severe) anemia • Improve functional outcomes	• Hemoglobin and hematocrit • Functional measures using standardized tool (eg, fatigue score, walk distance, grip strength) or self-report	• Control of symptoms • Better functional status in activities of daily living • Increased ability to participate in work or social and community interests
Platelet transfusion	• Improve symptoms of thrombocytopenia (patient experience of skin bruising and other bleeding) • Minimize major complications of (severe) thrombocytopenia • Improve functional outcomes	• Platelet count • Bleeding assessments (eg, standardized tool or self-report)	• Improved health-related QoL

use of alternatives where available," remind us to be patient-focused. Transfusion decisions and processes should be undertaken with the patient's active participation. We cannot simply apply a one-size-fits-all approach, but how do we determine the right approach and know how to advise an individual patient with MDS? Furthermore, hematologists are trying to balance care of each patient with efforts to minimize risks and costs and make best use of precious community blood supplies. Unfortunately, as outlined in Table 2, we still have many more questions than answers on how best to do this.

The need for high-quality data applicable to hematology/oncology, including MDS, was noted by the International Consensus Conference on PBM[22] and is reflected in current national and international clinical guidelines, where most recommend individualizing therapy but can provide only limited specific guidance on management, including hemoglobin and platelet transfusion thresholds.[15,16,23] The impact of this current uncertainty on MDS transfusion management was documented in a recent practice survey of Australian and New Zealand hematologists.[24]

Consider the following cases. How would you approach the transfusion decision for each patient? What factors would influence your decision?

Case 1: A 80-year-old woman with low- to intermediate-risk MDS who lives at home with her husband presents to clinic for review. She has no past history of cardiovascular disease, and her renal function is normal. She denies any symptoms of fatigue or dyspnea. She has previously had a trial of erythropoietin for management of anemia, with no response. Her most recent blood tests included a hemoglobin concentration of 85 g/L. You consider whether to administer an RBC transfusion.

Case 2: A 63-year-old man with low- to intermediate-risk MDS who lives at home with his wife presents to the clinic for review. His past history is significant for a myocardial infarction 1 year earlier. He has no chest pain, dyspnea, or fatigue. His most recent blood tests showed a hemoglobin concentration of 78 g/L and normal renal function. You consider whether to administer an RBC transfusion.

Case 3: A 68-year-old woman with intermediate-risk MDS who lives alone presents to the clinic for review. She reports exertional dyspnea and fatigue but says these features have been long-standing. She has no history of cardiovascular disease, and physical examination reveals no signs of cardiac failure. Her renal function is normal. Her last RBC transfusion was 4 weeks ago. At what hemoglobin threshold would you initiate an RBC transfusion?

Choosing hemoglobin thresholds for RBC transfusion

Although nearly 20,000 patients have been enrolled in randomized trials comparing different hemoglobin thresholds, these have been almost exclusively in the acute anemia setting, most often in critical care or cardiac surgery, and often aimed at addressing primary outcomes of short-term (eg, in hospital) mortality. It is inappropriate to extrapolate the results from these trials to recommend a "restrictive" policy in transfusion-dependent MDS.

Unfortunately, few transfusion trials have been conducted for patients with hematological malignancies, and no studies have looked at hemoglobin threshold or outcomes studies for chronically transfused patients with other blood diseases with

Table 2. Important questions that need answers in optimizing transfusion support for patients with MDS

Red cell transfusion
1. To what degree does anemia (hemoglobin below the reference range, a laboratory result) need to be corrected to see clinical benefit?
2. When should RBCs be transfused in MDS? What are the optimal hemoglobin thresholds and targets? Are they applicable to all patients, or are there subgroups, such as older patients or those with cardiovascular or respiratory comorbidities, who need special consideration?
3. What is the optimal RBC transfusion schedule? Is a more stable hemoglobin better (should we aim to avoid the peaks and troughs of hemoglobin), and if so, why, and how can we achieve it?
4. What is the optimal RBC product for transfusion in MDS (eg, improved oxygen delivery or lifespan, or degree of RBC antigen matching)?
Platelet transfusion
5. Does degree of thrombocytopenia correlate with health-related QoL? Are there outcomes other than bleeding that are important?
6. To what degree does thrombocytopenia (platelet count below the reference range, a laboratory result) need to be corrected to see clinical benefit?
7. What should be the recommended platelet transfusion thresholds and targets? Are they applicable to all patients, or are there subgroups who need special consideration?
8. What is the optimal platelet product for transfusion in MDS? How can platelet wastage (related to short shelf-life) be minimized?
9. What alternatives to platelet transfusions can be used to reduce bleeding?
All transfusions
10. What transfusion-related outcomes matter to patients, and how can they be measured (eg, using patient-reported outcomes) and prioritized?
11. How can patients be more meaningfully involved in transfusion decision-making, the transfusion process, and monitoring of transfusion outcomes, including adverse events?
12. What tools should we use to measure clinical need for and outcomes of transfusion?
13. What are the clinical and community burdens (including costs and complications) of anemia, thrombocytopenia, and transfusion in MDS?
14. How can the outpatient transfusion process be improved to optimize the patient experience, streamline health care delivery, and reduce costs?
15. Is home transfusion an option, and if so, when and for which patients?

cytopenias, such as aplastic anemia or myelofibrosis.[22,25] A recent study of adults undergoing hematopoietic stem cell transplantation showed that a restrictive (hemoglobin [Hb] <70 g/L) threshold for RBC transfusion was safe and delivered the same QoL outcomes as a liberal (Hb <90 g/L) strategy; however, patients were young (median age 57 years), the setting was primarily hospital-based (and median hospital length of stay was 23 days) with follow-up to 100 days, and patients had short-term transfusion needs.[26] There are challenges in extrapolating these trial data, or data from other chronically anemic patients (including those with renal impairment, now generally managed with ESAs) to patients with MDS in the community, because a significant number of patients with MDS have comorbidities such as cardiovascular disease. We do not know whether a more liberal transfusion policy is indicated for patients with cardiac disease, including in the setting of MDS specifically.[27]

With this uncertainty in mind, an international group recently conducted the Red Blood Cell Transfusion Schedule in Myelodysplastic Syndromes (REDDS) pilot trial in transfusion-dependent MDS. Patients were assigned to a restrictive (Hb 80 g/L, to maintain hemoglobin 85 to 100 g/L) or liberal (Hb 105 g/L, maintaining 110 to 125 g/L) threshold for RBC transfusion, with health-related QoL measured via the EORTC QLQ-C30 and EQ-5D-5L.[28] The primary outcome (adherence to assigned study arm) was shown to be feasible, and the protocol for outpatient transfusion was successfully implemented across multiple sites internationally. The investigators noted several additional points: Patients assigned to the liberal transfusion arm received about twice as many RBCs as the restrictive arm, and the time interval between transfusions was shorter for patients in the liberal arm. This has implications for both patients and transfusion services, given the need for more visits and more blood bank activities. A post hoc exploratory analysis suggested that the 5 main QoL domains were improved for participants in the liberal arm, supporting the need for additional research to elucidate the impact of different RBC transfusion policies. Finally, the REDDS analysis highlighted the need to consider not just hemoglobin concentration but also swings of amplitude, as demonstrated in an earlier modeling analysis.[29]

Additional research is needed, and a number of other studies are registered (Canada [NCT 02099669], France [NCT03643042], and the international REDDS2 trial [ACTRN12619001053112p]). However, larger studies of transfusion thresholds in MDS will be challenging, with limited numbers of potentially eligible patients at any given site.

If improved QoL is an aim of RBC transfusion in MDS, when and how should we measure it?

Most trials of RBC transfusion thresholds have used short-term mortality as the primary outcome. Trials of interventions to address anemia, such as ESAs, have mostly measured increase in hemoglobin as a trial outcome. Although this measurement is convenient and inexpensive, it does not tell the whole story, and we need to look at the clinical impact on patients, particularly functional outcomes and QoL, through relief of symptoms for which the intervention is being given. This also applies to RBC transfusion. Documentation of patient-reported outcomes (PROs) via patient-reported outcome measures (PROMs) is being included more routinely in clinical trials and practice. Functional outcomes are particularly important for outpatients who are managing their physical ADLs as well as the social and community aspects of their lives.

Table 3 summarizes clinical studies that have assessed QoL and functional outcomes related to RBC transfusion for patients with MDS.[28-34] In addition to the REDDS pilot trial,[28] a few observational studies have measured the impact of RBC transfusion on QoL or functional outcomes, and 1 small randomized trial compared RBCs of different storage age. In the RETRO study (which included patients with a range of hematology/oncology diagnoses including MDS), RBC transfusion was associated with improvement in some (fatigue, walk distance) but not all (dyspnea) outcomes, particularly if hemoglobin was maintained at ≥80 g/L 1 week after transfusion.[30] In a subsequent analysis from the RETRO study, Bruhn et al[31] studied the impact of RBC transfusions over 4 weeks on patient-reported fatigue via serial FACIT-Fatigue scores and noted improvement in early post-transfusion scores, without real change after that. Notably, responses varied widely between patients. Chan et al.[32] used a variety of tools to study QoL after RBC transfusion for medical patients, of whom some had MDS. Worse pretransfusion QoL scores predicted post-transfusion improvement. These observations are also supported by trials that have shown improved QoL for patients with MDS with higher hemoglobin, regardless of how it is achieved (eg, in the Nordic study where patients received darbepoetin with or without granulocyte colony-stimulating factor or transfusion, or both, to achieve a target Hb of ≥120 g/L).[35]

In addition to inclusion of PROs in clinical trials, implementation of PROMs in routine clinical practice is increasingly gaining interest. Use of PROMs to inform cancer care has been shown to improve outcomes including QoL, survival, and emergency department visits in other cancers. However, few studies have been done in hematological cancer, and to our knowledge none are specific to transfusion management. Extrapolating from other cancers, potential benefits from routine use of PROMs include improved accuracy of symptom assessment, improved patient–clinician communication, shared medical decision making, and improved QoL.[36] The choice of PROM is important for both clinical studies and practice, because there is different information to be gained from generic QoL tools such as the EQ5D (which are generally simple and fast to complete and permit comparisons across different conditions) with data obtained from condition-specific (eg, MDS) or symptom-specific (eg, anemia) tools, which tend to be more targeted and comprehensive but where data may be more difficult to obtain. We should also distinguish between information gained from completing a standard questionnaire compared with providing a description of experience in the patient's own words (eg, data that may be generated from individual interviews or focus groups).[4] Moreover, it should be recognized that patient and physician perceptions may differ substantially, either in emphasis (what is relevant to one may not be important to the other) or the importance attributed to it. For example, in one study physicians tended to give more positive scores about QoL than did their patients with MDS, and patients placed greater importance on the disruption to daily life by frequent hospital visits for transfusion than did physicians.[37]

The question of hemoglobin thresholds for RBC transfusion has been further highlighted by feedback from patients with MDS. A large, multicountry survey presented at ASH 2018 reported that 40% of patients with MDS wished they had received

Table 3. Studies of the impact of RBC transfusion on QoL and functional outcomes in MDS

Study	Patients	Study design	Intervention	Outcomes assessed	Comments
Bruhn et al[31] 2020	204 outpatients >50 y with hematological or cancer-related diagnosis (40 with MDS)	Observational study	Assessed before RBC transfusion and at days 3, 7, and 28 after RBC transfusion	FACIT-Fatigue	Patients with greater fatigue at baseline had early improvement in fatigue after RBC transfusion but no significant change between day 3 and day 28 after RBC transfusion
Caocci et al[29] 2007	32 patients with MDS, 20 received RBC transfusion	Observational study	Measured the association between amplitude of Hb fluctuations with QoL over 1 mo	EORTC QLQC30, patient self-report	Lower variation in Hb correlated with better QoL and lower fatigue; transfusion-free patients reported better QoL and less fatigue than transfused patients
Chan et al[32] 2018	101 patients receiving RBC transfusion (inpatients and outpatients), 40 with hematological diagnosis	Observational study	Measured QoL before RBC transfusion and day 1 and day 7 after RBC transfusion	Short Form 12 Version 2 FACT-Anemia	Greater increase in QoL observed in patients with worse baseline QoL scores; transfusion trigger was not associated with change in QoL
Hsia et al[33] 2016	20 transfusion-dependent adults (11 with MDS)	Randomized trial (n-of-1 design)	Fresh (<7 d of storage) vs standard-issue (up to 42 d of storage) RBC transfusion	FACT-An, 3 questions on a visual analog scale Patient self-report	No difference in QoL between fresh and standard-issue RBC; no clinically significant improvement in QoL after RBC transfusion (whether fresh or standard)
Jansen et al[34] 2020	19 patients with transfusion-dependent MDS	Randomized trial	Liberal (Hb transfusion trigger <97 g/L) vs restrictive (<73 g/L) RBC transfusion protocol	EuroQoL5D, Multidimensional Fatigue Inventory	Terminated prematurely because of slow recruitment No significant differences in symptoms, QoL scores, or cardiovascular outcomes
Oliva et al[7] 2005	39 patients with MDS	Observational, cross-sectional study	Cross-sectional study of cardiac and QoL assessment according to transfusion dependence	QOL-E, cardiac echocardiography	Worse QoL in transfused patients compared with nontransfused; higher rates of cardiac remodeling in transfusion-dependent group; cardiac remodeling associated with lower mean Hb levels and older age; each unit of Hb increase predicted a 49% reduction in risk of remodeling
St Lezin et al[30] 2019	221 outpatients >50 y with hematological or cancer-related diagnosis (40 with MDS)	Observational study	Assessed before RBC transfusion and 1 wk after RBC	FACIT-Fatigue Scale, FACIT-Dyspnea Scale, 6-min walk test (6MWT)	Clinically important improvement in fatigue or 6MWT but not dyspnea 1 wk after RBC in 70%; patients who maintained Hb 80 g/L at 1 wk, who had not received cancer therapy and who did not need hospitalization, showed clinically important increases in mean 6MWT distance
Stanworth et al[28] 2020	38 patients with transfusion-dependent MDS	Randomized trial	Liberal (maintain Hb 100-125 g/L) vs restrictive (maintain Hb 85-100 g/L) RBC transfusion protocol	EQ-5D EORTC QLQC30	Post hoc exploratory analysis suggested improved QoL (global health, physical functioning, fatigue, and dyspnea)

RBC transfusions at higher hemoglobin thresholds than they currently were, suggesting that patients are aware of and concerned about the impact of anemia and how it is managed.[38]

More research is needed to elucidate the relationship between anemia and thrombocytopenia and the range of symptoms being reported (including where there may be interactions) before and after transfusion. As this field develops, we can expect to see objectively recorded functional outcomes and PROs being considered absolutely central to the evaluations of both new agents and supportive care interventions, and we can also anticipate that new technologies such as wearable devices (eg, smart watches) will be more widely used for continuous monitoring and capture of these data.

The outpatient transfusion process

The recent REDDS trial illustrated the high frequency with which transfusion-dependent MDS outpatients need hospitalization.[28] Not surprisingly, the need for multiple visits is a major burden and disruption for transfusion-dependent patients with MDS. Many aspects of our current outpatient transfusion policies have been designed to fit the needs of busy clinics and transfusion departments rather than the needs of patients. However, the optimal way to deliver outpatient transfusion services is not known. The REDDS findings indicate the need for additional studies to evaluate the impacts and costs of changing transfusion policies, because changes would have important implications for patients (frequent travel and clinic visits) and hospitals alike.

For some patients, transfusions on weekends, or even at home, might be an option. It is unclear how widespread is the use of home transfusion services for patients with MDS; however, these have been described, and where adequately resourced they may help reduce the travel and attendance burden for selected patients.[39]

Other considerations

Transfusion complications in transfused patients with MDS range from common febrile reactions to uncommon but more serious adverse effects. Few reliable data are available on rates of these complications in patients with MDS, but many cases have been reported to hemovigilance programs, perhaps reflecting the high transfusion exposures in these patients. Transfusion-associated circulatory overload (TACO) is a particular concern for older adults, many of whom have cardiorespiratory comorbidities. Whether transfusing fewer units at a time, or more slowly, or accompanied by prophylactic diuretics reduces TACO risk for patients with MDS is not known.

Identifying and managing transfusion reactions in the outpatient setting can be difficult; these events are easily missed between visits or attributed to other causes, and patients need specific instructions on whom to contact and what to report. Many transfusion adverse events are process-related, and the complexity of the multiple interconnecting clinical and laboratory processes contributes to errors and delays. Efforts to simplify the outpatient transfusion process may reduce these hazards.

Alloimmunization to RBC antigens is a major concern, with most studies describing rates of 10% to 23% in transfusion-dependent patients with MDS, but much higher rates have been reported.[40-42] Alloimmunization appears to correlate with total RBC exposure and exposure to platelet units unmatched for RBC antigens, rather than inherent immunogenicity of the RBC units or the known immune dysregulation present in the underlying MDS, but it is probably multifactorial. A significant proportion of alloimmunized patients also develop autoantibodies, which may compound hemolytic complications.[40] A wide variety of RBC antibody specificities have been reported, but antibodies to K and the Rh system antigens (especially anti-E) are the most common; therefore, providing RBCs negative for these antigens may be sufficient to minimize alloimmunization for most patients.[42] However, more extensive matching, including genotyping, may confer additional benefit and is being increasingly used in routine practice before chronic transfusion programs.[43] In retrospective studies, azacitidine use has been reported to reduce rates of RBC alloimmunization.[44]

Iron overload is commonly identified in transfusion-dependent MDS, caused by the dual problems of ineffective hemopoiesis or deranged iron metabolism from MDS and iron loading from transfused RBCs.[1,9] The toxic effects of labile plasma iron seem to play an important role in end-organ damage. Ferritin levels have traditionally been monitored to document iron overload, and an elevated level has been shown to be an independent risk factor for mortality. However, hyperferritinemia is not specific for iron overload and is starting to be supplemented by monitoring of other parameters in trials and clinical practice.

Iron chelation has been reported to reduce transfusion requirements and should be considered early for patients who become transfusion dependent.[1] However, determining who is a candidate for chelation can be difficult; for instance, higher-risk patients needing greater transfusion intensity will become iron loaded more quickly but may not benefit from chelation because of their shorter survival. Furthermore, chelation success still depends on compliance with therapy, but delivering tolerable, clinically effective and cost-effective iron chelation has been challenging, particularly until the advent of oral agents, and all chelation agents carry costs and the risk of adverse effects.

Guidelines variably recommend commencing chelation at ferritin >1,000 µg/L or after receipt of 20 to 30 U of RBCs. The recent TELESTO trial randomly assigned 225 patients with low- to intermediate-risk MDS who had received 15 to 75 U of RBCs before study entry to deferasirox or placebo.[45] Because of slow recruitment, the trial was changed from a phase 3 to phase 2 trial. Event-free survival (worsening cardiac and liver function, need for hospitalization for congestive heart failure, acute myeloid leukemia transformation) was improved in the treatment group; adverse events were common. Longer follow-up in this study will be interesting, because few patients were followed at later time points, and it remains to be seen whether these results will be taken up in routine practice, because historically chelation rates have been low in MDS, especially for older and frail patients, even though chelation improves clinical outcomes.[46]

A few words about platelet transfusions in MDS

Up to 50% of patients need some platelet transfusion support. Routine prophylaxis (to prevent bleeding) with platelet transfusion for patients with MDS and thrombocytopenia who are not undergoing intensive therapy is not recommended, but few reliable data exist on how commonly this is performed in practice or how effective platelet transfusions are.[10-12] There is no international definition of platelet transfusion dependency in the MDS setting.

Dosing of platelet transfusion has largely been extrapolated from studies of other hematological malignancies. In a retrospective review of outpatient platelet transfusions, where 57% of the patients had leukemia or MDS, there was no clear advantage in transfusing 2 U of platelets compared with 1 U.[47] In a recent analysis from the South Australian MDS Registry, 9% of all patients (and 30% of women receiving DMT, compared with only 5% of men receiving DMT) who received a platelet transfusion developed immune-mediated refractoriness to platelet transfusions and needed HLA-matched platelets for future transfusion support, increasing the risks of bleeding and the costs and complexity of care.[12]

One approach to severe thrombocytopenia in MDS is to offer routine tranexamic acid. A number of randomized trials are testing the safety of tranexamic acid in acute settings of hematological malignancies, although none are specifically recruiting outpatients with MDS.

Conclusion—and the way forward

New understanding of the MDS disease process and the availability of novel therapies offer the prospect of major advances in management and outcomes for patients over the coming years. However, transfusion is still an important part of MDS supportive care. Although blood components are safe in most countries, they carry substantial risks and costs, and patients who are chronically transfused are recurrently exposed to these hazards. We need to make every management decision, including each transfusion decision, carefully, guided by the available evidence and the principles of PBM to deliver personalized MDS and transfusion therapy based on an individual patient's needs and with their participation.[48] We also need to monitor the impact of

our transfusion therapy, including the effects on QoL. Many questions remain, some of which are summarized in Table 2. By designing and contributing to high-quality, patient-centered research using a range of approaches, including clinical registries to provide real-world practice and outcome data, and performing well-conducted clinical trials incorporating PROs and functional assessments, we can strengthen the evidence base to guide our practice and improve outcomes for transfused patients with MDS.

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E.M.W. is president of the International Society of Blood Transfusion.

Z.K.M. is a member of the Scientific Advisory Committee of the Australasian Leukaemia and Lymphoma Group.

Off-label drug use

None disclosed.

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Social aspects of chronic transfusions: addressing social determinants of health, health literacy, and quality of life

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Chronic monthly transfusions are a lifesaving preventative therapy for many patients with sickle cell disease; however, the burden of this therapy for patients and families is high. In the United States, there is overlap in the population affected by sickle cell disease and those with the greatest burden of social needs. Hematology providers caring for patients with SCD have an opportunity to screen for and mitigate social determinants of health, especially in those receiving chronic transfusion therapy given the frequent interactions with the healthcare system and increased demand on already potentially limited resources. Given the complexity of the treatment and medication regimens, providers caring for patients receiving chronic transfusions should implement universal strategies to minimize the impact of low health literacy, as this therapy imposes a significant demand on the health literacy skills of a family. Despite the social and literacy burden of this intervention, it is reassuring that quality of life is preserved as patients with SCD on chronic transfusion therapy often report higher health related quality of life than their peers receiving other disease modifying therapies.

LEARNING OBJECTIVES

- Understand the impact of social determinants of health on a chronically transfused population with sickle cell disease, as well as available screening tools.
- Recognize the role of health literacy in complex medical decision-making and universal communication strategies to improve comprehension in patients of all health literacy levels.
- Identify health-related quality of life measures that are affected by chronic transfusion therapy.

Clinical case

The patient is a 2 year-old girl with hemoglobin SS. She has been followed in the hematology clinic since birth. She was born at home at an estimated 32 weeks to a 27-year-old G5P5 mother who had limited prenatal care. Her older siblings are all half-siblings and range from 2 to 12 years old. There are no other siblings with sickle cell disease (SCD). The patient has had 1 admission for acute chest syndrome and several admissions for fever and dactylitis. Her mother is unemployed and spends her time caring for her family at home. Her father is not involved in her care. The patient has Medicaid and is enrolled in the Special Supplemental Nutrition Program for Women, Infants, and Children. Her mother expressed concern about lead exposure at her 9-month hematology visit, given peeling paint in their home, but she subsequently failed to routinely follow up. Social workers connected with the patient's mother, who identified transportation as a significant barrier to attending clinic visits. The family received parking vouchers and gas cards but continued to miss appointments because of difficulty finding care for the other children in the home. Unfortunately, no extended family members were available to alleviate that burden, because many worked at the time when clinic was open. After she failed to come for her first transcranial Doppler, the medical team contacted child protective services with concerns about medical neglect.

Social determinants of health

Many facets of patient health are influenced by factors outside of the hospital or clinic setting. Health is heavily shaped by our families, homes, neighborhoods, and communities. These external factors are referred to as social determinants of health (SDHs), and they affect how patients interact with the health care system. In the United States, SDHs have been prioritized as part of the national health agenda in

Healthy People 2020, where the goal is to "create social and physical environments that promote good health for all."[1] The World Health Organization has also prioritized policies and prevention strategies that affect SDHs because 23% of deaths globally can be attributed to factors in the environment.[2]

SDHs are very broad and often overlap, but they can be organized into general categories. Healthy People 2020 divides the elements of SDHs into 5 domains: education, social and community context, health and health care, neighborhood and built environment, and economic stability.[1] SDHs such as financial stress, food insecurity, and housing instability have all been linked to poor health outcomes and early mortality,[3,4] and families often have resource needs across multiple domains.[5] Adversity experienced in the home may be categorized as a separate domain, because adverse childhood experiences (ACEs) have been shown to independently shape health outcomes later in life.[6] ACEs include exposure to child abuse, childhood neglect, and household dysfunction. They have been linked to health risk behaviors in a variety of cultural and economic settings.[7]

Poverty, or low socioeconomic position, underlies many social problems and increases the risk of experiencing ACEs.[8] In the United States, where structural inequity and systemic disenfranchisement have led to generational cycles of poverty that disproportionately affect racial and ethnic minorities, patients affected by SCD overlap with those at greatest risk for resource needs.[9] In a quality improvement study that implemented universal screening for SDHs in a US pediatric hematology clinic, 156 screens were completed, and 66% were positive for at least one unmet need. On average, families reported 1.2 unmet needs (range 0 to 5), with food insecurity, difficulty paying for utilities, and a desire for more education being the most common.[9] Our patient case highlights some of the domains affected at baseline by our patients and families, which may be further taxed upon starting chronic transfusion therapy given the intensity and frequency of therapy. Caregivers lose more days from work, have higher transportation costs, have additional medication costs, and have a high demand for childcare if other siblings cannot come to clinic visits. For pediatric patients, absences from school affect their educational attainment and interactions with peers.

Socioeconomic disparities, such as lack of transportation and inadequate health insurance, have been shown to affect access to comprehensive care for SCD. These factors prevent patients from receiving appropriate and timely screening for the adverse effects of SCD, increasing the medical burden and health care costs of this disease.[10] In a post hoc analysis of baseline data from the Silent Cerebral Infarction Transfusion trial, every $10,000 increase in reported household income was associated with 5% fewer annualized emergency room (ER) visits.[11] In a national sample of pediatric patients with SCD in the United States, children who visited the ER in the previous year were more likely to live in a household with a single mother; however, other SDHs included in the analysis were not significant predictors of ER use.[12] In that study, authors postulate that the generally high burden of social disadvantage among children with SCD may explain their results.

A number of available screening tools for SDHs query elements from a variety of domains. See Table 1 for additional information about SDH screening tools for pediatric patients and families. Implementation of such screening tools can quickly identify the patients and families at highest risk. However, it is unclear which SDH or SDH domain has the greatest influence on patient health outcomes and health care utilization. Also, the critical SDH domain probably varies by country and location, given the differing availability of resources. For example, health care access is more critical in the United States, where universal health care does not exist.

If patients or families declare a need, programs should be prepared to connect families with resources, because it is unethical to screen for SDHs without providing support[25]; however, it is unclear which interventions provide the greatest benefit. For concrete needs, such as food insecurity, referring a family to a food bank may provide immediate assistance. Some needs, such as a desire for more education, may require a broader network of support to be achievable. Additionally, resources vary significantly between locations, so any program attempting to implement a screening tool for SDHs should engage members of community support organizations to review the resources available in their area. In the United States, programs that identify local resources include the United Way (www.211.org) and the EveryONE Project's Neighborhood Navigator (www.familydoctor.org/NeighborhoodNavigator), sponsored by the American Academy of Family Physicians.

The workflow to implement SDH screening and referral also varies by clinical practice. Some clinics may have an embedded social worker, mental health counselor, or legal representative to provide direct support to patients and families who disclose need. Others clinics will need to develop partnerships with community organizations, maximizing the creation of a "health neighborhood."[25] Some interventions require additional personnel, such as a community navigator, to provide support for families[18]; however, other successful strategies have used minimal contact interventions, such as faxed referrals from clinics directly to community partners.[26] How to screen patients and families is also unclear. Some studies show improved reporting with anonymous computer or web-based surveys,[17] whereas others have integrated SDH screening directly into the electronic health record.[27] If screening tools are to be implemented in a clinical setting, they should be implemented universally to avoid stigma and perceived bias. It is also essential to build on a family's and community's strengths and elicit the input of the family as to which resources may provide the most critical benefit.

Many studies on SDH screening tools have focused on implementation outcomes such as how the questions were perceived by patients or families, time spent on the screening or intervention, or quantity of referrals provided. Other studies have assessed the effect of the intervention on SDHs such as employment rates at follow-up or enrollment in social services. Although data on outcomes are mixed, they have generally yielded positive effects, and screening and interventions are acceptable to patients and families. However, few studies have quantified the impact of SDH screening and interventions on health outcomes and health care utilization.[28]

Case part 2

The patient's initial transcranial Doppler was abnormal, and she was started on monthly chronic transfusions to reduce her risk of stroke. After several months, the patient is prescribed chelation therapy with written steps for connecting with the specialty pharmacy; however, at subsequent transfusion visits, the

patient's mother reports that she has not received the medication. The team provides verbal education about iron overload; however, several months pass without the child receiving chelation, and her ferritin continues to rise. At visits, the patient's mother understands that she is supposed to start the medication, but she is unable to articulate why she needs the medication or how to set up delivery.

Health literacy

Given the difficulty obtaining the medication, the team became concerned about the family's health literacy, which is another factor that has been postulated to be a mediator of health disparities. Health literacy is "the degree to which individuals have the capacity to obtain, process and understand basic health information and services needed to make appropriate health decisions."[29] In the United States, risk factors for low health literacy include low socioeconomic status, racial and ethnic minority status, lower educational attainment, poor English proficiency, older age, and birth outside the country.[30,31] It is estimated that 28% of parents in the United States have limited health literacy.[31] Among parents of children with SCD, low health literacy has been associated with lower disease-specific knowledge, although it was not associated with increased health care utilization, such as hospitalizations or ER visits.[32]

The greater the complexity of the tasks and decisions being placed before families, the greater the need for thorough understanding and comprehension, so adequacy of health literacy relies not only on the patients and families but also on the health care situation at hand. For patients with SCD receiving chronic transfusions, even those with the highest personal levels of health literacy may have difficulty with comprehension and decision making given the complex nature of the illness, the need to weigh multiple potential risks to the patient, and the many tasks set before them. In a cross-sectional study of health literacy among adult caregivers of children receiving chronic transfusion therapy and adolescents with SCD personally receiving chronic transfusion therapy, 34% of caregivers and 69% of adolescents had inadequate health literacy based on standardized testing, and low health literacy was associated with lower disease-specific knowledge. Knowledge scores for caregivers were weakly correlated with community-level median income and unemployment rates but not with insurance payor status.[33] The greatest gaps in disease-specific knowledge among those caring for or receiving chronic transfusion therapy were in questions related to red blood cell alloimmunization status and risks, the availability of curative treatments for SCD, and personal indications for receiving chronic transfusion therapy, indicating opportunities for improvement.[33]

One web-based decision tool that has been developed for patients with SCD considering chronic transfusion therapy or other therapeutic options is available at www.sickleoptions.org. It provides information, personal stories, and additional references as a decision aid for patients, using strategies that have been shown to overcome low health literacy. It is written at a ≤5th grade reading level and maximizes the use of graphics to improve understanding. In a randomized controlled trial, it was highly acceptable to patients and easy to use. Compared with patients who did not receive the additional decision supports, those who did had greater decisional self-efficacy, less decisional conflict, and better preparation for decision making.[34]

A number of validated tools are used to assess domains of health literacy, including disease-specific literacy. See Table 2 for additional information on a sample of commonly used health literacy screening and assessment tools. Additional resources are available through the Health Literacy Toolshed, available at https://healthliteracy.bu.edu/. The majority of these tools are intended for adults or adult caregivers; however, some tools have been validated for use in children and adolescents.

Simplifying the information, using plain language, and limiting the amount of information to 3 key messages at a time are evidence-based strategies that allow families to better comprehend the critical elements being conveyed to them.[43] If time allows, having patients demonstrate skills or teach back to the provider the instructions for medication or care are additional methods shown to improve comprehension and health-related outcomes. Information delivered via multiple different modalities, such as text, pictures, or video, and information that can be reviewed after the office visit have also been shown to improve understanding.[43]

It is incumbent on the whole health care system to improve communication in ways that overcome low health literacy and make it easier for all patients to navigate, understand, and interpret information relevant to their health and the health of their children.[43] Providers caring for patients with SCD should improve communication strategies on general topics critical for decision making and ensure that they are universally available. For patients on chronic transfusion therapy, providers should capitalize on the frequent encounters to teach and engage patients directly about their personal condition and complications so that patients can be empowered decision makers in the future.

Case part 3

The team implemented several teaching strategies, including limiting each education session to 3 critical points and providing written materials, to convey the risks of iron overload. The written information on how to set up medication delivery from the specialty pharmacy was enhanced to include specific contact numbers and expected timelines for when to hear back, as well as whom to call if medications were not delivered. Additionally, a case manager was available to reinforce that education, coordinate appointment scheduling, send reminders for medication refills and appointments, and ensure that any medications requiring prior authorizations were up to date. The patient has now been receiving regular transfusions for >18 months, and her ferritin is in the goal range. Her mother reports that the patient is "doing great." She has not had any admissions since she started on transfusion therapy. Her mother has regularly scheduled childcare for her other children during transfusion visits because those can be planned in advance.

Quality of life and well-being

For patients with chronic illnesses, improved quality of life is arguably the most important result of any therapy; however, historically, this measure has been overlooked as an outcome. As greater emphasis has been placed on patient-reported health-related quality of life (HRQOL), generic and disease-specific measures of HRQOL have been developed and validated to measure the influence of health on functional domains, such

Table 1. Examples of screening tools for SDHs

Screening tool	Administration method, time to complete	Summary of items evaluated	Additional notes	Age range (y)	References
Safe Environment for Every Kid Parent Screening Questionnaire	Paper and pencil or computer, 3-4 min, 16 or 20-item questionnaire with yes/no options	Food insufficiency Smoke alarm needed Poison control contact needed Parental stress and depression Parental intimate partner violence Parental drug or alcohol problems Tobacco use in home Help with the child is needed	Part of a larger intervention that includes training of clinicians, identification of community resources, and social work support.	0-18	13,14
HealthBegins Upstream Risks Screening Tool	Face-to-face interview, 6 min	Food insufficiency Difficulty making ends meet or basic needs Parental employment Parental education Concerns about the child's learning or behavior Parental physical activity and consumption of nutritious foods Housing and neighborhood concerns Religious or organizational affiliation Parental social support Parental marital status		NR	15
Health Related Social Problems Screener	Computer or tablet, 20 min	Food insufficiency Housing instability Parental employment Household income Insurance status Problems receiving health care Intimate partner violence Housing conditions	Survey used branching logic and ranged from 99 to 166 questions based on responses provided. Referrals were automatically generated. This format was highly acceptable to most families.	0-6	16
iScreen	Computer, face-to-face, or phone interviews, 10 min, 23 items assessed via Likert scale	Economic instability Concerns about lack of child care or services Physical or mental health coverage Concerns about tobacco smoke or physical activity Concerns about housing or transportation Immigration status Drug or alcohol problems in the home Family member incarceration Violence toward the child in the home	Informants using the computer-based platform disclosed more needs, both in quantity and as a higher degree of endorsement than those performed face-to-face.	0-18	17,18

Adapted from Sokol et al.[23] and Moen et al.[24]
NR, not reported.

Table 1. (Continued)

Screening tool	Administration method, time to complete	Summary of items evaluated	Additional notes	Age range (y)	References
Addressing Social Key Questions for Health Tool	Paper and pencil, 4-5 min, 13-question screen	Food insufficiency Housing insecurity Need for legal aid Parental education and employment Child witnessed violence and bullying Child physical and sexual abuse Child separation from caregiver Parental mental illness or substance abuse	Addresses ACEs and unmet social needs but low capture rate of ACEs.	0-18	19
WE CARE Survey Instrument	Paper and pencil, 4-5 min, 6 questions	Food insufficiency Housing instability Parental education and employment Difficulty paying bills Lack of child care		0-10	20,21
Family Needs Screening Program	Paper and pencil, 1-page questionnaire with mostly yes/no options	Housing instability Difficulty with paying bills, meeting basic needs, or getting public benefits Need for legal aid Parental education Help with child or elder care Health literacy Experiences of discrimination Available social supports Parental alcohol, drug, and tobacco use Housing conditions	Available in multiple languages targeting recent immigrants. Resource navigators supported families following a positive screen. Electronic referrals to community-based services also provided.	0-18	22

Adapted from Sokol et al.[23] and Moen et al.[24]
NR, not reported.

as physical quality of life (QOL), mental QOL, fatigue, pain, social engagement, relationships, and emotional distress. Unfortunately, adult and pediatric patients with SCD report lower HRQOL in nearly every domain compared with unaffected peers, especially in the areas of pain, fatigue, and physical functioning.[44] Female gender, older age, pain, socioeconomic factors such as insurance type, negative perceptions of SCD, and poor adherence to hydroxyurea increase the risk of lower reported HRQOL for patients with SCD.[45-50] See Table 3 for examples of HRQOL measurement tools used for pediatric patients with SCD.

Qualitative surveys have identified QOL domains that specifically affect pediatric patients receiving chronic transfusion therapy. These domains include physical and psychological aspects of pain, the positive and negative influence of chronic transfusion therapy on school and academics, fear of having a stroke, and an awareness of their disease burden and how it is influenced by their transfusion therapy.[57] However, as exemplified by the patient case, pediatric patients receiving chronic transfusion therapy appear to have better HRQOL than others with SCD, despite the burdens of the treatment. As part of the Silent Cerebral Infarction Transfusion trial, parents completed a QOL questionnaire, the Child Health Questionnaire Parent Form 50, at baseline before randomization, and at the child's last visit on the trial (36 months) or at the time of acute neurological event. There were no differences in baseline QOL scores, but at completion of the study, parents of patients on chronic transfusion therapy reported improved QOL in the domains of physical functioning, decreased pain, and improved overall health. In addition, the scores of those receiving transfusion therapy improved by a greater degree over time, reflecting improved perceptions of general health, physical functioning, and health compared with the year before.[58]

Using a self-reporting tool, the Pediatric Quality of Life Sickle Cell Disease module (PedsQL SCD), pediatric patients receiving chronic transfusion therapy reported less pain and fewer effects from pain on functioning when compared with peers. Overall scores of HRQOL were significantly higher among patients on chronic transfusion therapy than among those with SCD classified as having severe disease, and patients on chronic transfusions reported significantly less pain than peers classified as having both severe or mild disease. Importantly, a higher proportion of patients receiving chronic transfusion therapy scored

Table 2. Examples and characteristics of commonly used tools to assess health literacy

Screening tool	Questionnaires available, metrics for completion	Summary of literacy domains evaluated	Additional notes	References
Newest Vital Sign	6 items, 3 min	Written communication Medical numeracy, quantitation	Available in English and Spanish. Interpretation of a nutrition label. Tested in children as young as 10 y old.	35
Parental Health Literacy Activities Test (PHLAT)	20 items, 21 min PHLAT-10: 10 items, 13 min	Written communication Medical numeracy, quantitation	Queries 3 domains: nutrition, growth, development; injury and safety; and medical and preventive care.	36
Rapid Estimate of Adult Learning in Medicine	66 items, 2-3 min Short Form: 7 items, 1 min Teen Form: 66 items, 2-3 min Teen Short Form: 10 item, 20 s	Oral and written communication Vocabulary knowledge	Subjects are graded on their ability to pronounce medical terms.	37,38
Single Item Literacy Screener	1 item, 1 min	Written communication	Single question: "How often do you need to have someone help you when you read instructions, pamphlets, or other written material from your doctor or pharmacy?" Likert scale where 1 = Never, 5 = Always. Score >2 considered positive.	39
Test of Functional Health Literacy in Adults	67 items, 20 min Short Form: 40 items, 7 min	Written communication Medical numeracy, quantitation	Available in English and Spanish. Asks questions about instructions for prescriptions or medical scenarios. Includes fill-in-the-blank portion identifying the most contextually appropriate word. Validated in children 13-17 y old.	40
Health Literacy Skills Instrument	Computer based 25 items, NR Short Form: 10 items, 10 min	Print literacy Oral literacy Numeracy Internet navigation	Publicly available and can be self-administered using a computer.	41,42

Adapted from Morrison et al.[43]
NR, not reported.

at a level consistent with "high functioning" in the domains of pain and pain-related functioning, reflecting the improved clinical effect of chronic transfusions.[59] Despite the barriers and challenges to chronic transfusion therapy, HRQOL appears to be improved among pediatric patients who receive this life-saving treatment.

Optimizing care

Because the majority of patients with SCD in the United States are of African descent, cultural and racial barriers to care exist. SDHs and health literacy are mediators of health disparities that unjustly burden patients with SCD. Providers should be aware of the stigma that patients with SCD face and work to mitigate systemic disadvantages through resources offered to patients and through greater advocacy and policy efforts at the national level. The effects of SDH on health care utilization, disease burden, and mortality are high. For many pediatric patients with SCD, their hematologist becomes their medical home. For those on chronic transfusion therapy, the intensity of treatment may increase the burdens on an already stretched network of resources. But regular and frequent interactions with the health care system are opportunities to identify needs and refer patients and families for support. At initiation of chronic transfusion therapy, providers should screen for SDHs. More research is needed to identify ideal screening strategies, but screening tools as brief as 2 questions can predict who is at highest risk of unmet needs across multiple domains.[5] Resources should be identified before implementation of screening, so that if a family screens positive, they can receive support right away. The approach to screening and referral must be tailored to the resources available, workflow in clinic, and population served, but rescreening should be planned at regular intervals because SDHs

Table 3. HRQOL measures used in studies of pediatric patients with SCD

Screening tool	Questionnaires available, metrics for completion	Summary of domains evaluated	Additional notes	References
Child Health Questionnaire	Parent-Report Form: 50 questions, 10-15 min Parent-Report Short Form (CHQ_PF28): 28 questions, 5-10 min Child Self-Report Form (CHQ_CF87): 87 questions, 14 min Child Self-Report Short Form (CHQ_CF45): 45 questions, 11 min	Global general health Global general behavior General health Bodily pain Self-esteem Physical functioning Physical health summary Psychosocial health summary Role functioning: physical General behavior Role emotional: behavior Family activities Mental health Family cohesion Parental impact: emotional Parental impact: time	Appropriate for parental reporting for children 5-18 y old. Child self-report validated for children ≥8-10 y old. 1 mo recall, except change in health, which asks for 1 y recall. Responses vary from 4 to 6 level options. Parent report forms provide 2 summary scores for physical and psychosocial health.	51,52
Pediatric Quality of Life (PedsQL)	Can be facilitated or read to patient or family member PedsQL Generic Core Scales: 23 items, 4 min PedsQL Generic Core Short Form: 15 items	Generic Core Physical functioning Emotional functioning Social functioning School functioning Sickle Cell Disease Module	Provides summary scores for overall, physical, and psychosocial health. Parent report and child self-report forms separated into developmentally appropriate questions available for children 2-18 y old (5-18 y old if self-reporting). Different referent timeframes in the SCD module, 1 mo vs 7 d (acute).	49,53
PedsQL Sickle Cell Disease Module	PedsQL SCD: 43 items	Pain Pain-related functioning Pain management Health-related worries Treatment Communication with care providers		
Patient-Reported Outcomes Measurement Information System (PROMIS)	PROMIS Parent-Report PROMIS Child Self-Report	Global health measure including physical and mental health. Includes 22-25 domains related to physical, mental, and social health including: Physical functioning Pain Fatigue Emotional distress – anxiety, depression, anger Social functioning Family and peer relationships Physical activity	Self-report measures appropriate for children 8-17 y old. Parent reports available for children 5-17 y old. Shown to be responsive to acute pain in SCD. Available Proxy Profiles of varying length (25, 37, or 49 questions).	54-56

vary with time and circumstances. More research is needed to determine which interventions have the greatest impact on health outcomes; however, for the individual patient or family, asking about and providing resources for an unmet need not only builds trust in the medical team but may serve as an entry point for families to access additional support. Ideally, a multidisciplinary team that includes social work, case management, nursing, and psychology should collaborate to engage and support patients and families to achieve the best health outcomes. Clinics should adopt general strategies to overcome low health literacy in all communications with families, and implementation should be universal and culturally sensitive. Despite the many challenges posed by chronic transfusion therapy, patient QOL appears to be improved.

Conflict of interest disclosure

The author has no relevant conflicts of interest.

Off-label drug use

None disclosed.

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Can pregnancy-adapted algorithms avoid diagnostic imaging for pulmonary embolism?

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The low prevalence of pulmonary embolism (PE) among pregnant patients presenting with suspected PE implies that most of these patients will be found not have the disease. Given this low prevalence, excluding PE in this population has necessitated the use of sensitive and specific diagnostic imaging, such as computed tomography pulmonary angiography or ventilation-perfusion scanning. Recent studies suggest that a clinical prediction rule with D-dimer testing can also be used to exclude a subset of pregnant patients with suspected PE without the need for diagnostic imaging. The YEARS criteria, which consist of clinical signs and symptoms of deep venous thrombosis, hemoptysis, and PE as the most likely diagnosis (a subjective variable), combined with selective D-dimer levels, seem to safely exclude up to one-third of these patients without imaging. The revised Geneva rule using objective variables, combined with nonpregnancy cutoffs for D-dimer levels, offers some promise, although fewer patients avoided imaging (14%). These recent studies provide evidence in support of radiation avoidance for some patients; however, for most, imaging remains the only option. Future studies should focus on improving the safety and techniques of imaging modalities, in addition to improving the specificity of D-dimer testing and objective prediction rules. Studies assessing patients' and physicians' values, preferences, and risk perceptions are also required to assist clinicians in shared decision making when counseling pregnant patients with suspected PE.

LEARNING OBJECTIVES

- Learn to apply clinical decision rules and D-dimer results in pregnant women presenting with suspected pulmonary embolism, and avoid diagnostic imaging in some of these patients
- Recognize that improvements in safety and quality of imaging techniques are still required
- Explore patients' and physicians' values and risk tolerance to optimize decisions on diagnostic imaging use in these patients

Case

A 35-year-old woman pregnant for the first time presents to the emergency department at gestational age of 28 weeks complaining of shortness of breath lasting several days. She has no hemoptysis or clinical signs or symptoms of deep venous thrombosis (DVT). The emergency physician does not want to miss possible pulmonary embolism (PE) but is concerned about ordering computed tomography pulmonary angiography (CTPA) or a ventilation-perfusion (VQ) scan. You are asked about the safety of the tests.

Diagnostic imaging

Until recently, excluding PE in pregnant patients presenting with suspicious symptoms required diagnostic imaging.[1,2] Unlike in nonpregnant patients, specific prediction rules in pregnant patients have not been developed, and D-dimer tests with manufacturers' suggested cutoffs might not be specific enough to be used in pregnant patients.[3] In the absence of prospective studies defining the use of these ancillary tests to exclude PE, imaging diagnostic tests (ie, VQ scanning and CTPA) are the sole tests used to exclude PE. In addition to their performance, the major concerns surrounding these tests are radiation exposure of both maternal chest and developing fetus.

Several systematic reviews examining the performance of CTPA vs VQ scanning in pregnant women[4,5] have been published. In a recent review[4] that compared the diagnostic efficiency of both techniques, a total of 1270 patients who had a VQ scan were compared with 837 women who underwent CTPA. The negative predictive value of negative CTPA or VQ scan was 100% in the setting of low disease prevalence of 1% to 7%.[4] The sensitivity rates of

CTPA and VQ scanning in pregnancy, using clinical follow-up (3-24 months) as a surrogate for the reference standard, were 83% (95% confidence interval [CI], 0% to 100%) and 100% (95% CI, 0% to 100%), respectively.[5] The specificity of CTPA and VQ scanning for pregnant women with suspected PE will likely remain unknown. Although the specificity of these tests is assumed to be 100%, this is not accurate.[6,7]

These imaging studies do not always offer a result of certainty. The pooled rates of nondiagnostic tests of VQ scanning vs CTPA were similar in 1 review,[4] at 14% (95% CI, 10% to 18%) and 12% (95% CI, 6% to 17%), respectively. However, the authors acknowledged that the rates associated with nondiagnostic VQ scanning may have been overestimated. Moreover, the management of patients with a nondiagnostic VQ scan is vastly different from the management of those undergoing nondiagnostic CTPA. Patients with a nondiagnostic VQ scan might not require further imaging in the setting of low prevalence of disease.[8] In contrast, results from a nondiagnostic CTPA study,[9] in which the study protocol was inadequately modified to accommodate for the physiological changes of pregnancy, are problematic, and further imaging is recommended for definitive diagnosis.[1,2]

Above all, the major concern surrounding the use of any diagnostic imaging technique is the risk of maternal and fetal radiation exposure. In these studies, the risk of fetal radiation exposure with VQ scanning ranged from 0.32 to 0.73 mGy, whereas the risk with CTPA ranged from 0.03 to 0.66 mGy.[10] With exposure <50 mGy, the fetal risk of death, malformation, impaired neurodevelopment, or malignancy is likely minimal.[11] Beyond fetal radiation exposure, exposure of maternal chest to radiation with CTPA is substantial. The estimated mean effective radiation doses for CTPA and VQ scanning are 7 to 21 and 0.9 to 1.29 mSv, respectively, with breast-absorbed doses of 10 to 44 and 0.11 to 0.95 mGy, respectively.[10,11] Although the risk of early-onset breast cancer was not observed to increase in a large population cohort study[12] with median follow-up of 5.9 years in the computed tomography group and 7.3 years in the VQ scanning group, these findings are not yet reassuring because the length of follow-up is inadequate and the contribution of many confounders is unknown. More importantly, the cumulative effect of multiple chest diagnostic studies over a woman's lifetime was not explored.

As timely access to VQ scanning becomes less available in many centers, CTPA will likely become the diagnostic modality of choice for most pregnant women with suspected PE. Fortunately, the radiation dose to the maternal breast associated with modern CTPA techniques has also steadily declined over the last decade, with median exposure reported to be as low as 3 to 4 mGy.[13] Unfortunately, the number of studies performed to diagnose PE in pregnancy using CTPA has increased fourfold, without an increase in the rate of positive tests.[14] A prospective study is currently under way to evaluate the use of a low-dose CTPA protocol for pregnant women[15] to ensure that such an approach remains safe in excluding PE.

After your reassurance that imaging tests are safe, the patient wants to know if she really needs the tests?

In the last few years, 2 prospective management studies using D-dimer testing and the clinical prediction rule to exclude PE in pregnant women were published.[16,17] Guidelines incorporating the results of these 2 studies were also published.[18] The results from both these studies are summarized in Table 1.

In the first study, involving 395 patients, Righini et al[16] applied the revised Geneva score[18] and D-dimer testing to stratify pregnant women with suspected PE (defined as acute onset of new or worsening shortness of breath or chest pain without another obvious cause) into 2 groups: those who did and those who did not require further imaging (Figure 1). Pregnant women with high PTP for PE underwent CUS and CTPA (after negative CUS). Those with nonhigh PTP were further stratified using D-dimer levels into those who required CTPA (≥500 μg/L) and those who did not (<500 μg/L).

The revised Geneva prediction rule was adopted with no modification for pregnant women; most patients were classified as low or intermediate PTP (99.2%). Applying a D-dimer level cutoff of <500 μg/L among patients with low or intermediate PTP, 46 women (11.7%) did not undergo imaging. No VTE was diagnosed during follow-up in these patients, but 2 women received ongoing anticoagulation. Five patients with symptoms of DVT at presentation underwent bilateral CUS to identify DVT. Overall, CTPA was avoided in 14.2% of patients presenting with suspected PE in the study (ie, 85.8% required imaging). Of the 308 CTPAs performed, 5.2% were nondiagnostic; therefore, further imaging with subsequent VQ scanning was indicated.

The incidence of PE in the study by Righini et al[16] was 7.1% at study entry; the calculated incidence of PE among patients requiring CTPA was 6.1% (Table 1). The reported rate of symptomatic VTE in women with low or intermediate probability and a negative D-dimer test or a positive D-dimer test and negative or nondiagnostic imaging after completion of clinical follow-up

Table 1. Summary of findings reported from prospective studies of PE diagnosis in pregnancy

Study	Site of recruitment	Assessment of PTP	D-dimer assay and cutoff	Prevalence of PE at presentation, %	Prevalence of PE once CTPA was indicated, %	CTPA avoided, %	Subsequent VTE diagnosed during follow-up in those who avoided CTPA based on PTP and D-dimer
Righini et al[16]	Emergency	Revised Geneva	Vidas assay <500 μg/L	7.1	6.1	14.2	0 (0%) of 46* (95% CI, 0% to 8%)
van der Pol et al[17]	Emergency and obstetrical unit	YEARS	Various sensitive D-dimer assays using selective cutoffs at 500 and 1000 μg/L	4	5.4	39	1 (0.51%) of 195† (95% CI, 0.09% to 2.9%)

PTP, pretest probability; VTE, venous thromboembolism.
*Two patients were receiving anticoagulation.
†Unknown if any patient was receiving anticoagulation.

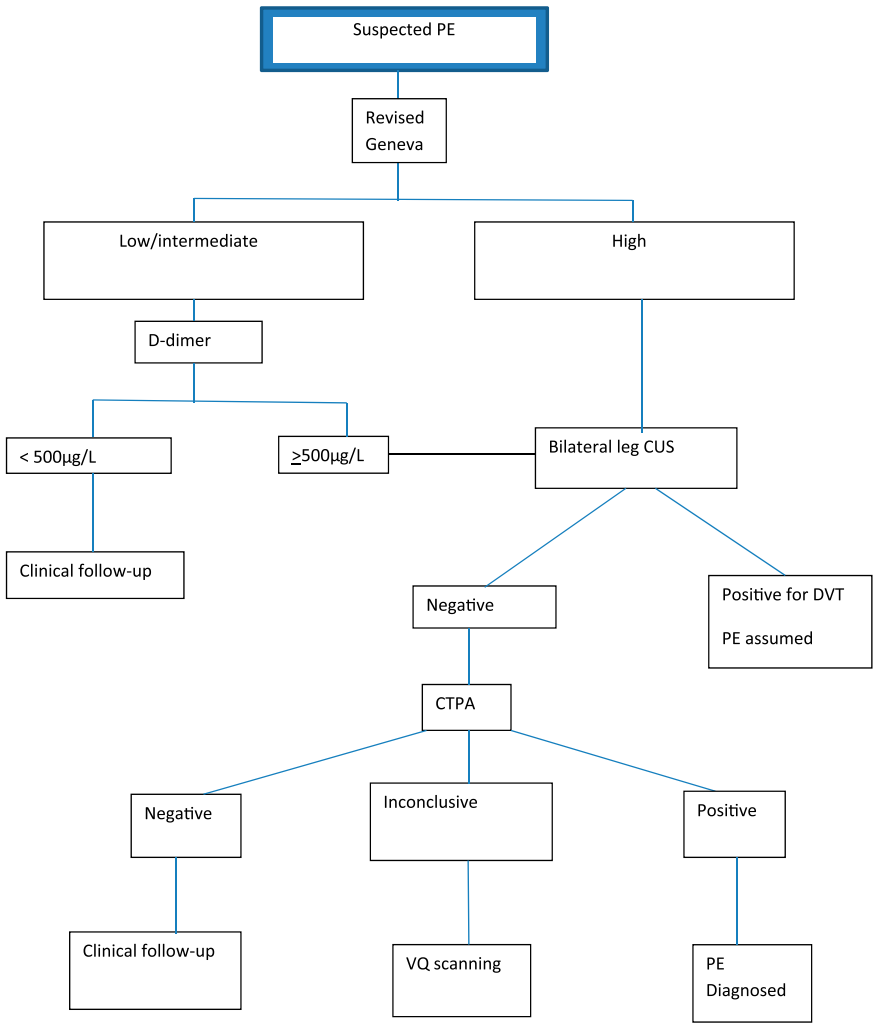

Figure 1. Diagnostic algorithm for the revised Geneva/D-dimer protocol for pregnant women with suspected PE. CUS, compression ultrasound.

was 0% (95% CI, 0% to 1.0%). The calculated risk of VTE on follow-up managed solely by PTP and D-dimer level alone was 0% (95% CI, 0% to 8%).

The revised Geneva/D-dimer study had several limitations: it was underpowered to evaluate the safety of excluding PE on the basis of ancillary tests alone; although the revised Geneva rule relied on objective variables, these were not specific to pregnancy; and a single unmodified D-dimer cutoff was used, limiting its utility beyond the earlier trimesters of pregnancy.

In the other prospective study of 498 patients, van der Pol et al[17] explored the use of selective D-dimer criteria and the YEARS prediction rule in the management of pregnant women with suspected PE. These women were assessed using the following variables[19]: clinical signs of DVT, hemoptysis, and PE as the most likely diagnosis. Management of these women with suspected PE is shown in Figure 2. At presentation, PTP was assessed using the YEARS criteria. Patients with clinical signs and symptoms of DVT underwent CUS. Selective D-dimer levels were used to stratify patients into those who required further imaging and those who did not. Patients not meeting YEARS criteria and with D-dimer <1000 µg/L and those with meeting YEARS criteria and with D-dimer <500 µg/L did not undergo CTPA and did not receive anticoagulation. All women with symptoms for DVT, and some without symptoms (not defined), underwent CUS testing.

With this approach, the authors avoided CTPA in 39% (95% CI, 35% to 44%) of all pregnant women presenting with suspected PE: 65% in the first and 32% in the third trimesters. The overall incidence of VTE on follow-up with the YEARS/D-dimer management strategy was 0.21% (95% CI, 0.04% to 1.2%). For the 195 patients who did not undergo CTPA, the incidence of VTE was 0.51% (95% CI, 0.9% to 2.9%).

Notably, of the 299 CTPA tests performed, there were no CTPA tests deemed inconclusive or nondiagnostic (verified with the author), following the standardization of CTPA with modification for pregnant women in all participating centers.[17]

Some limitations of the study included: the assessment of whether PE was the most likely diagnosis was the most decisive variable in the YEARS criteria, but how this subjective variable was determined and by whom are uncertain; PTP was not always assessed without knowledge of D-dimer level and might have influenced a clinician's determination of the likelihood of PE; and

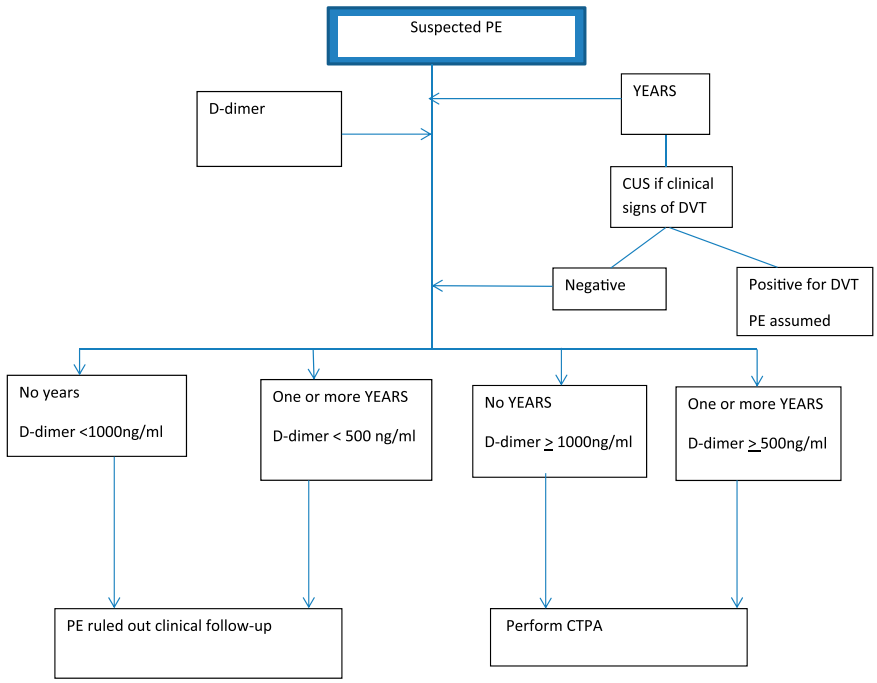

Figure 2. Diagnostic algorithm for the YEARS/D-dimer protocol for pregnant women with suspected PE.

the possible use of thromboprophylaxis in patients with a history of VTE (6%) and those with known thrombophilia (2.8%) was not detailed (this is especially relevant for VTE detection after initial assessment).

External validation of these 2 approaches was conducted in a UK cohort of 219 patients with PE diagnosed primarily via imaging.[20] The authors of the study raised some concerns that the strategies may not be safe; using the revised Geneva/D-dimer strategy would avoid imaging in 21% of women, including 3 of 12 patients with PE, whereas 44% would avoid imaging with the YEARS/D-dimer strategy, including 5 of 12 patients with PE. Sensitivity rates were calculated at 75% (95% CI, 42.8% to 93.3%) and 58.3% (95% CI, 28.6% to 83.5%) for the Geneva/D-dimer and YEARS/D-dimer strategies, respectively.

The failure of the 2 strategies in that specific UK cohort might have been secondary to the influence of the anticoagulation effect in lowering D-dimer levels. In addition, the UK study employed a different design (patients were identified with PE both retrospectively and prospectively), and a fixed diagnostic algorithm was not prescribed. Other factors affecting the performance of these strategies may include the test characteristics of the D-dimer assays used.[20]

Nevertheless, when the YEARS/D-dimer approach was applied to the revised Geneva/D-dimer cohort,[21] it was found that 21% (77) of the patients would not have required imaging, and no patient (0 of 77) would have been diagnosed with VTE on follow-up (95% CI, 0% to 3.9%).

Is CUS of lower extremities necessary as part of PE workup?

Many guidelines[1,2] recommend the use of bilateral CUS in the diagnostic management of pregnant women with suspected PE to possibly reduce the need for ionizing radiation; this was part of the diagnostic algorithm in both studies.[16,17] In the revised Geneva/D-dimer study,[16] CUS was performed in 75% of patients with suspected PE. DVT was diagnosed in 1.8% (7 of 328) of asymptomatic patients, compared with 8.8% (5 of 57) of patients with symptoms. In the YEARS/D-dimer study,[17] CUS was performed in 88% of the cohort; 7% (3 of 43) of patients with symptoms of DVT were positive, and 1.3% (1 of 79) of asymptomatic patients were diagnosed with DVT.

The reported rates of DVT in pregnant patients from these studies investigating pregnant women with suspected PE[16,17] were similar (7% to 8.8%) to those reported from prospective studies of pregnant women with suspected DVT (7.2% to 8.3%).[22,23] Therefore, CUS is clearly indicated if patients presenting with suspected PE have concurrent signs and symptoms of DVT. Even in the absence of DVT symptoms, DVT is diagnosed in 1% to 2% of patients; universal screening of these pregnant patients to avoid radiation exposure could still be considered. However, based on personal experience, obtaining CUS testing before timely diagnostic imaging is sometimes challenging.

Applying the diagnostic algorithm to this patient case and counseling the patient

The revised Geneva score for this specific patient would place her as low or intermediate PTP; her PTP based on YEARS criteria could be 1 or 0 based on the level of experience or risk aversion of the clinician who assessed her. Her D-dimer test was subsequently reported as 995 ng/mL.

Based on the study reported by Righini et al,[16] CTPA would be indicated after negative CUS. If the patient were assessed with no YEARS criteria, no further imaging would be required[17]; if she were assessed with 1 criterion, imaging would be indicated.

The subjectivity of this variable would likely be based on the experience and risk tolerance of the clinician. A notable observation from the 2 prospective studies in pregnancy[16,17] was the fact that the proportion of physicians who felt that PE was

the most likely diagnosis at presentation was 71% in the revised Geneva/D-dimer study[16] vs 44% in the YEARS/D-dimer study,[17] even though the participants in both studies were recruited from similar settings, and the proportion of patients diagnosed with PE was similar.

From the patient's perspective, the likelihood of diagnosed PE is low. At presentation, the incidence of PE is 4%[17]; if she were assessed as meeting 1 YEARS criterion and requiring imaging, the incidence of PE would marginally increase to 6%.[17] Given these low rates, the patient might opt not to comply with medical recommendations.

Indeed, protocol violations reported from the 2 prospective studies of PE diagnosis in pregnancy were fairly common.[16,17] Comparing the YEARS/D-dimer study in pregnant patients[17] with the corresponding study in nonpregnant patients,[19] protocol violations were observed more often among pregnant patients.[17] When CTPA was not indicated as per the protocol, 6% (12 of 199) of women had imaging, compared with 1.5% (24 of 1651) in the study of nonpregnant patients; when CTPA was indicated, 4.7% (14 of 299) of patients in the pregnancy study[17] versus 0% (0 of 1814) in the nonpregnancy study[19] did not undergo the test. Similarly, when CTPA was indicated in the revised Geneva/D-dimer study, 2.9% (10 of 342) of tests were not completed; in addition, 42% (14/33) of patients refused V/Q scanning when indicated.

The reasons for protocol violations are unclear; whether they are driven by physicians who are risk adverse or by patients who fear radiation exposure, or a combination of both, is not known. Not infrequently, a patient might also insist on imaging even if the risk of PE is low. Studies addressing patients' values, preferences, and risk perceptions would be helpful to aid clinicians in counseling these patients and arriving at an agreeable management decision. Further exploration of clinicians' assessments of the likelihood of PE vs common pregnancy symptoms of benign conditions could aid in more selective use of diagnostic imaging.

Conclusion

The prevalence of PE among pregnant women presenting with nonspecific symptoms of chest pain and dyspnea is low. Both D-dimer testing and clinical prediction rules can be used to assist in the management of pregnant women presenting with symptoms suspicious of PE, especially for those in the first 2 trimesters of pregnancy. The revised Geneva criteria are expectedly less specific for pregnant women, when compared with the YEARS criteria. Although the YEARS criteria performed better in negating the need for diagnostic imaging when combined with variable D-dimer levels, as compared with the revised GENEVA/D-dimer approach, the third variable used in the YEARS criteria (ie, that PE is the most likely diagnosis) is highly subjective. In addition to developing more specific PTP criteria for pregnant patients with suspected PE, future studies should explore how patients' values and risk tolerance and physicians' risk tolerance influence decision making.

Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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When I treat a patient with acute pulmonary embolism at home

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Home treatment is feasible and safe in selected patients with acute pulmonary embolism (PE) and is associated with a considerable reduction in health care costs. When establishing a PE outpatient pathway, 2 major decisions must be made. The first one concerns the selection of patients for home treatment. The second one involves dedicated outpatient follow-up including sufficient patient education and facilities for specialized follow-up visits. Current evidence points toward the use of either the Hestia criteria or Pulmonary Embolism Severity Index with/without assessment of the right ventricular function to select patients for home treatment, depending on local preferences. Results from ongoing trials are expected to enforce current guideline recommendations on home treatment and pave the way for more broad application of this elegant and cost-effective management option for patients with acute PE.

LEARNING OBJECTIVES

- Get an overview of all published literature on home treatment of acute pulmonary embolism
- Understand the evidence based risk stratification tools that can be used to select patients with acute PE for home treatment

Introduction

Acute pulmonary embolism (PE), the most severe presentation of venous thromboembolism (VTE), may be fatal if not diagnosed and treated in time.[1] Because of the associated high mortality risk, hospitalization has been the standard of care for all PE patients for monitoring and initiation of anticoagulant therapy. In the past decade, however, studies have shown that PE patients can be stratified into classes of higher or lower risk of adverse outcome based on clinical decision rules, biomarkers, and/or assessment of right ventricular (RV) function.[2] Guidelines now recommend formal risk stratification to guide the optimal therapeutic management, and it has been suggested that this may have led to a decrease in PE-related mortality.[3,4] This risk stratification cannot only be used to identity patients that benefit from reperfusion therapy but also to select patients who can be managed at home. Indeed, several large studies have been performed showing the safety of home treated PE patients and its benefits with regard to health care costs and patient satisfaction.[5-11] Here, we describe the current state of the art of selecting PE patients for home treatment and best practices with regard to PE outpatient pathways.

Case scenario

A 58-year-old woman was evaluated in our hospital because of acute dyspnea and pleuritic chest pain. Symptoms had started 1 week before presentation. She reported no provoking factors for PE nor symptoms suggestive of deep vein thrombosis. Her temperature was 37.2°C, heart rate was 85 beats/min, respiratory rate was 14 breaths/min, oxygen saturation at room air was 98%, and blood pressure was 136/72 mm Hg. Her physical examination and electrocardiogram were unremarkable. The attending physician considered the presence of acute PE. In absence of an alternative explanation, 1 YEARS item was awarded (PE most likely diagnosis), and a D-dimer test was ordered.[12] Because the D-dimer level was above the threshold (782 ng/mL; threshold, 500 ng/mL), a computed tomography pulmonary angiography was ordered showing a segmental PE in the left lower lobe. On confirmation of the diagnosis of acute PE, oral anticoagulant therapy was initiated. The attending physician now must decide on the optimal setting of treating this patient: does she require hospitalization or is she a candidate for home treatment?

Home treatment of acute PE

In the literature, outpatient management of acute PE has been referred to as home treatment, early discharge, and outpatient treatment, although a clear definition is lacking. Generally, home treatment is defined as a discharge within 24 hours of initial presentation and early discharge if patients leave the hospital within 3 days. Both home treatment and early discharge involve a much shorter hospitalization than the 7 to 14 days that has been described as the mean admission duration in several European countries.[13] In the United States, the median duration of hospital admission for PE was reported to be close to a week.[14]

There are many benefits of treating patients with acute PE at home. Mostly, patients are saved a hospital admission, which may lead to less anxiety, better quality of life, and higher patient satisfaction. Mostly, however, the health care costs are much lower if (unnecessary) admission is prevented. For instance, it was estimated that at least 25% of patients admitted for PE in the United States could be treated at home. Discharging those patients from the emergency ward would decrease health care costs by an estimated $1 billion each year.[15] In the Dutch setting, a recent post hoc analysis of the YEARS study identified a net cost reduction of €1.500 for each patient treated at home.

The severity of the PE and risk of adverse outcomes should largely determine clinical decision making with regard to initial home treatment. Other factors such as locoregional cultural and patient preferences and the structure of the health care system also play an important role. Because of this, major regional differences can be observed. For instance, practice-based studies have shown that 45% to 55% of hemodynamically stable PE patients are treated at home in Canada and the Netherlands, whereas in Spain and France, most patients are hospitalized.[13,16-20] The introduction of direct oral anticoagulants with a superior safety profile compared with vitamin K antagonists and many practical advantages have lowered the bar for home treatment of PE.[13,21] However, home treatment of PE has not (yet) become the standard of care in 2020. One of the main points of discussion is the threshold of safety (ie, which rate of complications in what time period would be acceptable to treat patients at home rather than in hospital). Although the exact answer to that question is subjective and may vary between individual physicians, patients, and policy makers, one thing is clear. Acute death from hemodynamic deterioration or major bleeding in the first few days after diagnosis is a price too high to pay. Patients at risk for such complications should be hospitalized. Other adverse outcomes such as death from comorbidities (eg, advanced cancer) within the first weeks after diagnosis can, however, not be prevented by hospital admission. Such patients may even prefer being at home surrounded by relatives over hospital admission. Hence, more than strictly adhering to rigid imaging or biomarker thresholds or only focusing on overall mortality, precision medicine is key, tailoring the optimal approach to the individual patient.

Landmark studies

In the last decade, several landmark studies have been published, demonstrating the safety of home treatment in selected low-risk PE patients. These studies are not easily comparable because of heterogeneous selection criteria and various definitions of home treatment. They nonetheless provide important information for the outcomes of home-treated PE patients across a wide range of patient categories and countries.

Table 1. PESI score[31]

Criteria	Score
Age	+1/y
Male sex	+10
History of cancer	+30
History of heart failure	+10
History of chronic lung disease	+10
Pulse > 110 beats/min	+20
Systolic blood pressure < 100 mm Hg	+30
Respiratory rate ≥ 30/min	+20
Body temperature < 36°C	+20
Altered mental status	+60
Arterial blood oxygen saturation < 90%	+20

Mortality risk: class I (<65 points), very low risk; class II (66-85 points), low risk; class III (86-105 points), intermediate risk; class IV (106-125 points), high risk; class V (>125 points): very high risk.

In the Outpatient Treatment of Pulmonary Embolism study, 344 PE patients (1557 screened for eligibility) were randomized to home treatment or hospitalization.[5] First, the Pulmonary Embolism Severity Index (PESI) score was used to identify patients with low mortality risk (Table 1): only patients with PESI class I and II were considered suitable for home treatment. In addition, patients had to fulfill several pragmatic criteria to rule out other factors necessitating hospital admission (ie, being independent from oxygen therapy and having an established support system at home). Patients randomized to home treatment left the hospital of a mean of 0.5 days, whereas patients randomized to hospitalization were discharged after a mean of 3.9 days. Noninferiority was shown in the incidence of recurrent VTE (0.6% vs 0%) and non-PE related death (0.6% vs 0.6%) after a 3-month follow-up period for home treatment and hospitalization, respectively. All patients were treated with a vitamin K antagonist. The incidence of major bleeding exceeded the noninferiority threshold in the home treatment group (1.8% vs 0%). Potential VTE-related medical resource use during follow-up was the same between groups.[5]

The Hestia study evaluated the efficacy and safety of home treatment in 297 PE patients using the Hestia criteria to identify eligibility for home treatment.[6] The Hestia criteria are pragmatic criteria of both risk of mortality and bleeding but also of other reasons for hospitalizing patients with acute PE such as hypoxemia, pain requiring analgesia, and bleeding risk (Table 2). Fifty-eight percent of the PE patients screened for study participation were eligible for home treatment, and 51% were treated at home. The 3-month incidence of recurrent VTE in these latter patients was 2.0% (95% confidence interval [CI], 0.8-4.3), of vitamin K antagonist–associated major bleeding was 0.7% (95% CI, 0.08-2.4), of PE-associated mortality was 0% (95% CI, 0-1.2), and of overall mortality was 1.0% (95% CI, 0.2-2.9).

The VESTA study was a noninferiority trial in which 550 patients with acute PE and none of the Hestia criteria were randomized between immediate home treatment and advanced risk stratification via n-terminal pro-brain natriuretic peptide testing. In the intervention group, patients were treated at home if the NT-proBNP was normal but hospitalized in case of

Table 2. Hestia criteria[6]

Criterion	
Is the patient hemodynamically unstable?	Yes/no
Is thrombolysis or embolectomy necessary?	Yes/no
Active bleeding or high risk of bleeding?	Yes/no
More than 24 h of oxygen supply to maintain oxygen saturation > 90%?	Yes/no
Is pulmonary embolism diagnosed during anticoagulant treatment?	Yes/no
Severe pain needing intravenous pain medication for more than 24 h?	Yes/no
Medical or social reason for treatment in the hospital for more than 24 h (infection, malignancy, no support system)?	Yes/no
Does the patient have a creatinine clearance of < 30 mL/min?	Yes/no
Does the patient have severe liver impairment?	Yes/no
Is the patient pregnant?	Yes/no
Does the patient have a documented history of heparin-induced thrombocytopenia?	Yes/no

If the answer to one of the questions is yes, the patient cannot be treated at home in the Hestia Study.

elevated NT-proBNP levels.[7] Only 12% of those randomized to NT-proBNP testing had elevated levels and were hospitalized. Noninferiority was shown for the composite outcome of PE- or bleeding-related mortality, cardiopulmonary resuscitation and intensive care unit admission, which occurred in 1.1% (95% CI, 0.2-3.2) and 0% (95% CI, 0-1.3), respectively. The incidence of recurrent VTE was also comparable between the 2 groups: 1.1% (95% CI, 0.2-3.2) for those in the standard of care arm vs 0.73% (95% CI, 0.1-2.6) in the NT-proBNP arm of the study. The most likely explanation for the low number of patients with elevated NT-proBNP is that the Hestia rule preselects patients with normal NT-proBNP levels.[7]

The eSPEED study was a controlled pragmatic trial designed to evaluate the effect of an integrated electronic clinical decision support system to facilitate risk stratification and decision making at the site of care for patients with acute PE.[8] The PESI was used as primary risk stratification tool. After the intervention, the proportion of patients treated at home increased considerably, with a relative increase of 61% (18% preintervention to 28% postintervention), whereas no change was found in the control sites (15% preintervention and 14% postintervention). Importantly, no increases were seen in 5-day return visits related to PE and in 30-day major adverse outcomes associated with clinical decision support system implementation: 12% (95% CI, 5.6-22) vs 6.2% (95% CI, 2.7-12) at the intervention sites vs 9.8% (95% CI, 3.7-20) and 5.1% (95% CI, 1.1-14) at the control sites, respectively.[8]

In the Low-Risk Pulmonary Embolism Prospective Management Study, 200 patients considered to have low-risk PE based on PESI (class I or II), echocardiography (no signs of right heart strain on echocardiogram), and whole-leg ultrasound of the legs (no proximal deep vein thrombosis) were treated at home with a direct oral anticoagulant.[9] Of the 1003 screened patients, 213 were in PESI class I or II and had no other exclusion criteria. Of those, 13 met 1 of the imaging exclusion criteria. The 90-day composite outcome of all-cause mortality, recurrent symptomatic VTE, and major bleeding occurred in 0.5 of patients (95% CI, 0.02-2.4). Patients indicated a high level of satisfaction with their care.[9]

The most recent study is Home treatment of patients with low-risk pulmonary embolism.[10] In total, 525 of 2854 screened patients with acute PE were treated with rivaroxaban and discharged early in the absence of any of the Hestia criteria, signs of RV dysfunction or free-floating thrombi in the right atrium or RV, and contraindications to rivaroxaban. The median length of hospitalization was 34 hours, and 12% of patients were discharged directly on confirmation of the PE diagnosis. The primary efficacy outcome was symptomatic recurrent VTE or PE-related death within 3 months of enrolment, which occurred in 0.6% of patients.[10] The incidence of major bleeding was 1.2%, and 2.3% of patients required hospitalization because of (suspected) PE-related complications.

Much more evidence is expected on short notice, notably for the HOME-PE study. In this randomized controlled noninferiority trial, 1975 normotensive PE patients are randomized to risk stratification by either the Hestia rule or the simplified PESI (sPESI) for determining the possibility of home treatment (#NCT02811237). The study will compare the safety and efficacy of both strategies, with the hypothesis that both study groups treated at home because of either none of the Hestia criteria or a low-risk classification by sPESI will have comparable rates of adverse events but that decision making based on the Hestia criteria leads to more patients selected for home treatment.

Best practice for PE outpatient pathways

When establishing a PE outpatient pathway, 2 major decisions must be made. The first one concerns the selection of patients for home treatment. The second one involves dedicated outpatient follow-up including sufficient patient education and facilities for specialized follow-up visits.

According to the literature discussed above, 2 triaging tools have been found adequate for selecting PE patients for home treatment: the Hestia criteria and PESI, with or without biomarker assessment or evaluation of the presence of RV overload. Of note, although the sPESI is much more user friendly than the PESI, well validated, and included in current guidelines, none of the landmark studies on home treatment of PE published to date applied this score.[22-24] Even so, it may be assumed that PESI can be substituted with sPESI. For the matter of RV overload, in the Hestia and VESTA studies, RV function evaluation (which is critical to the risk stratification as recommended by the European Society of Cardiology) was not part of standard baseline assessment. As a consequence, 30% of all patients treated at home had a RV/left ventricular (LV) diameter ratio > 1.0, without a higher incidence of adverse outcome: the combined 3-month incidence of recurrent VTE and all-cause death was 2.7% in patients treated at home with a RV/LV diameter ratio > 1.0 and 2.3% in patients with a normal RV/LV ratio.[25] Furthermore, high sensitive troponin-T (hsTnT) did not have an additional prognostic value on top of Hestia, as was the case for NT-proBNP in the VESTA study.[7,26] The adverse 30-day composite outcome of hemodynamic instability, intensive care unit admission, or death related to either PE or major bleeding occurred in 1.7% patients treated at home with post hoc measured elevated hsTnT levels compared with 0.70% with normal hsTnT (odds ratio, 2.5; 95% CI, 0.22-28). All-cause death occurred in 1.7% of patients in both groups (odds ratio, 1.0; 95% CI, 0.11-8.7).[26]

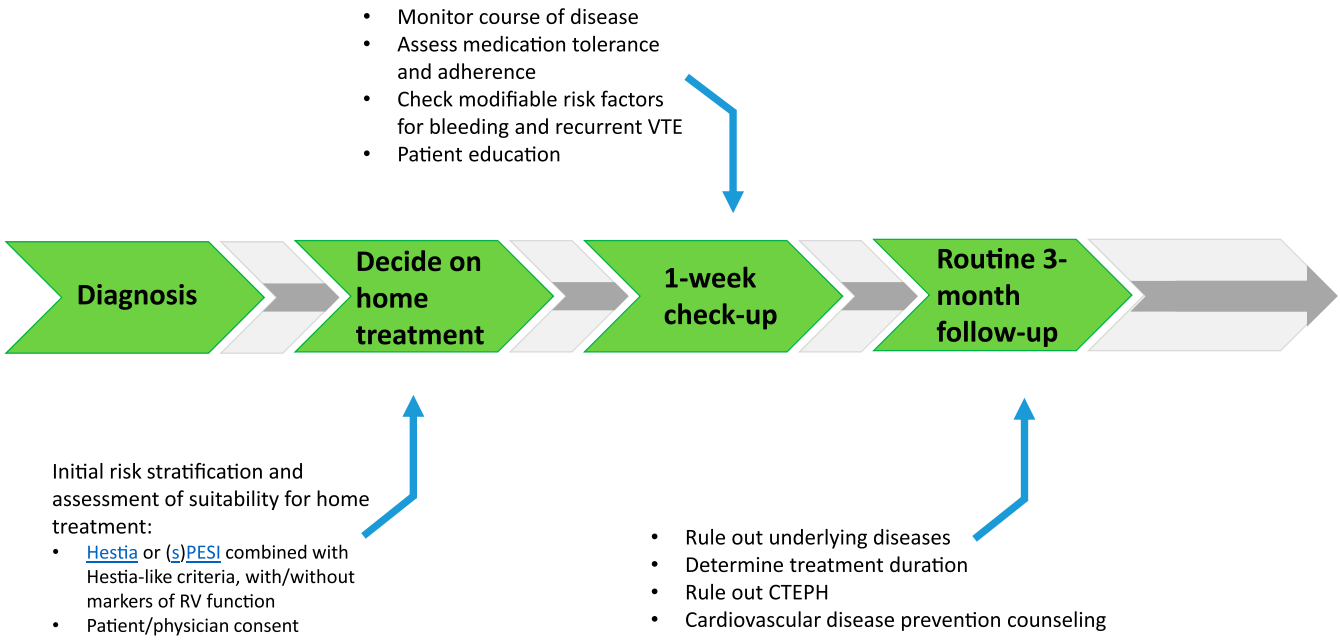

Figure 1. Outpatient pathway for acute pulmonary embolism.

These observations suggest that the hemodynamic profile of a patient (ie, the severity of RV overload and the resulting hemodynamic response) rather than just an abnormal RV/LV ratio or NT-proBNP is intrinsically taken into account in the decision to treat patients at hospital or at home when applying the Hestia criteria. Hence, in our practice, we use the Hestia criteria without further explicit (imaging) biomarkers. If PESI is used, parameters of the hemodynamic profile of the patients are included in the risk stratification, but RV function is not. Because PESI with/without measures of RV overload focuses on risk of early adverse alone and not on assessing the possibility of home treatment, PESI should always be combined with other Hestia-like criteria for this purpose as was done in the Outpatient Treatment of Pulmonary Embolism study.[5]

If patients are treated at home, a proper outpatient pathway should be in place (Figure 1). First of all, patients need to receive preferably written instructions on who and when to contact in case of alarm symptoms. Second, in most studies, patients were contacted by telephone or evaluated in an outpatient clinic in the first week after diagnosis. This is a very reasonable approach in practice-based conditions as well. At that moment, it is important to check the vital parameters, as well as whether the patient is doing well, follows the anticoagulant drug prescription, is aware of alarm symptoms, has received sufficient patient education, and has no untreated modifiable risk factors for complications such as major bleeding.[27-29] If the patient is recovering according to expectation and if no other interventions are necessary, the routine patient pathway can be followed, with additional visits to establish the optimal duration of anticoagulation and, if indicated, tests to rule out underlying disease. In general, outpatient pathways should be collaborative between general practitioners and thrombosis specialists, including fast exchange of a medical reports and/or discharge letters to all involved.[30]

Resolution to the case

The patient was hemodynamically stable and required no other treatment than (oral) anticoagulation. None of the Hestia criteria were present, and home treatment was discussed with the patient. She lived together with her husband who could take care of her, and she responded favorable to the suggestion of home treatment. Six days after immediate discharge from the emergency department, she visited our dedicated thrombosis outpatient clinic. A specialized nurse evaluated the initial course of disease, presence of complications, and risk factors for complications (eg, by measuring blood pressure and checking medication adherence). It was concluded that the patient was recovering well, had taken the medication in accordance with the prescription, and was at low risk of complications. Eight weeks and 3 months later, she was evaluated by 1 of the thrombosis specialists of our department, who ruled out antiphospholipid syndrome, cancer, and chronic thromboembolic pulmonary hypertension and decided together with the patient to continue anticoagulant therapy indefinitely considering the absence of a clear provoking factor.

Conclusion

Home treatment is feasible and safe in selected PE patients and is associated with a considerable reduction in health care costs. Current evidence points toward the use of either the Hestia criteria or PESI with/without assessment of the RV function to select patients for home treatment. Results from ongoing trials are expected to enforce current guideline recommendations on home treatment and pave the way for more broad application of this elegant and cost effective management option for patients with acute PE.

Conflict-of interest disclosure

F.A.K. received research grants from Bayer, Bristol-Myers Squibb, Boehringer-Ingelheim, MSD, Daiichi-Sankyo, Actelion, the Dutch Thrombosis Association, and the Dutch Heart Foundation. M.V.H. received research grants from ZonMW, Boehringer Ingelheim Bayer Health Care, and Pfizer-Bristol-Myers Squibb; and received

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Off-label drug use

None disclosed.

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Which patients are at high risk of recurrent venous thromboembolism (deep vein thrombosis and pulmonary embolism)?

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Recurrent venous thromboembolism (VTE, or deep vein thrombosis and pulmonary embolism) is associated with mortality and long-term morbidity. The circumstances in which an index VTE event occurred are crucial when personalized VTE recurrence risk is assessed. Patients who experience a VTE event in the setting of a transient major risk factor (such as surgery associated with general anesthesia for >30 minutes) are predicted to have a low VTE recurrence risk following discontinuation of anticoagulation, and limited-duration anticoagulation is generally recommended. In contrast, those patients whose VTE event occurred in the absence of risk factors or who have persistent risk factors have a higher VTE recurrence risk. Here, we review the literature surrounding VTE recurrence risk in a range of clinical conditions. We describe gender-specific risks, including VTE recurrence risk following hormone- and pregnancy-associated VTE events. Finally, we discuss how the competing impacts of VTE recurrence and bleeding have shaped international guideline recommendations.

LEARNING OBJECTIVES

- Understand why an evaluation of risk factors that were present at the time of the index venous thromboembolism (VTE) event (and whether these risk factors were transient or persistent) is essential when assessing a patient's future VTE recurrence risk
- Review data regarding additional predictors of VTE recurrence risk

Clinical case 1

A 45-year-old man who was diagnosed with an acute proximal deep vein thrombosis (DVT) and who has received therapeutic anticoagulation for 3 months attends your clinic. He has had no bleeding complications and has no identifiable risk factors for bleeding. Should he continue anticoagulation or stop?

Introduction

Recurrent venous thromboembolism (VTE; or DVT and pulmonary embolism [PE]) is associated with mortality and long-term morbidity. Conversely, anticoagulation confers potentially devastating bleeding risks. It is important to understand which patients are at highest VTE recurrence risk so that we can target awareness and prevention strategies to the right patients. Of critical importance are the circumstances in which a VTE event occurred: were risk factors present, and if so, what was their nature and are they persistent? These risk factors are central to decision making surrounding duration of anticoagulation.

In this review, we discuss which patients have the highest predicted VTE recurrence risk, including an update on emerging personalized strategies. Finally, we will take a deep-dive into VTE recurrence risk in women, with a focus on gender-specific challenges and knowledge gaps.

Predicting VTE recurrence risk: go back to the beginning!

VTE recurrence risk is determined by risk factors that were present at the time of the initial VTE event[1,2] (Figure 1). The predicted annual recurrence risk after an unprovoked VTE event is higher than that after a VTE provoked by a major transient risk factor (Table 1).[1-4] (Although the term unprovoked is no longer encouraged by European guidelines,[2] we do use it in this review to refer to "an index VTE

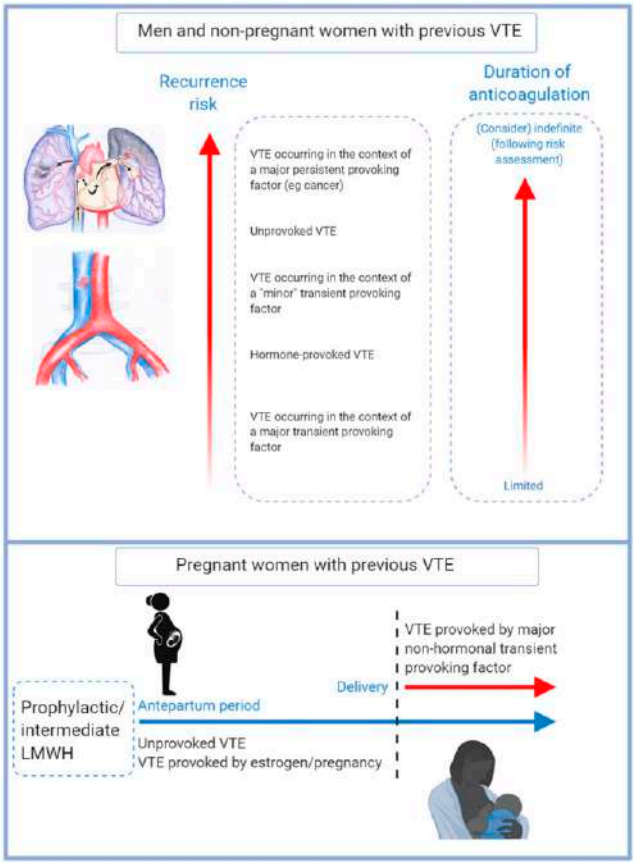

Figure 1. VTE recurrence risk and duration of anticoagulation. For men and nonpregnant women with an index VTE event, VTE recurrence risk is driven by risk factors that were present at the time of the initial VTE event.[1,2] The predicted annual recurrence risk after an unprovoked VTE event is higher than that after a VTE provoked by a major transient risk factor. VTE occurring in the context of minor risk factors are associated with higher predicted recurrence risk than those occurring in the context of major transient risk factors.[6] Recurrence risks for patients with major persistent risk factors (most notably active cancer) are among the highest of all.[1,2] Guideline recommendations on duration of anticoagulation are guided by data including VTE recurrence risk and bleeding risk on anticoagulation, with limited and indefinite duration anticoagulation being recommended for patients with low and high recurrence risks respectively.[2,43] For some patients including "cis" and transgender women whose VTE occurred in the context of hormone use, estimation of recurrence risk may be particularly challenging because of knowledge gaps, and these areas are important research priorities.[27] For pregnant women with prior VTE (especially those with an unprovoked or a hormone-provoked VTE,[21] predicted recurrence risk during pregnancy is sufficiently high to warrant both antenatal and postnatal thromboprophylaxis, whereas postpartum thromboprophylaxis only is recommended for women with lower predicted recurrence risks.[25] The optimal LMWH dose for women with prior VTE is currently being investigated in the ongoing Highlow study (NCT 01828697; highlowstudy.org).

episode that occurred in the absence of any identifiable risk factor" for simplicity).

In a recent systematic review, VTE recurrence rates at 24 months were reported to be 3.3% (95% confidence interval [CI], 2.8-3.9), 0.7% (95% CI, 0-1.5), 4.2% (95% CI, 2.8-5.6), and 7.4% (95% CI, 6.5-8.2) per patient year in patients with a transient risk factor, a surgical risk factor, a nonsurgical risk factor, and unprovoked VTE, respectively.[5] Guidelines from the European Society of Cardiology (ESC)[2] and the International Society on Thrombosis and Haemostasis (ISTH)[1] provide a framework for categorization of VTE risk factors (Table 1). The categorization of risk factors for the index VTE event in both guidelines are broadly similar, with the exception of differing terminology (the ESC avoids terms such as provoked, unprovoked, or idiopathic VTE[2]; and a focus on the presence of risk factors [categorizing these as transient or persistent] in the ISTH guideline as the key definition of risk category).[1]

Attention to the initial provoking factor is also essential; in a recent study incorporating data from 2 randomized trials, recurrence rates in patients whose initial VTE event occurred in the context of nonmajor (transient or persistent) risk factors were similar to those of patients whose initial event was unprovoked (hazard ratio [HR], 0.81; 95% CI, 0.56-1.16). In the placebo arms, recurrence rates during the study period for patients with minor (as judged by the authors) transient, minor persistent, and unprovoked VTE were reported to be 7.1%, 10.7%, and 10%, respectively.[6] Given the relatively small numbers of patients, firm conclusions on VTE recurrence risks associated with individual nonmajor risk factors will require future prospective clinical management studies.

Recurrence risks for patients with major persistent risk factors (most notably active cancer) are among the highest of all (Figure 1; Table 1).[1,2] Conversely, predicting recurrence risk and making decisions on duration of anticoagulation in patients whose initial VTE event occurred in the context of an intermediate risk factor, such as hormonal therapy or pregnancy, is particularly challenging, especially as anticoagulation poses additional, gender-specific risks.

Moreover, the site of the initial VTE appears important, with PE recurring more often as PE.[2] In the Tromsø population-based cohort study, patients with a first PE were 2.4-fold more likely to develop a second PE rather than a DVT.[7]

Unprovoked VTE

Up to 50% of all people with a first VTE episode have no identifiable cause for this event (Table 2).[2] A recent systematic review and meta-analysis estimated that the risk of recurrent VTE in patients whose initial event was unprovoked was 10%, 25%, and 36% at 1, 5, and 10 years after treatment, respectively.[8]

Cancer

People with active cancer are among those with the highest VTE recurrence risk, with a 2-9 fold increased risk compared with noncancer patients.[7,9,10] A recent meta-analysis reported recurrent VTE and fatal recurrent VTE rates of 23.7 (95% CI, 20.1-27.8) and 1.9 (95% CI, 0.8-4.0) per 100 patient-years, respectively.[10] In a cohort study including 543 patients, validated in an independent set of 819 patients, a proposed recurrent VTE prediction score was developed that included breast cancer (−1 point), tumor node metastasis stage I or II (−1 point), and female sex, lung cancer, and previous VTE (+1 point each).[11] Patients with a score of ≤0 and ≥1, respectively, had low (≤4.5%) and high (≥19%)

Table 1. VTE risk factor classification

ESC[2]			ISTH[1]		
RF category	**Recurrence risk**	**Examples**	**RF category**	**Recurrence risk or risk of index event**	**Examples**
			VTE provoked by a transient risk factor		
Major transient or reversible RF associated with >10-fold increased risk for the index VTE event (compared with patients without the risk factor)	Low recurrence risk* (<3%/y)	Surgery with GA for >30 min Confined to bed in hospital (only bathroom privileges) for ≥3 days because of an acute illness or acute exacerbation of a chronic illness Trauma with fractures	Major transient RF during the 3 mo before diagnosis of VTE	Half the risk of recurrent VTE (compared with if there was no transient risk factor), when the risk factor occurred up to 3 mo before the VTE or RF was associated with >10-fold increased risk of having a first VTE	Surgery with GA for >30 min Confined to bed in hospital (only bathroom privileges) for ≥3 days because of an acute illness Cesarean section
Transient or reversible factors associated with ≤10-fold increased risk for (index) VTE or Nonmalignant persistent risk factors Or No identifiable risk factor	Intermediate recurrence risk (3-8%/y)	Minor surgery (GA for <30 min) Admission to hospital for <3 days with an acute illness Estrogen therapy/ contraception Pregnancy or puerperium Confined to bed out of hospital for ≥3 days with an acute illness Leg injury (without fracture) associated with reduced mobility for ≥3 days Long haul flight Inflammatory bowel disease Active autoimmune disease No identifiable risk factor	Minor (yet important) transient RF during the 2 mo before diagnosis of VTE	Half the risk of recurrent VTE after stopping anticoagulant therapy (compared with if there was no transient RF), when the RF occurred up to 2 mo before the VTE or RF was associated with a 3- to 10-fold increase in the risk of having a first VTE	Surgery with GA for <30 min Admission to hospital for <3 days with an acute illness Estrogen therapy Pregnancy or puerperium Confined to bed out of hospital for ≥3 days with an acute illness Leg injury associated with reduced mobility for at least 3 days
			Unprovoked VTE		
				Recurrence risk individualized/stratified using other assessments†	No provoking factors (transient or persistent)
			VTE provoked by a persistent risk factor		
	High recurrence risk (>8%/y)	Active cancer At least 1 previous episode of VTE in the absence of a major transient or reversible factor Antiphospholipid antibody syndrome	Active cancer		Cancer is considered active if any of the following apply: (1) has not received potentially curative treatment; or (2) there is evidence that treatment has not been curative (eg, recurrent or progressive disease); or (3) treatment is ongoing
			Ongoing nonmalignant condition associated with at least a 2-fold risk of recurrent VTE after stopping anticoagulant therapy		Inflammatory bowel disease.

GA, general anesthesia; RF, risk factor.
*If anticoagulation is discontinued after the first 3 months.
†Described in text of guideline and in this current review.

Table 2. VTE recurrence risks in selected studies evaluating risk factors for recurrence

Study	Type of study/nature of initial VTE*	Cumulative recurrence risk for entire group	Predictors of VTE recurrence	VTE recurrence risk RR or HR (95% CI)
Heit et al 2000 [45]	Prospective cohort study: provoked and unprovoked first VTE	1 y: 12.9% 10 y: 30.4%	Male sex	Male vs. Female: HR 1.29 (1.06-1.57) Definite/probable VTE: 2.07 (1.60-2.67)
Kyrle et al 2004 [46]	Prospective cohort study: first unprovoked VTE	5 y: Men: 30.7% (23.8-37.6) Women: 8.5% (5.0-12.0)	Male sex	Male vs. Female: RR 3.6 (2.3-5.5)
Baglin et al 2004 [47]	Prospective single-center cohort study: provoked/unprovoked VTE	2 y: Men: 19.2% Women: 7.7%	Male sex	Male vs. Female: HR 2.66 (1.49-4.77)
Rodger et al 2008 [42]	Multicenter prospective cohort study: First unprovoked VTE	N/R	Male sex	Annual recurrence risk: Men 13.7% (10.8-17.0%) Women: 5.5% (3.7-7.8%); $P < .001$
Lijfering et al 2009 [48]	Post hoc analysis of pooled data from family cohort studies: Provoked and unprovoked VTE	N/R	Male sex	Male vs. Female: RR 1.6 (95% CI, 1.3-2.0)
Christiansen et al 2010 [33]	Prospective follow-up of case-control study; provoked and unprovoked first VTE	N/R	Male sex	Men: IR 41.2/1000 patient-years Women: IR 14.2/1000 patient -years HR 2.8 (1.4-5.7) (unprovoked VTE)
Douketis et al 2011 [49]	Patient-level meta-analysis (provoked and unprovoked VTE)	1 y: Men: 9.5% (7.9%-11.4%) Women: 5.3% (4.1%-6.7%) 3 y: Men: 19.7% (16.5%-23.4%) Women: 9.1% (7.3% −11.3%)	Male sex	Male vs. Female: HR 2.2 (1.7-2.8)
Khan et al 2019 [8]	Meta-analysis first unprovoked VTE	2 y: 16% (13-19%) 5 y: 25% (21- 29%) 10 y: 36% (28-45%)	Male sex	First year: Men: 11.9/100 patient-years (9.6-14.4) Women: 8.9/100 patient-years (6.8-11.3) Rate ratio 1.4 (1.3-1.6)
Palareti et al 2006 [50]	RCT first unprovoked VTE	N/R	D-dimer 1 mo after D/C AC	HR (abnormal vs normal D-dimer without AC) 2.49 (1.35–4.59)
Verhovsek et al 2008 [51]	Meta-analysis (first unprovoked VTE)	N/R	Abnormal D-dimer levels after anticoagulation completion	Positive D-dimer results: −8.9%/year (5.8%-11.9%) Negative D-dimer results: −3.5%/year (2.7%-4.3%)
Cosmi et al 2010 [52]	Prospective multicenter study (first unprovoked VTE)	N/R	D-dimer 1 mo after D/C AC	Persistently abnormal D-dimer: 27%/person-years (12-48) Persistently normal D-dimer: 2.9%/person-years (1-7) Adjusted HR 7.9 (2.1-30)

AC, anticoagulation; D/C, discontinued; HR, hazard ratio; N/R, not reported; OR: odds ratio; RR, relative risk.
*In these studies, VTE events included DVT and PE.

Table 2. (Continued)

Study	Type of study/nature of initial VTE*	Cumulative recurrence risk for entire group	Predictors of VTE recurrence	VTE recurrence risk RR or HR (95% CI)
Palaretti et al 2014 [53]	Prospective clinical management study (VTE associated with no/weak risk factors)	N/R	D-dimers after AC	1. Persistently negative D-dimer after D/C AC: 3.0 per 100 person-years (2.0-4.4) 2. Positive D-dimers, refused to resume AC: 8.8 per 100 person-years (5-14.1) HR 1 vs 2: 2.92 (1.87-9.72)
Kearon et al 2015 [29]	Prospective clinical management study (first unprovoked VTE)	N/R	D-dimers at end of AC: if second test negative >1 mo, AC not restarted in men/women	Persistently negative D-dimers: Overall: 6.7% (4.8-9.0%)/person-year Men: 9.7% (6.7-13.7%)/person-year Nonestrogen women: 5.4% (2.5-10.2%)/person-year Estrogen women: 0.0% (0.0-3.0%)/person-year
Tan et al 2011 [54]	Systematic review: studies including provoked and unprovoked first VTE	N/R	Residual vein thrombosis	Residual vein thrombosis vs. none: Overall: OR 2.02 (1.62-2.5), unprovoked VTE: OR 1.5 (1.12-2.01)
Carrier et al 2011 [55]	Systematic review (unprovoked and provoked first VTE)	N/R	Residual vein occlusion	Residual vein occlusion vs. none: Any VTE: OR 1.5 (1.1-2.0) unprovoked VTE: OR 1.24 (0.9-1.7)

AC, anticoagulation; D/C, discontinued; HR, hazard ratio; N/R, not reported; OR: odds ratio; RR, relative risk.
*In these studies, VTE events included DVT and PE.

recurrence rates during 6 months. A population-based cohort study also identified predictors for VTE recurrence in cancer patients including cancer type and the presence of metastases.[9]

Inherited thrombophilia/family history

Inherited thrombophilia is a recognized predisposing factor for an index VTE event.[3] However, the impact of inherited thrombophilia on VTE recurrence risk is less clear. In a prospective cohort study following unselected patients with a first VTE, 85% of whom underwent inherited thrombophilia screening, VTE recurrence rates were not significantly different in those with or without inherited thrombophilia (HR, 1.50; 95% CI, 0.82-2.77).[12] The risk of recurrent thrombosis was also determined in a prospective follow-up study of 474 patients who had participated in the Leiden Thrombophilia Study, a large population-based case-control study of risk factors for a first DVT.[3] There was no significance increase in thrombosis recurrence risk among 319 patients with ≥1 thrombophilic abnormality compared with those with none (HR, 1.4; 95% CI, 0.9-2.2). In particular, there was no increase in thrombosis recurrence risk in patients who were heterozygous for either factor V Leiden polymorphism or the prothrombin gene mutation, including after adjustment for age, sex, and duration of anticoagulation. In patients with anticoagulant protein deficiency (protein C, protein S, or antithrombin, the corresponding HR was 1.8 (95% CI, 0.9-3.7).

Antiphospholipid syndrome

Patients with antiphospholipid syndrome (APS) who have experienced a VTE event seem to have a high predicted recurrence risk.[13] An observational cohort study including 55% VTE patients, 55% of whom remained on therapeutic anticoagulation after their first event, reported a thrombotic recurrence rate of 49% after 10 years.[14] In a very recently published systematic review, the 2-year recurrent thromboembolism rate in patients with VTE who were and were not taking anticoagulant therapy was 0.054 (95% CI, 0.037-0.079) and 0.178 (95% CI, 0.150-0.209), respectively.[15] Given this high recurrence risk, international guidelines recommend indefinite anticoagulation for many VTE patients with a diagnosis of APS.[2,16] However, as in all clinical scenarios, individual risk assessment with careful attention to the nature of risk factors is essential to guide decision making. Current international guidelines do not recommend universal APS screening for all patients with unprovoked VTE.[17]

Toward personalization of recurrence risk?

Emerging data suggest that additional risk factors may modulate predicted VTE recurrence risk including male sex, D-dimer levels, and postthrombotic syndrome (Table 2). Knowledge of these determinants may be useful during shared decision making with patients, especially in circumstances where the optimal duration of anticoagulation is not clear, taking into account patient preferences (Figure 2). For example, studies have consistently reported an approximately twofold higher VTE recurrence risk for men compared with women (Table 2).

Risk prediction models for patients with unprovoked VTE: can we identify low-risk groups who may safely discontinue anticoagulation?

"Personalization" of risk profile could optimize benefit-to risk ratios by reliably identifying patients with lower and higher VTE recurrence risk, who could perhaps more safely discontinue or continue anticoagulation (Table 3). The recently published REVERSE II multinational management study aimed to determine whether patients with a low risk of recurrence can safely stop

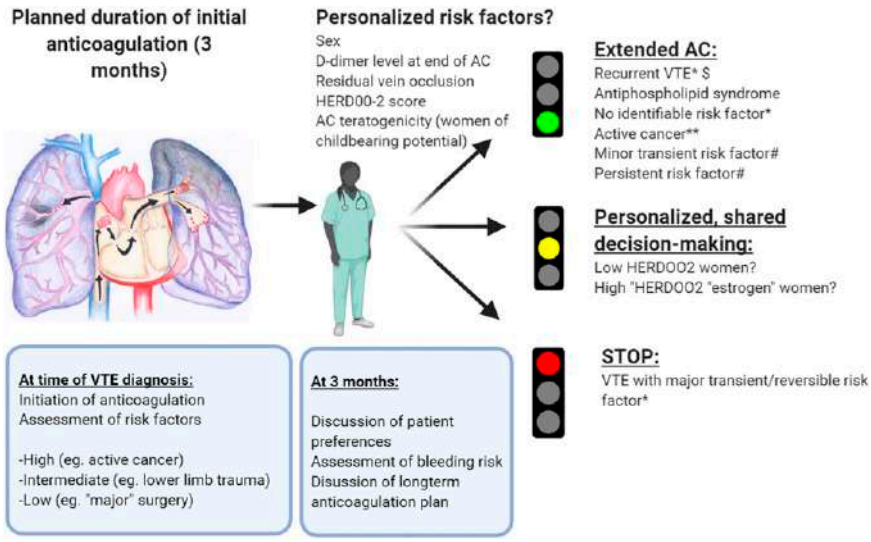

Figure 2. How we approach duration of anticoagulation in patients with a first VTE event. VTE recurrence risk is primarily informed by an initial evaluation of risk factors. We discuss an initial plan with the patient at the time of VTE diagnosis regarding the likely duration of anticoagulation, based on an evaluation of the circumstances in which the VTE occurred and bleeding risk. After a limited period of anticoagulation (typically 3 months), we schedule a consultation[44] to confirm whether extended anticoagulation is warranted based on initial risk factors, ongoing risk factors, bleeding risk, gender-specific considerations, comorbidities, potential additional personalized risk factors, and patient preferences, particularly in situations where the optimal duration of anticoagulation is less clear. This discussion guides our evidence-based shared decision making on duration of anticoagulation. Patients whose initial event occurred in the context of a major, transient/reversible provoking factor (such as major surgery or surgery with general anesthesia for >30 minutes) are recommended by international guidelines to receive limited-duration anticoagulation (red light).[2,43] In contrast, those with an unprovoked VTE event and other VTE events associated with a high predicted recurrence risk (and who have a low bleeding risk) are recommended by guidelines to receive extended or indefinite-duration anticoagulation (green light). Emerging data may in the future guide optimal management of patients with higher or lower personalized risk (orange light): for simplicity, in this figure the strength of recommendation, where indicated, is in accordance with the ESC 2019 Guideline on Acute PE.[2] *Class I B (ESC 'is recommended'; data derived from a single randomized clinical trial or large nonrandomized studies). **Class IIa level A (ESC 'should be considered'; data derived from multiple randomized clinical trials or meta-analyses). #Class IIa level C (ESC should be considered; consensus of opinion of experts and/or small studies, retrospective studies, registries), although the relative strengths of recommendations are in line with other similar guidelines. AC, anticoagulation. $At least 1 previous episode of VTE not related to a major transient or reversible risk factor. Low HERDOO2 risk: ≤1 HERDOO2 criteria (hyperpigmentation, edema, or redness in either leg; D-dimer level ≥ 250 μg/L; obesity with body mass index ≥ 30 kg/m^2; or older age ≥ 65 years).[18]

anticoagulant therapy after 5 to 7 months of treatment.[18] The authors evaluated a previously derived HERDOO2 rule (hyperpigmentation, edema, or redness in either leg; D-dimer level ≥ 250 μg/L; obesity with body mass index ≥ 30 kg/m^2; or older age, ≥65 years) in 2785 patients with a first unprovoked proximal DVT or PE who had completed 5 to 12 months of anticoagulation. The primary outcome was independently and blindly adjudicated recurrent VTE during 1 year and occurred in low-risk women at a rate of 3.0%/patient-year (95% CI, 1.8-4.8%) and in high-risk women and men who discontinued and continued anticoagulation at a rate of 8.1%, (95% CI, 5.2-11.9) and 1.6% (95% CI, 1.1-2.3), respectively.

Another model, the Vienna prediction model, was derived in a prospective cohort study including people with a first unprovoked VTE. Predictors included sex, the location and type of the VTE event, and D-dimer level.[19] Finally, a score termed DASH (D-dimer, Age, Sex, Hormonal therapy) was derived in a meta-analysis of individual patient data from prospective studies.[20] In this model, an abnormal D-dimer measured 3 to 5 weeks after discontinuation of anticoagulation, younger age, male sex, and hormone therapy were assigned scores of 2, 1, 1, and −2, respectively. Neither the Vienna prediction model nor the DASH score have been prospectively validated in a clinical management study.

As noted by the recently published ESC guidelines on acute PE, the therapeutic implications of risk prediction models such as the HERDOO-2 rule and the Vienna prediction and DASH scores may be less certain in the direct oral anticoagulant (DOAC) era,[2] and future studies will no doubt shed more light on the optimal risk assessment strategy.

Returning to the individual whose case was described in the opening paragraph, indefinite anticoagulation (with regular follow-up) was recommended and jointly agreed with the patient, given his predicted VTE recurrence risk and low bleeding risk, according to the strategy outlined in Figure 2.

VTE recurrence risk in women

Pregnant women

Case 2: A 25-year-old woman presents at 8 weeks of gestation. She experienced a PE while on a combined oral contraceptive pill 2 years ago and completed a limited duration of anticoagulation. Should she receive prophylactic anticoagulation to prevent VTE recurrence during pregnancy?

Table 3. Recurrence risk prediction models after a first unprovoked VTE

Study	Type of study	Predictors of VTE recurrence (vs no risk factor)	VTE recurrence risk or RR, HR (95% CI), or score in model
Rodger et al 2008 (HERDOO2 derivation study)[42]	Prospective cohort study	(1) Postthrombotic syndrome (HER) (2) Elevated D-dimer on anticoagulation (3) Obesity (4) Older age	(1) Men: RR 2.54 (1.48-4.38) (1) Women: RR 3.04 (1.40-6.60) (2) Women: RR 3.02 (1.41-6.51) (3) Women: RR 2.33 (1.14-4.74) (4) Women: RR 2.26 (1.12-4.56)
Eichinger et al 2010 (Vienna prediction model)[19]	Prospective cohort study	Male sex Proximal vs distal DVT PE vs distal DVT Elevated D-dimer	HR 1.91 (1.37-2.67) HR 2.76 (1.57-4.84) HR 3.15 (1.83-5.44) HR 1.24 (1.05-1.45)
Tosetto et al 2012 (DASH score)[20]	Patient-level meta-analysis	Elevated D-dimer Young age* Male sex Hormone use	Score 2 Score 1 Score 1 Score −2
Rodger et al 2017 (REVERSE; HERDOO2 rule)[18]	Prospective cohort management study	Low-HERDOO2-risk women Men and high-risk women: Continued AC D/C AC High-HERDOO-risk women who D/C AC	3% per 100 patient years (1.8-4.8) 1.6% per 100 patient years (1.1-2.3) 8.1% per 100 patient years (5.2-11.9) 7.4% per 100 patient years (3.0-15.2)

Low HERDOO2 risk, ≤1 HERDOO2 criteria (hyperpigmentation, edema, or redness [HER] in either leg; D-dimer level ≥ 250 µg/L; obesity with body mass index ≥ 30 kg/m^2; or older age, ≥ 65 years).
AC, anticoagulation; D/C, discontinued; HR, hazard ratio; RR, relative risk.
*First quartile (14-47 years) vs fourth quartile (>72 years).

Women with a personal VTE history have a high VTE recurrence risk during pregnancy[21,22] and require special attention, because VTE remains a leading cause of maternal death as a direct consequence of pregnancy.[2] Pregnant women who are at highest risk are those with a prior history of an unprovoked or a hormone-provoked VTE.[21] In a pooled analysis of 4 cohort studies, major antenatal VTE recurrence rates during pregnancy without prophylaxis were 1.1% (95% CI, 0.2-5.8) 6.4% (95% CI, 3.9-10.4), and 3.6% (95% CI, 1.4-8.9) for provoked (nonhormonal), estrogen-related, and unprovoked VTE, respectively.[23] It appears that this risk is reduced with low-molecular-weight heparin (LMWH).[23] Previous guidelines have suggested various approaches to VTE prevention in these women including a low prophylactic or an intermediate LMWH dose[24,25] (Figure 1). The optimal LMWH dose for women with prior VTE is currently being investigated in the ongoing Highlow study (NCT01828697; highlowstudy.org), a multinational randomized controlled trial (RCT) evaluating efficacy and safety of a fixed low dose of LMWH compared with an intermediate weight-adjusted dose in the prevention of VTE recurrence during pregnancy.

VTE recurrence risk in women whose initial VTE event was provoked by hormone use

Case 3: A 35-year-old woman was diagnosed with a proximal right DVT. She started a DOAC, and you now meet her after 3 months of anticoagulation. She complains of persistent right lower limb pain and swelling with erythema. She does not describe DOAC-related abnormal uterine bleeding or any other bleeding complications. Should she discontinue anticoagulation?

For women, VTE events occurring in the context of hormone use have traditionally been regarded as provoked events for the purposes of decision making surrounding duration of anticoagulation.[22] Studies evaluating the impact of hormone use on VTE recurrence risk have reported conflicting results (Table 4). Absolute reported recurrence risks in hormonal contraceptive users also vary widely between studies (Table 4). Hormone use is categorized as an intermediate VTE-provoking factor, with a 3% to 8% predicted associated recurrence risk, by ESC guidelines,[2] and as a minor (yet important) transient risk factor (with a 50% predicted recurrence risk compared with unprovoked VTE) by the ISTH.[1] Many current guidelines do not provide clear guidance on duration of anticoagulation in women experiencing a VTE in the context of contraceptive use, with the exception of the 2012 ISTH guidelines on duration of anticoagulation, which suggest that a duration of 3 months is sufficient, provided that hormone use is discontinued.[26] An ESC consensus statement addressing this topic will be published in 2020.

Recent data suggest that recurrence risk for women with hormone-provoked VTE may be modulated by additional risk factors, thus potentially increasing or decreasing predicted recurrence risk. Highlighting the importance of individual risk assessment, the REVERSE investigators reported a high annual recurrence risk (6.6%; 95% CI, 0.0-15.9 and 4.1%; 95% CI, 0.0-12.2, respectively) among estrogen hormone replacement therapy

Table 4. VTE recurrence risks in women with hormone exposure at the time of index VTE

Reference	Study type	Recurrence rates of hormone VTE	Groups compared (for HR, RR, or IRR)	HR, RR, or IRR (95% CI)
Heit et al 2000 [45]	Retrospective cohort	N/R for ♀ separately	COC- VTE ♀* vs all VTE HRT-related VTE vs all VTE	HR 0.3 (0.12-0.73) HR 0.7 (0.31-1.59)
Kyrle et al 2004 [46]	Prospective cohort	OC-VTE ♀: 5.9% at 5 y Non-OC ♀: 4.3% at 5 y	OC- VTE vs idiopathic-VTE ♀ HRT-VTE vs idiopathic-VTE ♀	RR 0.8 (0.1-4.0) RR 1.6 (0.4-6.0)
Baglin et al 2004 [47]	Prospective cohort	Estrogen-VTE: 8.7% at 2 y Other ♀: 8.7% at 2 y	Estrogen ♀ vs ♀ with other risk factors	HR 1.181 (0.35-4.03)
Cushman et al 2006 [56]	Data from PREVENT RCT	Hormone-VTE: 5% at 3 y Other ♀: 15% at 3 y	1.Hormone-VTE vs idiopathic 2.OC-VTE vs idiopathic 3.HRT-VTE vs idiopathic	1.aHR 0.54 (0.19-1.54) 2.aHR 0.43 (0.07-2.46) 3.aHR 0.62 (0.2-1.91)
Rodger et al 2008 [42]	Prospective cohort	N/R	1.OC-VTE vs non-OC-VTE ♀ 2. HRT-VTE vs non-HRT-VTE ♀	1.RR 0.37 (0.11-1.21) 2.RR 2.19 (0.86-5.55)
Le Gal et al 2010 [28]	Data from prospective cohort study	COC-VTE: 1.7% pa HRT-VTE: 10% pa Non-COC-VTE ♀ 5% pa High HERDOO2 OC-VTE 4% pa	1.COC-VTE v snon-COC VTE ♀ 2.HRT-VTE vs non-HRT VTE ♀	1.aHR 0.6 (0.1-2.8) 2.aHR 1.8 (0.6-5.2) (adjusted for age)
Douketis et al 2011 [49]	Patient-level meta-analysis	N/R	1. Hormone-VTE vs unprovoked VTE ♀ 2. OC-VTE vs unprovoked ♀ 3. HRT-VTE vs unprovoked ♀	1. HR 0.5 (0.3-0.8) 2. HR 0.39 (0.16-0.91) 3. HR 0.76 (0.39-1.49)
Tosetto et al 2012 [20]	Individual patient data (prospective studies)	N/R	Hormone-VTE vs non-hormone-VTE ♀	HR N/R (β coefficient −1.08; P = .002†)
Le Moigne et al 2013 [57]	Prospective single-center cohort	COC-VTE: IR 17.9/1000/y Non-COC: 17.6/1000/y	COC-VTE vs non-COC VTE ♀	IRR 0.7 (0.2-2.4)
Eischer et al 2014 [58]	Prospective cohort (all women)	Estrogen-VTE: 6% at 5 y Non estrogen ♀: 17% at 5 y	1.EC-VTE vs non-EC VTE ♀ 2.HRT-VTE vs non-HRT VTE ♀	1.RR 0.3 (0.1-0.5) 2.RR 0.7 (0.3-1.5)
Ljungqvist et al 2014 [59]	Prospective follow-up of case control study	Estrogen-VTE: 2% pa Non estrogen ♀: 3.2% pa	Hormone-VTE vs unprovoked ♀	HR 0.57 (0.36-0.9) aHR 0.7 (0.43-1.20)

Low HERDOO2 risk: ≤1 HERDOO2 criteria (hyperpigmentation, edema, or redness in either leg; D-dimer level ≥ 250 μg/L; obesity with body mass index ≥ 30 kg/m^2; or older age, ≥ 65 years).
AC, anticoagulation; aHR, adjusted hazard ratio; CHC, combined hormonal contraceptives; COC, combined oral contraceptive; EC, estrogen contraceptives (including nonoral formulations); FVL, factor V Leiden polymorphism; Gen, generation; HC, hormonal contraceptive; HR, hazard ratio; HRT, hormone replacement therapy; IR, incidence rate; IRR, incidence rate ratio; N/R, not reported; N/S, not specified; OC, oral contraceptives; pa, per annum; PP, postpartum; PPY, per patient year; PY, person year; RR, risk ratio.
*COC-VTE: initial VTE occurring in the context of COC.
†Recurrence risk modeled using multivariable Cox regression; β coefficient and P value after backward elimination is reported.

Table 4. (Continued)

Reference	Study type	Recurrence rates of hormone VTE	Groups compared (for HR, RR, or IRR)	HR, RR, or IRR (95% CI)
Kearon et al 2015 [29]	Prospective clinical management	Non-estrogen ♀: 5.4% PPY Estrogen ♀: 0.0% PPY All had persistent negative D-dimer at end of AC and off AC	Men, nonestrogen ♀, estrogen ♀	N/R (P = .001 for the 3-group comparison)
Rodger et al 2017 [18]	Prospective clinical management	Low HERDOO2 stopping AC; <50 y: Estrogen-VTE: 1.4%/100 PY Nonestrogen ♀: 3.1%/100 PY	N/R	N/R
Kiconco et al 2017 [60]	Retrospective population-based cohort study	Hormone-VTE: 37/1000 PY Non hormone ♀: 51/1000 PY	1.Hormone-VTE vs unprovoked ♀ 2. OC-VTE vs unprovoked ♀ 3. HRT-VTE vs unprovoked ♀	1. aHR 0.72 (0.58-0.88) 2. aHR 0.71 (0.52-0.96) 3. aHR 0.71 (0.53-0.95)

Low HERDOO2 risk: ≤1 HERDOO2 criteria (hyperpigmentation, edema, or redness in either leg; D-dimer level ≥ 250 μg/L; obesity with body mass index ≥ 30 kg/m^2; or older age, ≥ 65 years).
AC, anticoagulation; aHR, adjusted hazard ratio; CHC, combined hormonal contraceptives; COC, combined oral contraceptive; EC, estrogen contraceptives (including nonoral formulations); FVL, factor V Leiden polymorphism; Gen, generation; HC, hormonal contraceptive; HR, hazard ratio; HRT, hormone replacement therapy; IR, incidence rate; IRR, incidence rate ratio; N/R, not reported; N/S, not specified; OC, oral contraceptives; pa, per annum; PP, postpartum; PPY, per patient year; PY, person year; RR, risk ratio.
*COC-VTE: initial VTE occurring in the context of COC.
†Recurrence risk modeled using multivariable Cox regression; β coefficient and P value after backward elimination is reported.

and estrogen contraceptive users who were high risk by HERDOO2 criteria.[28] Conversely, it may be possible to estimate a group of women with a potentially lower recurrence risk: a multinational prospective clinical management study reported VTE recurrence rates of 5.4% (95% CI, 2.5-10.2) per patient-year in nonestrogen women and a very low rate (0.0%; 95% CI, 0.0-3.0 per patient-year) in estrogen women who had, importantly, a persistently negative D-dimer after discontinuation of anticoagulation.[29]

Collectively, these data are hypothesis generating. Most women with contraceptive-provoked VTE may discontinue anticoagulation after 3 months, provided that alternative contraceptive options are provided that do not increase thrombotic risk. However, future studies may further explore personalized benefit to risk ratio in women with hormone-provoked VTE. In the meantime, the consultation process (Figure 2) should include discussion of risk factors for VTE recurrence, sex-specific bleeding risk (including abnormal uterine bleeding), adequate contraception, methods and preconceptual counseling.[22]

VTE recurrence risk in nonpregnant women whose initial event was provoked by pregnancy

For women with a prior VTE event provoked by pregnancy or the postpartum period, the incidence of recurrent VTE has reported to be lower than in the case of unprovoked VTE, prompting guideline categorization of this risk factor as associated with an intermediate recurrence risk.[2] In a large retrospective study, the cumulative incidence of recurrent VTE up to 60 months after the index VTE event was significantly higher in women aged 18 to 46 years with unprovoked VTE compared with pregnancy (or postpartum)-associated VTE (10.4% and 5.8%, respectively; P < .02; adjusted HR, 0.6; 95% CI, 0.4-0.9).[30] More recently, the Registro Informatizado de Enfermedad TromboEmbólica (Computerized Registry of Patients with Venous Thromboembolism) investigators reported a 2-year VTE recurrence rate of 3.3% for women whose initial event was pregnancy (or postpartum) associated.[31] Collectively, these data suggest that limited duration anticoagulation may be appropriate for most women with prior pregnancy-associated VTE, after close follow-up and personalized assessment of risk factors as outlined in Figure 2.

VTE recurrence risks in people identifying as transgender

People identifying as transgender who have experienced a VTE face particular challenges.[32] Hormone therapy is a key component of the transition from male to female. In a cohort study, cis women (women genetically assigned female at birth who identify as female) whose initial event was hormone-associated and who continued oral contraceptives after discontinuation of anticoagulation had a fourfold increased risk of recurrent VTE compared with women who stopped oral contraceptives (incident rate ratio, 4.6; 95% CI, 1.9-11.5).[33] Although the recurrence risk for a transgender women with hormone-provoked VTE is not known, similar recurrence risks if hormones are continued without anticoagulation may potentially apply. However, discontinuation of hormones is often unacceptable. In a recent insightful review on the topic, Connors and Middeldorp[32] suggest a similar approach for transwomen who have experienced prior VTE to that taken for cis women during the phase of ongoing anticoagulation, with consideration given to extended anticoagulation if continued hormone therapy is chosen by the patient for quality-of-life reasons.[34]

What does this mean for decision making on duration of anticoagulation?

Anticoagulation reduces the risk of recurrent VTE; however, this benefit does not persist after discontinuation of anticoagulation.[35] When balancing benefit and harm for an individual patient, the case-fatality rates of bleeding and recurrent VTE and the probability of each are weighed. Case fatality rates in recent meta-analyses were 3.8% (95% CI, 2.0-6.1) for recurrent VTE,[8] 10.4% (95% CI, 6.6-15.4) for major bleeding during the initial VTE treatment phase with vitamin K antagonists (VKA), and 6.1% (95% CI,

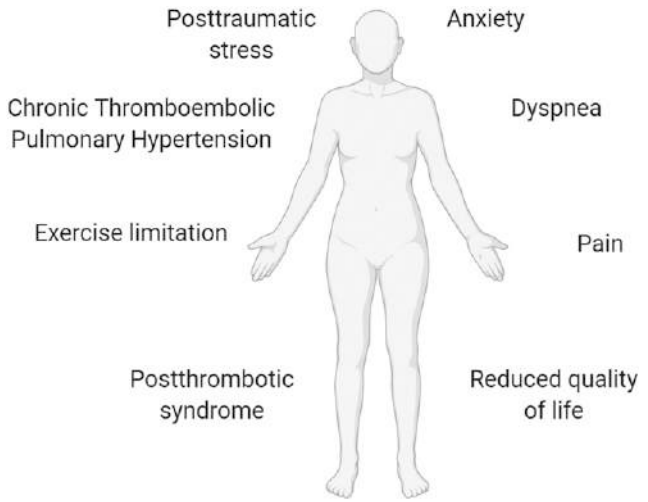

Figure 3. Long-term complications of recurrent VTE. Nonfatal, life-altering complications of VTE include postthrombotic syndrome (after DVT), chronic thromboembolic pulmonary hypertension (after PE), exercise intolerance, posttraumatic stress, dyspnea, anxiety, functional/exercise limitation, pain, reduced quality of life, and health care costs.[2,40] These symptoms may have a major impact on functional outcome.[40]

2.7-11.7) for DOAC therapy.[36] Case fatality rates for major bleeding during the secondary prevention phase of anticoagulation appear lower, at 0% (95% CI, 0.0-15.4) and 6.8% (95% CI, 1.4-18.6) for DOACs and VKA, respectively.[37] Although DOACs are associated with lower major bleeding rates than VKA therapy,[2] it should be noted that studies evaluating 2 different DOAC dosing strategies in comparison with either placebo[38] or aspirin[39] were not powered to compare outcomes between the 2 DOAC dosing strategies used (apixaban 2.5 mg and 5 mg twice daily in the AMPLIFY-EXT RCT[38] and rivaroxaban 10 mg and 20 mg once daily in the EINSTEIN CHOICE RCT[39]).

Nonfatal, life-altering complications of VTE should also be considered in this decision process, including postthrombotic syndrome and chronic thromboembolic pulmonary hypertension.[2] There is a new emerging awareness that nonfatal consequences of PE (collectively termed the post-PE syndrome) are more varied and common than had previously been realized and include exercise intolerance, anxiety, and functional limitation.[40] These symptoms can have a major impact on functional outcome, quality of life, and health care costs[40] (Figure 3).

An ISTH consensus statement previously suggested that it may be safe to discontinue anticoagulation if the risk of recurrent VTE is predicted to be less than 5% (with an upper bound of the 95% CI of 8%) at 1 year after discontinuation,[41] whereas studies aimed at deriving and validating clinical decision tools have sought to identify groups with predicted recurrence risks < 3%.[18,42] However, these thresholds may in the future be revaluated in light of an emerging awareness of long-term complications[40] and reported decreases in case-fatality rates of acute PE based on time-trend analyses in European, Asian, and North American populations.[2] Moreover, assessment and awareness of individualized bleeding risk is crucial during decision making: American College of Chest Physicians recommendations provide a superb framework to guide this risk assessment, which includes validated risk factors for bleeding while on anticoagulation including older age, comorbidities (including previous stroke and diabetes), recent surgery, frequent falls, alcohol abuse, cancer, metastatic disease, chronic renal or hepatic failure, thrombocytopenia, requirement for antiplatelet therapy, and a history of bleeding without a reversible cause.[43]

Collectively, these data have shaped recent international guidelines,[2,43] which recommend limited-duration anticoagulation for patients with a first VTE event that is provoked by a major transient/reversible risk factor (Figure 2). In contrast, indefinite anticoagulation is recommended for patients with recurrent unprovoked VTE or confirmed antiphospholipid syndrome. The strength of recommendation is somewhat weaker for those with a first unprovoked VTE, for those with persistent risk factors other than the antiphospholipid syndrome and for those with transient minor risk factors (as defined by Table 1), reflecting the lower level of certainty on the balance of benefit and harm in these patients: Consensus guidelines suggest that anticoagulation should be continued indefinitely in patients with unprovoked VTE who do not have a high bleeding risk. American College of Chest Physicians guidelines additionally note that personalized risk factors including "patient sex and D-dimer level measured a month after stopping anticoagulant therapy may influence the decision to stop or extend anticoagulant therapy."

Conclusions

VTE recurrence risk is largely determined by personalized risk factors, most notably the circumstances in which the index VTE event occurred (Figures 1 and 2). International guidelines recommend limited-duration anticoagulation for patients with a first VTE event that is provoked by a major transient/reversible risk factor who have a low predicted VTE recurrence risk (Figure 2), and indefinite anticoagulation for patients with, for example, recurrent unprovoked VTE or antiphospholipid syndrome who have a high predicted VTE recurrence risk (provided that their bleeding risk does not preclude this). Individualized discussion and shared decision making is important for those whose personalized risk factors are not yet addressed by high-quality data, and prospective clinical management studies and RCTs to address these knowledge gaps should be prioritized, notably in the area of cis-women's and transgender women's health.

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Off-label drug use

None disclosed.

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EVIDENCE-BASED MINIREVIEW

Advanced therapies and extracorporeal membrane oxygenation for the management of high-risk pulmonary embolism

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LEARNING OBJECTIVES

- Management of high-risk pulmonary embolism when systemic thrombolysis is contraindicated due to high risk of bleeding
- Indications for the use of extracorporeal membrane oxygenation in the management of pulmonary embolism

Clinical case

A 67-year-old man presented to the emergency room with progressively worsening shortness of breath and chest pain of 2 days duration. He has a history of hypertension, obesity and an ischemic stroke in the right middle cerebral artery, which was treated with systemic thrombolytics 4 weeks ago, and the patient was discharged on aspirin 81 mg daily. On arrival to the emergency room, the patient was tachycardic (heart rate 122 beats per minute) and hypotensive with a blood pressure of 88/60 mmHg. His respiratory rate was 24/minute, oxygen saturation was 88% on room air and improved to 92% on 2 liters of oxygen via nasal canula. Laboratory results were notable for a normal complete blood count and comprehensive metabolic panel, high-sensitivity troponin of 57 ng/L (normal 3-15 ng/L) and N-terminal pro-brain natriuretic peptide of 685 pg/ml (normal <100 pg/ml). CT angiogram of the chest showed large bilateral pulmonary emboli. Both bed-side echocardiogram and CT angiogram of the chest showed evidence of right heart strain. The patient was started on intravenous fluids, heparin infusion and the hospital's pulmonary embolism response team (PERT) was consulted for further management. Due to worsening respiratory status and persistent hypotension, he was intubated and started on inotropic support. Blood pressure remains low despite using maximal doses of norepinephrine and dobutamine. How should this patient be treated?

Introduction

The outcomes in patients with pulmonary embolism (PE) vary widely based on their clinical, imaging and laboratory parameters, and several prognostic scores have been developed to assess early PE-related mortality.[1] *High-risk* PE is characterized by the presence of shock or persistent hypotension (systolic blood pressure <90 mmHg, need for vasopressors, or a decrease in the systolic blood pressure by ≥40 mmHg from baseline for 15 minutes or longer despite resuscitation), and is associated with poor outcomes.[2,3] While low risk patients can be treated as out-patient, current guidelines recommend systemic thrombolysis with anticoagulation for patients with high-risk PE and hemodynamic compromise.[2,3] Thrombolytic therapy is associated with an increased risk of major bleeding, including intracranial hemorrhage, especially in the elderly, obese patients and those with comorbidities.[4] Advanced treatments or adjunctive treatments, including catheter based therapies and extracorporeal membrane oxygenation (ECMO), respectively, are being increasingly used for management of high-risk PE, though their optimal use remains uncertain. In this review, we discuss the management of high-risk PE when systemic thrombolysis is contraindicated due to a high risk of major bleeding, with an emphasis on the role for ECMO.

Initial treatment of high-risk PE

Supportive therapy including oxygen, mechanical ventilation, volume optimization, and use of vasopressors and inotropic support is often required in conjunction with anticoagulation as initial management of high-risk PE patients. Parenteral anticoagulation is recommended in patients who are hemodynamically compromised or at high risk of decompensation, and oral anticoagulants should be avoided in the acute phase.[3] Low molecular weight heparin and fondaparinux carry a lower risk of major bleeding and heparin induced thrombocytopenia, and unless contraindicated, are

Table 1. Selected studies on patients with acute, high-risk PE on ECMO support

Study, year	Patients, n*	Cardiac arrest, n (%)	Thrombolytic therapy, n (%)	Mechanical removal of PE, n	Duration of ECMO, average (range)	Complications/ Bleeding	Survival, %
Kawahito et al., 2000[38]	7	4 (71%)	7 (100%)	3 surgical embolectomies	18-168 hours	None	57
Maggio et al., 2007[39]	21 (19 VA ECMO and 2 VV ECMO)	8 (38%)	6 (29%)	4 surgical and 7 suction embolectomies	5.4 days (5 hours to 12.5 days)	4 ICH, 1 multiorgan failure	62
Sakuma et al., 2009[40]	193	NR	120 (62%), 20 patients got only heparin with ECMO	68 (35%) surgical embolectomies, 46 (24%) catheter therapy	NR	ICH or infarction in 24%	73
Malekan et al., 2012	4	NR	None, heparin with ECMO for all	1 suction pulmonary embolectomy	5.3 days	None	100
Munakata et al., 2012[25]	10	9 (90%)	10 (100%)	9 suction embolectomies	NR	2 major bleeding	70
Omar et al., 2013[41]	4	2 (50%)	1 (25%)	1 suction and 2 surgical pulmonary embolectomies	NR	NR	25
Maj et al., 2014[42]	6	6 (100%)	4 (66%)	1 surgical embolectomy, 2 CDT	4.5 days (1 to 11 days)	3 major bleeding	33
Cho et al.,2016[43]	13	NR	2 (15%)	11 surgical embolectomies	NR	NR	NR
Dolmatova et al.,2017[31]	5	4 (80%)	None	1 surgical embolectomy, 2 CDT	NR	NR	60
Corsi et al., 2017[27]	17	15 (88%)	8 (47%)	1 suction and 1 surgical embolectomy	4 (1 to 12) days	15 major bleeding	47
George et al., 2018[44]	32	15 (47%)	5 (16%)	11 CDT, 2 surgical embolectomies, 4 aspiration thrombectomies	NR	11 any bleeding, 1 ICH	53
Swol et al., 2018[30]	5	5 (100%)	3 (60%)	1 surgical pulmonary embolectomy	48.6 hours (1.5 to 100)	2 major bleeding	40
Moon et al., 2018[28]	14	11 (79%)	1 (7%)	1 surgical pulmonary embolectomy	7.9 days	7 moderate to severe bleeding	36
Meneveau et al., 2018[35]	52	39 (75%)	20 (38%)	17 surgical embolectomies, 18 anticoagulation alone with ECMO	NR	20 major bleeding	38% at 30 days, 35% at 90 days
Pasrija et al., 2018[34]	20	5 (25%)	7 (35%)	1 (5%) CDT, 11 (55%) surgical embolectomies, 8 (40%) anticoagulation alone with ECMO	5.1 days (3.7 to 6.7)	2 bleeding requiring transfusion	95

Abbreviations: VV ECMO, veno-venous ECMO; VA ECMO, veno-arterial ECMO; ICH, intracranial hemorrhage; NR, not reported; CDT, catheter directed treatment.
*Indicates VA ECMO support unless indicated.

Table 1. (Continued)

Study, year	Patients, n*	Cardiac arrest, n (%)	Thrombolytic therapy, n (%)	Mechanical removal of PE, n	Duration of ECMO, average (range)	Complications/ Bleeding	Survival, %
Pasrija et al., 2018[29]	Protocol: 27	6 (22%)	6 (22%)	12 surgical embolectomies, 15 anticoagulation alone with ECMO	5.8 days (4.3 to 6.7)	4 bleeding (15%)	96% at 1 year
	Historic controls: 6/27	6	NR	6 surgical embolectomy	NR	3 bleeding (50%)	73
Al-Bawardy et al., 2019[45]	13	13 (100%)	8 (62%)	3 (23%) CDT, 4 (31%) surgical embolectomy	5.5 days (2 to 18)	7 (54%) major bleeding	69% at 30d, 46% at 1 year
Ius et al., 2019[46]	36	15 (42%)	19 systemic or CDT	20 surgical embolectomy, 16 anticoagulation with ECMO	Variable	1 leg ischemia, 2 bleeding	67
Kjaergaard et al., 2019[47]	22	22 (100%)	12 (55%)	5 surgical thrombectomy, 1 suction thrombectomy, 10 anticoagulation with ECMO	0.5 to 168 hours	2 intrabdominal bleeds, 2 ICH	55% at 1 month and 45% at 1 year
Oh et al., 2019[48]	16	12 (75%)	7 (43.8%)	9 (56.3%)	1.5 days (0 to 4.5)	Bleeding in 9 patients	67
Kmiec et al., 2020[37]	75 (46 VA ECMO, 29 VV ECMO)	49 (65%)	30 (40%)	28 anticoagulation alone with ECMO, 8 interventional thrombectomies, 10 surgical embolectomies	NR	NR	47
Guliani et al., 2020[32]	17	10 (59%)	None	3 CDT	86 hours	Bleeding in 4 patients	76
Ghoreishi et al., 2020[33]	41	12 (29%)	10 (24%)	11 surgical embolectomies, 30 anticoagulation with ECMO	6 days	NR	98

Abbreviations: VV ECMO, veno-venous ECMO; VA ECMO, veno-arterial ECMO; ICH, intracranial hemorrhage; NR, not reported; CDT, catheter directed treatment.
*Indicates VA ECMO support unless indicated.

the preferred anticoagulant agents for the initial treatment of PE.[2,3] However, unfractionated heparin may be considered in patients with hemodynamic decompensation, due to the anticipated necessity for reperfusion treatment.[3] Patients in whom initial therapy fails to improve hemodynamic parameters and have persistent cardiogenic shock or develop cardiac arrest may require advanced therapies as described below. Anticoagulation should be continued to prevent new thrombosis while these therapies are administered. In massive PE, a significant reduction in recurrent PE or mortality was noted from 19% with heparin alone to 9.4% with full dose fibrinolysis.[5] However, systemic thrombolysis with anticoagulation is associated with a 3 fold increased risk of major bleeding compared to anticoagulation alone, with a 9.9% incidence of major bleeding and 1.7% incidence of intracerebral or fatal bleeding.[6-8]

Treatment of PE when systemic thrombolytics are contraindicated

In patients receiving anticoagulation, a history of stroke is known to increase the risk of major bleeding,[9,10] and ischemic stroke within 3 months is a major contraindication for systemic or locally administered thrombolysis.[2] Absolute and relative contraindications to fibrinolysis are provided by several society guidelines,[2,3,11] and other major contraindications include structural intracranial disease, hemorrhage, recent brain or spinal surgery, brain injury, bleeding diathesis and active bleeding.[2] A risk stratification score to assess the risk of intracranial hemorrhage in PE patients receiving thrombolytics, the PE-CH score, was developed including 4 independent prognostic factors: preexisting peripheral vascular disease, age >65 years, prior stroke with residual deficit, and prior myocardial infarction.[12] In patients with high-risk PE and perceived high risk of bleeding, surgical embolectomy, mechanical catheter based treatments and ECMO could be considered.

Surgical pulmonary embolectomy

Surgical embolectomy with cardiopulmonary bypass is an effective strategy when thrombus removal is indicated but there is an absolute contraindication for thrombolytic therapy, or to rescue patients that are refractory to thrombolysis. [13] In patients with massive PE who do not respond to thrombolysis within the

first 36 hours as evident by persistent clinical instability and residual echocardiographic right ventricular dysfunction, rescue surgical embolectomy led to better in-hospital course when compared with repeat thrombolysis, and was associated with lower recurrent PE and mortality.[14] A recent meta-analysis involving 1,579 patients who underwent surgical embolectomy showed an in-hospital all-cause mortality of 26.3% and a long-term all-cause mortality rate of 6.5 deaths per 100 person-year. Among these patients, 36% had preoperative contraindications to systemic thrombolysis, 33.9% suffered preoperative cardiac arrest, and 27% required the use of ECMO.[15]

Catheter-directed therapies

Interventional catheter-based treatments for acute PE include catheter directed therapies (CDT) with or without thrombolytics, and should be offered to patients who have a moderate to high risk of bleeding with systemic thrombolysis, and have access to the expertise and resources required to perform these procedures.[2,3] CDT achieves a high local concentration of thrombolytic drug by infusing drug directly into the PE, and can be combined with thrombus fragmentation resulting from placement of the infusion catheter and additional maneuvers, or ultrasound delivered via the catheter. The SEATTLE II study evaluated the safety and efficacy of ultrasound-facilitated, catheter-directed, low-dose fibrinolysis in massive and submassive PE, and showed moderate bleeding in 10% of participants though none had intracranial hemorrhage.[16] In pooled data from 348 patients, clinical success with percutaneous therapy alone for patients with acute massive PE was 81% (aspiration thrombectomy 81%; fragmentation 82%; rheolytic thrombectomy 75%) and 95% when combined with local infusion of thrombolytic agents (aspiration thrombectomy 100%; fragmentation 90%; rheolytic thrombectomy 91%).[17]

Although bleeding rates have not been directly compared in the contemporary era with advanced CDT, the incidence of bleeding may be lower than that reported in studies of systemic agents (approximately 0 to 4% vs 10 to 20% for major bleeding and <1% vs 2 to 5% for intracranial hemorrhage respectively).[7,16,18,19] However, since the evidence comes from prospective cohorts and registries that included small number of patients with massive PE, the efficacy and safety of these techniques in high-risk PE patients is uncertain.[16,20-22]

Extracorporeal membrane oxygenation

For patients with life-threatening PE at high risk of bleeding, catheter-directed thrombolytic therapy is relatively contraindicated, especially since the associated risk of major bleeding is unknown. Mechanical cardiopulmonary support, mostly with veno-arterial ECMO, is helpful in high-risk PE to maintain the circulation and oxygenation of organs during acute right ventricular failure and cardiogenic shock. Multiple case series and retrospective studies have shown good outcomes with ECMO for massive PE, but there are no randomized controlled trials comparing ECMO to other treatments.[23] ECMO has been used in different clinical scenarios, to rescue patients when thrombolytic treatment fails or as a temporary hemodynamic support prior to surgical[24] or catheter-based embolectomy,[25] and in patients with refractory cardiogenic shock or cardiac arrest. Surgical embolectomy requires sternotomy and cardiopulmonary bypass and carries a significant morbidity and mortality in patients with advanced shock and multiorgan failure. In these patients, heparin therapy with ECMO may offer a rapid and effective alternative treatment option until heparin-induced and endogenous thrombolysis permits weaning-off support, often within few days.[26-28] Pasrija et.al., reported that implementation of a protocolized strategy of triaging patients with massive PE based on an algorithmic approach rather than aggressive early surgical approach reduces morbidity and mortality.[29]

Consensus regarding the optimal management of PE patients with persistent shock and when thrombolysis is contraindicated is lacking. It is unclear if these patients benefit from ECMO first or embolectomy first approach, and ECMO is often used following failure of other therapeutic options.[30,31] Mortality rates are high when ECMO is used as a salvage therapy in patients who have failed other advanced therapies, and ECMO with therapeutic anticoagulation is emerging as a promising initial support strategy and as a bridge to recovery or surgical pulmonary embolectomy at several centers.[29,32-34] In a protocol using ECMO support for 3 to 5 days followed by reevaluation of right ventricular function, 73% of the patients responded to anticoagulation alone and 27% required subsequent surgical embolectomy. Prolonged shortness of breath, elevated N-terminal pro-brain natriuretic peptide, enlarged pulmonary artery diameter, and previous history of venous thromboembolism were associated with lack of right ventricular recovery, and early surgical intervention may be considered in these patients. The use of ECMO requires full dose anticoagulation to maintain the functionality of the system; hence, is associated with complications such as bleeding and infection, and judicious patient selection and center experience play a role. In a multicenter study assessing outcomes of 52 patients treated with ECMO, patients who failed fibrinolysis and those who did not receive reperfusion therapy had unfavorable prognosis compared with ECMO performed in addition to surgical embolectomy.[35] Among patients with high-risk PE who require anticoagulation and treatment with ECMO, several patients also received thrombolysis in the published literature (Table 1). The bleeding rate was higher in patients who had thrombolysis or surgery combined with ECMO, than those with ECMO alone.[35] Considering that heparin-induced clot dissolution and spontaneous fibrinolysis allows ECMO weaning after only a few days on support, the benefit of additional mechanical clot-removal therapies, catheter-based or surgical thrombectomy on ECMO, warrant further investigation. The appropriateness of these recommendations for a specific patient may vary depending on several factors and should be best judged by the clinician. Optimal medical decisions must incorporate factors such as age, comorbidities, life expectancy, patient wishes, and quality of life. Establishment of multidisciplinary PE response teams (PERTs) is encouraged, as they address the needs of modern systems-based healthcare (Class IIa recommendation, Level C).[36]

Recent guidelines from the European Society of Cardiology recommend surgical embolectomy (Class I recommendation, Level C) or percutaneous catheter directed treatment (Class IIa recommendation, Level C) in patients with massive PE at high risk of bleeding, if they have access to the expertise and resources. ECMO may be considered in conjunction with these treatments in patients with refractory cardiogenic shock or cardiac arrest (Class IIb recommendation, Level C).[3] Level of evidence for embolectomy is probably at the same level as for

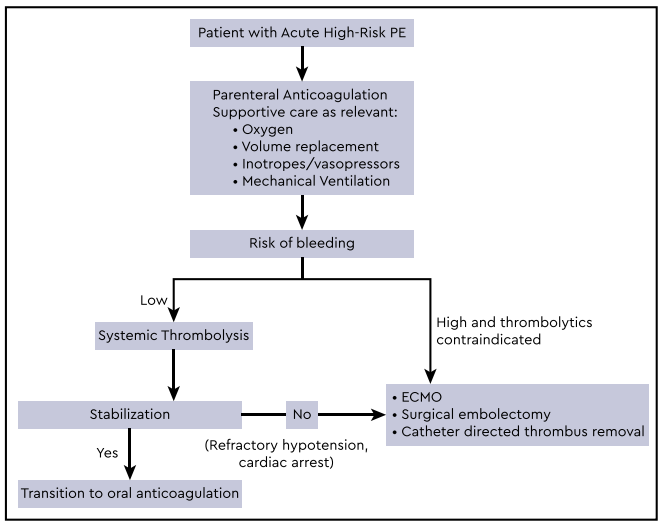

Figure 1. Management of acute high-risk pulmonary embolism.

CDT and ECMO in the current era, as more studies show benefit of ECMO with heparin as a stand-alone therapy.[37] Following hemodynamic stabilization, patients recovering from high-risk PE can be switched from parenteral to oral anticoagulation. As these patients were excluded from the direct acting oral anticoagulant clinical trials, the optimal time point for this transition and their efficacy has not been determined by existing evidence.

In summary, the use of ECMO, whether utilized as a bridge to embolectomy or as a therapeutic intervention, seems promising in high-risk PE patients who have contraindication for systemic thrombolysis. Further randomized studies to compare ECMO to other interventions to determine their efficacy and safety are warranted.

Resolution to the Patient Case

In our patient who is elderly with multiple co-morbidities and has high-risk PE due to persistent hypotension, history of recent stroke is an absolute contraindication for systemic thrombolysis. Due to refractory cardiogenic shock, PERT team recommended treatment with heparin anticoagulation and ECMO, and cardiovascular parameters improved after 4 days. He was transferred to the floor after a week and discharged home on apixaban 5 mg twice daily. At a 1 month follow up visit, he reported no complications from anticoagulation and was recovering well.

Conclusion

The use of ECMO with anticoagulation seems promising in high-risk PE when systemic thrombolysis is contraindicated, though quality of evidence is low and future studies are urgently needed to better define its optimal use.

Conflict-of-interest disclosure

R.G. serves as a consultant for Sanofi Genzyme for cold agglutinin disease. This is not relevant to the current article. F.A.K. reports research grants from Bayer, Bristol-Myers Squibb, Boehringer-Ingelheim, Daiichi-Sankyo, MSD and Actelion, the Dutch Heart Foundation, the Netherlands Organization for Health Research and Development, and the Dutch Thrombosis Association, all outside the submitted work.

Off-label drug use

None disclosed.

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Informed consent for genetic testing in hematology

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Informed consent is a fundamental component of modern health care. All competent adult patients have the legal and ethical authority to accept (consent) or refuse (dissent) recommended health-related interventions. Various models of informed consent have been described, and herein I introduce a model that divides informed consent into 7 distinct elements: competence, voluntariness, disclosure, recommendation, understanding, decision, and authorization. Genetic testing, which is rapidly becoming a common feature of both clinical care and research in hematology, adds additional layers of complexity to each of these consent elements. Using the example case of Mr. Smith, a man with newly diagnosed acute myeloid leukemia whose clinicians offer him genetic testing of the leukemia through a clinical trial, I highlight the challenges and controversies of informed consent for genetic testing, focusing on each consent element as it pertains to genetic testing in such a setting. Ultimately, given the growing importance of genetic testing for hematologic disorders, clinicians, and researchers in hematology should be facile at participating in all aspects of informed consent for genetic testing.

LEARNING OBJECTIVES

- Identify the fundamental role of informed consent in health care and the different elements that comprise informed consent
- Understand why informed consent for genetic testing is complex and how genetic testing in hematology complicates each consent element

Clinical case

Consider the fictional case of Mr. Smith, a 50-year-old man who has just been diagnosed with CD33⁻ acute myeloid leukemia (AML) with favorable-risk cytogenetics. Otherwise healthy, he met with his clinical team to discuss treatment options. His clinicians recommend standard induction chemotherapy with cytarabine and daunorubicin ("7+3").[1,2] They also recommend that he participate in a clinical trial that is currently enrolling patients. In this trial, genetic sequencing would be performed on his leukemia cells to see if there are any targetable alterations that could inform further antileukemia therapy. "Sounds good to me," Mr. Smith responds immediately. "That certainly sounds like my best chance for a cure."

Introduction

Genetic testing is rapidly becoming more commonplace throughout medicine, and since the advent of tyrosine kinase inhibitors,[3] its use in hematology has increased similarly.[4] Genetic and genomic testing provide great hope and opportunity to clinicians, patients, families, and scientists, but it also carries with it significant ethical complexity.[5] This article focuses on the unique complexities surrounding informed consent for genetic testing in hematology. I will discuss single tests ("genetic tests"), as well as panel testing and next-generation sequencing ("genomic tests"). For the sake of simplicity, I will refer to these terms together as genetic testing but will specify when particular considerations apply to one type. Genetic testing, even when performed as a clinical (rather than research) test, has numerous unique characteristics in clinical hematology, warranting particular focus on informed consent in this setting. A comprehensive discussion of these unique features is beyond the scope of this article, but they include the possibility of identification of germline variants when performing somatic sequencing, the great uncertainty inherent in genetic testing, and the high rate and potential impact of incidental findings in this type of testing.[5]

Informed consent

Informed consent is a core component of ethical practice of both clinical care and clinical research,[6,7] and many unique features of genetic testing add ethical complexities to informed consent. Importantly, documentation of consent (eg, signature on an informed consent form or vocalization of consent to proceed with an intervention) is only 1 element of informed consent. Various ways of defining informed consent have been proposed, but a widely accepted model divides consent into 7 individual elements, grouped into 3 larger categories: threshold elements, information elements, and consent elements (Table 1).[6] In this article, I will describe these elements and examine the unique complexities inherent in each regarding consent for genetic testing in hematology. Importantly, these elements also apply to informed *refusal*, the autonomous decision to forgo a particular treatment or intervention, although this will not be the main focus of this article. All competent adults have the legal and ethical right to accept or refuse a given medical intervention. The sections that follow describe how to determine whether that acceptance (consent) or refusal (dissent) is legally and ethically sound.

Threshold elements: competence and voluntariness

Competence, the first of 2 threshold elements of informed consent, refers to an individual's ability to understand and appreciate the situation, to weigh the risks and benefits of treatment options (reasoning), and to communicate a choice (Table 2).[8,9] Here, the terms competence and capacity are used interchangeably, though some identify minor distinctions between these concepts. Competence is defined for a given domain and a task within that domain; an individual, for example, may be competent to choose whether to have blood drawn from the left or right arm but may not be competent to decide whether to undergo a surgical procedure. Generally, greater competence is expected for more complex and/or risky health care decisions.[10] In addition, competence can vary over time, as many factors can influence an individual's competence (eg, effect of the underlying disease, medications and treatments, and comorbidities). Importantly, various hematologic disorders could affect an individual's ability to make a competent decision (eg, significant intellectual disability after a stroke due to a hypercoagulable state, postictal state after a seizure from an adverse event during chemotherapy, and age-related dementia unrelated to the underlying hematologic disorder), independent of the decision itself, which will be addressed further later. In this case, Mr. Smith's competence could be affected by several factors related or unrelated to leukemia. The presence of a clinically significant intracranial chloroma, for example, may affect his level of consciousness and ability to make competent decisions. It is also possible that, later in treatment, his competence could be affected by disease progression, treatment toxicities, or other factors, that may predict a change in his competence over time and with changing circumstances.

Voluntariness, the second threshold element, refers to an individual's ability to make a choice independent of outside influence.[6,11] The standard for voluntariness typically is high: a decision is only *involuntary* if it is coerced or made under a credible and intended threat.[11] In health care, such an occurrence is rare. More common, however, are subtle forms of influence that do not reach the level of coercion, such as persuasion or manipulation. There is a great debate about how much influence is too much, but it depends significantly on the level of vulnerability of a patient.[11] When considering compensation for participation in a clinical trial, for example, a large monetary payment for participation may be differentially influential, depending on an individual's financial means. In this way, even though such a large payment may not be coercive, it could be seen to take advantage of an individual's vulnerability.

Given the potential vulnerability of patients undergoing genomic sequencing, this is a significant concern for patients

Table 1. Elements of informed consent

Consent category	Consent element	Case example
Threshold elements (preconditions)	Competence/ capacity	In this case, Mr. Smith is assumed to be competent to make a decision about treatment of his leukemia, including the genetic testing that has been recommended. It is possible, however, that if he had a large intracranial chloroma or large burden of CNS disease (or unrelated neurologic dysfunction) that he may not be able to competently make these decisions and provide informed consent for his treatment plans.
	Voluntariness	Mr. Smith must be given the opportunity to make a voluntary decision about how to proceed in treating his acute myeloid leukemia, free of coercion.
Information elements	Disclosure	The clinician-investigators must disclose risks and benefits of the proposed interventions, including risks and benefits of leukemia sequencing, to Mr. Smith, as well as other disclosures relevant to Mr. Smith's decision (including that the sequencing is part of a research study, not standard clinical care).
	Recommendation	To help Mr. Smith make an informed choice about whether to enroll on the sequencing trial, his clinician-investigators should recommend to him the intervention they feel best aligns with his values and beliefs, given his present clinical condition and circumstances.
	Understanding	In order to proceed, Mr. Smith should be able to demonstrate to his team that he understands the information about the clinical trial (including the sequencing that is part of it) that has been disclosed to him and the plan that his team has recommended.
Consent elements	Decision	Mr. Smith should be able to clearly state which available option he has chosen and why that is his choice.
	Authorization	Prior to initiation of therapy (and enrollment on the trial, if he opts to enroll), Mr. Smith should confirm that he authorizes his team to proceed and to carry out his stated decision.

Adapted from Beauchamp and Childress.[6]

Table 2. Required elements of decisional capacity and competence

Decisional element	Definition	Case example
Understanding	The patient's ability to grasp the meaning of information communicated by the physician and other caregivers.	Ms. Haverford has just been diagnosed with an advanced hematologic malignancy, but she has been completely silent while her physician explains her the diagnosis and recommended treatment. Further inquiry is necessary to ensure that she understands the information that has been communicated to her.
Appreciation	The patient's ability to appreciate the consequences of their situation (medical condition, need for treatment [when applicable], and likely benefits and harms of each possible treatment).	Ms. Haverford is a highly educated patient who has clearly understood the information conveyed, but she seems to question how certain it is that she really has cancer and thus whether any treatment is actually needed. Exploration of her appreciation of his condition is clearly needed.
Reasoning	Patient can weigh risks and benefits within/across treatment options and arrive at a decision that is consistent with their starting premise(s).	Ms. Haverford has been clear that she places great value on comfort but elects a treatment approach that is likely to cause substantial distress. This discordance deserves a careful inquiry into the reasoning underlying that decision.
Communicating a choice	Patient can clearly indicate the preferred treatment option and maintain that choice for a sufficient period of time for it to be implemented.	Ms. Haverford demonstrates great ambivalence about a treatment choice, not clearly embracing any option but shifting among them. The basis for that ambivalence should be explored and, if possible, resolved.

The table describes the fictional case of Ms. Haverford, who was recently diagnosed with an advanced hematologic malignancy. Whereas Mr. Smith's team appeared to have no concerns about his capacity and competence, this table describes the fundamental elements of decisional capacity and competence, and how these might apply in a case such as that of Ms. Haverford.
 Adapted from Marron et al, 2020.[9]

with hematologic disorders. Even though Mr. Smith's clinical team did not intend to mislead or manipulate him, it is possible that, given the gravity of his diagnosis, he felt compelled to participate in the clinical trial, as exemplified by his remark that the trial sounds like his best chance of a cure. Although this scenario would be unlikely to meet the standard of coercion, it could nonetheless raise concerns about whether Mr. Smith's decision was influenced by his vulnerable status. It also speaks to the possibility of therapeutic misconception,[12] which will be discussed in greater detail later.

When examining voluntariness regarding genomic sequencing, it is also important to consider the possible discovery of information about those other than the individual who provides consent for testing. Were Mr. Smith to undergo sequencing and learn that he had a germline alteration that predisposed him to leukemia, it could have implications for his relatives as well (assuming the mutation was not de novo). Some have argued that individuals have a duty to warn others upon learning of possible risks to them, such as a germline risk similar to this one.[13] It remains controversial, however, whether and how relatives should provide consent (or at least be alerted) before testing, counseling, and disclosure. This is of particular importance given the growing role of cascade testing in identifying those at risk of genetic disorders who have an affected relative.[14] An added complexity is that related donor hematopoietic stem cell transplantation is often considered for those with hematologic malignancies (particularly those resulting from a germline predisposition). Before transplantation, however, it is important to be sure that the potential donor does not have the same germline alteration as the index patient, although best practices in this setting remain controversial.[15] Assuming that Mr. Smith provides consent for sequencing, is there an expectation that he will confirm that his first-degree relatives also provide consent? And if he learns that he indeed has a predisposition for germline cancer, how should this subject be broached with his relatives, who may involuntarily (or at least unwittingly) receive medical information about themselves, without their explicit consent? And if he has such a predisposition, how should family members be approached to serve as a potential related stem cell donor, understanding that they also would be likely to have to undergo sequencing to ensure that they do not have the same predisposition? Further work is necessary to answer these complex, challenging questions.

Information elements: disclosure, recommendation, and understanding

Disclosure, the first of 3 information elements of consent, refers to the ethical obligation of complete and comprehensive disclosure of the risks and benefits of a given intervention and disclosure of any potential conflicts of the clinician.[6] The former is generally more salient regarding genetic testing, but conflicts of interest and commitment also warrant consideration, particularly given that many clinicians and researchers have a financial stake in testing that they may order or recommend (either at their own institution or via private testing facilities). Returning to the former, clinicians disclose risks and benefits as a matter of daily practice with regard to new treatments, surgeries, and other interventions, and similar disclosure is important with regard to genetic testing. Unlike invasive procedures, the physical risk of such testing typically is minimal, but there are unique risks related to genetic testing. A growing body of literature highlights the potential psychological implications of genetic testing, including increased stress and anxiety and effects on overall psychological well-being.[16,17] Furthermore, although the Genetic Information Nondiscrimination Act and Affordable Care Act protect against genetic discrimination on the part of some types of insurance and in a subset of other areas, despite these protections, there remains a risk of discrimination based on genetic and genomic findings.[18]

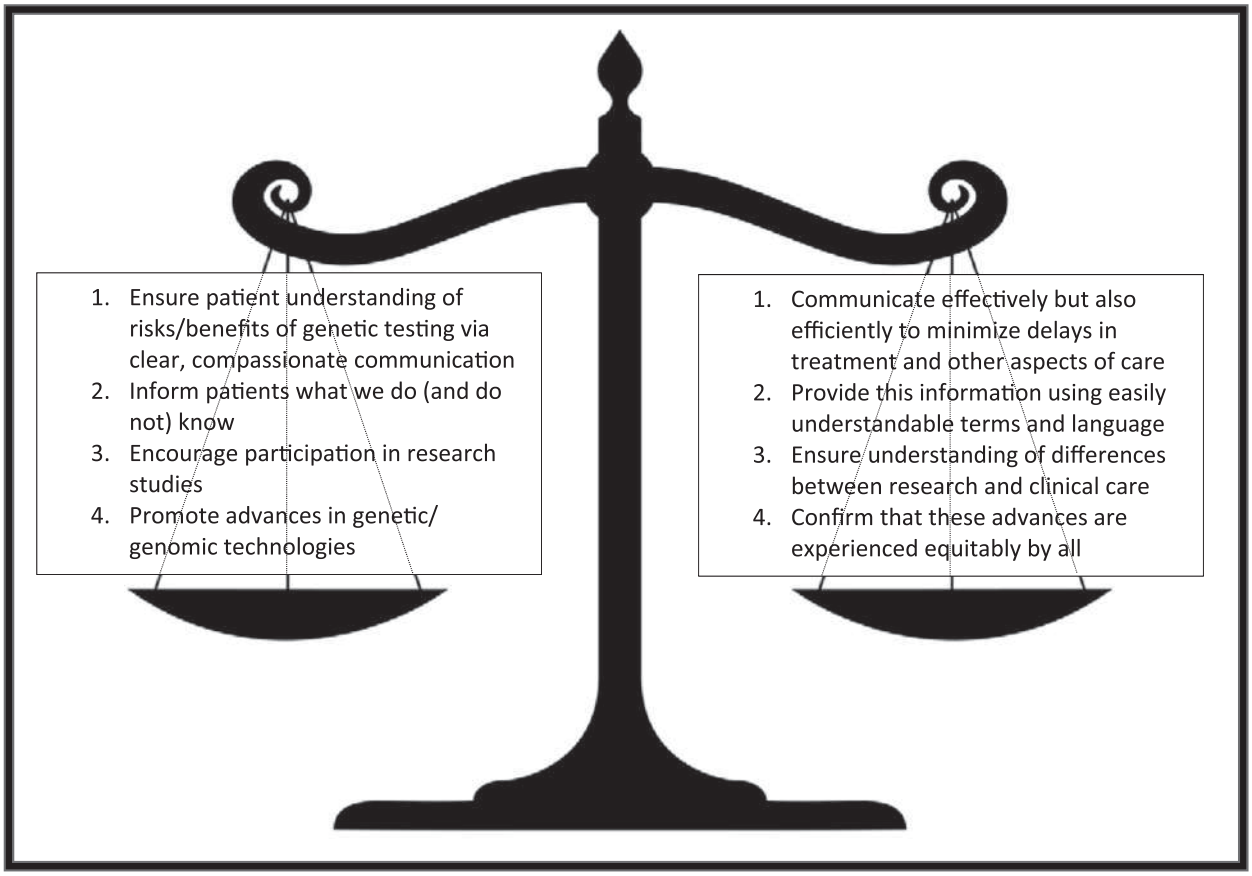

Figure 1. The balancing act of informed consent for genetic testing in hematology.

An additional important consideration regarding disclosure in genetic testing is that of whether the testing is part of clinical care or research. Typically, this distinction is clear, and until relatively recently, testing (particularly next generation sequencing) was performed only as part of a research study. Today, however, as genetic testing rapidly moves from the laboratory to the clinic, this distinction can become less clear. Classically, therapeutic misconception has described the conflation of understanding of the goals of research (generalizable knowledge) and clinical care (individualized patient-level benefit) and is seen in ~60% of research participants.[12] Mr. Smith's response speaks to why this distinction is important; his belief that genetic testing represents his best chance of a cure seems to imply that he thinks the testing is intended, first and foremost, to improve his clinical care. Further details of the research study are necessary to be certain, but because his sequencing would be part of a clinical trial, the truth may not be so straightforward. Not surprisingly, many participants in research on pediatric and adult genetic testing express similar misunderstanding regarding whether the testing is intended primarily to help them (clinical) or develop generalizable knowledge (research) and may overestimate the expected impact of genetic testing on their care and clinical outcome.[16,19,20] This speaks to the importance of pretest counseling about genetic testing. Unfortunately, however, many clinicians express low confidence in their ability to provide adequate counseling about genetic results,[21,22] and many institutions lack sufficient genetic counselors for this purpose.[23] This insufficiency is further complicated by variability in the role played by genetic counselors across institutions[24] and the need for greater education of genetic counselors about germline predisposition syndromes,[25] with the latter likely to be even more prevalent regarding predisposition to hematologic and hematopoietic disorders.

The final 2 information elements of informed consent, recommendation and understanding, will be discussed together, as they are inextricably linked.[6] Clinicians' recommendations carry great weight with many individuals; as a result, a clinician's recommendation is likely to significantly influence a patient's choice about a given intervention. The patient's choice also relies on having an understanding of that choice, which may be the most challenging aspect of informed consent for genetic testing. The public's health literacy and numeracy are known to be poor,[26] and given the probabilistic nature of genetics, it is not surprising that low levels of genetic knowledge are prevalent.[27] Unfortunately, disparities in knowledge about genetics have been reported according to race, education, and age,[16,27] which has troubling implications for both consent for genetic testing and for the use of genetic results.

As mentioned earlier, another important component of understanding is whether a genetic test is performed for clinical or research purposes. In our case, Mr. Smith demonstrates some confusion about this distinction, sometimes referenced as therapeutic misconception.[12] Although some individuals' decisions may not change if they are testing for clinical vs research purposes, the distinction is an important one for many and warrants

clarification. Further, a patient who provides truly informed consent for genetic testing must understand both what the test is likely to offer and what it will not, which may differ in clinical vs research settings. Full disclosure of risks and benefits of testing helps delineate what testing offers, but the nature of genetic testing makes such disclosure (and resultant understanding based on it) difficult. We do not know how Mr. Smith's team described the testing to him, but clinicians and researchers often use ill-defined (or at least variably defined) terms such as "actionable results." Discussions about potential outcomes of testing are challenging as well; to support informed decision making, the patient should know how likely testing is to find an actionable result (and what that means), to lead to a change in treatment, and to improve a patient's chance of survival and cure. Research demonstrates that patients and families commonly misunderstand these and other features of genetic testing.[16,19]

The potential for misunderstanding of these complex concepts speaks to the importance of ensuring that patients understand all aspects of the genetic testing being proposed, for them to make informed decisions. Explanations can take time, but it is time well spent. Clear communication alone does not always impart improved understanding, however, as has been seen regarding communication about such concepts as prognosis.[28,29] Recent work examining specific strategies for improving a patient's understanding regarding genetic testing shows promise,[30,31] but more work is needed in this area.

Consent elements: decision and authorization

These final 2 elements of informed consent, decision and authorization, refer to an individual's decision among proposed treatment options and authorizing (or refusing) one of them.[6] Unfortunately, many think of consent as a signature on a form that documents consent; importantly, this form serves only as legal proof that a person has opted to move forward with a given intervention and authorizes the clinician to proceed. This consent document is an important legal record, but the entire consent process (including all elements described to this point) remains ethically required. Consent is much more than a signature on a form, but the signature (literal in some cases, figurative in others), representing an individual's decision and authorization to proceed, is a necessary element of valid informed consent.

Importantly, many aspects of medical care are thought not to require explicit consent. A clinician does not ask for consent to listen to a patient's heart, for example, or for sending off a particular laboratory test. Genetic testing, however, is far more complex and nuanced than such standard aspects of medical care,[5] leading most to argue that a more formal consent process (including some form of documentation of the patient's decision and authorization) is recommended for such assessments. There is a robust body of literature regarding what aspects of medical care require explicit consent,[6,32,33] but a full analysis of the subject is beyond the scope of this article.

Consent disparities and conclusions

Informed consent is a core component of modern medical practice, and its importance is particularly noteworthy, and particularly complex, in genetic testing.[5] Consent has 7 elements, each of which demonstrates added complexity when the consent is for genetic testing. The case of Mr. Smith, although rather straightforward medically, highlights some of these complexities, ranging from difficulty defining and communicating the potential risks and benefits (and uncertainties) of genetic testing, to the difficulty in verifying the understanding of these. Without question, however, as genetic testing becomes more common in both research and clinical practice in hematology, ensuring that patients provide informed consent for testing will become all the more important.

As the global community becomes more aware of unfortunate disparities in health care access and outcomes, disparities related to genetic testing warrant mentioning. Work with genomic repositories such as The Cancer Genome Atlas demonstrates that much more is known about genetic and genomic diversity (in both healthy and disease states) among Whites than among those of racial and ethnic minorities.[34] This disparity imparts the possibility that genomic knowledge will disproportionately benefit those about whom we know more, which has been confirmed in several recent analyses.[35,36] These inequities are only beginning to be understood, but recent work identifies that this may translate into disparate outcomes from genetic and genomic advances. The use of tyrosine kinase inhibitors in chronic myeloid leukemia, for example, appears to disproportionately benefit those of European background over African Americans.[37,38] Compounded by decreased availability of genomic testing for those with lower income,[39] lower rates of enrollment by those of minority background in genetic and genomic research studies,[40,41] and reports of less knowledge about genetics among those of minority background and with less education,[16,27] we are at a crossroads to ensure that future advances in genetic testing and genetic technologies are equitably accessible to all.[42]

Ultimately, informed consent is a complex balancing act (Figure 1) that serves to support and demonstrate respect for an individual's autonomous choices. The importance of supporting and respecting this choice is readily apparent when considering consent for genetic testing in hematology in both research and clinical settings. As the role of genetic testing in hematology grows further, to provide optimal and equitable care to their patients, all hematologists must exhibit practical knowledge of the elements of informed consent as they relate to genetic testing in hematology.

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Off-label drug use

None disclosed.

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Identifying potential germline variants from sequencing hematopoietic malignancies

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Next-generation sequencing (NGS) of bone marrow and peripheral blood increasingly guides clinical care in hematological malignancies. NGS data may help to identify single nucleotide variants, insertions/deletions, copy number variations, and translocations at a single time point, and repeated NGS testing allows tracking of dynamic changes in variants during the course of a patient's disease. Tumor cells used for NGS may contain germline, somatic, and clonal hematopoietic DNA alterations, and distinguishing the etiology of a variant may be challenging. We describe an approach using patient history, individual variant characteristics, and sequential NGS assays to identify potential germline variants. Our current criteria for identifying an individual likely to have a deleterious germline variant include a strong family history or multiple cancers in a single patient, diagnosis of a hematopoietic malignancy at a younger age than seen in the general population, variant allele frequency > 0.3 of a deleterious allele in a known germline predisposition gene, and variant persistence identified on clinical NGS panels, despite a change in disease state. Sequential molecular testing of hematopoietic specimens may provide insight into disease pathology, impact patient and family members' care, and potentially identify new cancer-predisposing risk alleles. Ideally, individuals should give consent at the time of NGS testing to receive information about potential germline variants and to allow future contact as research advances.

LEARNING OBJECTIVES

- Know when to suspect germline cancer predisposition and how it may impact a patient's disease and clinical course
- Understand how clinical next-generation sequencing of tumor cells may be used to identify potential germline cancer-predisposition variants
- Comprehend the evolving nature of genomics pertaining to germline predisposition, implications when considering hematopoietic stem cell transplants using related donors, and informed consent for clinical testing of diseased tissue

Clinical case

A 73-year-old Caucasian woman presented with fatigue, and a complete blood cell count revealed a total white cell count of 2400 per microliter, hemoglobin of 11.4 g/dL, and a platelet count of 104 000 per microliter. The patient was referred to a hematologist who took a detailed personal and family history. Her past medical history was notable for gastric cancer that was treated with a partial gastrectomy followed by oxaliplatin/folinic acid/fluorouracil (FOLFOX) chemotherapy. Although 6 cycles were recommended, the patient stopped chemotherapy after the third cycle because of delayed blood cell count recovery. Her family history included a father diagnosed with "some kind of blood cancer" at ~75 years of age and a paternal grandmother with "head and neck cancer" at ~60 years of age (Figure 1A). She noted that her grandmother had never smoked cigarettes or drunk alcohol. A bone marrow (BM) biopsy demonstrated a myelodysplastic syndrome (MDS) characterized by refractory cytopenias with multilineage dysplasia, 15% cellularity, and 14% blasts. Cytogenetic analysis showed a normal karyotype, and molecular profiling using a next-generation sequencing (NGS) panel reported 171 unique variants, including 2 deleterious *DDX41* variants: p.Asp140Gfs*2 (p.Asp140fs), initially with a variant allele frequency (VAF) of 0.53, and a p.Arg525His variant with a VAF of 0.27 (Figure 1B). The hematologist recognized the DDX41 p.Asp140fs variant as potentially germline, which is most common in the non-Finnish European

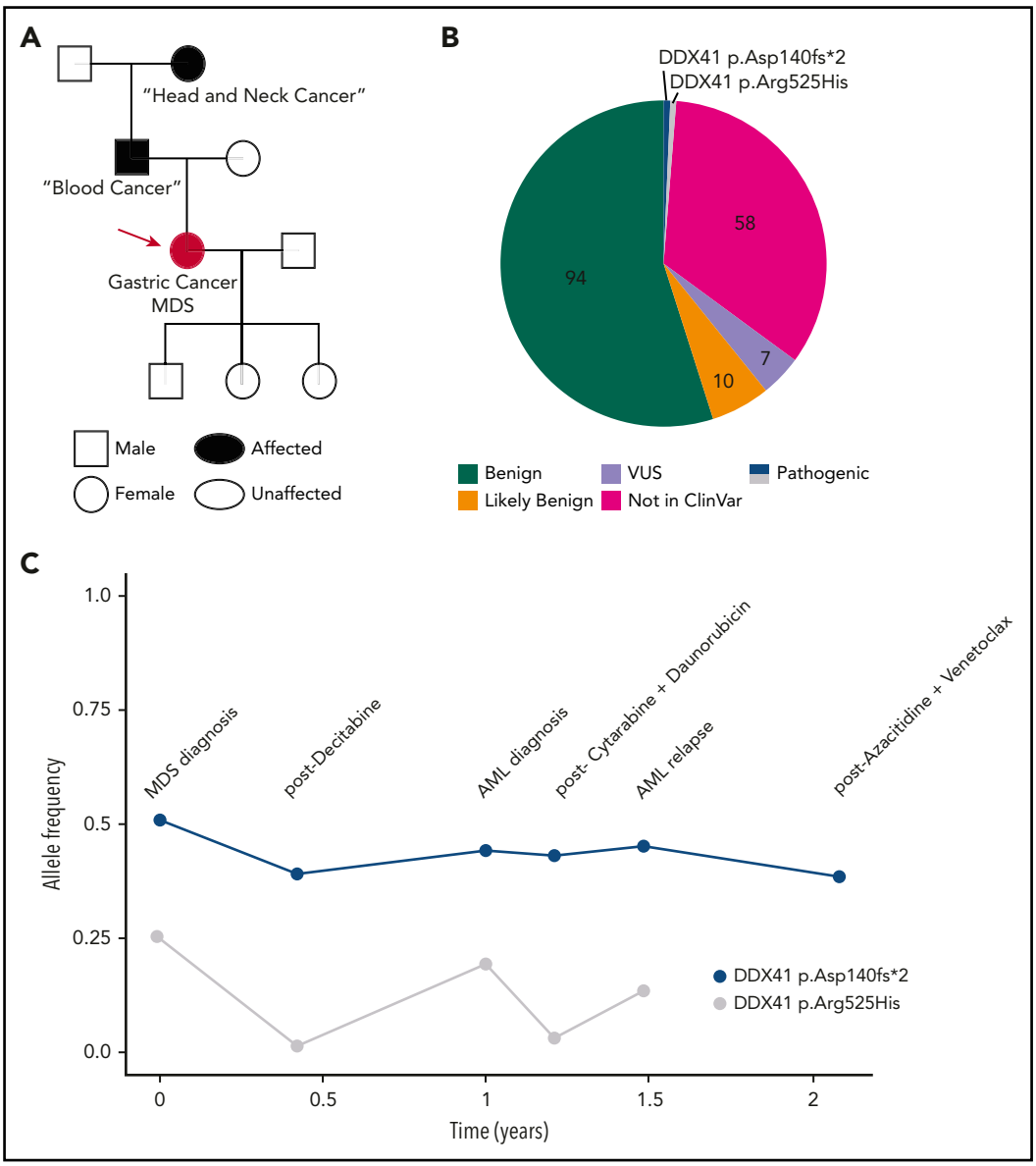

Figure 1. Illustrative case of deleterious *DDX41* variants identified during clinical evaluation of a hematopoietic malignancy. (A) Family history revealed 2 cancers in the patient/proband (red circle), a blood cancer of unclear nature in the father, and a head and neck cancer in the paternal grandmother. (B) Molecular profiling via a 150-gene clinical NGS panel identified 171 total variants. After annotation, filtering for clinical relevance, and individual verification by the in-house pathologist, 7 (4%) were reported as variant of uncertain significance, and the 2 (1.1%) *DDX41* variants were reported as pathogenic on a final document provided to the treatment team. (C) *DDX41* allele VAF is graphed throughout the patient's clinical course for the 2 variants identified. AML, acute myeloid leukemia.

population, and the p.Arg525His variant as a hotspot acquired mutation in myeloid malignancies in those with deleterious germline *DDX41* variants. She referred the patient to a dermatologist for a skin biopsy.

Identifying potential germline alterations

Suspicion for germline predisposition may arise from a family history of bleeding, low blood cell counts/function, and/or a personal/family history of cancers. Examples include young age of diagnosis for a specific malignancy, multiple malignancies in the proband, and/or the presence of a hematopoietic or young-onset (<50 years of age) solid tumor within 2 generations of the proband.[1] An advanced age at cancer diagnosis does not exclude the potential for a germline-predisposition syndrome. For instance, the average age of diagnosis of a myeloid malignancy in patients with germline *DDX41* mutations is ~70 years.[2] Additionally, some inherited predisposition syndromes may be caused by de novo gene mutations (ie, arising during embryogenesis and present in the germline of affected individuals but not the parents), such as *GATA2*, *BRCA1*, and *BRCA2*, among others.[3,4]

Cultured skin fibroblasts or hair bulbs are considered to the best source for germline DNA.[5,6] Skin biopsies may be performed simultaneously with a BM biopsy, when the skin is sterile and anesthetized. Unfortunately, culturing fibroblasts requires several

weeks and may be technically challenging, and hair bulbs may be scarce in oncology patients and do not yield large quantities of DNA, limiting downstream testing. In addition to determining the germline status of a particular allele via sequencing from these DNA sources, an allele identified in ≥2 related individuals defines its germline status. Although tempting, peripheral blood (PB) or a lymph node without evidence of tumor as a comparative "normal" sample may confound germline predictions as a result of clonal hematopoiesis (CH) and other rearrangements. Buccal swabs and fingernails may be contaminated with hematopoietic cells.[6] Unfortunately, even if the proper material is selected, NGS panels for germline variants differ with regard to which genes are included and the types of variants detected.[7] Therefore, identifying a panel that provides comprehensive testing for relevant genes and variant types is essential.

Curation of gene variants is the systematic evidenced-based process by which functional data, population frequency, disease phenotype, familial pedigree, and biologic evidence are integrated to assign clinical significance using the standardized 5-tier system outlined by the American College of Medical Genetics and Genomics and the Association of Molecular Pathology. Tiers include pathogenic, likely pathogenic, variant of uncertain significance, likely benign, or benign.[8,9] Formal germline variant deposition occurs in the open access platform ClinVar (ncbi.nlm.nih.gov/clinvar), with curation mediated by expert panels within ClinGen (clinicalgenome.org). Curation rules for germline *RUNX1* variants are available from the Myeloid Malignancy Variant Curation Expert Panel (clinicalgenome.org/affiliation/50034/) and are being applied to *RUNX1* variants within ClinVar with U.S. Food and Drug Administration recognition.[10,11] The ClinGen curation process undergoes reassessment every 2 years to allow updating based on recent literature and revised ClinGen recommendations.

Identifying potential germline alleles from NGS assays of tumor samples

Molecular profiling of specimens containing tumor cells, including tumor/BM biopsies and PB samples, is performed with increasing frequency. All sample types potentially contain germline and somatic variants, and tumor biopsies may also contain PB or infiltrating leukocytes containing independent acquired CH variants within hematopoietic tissue.[1] DNA aberrations may arise from several distinct etiologies. Somatic alterations

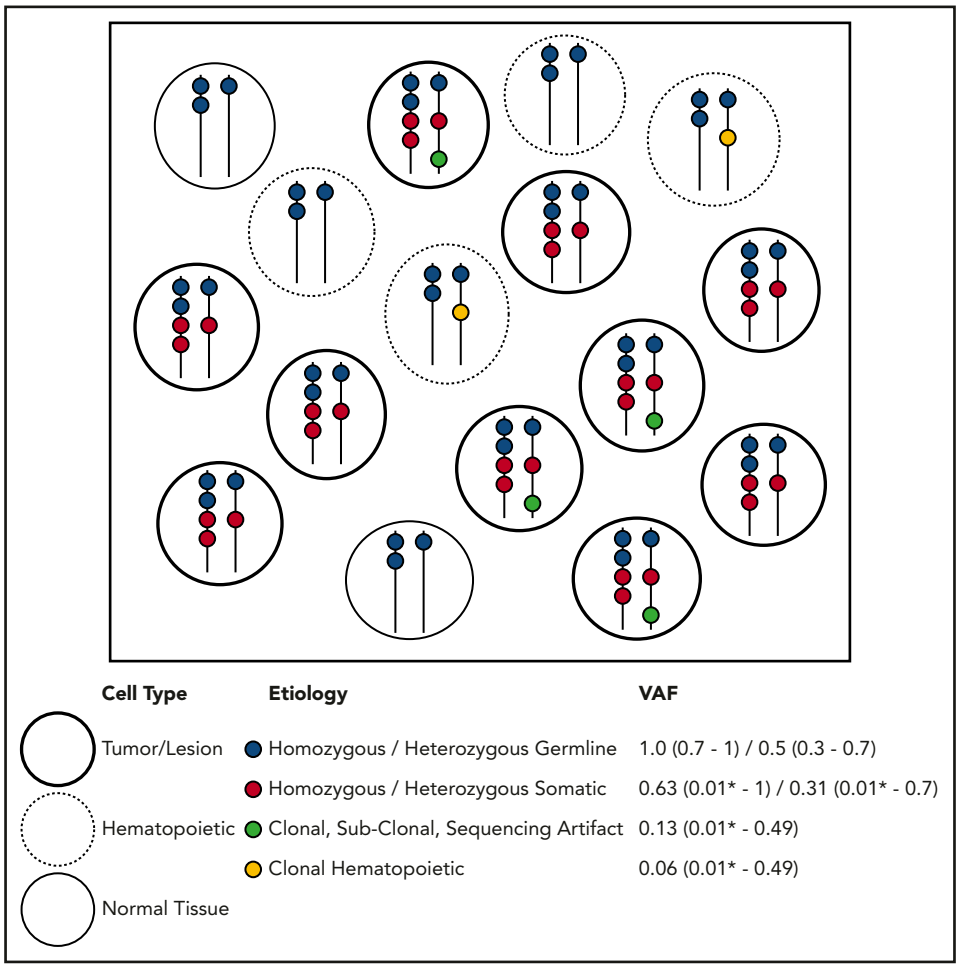

Figure 2. Etiology of DNA alterations in a representative sample of NGS sequencing. The box contains a population of cells (each circle) undergoing bulk NGS. Each color represents a DNA alteration of different etiology. VAF for the representative example is shown with typical ranges observed on clinical NGS panels. This example does not account for CNVs or DNA structural aberrations. *Lower detection limit depends on the depth and platform of NGS.

are unique to the tumor itself, CH alterations are derived from the clonal expansion of hematopoietic stem or progenitor cells, and germline variants are present in all nongerm cells in the body (Figure 2). When a population of cells undergoes bulk NGS, each aberration identified is reported with a single VAF, making it difficult to infer the nature of the variant directly. Ideally, germline variants have VAF ~ 0.5 if heterozygous or VAF ~ 1.0 if homozygous (Figure 2). However, the VAF must be interpreted relative to germline mosaicism, loss of heterozygosity, copy number variations (CNVs) in tumor cells, insertions/deletions, structural rearrangements, and sequencing artifacts, including statistical fluctuation particularly with shallow sequencing depths. Specific NGS tests (eg, exomes, genomes, targeted gene panels, hot spot panels, and CNV tiling), sequencing technologies, and data processing, alignment, and variant calling/annotation methods may affect the variants identified and the VAF calculated. Therefore,

Table 1. Genes recommended as yielding clinically actionable results by the American College of Medical Genetics and Genomics, the National Comprehensive Cancer Network, and the World Health Organization

Disorder/syndrome	Genes
Hereditary breast and ovarian cancer	*BRCA1*, *BRCA2*
Li-Fraumeni syndrome	*TP53*
Peutz-Jeghers syndrome	*STK11*
Lynch syndrome	*MLH1*, *MSH2*, *MSH6*, *PMS2*
FAP	*APC*
MYH-associated polyposis; adenomas, multiple colorectal, FAP type 2; colorectal adenomatous polyposis, autosomal recessive, with pilomatricomas	*MUTYH*
Juvenile polyposis	*BMPR1A*, *SMAD4*
von Hippel–Lindau syndrome	*VHL*
Multiple endocrine neoplasia type 1	*MEN1*
Multiple endocrine neoplasia type 2	*RET*
Familial medullary thyroid cancer	*RET*
PTEN hamartoma tumor syndrome	*PTEN*
Retinoblastoma	*RB1*
Hereditary paraganglioma- pheochromocytoma syndrome	*SDHAF2*, *SDHB*, *SDHC*, *SDHD*
Tuberous sclerosis complex	*TSC1*, *TSC2*
WT1-related Wilms tumor	*WT1*
Neurofibromatosis type 2	*NF2*
Ehlers-Danlos syndrome, vascular type	*COL3A1*
Marfan syndrome, Loeys-Dietz syndrome, and familial thoracic aortic aneurysms and dissections	*ACTA2*, *FBN1*, *MYH11*, *SMAD3*, *TGFBR1*, *TGFBR2*
Hypertrophic cardiomyopathy, dilated cardiomyopathy	*ACTC1*, *GLA*, *LMNA*, *MYBPC3*, *MYH7*, *MYL3*, *PRKAG2*, *TNNI3*, *TNNT2*, *TPM1*, *MYL2*
Catecholaminergic polymorphic ventricular tachycardia	*RYR2*
Arrhythmogenic right ventricular cardiomyopathy	*DSC2*, *DSG2*, *DSP*, *PKP2*, *TMEM43*
Romano-Ward long-QT syndrome types 1, 2, and 3; Brugada syndrome	*KCNH2*, *KCNQ1*, *SCN5A*
Familial hypercholesterolemia	*APOB*, *LDLR*, *PCSK9*
Wilson disease	*ATP7B*
Ornithine transcarbamylase deficiency	*OTC*
Malignant hyperthermia susceptibility	*CACNA1S*, *RYR1*
Familial MDS/AML	*ANKRD26*, *CEBPA*, *DDX41*, *ETV6*, *GATA2*, *MBD4*, *MECOM/EVI1* *PTPN11*, *RUNX1*, *SAMD9*, *SAMD9L*, *SRP72*, *TET2*
Inherited BM failure syndromes with germline predisposition to myeloid neoplasms	*DKC1*, *DNAJC21*, *ELANE*, *EFL1*, *ERCC6L2*, *FANC* genes, *GFI1*, *HAX1*, *NAF1*, *NPM1*, *RAD51C*, *RECQl4*, *RTEL1*, *SBDS*, *SRP72*, *TERT*, *TERC*
Inherited plasma cell disorders	*ARID1A*, *DIS3*, *KDM1A*, *USP45*
Inherited syndromes associated with myeloid neoplasms	*ATG2B/GSKIP*, *BLM*, *BRCA1*, *BRCA2*, *CBL*, *KRAS*, *NF1*, *PTPN11*, *TP53*

AML, acute myeloid leukemia; FAP, familial adenomatous polyposis.
Recommendations were collected from Trottier et al,[1] Arber et al,[34] Kalia et al,[35] and Greenberg et al.[36]

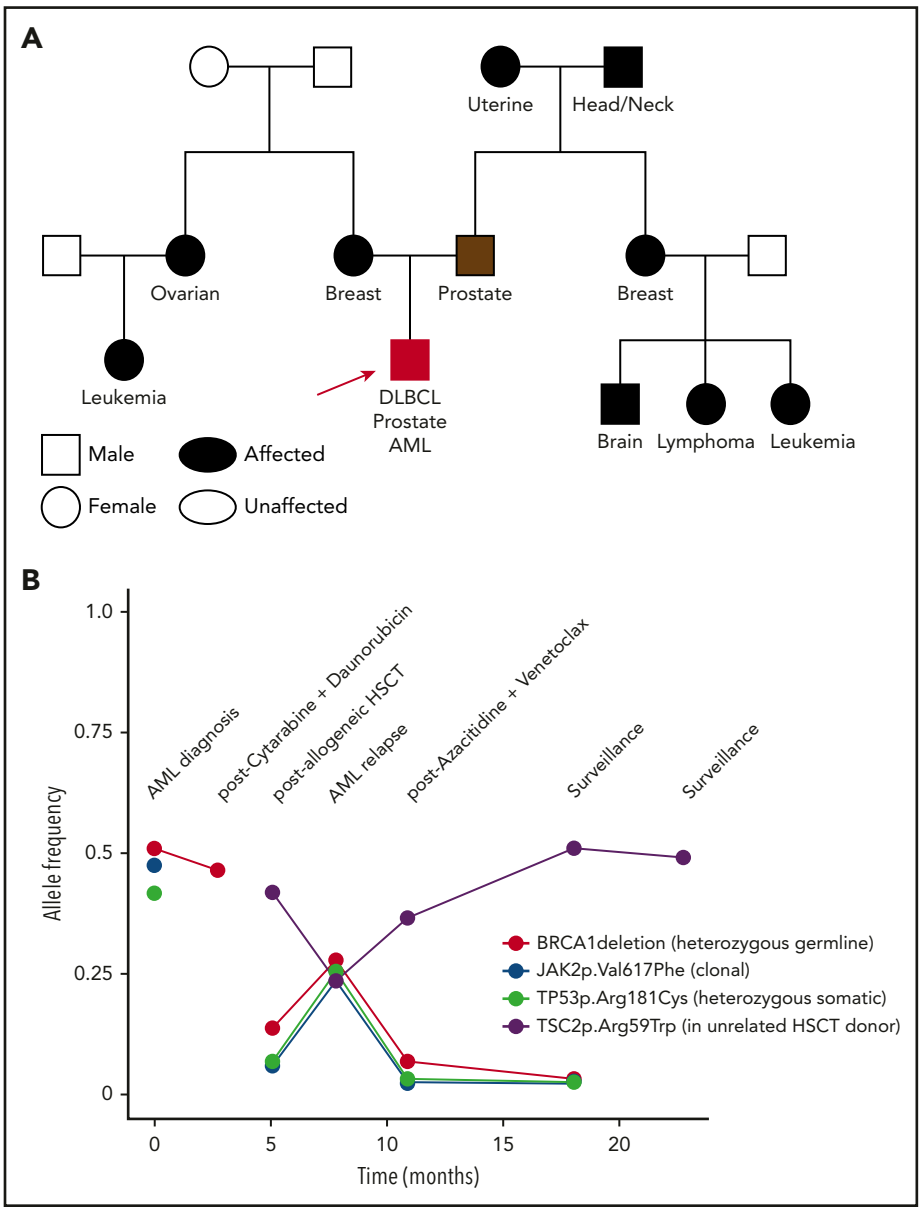

Figure 3. A complex clinical case and VAF of deleterious variants seen over time. A 51-year-old white man had an 8 × 10–cm mass that was determined to be diffuse large B-cell lymphoma (DLBCL). He received 6 cycles of rituximab/cyclophosphamide/doxorubicin/vincristine/prednisone (R-CHOP), 2 cycles of etoposide/methylprednisolone/high-dose cytarabine/cisplatin (ESHAP), and radiation to the mediastinum, ultimately achieving a complete response. At age 57, a screening prostate-specific antigen (PSA) was 12.4 ng/mL. Prostate biopsy showed a 4 + 4 = 8 Gleason score adenocarcinoma, and the patient had a prostatectomy with normalization of his PSA. At age 61 years, he was diagnosed with essential thrombocytosis, with JAK2 p.Val617Phe. He eventually progressed to AML, when a detailed family history was obtained. (A) Family history revealed numerous relatives with cancer: mother, breast cancer (55 years old); father, prostate cancer (69 years old); maternal aunt, ovarian cancer (37 years old); maternal cousin, unknown type of leukemia; paternal grandmother, uterine cancer; paternal grandfather, head and neck cancer; paternal aunt, breast cancer (70 years old); paternal cousin, brain tumor (75 years old); paternal cousin, lymphoma (70 years old); paternal cousin, unknown type of leukemia (12 years old). Molecular profiling at AML diagnosis showed a complex karyotype, including deletions of the long arms of chromosomes 5 and 7. NGS of predominantly leukemia cells from a BM biopsy showed a *TP53* mutation and a deletion within *BRCA1*. The patient underwent induction chemotherapy, and molecular profiling at clinical remission demonstrated persistence of the *BRCA1* deletion and loss of the *TP53* mutation. Germline genetic testing on DNA derived from the patient's cultured skin fibroblasts confirmed a germline *BRCA1* deletion. He underwent an allogeneic HSCT using an unrelated donor, given the potential risk of the familial *BRCA1* deletion, which had been found in an HLA-matched sibling. (B) The VAF of DNA alterations are plotted over time and show persistence of the germline *BRCA1* deletion at a relatively high VAF prior to HSCT; the acquired clonal *JAK2* and *TP53* variants prior to HSCT; and an acquired *TSC2* variant post-HSCT of donor origin. Lessons from this case include: (1) The patient was diagnosed with 3 cancers by the time germline testing was performed: DLBCL, prostate cancer, and AML. Genetic counseling and testing were warranted at the time of his first cancer based on his extensive family cancer history. (2) *BRCA1* and

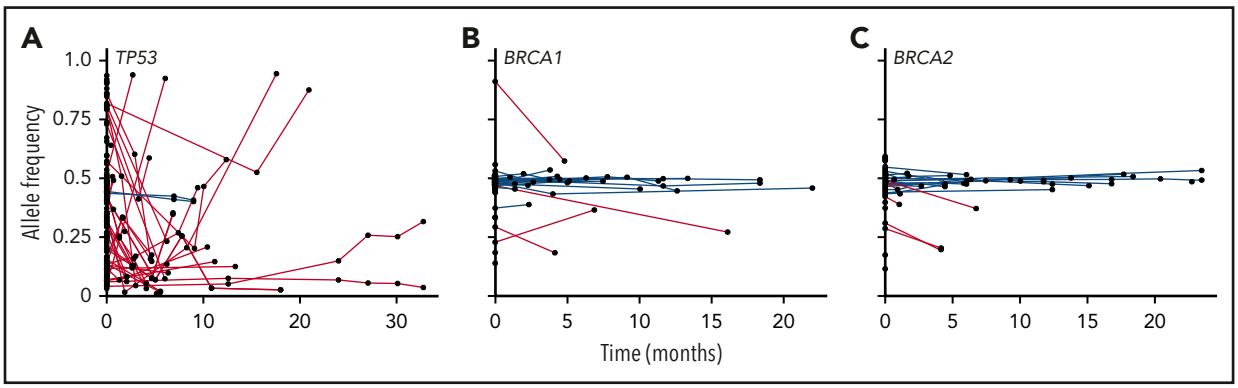

Figure 4. VAFs for *TP53*, *BRCA1*, and *BRCA2* and over time in patients with hematopoietic malignancies. VAFs of DNA alterations in *TP53* (A), *BRCA1* (B), and *BRCA2* (C) in individual patients at the University of Chicago are graphed over time. Each point indicates an individual variant identified in an in-house NGS assay, and red lines connect likely somatic variants; likely germline variants are shown in blue.

careful interpretation of NGS data from clinical samples is needed. A suggested preliminary screening approach includes determining likely germline vs somatic status considering the gene, VAF, purity, and ploidy and then, among the likely somatic alterations, determining whether it is likely derived from tumor or common CH variants from contaminating hematopoietic cells.

If NGS panels include genes that confer germline risk for BM failure or cancer (Table 1), they may provide an opportunity to detect germline variants in patients with hematopoietic malignancies, even when DNA from tumor cells is used.[12] For some genes, detection of particular alleles should raise suspicion of germline origin. For example, some germline *DDX41* variants are commonly observed in certain populations (opening case), and all truncating variants reported to date are germline.[13] For other alleles, such as many *RUNX1* or *TP53* variants, etiology is difficult to surmise, because the same variant can be somatic or germline. Importantly, suspicion of a germline variant should be confirmed by testing true germline DNA.

Many studies examine the frequency of germline variants identified in tumor-based sequencing and provide a conservative updated list of genes in which germline variants drive tumorigenesis (Table 1).[12,14-16] Germline variants are identified on ~7% to 25% of tumor-only panels, but most are not deleterious.[12,14-16] The most frequent genes with germline variants in solid tumors and hematopoietic malignancies include *ATM*, *BRCA1*, *BRCA2*, *CHEK2*, *DDX41*, *GATA2*, *MUTYH*, and *TP53*.[12,14,16]

Some bioinformatic algorithms may suggest germline etiology using single NGS assays.[17,18] However, additional insight is gained by examining tests over time. This is illustrated by a second case that highlights an individual with a personal history of diffuse large B-cell lymphoma, prostate cancer, and acute myeloid leukemia (AML), as well as a strong family history of cancer, who was found to have a *BRCA1* deletion with VAF ~ 50% on multiple NGS assays that ultimately was shown to be germline (Figure 3). The patient's AML had acquired *JAK2* and *TP53* variants, potentially as a consequence of underlying germline predisposition and environmental risk factors.[19] A *TSC2* variant was seen after allogeneic hematopoietic stem cell transplantation (HSCT). As this case highlights, a germline variant typically remains detectable over time with a relatively consistent VAF (Figure 4, blue lines), whereas the VAF of somatic variants is prone to change with disease status (Figure 4, red lines). Some gene mutations, like those in *TP53*, occur most commonly as somatic alleles (Figure 4A, red line). Rarely, individuals who may not have met clinical criteria for Li-Fraumeni syndrome are found to have germline *TP53* mutations by tracking the VAF over time or in multiple assays (Figure 4A, blue lines). In our experience, deleterious *BRCA1/2* variants are more commonly germline alterations (Figure 4B-C). Following VAF over time is an efficient way to identify potential germline variants using data already collected for diagnostic and prognostic purposes. Other conditions that may yield a stable VAF ~ 50% include persistent disease, CH, CNVs, and loss of heterozygosity. It is important to remember that germline variants can be deleterious, benign, or of uncertain significance, so the germline nature of a variant should not be equated with being pathogenic. Figure 5 shows an algorithm for identifying germline variants and guiding when to send comprehensive testing. At the University of Chicago, we compare new NGS test results from PB and BM for all patients with hematopoietic malignancies with previous data obtained from that individual. In a typical month, we review data from ~60 to 85 patients with hematopoietic malignancies who have had multiple NGS tests and identify 1 to 3 (1% to 5%) patients with potentially pathogenic germline variants that were not otherwise detected. This information is conveyed to the patient's primary oncologist who facilitates genetic counseling and, when appropriate, testing to confirm germline status (Figure 5).

Figure 3 (continued) *BRCA2* are Fanconi anemia-like genes,[37] encoding proteins important for DNA repair pathways active in the BM. Individuals with BRCA pathway mutations are at increased risk for the development of hematopoietic malignancies.[38] In fact, cancer predisposition syndromes generally thought of as predisposing to solid tumors also increase the risk for hematopoietic malignancies, such as Lynch and Li-Fraumeni syndromes.[39,40]

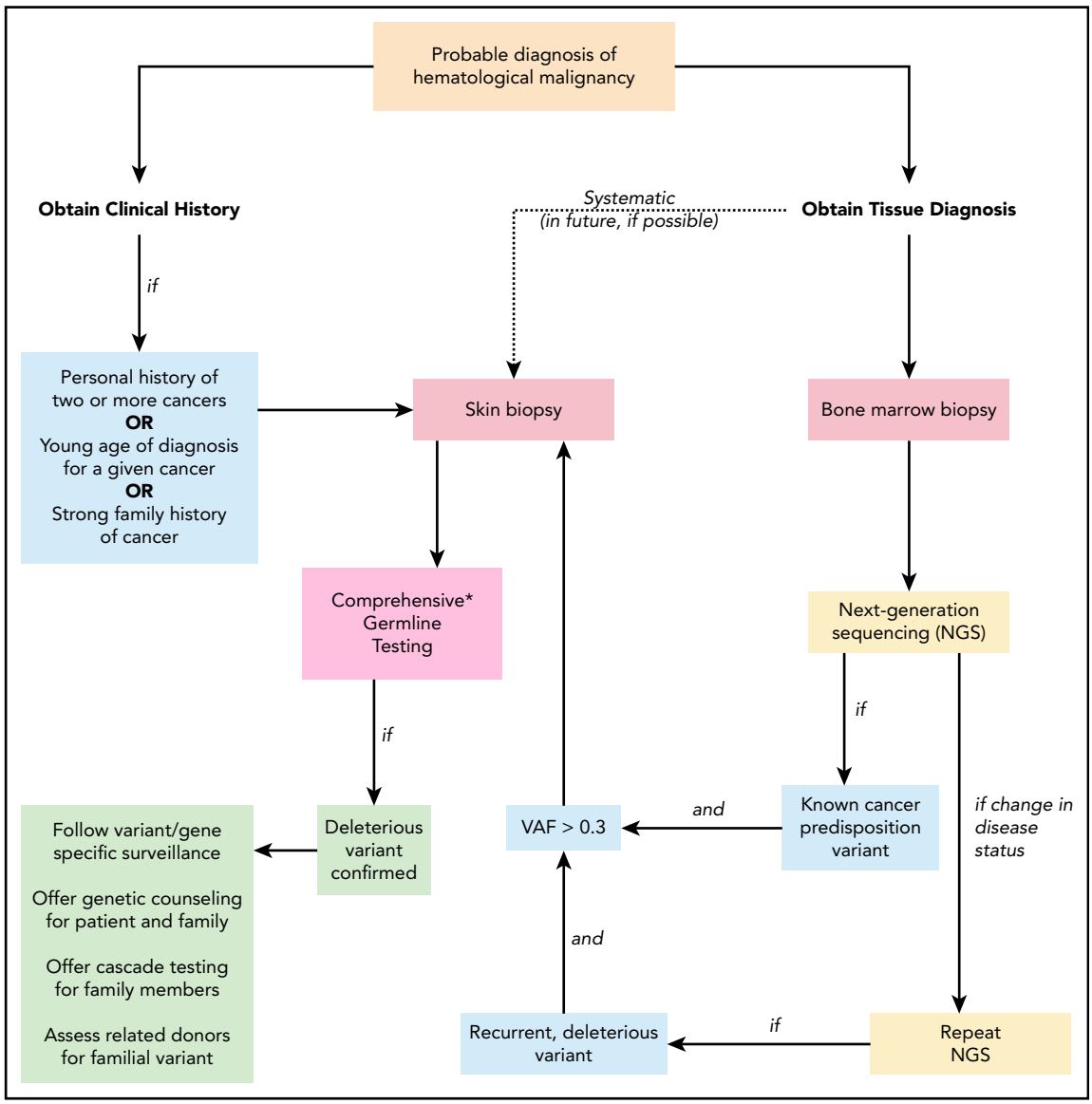

Figure 5. Suggested algorithm for identifying patients with a deleterious germline cancer predisposition variant. When a patient is diagnosed with a hematopoietic malignancy, clinical history and tumor biopsies are performed. Personal history of prior cancer (1 other hematopoietic malignancy or solid tumor, including melanoma in an individual younger than 50 years of age), diagnosis at a younger age than seen in the general population for a given cancer, or a strong family history of cancer (relative diagnosed with cancer within 2 generations of the patient) should prompt a skin biopsy and comprehensive germline testing. If tumor-only NGS identifies a known cancer-predisposition variant and the VAF is > 0.3, germline testing of the variant should follow. As additional NGS tests are performed to monitor the patient's clinical course, persistent deleterious variants with VAF > 0.3 should prompt consideration of germline status. This is especially warranted if the deleterious variant is present in a gene associated with cancer risk. In the future, systematic collection of a skin biopsy at the time of the initial BM biopsy and culturing of fibroblasts to obtain germline DNA may become standard (dotted line). Once a deleterious germline variant is confirmed, variant/gene-specific surveillance should be followed for the patient (including a risk assessment for cancer involving organs outside the BM), genetic counseling and germline testing should be offered to appropriate family members, and potential risks should be considered if the patient were to undergo related HSCT from a family member sharing the allele. NGS, next-generation sequencing; VAF variant allele frequency. *Comprehensive testing that includes all genes and variant types that confer cancer risk is not standardized and requires careful review of testing options.

Testing related HSCT donors

Allogeneic HSCT is often considered for patients with hematopoietic malignancies. Determining whether a germline variant is pathogenic is critical for choosing the donor, because family members are generally preferred.[20] When related donors have been used who were found retrospectively to have deleterious germline variants in *RUNX1* or *CEBPA*, poor outcomes are observed, including poor hematopoietic stem cell mobilization by the donor,[21,22] failure or delay in engraftment,[21,23] poor immune function,[21,23] early relapse,[23] donor-derived leukemias,[22,24,25] and new diagnosis of leukemia in the related donor after stem cell mobilization/collection.[24,25] Thus, using related donors with deleterious germline *RUNX1* or *CEBPA* mutations is not advised, but our knowledge about other genes is limited. As research expands to assess the impact of deleterious germline variants in HSCT donors and recipients, we may learn which are permissive for successful transplantation, which are prudent to avoid, and whether mobilization with granulocyte colony-stimulating factor and other agents confers additional risk. Furthermore, matched unrelated donors within close-knit populations, such as the Ashkenazi Jewish population, may also harbor potential pathogenic germline alleles. In fact, all donors, including cord blood units, may have deleterious germline variants, but this potential hazard is not assessed routinely. Currently, there is a range of criteria in clinical practice, with some transplant centers mandating donor testing and rejecting any donor with a deleterious germline variant in any cancer risk gene, whereas others forgo testing altogether. Overall, more data are needed to determine whether there are deleterious variants permissive for HSCT (as highlighted in the second case).

Patient consent

As the clinical utility of NGS sequencing becomes commonplace, it is important to consider the dynamic nature of the field. The number of actionable variants is increasing, and investigators are enhancing ways to use these data.[26-28] Personalized medicine trials are underway, and researchers are developing inventive new ways to interpret NGS findings, such as monitoring measurable residual disease, tracking clonal expansion to understand tumor evolution, and identifying novel germline cancer-predisposition syndromes.[29-31] Health care providers and patients should understand the limitations, the benefits, and the potential that information may change with advances in NGS and variant interpretation. Some institutions consent to patient understanding that NGS testing may reveal potential germline variants, some give patients the option to receive such data, and other centers forgo consent altogether. Overall, it may be important to discuss the challenges and limitations and to disclose the evolving nature of knowledge with patients as we perform more clinical NGS panels.[32,33]

Returning to the opening case

Testing DNA from cultured skin fibroblasts confirmed the germline status of the DDX41 p.Asp140fs allele, as suspected. The patient shared this information with her three children, who considered cascade testing. Over the next 2 years, the patient progressed to AML and received multiple chemotherapy regimens. Molecular testing revealed the germline variant repeatedly, with the VAF dropping to 0.39 on 1 occasion (Figure 1C), highlighting variability in technical assay performance. The VAF of the p.Arg525His allele varied widely in parallel with the BM blast percentage (Figure 1C).

Key points include:

- The diagnosis of 2 cancers (ie, gastric cancer and MDS in this case) should prompt a skin biopsy at the time of the diagnostic BM biopsy (Figure 5).
- Careful selection of NGS panels is required to ensure comprehensive testing. Importantly, *DDX41* is missing from many commercial NGS platforms.[7] Each assay is unique in design and implementation, resulting in particular technical limitations and variabilities.
- Initial descriptions of cancer-predisposition syndromes may be based on small case numbers, and expansion of the phenotype may occur over time. This case raises the question as to whether some solid tumors may occur more frequently in individuals/families with deleterious germline *DDX41* variants.
- Repeating NGS assays throughout a patient's disease provides a means to identify potential germline variants. Germline status should be considered when a deleterious variant is identified in a known cancer-predisposition gene with a VAF > 0.3, if the variant is usually of germline nature (opening case) or persists over time (Figures 1C, 3B, and 5, and the second case).

Conclusions

NGS panels of PB, BM, or tumor specimens are used to identify therapeutically actionable aberrations, for risk stratification, and to provide insights into disease biology. Interpretation may be challenging because somatic, CH, or germline findings may be present. If systematic germline testing is not available, a family history or multiple cancer diagnoses, early age of diagnosis, the presence of known germline predisposition gene variants, or persistent DNA alterations on sequential assays over time should raise concern for germline predisposition. Repeating clinical NGS panels may be an opportunity to distinguish variant etiology and should be considered throughout a patient's clinical course, although this does not replace comprehensive germline testing. Frequent reevaluation of NGS results may be important as research progresses, and germline testing of related HSCT donors should be considered if a patient harbors a pathogenic germline variant. Outcomes after HSCT using donors with germline variants warrant careful study, and discussions at the time of NGS on tumor cells ideally includes the indication of patient preference for disclosure of possible germline alleles.

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Conflict-of-interest disclosures

L.A.G. receives royalties from UptoDate, Inc. for an article on inherited hematopoietic malignancies. I.L.K. declares no competing financial interests.

Off-label drug use

None disclosed.

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First-generation vs second-generation tyrosine kinase inhibitors: which is best at diagnosis of chronic phase chronic myeloid leukemia?

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In 2020, for the great majority of patients with chronic phase chronic myeloid leukemia (CML), life expectancy is unaffected by a diagnosis of CML because of the unparalleled efficacy of ABL-targeted tyrosine kinase inhibitors (TKIs) in halting disease progression. A wealth of choices exist for first-line treatment selection, including the first-generation TKI imatinib and the second-generation TKIs bosutinib, dasatinib, and nilotinib. How I select first-line therapy between first-generation and second-generation TKIs is discussed in the context of patient-specific CML disease risk, therapy-related risks, and treatment goals. Although rare, identifying patients with CML at higher risk for disease progression or resistance is important and influences first-line TKI selection. I review the impact of first-generation vs second-generation TKI selection on treatment response and outcomes; the ability to achieve, as well as the timing of, treatment-free remission; and the impact of specific TKIs on longer-term health.

LEARNING OBJECTIVES

- Identify disease-specific risk factors at chronic myeloid leukemia diagnosis that influence first-line tyrosine kinase inhibitor (TKI) selection
- Delineate patient comorbidities that impact first-line TKI selection
- Examine how first-line TKI selection impacts treatment-free remission

Introduction

Chronic myeloid leukemia (CML) while in chronic phase (CP) is driven by the constitutively active BCR-ABL tyrosine kinase resulting from the translocation t(9;22)(q34;q11). The ABL-targeted tyrosine kinase inhibitors (TKIs) imatinib, bosutinib, dasatinib, and nilotinib have transformed leukemia with poor overall survival (OS) into a disease in which life expectancy for most individuals is not impacted by CML, and many are now living with a CML diagnosis. I discuss how I select first-line TKI therapy for patients with CP CML, weighing CML risk factors, patient age, medical history, and treatment goals. Treatment-free remission (TFR), as discussed by Dr. Delphine Rea, is among the most important patient-described goals and is a strategy, if successful, that can limit health care costs. However, ~80% of patients remain on TKI therapy in the longer term. How to promote safe management in patients with comorbidities, as discussed by Dr. Jorge Cortes, while aiming for TFR and optimal quality of life through appropriate TKI selection is a discussion between patients and health care providers that begins at diagnosis.

Clinical case 1

Patient 1 is a 47-year-old woman with no significant past medical history who presented with left upper quadrant abdominal pain, 8-pound weight loss, and fatigue. Her only medication is a daily multivitamin. She does not use tobacco or have a history of tobacco use, and she does not use alcohol. Her 10-year atherosclerotic cardiovascular disease (ASCVD) risk score is 0.8% (low; http://tools.acc.org/ASCVD-Risk-Estimator-Plus/#!/calculate/estimate/). Results of her evaluation are listed below:

- Complete blood count: white blood cell count, 183 000/μL; 8% blasts; 9% basophils; 2% eosinophils; platelet count, 520 000/μL
- Her spleen was palpable 9 cm below the costal margin.
- Her peripheral blood *BCR-ABL1* was 110%.
- Bone marrow evaluation: hypercellularity (80%); 1+/3 focal areas of increased reticulin fibrosis; blasts 9%; chromosome banding analysis showing 46,XX, t(9;22)(q34;q11.2)[20]/46; no additional chromosome abnormalities (ACA) noted

- Sokal score, 1.84, high risk (>1.2)
- European Treatment and Outcome Study Long-Term Survival (ELTS) score (https://www.leukemia-net.org/content/leukemias/cml/project_info/index_eng.html), 2.2239, high risk (>2.2185)

Clinical case 2

Patient 2 is 61-year-old woman with a past medical history notable for hypertension and type 2 diabetes mellitus (T2DM) who presented with fatigue to her primary physician. Her medications include metformin and lisinopril. She has no tobacco or history of tobacco use and drinks one glass of wine per week. Her 10-year ASCVD risk score is 15.8% (intermediate). Results of her evaluation are listed below:

- Complete blood count: white blood cell count, 28 900/μL; 1% blasts; 4% basophils; 2% eosinophils; platelet count, 602 000/μL
- Her spleen was palpable 1 cm below the costal margin.
- Her peripheral blood *BCR-ABL1* was 44.9%.
- Bone marrow evaluation: hypercellularity (100%); blasts 1%; chromosome banding analysis showing 46,XX, t(9;22)(q34; q11.2)[19]/46, XX[1]; no ACAs noted
- Sokal score, 0.91, intermediate risk (0.8-1.2)
- ELTS score, 1.2632, low risk (≤1.5680)

How do I identify risky patients at diagnosis?

Ten-year updates of the imatinib first-line registration study IRIS[1] demonstrate excellent longer-term OS: 91.1% vs 85.3% in patients with vs without major molecular response (MMR; *BCR-ABL1*, <0.1%) achieved at 12 months, respectively. Expected treatment responses are defined and reviewed in Tables 1 and 2. Although many patients with CP CML do well, identifying the small group of patients at risk for poor outcomes at diagnosis is highly clinically relevant. These patients require close monitoring to ensure that they achieve treatment milestones and rapid transition to alternative therapies when they do not. Clinical multivariate prognostic risk models (eg, Sokal, Euro/Hasford) remain valuable for risk stratification and identify patients at risk for poorer OS, progression-free survival (PFS), and event-free survival, as well as disease progression. In the IRIS study, in patients with high Sokal risk scores, estimated 10-year OS was inferior at 68.6% vs 80.3% (intermediate risk) and 89.9% (low risk), with similar observations made in the German CML Study IV (Figure 1).[1,2] A newer risk model, the ELTS score, which, like the earlier Sokal score, includes the variables age, spleen size by manual palpation, platelet count, and blast percentage, was specifically derived in imatinib-treated patients to discriminate more accurately the risk for CML-related death.[3] Reflecting good outcomes in older individuals, increasing age has a more limited negative impact on prognosis. Among 1120 out-of-study imatinib-treated patients used for validation, the 5-year cumulative incidence probability of dying of CML was 3% (95% confidence interval [CI], 2% to 5%) in low-risk patients (eg, patient 2), 4% (95% CI, 3% to 7%) in intermediate-risk patients, and 15% (95% CI, 10% to 22%) in high-risk patients (eg, patient 1).[3] Retrospective analyses, including in "real-world" and second-generation TKI-treated patients, have validated the original observations, including in children and young adults.[4,5]

Although this is rare, ~5% of newly diagnosed patients harbor ACAs in Philadelphia (Ph) chromosome–positive cells, which technically also support a diagnosis of accelerated phase (AP) CML. Retrospective analyses from the German CML Study IV originally identified ACAs termed "major route" (trisomy 8, second Ph chromosome, isochromosome [17q], or trisomy 19) that were associated with poorer PFS and OS than in patients without ACAs or those with rarer ACAs (termed "minor route").[6,7] Another study identified only isochromosome (17q), −7/del7q, and 3q26.2 in association with poorer treatment responses and OS.[8] Adding to the debate, a report of 603 patients treated with frontline therapy in various prospective clinical trials found no differences in cumulative complete cytogenetic response or MMR rates, PFS, or OS between patients with and those without ACAs.[9] However, a caveat is that no patients in this study had isochromosome (17q), 3q26.2, or complex aberrant karyotypes associated with poorer outcomes in other series. In keeping with panel recommendations, I consider patients with trisomy 8, second Ph chromosome, trisomy 19, isochromosome (17q), −7/del7q, 11q23, 3q26.2 aberrations, or complex aberrant karyotypes as being at high risk, but I recognize that the first three abnormalities may be less worrisome.[10,11]

The p190 BCR-ABL protein isoform (e1a2 transcript), seen in acute lymphoblastic leukemia, is present in ~1% to 2% of cases and is associated with inferior outcomes.[12] Although recent updates are limited, I consider the p190 isoform to confer high risk. A difference in prognosis initially reported between *BCR-ABL1* transcript types e13a2 and e14a2 (p210 protein isoform) has not been corroborated in more recent reviews.[13,14] For other rare

Table 1. Chronic myeloid leukemia molecular responses defined

Response	Definition
Early molecular response	*BCR-ABL1* ≤10% (PB)
BCR-ABL1 < 1%	Molecular equivalent of CCyR
Major molecular response	*BCR-ABL1* ≤0.1% (PB) (common trial endpoint)
Deeper molecular responses (MR)	*BCR-ABL1* ≤0.01% (MR 4) (PB)
	BCR-ABL1 ≤0.0032% (MR 4.5) (PB)
	Or undetectable *BCR-ABL1* with specific detection of control gene*

CCyR, complete cytogenetic response (no Philadelphia chromosome–positive metaphases by bone marrow examination); PB, peripheral blood.
*Control genes include *ABL*, *BCR*, and *GUSB*. For example, specific control gene detection for MR4 requires a minimum of 10 000 *ABL1* transcripts or 24 000 *GUSB* transcripts, and for MR4.5, it requires a minimum of 32 000 *ABL1* transcripts or 77 000 *GUSB* transcripts.

Table 2. European LeukemiaNet and National Comprehensive Cancer Network recommendations

	ELN optimal	ELN warning	ELN failure
Baseline	NA	High-risk ACA, high-risk ELTS score	NA
3 mo	≤10%	>10%	>10% if confirmed within 1-3 mo
6 mo	≤1%	>1%-10%	>10%
12 mo	≤0.1%	>0.1%-1%	>1%
Any time	≤0.1%	>0.1%-1%, loss of ≤0.1% (MMR)	>1%, resistance mutations, high-risk ACA
	NCCN 3 months	**NCCN 6 months**	**NCCN 12 months**
>10%	NCCN Possible TKI Resistance	NCCN TKI-resistant	NCCN TKI-resistant
>1% - 10%	NCCN TKI-sensitive	NCCN TKI-sensitive	NCCN Possible TKI Resistance
>0.1 - 1%	NCCN TKI-sensitive	NCCN TKI-sensitive	NCCN TKI sensitive*
≤ 0.1%	NCCN TKI-sensitive	NCCN TKI-sensitive	NCCN TKI-sensitive

ELN and NCCN milestone recommendations in 2020 are similar, although how each panel chooses to highlight timing of response and achievement of deeper molecular responses is slightly different. Absence of early molecular response at 6 months is considered failure by ELN recommendations and TKI resistant by NCCN recommendations. NCCN and ELN highlight achievement of <1% *BCR-ABL1*, which is associated with significant progression-free survival benefits. Not achieving this milestone denotes failure or TKI resistance. Both recognize that milestones such as major molecular response (MMR, BCR-ABL1 < 0.1%) must be achieved in patients aiming for treatment-free remission (TFR) and that there is a high likelihood of achieving a subsequent deep molecular response (MR4) for patients achieving MMR at 12 months. *Achievement of BCR-ABL1 >0.1-1% is associated with improved long-term overall survival even if MMR is not achieved. For ELN recommendations a change of treatment may be considered if MMR is not reached by 36 to 48 months, which may facilitate future achievement of TFR. NCCN recommendations also endorse shared decision-making with patients if MMR is not achieved. Assessment for ABL mutations are recommended for ELN warning/failure and NCCN possible TKI resistance/TKI resistance.

ACA, additional chromosome abnormalities in Philadelphia chromosome–positive cells; ELN, European LeukemiaNet; ELTS, European Treatment Outcome Study Long-Term Survival; MMR, major molecular response; NA, not applicable; NCCN, National Comprehensive Cancer Network; TKI, tyrosine kinase inhibitor.

transcript variants, the impact on prognosis is unclear; these are not detected by quantitative polymerase chain reaction assays that measure only e1a2, e13a2, and e14a2 transcripts, which are commonly used at diagnosis, but they can be detected by qualitative *BCR-ABL1* assays.

In other myeloid malignancies, next-generation sequencing (NGS) has identified specific myeloid mutations at diagnosis and examined clonal selection and new mutation development with therapy and disease progression. As recently reviewed, mutations in *RUNX1*, *IKZF1*, and *ASXL1* are rarely detected in CP patients but are more common in AP and blast phase (BP) patients.[15,16] A study of 100, predominantly CP, patients delineated patterns of mutation kinetics and new mutation acquisition.[15] Some did not impact excellent TKI responses; however, treatment failure was common in the rare patients who acquired *TP53*, *KMT2D*, and *TET2* mutations.

What are the benefits of first-line second-generation TKI use?

To date, when looking at all patients, no statistically significant improvement in OS or PFS has been reported for any second-generation TKI, used at recommended first-line doses, as compared with imatinib.[17-19] However, first-line phase 3 randomized registration studies of dasatinib, nilotinib, and bosutinib vs imatinib (DASISION[17], ENESTnd[18], and BFORE[19], respectively) have observed that (1) patients receiving second-generation TKIs achieve more rapid molecular responses; (2) dasatinib- and nilotinib-treated patients develop fewer mutations conferring TKI resistance and achieve responses allowing TFR consideration more rapidly; and (3) although rare, nilotinib-treated patients have fewer progression events to AP or BP. Second-generation TKI use (summarized in Tables 3 and 4 and by risk score in Tables 5 and 6) resulted in higher probability of achieving early molecular response (EMR; *BCR-ABL1*, ≤10%), MMR, and deeper molecular responses (MR4 and MR4.5; *BCR-ABL1*, ≤0.01% and ≤0.0032%, respectively).[17-19] Achieving EMR at 3 or 6 months is associated with improved OS and PFS, a benefit of ~10% to 15%, regardless of TKI. Although fewer imatinib-treated patients achieve EMR at 3 months, data suggest that the absence of EMR at 6 months is the stronger indicator of poor PFS and OS.[20,21] In addition, relevant to TFR, among 1442 evaluable patients treated with imatinib and with imatinib in combination in the German CML Study IV, the cumulative incidences of MR4 and MR4.5 were 68% and 53%, respectively, by 5 years and 81% and 72%, respectively, by 10 years.[11]

A clinically relevant question is whether sequencing a second-generation TKI after imatinib rather than starting a first-line second-generation TKI misses a critical treatment window. Across various studies of imatinib ~25% to 30% of patients have been reported either not to achieve a response or to lose response. For many patients with appropriate monitoring, switching from imatinib to a second-generation TKI for resistance (Table 2) will result in good outcomes.[22-25] In 2020, patients are more likely to switch therapy and to do so earlier in the treatment course, which may be beneficial. However, for some high-risk patients, it may matter. For me, "hitting" high-risk CML "hard" up front with a second-generation

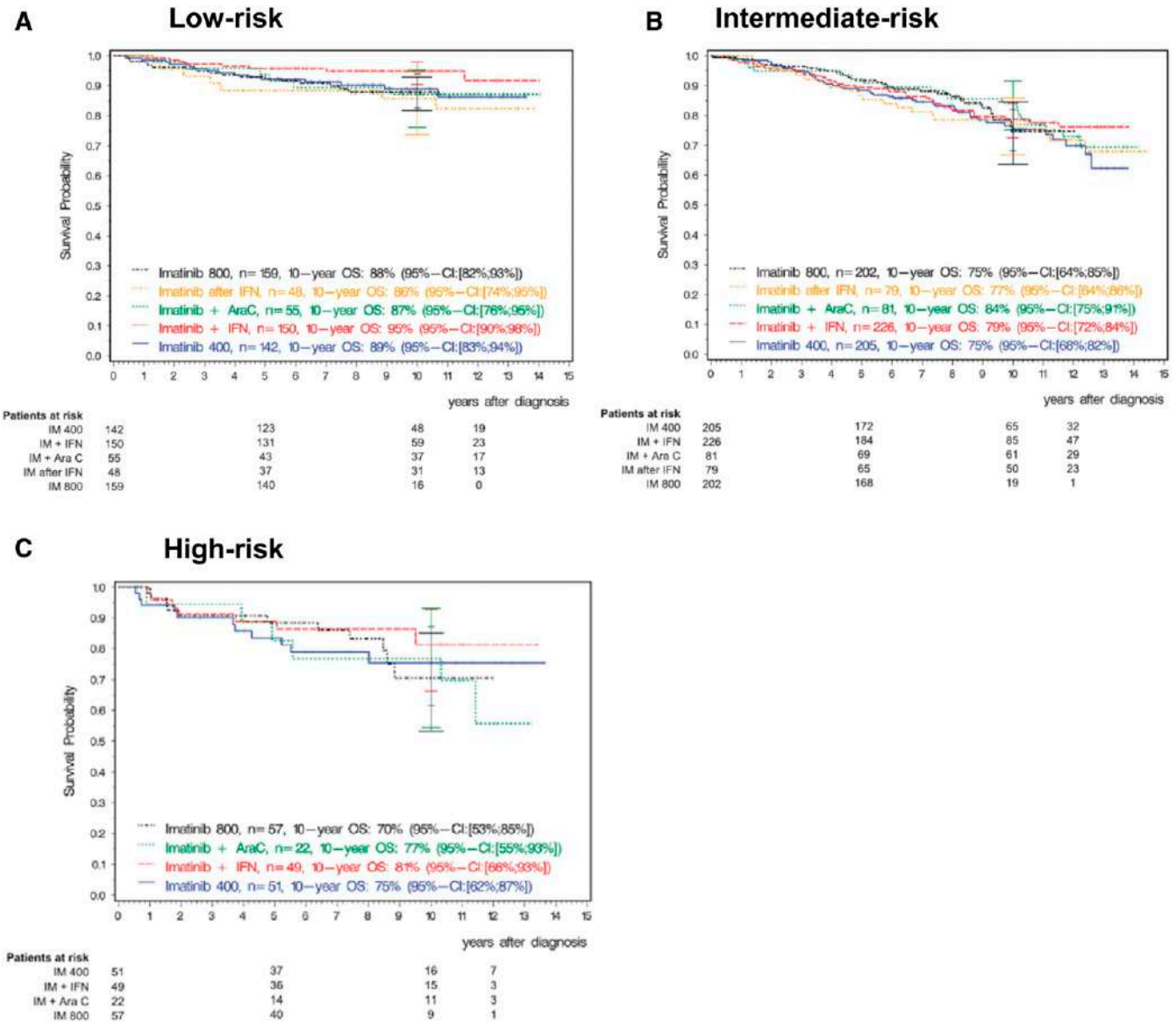

Figure 1. Overall survival by Euro (Hasford) score with 10-year follow-up from the German CML Study IV. A total of 1551 patients with chronic phase CML were treated with imatinib 400 mg daily or 800 mg daily or with imatinib in combination with cytarabine or with interferon-α or with imatinib after interferon-α. Overall survival is shown by Euro risk score. (A) Low risk. (B) Intermediate risk. (C) High risk. Reprinted from Hehlmann et al[2] with permission. AraC, cytarabine; IFN, interferon-α; IM, imatinib; OS, overall survival.

TKI is appealing because the longer the time the patient spends with higher levels of unopposed *BCR-ABL1*, the more likely it is that new ABL-independent genetic and molecular changes will appear.

Case 1

For patient 1, Sokal and ELTS risk scores were calculated as high risk; no other risk features (eg, ACA) were observed at diagnosis. The data supporting second-generation TKI use in high-risk score patients are most clearly delineated for nilotinib.[18] Five-year OS was 88.8% in high-risk Sokal score patients treated with nilotinib at 300 mg twice daily vs 84.2% for imatinib-treated patients (Tables 5 and 6). In ENESTnd, rates of progression to AP or BP while in the study or during follow-up occurred in 10 of 282 patients (3.5%; nilotinib 300 mg twice daily) vs 6 of 281 patients (2.1%; nilotinib 400 mg twice daily) vs 21 of 283 (7.4%; imatinib).[18] With 5-year follow-up for DASISION and looking specifically at deaths, 9 (34.6%) of 26 patients vs 17 (65.4%) of 26 patients in the dasatinib and imatinib treatment arms, respectively, died of CML-related causes.[17] However, because OS does not differ substantially, it is possible that second-generation TKIs contribute to greater drug-related fatal complications, such as cardiovascular complications. For bosutinib, for which follow-up is shorter, no significant difference in progression events has yet been reported.[19]

Patient 1 had no significant comorbidities and was started on nilotinib 300 mg twice daily. EMR was not achieved by 6 months (*BCR-ABL1*, 20%). Three months later, during follow-up, circulating myeloid blasts were detected. No mutations in ABL were identified. Her bone marrow examination revealed 35% myeloid blasts, 14 of 20

Table 3. Molecular response with long-term follow-up

Trial	Study arms	No. of patients	Median follow-up	EMR at 3 mo	CCyR by 12 mo	MMR 12 mo*,†	MMR by 2 y	MR4 by 2 y	MR4.5 by 2 y	MR4 by 5 y	MR 4.5 by 5 y	MR4 by 10 y	MR4.5 by 10 y
IRIS‡	Imatinib (400 mg)	553	11 y	—	69%*	39%*	—	—	—	—	40.2%§	—	63.2%§
	Interferon/ cytarabine	553		—			—	—	—	—	—	—	—
German CML Study IV	Imatinib (400 mg) arm (all)	400 (1551)	9.5 y	68.5%||	67.5%	36.7%	—	—	—	65.7%	49.4%	81%	67.2%
DASISION¶	Dasatinib (100 mg)	259	5 y	84%	83.0%	46.0%	64.0%	—	17.0%	—	42.0%	—	—
	Imatinib (400 mg)	260		64%	72.0%	28.0%	46.0%	—	8.0%	—	33.0%	—	—
ENESTnd#	Nilotinib 300 mg twice daily	282	5 y	91%	80.0%	44%†	71.0%	39.0%	25.0%	65.6%	53.5%	73%	64%
	Nilotinib 400 mg twice daily	281		89%	78.0%	43%†	67.0%	33.0%	19.0%	63.0%	52.3%	—	—
	Imatinib (400 mg)	283		67%	65.0%	22%†	44.0%	18.0%	9.0%	41.7%	31.4%	56%	44%
BFORE#,**	Bosutinib (400 mg)	268	2 y	75%	77.2%	47.2%†	61.2%	32.8%	13.1%	—	—	—	—
	Imatinib (400 mg)	268		57%	66.4%	36.9%†	50.7%	25.7%	10.8%	—	—	—	—

Data from 4 first-line phase 3 randomized registration studies (IRIS, DASISION, ENESTnd, and BFORE) and the first-line imatinib 400 mg daily arm of the German CML Study IV are shown. MRs at various time points are shown. These trials cannot be directly compared, because different methods of trial evaluation were used (eg, rates at a specific time point vs cumulative incidence estimates).
CCyR, complete cytogenetic response (no Philadelphia chromosome–positive metaphases by bone marrow examination); EMR, early molecular response; MMR, major molecular response; MR, molecular response.
*Estimated rate.
†Rate at 12 months (ie, not cumulative).
‡The primary endpoint for IRIS was event-free survival (survival without transformation to accelerated phase/blast phase, loss of complete hematologic response, loss of major cytogenetic response, or increased white blood cell count); survival outcomes include 363 patients who crossed over to imatinib.
§Rate at the specific time point (eg, at 5 years and at 10 years).
||Includes all patients in all arms.
¶The primary endpoint for the DASISION study was confirmed complete cytogenetic response by 12 months.
#The primary endpoint of the ENESTnd and BFORE studies was MMR rate at 12 months.
**Twenty-four-month BFORE trial updates have been presented in abstract format.[41]

cells were Ph+, and 13 of these cells had evidence of new monosomy 7. NGS revealed a mutation in the runt homology domain of *RUNX1* (p.Arg107Cys; not germline). Although NGS assessment is increasingly available at some centers, there are no formal recommendations to select treatment on the basis of detection at diagnosis; however, emerging data may support mutations in *RUNX1* are worrisome. Patient 1 received induction chemotherapy with ponatinib followed by a matched related donor allogeneic hematopoietic cell transplant. This is not a typical case but highlights a rare but very risky group of patients with no EMR receiving first-line second-generation TKIs who may benefit from an early switch to the powerful third-generation TKI ponatinib and early consideration for allogeneic hematopoietic cell transplant.

What are the risks of second-generation TKI use?

Each TKI has unique toxicities that, in combination with patient-specific medical history, inform selection. Despite early warnings regarding reduced cardiac ejection fraction for imatinib, no significant irreversible toxicities have been identified, and consequently its long-term safety profile is excellent. Early data support that responses to generic imatinib after imatinib mesylate (Gleevec) are stable or improving.[26] Fewer data are available for patients starting generic imatinib as first-line treatment; however, it is reasonable to expect that generic drugs meeting standards of production quality and bioavailability will have similar efficacy, although potentially different side effects. For nilotinib, the most significant and potentially irreversible complications are cerebrovascular, cardiovascular, and peripheral arterial occlusive disease.[27-30] Across retrospective studies, events have occurred in 10% to 20% of nilotinib-treated patients. A recent update to ENESTnd with ≥10 years of follow-up reported that among non–CML-related deaths (90% of all deaths), 7 (19%) of 37 patients died of cardiac or vascular disorders, and events continued over time.[30] I avoid use of nilotinib in patients with diabetes mellitus, cardiovascular disease, metabolic syndrome, or hyperlipidemia. Unique dasatinib risks include pleural effusion (a risk that increases

Table 4. Disease progression, progression-free survival, and overall survival with long-term follow-up

Trial	Study arms	No. of patients	Median follow-up	Disease progression, n (%)	PFS	OS
IRIS*	Imatinib (400 mg)	553	11 y	38 (6.9%)	92.1%	83.3%
	Interferon/cytarabine	553		71 (12.8%)	—	78.8%
German CML Study IV	Imatinib (400 mg) arm (all)	400 (1551)	9.5 y	17 (4.2%)	80.0%	80.0%
DASISION	Dasatinib (100 mg)	259	5 y	12 (5%)	85.0%	91.0%
	Imatinib (400 mg)	260		19 (7%)	86.0%	90.0%
ENESTnd†	Nilotinib 300 mg twice daily	282	5 y	10 (4%)	92.0%	94.0%
	Nilotinib 400 mg twice daily	281		6 (2%)	96.0%	96.0%
	Imatinib (400 mg)	283		21 (7%)	91.0%	92.0%
BFORE‡	Bosutinib (400 mg)	268	2 y	6 (2%)	—	99.2%
	Imatinib (400 mg)	268		7 (3%)	—	97.0%

Data from 4 first-line phase 3 randomized registration studies (IRIS, DASISION, ENESTnd, and BFORE) and the first-line imatinib 400 mg daily arm of the German CML Study IV are shown. PFS, OS, and disease progression (defined as progression to accelerated phase or blast phase) are shown. OS, overall survival; PFS, progression-free survival. Adapted with permission from the NCCN Guidelines® for Chronic Myeloid Leukemia V.1.2021. © 2021 National Comprehensive Cancer Network, Inc. All rights reserved. The NCCN Guidelines and illustrations herein may not be reproduced in any form for any purpose without the express written permission of the NCCN. NCCN makes no warranties of any kind whatsoever regarding their content, use or application and disclaims any responsibility for their application or use in any way.
*Survival outcomes include 363 patients who crossed over to imatinib.
†Progression to accelerated phase/blast phase during the study.
‡Twenty-four-month BFORE trial updates have been presented in abstract format.[41]

with age); mild platelet dysfunction that can result in bleeding; and, more rarely, pericardial effusion and pulmonary arterial hypertension (PAH). A recent retrospective review of a pooled population of 11 trials, DASISION, and 034/dose optimization studies (N = 2,712) demonstrated that the annual risk of pleural effusion is ~5% to 15% and was 28% at 5 years for the first-line DASISION study.[31] Diarrhea is the most common and annoying bosutinib-related toxicity, reported in ~70% of patients, and bosutinib use can be problematic in patients with irritable bowel syndrome.[19] However, grade 3 diarrhea is rarer (7.8%), and symptoms will often improve over time and respond to dose adjustments.[32] Drug-induced liver injury has been reported with bosutinib but is rare, and risks for pleural effusion and cardiovascular events are low.

Case 2

Sokal score stratified patient 2 as being at intermediate risk, and her ELTS score classified her as low risk. Because of hypertension and T2DM, nilotinib was a less than ideal choice, and she started dasatinib at 100 mg daily. Her *BCR-ABL1* at 3 months was 1.8% and by 15 months was undetectable and remained undetectable. Approximately 34 months after starting dasatinib, she presented with shortness of breath and cough. Her chest x-ray revealed a large right pleural effusion. The pleural fluid was exudative. Her echocardiogram demonstrated a normal ejection fraction (61%) and no wall motion abnormalities; however, a small circumferential pericardial effusion was noted, and her pulmonary artery systolic pressures (PASPs) were severely elevated at 76 to 81 mm Hg. The findings of right heart

Table 5. Molecular response rates by risk score

Trial	Study arms	Low risk		Intermediate risk		High risk	
		MMR	MR4.5	MMR	MR4.5	MMR	MR4.5
DASISION	Dasatinib (100 mg)	90%	55%	71%	43%	67%	31%
	Imatinib (400 mg)	69%	44%	65%	28%	54%	30%
ENESTnd	Nilotinib 300 mg twice daily	—	53%	—	60%	—	45%
	Nilotinib 400 mg twice daily	—	62%	—	50%	—	42%
	Imatinib (400 mg)	—	38%	—	33%	—	23%
BFORE	Bosutinib (400 mg)	58%	—	45%	—	34%	—
	Imatinib (400 mg)	46%	—	39%	—	17%	—

The MMR and MR4.5 rates of these trials cannot be directly compared, because different methods of trial evaluation and different time points were presented in published data. For DASISION, MMR and MR4.5 are reported at any time with 5-year follow-up by Hasford (Euro) score. For ENESTnd, MMR and MR4.5 are rates are reported by 5-year by Sokal score. For BFORE, MMR rates are reported at 12 months by Sokal score.
MMR, major molecular response; MR, molecular response.

Table 6. Outcomes by risk score

ENESTnd study arms	Low risk			Intermediate risk			High risk		
	Disease progression, n (%)	PFS	OS	Disease progression, n (%)	PFS	OS	Disease progression, n (%)	PFS	OS
Nilotinib 300 mg twice daily	1 (1%)	96.0%	97.0%	2 (2%)	92.9%	93.8%	7 (9%)	86.2%	88.8%
Nilotinib 400 mg twice daily	1 (1%)	99.0%	99.0%	1 (1%)	96.9%	96.9%	4 (5.1%)	90.0%	91.5%
Imatinib (400 mg)	0	100.0%	100.0%	10 (9.9%)	87.9%	88.5%	11 (14.1%)	82.6%	84.2%

Estimated 5-year PFS and OS and progression to accelerated phase or blast phase on study for ENESTnd are shown. The ENESTnd data show that disease progression occurs more frequently in high-risk patients and that for nilotinib-treated intermediate- and high-risk patients, the risk of progression was lower with nilotinib than with imatinib.

OS, overall survival; PFS, progression-free survival.

catheterization correlated with the echocardiogram findings. Dasatinib was stopped. After 3 years, her PASP returned to nearly normal at 38 mm Hg, an observation in keeping with a retrospective review of PAH cases in which improved or normalized PASPs were observed in 34 (94%) of 36 patients after dasatinib cessation.[33,34] Patient 2 remains in TFR (4 years). Her excellent *BCR-ABL1* response allowed early TFR, but she developed significant dasatinib-associated complications requiring years to improve. I avoid use of dasatinib in patients with pulmonary or pericardial disease and use a lower starting dose (50 mg daily) in older patients on the basis of a recent report.[35] However, whether effusion or PAH risks are lower requires longer follow-up. The topic of lower first-line doses and dose de-escalation after MMR or deeper responses is an important area with emerging data.[36,37] Relevant to patient 2 with T2DM and an ASCVD risk score of 15.8%, recent reports suggest that cardiovascular risk is also higher with dasatinib.[27] Whether intermediate-risk patients should receive first-line second-generation TKIs is an area of debate. On the basis of age and comorbidities, I favor imatinib in patients such as patient 2. Last, her response was in keeping with the ELTS low-risk prediction. Although it has not been tested prospectively, I also calculate ELTS score at diagnosis to guide first-line TKI decision making.

How I choose between first- and second-generation TKIs as first-line therapy

Life expectancy not impacted by CML is the overarching goal, and my general approach is shown in Figure 2. This approach is in no way definitive, and individual patient goals and preferences matter. I use second-generation TKIs whenever feasible for patients at higher risk for treatment failure (eg, high clinical risk scores, high-risk ACA, and p190), although an OS benefit has not clearly been established. Response monitoring, particularly for EMR achievement, is critical. New molecular tools for risk stratification at diagnosis, including somatic mutation panels and a recently reported 17-gene expression signature associated with absence of EMR and poorer event-free survival and OS, are becoming available[38] and may help clarify who benefits from second-generation TKIs as first-line treatment. For younger patients, particularly women who have not embarked on family planning, second-generation TKI use with the goal of TFR as rapidly as possible is reasonable, and I typically select second-generation TKIs, which may also be tolerated better. Because cardiovascular complications are age related, for older patients (aged >50) without comorbidities, the discussion is more nuanced and includes ASCVD risk calculation. However, it is difficult to predict who will experience events. As patients age, my enthusiasm for first-line second-generation TKIs declines, and a switch, if needed, due to resistance or intolerance is my typical approach, particularly for low-risk patients. In addition, I am cautious when using second-generation TKIs in patients with specific comorbidities (Figure 2). Aiming for TFR is an expressed goal of almost all my patients, although those in successful TFR are still a minority, and safety with long-term drug use matters. Imatinib-treated patients achieve deeper molecular responses, as indicated by IRIS and German CML Study IV updates, although the timelines are longer. Although careful discussion of first-line therapy selection is appropriate, for some, choices are either not available or not affordable. TKI costs are a significant burden on patients and health care systems. Although significantly less expensive in some countries, the cost of generic imatinib has been high in the United States. In a recent Kaiser Family Foundation report of older patients enrolled in Medicare part D who are not eligible for low-income subsidies, median out-of-pocket costs in 2019 for generic imatinib were above the catastrophic threshold (by $3883).[39] The cost of second-generation TKIs is even higher. Although generic drug prices are declining and the possibility of generic dasatinib is on the horizon, a recent cost-effectiveness analysis simulating 10 years of CML treatment identified that an imatinib-first approach was the most cost-effective one, even when TFR was considered.[40]

Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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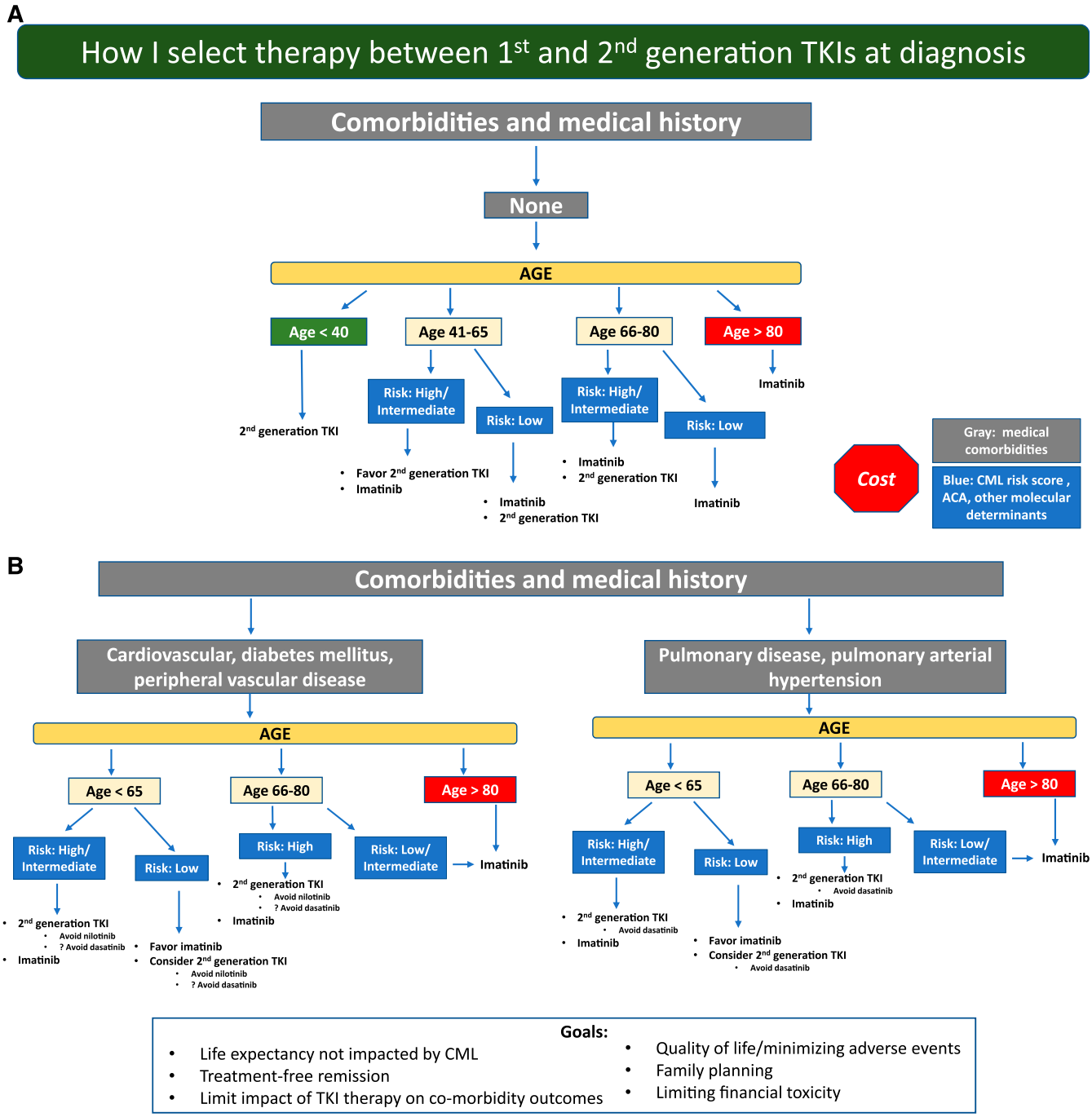

Figure 2. A general overview of my approach is shown. A first step is to assess patient medical comorbidities because this influences the risks and benefits of imatinib vs specific second-generation tyrosine kinase inhibitors (TKIs). (A) Patients without comorbidities. (B) Patients with particular comorbidities. Because comorbidities, in particular cardiovascular comorbidities, increase with age, selecting a second-generation TKI in older individuals with or without comorbidities requires a careful discussion and monitoring. Patient goals and preferences are also important and influence decision making.

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How to manage CML patients with comorbidities

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Patients with chronic myeloid leukemia (CML) often have comorbidities, at an incidence that might be higher than in the general population. Because of the favorable outcome of most patients with CML treated with tyrosine kinase inhibitors (TKIs), a greater number of comorbidities might be the most significant adverse feature for long-term survival. The presence of comorbidities may also affect the risk of developing adverse events with TKIs. This effect is perhaps best exemplified by the risk of developing arterio-occlusive events, which is greatest for patients who have other risk factors for such events, with the risk increasing with higher numbers of comorbidities. The coexistence of comorbidities in patients with CML not only may affect TKI selection but also demands close monitoring of the overall health condition of the patient to optimize safety and provide the opportunity for an optimal outcome to such patients. With optimal, holistic management of leukemia and all other conditions afflicting them, patients with CML and comorbidities may aim for a near-normal life expectancy, just as the more select patients enrolled in clinical trials now enjoy.

LEARNING OBJECTIVES

- Discuss the impact of the presence of comorbidities with CML on patients' long-term outcomes and the selection of TKIs
- Review the management of patients with CML and comorbidities and suggest recommendations to address the most common needs

The clinical benefits of treatment with tyrosine kinase inhibitors (TKIs) for patients with chronic myeloid leukemia (CML) are unquestionable. The ability for some patients to achieve deep molecular responses, to prevent transformation, to return to near-normal life expectancy, and more recently to discontinue therapy successfully are well documented. There are currently 5 TKIs approved for CML, although approval and availability vary widely worldwide. TKIs are generally considered safe, albeit with some variability in their association with the most commonly observed adverse events (AEs). Still, most patients experience some AEs, most frequently mild and often transient, but serious AEs may occasionally be observed.

Our understanding of the general benefit of TKIs comes largely from clinical trials. A common feature of clinical trials is the patient selection, with multiple inclusion and exclusion criteria. This is done for safety purposes, particularly important in the early stages of the development of a drug. But exclusions can often be extensive. For example, the clinical trials for frontline therapy with dasatinib (DASISION)[1] or nilotinib (ENESTnd)[2] compared with imatinib had 18 and 21 exclusion criteria, respectively (some of them listing several subcategories within 1 main category). Because they are excluded from clinical trials, information about how to manage patients with some comorbidities or recent events (eg, cardiovascular or cancer), or whose who are receiving many of the concomitant medications used to manage such conditions, is scant and available only from small series or anecdotal reports.

Patients with CML often have comorbidities, many of which have made them ineligible for clinical trials. One report of patients with CML showed that, at baseline, cardiovascular risk factors included obesity and hypertension in ~30% of patients, diabetes in 11%, and dyslipidemias in 18%. This finding led to a Framingham score of 12.8% (compared with 8.7% average for the US population), and 17% had a history of coronary heart disease.[3] Others have reported similar data,[4,5] with the incidence of comorbidities and number of concomitant medications increasing with age.[6] For example, hypertension was reported for 62% of patients age >75 years. Comorbidities not only increase the risk of developing AEs such as arterio-occlusive events (AOEs)[7] but are now the main cause of death for patients with CML treated with TKIs.[8] In a study of 1,519 patients participating in CML Study IV, 40% had comorbidities, with 61% having a Charlson Comorbidity Index (CCI) of ≥3 (22% had a score of ≥5). The 8-year

survival for patients with a CCI ≥5 was 48%, compared with 91% for those with a CCI of 2.[8] Thus, the presence of comorbidities is an important element of the management of patients with CML.

The patient

Our patient is a 59-year-old construction worker with an 8.5 pack-year smoking history, obesity, and hypertension treated with lisinopril but still poorly controlled. He has been found to have hyperglycemia on several visits to his primary doctor but has never been told he has diabetes or started on specific therapy. His total cholesterol level is 232 mg/dL (5.99 mmol/L). He is now diagnosed with chronic phase CML with a high Sokal risk score. This is not an uncommon scenario, a patient with various comorbidities, often undiagnosed, uncontrolled, or unmanaged. First, we need to discuss thoroughly with the patient the diagnosis, the treatment options, the goals of therapy, and the risks and benefits of each treatment option.

Cardiovascular risk and comorbidities

There are 2 elements to treating a patient with several comorbidities: Deciding what the best TKI option is (Table 1) and managing the comorbidities. Perhaps the greatest risk for this patient is the development of AOEs. Since the first reports of AOEs with ponatinib, it has become evident that this is a risk shared by most TKIs, although at different levels of risk. It is difficult to precisely quantitate the AOE risk with each TKI because the available descriptions from different TKIs report AOEs in various forms, some with a precise inclusion of only specific events, and others with a wider search for various Medical Dictionary for Regulatory Activities (MedDRA) terms that could possibly reflect an AOE. The latter approach documents more events but may include false positives. There is no direct comparison between the second-generation tyrosine kinase inhibitors (2GTKIs), but the randomized trials of dasatinib, nilotinib, and bosutinib versus imatinib allow us to analyze the relative incidence using imatinib as a "control." The risk of cardiovascular events is significantly higher with 2GTKIs (at least dasatinib and nilotinib) than with imatinib, with a 5-year risk of cardiovascular ischemic events 2 times higher with dasatinib and nilotinib (both reporting 4%) than with imatinib (2% in both studies)[9,10] (Table 2). One analysis that adjusted for the variability in reporting by using the same method to identify these events in separate trials with various TKIs suggests a similar trend (risk 1.5–2 times higher with nilotinib or dasatinib than with imatinib),[7] and a meta-analysis of published literature also suggests a higher risk with all TKIs than with imatinib (statistically significant for all except bosutinib, albeit with smaller sample size and shorter follow-up).[11] In the Swedish registry the incidence of myocardial infarction for patients treated with nilotinib or dasatinib is 3.6 and 2.4 times higher than for those treated with imatinib.[12] Thus, imatinib seems to confer the lowest risk, although patients with CML overall have a risk of arterial or vascular events 1.7 times higher than in the general population.[12] Patients with high-risk features for AOEs could be treated preferentially with imatinib. However, this suggestion should be weighted with the patient's risk and goals. Patients with higher Sokal risk scores have a poor response to imatinib, and patients interested in eventually considering treatment discontinuation have a higher probability of achieving that goal with a 2GTKI. In this case, a 2GTKI, possibly bosutinib, might be preferable.

It is important to underscore the role comorbidities play in the risk of AOEs. With ponatinib, the risk of developing AOEs was 27% for patients with history of hypertension (53% of all patients) and 12% for those without hypertension (ie, relative risk 2.1 for patients with hypertension). Similarly, patients with history of heart disease, diabetes, hypercholesterolemia, or obesity had a higher risk of developing such events.[13] The more risk factors a patient has, the greater the risk.[7,13] Among patients receiving frontline therapy with a TKI, patients with 1 risk factor had an incidence rate ratio for AOEs of 1.7 compared with those without known risk factors. This ratio increased to 2.31 with 2 risk factors and to 3.08 with ≥3.[7] In ENESTnd, the risk of developing any AOE correlated strongly with the Framingham risk category, with AOEs reported in 1.7%, 12.2%, and 17.5% of patients treated with nilotinib in the low-, intermediate-, and high-risk Framingham categories.[10] The Systematic Coronary Risk Evaluation was predictive of the risk of AOEs for patients treated with ponatinib.[14] Therefore, in a patient like ours it is important to aggressively control all risk factors. This includes properly managing underlying conditions such as hypertension and making the lifestyle changes necessary to decrease risk (eg, stop smoking, lose weight, exercise). Aspirin is sometimes recommended in this setting, but its benefit has not been studied prospectively, and it could be questioned considering that ponatinib,[15] like dasatinib,[16] may inhibit platelet aggregation. An intriguing mechanism by which ponatinib may contribute to these events is through vascular toxicity mediated by von Willebrand factor–mediated platelet adhesion. This effect would not be expected to respond to antiplatelet therapies, but it opens the even more intriguing prospect of interventions such as use of N-acetyl cysteine to help ameliorate this effect (as it did in preclinical models).[17] This prospect remains hypothetical and awaits clinical testing. Guidelines have been

Table 1. Suggestions for TKI selection for frontline therapy based on selected comorbidities

Comorbidity	Preferred	Less preferred
Diabetes	Imatinib, dasatinib, bosutinib	Nilotinib
Pulmonary disease/pulmonary arterial hypertension	Imatinib, bosutinib, nilotinib	Dasatinib
Gastrointestinal issues	Nilotinib, dasatinib	Imatinib, bosutinib
Cardiovascular	Imatinib, bosutinib	Nilotinib, dasatinib
Peripheral arterial	Imatinib, bosutinib (dasatinib?)	Nilotinib
Liver	Imatinib, dasatinib (nilotinib?)	Bosutinib
Renal	Nilotinib, dasatinib	Imatinib, bosutinib

Table 2. AOEs reported in frontline randomized trials with ≥4 y follow-up

	DASISION[9]*				ENESTnd[10]†				BELA[40]‡			
	Dasatinib		Imatinib		Nilotinib		Imatinib		Bosutinib		Imatinib	
	Any grade	Grade 3–5	Any grade	Grade 3–5	Any grade	Grade 3–5	Any grade	Grade 3–5	Any grade	Grade 3–5	Any grade	Grade 3–5
Any ischemic event	4.65	3.49	2.32	1.55	7.8	4.6	2.1	1.8	4.84	1.21	3.59	1.59
Cardiovascular	3.88	2.71	1.55	1.16	3.9	2.2	1.8	1.4	2.42	0.81	1.99	0.40
Cerebrovascular	0.78	0.78	0	0	1.4	1.1	0.4	0.4	0.81	0.40	0.80	0.80
Peripheral arterial disease	0	0	0.77	0.39	2.5	1.4	0	0	1.61	0	0.80	0.40

*5-y follow-up report. Cardiovascular events include myocardial infarction, angina pectoris, coronary artery disease, and acute coronary syndrome; cerebrovascular events included only transient ischemic attack; peripheral arterial disease not specified.
†Minimum 5-y follow-up. Nilotinib dosage was 300 mg twice daily. Ischemic heart disease was defined as any AE reported under any preferred term (PT) in the standardized MedDRA queries (SMQ) narrow terms for *ischemic heart disease*. Ischemic cerebrovascular event was defined as any AE reported under any PT in the SMQ narrow terms for *ischemic cerebrovascular conditions*. An SMQ for peripheral artery disease (PAD) does not currently exist; therefore, PAD events were identified by the following PTs: *arterial occlusive disease*, *arterial stenosis*, *femoral artery occlusion*, *intermittent claudication*, *ischemic limb pain*, *peripheral arterial occlusive disease*, *peripheral artery angioplasty*, *peripheral artery bypass*, *peripheral artery restenosis*, *peripheral artery stenosis*, *peripheral artery stent insertion*, *peripheral artery thrombosis*, *peripheral coldness*, *peripheral ischemia*, *peripheral revascularization*, *peripheral vascular disorder*, *poor peripheral circulation*, and *Raynaud's phenomenon*.
‡Bosutinib 500 mg once daily was used in this study. Follow-up was ≥48 mo. Analysis was based on MedDRA PTs "likely indicating vascular or cardiac toxicities"; cerebrovascular treatment-emergent adverse events (TEAEs) (related high-level terms under the high-level group terms *central nervous system* [CNS] *vascular disorders*, including *CNS hemorrhages* and *cerebrovascular accidents*, *CNS vascular disorders not elsewhere classified* [NEC], and *transient cerebrovascular events*), cardiovascular TEAEs (all PTs under the high-level terms *ischemic coronary artery disorders* and *coronary artery disorders NEC*), and peripheral vascular TEAEs (related terms under the high-level group terms *arteriosclerosis*, *stenosis*, *vascular insufficiency and necrosis*, *embolism and thrombosis*, *vascular disorders NEC*, *cardiac and vascular investigations excluding enzyme tests*, and *vascular therapeutic procedures*).

proposed for the management of these cases.[18,19] However, these guidelines are often unbalanced in their recommendations with assessments such as blood pressure checks, fasting glucose, and lipid levels recommended for patients on nilotinib but not those on ponatinib.[19] These should be standard assessments for all patients based on the aforementioned similar risk of AOEs and certainly for all patients at higher risk, such as our patient. The ABCDE approach,[20] recommended for the general population, can be considered for patients receiving TKIs, particularly those with comorbidities, and those with the highest-risk disease (eg, by Sokal) for whom control of the disease is better served by a 2GTKI despite the higher risk of AOEs. (Table 3).

Glucose and lipids

Other considerations also play a role in the treatment selections for our patient. The patient has hyperglycemia and hypercholesterolemia. These are risk features for AOEs, and TKIs may have an effect on them. Nilotinib is associated with hyperglycemia and hyperlipidemia. In ENESTnd, 7% of patients treated with nilotinib experienced grade 3–4 hyperglycemia, compared with 0.4% of those treated with imatinib.[10] Despite these frequent abnormalities, development of diabetes, impaired fasting glucose, or metabolic syndrome, according to the criteria of the American Diabetes Association, do not seem to be more common with nilotinib than with dasatinib or imatinib.[21] Still, patients receiving nilotinib should have glucose levels and lipid profile monitored before, during, and after treatment.[22] For diabetic patients, self-monitoring and regular assessment of hemoglobin A1c are recommended.[22]

Hyperlipidemia is another risk factor for AOEs, and our patient has an elevated cholesterol level. Imatinib may improve the lipid profile of patients, and nilotinib may worsen it, usually soon after treatment starts.[23] In a report of nilotinib-treated patients, by 3 months the proportion of patients with "nonoptimal" low-density lipoprotein cholesterol increased from 48% to 89%. A similar effect was observed for high-density lipoprotein cholesterol.[23] The risk with dasatinib appears lower than with nilotinib.[24] Patients should have a lipid profile assessment before the start of therapy and at regular intervals (eg, every 3–6 months for patients at higher risk) during therapy with TKIs. Management should be instituted with attention to the possible drug–drug interaction with some statins. Coadministration of TKIs induces increased exposure to atorvastatin and simvastatin; there is less interaction with pravastatin and rosuvastatin.[25] Guidelines for management of glycemia and lipids have been proposed by a panel of expert endocrinologists that can be used for patients at highest risk. Although reasonable, prospective validation of such recommendations is not currently available (Table 3).

Pleural effusion

This patient may also have an increased risk of developing pleural effusion with dasatinib. Hypertension may increase the risk of developing pleural effusion,[26] although in a multivariate analysis age was the dominant risk factor.[26,27] From DASISION, the median age for patients who developed pleural effusion was 56 years, compared with 41 years for those who did not.[27] Dosage and schedule are important factors in the risk of developing pleural effusion, with 100 mg once daily associated with lower risk than the originally approved dosage of 70 mg twice daily.[28] Although lower dosages of dasatinib have not been directly compared with the standard 100 mg daily, a recent report of dasatinib starting at 50 mg once daily for patients with newly diagnosed chronic phase CML reported an incidence of pleural effusion of only 6% after a median follow-up of 24 months.[29] Longer follow-up is needed to determine the actual incidence because the first pleural effusion event may occur some years after the start of therapy, but it suggests that lower dosages may decrease the incidence and could be considered for patients at

Table 3. Suggested follow-up of patients with comorbidities for selected health conditions based on recommendations from an expert panel of cardiology[20] and endocrinology[22] experts

Monitoring and management of cardiovascular risk[19,20]		Comment
A	Assessment of risk	At baseline and throughout TKI therapy.
	Antiplatelet therapy	No available data in context of TKIs; consider risk of bleeding (eg, with dasatinib).
B	Blood pressure	Monitor and manage optimally; ponatinib is associated with high blood pressure (VEGFR inhibition)[13] but has been reported with other TKIs.[7]
C	Cholesterol	Reported more frequently elevated with nilotinib.
	Cigarette or tobacco cessation	
D	Diet and weight management	Rule out (and manage if appropriate) weight gain from fluid retention.
	Diabetes prevention and management	Hyperglycemia more common with nilotinib; lower glucose levels with imatinib.
E	Exercise	May help manage fatigue.
Monitoring and management of glycemia[22]		**Comment**
TKI treatment may lead to hyperglycemia or hypoglycemia.		Hyperglycemia more common with nilotinib; hypoglycemia rare (case reports with imatinib).
In case of diabetes under TKI, metformin should be used.		
Hemoglobin A1c target for TKI-induced diabetes <8%; should be personalized.		
Diagnosis of diabetes under TKI does not contraindicate continuation.		
In hypoglycemia under TKI in patients with prior therapy for diabetes, treatment may need to be adapted or interrupted.		
Assess glucose before initiation of TKI.		
If preexisting diabetes, achieve good glucose balance before initiation of TKI.		
Nondiabetic patients, glucose assessed every 2 wk during 1st month, then monthly.		Most important while on nilotinib.
If moderate hyperglycemia or diabetes before TKI, close glucose self-monitoring and education; hemoglobin A1c every 3 mo.		
Upon TKI termination, 4-wk glucose monitoring to adapt antidiabetic therapy.		
Monitoring and management of dyslipidemia[22]		**Comment**
Adapt lipid targets to general health status and prognosis.		Consider drug–drug interactions if therapy is needed.
Assess together with thyroid assessment; hypothyroidism should be treated before start of TKI.		
Total cholesterol, high-density lipoprotein, low-density lipoprotein, triglycerides before start of TKI.		
Lipid assessment every 6 mo.		
Upon TKI discontinuation, stop lipid reduction therapy and re-assess at 2 mo if no prior therapy or reassess optimal dosage if prior therapy.		

These recommendations may be valuable particularly for the highest-risk patients. Prospective validation of these recommendations is not available at the time of this writing.

higher risk for this AE. Recommendations for the treatment of patients with pleural effusion have been recently published.[30]

Renal dysfunction

Patients like ours will probably have some borderline or decreased renal function. A decrease in glomerular filtration rate has been reported for patients treated with imatinib and bosutinib.[31] The decline is modest and similar with both agents, but it must be monitored. Dasatinib and nilotinib do not seem to be associated with such declines.[32] Changes in glomerular filtration rate with imatinib have been linked to the development of cardiovascular events.[33] For patients with modest levels of kidney dysfunction (or liver dysfunction) at baseline, imatinib, dasatinib, and nilotinib have been shown to be safe when administered at the standard dosages.[34,35] Efficacy is maintained, and although some patients may experience further decline in kidney function, it seems to be transient and manageable with treatment interruptions and hydration. Dosage reductions are needed more frequently for such patients.[34,35] Therefore, these patients can be treated with a TKI that is considered most

appropriate and closely monitored, and dose adjustments can be used when needed. For patients with chronic kidney failure undergoing dialysis, there is only limited, anecdotal evidence. There are few instances of safe administration and plasma levels within the levels in the general population with imatinib,[36] and there is 1 report of a patient with increased trough plasma levels of dasatinib.[37] Thus, patients undergoing dialysis may perhaps be treated as clinically indicated based on their disease risk score, with imatinib possibly being safest, with close monitoring for AEs.

Salvage therapy

Let us assume our patient received imatinib with no complete cytogenetic response after 12 months, and then bosutinib with a transient major molecular response but now lost complete cytogenetic response. He has no mutations. We face again the dilemma of what TKI to use next. In my view, this patient should be treated with what we think gives him the best opportunity for a durable response. For third-line treatment, the best data available among TKIs approved at the time of this writing are for ponatinib, with major cytogenetic response reported for 60% of such patients. The risk of AOEs is an issue for our patient because of his comorbidities. However, after 2 prior TKIs failed, the risk of progression and death from CML is greater (expected 5-year survival 80% after failure of 2 prior TKIs[38]) than the risk of dying of an AOE with ponatinib (1% of patients died of AOEs after 5 years[13]). The exposure-adjusted incidence of AOEs, based on a very broad definition of these events, was 14.1 per 100 patient-years.[13] Our patient should be carefully monitored and the comorbidities controlled, with particular attention to hypertension, which is more common with ponatinib and may necessitate treatment adjustments. The starting dosage is an important consideration. The standard dosage is 45 mg daily. A recent randomized study suggests there is a correlation of dosage with efficacy and safety. The probability of achieving BCR-ABL1/ABL1 ≤1% is greater with 45 mg daily (39%) than with 30 mg (27%) or 15 mg (27%), with AOEs reported in 5%, 4% and 1%, respectively.[39] Thus, I would probably use 45 mg and reduce to 15 mg once transcript levels of ≤1% are achieved, with adequate monitoring and management of comorbidities.

Conclusion

In summary, patients with comorbidities have a higher risk of developing AEs with TKIs. Still, with adequate patient education and proper attention to their overall health, these risks can be managed, and patients should receive the TKI that best suits their needs depending on the stage, prior therapies, and patient goals. Throughout therapy, monitoring should include not only polymerase chain reaction but also review of other health conditions, whether they were present at the start of therapy or developed during therapy, to provide optimal management. Management may include referral to other specialists (eg, onco-cardiologists, endocrinologists) when appropriate to optimize care. This co-management should be considered for patients at the highest risk of cardiovascular or other complications and those with more risk factors and comorbidities. If we do this, patients with comorbidities may have a reasonable opportunity to have a good overall treatment outcome.

Conflict-of-interest disclosure

J.C. is a consultant (with compensation) for Novartis, Pfizer, and Takeda, and his institutions (current and/or former) have received research support from Novartis, Pfizer, Takeda, BMS, and Sun Pharma.

Off-label drug use

None disclosed.

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Handling challenging questions in the management of chronic myeloid leukemia: when is it safe to stop tyrosine kinase inhibitors?

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The paradigm for managing patients with chronic myeloid leukemia is evolving. In the recent past, restoring a normal life expectancy while patients are receiving never-ending targeted therapy with BCR–ABL1 tyrosine kinase inhibitors through prevention of progression to blast phase and mitigation of iatrogenic risks was considered the best achievable outcome. Now, long-term treatment-free remission with continued response off tyrosine kinase inhibitor therapy is recognized as the most optimal benefit of treatment. Indeed, numerous independent clinical trials provided solid proof that tyrosine kinase inhibitor discontinuation was feasible in patients with deep and sustained molecular responses. This article discusses when tyrosine kinase inhibitors may be safely stopped in clinical practice on the basis of the best and latest available evidence.

LEARNING OBJECTIVES

- Know factors influencing deep molecular response achievement
- Understand appropriate selection criteria of tyrosine kinase inhibitor discontinuation
- Understand safety aspects after end of treatment

Introduction

During treatment with tyrosine kinase inhibitors (TKIs) targeting BCR-ABL1, the driving oncoprotein of chronic myeloid leukemia (CML), obtaining an at least 3-log reduction in *BCR-ABL1* transcripts, which defines a major molecular response (MMR) (MMR/*BCR-ABL1* internationally standardized [IS] ratio, ≤0.1%), is an important step toward a favorable outcome. Indeed, stable MMR represents a robust surrogate marker for long-term progression-free survival.[1] However, patients in MMR but not achieving deep molecular responses (DMRs), such as a 4-log (MR4), 4.5-log (MR4.5), or even 5-log-(MR5) reduction in leukemia load, must receive TKIs continuously to maintain CML under control because treatment-free remission (TFR) is unlikely (Table 1).[2,3] On the contrary, a large body of clinical research has established that the long-term success rate of TKI discontinuation in patients with sustained DMR was ≥50%, with success defined as remaining in DMR or MMR.[4-8] Furthermore, it was demonstrated that, provided proper residual disease monitoring and rules for resuming therapy were followed, CML sensitivity to TKIs was largely preserved. DMR was restored soon after treatment reintroduction in almost all patients with molecular relapse. TFR is now a new goal of CML therapy, although with the current TKI arsenal and standard treatment-switching strategies, only 10% to 30% of patients with CML may achieve TFR.[9] Nonetheless, when TFR is set as a high-priority objective, DMR is a prominent clinically meaningful endpoint of treatment.

The European LeukemiaNet (ELN), the National Comprehensive Cancer Network (NCCN), and other cooperative working groups have built clinical practice recommendations to guide physicians regarding selection of patients for TKI discontinuation.[10] The ELN set minimal criteria for safely stopping TKIs as follows: (1) CML in first chronic phase (CP); (2) TKI provided as first- or second-line treatment, provided that the treatment change was driven by intolerance; (3) ≥5 years of treatment with the first-generation TKI imatinib or 4 years with second-generation TKI dasatinib, nilotinib, or bosutinib; and (4) ≥2 years of sustained MR4 or better (Table 2). In the NCCN version 3.2020 guidelines for TKI discontinuation, slightly less stringent selection criteria than those of the ELN were chosen.[11] At least 3 years of treatment are requested, including ≥2 years of sustained MR4 or better, and patients with DMR to salvage TKI for resistant CP-CML are not excluded from TKI discontinuation attempts (Table 3). In the

Table 1. Definition of molecular responses by peripheral blood real time quantitative polymerase chain reaction

Deep molecular response levels	Definition
MR4	*BCR-ABL1* IS ratio ≤0.01% or undetectable transcripts with ≥10 000 copies of *ABL1* or ≥24 000 copies of *GUS*
MR4.5	*BCR-ABL1* IS ratio ≤0.0032% or undetectable transcripts with ≥32 000 copies of *ABL1* or ≥77 000 copies of *GUS*
MR5	*BCR-ABL1* IS ratio ≤0.001% or undetectable transcripts with ≥100 000 copies of *ABL1* or ≥240 000 copies of *GUS*

IS, internationally standardized; MR4, 4-log molecular response; MR4.5, 4.5-log molecular response; MR5, 5-log molecular response.

latter situation, expected TFR rates are less favorable than when DMRs are readily obtained but patient safety seems preserved.[12,13] As an illustration, the 4-year cumulative incidences of MMR loss after dasatinib or nilotinib discontinuation in the STOP 2G-TKI study were 76.9% in patients with prior suboptimal response or resistance to imatinib and only 35.5% in those lacking such a history, but all relapses were successfully controlled after recommencing the original second-generation TKI.[13]

Clinical case

A 34-year-old woman complaining of fatigue was referred for absolute leukocytosis of 44 000/μL. Blood and marrow smear, cytogenetics, and molecular biology tests revealed CP-CML. The Philadelphia chromosome was not accompanied by additional cytogenetic abnormalities; *BCR-ABL1* transcripts were of the p210 e13a2 type; and the patient's Sokal risk group was low. The therapeutic goal and the principle of using *BCR-ABL1* TKIs were explained, and one of the pressing questions raised by the patient was when therapy would end. At the time of CML diagnosis, forecasting on an individual basis if and when TKIs may be stopped is not possible. Nevertheless, maximizing chances of achieving DMR through individualized TKI selection and dynamic molecular response–based switching strategies may open the door for removal of therapy.

DMR as a key milestone in the path to TKI discontinuation: first-line treatment choices

In the frontline setting, the likelihood of gaining DMR depends on ≥3 parameters: TKI generation, CP-CML risk score, and early molecular responses (EMRs), as detailed below.

TKI generation and DMR

Second-generation TKIs produce significantly higher rates of DMR than standard-dose imatinib in newly diagnosed CP-CML. In the phase 3 DASISION trial, the cumulative incidences of MR4.5 with first-line imatinib were 3% by 1 year, 8% by 2 years, 13% by 3 years, 23% by 4 years, and 33% by 5 years.[14] The cumulative incidences of MR4.5 obtained in the first-line dasatinib 100 mg daily arm were 5% by 1 year, 19% by 2 years, 24% by 3 years, 34% by 4 years, and 42% by 5 years. In the phase 3 ENESTnd study, the cumulative incidences of MR4.5 on imatinib were 1% by 1 year, 9% by 2 years, 15% by 3 years, 23% by 4 years, 31% by 5 years, and 45.2% by 10 years.[15,16] The cumulative incidences of MR4.5 obtained in the nilotinib 300-mg twice-daily arm were 11% by 1 year, 25% by 2 years, 32% by 3 years, 40% by 4 years, 54% by 5 years, and 63.8% by 10 years. The phase 3 BFORE trial comparing bosutinib 400 mg daily with standard-dose imatinib in the frontline setting is not mature enough to draw informative conclusions.[17]

CP-CML risk scores and DMR

In DASISION and ENESTnd, the best DMR rates were obtained with second-generation TKIs, regardless of baseline CP-CML risk.[14,15] As an example, in ENESTnd, the 5-year cumulative incidences of MR4.5 were 53.4% with nilotinib 300 mg twice daily versus 36.5% with imatinib 400 mg daily in patients with a low Sokal score, 60.4% with nilotinib 300 mg twice daily versus 32.7% with imatinib in patients with an intermediate Sokal score, and 44.6% with nilotinib 300 mg twice daily versus 23.1% with imatinib in patients with a high Sokal score.[15]

EMR to TKIs and DMR

The 3-month evaluation of response to TKIs is an important step during CML management. Achievement of an optimal EMR, corresponding to *BCR-ABL1* transcript levels ≤10% IS, indicates a favorable overall and progression-free survival as well as a very low risk of transformation.[14,15] Furthermore, several studies found that EMR was an early predictor of DMR, regardless of first-line TKI type and CP-CML risk score.[14,15,18,19] In ENESTnd, cumulative incidences of MR4.5 by 5 years in patients with <1%, between 1% and 10%, and >10% *BCR-ABL1* IS at 3 months were 70%, 51.7%, and 8.3%, respectively, with nilotinib 300 mg twice daily and 67.4%, 33.8%, and 15.9%, respectively, with imatinib.[18] In a large single-center study, Sasaki et al[20] found that best fit average real time quantitative polymerase chain reaction values for sustained MR4.5 for ≥2 years at any time during first-line TKI treatment were 0.051% IS at 3 months, 0.019% IS at 6 months, 0.007% IS at 9 months, and 0.003% IS at 12 months. Minimum

Table 2. European LeukemiaNet 2020 recommendations: requirements for tyrosine kinase inhibitor discontinuation

Mandatory	Minimal	Optimal
CP-CML	First-line TKI or second-line TKI if motivated by intolerance to first-line drug	Duration of therapy >5 y
Real time quantitative polymerase chain reaction on the IS scale	Typical e13a2 or e14a2 *BCR-ABL1* transcripts	DMR duration >3 y if MR4
Patient motivation and adherence	Duration of therapy >5 y if imatinib >4 y if second-generation TKI	DMR duration >2 y if MR4.5
	DMR duration (MR4 or M4.5) >2 y	

CML, chronic myeloid leukemia; CP, chronic phase; DMR, deep molecular response; IS, internationally standardized; MR4, 4-log molecular response; MR4.5, 4.5-log molecular response; TKI, tyrosine kinase inhibitor.

Table 3. National Comprehensive Cancer Network 2020 guidelines: criteria for discontinuation of tyrosine kinase inhibitor therapy

TKI discontinuation may be considered if all criteria below are met
Age ≥18 y
CP-CML, no history of accelerated or blast phase CML
Quantifiable *BCR-ABL1* transcripts
TKI therapy for ≥3 y
Stable MR4 for ≥2 y
Access to real time quantitative polymerase chain reaction with sensitivity of at least MR4.5

CML, chronic myeloid leukemia; CP, chronic phase; MR4.5, 4.5-log molecular response; TKI, tyrosine kinase inhibitor.

acceptable RT quantitative polymerase chain reaction values for sustained MR4.5 for ≥2 years at any time during first-line TKI treatment were 1.561% IS at 3 months, 0.592% IS at 6 months, 0.295% IS at 9 months, and 0.085% IS at 12 months (95th percentile). Altogether, these findings suggest that a drastic *BCR-ABL1* reduction strategy soon after TKI onset may increase the probability of and reduce the time to TKI discontinuation.

DMR as a key milestone in the path to TKI discontinuation: switching strategies

For patients incapable of reaching DMR or deemed to have a low likelihood of DMR, a change of TKI or a combination therapy as a way to achieve DMR is being explored. Such strategies are not approved by health authorities and remain within the scope of research. The randomized ENESTcmr trial showed that in patients lacking DMR after ≥3 years of first-line imatinib, a switch to nilotinib was more efficient at inducing DMR than was remaining on imatinib.[21,22] By 2 years, MR4.5 was obtained in 42.9% of patients who received nilotinib and in 20.8% of patients who stayed on imatinib.[21] The randomized DASCERN trial assessed the benefit of a switch from imatinib to dasatinib in patients lacking EMR on first-line imatinib, and DMR achievement was explored as a secondary endpoint. By 3 years, MR4 but not MR4.5 was more frequently attained with dasatinib (42%) than with imatinib (26%).[23] Importantly, nilotinib exposes patients to ischemic cardiovascular events, and dasatinib is well known to frequently cause pleural effusion; thus, a balanced evaluation of potential benefits and harms of switching approaches to achieve DMR is necessary.[14,15] For patients lacking DMR on a second-generation TKI, using the more potent third-generation TKI ponatinib has some theoretical interest; however, this strategy has not been considered, owing to the cardiovascular toxicity profile of ponatinib.[24] Other approaches are underway in the context of clinical trials, such as combining adenosine triphosphate–competitive TKIs with the allosteric TKI asciminib or with other therapies targeting residual CML cells or boosting the antileukemic immune response.[25]

Clinical case: follow-up

The patient had no prohibitive comorbid condition that could adversely affect TKI safety. It was thus decided to start a first-line second-generation TKI. We must recognize that second-generation TKIs do not offer an overall survival advantage over imatinib and that these drugs mostly benefit to intermediate- or high-risk patients with CP-CML in terms of reduction of progression events. Nevertheless, second-generation TKIs benefit low-risk patients because they significantly enhance chances of DMR and speed up DMR time as compared with imatinib. The patient obtained an MMR at 3 months, an MR4 at 6 months, then an MR4.5 at 12 months. Once achieved, the estimated durability of DMR is ~70%.[26] The patient maintained MR4.5 after 2 more years of continuous treatment, thus fulfilling minimal criteria for TKI discontinuation.

When is the right time to discontinue TKIs in patients with DMR?

Although some patients may obtain DMR rapidly, stopping TKIs before the third year of therapy is not advisable, because most CML progression events occur during the first 2 to 3 years of treatment. In the NCCN guidelines, a minimum of 3 years of TKI exposure, including 2 years in MR4 or better, is sufficient to envisage treatment discontinuation.[11] For the ELN, the optimal duration of treatment is ≥5 years, including ≥3 years in MR4 or ≥2 years in MR4.5 (Table 2).[10] Differences between ELN recommendations and NCCN guidelines highlight the fact that optimal durations of TKI therapy and DMR and best DMR levels before TKI discontinuation remain under debate. Possible predictors of TFR have been investigated because these might guide decision making regarding if and when to stop treatment on an individual basis. DMR level and total duration of TKI and, more important, that of DMR appear to play an important role.[27] The international EUROSKI trial revealed that the estimated risk of molecular relapse after imatinib removal continuously decreased as DMR duration increased.[28] To what extent this holds true for second-generation TKIs remains to be determined. Overall, accurately foreseeing TFR chances in individual patients remains difficult, and the choice between minimal stopping criteria or postponing TKI discontinuation until optimal conditions are obtained requires weighing the benefits against potential collateral damage of extended TKI therapy. Patient preferences may be taken into account as well.[29] In the future, biomarkers such as immunological parameters may help predict the success of TKI cessation.[30]

Risks associated with TKI discontinuation

Patients in DMR while receiving therapy have a negligible risk of secondary resistance, disease progression, or CML-related death; thus, the safety of TKI discontinuation is of utmost importance. Both the ELN and the NCCN have agreed to consider that a loss of MMR after TKI removal appropriately defines a molecular relapse and warrants clinical intervention in a timely fashion, namely within 4 weeks.[10,11] About 85% of molecular relapses occur within a short time window of 3 to 12 months and are characterized by a 0.5- to 1-log increase per month in the leukemia load, suggesting that hematological relapses will likely follow molecular relapses in the absence of rapid TKI resumption. Molecular relapses occurring beyond the first 12 months usually display slower kinetics.[31] Thus, surveillance in the post-treatment setting relies on adaptation of *BCR-ABL1* transcript assessment to the time to onset of molecular relapse (Table 4). Those with molecular relapse are sensitive to the same TKI as the one used before discontinuation because MMR and DMR are regained within a median time of 3 to 6 months with a few exceptions, underscoring the importance of *BCR-ABL1* transcript monitoring after treatment resumption.[31] The feasibility of

Table 4. National Comprehensive Cancer Network 2020 guidelines: *BCR-ABL1* transcript monitoring policy after tyrosine kinase inhibitor removal

NCCN 2020
Monthly during the first year
Every 2 mo during the second year
Every 3 mo after the second year
After TKI reintroduction in case of molecular relapse: monthly until MMR recovery, then every 3 mo

MMR, major molecular response; NCCN, National Comprehensive Cancer Network; TKI, tyrosine kinase inhibitor.

second TKI discontinuation attempts in patients recovering sustained DMR is currently under investigation but is not advisable yet in clinical practice.[32] Occasional cases of CML transformation have been reported either during the treatment-free phase or just after therapy restart and resemble sudden blast phase.[33] Although these are exceptional cases, they do occur in optimal responders to TKIs. Thus, vigilance is required because any risk level exceeding that existing in optimal responders while receiving treatment may put into question real-life TKI discontinuation opportunities.

The disappearance of possible drug-related adverse events is an obvious expectation after TKI removal, and, indeed, most regress during the treatment-free phase.[34] However, ~30% of patients may experience newly occurring or worsening of preexisting musculoskeletal pain within several weeks after TKI discontinuation and for up to several months.[35,36] Although unrelated to the molecular status, information about this so-called TKI withdrawal syndrome is important to communicate to patients because quality of life may be transiently altered, and painkillers may be needed. Whether this phenomenon may be minimized by tapering TKI doses over several months before discontinuation is an open question. Other aspects of patient safety after TKI removal should also be looked at, such as relevant effects of the suppression of TKI either on selected biological parameters, such as glycemia in patients with diabetes stopping imatinib, or on other-drug metabolism.[37]

Conclusion

Twenty years after approval of the first TKI against CML, followed by the expansion of the lifesaving *BCR-ABL1* TKI arsenal, integration of TFR as a new goal of CML management represents a huge step toward a cure. Of course, there is significant room for improvement in determining durable TFR predictability and achievability. Currently, it seems reasonable to wait until optimal conditions are met before stopping TKIs, namely ≥4 to 5 years of treatment and ≥2 to 3 years of DMR, in line with current recommendations and in the absence of iatrogenic issue. There is a long way to go before all patients may be eligible for TKI cessation. First-line adenosine triphosphate–competitive TKIs in combination with pegylated interferon are being compared with TKI monotherapy as a potential way to increase DMR and TFR.[38,39] Investigating the effect of therapeutic interventions on the basis of EMR levels on DMR achievement, such as early switches in favor of a more potent TKI or early add-on strategies, may also be of clinical interest. In addition, the issue of when *BCR-ABL1* transcript monitoring in patients who do not relapse may be stopped needs to be resolved because very long-term TFR data are sparse. Challenges over the coming years also include unraveling mechanisms of TFR despite apparent leukemic stem cell persistence and understanding reasons underlying divergent outcomes after TKI discontinuation.

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Conflict-of-interest disclosure

D.R. has received honoraria from and served on advisory boards for Pfizer, Novartis, and Incyte and has been a member of clinical trial steering committees for Bristol Myers Squibb and Novartis.

Off-label drug use

None disclosed.

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Sequencing multiple myeloma therapies with and after antibody therapies

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In multiple myeloma (MM), treatment selection and sequencing become increasingly complex with the increasing number of therapeutic options, including antibodies. Choice of treatment is dependent on various factors including patient- and tumor-related features. In addition, treatment-related factors, such as type and response to prior therapy, are also critical in terms of the selection of a new treatment regimen. Furthermore, approval status and reimbursement policies influence treatment choice. At the time of first relapse, patients who received a bortezomib-based regimen can switch to lenalidomide-based treatment, whereas patients who received lenalidomide until progression can switch to a proteasome inhibitor–based therapy. Alternatively, there is increasing evidence that pomalidomide-based triplets are also effective following the development of lenalidomide-refractory disease both in early and later relapse settings. Patients who become refractory to immunomodulatory drugs, proteasome inhibitors, and CD38 antibodies have a poor prognosis. These triple-class refractory patients may benefit from novel, recently approved agents such as XPO1 inhibitors or from participation in a clinical trial. Furthermore, retreatment with agents that were received in previous lines of therapy can also be considered in heavily pretreated patients, for example, in combination with classic cytotoxic drugs. Importantly, with the increasing use of CD38 antibodies in newly diagnosed and early relapsed/refractory MM, more information is needed on the potential value of retreatment with CD38 antibodies. With the introduction of new immunotherapies with novel modes of action, we also need a better understanding of sequencing of immunotherapeutic agents by taking into account the effect of prior therapy on immune function.

LEARNING OBJECTIVES

- Understand the recent changes in the treatment landscape of newly diagnosed multiple myeloma
- Understand how various patient-, disease-, and treatment-related features have an impact on treatment choice at diagnosis and subsequent treatment lines
- Understand the activity and safety profile of approved relapse regimens for the treatment of multiple myeloma
- Understand how a treatment regimen can have an impact on the subsequent line of therapy, both beneficial and detrimental, via affecting the frequency and function of immune cells

Clinical case

A 74-year-old patient with multiple myeloma (MM) was seen in the outpatient clinic for management of his first relapse. He was diagnosed with immunoglobulin G-κ MM in 2011 with bone disease, anemia, and hypercalcemia. Cytogenetic evaluation at diagnosis by fluorescence in situ hybridization showed hyperdiploidy, but no adverse-risk cytogenetic aberrations. He was enrolled in the EMN02 study and received induction therapy with bortezomib-cyclophosphamide-dexamethasone (very good partial response [VGPR] after 4 cycles), followed by high-dose melphalan (HDM) and autologous stem cell transplantation (auto-SCT). He achieved a complete response and continued with lenalidomide maintenance. In 2016, he developed progression during lenalidomide maintenance (10 mg) with a rapidly increasing M-protein and whole-body low-dose computed tomography scan that revealed new bone lesions. How should this patient be treated?

Introduction

The landscape of how to treat newly diagnosed (ND) MM (NDMM) is rapidly evolving with introduction of novel drugs and new therapeutic strategies. Based on patient and tumor characteristics, NDMM patients are treated with a combination of drugs that provides an optimal balance

between anti-MM activity and safety, whereby the goal is to achieve a deep remission. To this end, I use the best drugs upfront because it has been clearly shown in several studies that introduction of new drugs in upfront regimens improves depth of response, including minimal residual disease (MRD) negativity, which translates into superior progression-free survival (PFS) and overall survival (OS), and, especially for transplant-eligible patients, the proportion of patients with operational cure.[1,2] Moreover, a substantial fraction of patients do not receive second-line or third-line therapy because of death due to disease progression or therapy-related complications.[3,4] This is more common in elderly patients, and those who have comorbidities and previously experienced adverse events.[4] Furthermore, quality of life is best preserved during earlier therapy lines, whereas at later treatment lines, quality of life is reduced due to MM and therapy-related complications.[4]

Because first-line treatment dictates, in part, which therapy can be effectively given at the time of progression, I first briefly describe changes in first-line treatment of NDMM patients.

Changes in frontline treatment

Lenalidomide is increasingly used in patients with ND disease in both transplant-ineligible patients (lenalidomide-dexamethasone [Rd] and bortezomib-lenalidomide-dexamethasone [VRd]) and transplant-eligible patients (VRd induction and lenalidomide maintenance).[5-8] Lenalidomide is frequently administered as continuous treatment until progression. This indicates that an increasing fraction of patients will have lenalidomide-refractory disease and prior bortezomib exposure at the time of first progression.

The second important development is the increased use of CD38 antibodies as part of first-line regimens (Table 1). CD38 antibodies were initially evaluated in heavily pretreated patients as single agents. Based on the high activity as monotherapy (at least partial response [PR], 30%) and favorable toxicity profile (mainly infusion reactions, mostly occurring during the first infusion),[9] CD38 antibodies were also tested in combination with standard-of-care regimens in earlier lines of therapy. Based on these pivotal studies, several CD38 antibody-based combination therapies are now approved for the treatment of patients with both relapsed/refractory disease or NDMM (Figure 1).

Transplant-ineligible NDMM patients can be treated with daratumumab combined with Rd or bortezomib-melphalan-prednisone (VMP) based on the results from the MAIA and ALCYONE studies, respectively. In both studies a higher frequency of deep responses, including MRD negativity, was achieved by adding daratumumab to Rd or VMP, which translated into a superior PFS.[10-12] Importantly, in the ALCYONE study, there is also an OS advantage for daratumumab-VMP, compared with VMP. However, only 10% of the patients treated with VMP received a daratumumab-based regimen at the time of first relapse, which may partially explain the better survival in the daratumumab-treated group. Importantly, in both studies, patients ≥75 years of age had similar benefit from daratumumab, when compared with younger patients, which underlines the good tolerability profile of CD38 antibodies.[10-12] Other studies with CD38 antibody-based regimens are under way in this patient population (eg, daratumumab or isatuximab + VRd).

Recently, the US Food and Drug Administration (FDA) and European Medicines Agency (EMA) approved the first antibody-based induction and consolidation regimen for transplant-eligible patients based on the results from the CASSIOPEIA study. This study showed that adding daratumumab to bortezomib-thalidomide-dexamethasone (D-VTd) induction before and consolidation after transplantation improves response rate and PFS.[13] The GRIFFIN study showed, in a similar patient population, that daratumumab combined with VRd resulted in deeper responses, but with relatively short follow-up, not yet a PFS benefit. In Europe, the PERSEUS study is evaluating daratumumab-VRd in a larger number of transplant-eligible patients.

Second-line therapy

Although survival of MM patients has markedly improved, eventually most patients develop progressive disease. If a patient develops progression, treatment should be initiated in case of MM-related symptoms (clinical relapse) or a significant and rapid M-protein increase (eg, doubling of M-protein within 2 months; biochemical relapse).

Treatment selection and sequencing become increasingly complex with the increasing number of therapeutic options. The optimal sequence and choice of drugs is not established, and selection of treatment is dependent on patient- and tumor-related factors. Furthermore, treatment-related factors, such as type and response to prior therapy, are critical in terms of the selection of a new treatment regimen. Also, the availability of drugs, which varies considerably between countries, is an important determinant of treatment choice (Figure 2).

Several randomized phase 3 studies in patients with relapsed/refractory MM have demonstrated the superiority of triplet regimens over doublet regimens in terms of response rate and PFS. An OS benefit with a triplet regimen was observed in some studies with long follow-up. There are currently 4 lenalidomide-based triplets approved by the FDA and the EMA: 2 with an antibody as partner drug (daratumumab-Rd and elotuzumab-Rd) and 2 with a proteasome inhibitor (PI) as partner drug (carfilzomib-Rd [KRd] and ixazomib-Rd) (Table 2). Moreover, there are 3 bortezomib-dexamethasone (Vd)-based triplets approved by the FDA and the EMA: Vd plus daratumumab (DVd), pomalidomide (PVd), and panobinostat (Table 3).

These phase 3 studies have led to the increased application of 3-drug regimens in patients with relapsed/refractory (RR) MM. However, a (dose-adjusted) doublet regimen with reduced toxicity can be the best option in frail patients. Assessment of comorbidity is also critical in selecting the best personalized treatment option for the patient. Carfilzomib is the preferred PI in patients with peripheral neuropathy, whereas this drug should be used with caution in patients with a history of cardiovascular disease.[14] Dose adjustment is needed for certain drugs (eg, lenalidomide) in patients with reduced renal function to avoid toxicity. In addition, route and frequency of administration may affect quality of life and should also be considered in treatment selection. Ixazomib-Rd is an active and well-tolerated fully oral triplet combination, which may be the preferred option for elderly patients with restricted mobility or those who are working.[15] Novel administration strategies may also improve convenience for patients and reduce health care burden. For example, subcutaneous daratumumab, which is recently approved, has a markedly reduced administration time (5 minutes) and results in a significant reduction in infusion-related reactions, compared with IV administration.[16] Furthermore, once-weekly carfilzomib provides a more convenient dosing regimen, when compared with twice-weekly administration.[17] Potent triplet

Table 1. Frontline phase 3 studies with CD38 antibody-based treatment

	MAIA		ALCYONE		CASSIOPEIA		GRIFFIN	
Regimen	Dara-Rd	Rd	Dara-VMP	VMP	Dara-VTd	VTd	Dara-VRd	VRd
					HDM	HDM	HDM	HDM
					Dara-VTd	VTD	Dara-VRd	VRd
					R2: observation or dara maintenance (2 y)	R2: observation or dara maintenance (2 y)	Dara + len maintenance (Dara 2 y)	len maintenance
No. of patients	368	369	350	356	543	542	104	103
Patient population	NDMM, ineligible for transplant		NDMM, ineligible for transplant		NDMM, eligible for transplant		NDMM, eligible for transplant	
	ECOG PS 0-2		ECOG PS 0-2		ECOG PS 0-2		ECOG PS 0-2	
	Creatinine clearance: ≥30 mL/min		Creatinine clearance: ≥40 mL/min		Creatinine clearance: ≥40 mL/min		Creatinine clearance: ≥30 mL/min	
Median follow-up, mo	28		40.1		18.8		22.1	
≥PR	92.9	81.3	90.9	73.9	92.6*	89.9*	99.0*	91.8*
(s)CR	47.6	24.9	46	25	39*	26*	51.5*	42.3*
MRD negativity (10^{-5})	24.2	7.3	28	7	64	44*	51.0	20.4
HR PFS (95% CI)	0.56 (0.43-0.73)		0.42 (0.34-0.51)		0.47 (0.33-0.67)		Not reported	
Median PFS (months)	NR	31.9	36.4	19.3	NR	NR	NR	NR
HR OS (95% CI)	Not reported		0.61 (0.46-0.80)		0.43 (0.23-0.80)		Not reported	
Median OS (months)	NR	NR	NR	NR	NR	NR	NR	NR
Grade ≥ 3 neutropenia	50.0	35.3	40†	39†	28	15	41	22
Grade ≥ 3 infections	32.1	23.3	22†	15†	22	20	23	22
Grade ≥ 3 Pneumonia	13.7	7.9	11†	4.2†	Not reported	Not reported	13‡	15‡
Infusion-related reactions (all grade)	40.9	NA	27.7	NA	35	NA	42	NA

CR, complete response; dara, daratumumab; Dara-Rd, daratumumab-lenalidomide-dexamethasone; Dara-VMP, daratumumab-bortezomib-melphalan-prednisone; Dara-VRd, daratumumab-bortezomib-lenalidomide-dexamethasone; Dara-VTd, daratumumab-bortezomib-thalidomide-dexamethasone; HR, hazard ratio; len, lenalidomide; NA, not applicable; NR, not reached; PR, partial response; R2, second randomization; Rd, lenalidomide-dexamethasone; VMP, bortezomib-melphalan-prednisone; VRd, bortezomib-lenalidomide-dexamethasone; VTd, bortezomib-thalidomide-dexamethasone;
*Response after consolidation.
†During cycles 1-9.
‡Any grade pneumonia..

regimens (eg, KRd and CD38-based regimens) are recommended in case of aggressive relapse with rapidly developing clinical symptoms. Importantly, triplet regimens also improve the outcome of patients with high-risk cytogenetic abnormalities, but poor risk is not fully abrogated.

Current treatment pathways often sequence drugs with different modes of action, but reusing a drug can also be considered based on prior response and treatment-free interval. Patients who received frontline bortezomib-based therapy can be effectively treated at the time of relapse with a lenalidomide-containing regimen, whereas patients who develop disease progression during lenalidomide treatment can receive a PI-based therapy. Furthermore, there is increasing evidence that despite acquired resistance to lenalidomide, a pomalidomide-containing regimen directly after lenalidomide can also be effective.[18,19] Although cross-trial comparisons have limitations due to differences in trial design, inclusion criteria, and prior lines of therapy, a recent network meta-analysis indicates that CD38 antibody-based triplets rank among the most effective treatment options.[20] The overall safety profile of CD38 antibody-based triplets is favorable, even in elderly patients.[21] However, addition of a CD38 antibody to standard of care is associated with a modest increase in neutropenia and (respiratory) infections. Altogether, I favor a CD38 antibody-based regimen in patients who did not receive a CD38 antibody in prior lines of therapy, whereby the partner drugs are dependent on prior therapy: Vd

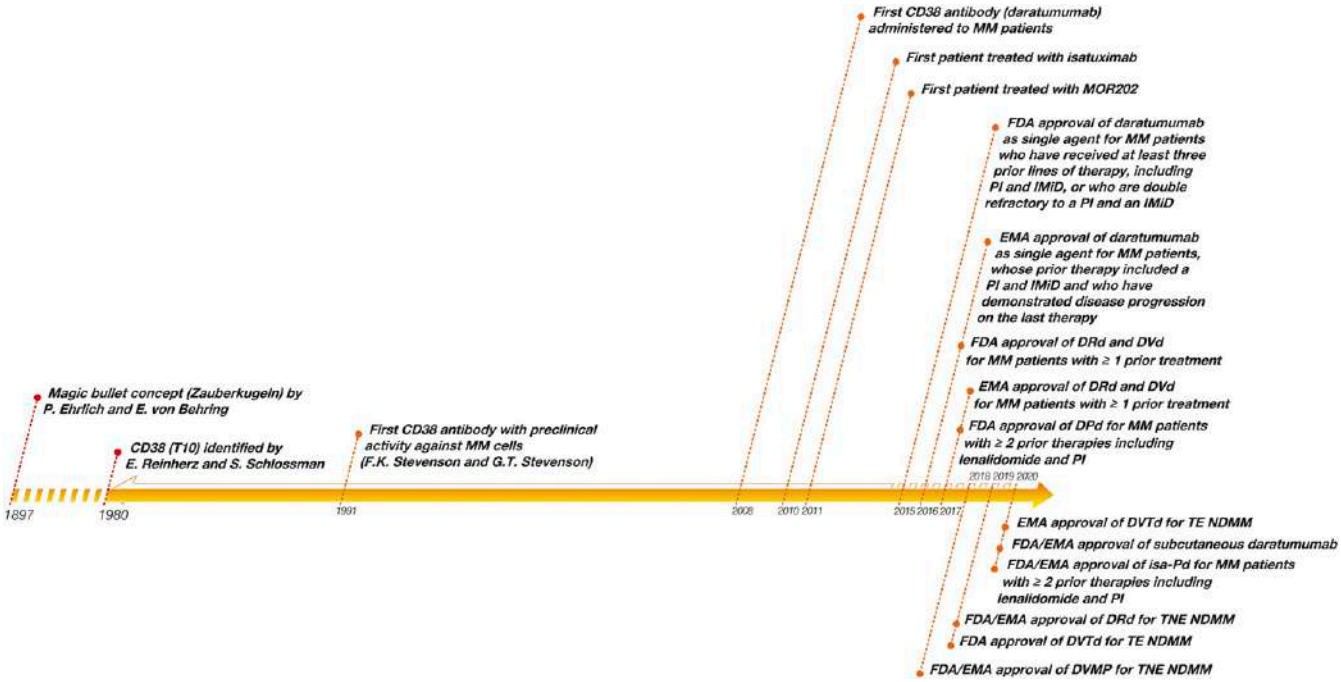

Figure 1. History of CD38-targeting antibodies in MM. Adapted from van de Donk et al[59] with permission.

(or carfilzomib-dexamethasone [Kd]/pomalidomide-dexamethasone [Pd] if available) in patients who developed lenalidomide-refractory disease and Rd in patients without lenalidomide-refractory MM.

After reinduction therapy, consolidation with high-dose therapy plus auto-SCT should be considered in patients who did not receive upfront auto-SCT, or after a previous auto-SCT with response of at least 24 months (36 months in patients who received maintenance therapy). The Myeloma X study showed that consolidation with HDM plus auto-SCT was superior to weekly cyclophosphamide after previous auto-SCT.[22] In the German ReLApsE trial, there was a trend for improved PFS, as well as superior OS, in patients who received HDM plus salvage auto-SCT followed by lenalidomide maintenance, when compared with continuous treatment with Rd.[23] However, the role of salvage auto-SCT in the era of continuous novel agent–based triplet therapies (effective alternatives for salvage auto-SCT in the current treatment landscape) remains unclear.

Early relapse following frontline therapy carries a very poor outcome regardless of cytogenetic risk, and, although outcomes have improved over time due to increased availability of novel classes of drugs, it still represents an unmet medical need.[24,25] Allogeneic stem cell transplantation can be considered in these patients, preferentially in the setting of a clinical trial, but is associated with substantial morbidity and mortality due to infectious complications and graft-versus-host disease. Because of the rapid development of resistance to all available drugs, these patients may also benefit from new agents with novel modes of action such as novel T-cell–redirecting therapies such as B-cell maturation antigen (BCMA)-targeting chimeric antigen receptor (CAR) T cells or bispecific antibodies.

Refractory to lenalidomide

Because of the increased use of lenalidomide in NDMM, there is an increasing fraction of patients who present with lenalidomide-refractory disease at the time of first relapse (Figure 2). There is no clear data available about the efficacy of increasing the dose of lenalidomide or adding dexamethasone in patients with biochemical progression during lenalidomide maintenance.[26] Furthermore, lenalidomide-refractory patients were excluded from the phase 3 trials evaluating a lenalidomide-based triplet in early RRMM. Therefore, lenalidomide-refractory patients are typically switched to a PI-based regimen. However, in several studies evaluating PI-based triplets the proportion of lenalidomide-refractory patients was very low, and specific data for patients progressing on frontline lenalidomide are not always available. The median PFS for lenalidomide-refractory patients, irrespective of number of prior treatment lines, was 8.6 months for Kd, 4.9 to 6.6 months for Vd, and 7.8 months for DVd.[26] This is inferior to what is observed in the whole study population with a median PFS of 18.7 months for Kd,[14] 7.1 to 9.4 months for Vd,[14,27] and 16.7 months for DVd.[27]

Importantly, the OPTMISMM study enrolled patients who were all previously lenalidomide exposed (69.9% were lenalidomide-refractory). There was a superior outcome with PVd compared with Vd in lenalidomide-refractory patients with 1 prior therapy (median PFS, 17.8 vs 9.5 months).[19] The phase 3 ICARIA study also showed an advantage of a pomalidomide-containing triplet (isatuximab combined with Pd) over Pd alone in lenalidomide-refractory patients with ≥2 prior lines of therapy.[28] In addition, several phase 2 studies (most in more advanced MM; Table 4) have shown activity of other pomalidomide-based combinations, such as Pd with cyclophosphamide,[29] carfilzomib,[30] ixazomib,[31] daratumumab,[18,32] or elotuzumab,[33] in patients with lenalidomide-refractory disease. The EMN011 trial treated patients with refractory disease or first progression after inclusion in the EMN02 study (all patients received lenalidomide maintenance until progression in EMN02) with carfilzomib-pomalidomide-dexamethasone (KPd; median PFS, 18 months).[30] A recent phase

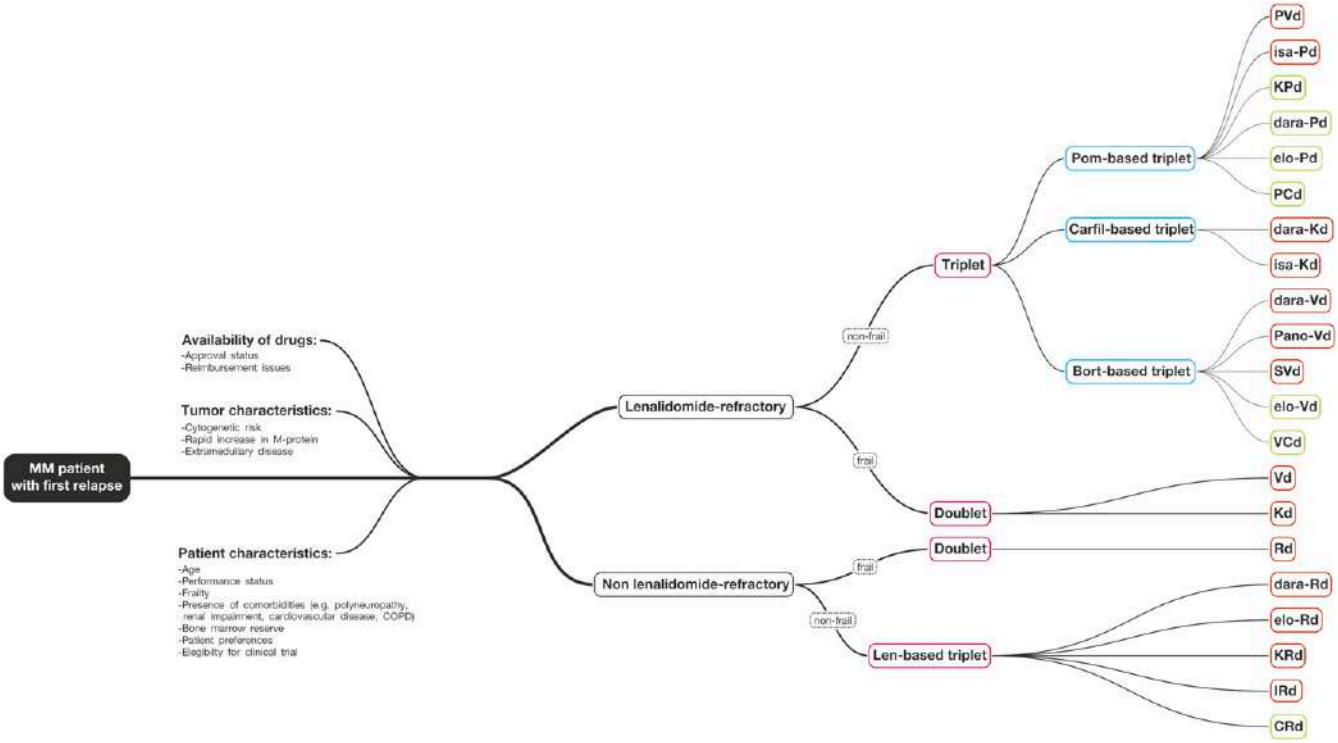

Figure 2. Treatment of MM patients with first relapse. Choice of treatment of patients with first relapse is dependent on patient-, tumor-, and treatment-related features. Patients, who progress on lenalidomide, can be treated with proteasome inhibitor–based therapy or a pomalidomide-based regimen. Patients who are not lenalidomide-refractory can be treated with a lenalidomide-based regimen. Alternatively, these patients may also receive retreatment with proteasome inhibitors if there is a treatment-free interval of >6 months. Regimens with red contour were evaluated in phase 3 trials; regimens with green contour were evaluated in phase 1 or 2 trials. Bort, bortezomib; Carfil, carfilzomib; CRd, cyclophosphamide-lenalidomide-dexamethasone; dara-Kd, daratumumab-carfilzomib-dexamethasone; dara-Pd, daratumumab-pomalidomide-dexamethasone; dara-Rd, daratumumab-lenalidomide-dexamethasone; dara-Vd, daratumumab-bortezomib-dexamethasone; elo-Pd, elotuzumab-pomalidomide-dexamethasone; elo-Rd, elotuzumab-lenalidomide-dexamethasone; elo-Vd, elotuzumab-bortezomib-dexamethasone; IRd, ixazomib-lenalidomide-dexamethasone; isa-Kd, isatuximab-carfilzomib-dexamethasone; Isa-Pd, isatuximab-pomalidomide-dexamethasone; Kd, carfilzomib-dexamethasone; KPd, carfilzomib-pomalidomide-dexamethasone; Len, lenalidomide; Pano-Vd, panobinostat-bortezomib-dexamethasone; PCd, pomalidomide-cyclophosphamide-dexamethasone; Pom, pomalidomide; PVd, pomalidomide-bortezomib-dexamethasone; Rd, lenalidomide-dexamethasone; SVd, selinexor-bortezomib-dexamethasone; VCd, bortezomib-cyclophosphamide-dexamethasone; Vd, bortezomib-dexamethasone.

2 study showed that sequencing daratumumab-pomalidomide-dexamethasone (DPd) immediately after development of lenalidomide-refractory disease in patients with 1 or 2 prior lines of therapy was effective.[18] The ongoing phase 3 APOLLO study, which evaluates DPd, also enrolled lenalidomide-refractory patients with 1 prior line of therapy.

Kd is another backbone to which a third drug can be added to improve anti-MM activity. The CANDOR study enrolled a substantial fraction of lenalidomide-exposed or refractory patients (42.3% and 33.0%, respectively). Addition of daratumumab to Kd markedly improved the outcome of these patients.[34] Similarly, addition of isatuximab to Kd significantly improved depth of response and PFS in the IKEMA study.[35] No data are currently available in both studies for the subgroup of patients who progressed on frontline lenalidomide treatment. These regimens are not yet approved by FDA/EMA.

Refractory to CD38 antibody

Patients with first relapse and prior daratumumab exposure represent a growing population of patients. Although the majority of phase 3 trials evaluating triplet regimens in early relapsed MM did not enroll daratumumab-exposed patients (Tables 2 and 3), nondaratumumab-containing regimens, such as KRd or PVd, can be used to treat these patients because cross-resistance with such regimens and daratumumab is not expected. Choice of regimen is also dependent on whether the patient is progressing during treatment with other drugs (eg, lenalidomide), as well as on patient and tumor characteristics as discussed in the Second-line therapy section above.

However, there are still several open questions, including the impact of natural killer (NK)-cell depletion by daratumumab[36] on a subsequent elotuzumab-containing regimen. The effect of NK-cell reduction may be limited because elotuzumab eliminates MM cells not only via NK cells, but also through a monocyte-mediated killing mechanism.

At this moment, there is not sufficient evidence to support retreatment with a CD38 antibody. Because daratumumab treatment results in CD38 reduction on the MM cell surface and depletion of NK cells, a treatment-free interval of 3 to 6 months may be needed to allow for recovery of CD38 and NK cells to baseline levels. An ongoing randomized phase 2 trial is

Table 2. Phase 3 studies evaluating Rd-based triplets in early RRMM

	POLLUX[62-64]	ELOQUENT-2[65,66]	ASPIRE[67,68]	TOURMALINE-MM1[15]
No. of patients	569	646	792	722
Regimen	dara-Rd vs Rd	elo-Rd vs Rd	KRd vs Rd	IRd vs Rd
Patient population	• At least 1 prior line of therapy; len-refractory patients were excluded • Creatinine clearance ≥30 mL/min	• 1-3 prior lines of therapy; len-refractory patients were excluded • Creatinine clearance ≥30 mL/min	• 1-3 prior lines of therapy; len-refractory and PI-refractory patients were excluded • Creatinine clearance ≥50 mL/min	• 1-3 prior lines of therapy; len-refractory and PI-refractory patients were excluded • Creatinine clearance ≥30 mL/min
Prior len % / len-refractory %	17.6 / NA	6 / NA	19.8 / 7.2	12 / 0.1
Prior bort % / bort-refractory %	84.2 / 20.6	70 / NA	65.8 / 14.9	69 / 2*
CD38 antibody exposed, %	0?	0 ?	0?	0?
Median follow-up, mo	44.3	46	For PFS: 32.3 For OS: 67.1	14.8
≥PR, %	92.9 vs 76.4	79 vs 66	87.1 vs 66.7	78.3 vs 71.5
≥CR, %	56.6 vs 23.2	5 vs 9	31.8 vs 9.3	14.2 vs 6.6
HR PFS (95% CI)	0.44 (0.35-0.55); *P* < .0001	0.71 (0.59-0.86); *P* = .0004	0.69 (0.57-0.83); *P* = .0001	0.74 (0.59-0.94); *P* = .01
HR OS (95% CI)	HR for OS not reported; 42-mo OS: 65% vs 57%	0.78 (0.63-0.96); *P* = not reported	0.79 (0.67-0.95); *P* = .0045	No OS benefit shown

bort, bortezomib; CI, confidence interval; CR, complete response; dara-Rd, daratumumab-lenalidomide-dexamethasone; elo-Rd, elotuzumab-lenalidomide-dexamethasone; HR, hazard ratio; IRd, ixazomib-lenalidomide-dexamethasone; KRd, carfilzomib-lenalidomide-dexamethasone; len, lenalidomide; NA, not available; PI, proteasome inhibitor; PR, partial response; Rd, lenalidomide-dexamethasone.
*Refractory to any PI.

evaluating the value of adding daratumumab to Kd in patients with prior daratumumab treatment with a treatment-free interval of ≥3 months. When considering retreatment, prior response, treatment-free interval, costs, and alternative treatment options should be taken into account.

There are also small case series showing that continuation of daratumumab, with either addition or switch in class of partner drug, may be beneficial.[37-39] In addition, preclinical studies have increased our understanding of development of resistance to CD38 antibodies, which has led to the development of several interventions that are currently explored in clinical trials. This includes the administration of agents, such as all-*trans* retinoic acid and histone deacetylase inhibitors, which increase CD38 expression on the tumor cell surface.[40] Also, adoptive transfer of CD38^{low} NK cells, is explored as a novel strategy to enhance anti-MM activity of CD38-targeting antibodies. Furthermore, next-generation CD38-targeting drugs may be of value in case of resistance to daratumumab or isatuximab (eg, CD38 hexabodies or CD38-targeting bispecific/trispecific agents).[41] Alternatively, CD38 antibody-refractory patients may also benefit from immunotherapies targeting other tumor antigens such as BCMA or GPRC5D.

Clinical case continued

Our patient developed symptomatic progression during lenalidomide maintenance therapy. Cytogenetic analysis showed that he had acquired del(17p). He had also suffered from a myocardial infarction 9 months ago, but was currently biking and walking every day in his city. Because of the poor risk conferred by del(17p) and patient's good performance status, he started treatment with a PI-based triplet regimen. We chose a bortezomib-dexamethasone backbone because his cardiac history increases the risk of carfilzomib-related cardiovascular adverse events. As the third drug, we added daratumumab, based on the high activity of DVd in patients with first relapse. DVd is also beneficial in high-risk patients, but poor risk is not completely abrogated. With the recommended pre- and post-infusion medication, he experienced a mild infusion-related reaction during the first daratumumab infusion. When he developed mild peripheral neuropathy, we administered bortezomib once weekly instead of twice weekly, resulting in stabilization of the neuropathy. He achieved a VGPR, which lasted 2 years.

Double-refractory disease

Pd is approved for the treatment of patients with ≥2 prior lines of therapy, including lenalidomide and a PI. Activity of this regimen was significantly improved by adding a third drug. Three antibody-based regimens are currently approved in a similar patient population (DPd,[32] isatuximab-Pd,[28] and elotuzumab-Pd[33]). Alternatively, when these antibodies are not available, cyclophosphamide can be added to pomalidomide-dexamethasone (PCd; Table 4) or to pomalidomide-prednisone (PCP).[29,42] These regimens represent a fully oral, relatively cheap treatment options.[29,42]

Table 3. Phase 3 studies evaluating PI-based triplets in early RRMM

	CASTOR[27,69]	OPTMISSM[19]	PANORAMA 1[70,71]	CANDOR[34]	IKEMA[35]	BOSTON[72]
No. of patients	498	559	768	466	302	402
Regimen	dara-Vd vs Vd	PVd vs Vd	Pano-Vd vs Vd	dara-Kd vs Kd	isa-Kd vs Kd	SVd vs Vd
Patient population	• At least 1 prior line of therapy; bort-refractory patients were excluded • Creatinine clearance ≥20 mL/min	• 1-3 prior lines, including ≥2 cycles of lenalidomide • Patients with severe renal impairment requiring dialysis were excluded	• 1-3 prior lines of therapy; PI-refractory patients were excluded • Creatinine clearance ≥60 mL/min	• 1-3 prior lines of therapy • Creatinine clearance ≥20 mL/min	• 1-3 prior lines of therapy • No prior therapy with carfilzomib • Creatinine clearance ≥15 mL/min	• 1-3 prior lines of therapy • At least a PR to a prior PI, if received • Creatinine clearance ≥20 mL/min
Prior len % / len-refractory %	42.0 / NA	100 / 69.9	20.4 / NA	42.3 / 33.0	NA / 32.8	38.3 / NA
Prior bort % / bort-refractory %	65.5 / NA	72.3 / 10.0	43.0 / NA	90.3 / 29.0	89.7 / 33.1*	69.4 / NA
CD38 antibody exposed %	0?	0?	0?	0?	0?	4.2
Median follow-up, mo	19.4	15.9	For PFS: 6.5 For OS: not reported	16.9	20.7	13.2 and 16.5 mo in SVd and Vd arms, respectively
≥PR %	83.8 vs 63.2	82.2 vs 50.0	60.7 vs 54.6	84.3 vs 74.7	86.6 vs 82.9	76.4 vs 62.3
(s)CR %	28.8 vs 9.8	15.7 vs 4.0	11 vs 6	28.5 vs 10.4	39.7 vs 27.6	16.9 vs 10.6
HR PFS (95% CI)	0.31 (0.24-0.39); $P < .0001$	0.61 (0.49-0.77); $P < .0001$	0.63 (0.52-0.76); $P < .0001$	0.63 (0.46-0.85); $P = .0014$	0.53 (0.32-0.89); $P = .007$	0.70 (0.53-0.93); $P = .0075$
HR OS (95% CI)	OS data immature	OS data immature; 0.98 (0.73-1.32); $P = .89$	0.94 (0.78-1.14); $P = .54$	0.75 (0.49-1.13); $P = .084$	OS data immature	0.84 (0.57-1.23); $P = .19$

bort, bortezomib; CI, confidence interval; CR, complete response; dara-Kd, daratumumab-carfilzomib-dexamethasone; dara-Vd, daratumumab-bortezomib-dexamethasone; HR, hazard ratio; isa-Kd, isatuximab-carfilzomib-dexamethasone; Kd, carfilzomib-dexamethasone; NA, not available; Pano-Vd, panobinostat-bortezomib-dexamethasone; PI, proteasome inhibitor; PR, partial response; PVd, pomalidomide-bortezomib-dexamethasone; SVd, selinexor-bortezomib-dexamethasone; Vd, bortezomib-dexamethasone.
*Prior PIs/PI-refractory.

Table 4. Randomized phase 2 and 3 studies evaluating pomalidomide-based triplets in patients with RRMM

	isa-Pd vs Pd[28]	PCd vs Pd[29]	elo-Pd vs Pd[33]
Phase	Randomized phase 3	Randomized phase 2	Randomized phase 2
No. of patients	307	70	117
Median of prior lines	3	4	3
Len-refractory, %	93	100	87
Bort-refractory, %	76*	74	80*
Median follow-up, mo	11.6	Not reported	Minimum follow-up of 9.1 mo
≥PR, %	60 vs 35	65 vs 39	53 vs 26
≥VGPR, %	32 vs 9	12 vs 14	20 vs 9
Median PFS, mo	11.5 vs 6.5	9.5 vs 4.4	10.3 vs 4.7 mo
Median OS, mo	1-y OS: 72% vs 63%	Not reached vs 16.8	Not reached in both arms
Approval	FDA/EMA	—	FDA/EMA

—, no approval by FDA/EMA; Bort, bortezomib; elo-Pd, elotuzumab-pomalidomide-dexamethasone; isa-Pd, isatuximab-pomalidomide-dexamethasone; Len, lenalidomide; PCd, pomalidomide-cyclophosphamide-dexamethasone; Pd, pomalidomide-dexamethasone.
*Refractory to at least 1 PI.

Kd is also effective in patients who are exposed to lenalidomide and bortezomib.[17] Further improvement can be obtained by adding a third drug such as daratumumab.[43] In addition, the combination of carfilzomib with pomalidomide-dexamethasone (KPd) is also effective in heavily pretreated patients.[44]

Triple-class refractory disease

Patients frequently experience multiple relapses with decreasing remission duration and length of the treatment-free interval with increasing lines of therapy.[4] Eventually, patients develop disease refractory to immunomodulatory drugs (IMiDs), PIs, and CD38 antibodies (triple-class refractory), which carries a very poor survival of <12 months.[45] The subgroup of patients refractory to lenalidomide, pomalidomide, bortezomib, carfilzomib, and a CD38 antibody (pentarefractory patients) has the worst outcome with a median OS of only 5.6 months.[45] Because of the poor prognosis, these patients should be considered for participation in a clinical trial. Studies have shown promising results for iberdomide,[46] CC-92480,[47] and melflufen[48] in triple-class refractory patients. In addition, belantamab mafodotin, an antibody-drug conjugate directed against BCMA, induces at least PR in 30% to 34% of triple-class refractory patients.[49] Median PFS was 2.9 and 4.9 months in the 2.5 mg/kg and 3.4 mg/kg cohort, respectively.[49] Furthermore, several studies with BCMA-specific CAR T cells demonstrate encouraging results in heavily pretreated MM patients. Ide-cel, a CAR T-cell product expressing a murine BCMA-targeting single-chain variable fragment, was evaluated in 128 triple-class exposed patients with at least complete response (CR) in 33% of patients and median PFS of 8.8 months.[50] JNJ-4528 is a CAR T-cell therapy with 2 BCMA-targeting domains that confers high-avidity binding. Preliminary results from the CARTITUDE-1 study (29 patients; 86% triple-refractory) showed a 100% response rate with CR in 86%, and a 9-month PFS rate of 86%.[51] Both CAR T-cell products received FDA breakthrough designation for RRMM. In contrast to CAR T cells, T-cell–redirecting bispecific antibodies and bispecific T-cell engager are directly "off the shelf" available. Several BCMA-targeting bispecific antibodies or BiTEs are currently evaluated in phase 1 studies with high response rates (at least PR, 67% to 89%) reported at the higher dose levels.[52-54] However, patients considered for trial participation often do not fulfill the criteria for enrollment, due to aggressive relapse with development of thrombocytopenia and renal failure and necessity to start therapy directly. Also, patients with nonsecretory disease can frequently not participate in clinical trials due to absence of measurable disease.

Outside of clinical trials, these patients can now receive selinexor (an exportin-1 inhibitor) in combination with dexamethasone. This regimen was recently approved for the treatment of triple-class refractory patients by the FDA, based on a 26% overall response rate and median PFS of 3.7 months in this patient population.[55] Treatment interruptions and dose reductions were common, as a result of nausea, anorexia, diarrhea, hyponatremia, thrombocytopenia, and fatigue. Alternatively, retreatment with agents that were received in previous lines of therapy can be considered in heavily pretreated patients, especially after a long-lasting remission and in combination with other drugs. The combination of novel agents with classic cytotoxic drugs, such as cyclophosphamide, anthracyclines, or bendamustine, can also be effective in patients with advanced MM. For example, lenalidomide combined with continuous low-dose oral cyclophosphamide and prednisone is effective and well tolerated in lenalidomide-refractory MM patients (at least PR, 67%; median PFS, 12.1 months).[56]

Sequencing immunotherapy

With the increasing use of immunotherapy in MM, more studies are needed to increase our understanding of the best sequence of anti-MM drugs to maximize patient benefit. PIs, alkylating drugs, and steroids reduce the number and function of T cells, and thereby these agents potentially reduce the activity of subsequent therapy with T-cell–engaging bispecific antibodies. Exposure to these drugs can also reduce the fitness of T cells in the apheresis product and thereby impair the efficacy of CAR T-cell therapy. In contrast, preclinical studies have shown that prior daratumumab treatment enhances the efficacy of

BCMA-targeting T-cell engaging bispecific antibodies by virtue of its immunomodulatory effects, such as the elimination of CD38$^+$ regulatory T cells and myeloid-derived suppressor cells.[57-59] On the other hand, because activated T cells upregulate CD38, antibodies targeting CD38 may also have a negative impact on these cells. However, we have previously demonstrated that daratumumab treatment results in a rapid downregulation of CD38 on T cells, which is mediated through the trogocytic transfer of CD38/daratumumab complexes from T cells to monocytes and granulocytes.[60] Despite reducing CD38 expression on T cells, daratumumab promotes T-cell expansion and increases their functional activity.[58,60] Ongoing studies will demonstrate whether CD38 antibodies can be effectively combined with T-cell–redirecting therapies. The immune-stimulating effects of daratumumab, which possibly persist into the subsequent line of therapy because of its long half-life,[57] may also explain the efficacy of retreatment with an IMiD following development of progression during daratumumab.[61] Importantly, IMiDs can still be immune stimulating, when the tumor is resistant to the drug.[56] Improved understanding of the impact of prior therapy on subsequent immunotherapy will also be relevant when T-cell–redirecting therapy will be used as part of first-line therapy (eg, to convert patients from MRD$^+$ to MRD$^-$). Another open question is the impact of reducing tumor burden prior to T-cell–redirecting therapy (optimizing effector-to-target ratio). More information is also needed about the best treatment strategy for patients relapsing after BCMA-targeted therapy: how effective is sequencing of agents that target BCMA vs using immunotherapies targeting other tumor antigens such as GPRC5D or SLAMF7? We also need clinical trials to evaluate novel combinations of immunotherapeutic agents. This includes studies to find the best partner drug for bispecific antibodies and to evaluate drugs that can improve the persistence of CAR T cells without increasing toxicity, such as cytokine-release syndrome. Potential candidates include T-cell stimulatory agents such as IMiDs, iberdomide, and CD38 antibodies.

Conclusions

Treatment selection and sequencing become more and more complex with the increasing number of therapeutic options that are available in MM. Also, the increased use of lenalidomide and CD38 antibodies as part of first-line regimens has major impact on treatment of first relapse. There are several important open questions, such as whether patients with prior exposure to CD38 antibodies can be retreated with or without a washout period. Furthermore, because of the introduction of new agents with a novel mechanism of action (such as T-cell–redirecting agents) we need a better understanding of how all available drugs can be sequenced in the most optimal way to maximize patient survival and minimize toxicity. Because of the introduction of new immunotherapies in MM, sequencing should, more than ever, take into account the potential harmful or beneficial impact of drugs on the immune microenvironment, and how that influences the efficacy of subsequent lines of therapy.

Conflict-of-interest disclosure

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Off-label drug use

None disclosed.

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Bispecifics, trispecifics, and other novel immune treatments in myeloma

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Despite recent advances in treatment, relapses in multiple myeloma (MM) are inevitable. Off-the-shelf immunotherapeutics represent a promising avenue for research, with various classes of agents under development and several demonstrating deep and durable responses in patients who have exhausted all available therapies. Antibody-drug conjugates (ADCs) seek to improve on naked monoclonal antibodies by delivering a cytotoxic payload directly to tumor cells while largely limiting systemic effects. Belantamab mafodotin, a B-cell maturation antigen (BCMA)–targeted ADC, has shown response rates >30% in a phase 2 trial of highly refractory patients and is being investigated in a variety of settings and combinations. Several other ADCs are in earlier stages of development that target cell surface antigens that are internalized, including BCMA, CD38, CD46, CD56, CD74, and CD138. Bispecifics are designed to bring cytotoxic immune effector cells into proximity with tumor cells, and several agents have shown high response rates in early trials. Current targets include BCMA, CD38, GPRC5d, and FCRH5, and all of these seek to engage T cells through CD3. Bispecifics targeting natural killer (NK) cells through CD16 are still in preclinical development. Trispecific antibodies may represent an advance over bispecifics by providing a T-cell costimulatory signal such as CD28, or alternatively, dual MM antigens to increase specificity of NK or T-cell targeting. This is an area of active preclinical research at this time. Lastly, designed ankyrin repeat proteins, which are small antibody-mimetic proteins with high target-binding affinity, have the potential to block multiple pathways at once and provide stimulatory signals to the immune system.

LEARNING OBJECTIVES

- Learn the myriad of targets under investigation for off-the-shelf immunotherapeutic approaches in the treatment of myeloma
- Interpret the emerging clinical data from early-phase studies based on the differences in structure and function of classes of immunotherapeutic agents

Clinical case

A 75-year-old woman with IgA-κ relapsed refractory multiple myeloma (RRMM) was diagnosed with MM 15 years ago and underwent 7 lines of therapy, including 2 autologous stem cell transplants. She was refractory to 3 immunomodulatory (IMiD) drugs (thalidomide, lenalidomide, and pomalidomide), 2 proteasome inhibitors (PIs; bortezomib and carfilzomib), and an anti-CD38 monoclonal antibody (daratumumab) and had recently progressed through selinexor. What novel off-the-shelf immune therapies are available in clinical trials for this patient?

Introduction

Despite many recent drug approvals, relapses in multiple myeloma (MM) are inevitable. Patients who are pentarefractory (refractory to 2 IMiDs, 2 PIs, and an anti-CD38 monoclonal antibody) have particularly poor outcomes, with median overall survival (OS) of 5.6 months in 1 study.[1] Several novel immunotherapeutic approaches are under development to harness the patient's immune system to attack the malignant plasma cells. Although there are 3 naked monoclonal antibodies (mAbs) approved for treatment of MM targeting CD38 or SLAMF7, many other known myeloma antigens could serve as therapeutic targets (Figure 1). Several off-the-shelf novel immune approaches using these targets are under investigation for MM, including antibody-drug conjugates (ADCs), bispecific antibodies, trispecific antibodies, and designed ankyrin repeat proteins (DARPins). ADCs and bispecifics, in particular, have demonstrated single-agent activity in RRMM and belantamab mafodotin, an anti-BCMA ADC, was approved by the FDA in August 2020 for RRMM with 4 prior

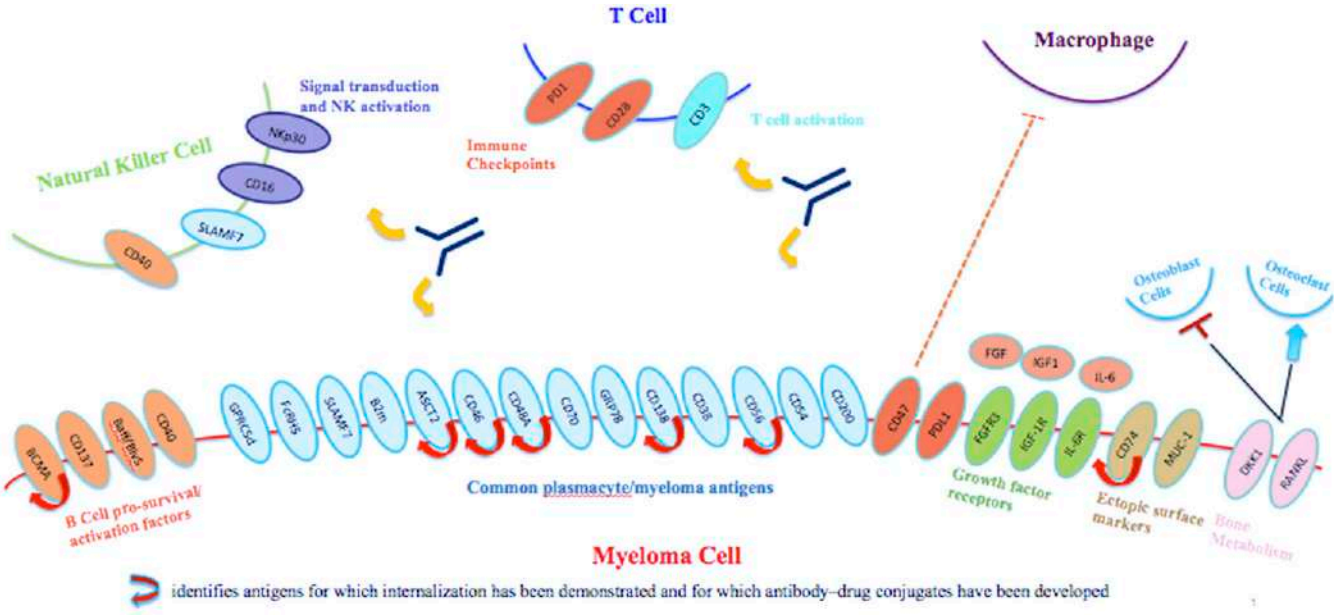

Figure 1. Antibody targets in multiple myeloma. There are numerous myeloma cell targets under investigation for immunotherapeutic approaches. The schematic is simplified, because not all of these targets are necessarily expressed on the cell surface, although those noted as undergoing internalization make ideal targets for ADCs. Other targets are expressed on cells comprising the immune microenvironment, including T cells, NK cells, and macrophages.

lines of therapy. We review the state of development of each class and the data presented to date.

Antibody-drug conjugates

Antibody-drug conjugates (ADCs) enhance naked antibodies by attaching a potent cytotoxic agent to the mAb via a stable linker (Figure 2). After the antibody binds to a cell surface antigen and undergoes receptor-mediated endocytosis, the ADC is trafficked to the lysosome where the linker is cleaved and the cytotoxic agent is released intracellularly.[2] The agent (the amount determined by the drug/antibody ratio [DAR]) accumulates in antigen-expressing cells while sparing other cells and limiting systemic toxicities. Belantamab mafodotin, an anti-BCMA ADC, was approved by the FDA in August 2020 for RRMM with at least 4 prior lines including an IMiD, PI, and anti-C38 mAb, while several other ADCs are in clinical trials for MM (Table 1).

The most advanced ADC in development for MM is belantamab mafodotin (GSK2857916), which consists of a humanized IgG-1 anti-B-cell maturation antigen (BCMA) mAb linked to the microtubule inhibitor monomethyl auristatin F (MMAF) at a DAR of 4.[3] The cysteine linker is not cleavable by proteases, making the ADC stable in the circulation. Belantamab mafodotin binds to BCMA, a member of the tumor necrosis factor superfamily expressed primarily on plasma cells, yet virtually absent on naive and memory B cells. Upon internalization, the ADC releases its payload, MMAF, to cause direct cytotoxicity. In addition, the Fc portion has been afucosylated to enhance antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis.

In DREAMM-1, a multicenter phase 1 trial,[4] 38 patients with RRMM were given belantamab mafodotin at 0.03 to 4.60 mg/kg in a 1-hour IV infusion every 3 weeks. No dose-limiting toxicities (DLTs) were observed, and the maximum tolerated dose (MTD) was not reached. Corneal events, thrombocytopenia, and anemia were the most common adverse events (AEs). In 35 heavily pretreated patients given 3.40 mg/kg,[5] the overall response rate (ORR) was 60%, although in 13 patients with prior daratumumab exposure, the ORR was lower at 39%.

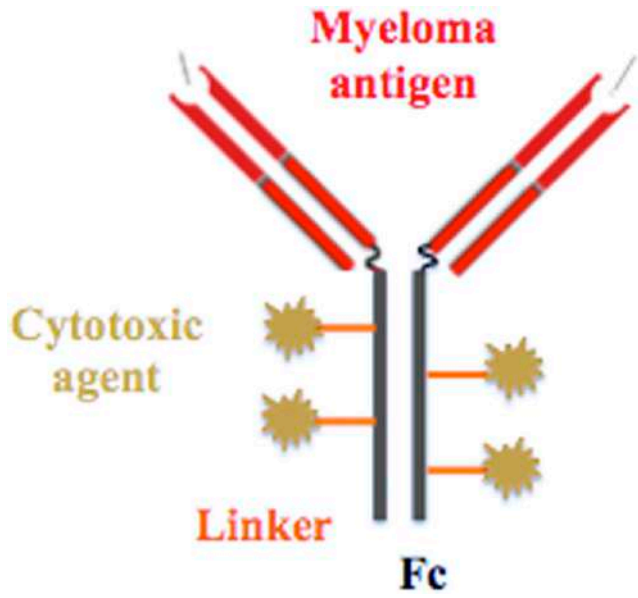

Figure 2. Antibody-drug conjugate. In addition to the antibody structure with antigen-binding domains, there are noncleavable linkers attaching the cytotoxic drugs to the Fc portion of the antibody. The drug-antibody ratio varies by agent and can affect cytotoxicity, stability in the circulation, and immunogenicity.

Table 1. Clinical trials for antibody-drug conjugates in MM

Agent	Target	Toxin	Phase	Clinical trial number	Status
Belantamab mafodotin (GSK2857916)	BCMA	MMAF	1/2	NCT03715478	Ongoing
			1/2	NCT03848845	Ongoing
			1/2	NCT03544281	Ongoing
			1/2	NCT04126200	Ongoing
			1	NCT04177823	Ongoing
			1	NCT03828292	Ongoing
			1	NCT04398680	Not yet recruiting
			1	NCT04398745	Not yet recruiting
			2	NCT03525678	Completed
			1	NCT02064387	Completed
AMG224	BCMA	Mertansine (DM1)	1	NCT02561962	Completed
Medi2228	BCMA	Pyrrolobenzodiazepine	1	NCT03489525	Ongoing
CC99712	BCMA	MMAE	1	NCT04036461	Ongoing
TAK-573	CD38	Attenuated interferon-α	1/2 1	NCT03215030 NCT03215030	Ongoing Not yet recruiting
TAK-169	CD38	Shiga-like toxin	1	NCT04017130	Ongoing
FOR46	CD46	MMAE	1	NCT03650491	Ongoing
Lorvotuzumab mertansine (1MGN901)	CD56	Mertansine (DM1)	1 1	NCT00991562 NCT00346255	Completed Completed
STRO-001	CD74	Maytansinoid	1	NCT03424603	Ongoing
Indatuximab ravtansine (BT062)	CD138	Ravtansine (DM4)	1/2a 1/2a 1	NCT01638936 NCT01001442 NCT00723359	Completed Completed Completed

PBD (DM1), pyrrolobenzodiazepine.

The multicenter phase 2 study, DREAMM-2, included 196 patients with RRMM with at least 3 prior lines of therapy and refractory to a PI, IMiD, and refractory to or intolerant of an anti-CD38 monoclonal antibody.[6] Patients were randomized to receive either 2.5 or 3.4 mg/kg of belantamab mafodotin. After a 13-month follow-up, the ORRs were 31% and 35% in a population that underwent a median of 7 or 6 lines of therapy, respectively, including ~5% complete response (CR). The median progression-free survival (PFS) was 2.8 and 3.9 months; however, the median duration of response (DOR) was encouraging at 11 and 6.2 months, and the median OS at 14.9 and 14.0 months. respectively. Efficacy outcomes were comparable for high-risk (~45% of the population) and standard-risk patients.

Keratopathy microcystlike epithelial changes in the 2 study arms occurred in 72% and 77% of patients, including grade 3 and 4 events in 46% and 42%, respectively. Corticosteroid eye drops were ineffective, and the changes were managed with artificial tears and dose delays (median, ~80 days) or modifications. The keratopathy was attributed to MMAF, given that ocular toxicities had not occurred with other cytotoxic conjugates. Many patients experienced blurred vision, which may have significant implications for quality of life. Thrombocytopenia (38% and 57%) and anemia (21% and 27%) were the next most common grade 3 and 4 AEs, whereas primarily grade 1 and 2 infusion reactions occurred in 21% and 16%, respectively, mostly during the first infusion. In comparison with bispecific anti-BCMA agents, grade 3+ infections occurred infrequently in 6% and 11% of the 2 groups, respectively. AEs led to treatment discontinuation, dose reduction, and dose delays occurred in ~10%, 40%, and 60% of patients.

Results of the addition of belantamab mafodotin 2.5 mg/kg to a bortezomib-dexamethasone backbone (DREAMM-6) in 18 patients were recently presented,[7] and whereas the ORR of 78% was encouraging, grade 3 keratopathy occurred in 10 patients (55%) and grade 3+ thrombocytopenia in 11 (61%). Additional trials evaluating belantamab mafodotin in combination with both approved and investigational agents are ongoing.

To date, 2 other ADCs are in clinical development, with reports of early-phase clinical data. Preliminary phase 1 results of STRO-001, an anti-CD74 mAb linked to a maytansinoid payload in a 2:1 DAR, have been reported in 25 patients with B-cell malignancies.[8] Of those, 14 were patients with MM, and 1 was reported to have SD. The main AEs included fatigue, pyrexia, cough, nausea, headache, and infusion reactions, with 2 DLTs (2 thromboembolic events). No ocular toxicity was reported.

AMG224, an anti-BCMA mAb linked to mertansine (DM1), showed a 23% ORR in RRMM. The main AEs included thrombocytopenia, anemia, neutropenia, aspartate aminotransferase

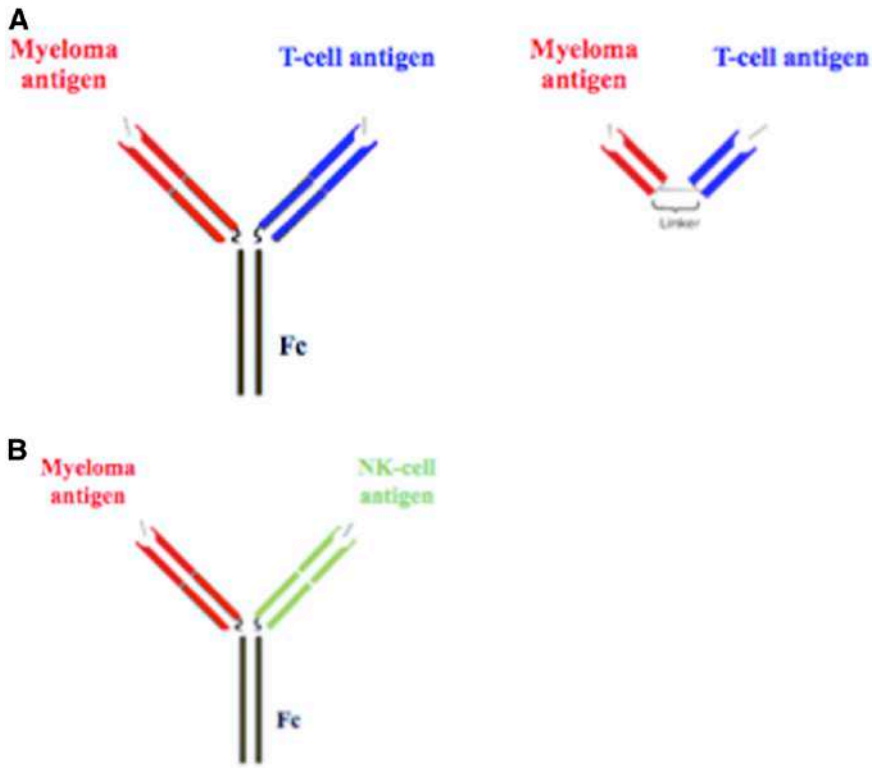

Figure 3. Bispecific antibodies. (A) Bispecific T-cell engagers bring CD3+ T cells in proximity to cells expressing tumor antigen, to form an immunologic synapse and promote cell-mediated cytotoxicity. The Fc portion provides stability in the circulation, allowing for intermittent rather than continuous dosing, and can also promote antibody-dependent cellular cytotoxicity and complement activation. These constructs vary widely by agent, and the schematics shown are only representative. There can be variability in antigen-binding domains and dimerization (homodimers vs heterodimers), resulting in differences in antigen-binding sites (valency), geometry, size, and flexibility, all of which can result in different pharmacokinetic and pharmacodynamic properties. (B) Bispecific NK-cell engager, with 1 binding domain for the myeloma antigen and 1 for NK antigens leading to signal transduction and NK-cell activation.

elevation, myalgias, and ocular toxicities.[9] Other ADCs targeting BCMA (MEDI2228,[10] CC99712), CD46 (FOR46[11]), and CD38 (TAK-573[12] and TAK-169[13]) are currently in phase 1 trials. Lorvotuzumab mertansine (IMGN901), an anti-CD56 mAb linked to mertansine, and indatuximab ravtansine (BT062), an anti-CD138 mAb linked to the microtubule inhibitor DM4,

Table 2. Clinical trials for bispecific antibodies in multiple myeloma

Agent	Targets	Phase	Clinical trial number	Status
AMG420	BCMAxCD3	1	NCT03836053	Completed
AMG701	BCMAxCD3	1/2	NCT03287908	Ongoing
CC-93269	BCMAxCD3	1	NCT03486067	Ongoing
PF-06863135	BCMAxCD3	1	NCT03269136	Ongoing
REGN5458	BCMAxCD3	1/2	NCT03761108	Ongoing
JNJ-64007957	BCMAxCD3	1b 1	NCT04108195 NCT03145181	Ongoing Ongoing
TNB-383B	BCMAxCD3	1	NCT03933735	Ongoing
GBR1342	CD38xCD3	1/2	NCT03309111	Ongoing
AMG424	CD38xCD3	1	NCT03445663	Ongoing
JNJ-64407564	GPRC5dxCD3	1b 1	NCT04108195 NCT03399799	Ongoing Ongoing
BFCR4350A	FCRH5xCD3	1	NCT03275103	Ongoing

Table 3. Comparison of anti-BCMA modalities

	CAR-T cells	Bispecific antibodies	ADCs
Pros	Unprecedented response rates, including MRD negativity in heavily pretreated patients	Off the shelf	Off the shelf
	One-time intervention; long chemotherapy holiday, resulting in median PFS ~1 year	Deep responses	Encouraging response rates
		Limited severe CRS; ? elderly	1-hour infusion every 3 weeks
		Can be given in community settings	No CRS
			Can be given in community settings
Cons	Manufacturing time makes it impractical for patients with aggressive or rapidly progressing disease	? Need for admissions with initial doses until CRS risk is low	Ocular toxicity; requires close collaboration with ophthalmology and may negatively impact quality of life
	Requires complex infrastructure, with a stem cell laboratory and nursing and ICU/ER training; thus restricted to accredited centers	Limited data in triple class/ pentarefractory	Thrombocytopenia
	CRS; ? role in elderly and frail patients	Dosing/schedule to be determined	Need for continuous treatment until progression
	Impact of bridging chemotherapy on duration of remission	Need for continuous treatment until progression	Modest ORR and PFS in triple class/ pentarefractory
	Cost, given relapses occur, even in MRD^{-} patients	Toxicities require further study; neuropathy, infections	
	Low white cells and platelets after CAR-T requiring ongoing/frequent monitoring and treatment		
	Management of CAR-T relapses challenging, especially if soon after fludarabine/cyclophosphamide, given impact on T cells		

ICU/ER, intensive care unit/emergency room.

showed modest activity in early-phase trials, and their development has been discontinued.[14-17]

Bispecific antibodies

Bispecific antibodies are designed to bind a tumor antigen while binding cytotoxic immune effector cells, usually T cells and sometimes NK cells, which are then activated to kill the nearby tumor cells (Figure 3).[18] Although there are many different bispecific constructs, the 2 major classes are those with an Fc region and those without. Bispecifics without an Fc region are small and easily penetrate tumor tissues.[19] The main drawback is a short half-life, requiring frequent or continuous dosing. Various "half-life extenders" can be added, including polyethylene glycol, polyethylene glycol–mimetic polypeptides, or albumin-binding moieties. Fc-containing bispecific antibodies are larger and more stable in the circulation and have the added advantage of Fc-mediated effector functions including ADCC and complement fixation.[20] At present, blinatumomab (a CD19xCD3 bispecific T-cell engager) is the only bispecific agent approved for use in cancer; however, the field is rapidly evolving, and many agents are in trials for MM (Table 2).

AMG420, targeting BCMAxCD3, was the first bispecific to have data reported on treatment of MM.[21] In a phase 1 study, it was administered as a continuous IV infusion for 4 weeks followed by 2 weeks off (6-week cycles). Of the 42 patients enrolled, 16 (38%) experienced cytokine release syndrome (CRS), with 1 grade 3 toxicity. The most common serious AEs were infection (14; 33%), including 5 central line infections and 2 cases (5%) of polyneuropathy (PN). Because of a grade 3 CRS and a grade 3 PN at a dose of 800 μg/d, the MTD was 400 μg/d. Two AE- but nontreatment-related deaths were from influenza/aspergillosis and adenovirus-related hepatitis. Patients had a median of 5 lines of prior therapy, although only 38%, 48%, and 21% were refractory to PIs, IMiDs, and daratumumab, respectively. At the MTD, the ORR was 70% (7 of 10), including 5 minimal residual disease (MRD)–negative CRs (MRD measured at a sensitivity of 10^{-4} by flow cytometry), 1 very good PR (VGPR), and 1 PR. Median time to response was 1 month and median DOR was 9 months. Although the efficacy results are encouraging, given the impracticality of continuous IV dosing, AMG701, a BCMAxCD3 bispecific with a half-life–extending Fc domain that allows for weekly dosing, is currently in a phase 1/2a trial.[22]

The largest BCMAxCD3 study presented to date is teclistamab (JNJ-64007957),[23] a humanized IgG-4 bispecific. In a phase 1 study of IV doses ranging from 0.3 to 720 μg/kg, predominantly with step-up dosing in 78 patients, 56% experienced CRS, none of which were grade 3+ and all of which were generally confined to initial doses. Two DLTs were grade 4 delirium (n = 1) and grade 4 thrombocytopenia (n = 1). Infections occurred in 65% of patients; 21% were grade 3+. Two deaths from AEs were grade 5 respiratory failure in the setting of pneumonia (deemed unrelated), and 1 death was caused by COVID-19. In a population with

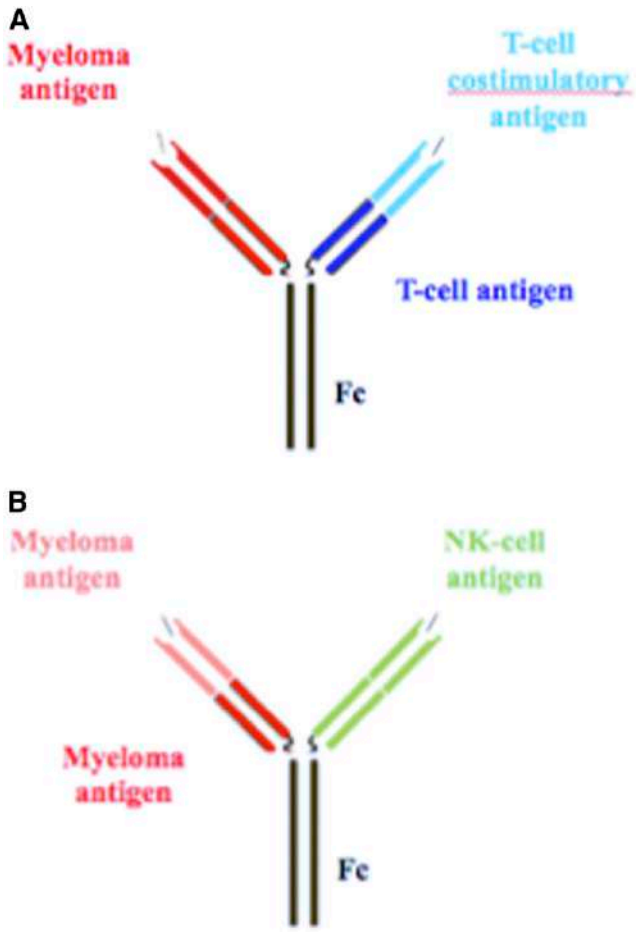

Figure 4. Trispecific antibodies. (A) Trispecific T-cell engagers, with 1 binding domain for the myeloma antigen and 2 for the T-cell antigens, which include CD3 and a costimulatory antigen. These schematics are representative of trispecifics and not the actual constructs. (B) Trispecific NK-cell engager with 2 myeloma antigen-binding domains and 1 NK-cell antigen domain.

a median of 6 lines of prior therapy and 80% triple-class refractory, the ORR at weekly dosing of 38.4 to 180 μg/kg (n = 44) was 30%. At 270 μg/kg (n = 12) the ORR was 67% (50% ≥VGPR), including 4 of 5 evaluable patients who were MRD$^-$ at 10^{-6}. Ongoing response was noted in 16 of 21 patients. Subcutaneous administration is also being studied.

A recent report of a phase 1 trial of CC-93269, an asymmetric 2+1 bispecific with bivalent BCMA binding, monovalent CD3 binding, and a half-life–extending Fc domain, showed early promising results.[24] Nineteen patients were enrolled, with a median of 6 prior therapies and most refractory to a PI, IMiD, and daratumumab. Grade 3 or 4 neutropenia (53%), anemia (42%), infections (26%), and thrombocytopenia (21%) were common. CRS was seen in most (90%) patients, and all but 1 were grade 1 or 2. In the 12 patients who received at least 6 mg as the initial dose, ORR was 83%, including 4 (33%) stringent CRs and 9 (75%) MRD$^-$ at 10^{-5} by flow cytometry. All responses were ongoing at a short median follow-up of 2.1 to 4.7 months.

Several other BCMAxCD3 bispecifics are under development, but data are limited. PF-06863135 was tested in 17 heavily pretreated patients, with a median of 11 prior therapies, and 5 patients with prior anti-BCMA (bispecific antibody or chimeric antigen receptor T-cell [CAR-T]) therapy.[25] There was only 1 MR and 5 SD, which may reflect the overall poor health of the T-cells of these heavily treated patients. Over 40 patients have now been treated, with dose-dependent responses and CRS as the main toxicity. Data on REGN5458 are available in just 3 elderly, heavily treated patients, showing a VGPR and an SD at the first dose level,[26] and TNB-383B[27] is in a phase 1 trial with no data reported yet.

There are limited data on the mechanisms of disease progression after BCMA-targeted treatments. However, recently, whereas the expression of BCMA on residual MM cells decreased more in patients responding to anti-BCMA CAR-T-cell therapy, BCMA expression increased at progression in most patients.[28] Therefore, such patients could receive other anti-BCMA therapies, although the kinetics of T-cell recovery after fludarabine/cyclophosphamide lymphodepleting chemotherapy used before CAR-T may be important for the efficacy of bispecific antibodies. The sequencing of these therapies is an area of active investigation. For now, the choice of modality depends on the disease characteristics, side-effect profiles, and practical considerations outlined in Table 3.

As BCMA-targeted therapies move closer to approval and widespread use, there will be an unmet need for BCMA-refractory patients. Bispecifics with several other antigen targets are currently in phase 1 trials. The orphan G protein–coupled receptor, class C group 5 member D (GPRC5D), is a 7-transmembrane G protein–coupled receptor whose ligand and signaling mechanisms are unknown. However, GPRC5D messenger RNA is primarily expressed in plasma cells and all neoplastic plasma cells. There is low expression in normal tissues, except in hair follicles, and in MM, overexpression correlates with worse OS.[29] Results from a phase 1 trial of the humanized immunoglobulin IgG4 GPRC5dxCD3 talquetamab (JNJ-64407564) involving >100 patients (to be presented at the 2020 American Society of Hematology annual meeting) include typically low-grade CRS in initial doses, as with other bispecific antibodies. Although efficacy has been seen at a variety of doses, including stringent CRs and durable responses, dose escalation is ongoing.

FCRH5, a B-cell lineage marker present universally on malignant plasma cells,[30] is the target (along with CD3) of BFCR4350A.[31] BFCR4350A has been tested in >40 patients, with CRS as the expected toxicity and responses seen at multiple dose levels. Two bispecifics, GBR1342[32] and AMG424,[33] target CD38xCD3; it will be interesting to see how efficacy and safety compare with currently available anti-CD38 naked mAbs. Although still in preclinical development, bispecific anti-BCMA NK-cell engagers (for example, AFM-26 targeting CD16[34] and CTX-4419[35] or CTX-8573[36] targeting NKp30) may prove to have

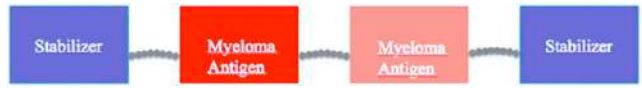

Figure 5. Designed ankyrin repeat proteins (DARPins), with 2 DARPins serving as stabilizers in the circulation and 2 targeting myeloma antigens. DARPins are much smaller than antibodies and can be linked in various numbers and combinations.

more efficient ADCC with potentially less CRS and may represent another promising avenue of research.

Trispecific antibodies

Although still in the preclinical stages of development, trispecific antibodies provide an intriguing future approach to the treatment of MM (Figure 4). Bispecifics typically target a tumor antigen and CD3 to bring cytotoxic T cells into proximity and form an immunologic synapse with malignant cells, leading to T-cell activation against the tumor. However, in the absence of costimulation, there is a higher likelihood of anergy,[37] leading to a suboptimal antitumor response. Wu, et al. recently demonstrated that a trispecific antibody targeting CD38, CD3, and CD28, a well-known costimulatory protein on T cells, was feasible to produce and showed very potent killing of CD38$^+$ myeloma cell lines, 3- to 4-log higher than daratumumab.[38] The trispecific agent suppressed myeloma growth in mice and promoted proliferation of memory and effector T cells and downregulation of regulatory T cells in primates. Similarly, efforts are ongoing to create trispecific NK-cell engagers, targeting CD16A and the MM antigens BCMA and CD200. Clinical trials of trispecific antibodies are eagerly awaited.

Designed ankyrin repeat proteins

Designed ankyrin repeat proteins (DARPins) are a class of genetically engineered antibody-mimetic proteins derived from ankyrin proteins, which are among the most common binding proteins in nature. They have high binding affinity and specificity for their designated targets.[39] They are typically much smaller (<20 kDa) than antibodies and can be linked to inhibit multiple pathways at once (Figure 5).[40] MP0250 is the first DARPin product to be tested in MM and contains vascular endothelial growth factor A and hepatocyte growth factor–neutralizing DARPins, although without an immunostimulatory component.[41] Preliminary results from a phase 2 trial combining MP0250 8 mg/kg IV every 21 days with bortezomib and dexamethasone were recently reported[42]; common grade 3+ events were hypertension (40%), thrombocytopenia (25%), proteinuria (20%), and anemia (20%). All 20 patients had been exposed to an IMiD and PI, and the ORR was 40% (67% in patients who had received a PI with the prior regimen). These results show that DARPins are a feasible technology in MM, and the addition of immunostimulatory components may further increase the effectiveness of this class of therapy.

Clinical case and conclusions

The patient was enrolled in a clinical trial with the ADC belantamab mafodotin. She experienced progression of disease after 2 cycles and was then treated with 1 cycle each of bortezomib-dexamethasone-cyclophosphamide-etoposide-cisplatin (V-DCEP) infusional chemotherapy and daratumumab-carfilzomib-thalidomide-dexamethasone with no response. After 1 cycle of the GPRC5dxCD3 bispecific talquetamab clinical trial, she achieved a VGPR, and after 5 cycles, she achieved and maintained a stringent CR, amounting to 10+ months of deep, durable disease control.

The treatment landscape of MM is rapidly changing, with 8 drugs approved by the US Food and Drug Administration in the past 8 years. Despite this encouraging progress, relapses of MM remain inevitable, and novel treatment approaches are urgently needed. Through various distinct technologies and classes of agents, immunotherapy holds promise as the next wave of novel therapies for MM.

Conflict-of-interest disclosure

J.R. has been on the Speakers Bureau of Celgene and Janssen and served on advisory boards and consulted for Celgene, Janssen, BMS, Takeda, Oncopeptides Adaptive Biotechnologies, Secura Bio, X4 Pharmaceuticals, Sanofi, Karyopharm, and Antengene. A.C. received research funding from and served as a consultant and on advisory boards for Janssen, Clegene, Novartis, and Amgen; consulted for Bristol-Myers Squibb; served on advisory boards for Karyopharm, Sanofi, and Oncopeptides; received research funding from and served on advisory boards for Seattle Genetics and Millennium Pharmaceuticals/Takeda; and received research funding from Pharmacyclics. G.L. declares no competing financial interests.

Off-label drug use

None disclosed.

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Future of CAR T cells in multiple myeloma

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Despite the significant improvement in survival outcomes of multiple myeloma (MM) over the past decade, it remains an incurable disease. Patients with triple-class refractory MM have limited treatment options and a dismal prognosis. Chimeric antigen receptor (CAR) T-cell therapy targeting B-cell maturation antigen has transformed the treatment armamentarium of relapsed/refractory MM (RRMM), with unprecedented overall response rates in this difficult-to-treat patient population. However, a significant proportion of patients ultimately relapse despite achieving deep remission. Several innovative approaches, including alternative/dual-antigen–specific CAR T-cell constructs, genetically engineered "off-the-shelf" CAR T cells, and strategies to counteract an immunosuppressive microenvironment, may dramatically reshape the field of CAR T-cell therapy in the future. These strategies are being actively investigated in preclinical and early clinical trial settings with the hopes of enhancing the durability of responses and, thereby, improving the overall survival of RRMM patients after CAR T-cell therapy.

LEARNING OBJECTIVES

- Summarize the current landmark clinical trials of CAR T cells for RRMM
- Describe the underlying mechanism of failure in patients with RRMM treated with CAR T-cell therapy
- Discuss the ongoing investigational strategies to overcome current barriers and enhance CAR T-cell efficacy in RRMM

Clinical case

A 65-year-old female was diagnosed with high-risk immunoglobulin G λ multiple myeloma (MM), International Staging System (ISS) stage III, in March of 2014. Bone marrow study at the time of diagnosis revealed extensive involvement by monoclonal plasma cells (90%) with fluorescence in situ hybridization cytogenetics analysis positive for +1q and −13q. She underwent induction therapy and autologous stem cell transplant in September of 2014 and achieved a partial response (PR), followed by lenalidomide maintenance. Her disease progressed in August of 2015. Since then, she relapsed after multiple lines of therapy, consistent with triple-class refractory myeloma. Ultimately, in September of 2017, she was evaluated for anti–B-cell maturation antigen (BCMA) chimeric antigen receptor (CAR) T cells. Bone marrow pathology revealed clonal plasma cells of 30%. She was treated with anti-BCMA CAR T-cell therapy, MCARH171 (dose, 450 × 10^6 total CAR T cells) after receiving fludarabine-cyclophosphamide lymphodepletion (LD) chemotherapy. The end-of-treatment evaluation at day 30 postinfusion showed a 63% reduction in monoclonal protein (from 1.16 g/dL to 0.43 g/dL), an undetectable free light chain, and no evidence of abnormal plasma cells in bone marrow, consistent with PR.

Introduction

Over the past decades, the treatment landscape for patients with MM has evolved significantly. The incorporation of several novel therapies, including immunomodulatory agents, proteasome inhibitors, and, more recently, monoclonal antibodies, to the MM treatment paradigm has improved the response rate and survival of these patients. However, MM generally remains an incurable disease. Historically, patients who fail to respond or relapse early after these novel-based treatments carry a dismal prognosis and ultimately die of disease progression.[1]

CAR T-cell therapy for relapsed/refractory MM

Recently, clinical trials of CAR T-cell therapy against MM-associated antigens have demonstrated promising clinical activity, providing unprecedented response rates in these heavily pretreated patients. The target of most active CAR T-cell trials in MM is B-cell maturation antigen (BCMA).

BCMA, a member of the tumor necrotic factor receptor superfamily, is highly specific to and expressed on the surface of plasmablasts, plasma cells, and activated B cells; thus, it is an attractive target for cellular immunotherapy of MM.[2] In all studies, patients received LD chemotherapy with fludarabine and cyclophosphamide. In 1 such study, Raje et al investigated idecabtagene vicleucel (Ide-cel; previously bb2121), lentiviral vector–based 4-1BB-CD3ζ BCMA-targeted CAR T cells.[3] The initial phase 1 report was of 33 patients with heavily treated relapsed/refractory MM (RRMM). The overall response rate (ORR) was 85%, with a complete response (CR) rate of 45%. Sixteen patients achieved minimal residual disease (MRD)-negative status at a sensitivity of $\leq 10^{-4}$ cells. Most patients attained a response early after infusion, with a median time to first PR or better of 1.0 month. The incidence of cytokine release syndrome (CRS) was high (25 patients, 76%), but severe (grade ≥ 3) CRS only occurred in 2 patients. Recently, Munshi et al reported initial results of the follow-up phase 2 open-label KarMMa trial of 128 RRMM patients treated with Ide-cel at a dose of 150 to 450 × 10^6 CAR T cells.[4] The study confirmed the efficacy of Ide-cel with an ORR and CR rate of 73% and 33%, respectively. Among patients who attained CR, 33% achieved MRD negativity at a sensitivity of 10^{-5} nucleated cells. Several other groups have reported results for BCMA-directed CAR T cells. A handful of studies of BCMA CAR T cells in RRMM have demonstrated remarkable response rates and well-tolerated adverse event profiles (Table 1). In addition to Ide-cel, JNJ-68284528 (previously known as LCAR-B38M, cilta-cabtagene autoleucel, lentiviral; CAR T-cell product containing 2 BCMA-targeting single domain nanobodies) and JCARH125 (orva-cabtagene autoleucel, lentiviral; fully human 4-1BB-CD3ζ CAR) are among several BCMA CAR T-cell products that have advanced into later stages of clinical trials. It is worth noting that the difference in safety and efficacy profiles between trials could be attributed to several factors (eg, CAR T-cell constructs, LD chemotherapy, patient's characteristics). Although the data from the original bb2121 study showed a low response rate (33%) in the 50 × 10^6 CAR T-cell cohort, and at least a very good partial remission (VGPR) was observed only in ≥150 × 10^6 cell cohorts, deep responses were seen in the 50 × 10^6 cell cohorts in the trials using JCARH125 and FCARH143 (lentiviral vector transduced fully human 4-1BB-CD3ζ CAR T cells with a defined ratio of $CD4^+$/$CD8^+$ lymphocytes in the final product) while still being able to safely dose escalate to similarly high doses; however, the clinical relevance of an optimal CAR T-cell dose remains unknown.[5,6] Recently, the phase 1b/2 CARTITUDE-1 study investigating LCAR-B38M BCMA CAR T cells, the identical BCMA CAR T-cell product used in the LEGEND-2 study,[6,7] reproduced an ORR of 100% in this heavily pretreated RRMM setting (CR rates of 74% and 86% in updated results from LEGEND-2 and CARTITUDE-1, respectively).[7-9] The updated results from the EVOLVE study (JCARH125, 300 to 600 × 10^6 cell cohorts) demonstrated a high response rate (ORR 92%, CR 36%) and an excellent safety profile. Several phase 3 clinical trials comparing BCMA CAR T cells with standard-of-care treatment options in earlier disease settings (eg, Ide-Cel in KarMMa-3 [NCT03651128] and LCAR-B38M in CARTITUDE-4 [NCT04181827]) are enrolling patients.

Clinical case (continued)

The patient continued to do well on posttreatment surveillance. Response assessment at 6 months after infusion showed M protein of 0.08 g/dL (93% reduction from pre–CAR T-cell treatment) and normal free light chain ratio, consistent with VGPR. There was no evidence of abnormal plasma cells in the bone marrow, including negative MRD by multiparametric flow cytometry (sensitivity 10^{-5} nucleated cells). At 1 year, serum protein electrophoresis showed undetectable monoclonal protein, but a persistent monoclonal band on immunofixation was present, consistent with a persistent MRD-negative VGPR.

Response kinetics and durability

Delayed clearance of monoclonal protein is commonly observed after BCMA CAR T-cell therapy, thus translating into a prolonged duration until maximal response is achieved. Treatment response in CAR T-cell clinical trials is based upon the reduction of monoclonal protein and resolution of extramedullary plasmacytoma, according to International Myeloma Working Group criteria. The depth of response by monoclonal protein in MM patients and its prognostic value depend on the time of assessment.[10] In recent years, disease assessment using highly sensitive methods to detect MRD was integrated into the International Myeloma Working Group response criteria. A negative MRD status is strongly associated with superior outcomes in patients achieving at least a VGPR.[11,12] The discordance between serum and bone marrow response could reflect a difference in test sensitivity, significance of the assessment time point, and/or potential sampling error. The updated long-term results of the CARTITUDE-1 trial showed that the median time to CR was 3 months, indicating that a more profound response can be achieved over time.

The median progression-free survival (PFS) after BCMA CAR T-cell therapy was ~12 months (range, 6-15 months), depending upon the study. In the updated Ide-cel BCMA CAR T-cell study, the median PFS of all treated patients was 8.8 months (95% confidence interval, 5.5-11.6), with increased median PFS in higher-dose cohorts (5.8 months in the 300 × 10^6 cell cohort and 11.3 months in the 450 × 10^6 cell cohort; 8.6 months for the whole cohort).[4] PFS outcomes for other CAR T-cell trials in RRMM are shown in Table 1.

Clinical case (continued)

The patient remained in remission for 18 months after BCMA-targeted CAR T-cell therapy. However, in May of 2019, laboratory results showed progressively increased serum free light chain. A bone marrow study showed 10% abnormal plasma cells, consistent with relapse.

Current limitations and potential strategies to overcome treatment failure

Despite an exceptional response rate observed across several BCMA-targeted CAR T cells, response durability has remained an ongoing clinical dilemma, because a significant proportion of patients eventually relapse.

Similar to $CD19^+$ B lymphoid malignancies, the mechanisms of CAR T-cell therapy failure in MM are multifactorial, involving patient-, malignancy-, and immune-associated factors. Tumor with low or negative antigen that evades CAR T-cell eradication (antigen escape) is 1 underlying mechanism of relapse after cellular immunotherapy. Downregulation or loss of BCMA expression was observed in patients who relapsed after CAR T-cell therapy.[6,13,14] However, unlike $CD19^+$ lymphoid malignancy, mutations at the DNA level have not been reported. CAR T-cell–mediated trogocytosis, a process by which malignancy-associated surface proteins are extracted from the cell surface via lymphocyte-tumor engagement, is another mechanism that could result in

Table 1. Selected landmark clinical trials of BCMA-targeted CAR T cells in RRMM (with n > 10)

Study	n	Phase	Vector	Product	Costimulatory domain	LD chemotherapy	CAR T-cell dose	Lines of prior treatment, median (range), n	Triple class refractory, %	Previous ASCT, %	CRS any grade, %	CRS grade ≥3, %	ICANS grade ≥3, %	Anti–IL-6 therapy, %	ORR, %	≥VGPR, %	CR, %	MRD negative, %*	PFS, median	OS, median
CRB-401[3]	33	1	Lenti	Ide-cel (bb2121)	4-1BB	Flu/Cyc	50/150/450/800 × 10^6 cells	7 (3-23)	N/A	97	76	6	3	21	85	72	45	94 (15/16; ≥PR patients)	11.8 mo	N/A
KarMMA[4]	128	2	Lenti	Ide-cel (bb2121)	4-1BB	Flu/Cyc	150/300/450 × 10^6 cells	6 (3-16)	84	94	84	6	3	2	73	53	33	33 (26/128; CR patients)	8.8 mo	19.4 mo
LEGEND-2[9]	57/74	1	Lenti	LCAR-B38M (JNJ68284528)	4-1BB	Cyc	0.5 × 10^6 (0.07-2.1) cells/kg	3 (1-9)	N/A	18	90	7	0	46	89	78	74	68.4 (39/57; CR patients)	19.9 mo	36.1 mo
CARTITUDE-1[7,50]	29	1b/2	Lenti	LCAR-B38M (JNJ68284528)	4-1BB	Flu/Cyc	0.75 × 10^6 (0.5-1.0) cells/kg	5 (3-18)	86	86	93	7	3	76	100	97	86	81 (13/16; CR patients)	87% (9 mo)	N/A
EVOLVE[5]	44	1	Lenti	Orvacabtagene autoleucel (JCARH125)	4-1BB	Flu/Cyc	50/150/450 × 10^6 cells	7 (3-23)	N/A	68	80	9	7	34	82	48	27	67 (6/9) at day 29 (≥PR patients)	N/A	N/A
EVOLVE[47]	62	1	Lenti	Orvacabtagene autoleucel (JCARH125)	4-1BB	Flu/Cyc	300/450/600 × 10^6 cells	6 (3-18)	94	94	89	3	3	76	92	68	35	96 (21/25) at 3 mo (≥PR patients)	N/A	N/A
NCI[13]	16	1	Retro	N/A	CD28	Flu/Cyc	9 × 10^6 cells/kg	9.5 (3-19)	N/A	N/A	94	38	19	31	81	63	13	100 (≥PR patients)	31 wk	N/A
UPENN[14]	25	1	Lenti	N/A	4-1BB	None or Cyc	10/50/100/500 × 10^6 cells	7 (3-13)	44	92	88	32	12	28	63	28	8	33 (≥PR patients)	65-125 d	502 d
P-BCMA-101[45]	23	1/2	PiggyBac transposon	P-BCMA-101	4-1BB	Flu/Cy	51/152/456/845/1143 × 10^6 cells	6 (3-11)	N/A	83	10 (2/21)	0	5 (1/21)	5 (1/21)	63 (12/19)	26 (5/19)	N/A	N/A	N/A	N/A
FHCRC[6]	11	1	Lenti	FCARH143	4-1BB	Yes (not specified)	50/150 × 10^6 cells	11 (8-20)	91	82	18	0	0	18	100	82	36	N/A	N/A	N/A
CT053[51]	24	1	Retro	CT053	4-1BB	Flu/Cyc	150 × 10^6 cells	4.5 (2-11)	N/A	42	63	0	4	53 (8/15)	88	83	79	85 (17/20)	N/A	N/A
CT103A[52]	18	1	Lenti	CT103A	4-1BB	Flu/Cyc	1/3/6/8 × 10^6 cells/kg	4 (3-6)	N/A	39	94	22	0	N/A	100 (17/17)	88 (15/17)	71 (12/17)	100 at 10^{-4} (≥PR patients)	N/A	N/A

ASCT, autologous stem cell transplant; Cyc, cyclophosphamide; Flu, fludarabine; ICANS, immune effector cell associated neurotoxicity syndrome; IL-6, interleukin-6; Lenti, lentivirus; N/A, not available or not applicable; OS, overall survival; PFS, progression-free survival; Retro, retrovirus.
*MRD negative at a sensitivity of 10^{-5} cells.

decreased target antigen density.[15] Lack of CAR T-cell persistence is likely another contributing factor to relapse in these patients. In addition, the immunosuppressive effects of the tumor microenvironment (TME) and malignant plasma cells on the function of CAR T cells potentially play a role in the resistance to immune-based therapy in patients with MM.

Overcoming antigen loss: beyond BCMA and polyspecific CAR T-cell constructs

The potential strategy to overcome an antigenic loss in relapse after CAR T-cell therapy includes sequential/combined infusion with CAR T cells against targets other than BCMA, CAR T cells with novel dual-targeting vector design, and BCMA expression upregulation. In addition to BCMA, several antigens have been identified and explored as potential targets of immunotherapy, including adoptive cellular therapy for MM (Figure 1). These antigens include, but are not limited to, CD138, G-protein–coupled receptor class C group 5 member D (GPRC5D), transmembrane activator and calcium-modulator and cyclophilin ligand, signaling lymphocytic activation molecule family 7, natural killer group 2 member D (NKG2D) ligands, CD229, and integrin β7.[16] Most of these non-BCMA–targeted CAR T cells are in early-stage clinical trials or preclinical phase studies. Our group demonstrated that GPRC5D is expressed on the surface of CD138$^+$ multiple myeloma cells, independent of BCMA expression, but it is minimally expressed in other cell lines, with the exception of hair follicles; thus, it is a potential target of engineered immune effector cell–based therapy.[17]

In addition to CAR T cells targeting antigens other than BCMA, engineering dual-targeted T cells is actively being investigated. Recently, our group reported preclinical data investigating dual-targeting approaches for CAR T-cell therapy, using BCMA and GPRC5D as a model. The study showed a superior antimyeloma response using a bicistronic construct encoding 2 independent 4-1BB CARs in preclinical models of MM with varying antigen expression compared with coinfusion of separate CAR T cells or a single-stalk tandem single-chain variable fragment (scFv) CAR design.[18] In contrast to the results of this study, Zah and colleagues recently reported superior results using a tandem scFv "single-stalk" CAR design targeting BCMA and CS1.[19] Transduction efficiency and gene expression were the limiting factors of the bicistronic approach; these challenges were not encountered in our study. Both studies revealed a trade-off between targeting 1 or the other antigens with a tandem single-stalk CAR design. There is reason to be hopeful that both approaches will enhance efficacy in patients, and it will be important to see how these strategies impact the durability of responses in the clinic.

One of the first clinical trials exploring such a dual-targeted approach was reported by Li et al, who evaluated a BCMA/CD38 tandem single-stalk CAR.[20] Results from 22 patients with ≥2 prior lines of therapy included an ORR of 91% and a CR rate of 54.5%. Clinical trials using a split apheresis product transduced with unique vectors targeting distinct antigens, a so-called "CAR pool approach," are also underway. Yan et al reported a phase 2 study of combined treatment with anti-CD19 and anti-BCMA CAR T cells in 21 RRMM patients.[21] The median lines of prior therapies was 6 (range, 4-17), and 3 patients (14%) underwent autologous stem cell transplantation before CAR T-cell therapy. The investigators indicated that this strategy is a safe and active approach, with ORR, VGPR or better, CR or better, and MRD negativity rates of 95%, 81%, 57%, and 81%, respectively. The median PFS of patients who achieved a VGPR or better was 8 months (NCT04162353).

As discussed above, data from BCMA monotargeted CAR T-cell therapies have demonstrated decreased BCMA expression density after anti-BCMA CAR T-cell treatment.[6,13,14] Preliminary data showed that γ-secretase inhibitor (GSI) inhibited the cleavage of BCMA and increased its expression on the plasma cell surface. It was hypothesized that this might improve the efficacy of BCMA CAR T cells in the future.[22,23] Administration of an oral GSI with BCMA CAR T cells is being explored (NCT03502577).[24] Cowan et al reported the preliminary results of this approach in patients with RRMM, with an ORR of 100% among 6 evaluable patients.[24] All patients had increased BCMA expression on the plasma cell surface on serial bone marrow biopsies after receiving the GSI.

Impeding host immune response: decreasing CAR antigenicity and combating suppressive TME

An antimurine host immune response to CAR is a potential insult that can result in compromised in vivo CAR T-cell persistence.

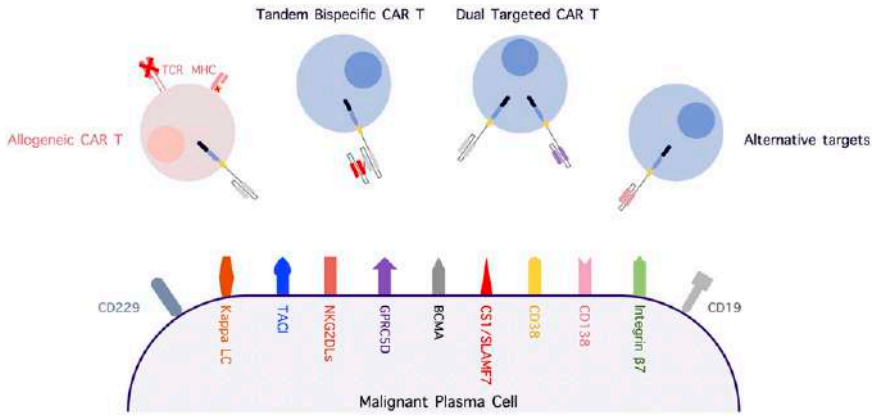

Figure 1. Alternative myeloma-associated targets for immune-based therapy and strategies involving novel CAR T-cell constructs. CS1, CD2 subset 1; LC, light chain; MHC, major histocompatibility complex; NKG2DLs, NKG2D ligands; SLAMF7, signaling lymphocytic activation molecule family 7; TACI, transmembrane activator and CAML interactor; TCR, T-cell receptor.

This was shown to be a clinically relevant concern for CD19-targeted CAR T-cell therapy in large cell lymphoma,[25] and it was recently found to be a potential concern for BCMA-targeted CAR T-cell therapies incorporating the murine-derived 11D5-3 scFv.[26] Engineering novel CAR T cells with humanized or a fully human CAR construct is an area of active research being explored by many groups and may ultimately be critical to providing long-term durability.[21,27]

The immunosuppressive TME in bone marrow may also play an important role in resistance, immune escape, and progression of MM following CAR T-cell therapy. Preclinical and clinical data revealed a highly concentrated immune-resistant cytokine milieu and an increased number of immunosuppressive cells, including regulatory T cells, T helper 2 cells, myeloid-derived suppressor cells, cancer-associated fibroblasts (CAFs), tumor-associated macrophages, and osteoclasts (Figure 2).[28] The immunosuppressive effect of myeloma cells and TME, along with an ongoing T-cell stimulation, contributes to T-cell dysfunction and activation-induced T-cell death. Therefore, targeting TME can alleviate some essential resistance pathways of MM to CAR T cells. Sakemura and colleagues conducted a preclinical study exploring CAR T-cell product targeting of fibroblast associated protein (FAP) and BCMA/CS1 in a CAF-enriched environment. Inhibition of CAFs by FAP CAR resulted in a superior myeloma killing effect of BCMA/CS1 target CAR T cells.[29] An "armored" CAR T cell is among several approaches aiming to improve functions of engineered CAR T cells by preventing T-cell exhaustion, overcoming immunosuppressive TME, or enhancing killing function and T-cell persistence. Engineering CAR T cells to secrete programmed cell death protein 1 (PD-1) or programmed death ligand 1 (PD-L1) antibody, which selectively binds to PD-L1 expressed by various cells in TME, thus preventing endogenous PD-1/PD-L1 axis activation, may alleviate TME-induced immune escape.[30,31] Currently, an ongoing phase 1 clinical trial is exploring the safety of BCMA CAR T cells secreting a mutant PD-1 Fc fusion protein in RRMM (NCT04162119). The CAR T-cell construct inheriting an additional gene that leads to constitutive interleukin-12 secretion is another novel CAR T-cell model that was shown to have improved tumor-killing effect and overcome the immunosuppressive effect of TME in the preclinical models.[32] Silencing immune checkpoint signaling using a genome-editing technique to enhance the anti-tumor killing effect and prevent T-cell exhaustion/activation-induced cell death was tested in a preclinical model.[33,34] Recently, Stadtmauer et al presented the data from a phase 1 study of NY-ESO-1–targeted engineered T cells with a disrupted PDCD1 gene using a clustered regularly interspaced short palindromic repeats (CRISPR) gene-editing technique in 3 patients with refractory cancers, 2 of which were MM.[35] The study showed a robust in vivo expansion with durable persistence and evidence of intratumoral infiltration of engineered T cells in all 3 patients. Although the treatment response observed in this study was modest, this finding proved the feasibility and safety of immune checkpoint disruption as a platform to improve the persistence of adoptive T cells. Rafiq et al demonstrated an enhanced CAR T-cell function and trafficking of CAR T cells to tumor sites in a preclinical model of PD-1 blocking scFv-secreting CAR T cells.[30] Combining immunomodulatory agents (ie, lenalidomide) with CAR T-cell therapy was shown to enhance CAR T-cell function in the immunosuppressive TME. Works et al found that lenalidomide could potentiate cytokine production and cytolytic activities.[36] In addition, lenalidomide prevented exhaustion of CAR T cells under low-antigen or immunosuppressive environments in a xenograft model.[37]

Universal adoptive engineered cellular therapy: allogeneic and iPSC-derived immune effector cells

In addition to data available from autologous CAR T cell trials, manufacturing CAR T cells using lymphocytes from allogeneic donors has long been investigated in several types of malignancy, including MM. Several novel bioengineering methods (ie, knocking out the T-cell receptor and major histocompatibility complex expression using various gene-editing techniques) have been implemented to moderate potential graft-versus-host toxicity and host rejection. The phase 1 UNIVERSAL (NCT04093596) and MELANI-01 (NCT04142619) trials are investigating the safety and feasibility of 2 allogeneic CAR T cells in RRMM patients (Table 2). In addition to donor-derived immune effector cells, induced

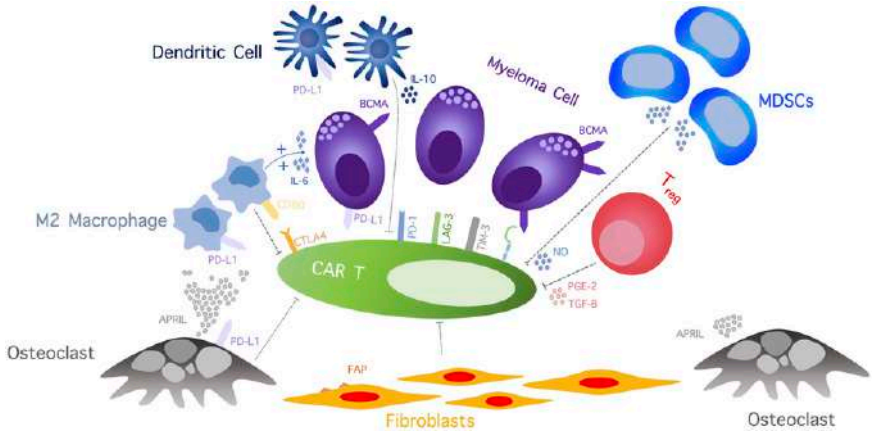

Figure 2. The complicated immunosuppressive TME effect on CAR T cells includes a wide array of cellular network and cytokines that induce CAR T-cell exhaustion, inhibit CAR T-cell function, and promote CAR T-cell apoptosis. APRIL, a proliferation-inducing ligand; IL, interleukin; LAG-3, lymphocyte-activation gene-3, MDSCs, myeloid-derived stem cells; NO, nitric oxide; PGE-2, prostaglandin E2; TGF-B, transforming growth factor β; TIM-3, T-cell immunoglobulin mucin-3; T_{reg}, regulatory T cell.

pluripotent stem cell (iPSC)-derived immune cells are a promising platform for adoptive cellular therapy. In addition to their "off the shelf" availability, iPSC-derived lymphocytes offer a unique advantage via clonal selection: a highly selected, multiply gene-edited, and consistent tumor-specific immune cell product can be produced.[38] Combining advanced techniques in developmental biology and novel genetic engineering, iPSC-derived T cells exhibit potent antitumor activity similar to conventional CAR T cells, but they tend to maintain the innate phenotype, which can translate into fewer concerns about graft-versus-host disease.[39] Several iPSC-derived CAR immune cells are currently under investigation in hematologic malignancies. Recently, Bjordahl et al reported preclinical data using FT576 cells, a novel dual-target CAR iPSC-derived natural killer (NK) cell against BCMA and CD38 that shows high cytotoxic activity against myeloma cell lines.[40] The additional hypothesized advantage of CAR NK cells is the absence of graft-versus-host disease development and a potentially lower risk for CRS compared with conventional CAR T cells. Several preclinical studies demonstrated cytotoxic activity and myeloma cell growth inhibition using CAR NK cells against various targets, including CS1, CD138, BCMA, and NKG2D ligands.[41] A phase 1/2 study of BCMA CAR NK cells in RRMM is ongoing (NCT03940833).

In addition to "off the shelf" availability, a major advantage of gene-modified allogeneic and iPSC-derived immune effector cells is the potential for superior fitness of healthy donor lymphocytes over autologous cells obtained from heavily treated patients, which can translate into better efficacy and survival outcomes. Garfall and colleagues demonstrated the influence of T-cell fitness on the function of CAR T cells as a clinically meaningful attribute of cellular therapies.[42] Pheresis products collected from patients after initial induction therapy had a higher proportion of $CD8^+CD45RO^-CD27^+$ memory T cells and $CD4^+/CD8^+$ ratio than from patients with heavily treated RRMM, which were predictors associated with clinical response in patients with RRMM treated with BCMA CAR T cells.[14] The result of this study was similar to the finding in chronic lymphocytic leukemia (CLL) patients treated with CD19 CAR T cells.[43]

Strategies to overcome intrinsic T-cell defects

In concordance with data from B lymphoid malignancies, individual T-cell subsets have different replication potential and cytotoxic capacity, which play a critical role in the function of immune effector cells. Stem cell memory T cells and other less differentiated T cells carry a high potential for in vivo expansion, survival, and persistence and may be less susceptible to activation-induced exhaustion.[44] The bb21217 anti-BCMA CAR T-cell product is generated by manufacturing T cells with phosphoinositide 3-kinase inhibitor, bb007, during the culture process to enrich the "memory-like" T-cell composition. This product induced an ORR of 83% and a toxicity profile comparable with other trials in 22 patients with RRMM.[8] P-BCMA-101 is a nonviral-based BCMA targeted CAR T-cell product using the piggyBac transposon-based manufacturing system. The product contains a high proportion of CAR T cells with a stem cell memory T cell phenotype,[45] which is hypothesized by the investigators to improve response rate and durability. Refining the ratio of $CD4^+/CD8^+$ in CAR T products is another approach that is being actively explored.[46] Examples of CAR T-cell clinical studies focusing on this approach include FCARH143, a BCMA-targeted CAR T-cell product with separate $CD4^+$ and $CD8^+$ manufacturing and reinfusion at a fixed ratio of $CD4^+/CD8^+$ T lymphocytes, as well as JCARH125, a BCMA-targeting CAR T-cell product with a single-track manufacturing process and cytokine cocktail designed to result in a consistent $CD4^+/CD8^+$ ratio, with enrichment of CAR T cells with central memory phenotype in the final product. Both of these trials demonstrated high response rates (ORR > 90%) in heavily treated RRMM.[6,47]

Other actively investigated preclinical approaches to enhance CAR T-cell persistence and function include modifying the immunoreceptor tyrosine-based activation motifs (ITAMs) in the CD3ζ chain of the CAR endodomain and constructing T-cell receptor α constant–specific CAR using various genome-editing techniques.[48,49] The typical construct of CD3ζ ITAMs in CAR T cells consists of 3 domains (ITAM1, ITAM2, ITAM3). Modulating activation potential by decreasing the expression of ITAMs affects T-cell signaling and function and controls T-cell fates. Feucht et al demonstrated improved CAR T-cell persistence in 1928ζ CAR T cells with a single ITAM domain.[48] In a murine model, generating T-cell receptor α constant–specific CAR using CRISPR/Cas9 strengthened 1928ζ T-cell potency and elicited superior tumor-killing effect compared with conventional γ-retrovirus vector CAR T cells that result in multiple integration sites.[49]

Table 2. Available allogeneic CAR-expressed immune effector cells in MM

Product	Trial	ClinicalTrials.gov identifier	Phase	Type	Target	Vector	Gene editing event	Inclusion	n (estimated)	Status
UCARTCS1	MELANI-01	NCT04142619	1	CAR T	CS1	Lentivirus	TALEN	RRMM	18	Recruiting
ALLO-715	UNIVERSAL	NCT04093596	1	CAR T	BCMA	Lentivirus	TALEN	RRMM	90	Recruiting
PBCAR269A	PBCAR269A-01	NCT04171843	1/2a	CAR T	BCMA	Adenovirus	ARCUS endonuclease	RRMM	48	Recruiting
CTX120	Unnamed	NCT04244656	1	CAR T	BCMA	CRISPR/Cas9	CRISPR/Cas9	RRMM	80	Recruiting
BCMA-UCART	Unnamed	NCT03752541	1	CAR T	BCMA	Unknown	Unknown	RRMM	20	Recruiting

ClinicalTrials.gov access date was 30 May 2020.
CS1, CD2 subset 1; TALEN, transcription activator-like effector nucleases.

Conclusions

In 2020, CAR T-cell therapy has reached a therapeutic milestone, offering great promise to patients with RRMM. However, despite an exceptional ORR, response durability remains a significant challenge. Further studies are needed to decipher current therapeutic dilemmas and to advance CAR T-cell therapy to additional disease settings for patients with MM.

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Conflict-of-interest disclosure

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Off-label drug use

None disclosed.

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EVIDENCE-BASED MINIREVIEW

Should all newly diagnosed MM patients receive CD38 antibody–based treatment?

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CD38 antibodies were first evaluated in extensively pretreated patients with multiple myeloma (MM). Currently, there are 3 CD38 antibody–based regimens approved for the treatment of both transplant-eligible (daratumumab plus bortezomib-thalidomide-dexamethasone [D-VTd]) and transplant-ineligible (daratumumab plus lenalidomide-dexamethasone [D-Rd] or daratumumab plus bortezomib-melphalan-prednisone [D-VMP]) patients with newly diagnosed MM (NDMM). The phase 3 studies that evaluated these regimens uniformly showed that the addition of daratumumab to backbone regimens improved the depth of response, which translated into improved progression-free survival and also overall survival in 2 of the studies. Importantly, elderly patients age 75 years or older benefit from these regimens, indicating that these regimens have an acceptable safety profile. Although the number of patients with high-risk cytogenetics was relatively small, these patients also experienced benefit from the addition of daratumumab to standard-of-care regimens, but poor risk conferred by the cytogenetic aberrations is not completely abrogated. Altogether, daratumumab-based regimens have high anti-MM activity and a favorable toxicity profile and therefore represent new standards of care for patients with NDMM.

LEARNING OBJECTIVES

- Review the evidence for the use of CD38 monoclonal antibodies in patients with NDMM
- Understand the benefit of CD38 antibody-based therapy in several subsets of patients, including elderly patients and patients with high-risk disease

Clinical case

A 75-year-old man presented with bone disease and anemia and was diagnosed with multiple myeloma (MM) in 2017. Additional staging revealed International Staging System (ISS) stage II disease without poor-risk cytogenetic features. He also suffered from diabetes mellitus and grade 2 diabetic neuropathy. He walked 4 to 8 km with his dog every day and had no limitations in (instrumental) activities of daily living. At that time, he came to the office to discuss his first-line treatment options.

Introduction

The treatment landscape of MM is rapidly changing with the incorporation of CD38 antibodies in first-line regimens. Daratumumab is a first-in-class, fully human, CD38-targeting antibody that showed marked activity and a favorable toxicity profile when it was first evaluated as a single agent in heavily pretreated MM patients. This resulted in the evaluation of CD38 antibodies in early relapsed MM and subsequently in newly diagnosed (ND) disease. There are currently 3 CD38 antibody-based regimens approved by the US Food and Drug Administration and European Medicines Agency for the treatment of NDMM: 2 for transplant-ineligible patients (daratumumab plus bortezomib-melphalan-prednisone [D-VMP] and daratumumab plus lenalidomide-dexamethasone [D-Rd]), and 1 for transplant-eligible patients (daratumumab plus bortezomib-thalidomide-dexamethasone [D-VTd]). We will review the efficacy of these CD38 antibody–based combination regimens and then describe the value of CD38 antibody–based combination therapy in specific MM subgroups, with a focus on elderly patients and those with high-risk cytogenetic aberrations.

CD38 antibody–based therapy for transplant-ineligible patients

Established treatment options for transplant-ineligible NDMM patients include combination therapies such as lenalidomide-dexamethasone (Rd), bortezomib-melphalan-prednisone (VMP), and bortezomib-lenalidomide-dexamethasone (VRd).

In addition, daratumumab-containing regimens are being used more often on the basis of the results from 2 randomized phase 3 trials. The ALCYONE study evaluated VMP with or without daratumumab in transplant-ineligible NDMM patients (median age, 71 years).[1,2] Patients treated with daratumumab achieved a deeper response and had improved progression-free survival (PFS) compared with patients who were treated with VMP alone. With longer follow-up, the addition of daratumumab to VMP also resulted in an overall survival (OS) benefit. However, it should be acknowledged that only 10% of the patients who developed disease progression in the VMP arm were treated with a daratumumab-based relapse regimen, which would negatively impact OS in the VMP arm.[1]

In a comparable patient population, the MAIA study (median age, 73 years) showed that adding daratumumab to continuous Rd improved the depth of response, PFS, and also PFS2 (defined as the time from random assignment to progression on the next line of therapy or death).[3,4] At a median follow-up of 36.4 months, there was no difference in OS between treatment arms, and follow-up for long-term survival is ongoing.[3,4] Importantly, faster and sustained improvement in health-related quality of life was observed in patients treated with daratumumab plus Rd compared with Rd alone.[5]

CD38 antibody–based therapy for transplant-eligible patients

The CASSIOPEIA study demonstrated that addition of daratumumab to bortezomib-thalidomide-dexamethasone (VTd, a standard-of-care induction regimen in Europe) before (induction) and after transplantation (consolidation) improved the depth of response, PFS, and although follow-up is still short, also OS.[6] In that study, 100 days after transplantation, there was a second random assignment to either observation or daratumumab maintenance (every 8 weeks until disease progression or for a maximum of 2 years).[6] Results from the second random assignment for maintenance therapy are not yet available. Several other phase 3 trials are also evaluating the value of daratumumab as maintenance therapy after auto-SCT, including the AURIGA and DRAMMATIC studies, which are evaluating maintenance with daratumumab plus lenalidomide vs lenalidomide alone in NDMM patients after auto-SCT.

Management aspects

Daratumumab-based regimens are generally well tolerated. However, adding daratumumab to standard-of-care regimens increases the frequency of infections, especially respiratory infections, probably because of a higher frequency of neutropenia, as well as induction of hypogammaglobulinemia and depletion of natural killer cells. This highlights the importance of providing adequate supportive care, including growth factor support, prophylactic antibiotics, immunoglobulin replacement therapy, and vaccination.[7] Furthermore, ~30% to 40% of patients experience a generally mild infusion reaction, mostly during the first daratumumab infusion. A big step forward is the approval of the subcutaneous formulation of daratumumab, which reduces the time of administration to 3 to 5 minutes.[8] CD38 antibodies also affect certain laboratory assays. CD38-targeting antibodies interfere with blood group compatibility testing because these antibodies also bind to CD38 molecules present on reagent or donor red blood cells.[9] Several strategies are available to negate CD38 antibody interference with blood bank compatibility tests.[9] Furthermore, CD38 antibodies can be detected as a small monoclonal band by serum protein electrophoresis and serum immune fixation electrophoresis, which may interfere with response evaluation in case the patient's M-protein and the CD38 antibody have the same heavy- and light-chain isotype and comigrate into the same region.[9] The daratumumab-specific immune fixation electrophoresis reflex assay can be used to discriminate between residual M-protein and daratumumab.[10]

Specific subgroups

Elderly patients

MM has a median age at presentation of ~70 years, and elderly patients benefit less from novel agents, probably because of a reduced ability to tolerate the therapy, which then leads to treatment discontinuation. Subgroup analyses also show that patients age 75 years or older benefit from daratumumab-based regimens with improved response rates and better PFS (Table 1).[1-3,11] The ALCYONE study also demonstrated improved OS in elderly patients who received daratumumab added to VMP, but this improvement did not reach statistical significance (Table 1).[1] These studies also show that daratumumab-based therapy is well tolerated in elderly patients. However, the elderly population is very heterogeneous, and frailty assessment is needed to further define their frailty profile. Data on frailty are not currently available in the MAIA and ALCYONE studies.

Nevertheless, there is already preliminary evidence from a phase 2 study that daratumumab-based therapy is feasible in frail patients. The HOVON143 study showed that daratumumab combined with ixazomib and dexamethasone is effective in unfit and frail NDMM patients.[12] Importantly, the Intergroupe Francophone du Myelome has a phase 3 study that is evaluating the efficacy and tolerability of subcutaneous daratumumab plus lenalidomide without dexamethasone vs lenalidomide plus dexamethasone in a frail NDMM patient population. A dexamethasone-free regimen will probably reduce the frequency of adverse events, such as infections, psychiatric adverse effects, and diabetes mellitus and thereby prevent treatment discontinuations, which have a major impact on survival.

High-risk disease

Interpreting the value of CD38 antibody–based therapy for patients with high-risk cytogenetic aberrations is challenging because of the relatively small numbers of patients with high-risk disease in the different studies (Table 2). However, a recent meta-analysis of randomized phase 3 trials showed that incorporating daratumumab into backbone regimens was associated with a significantly improved PFS among patients with high-risk disease (presence of del(17p), t(4;14) or t(14;16)) in both the ND and relapsed/refractory setting.[13] PFS data for all individual phase 3 studies evaluating CD38 antibodies in NDMM and relapsed/refractory MM are provided in Table 2. Importantly, poor risk conferred by high-risk cytogenetic abnormalities is not completely abrogated by adding a CD38 antibody to backbone regimens (Table 2).[1-3,6]

We will soon learn whether other regimens, such as daratumumab or isatuximab combined with VRd or carfilzomib-lenalidomide-dexamethasone (KRd) further improve the poor outcome conferred by high-risk cytogenetic aberrations. Alternatively, these patients may also benefit from new strategies that incorporate agents with novel mechanisms of action such as T-cell redirecting therapies. Daratumumab added to VMP/Rd/VTd also improved PFS in other subgroups with

Table 1. Comparison of ALCYONE and MAIA studies

	ALCYONE[1,2,11]						MAIA[3,4,20]					
	All patients		Age <75 y		Age ≥75 y		All patients		Age <75 y		Age ≥75 y	
	D-VMP	VMP	D-VMP	VMP	D-VMP	VMP	D-Rd	Rd	D-Rd	Rd	D-Rd	Rd
No. of patients	350	356	246	249	104	107	368	369	208	208	160	161
Median follow-up, mo	40.1*		40.1*		40.1*		36.4†		36.4†		36.4†	
PR or better (%)	90.9	73.9	92.3	75.5	87.5	70.1	93	82	95	82	90	81
CR or sCR (%)	46	25	48	26	41	24	50	27	52	25	41	25
MRD negativity (10^{-5}) (%)	28	7	22	6	24	8	29	9	27.9	7.2	19.4	7.5
PFS												
HR	0.42		0.49		0.53		0.56		0.49		0.62	
95% CI	0.34-0.51		0.36-0.68		0.32-0.85		0.44-0.71		0.35-0.69		0.44-0.87	
OS												
HR	0.61		0.56		0.71		NR		NR		NR	
95% CI	0.46-0.80		0.40-0.79		0.44-1.13							
Grade ≥3 (%)												
Neutropenia	40	39	35	38	52	42	51	35	43	31	60	41
Infections	23	15	21	13	28	20	36	27	32	23	33	24
Infusion-related reactions (all grade) (%)	27.7	NA	24	NA	36	NA	40.9	NA	44.9	NA	35.7	NA

Patient population: NDMM, ineligible for transplant; Eastern Cooperative Oncology Group performance status, 0-2; creatinine clearance, ≥40 mL/min in ALCYONE and ≥30 mL/min in MAIA.
HR, hazard ratio; NA, not applicable; NR, not reported; PR, partial response; sCR, stringent complete response.
*PFS and MRD data for the subgroups and toxicity data are based on the analysis with a follow-up of 16.5 months.[2]
†Response and toxicity data for the subgroups is based on the analysis with follow-up of 28.0 months.[20]

high-risk features, such as in patients with reduced renal function or ISS stage III disease.[1-3,6]

First-line CD38-based treatment

Altogether, these studies indicate that the efficacy and safety of daratumumab in combination with standard-of-care regimens is now established in NDMM, and this supports the use of daratumumab-based regimens at diagnosis for both young and elderly patients. However, there are several open questions. First, in transplant-ineligible patients, D-Rd and D-VMP have not been compared head-to-head with VRd, which is commonly used in the United States and was also recently approved in Europe for transplant-ineligible patients. The SWOG S0777 study (median age, 63 years) showed an improved PFS and OS in patients treated with VRd compared with Rd.[14] In that study, only 43% of the patients were age 65 years or older because both transplant-ineligible patients and younger patients who were not planned for immediate first-line transplantation could be enrolled.

Although comparisons between these trials should be made with caution because of major differences in age distribution, the median PFS with VRd was 34 months in patients age 65 years or older,[14] whereas median PFS was 36.4 months with D-VMP, and PFS at 3 years was 68% with D-Rd. This indicates that, next to the new daratumumab-based regimens, VRd continues to represent an appropriate standard-of-care treatment whereby dose-adjusted VRd can be used in older patients to improve tolerance.[15] Treatment choice is also dependent on the overall tolerability profile of the available therapeutic regimens. An advantage of the D-Rd regimen is the low rate of treatment-emergent neuropathy compared with the bortezomib-containing regimens.[2,3,16] Other factors, including patient characteristics (eg, presence of comorbidities, frailty, and age), patient preferences, and reimbursement and availability issues, also have an impact on treatment selection. Importantly, bortezomib is given for a fixed period of time in the VRd regimen, whereas daratumumab is given until progression in the D-Rd and D-VMP regimens, which has important financial implications. Ongoing phase 3 trials are evaluating whether adding a CD38 antibody to the VRd regimen (daratumumab in the CEPHEUS trial and isatuximab in the IMROZ trial) results in additional survival benefit. Furthermore, less intensive 2-drug regimens such as Rd remain important options for frail patients.

Second, in many countries, VRd is the preferred regimen for transplant-eligible patients, and there are no studies comparing D-VTd with VRd before and after transplantation. The randomized phase 2 GRIFFIN study evaluates the value of the addition of daratumumab to VRd in transplant-eligible patients. Preliminary results show an improved depth of response including complete response and minimal residual disease (MRD) negativity with daratumumab added to VRd.[17,18] At a median follow-up of 22.1 months, there was not yet a PFS or OS benefit for patients treated with daratumumab; follow-up for long-term survival is ongoing.[17,18] Several studies are also evaluating carfilzomib, a second-generation proteasome inhibitor, plus lenalidomide-dexamethasone as a backbone for CD38 antibody–based combinations, aiming at further increasing the proportion of patients with sustained MRD negativity.

Table 2. Impact of high-risk cytogenetic aberrations on PFS

Definition of high-risk cytogenetic profile	Studies in patients with NDMM						Studies in patients with RRMM											
	MAIA[4]		ALCYONE[21]		CASSIOPEIA[6]		POLLUX[22]		CASTOR[23]		CANDOR[24]		ICARIA[25]		IKEMA[26]	
	t(4;14), t(14;16), or del(17p) by FISH or karyotype analysis		t(4;14), t(14;16), or del(17p) by FISH or karyotype analysis		del(17p) with cutoff 50% or t(4;14) with cutoff 30% by FISH		t(4;14), t(14;16), or del(17p) by FISH or karyotype analysis		t(4;14), t(14;16), or del(17p) by FISH or karyotype analysis		del(17p), t(14;16) or t(4;14) by FISH		del(17p) with cutoff of 50%; t(14;16) with cutoff 30%; or t(4;14) with cutoff 30% by FISH		del(17p) with cutoff of 50%; t(14;16) with cutoff 30%; or t(4;14) with cutoff 30% by FISH	
Cytogenetic risk category	**Standard**	**High**	**Standard**	**High**	**Standard**	**High**	**Standard**	**High**	**Standard**	**High**	**Standard**	**High**	**Standard**	**High**	**Standard**	**High**
No. of patients	550	92	518	98	914	168	369	70	258	95	156	74	181	60	192	73
Median follow-up, mo	36.4	36.4	27.8	27.8	18.8	18.8	44.3	44.3	50.2	50.2	16.9	16.9	11.6	11.6	20.7	20.7
PFS																
HR	0.50	0.57	0.34	0.78	0.41	0.67	0.43	0.34	0.25	0.41	0.55	0.58	0.62	0.66	0.44	0.72
95% CI	0.38-0.65	0.32-1.04	0.26-0.45	0.49-1.26	0.26-0.62	0.35-1.30	0.32-0.57	0.16-0.72	0.18-0.35	0.21-0.83	0.31-0.97	0.30-1.12	0.42-0.93	0.33-1.28	0.27-0.73	0.36-1.45

FISH, fluorescence in situ hybridization; RRMM, relapsed/refractory multiple myeloma.

Furthermore, there are no trials comparing the strategy of first-line daratumumab vs CD38 antibody–based therapy at the time of first relapse. Although patients can receive a daratumumab-based regimen at the time of first relapse, we favor the use of CD38 antibodies for first-line therapy because in real-world clinical practice, only two-thirds of patients receive more than 1 line of therapy, which is more common in elderly patients.[19] We also favor the use of the best drugs, including daratumumab, for first-line therapy to induce the deepest response possible, because clinical outcomes correlate with depth of response. Importantly, quality of life is best preserved during the first remission and gradually diminishes with each subsequent progression because of a cumulative burden of therapy and disease-related complications (eg, vertebral fractures). In addition, if results of a large daratumumab retreatment study are positive (NCT03871829), the design of the phase 3 studies in transplant-eligible patients allows for retreatment (eg, fixed duration of daratumumab in CASSIOPEIA, and stopping daratumumab in patients with sustained MRD negativity in PERSEUS).

Clinical case (continued)

We discussed with the patient the different treatment options inside and outside a clinical trial. Because the patient was an intermediate-fit 75-year-old man, our treatment goal was to induce a deep and durable response with a triplet or quadruplet regimen. Bortezomib-based regimens, including D-VMP and VRd, were not preferred options because bortezomib may aggravate the diabetic neuropathy. After counseling, he initiated treatment with D-Rd in the setting of a clinical trial, with aspirin as thrombosis prophylaxis and cotrimoxazol as antibacterial prophylaxis. After 6 months, dexamethasone was tapered and later stopped because of adverse events including insomnia. After 9 months, the dose of lenalidomide was eventually reduced from 25 mg to 10 mg because of fatigue. He achieved a complete response with no recurrence of disease up to this point, and daratumumab was continued according to schedule.

In conclusion, CD38 antibodies have transformed MM treatment with several phase 3 studies showing that both transplant-eligible and transplant-ineligible NDMM patients benefit from daratumumab-containing triplet or quadruplet regimens. Importantly, patients age 75 years or older also experience clinical benefit from adding daratumumab to VMP or Rd, which points to the favorable toxicity profile of this drug. Patients with high-risk cytogenetics also benefit from these new treatment strategies, but to a lesser extent than standard-risk patients, highlighting the need for the earlier incorporation of new drugs into their first-line treatment. Altogether, CD38 antibody–based combinations represent the new standard of care for NDMM patients, based on both high antitumor activity and an acceptable safety profile. The use of these regimens should, therefore, be considered as a first treatment option for both transplant-eligible and transplant-ineligible MM patients.

Conflict-of-interest disclosure

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Off-label drug use

None disclosed.

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Improving symptom control and reducing toxicities for pediatric patients with hematological malignancies

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The continuing improvement in pediatric cancer survival over time is largely attributable to the availability of intensive therapies. Increasing attention has been focused on addressing the physical and psychosocial impacts of cancer and cancer treatments. Evidence from adult oncology suggests that routine symptom screening and feedback to health care providers can improve patient-clinician communication, reduce distress, and improve quality of life and may even increase survival. Many questions remain regarding implementation of routine symptom screening in pediatric cancer care, including the best symptom assessment instrument and the reporter type and feasibility of integration with electronic health records (EHRs). Nonsymptom adverse events are also important, for both routine clinical care and adverse event reporting for patients enrolled in clinical trials. However, traditional mechanisms for reporting adverse events lead to substantial inaccuracies and are labor intensive. An automated approach for abstraction from EHRs is a potential mechanism for improving accuracy and reducing workload. Finally, identification of symptom and nonsymptom toxicities must be paired with prophylactic and therapeutic strategies. These strategies should be based on clinical practice guidelines that synthesize evidence and use multiprofessional, multidisciplinary expertise to place this evidence in clinical context and create recommendations. How best to implement clinical practice guidelines remains a challenge, but EHR order sets and alerts may be useful. In summary, although survival is excellent for pediatric patients receiving cancer therapies, more focus is needed on identification of symptoms and nonsymptom toxicities and their management. The EHR may be useful for promoting better supportive care through these mechanisms.

LEARNING OBJECTIVES

- Understand the importance of routine symptom screening for pediatric patients with hematological malignancies
- Understand the importance of rigorously developed supportive care clinical practice guidelines

Clinical case

A 12-year-old girl who had completed chemotherapy for acute myeloid leukemia (AML) 6 months ago presented with bone marrow relapse and was enrolled in a clinical trial. She stated that she felt "terrible" during most of her initial treatment and asked whether this next treatment would make her feel even worse. Her parents were with her and asked whether there was anything that could be done to help her cope with the toxicities that they were expecting with the new treatment plan. During the first cycle of chemotherapy, she developed bacteremia and most likely had pulmonary aspergillosis. The clinical research associate was uncertain how to report these toxicities accurately.

Introduction

Pediatric cancer care has benefitted from decades of successive clinical trials, resulting in continual improvement in survival for children and adolescents with cancer. Currently, >82% of pediatric patients with cancer survive at least 5 years after diagnosis. Although precision medicine and targeted therapies are[1] promising approaches, the foundation of pediatric cancer treatment remains [2]conventional chemotherapy, surgery, and radiotherapy. There has been increasing recognition that these therapies have an adverse impact on pediatric patients and negatively affect their quality of life. Consequently, increasing attention has been turned toward addressing the physical and psychosocial effects of cancer and cancer treatments.

This article focuses on measuring symptoms and nonsymptom toxicities, in the context of both routine care and clinical trials, and also addresses the implementation of evidence-based prophylactic and therapeutic strategies to reduce these toxicities.

Symptom screening in routine care

Evidence supporting the hypothesis that routine symptom screening could improve outcomes first emerged in the adult cancer literature. Observational studies suggested that routine collection of patient-reported outcomes would improve patient-clinician communication,[4] reduce distress,[5] and improve quality of life.[6] The strongest evidence supporting the importance of routine symptom screening came from randomized trials. In one trial, 766 adults with metastatic solid tumors were randomized into a routine symptom screening group or a standard-of-care group. The routine symptom screening group reported 12 common symptoms at clinic visits, and those with computers also received weekly e-mail prompts. Patients in the intervention group had significantly improved quality of life, fewer emergency department visits, and fewer hospitalizations than those in the standard-of-care control group.[6] In a follow-up analysis, median overall survival was 31.2 months (95% confidence interval, 24.5-39.6) in the routine symptom screening group vs 26.0 months (95% confidence interval, 22.1-30.9) in the standard-of-care group (P = .03).[7] These studies set the stage for routine symptom screening in pediatric patients with cancer.

The choice of instrument to use for routine symptom screening has been addressed in 3 systematic reviews of system assessment scales used in pediatric patients with cancer.[8-10] Scales most commonly used were the Memorial Symptom Assessment Scale (MSAS) 10-18, the MSAS 7-12, the Symptom Distress Scale, and the Symptom Screening in Pediatrics Tool (SSPedi). In the most recent systematic review,[9] more than half of the identified studies involved electronic administration of the symptom assessment scale, which is likely to be well received in pediatric populations.

SSPedi is a self-reported, 15-item symptom screening tool created specifically for children receiving cancer treatments (Figure 1). It measures the extent of discomfort of 15 symptoms as follows: disappointment or sadness, fear or worry, cranky or angry disposition, problems with concentration or memory, bodily or facial changes, fatigue, mouth sores, headache, other pain, tingling or numbness, vomiting, changes in appetite, changes in taste, constipation, and diarrhea. SSPedi is available in paper-based and electronic formats, with the latter having an audio feature that provides oral reading of the entire instrument or specific questions. In a multicenter study conducted in Canada and the United States, SSPedi was reliable (internal consistency, test-retest reliability, and interrater reliability), valid (construct validity), and responsive to change in 502 English-speaking children 8 to 18 years of age who were receiving cancer therapies.[3] It has been translated into Spanish and French.[11] The most commonly reported bothersome symptoms are shown in Figure 2. A self-report version for children 4 to 7 years of age (mini-SSPedi[12]) and a proxy-report version for children 2 to 18 years of age[13] have also been developed.

Once an instrument has been identified, an important question is whether it is the best reporter of the pediatric patient's symptoms for clinical implementation. It has long been recognized that the patient is the best reporter of symptoms.[14] However, in pediatric cancer care, there are many scenarios where pediatric patients either cannot or will not report their symptoms. Such scenarios include children who are too young, those with cognitive impairment, or those who are too ill.[15] Thus, an ideal symptom screening approach must be flexible enough to allow for different types of reporter. We have proposed a novel method for symptom reporting that involves a structured dyadic approach. This approach may be particularly useful in young children.[16] Whether the approach is feasible and valid, however, remains to be answered.

Another important issue is the mechanism by which symptoms are collected and reported to health care professionals, with the 2 broad options being stand-alone systems or integration into electronic health records (EHRs; Table 1). There are many advantages of incorporating symptom data into EHRs, such as efficiency of integration into the workflow of clinicians and ability to link documented symptoms to orders for intervention. Some vendors (eg, Epic Systems Corporation; Verona, WI) have developed programs that enable the capture of patient-reported outcomes. However, these programs generally have not had modules that are specific to pediatric patients. Advantages of using stand-alone systems include agility in development and modification and compatibility across health systems with programs from different EHR vendors.

Finally, many practical questions remain to be addressed, including the ideal frequency for reminders, treatment periods during which symptoms should be elicited, and the best approach to providing results of symptom screening to health care professionals. These questions are likely to be addressed through the next generation of clinical trials. Nonetheless, the status of symptom screening in pediatric cancer is currently at the interface between research and clinical implementation, and this pendulum is likely to swing toward clinical implementation in the future.

Capturing nonsymptom toxicities in clinical practice and clinical trials

Capture of nonsymptom toxicities presents a range of different challenges. Documentation of toxicities by clinicians is crucial for understanding a patient's experience during treatment. Identification of adverse events (AEs) has been guided for many trials and patients by the United States National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE).[17] The CTCAE was developed in 1983 to standardize reporting of AEs across clinical trials.[18-20] However, over time, it has increased in complexity, and the most recent version, CTCAE v5, includes more than 800 AEs.[17,20] Although this complexity increases the potential for more granular reporting, it also raises concern about potential variation in approaches to identifying toxicities. Some individuals may report specific signs or symptoms, whereas others may report a syndrome that encompasses many individual toxicities. For example, tumor lysis syndrome could be reported as the syndrome; as individual components of hyperkalemia, hyperphosphatemia, hypocalcemia, and hyperuricemia; or as both. CTCAE may also be inappropriately used, depending on the subjective or complex nature of the definitions.[21] Variation in reporting approaches and interpretation causes difficulties in determining accurate toxicity rates for specific chemotherapy regimens. Further, CTCAE definitions cause particular challenges for pediatric patients, despite the addition of pediatric-specific criteria beginning with CTCAE

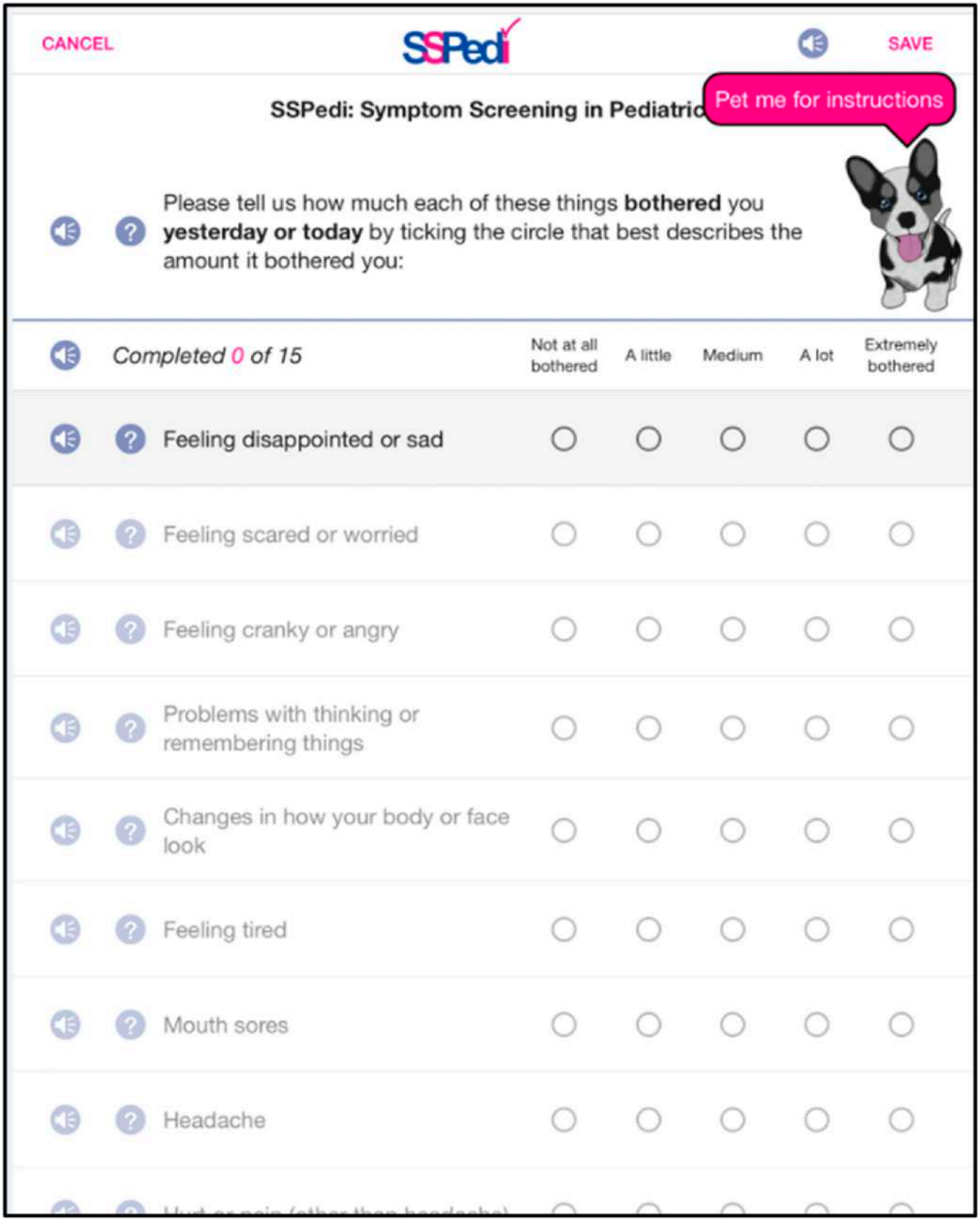

Figure 1. **Symptom screening in a pediatrics tool.**

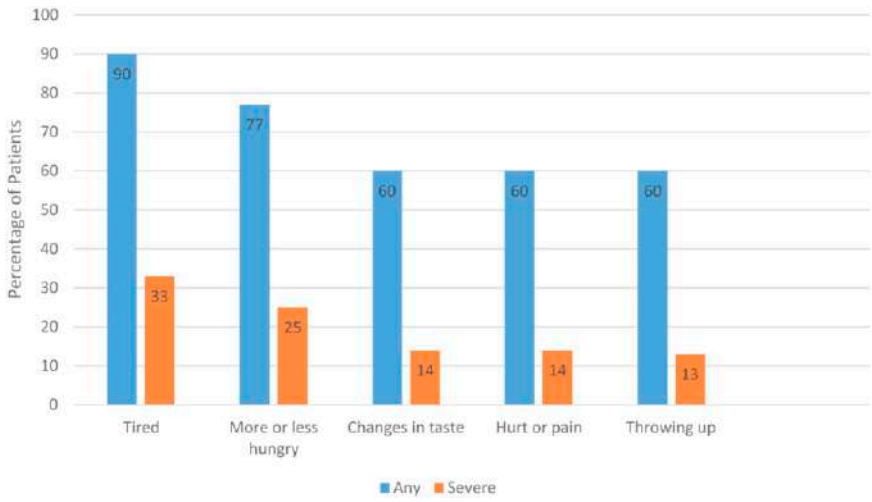

Figure 2. Most common severely bothersome symptoms among inpatients 8 to 18 years of age. Adapted from Johnston et al.[39]

v3.0.[20] For example, many CTCAE definitions rely on activities of daily living, which vary widely by age and patient, especially among children who meet developmental milestones at different ages.[20]

For patients enrolled in National Cancer Institute cooperative group trials, reporting of AEs is mandatory. AE rates reported from clinical trials may be the only source of information regarding the toxicity of specific chemotherapy combinations. In these circumstances, trial AE rates are the only data source that guides clinicians and patients regarding potential treatment-related toxicities. On clinical trials, AE capture is typically performed via manual medical record review and reporting by clinical research associates (CRAs). This process is labor intensive and is only one of many responsibilities that CRAs have.[22] Studies have shown that, despite the effort devoted to it, toxicities are underreported.[23,24] One study demonstrated that AE reports in a trial for pediatric AML had less than 50% sensitivity for 8 of 12 clinically relevant AEs when compared with the gold-standard chart abstraction.[25] This underreporting means that clinicians do not have an accurate understanding of toxicity rates and therefore cannot truly prepare patients for potential toxicity during therapy. Further, phase 3 clinical trials typically report the highest grade of toxicity experienced during each chemotherapy course, but the grade may not fully represent the experience of the patient, especially those receiving prolonged oral chemotherapy, such as children with acute lymphoblastic leukemia who are in maintenance therapy. Efforts must be implemented to capture and describe toxicity profiles over time.

Similar to the benefits of using EHRs to collect symptom data from patients, EHRs may be leveraged to improve capture of nonsymptom toxicities recorded by clinicians (Table 1). Automated ascertainment that extracted laboratory data directory from the EHR, cleaned and processed the data, and graded laboratory-identified AEs according to CTCAE criteria had high accuracy at a single institution.[26] This automated R package, ExtractEHR, was implemented at 3 hospitals to obtain laboratory result data and described accurate rates of laboratory-identified AEs by chemotherapy course for pediatric patients undergoing therapy for acute lymphoblastic leukemia or AML.[27] Work is ongoing using the same package to capture non–laboratory-identified toxicities in pediatric patients with leukemia across multiple centers. Some institutions have integrated AE capture systems into the EHR to alert clinicians and track AEs over time, although no study on this approach has been published from pediatric centers.[28] Trigger tools to alert clinicians to AEs have also been tested, but a recent study reported low positive predictive value when using a medication-based trigger.[29] Further tailoring of such tools is needed realize clinical benefit.

Table 1. Leveraging EHRs for symptom screening, identification of nonsymptom toxicities, and improving CPG-concordant care

Target areas	Components
Facilitate symptom screening	Allow patient self-report or proxy report to track symptoms
	Allow health care professionals to view symptom scores
Automated capture of toxicities	Extraction of data directly from the EHR
	Clean data to ensure complete data capture
	Remove false results
	Grade toxicities according to standard grading systems
Enhance guideline-concordant supportive care	Order sets consistent with CPGs
	Build alerts when symptoms or other toxicities are identified
	Incorporate management recommendations into alerts

Accurate capture of nonsymptom toxicities is crucial for understanding the patient's experience and ultimately implementing measures to reduce toxicity. EHR-based ascertainment has the potential to standardize approaches to capture of AE and reduce the manual effort required for reporting. If implemented widely, this approach would improve accuracy and efficiency and free CRAs to capture more complex toxicities or to perform other responsibilities. This improved knowledge of AEs would also educate clinicians, improve identification of AEs for patients in clinical practice, and provide a resource clinicians can use to guide patients regarding toxicities that may occur during therapy.

Interventions to improve symptoms

Preventing toxicity and relieving symptoms is essential in the holistic care of children undergoing cancer therapy and their families. Delivering this care, triggered by symptom scores or toxicity assessment or by a predicted high probability that problems will occur, should be informed by the same high quality of evidence that underpins cancer care. This belief is the basis for the development of high-quality supportive care clinical practice guidelines (CPGs).

CPGs are implementable pieces of evidence synthesis[30] (Figure 3). They define the clinical situation to be addressed; use explicit and comprehensive methods to search for and appraise the risk of bias of studies that address clinical situations; and with multiprofessional, multidisciplinary expertise, place these elements in clinical context to aid in recommending action (or inaction) and in defining areas of needed research. Guidance documents, from narrative reviews to expert position statements, vary in the rigor of their development and in the transparency in decision making. They require experts to offer their expertise during development, but their views are considered in the context of the evidence, and the trail of thinking is laid bare, rather than hidden in wise pronouncements. CPGs are not just meta-analyses, although high-quality evidence synthesis with systematic reviews and similar studies are the bricks from which they are built.

CPGs should be patient centered, valid, accessible, and practical. Assessing which outcomes and experiences are of greatest importance to families and clinicians has been prioritized in the development of many supportive care CPGs, including the following: avoiding death from toxicity[31]; shortening the hospital stay[32]; and minimizing pain,[33] mucositis,[34] nausea,[35] and fatigue.[36] CPG validity can be assessed by using a formal assessment tool (such as AGREE II [Appraisal of Guidelines for Research and Evaluation])[37] or by using guidelines endorsed by an organization that has undertaken such an assessment.

Implementation of guidelines, that is, converting their bald academic recommendations into something the clinical team can act on, is a separate skill. Local knowledge and significant leadership are needed to change the behaviors of a clinical team. A series of studies have been undertaken to assess how well CPGs effect change and the best techniques for the assessment, but much more research is needed.[38] Tools such as integrated care pathways, prepopulated test order sets, and 1-touch prescription protocols linked to other elements of the EHR can help (Table 1). Patient-led interaction and high-quality education linked to emotional motivation may be effective as well. Printing a flowsheet, e-mailing a 20-page document to the

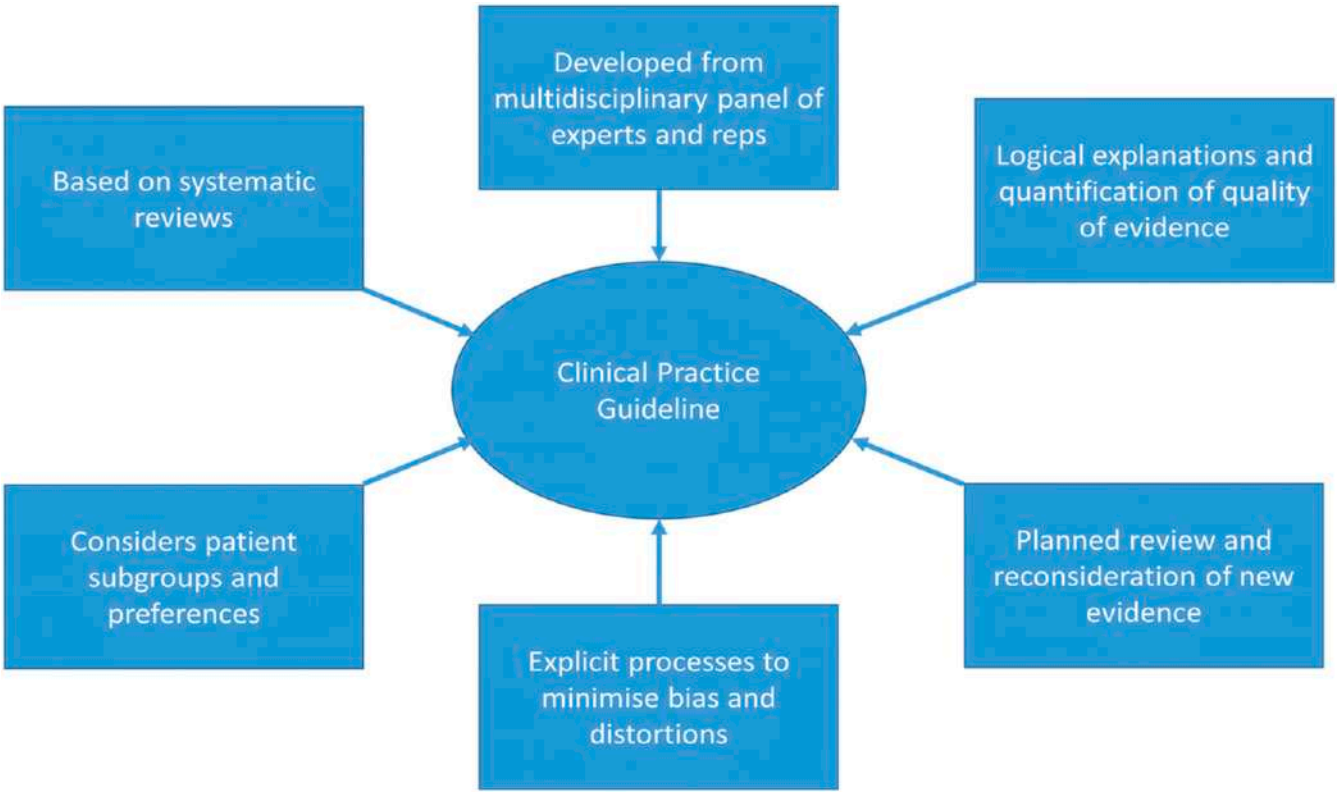

Figure 3. Definition of a CPG. Adapted from Graham et al.[40]

clinical team, or silently placing it in an online library wastes the clinician's time and breaks the hearts of the EHR developers, as well as reducing the chances of it being clinically implemented an improving symptoms.

Back to the clinical case

The patient's hospital had adopted a systematic approach to routine symptom screening in the ambulatory and inpatient settings. Fatigue, nausea, and changes in taste were quickly deemed extremely bothersome. Based on the hospital's clinical pathways, routine physical exercise with a physiotherapist was implemented. Adherence to a CPG for prevention of nausea and treatment guidance was closely monitored. Several approaches to managing the changes in taste were provided, although this symptom was less well controlled. The hospital also implemented an automated AE capture system, and reports were validated by the patient's attending physician. She completed relapse therapy and reported her experience was much better in comparison with the initial therapy, even though the second treatment had been more intensive.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

Off-label drug use

None disclosed.

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How do I sequence therapy for follicular lymphoma?

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In the past decade, many new agents have been introduced for the management of follicular lymphoma, and therapeutic strategies have evolved over time. The clinical benefits of the different treatments vary and, at the time of progression, are influenced by patient and disease characteristics, the duration of the interval from last treatment, and the nature of the treatments previously administered. Altogether, this results in a marked heterogeneity of clinical situations encountered during the treatment of these patients. Despite numerous trials performed in the field, there is no single standard of care for patients undergoing second-line treatment or beyond. Furthermore, patients recruited in these studies have characteristics that rarely represent the full spectrum of possible clinical presentations. Therefore, to optimally individualize treatment, all of the risks (short- and long-term) and benefits of the available options should be well known. Discussing the goals of therapy with the patient at each intervention is also critical in providing an optimal sequence of therapy.

LEARNING OBJECTIVES

- Describe the new agents or regimens available for patients with relapsed or refractory follicular lymphoma, their efficacy, and their side effects
- Understand how the strategy chosen for the first line of therapy influences subsequent lines
- Discuss the parameters to be considered for the different lines of therapy in order to improve outcomes without reducing the patient's quality of life

Introduction

The life expectancy of patients with follicular lymphoma has substantially increased in the last 3 decades. For a significant proportion of patients, prolonged response can be achieved (≥10 years) without the need for additional treatment through the use of effective therapy, which often comprises a combination of an anti-CD20 antibody and chemotherapy (possibly consolidated by antibody maintenance).[1] However, several points must be considered when one is discussing the sequence of therapy to achieve optimal treatment of patients with disease progression. First, this indolent disease can be asymptomatic for years, not only before therapy is initiated but also years later, even for some patients who have experienced several episodes of disease progression and have been exposed to multiple lines of therapy. This lack of symptoms might reflect the biological heterogeneity of this disease, with each clinical progression possibly emerging from related but divergent lymphoma clones.[2] Therefore, disease progression does not necessarily warrant retreatment. Second, although the majority of patients with follicular lymphoma die of their disease, other causes of death, including treatment-related toxicities, are present.[3] Avoiding treatment sequelae that will decrease the quality of life of these patients, or their life expectancy, is an important goal. Third, patients with histological transformation have a dramatic cumulative risk of lymphoma-related death, whereas patients without histological transformation have a lower risk of lymphoma-related death.

Therefore, when making therapeutic decisions for a patient presenting with follicular lymphoma, we should always weigh the benefits and risks of each available option and define the goals of therapy. Several parameters should be evaluated: the clinical need to initiate therapy, such as presence of disease-related symptoms or threat of organ function, Groupe d'Etude des Lymphomes Folliculaires criteria (Table 1), and pace of lymphoma growth; prognostic parameters that may help predict long-term outcomes; age and comorbidities; and the patient's wishes and expectations.

Mutation[4] or gene expression profiling[5] data have been proposed as new prognostic parameters for patients

undergoing first-line therapy, but they lack robust reproducibility with different therapeutic strategies and cannot inform us about treatment selection. Similarly, although some biological and clinical differences exist between patients with histological grade 1 to 2 vs grade 3A follicular lymphoma,[6] management is broadly similar between the two groups.

Although the need to address treatment sequencing implies that the first line of therapy has failed, we will briefly review the options initially adopted, because they also determine the choice of the next sequences (Figure 1). The three clinical cases below will help us walk through these different sequences of therapy.

Patient 1: On the basis of a biopsy of a 3-cm cervical lymph node, an asymptomatic 58-year-old woman received a diagnosis of follicular lymphoma grade 1 to 2, Ann Arbor stage I. She received involved-site radiation (24 Gy) in 2000 and had disease relapse in 2010, with small (1- to 1.5-cm) cervical and axillary nodes and a low tumor burden according to Groupe d'Etude des Lymphomes Folliculaires criteria. She remained asymptomatic.

Patient 2: A 45-year-old man with follicular lymphoma grade 2, Ann Arbor stage III, and a high tumor burden was treated in a clinical trial with rituximab–cyclophosphamide, hydroxydaunorubicin, vincristine sulfate, and prednisone (R-CHOP) followed by 2 years of rituximab maintenance in 2005. He presented with a disseminated disease recurrence 16 months after initiating the maintenance. The new biopsy showed follicular lymphoma grade 3A; the bone marrow biopsy showed no lymphoma infiltration.

Patient 3: A 67-year-old woman was treated in 2014 for follicular lymphoma grade 1, Ann Arbor stage IV, with 6 cycles of bendamustine and rituximab. She presented with a disseminated disease recurrence 5 years later. A new biopsy confirmed follicular lymphoma with an unchanged grade.

Current options for the first therapeutic sequence

At diagnosis, we can face three different situations:

1. Patients with strictly localized disease
2. Patients with disseminated disease but low tumor burden and no immediate treatment indication
3. Patients with a clear indication for systemic treatment or high tumor burden

Patients with localized disease

For patients with stage I or contiguous stage II disease without tumor bulk or biological pejorative features who were adequately staged with positron emission tomography–computed tomography (PET-CT) scans and bone marrow biopsy, involved-site radiation therapy is the preferred option according to the National Comprehensive Cancer Network[7] and European Society for Medical Oncology[8] guidelines, with the expectation that it will be curative. Data from the LymphoCare observational study suggested that the interval without lymphoma progression was similar between patients who underwent radiation therapy and patients who underwent watchful waiting.[9] The same study indicated that combined therapy (radiation therapy and systemic therapy) might be the best option to achieve a longer progression-free interval. In line with this observation, a recent randomized study indicated that systemic treatment after radiation therapy for patients with localized disease significantly increased 10-year progression-free survival (PFS) from 41% without adjuvant systemic therapy to 59% with adjuvant cyclophosphamide, vincristine, and prednisone (CVP) or rituximab-CVP (R-CVP).[10] Although this study has several limitations (heterogeneity of histological profiles and therapies, length of accrual), it provides further evidence to support systemic treatment in addition to radiation therapy.[11,12] Which regimen to administer (rituximab alone or in combination with chemotherapy), when to sequence it (before or after radiation therapy), and how to administer it (schedule and length) remain unanswered. What is likely, however, is that more patients with localized disease will be treated with combined modality therapy in the near future.

Table 1. Groupe d'Etude des Lymphomes Folliculaires criteria

The presence of any 1 of these criteria defines a patient with a high tumor burden
A lymphoma tumor ≥7 cm in diameter*
Three nodes in 3 distinct areas, with each node ≥3 cm in diameter
Presence of systemic symptoms
Symptomatic spleen enlargement
Ascites or pleural effusion
Cytopenias (neutrophil counts <1 G/L or platelet counts <100 G/L)
Circulating lymphoma cells (>5.0 G/L)

*Except spleen.

Patients with a low tumor burden

For patients with no formal indication for treatment (low tumor burden, asymptomatic, and no rapid clinical progression), watchful waiting remains the standard course. However, rituximab single-agent therapy has become a popular option for these patients on the basis of multiple phase 2 studies and 1 randomized study showing a significant increase in PFS after 4 single infusions of rituximab.[13] There is no evidence that early administration of rituximab impairs the efficacy of second-line therapy. However, it is questionable to use this strategy with the goal of delaying the use of immunochemotherapy (cytotoxic agent plus an anti-CD20 antibody), because the application of this combination has clearly demonstrated a significant reduction in the risk of death for patients with follicular lymphoma.

Patients with high tumor burden

Combinations of either bendamustine, CHOP, or CVP with rituximab (R-benda, R-CHOP, and R-CVP, respectively) or obinutuzumab (O-benda, O-CHOP, and O-CVP) make up the current standard treatment options for patients with a high tumor burden. Bendamustine has been widely adopted in different regions of the globe, given its lower toxicity profile, in terms of neutropenia, and the lack of drug-induced alopecia. The use of bendamustine also spares administration of anthracyclines, which can be useful in later lines, specifically if histological transformation occurs. However, data from the GALLIUM study indicated that the use of R- and O-benda followed by 2 years of antibody maintenance was associated with a significant risk of adverse events, including fatal ones.[14] In the same study, obinutuzumab with the different chemotherapy backbones was shown to increase PFS,

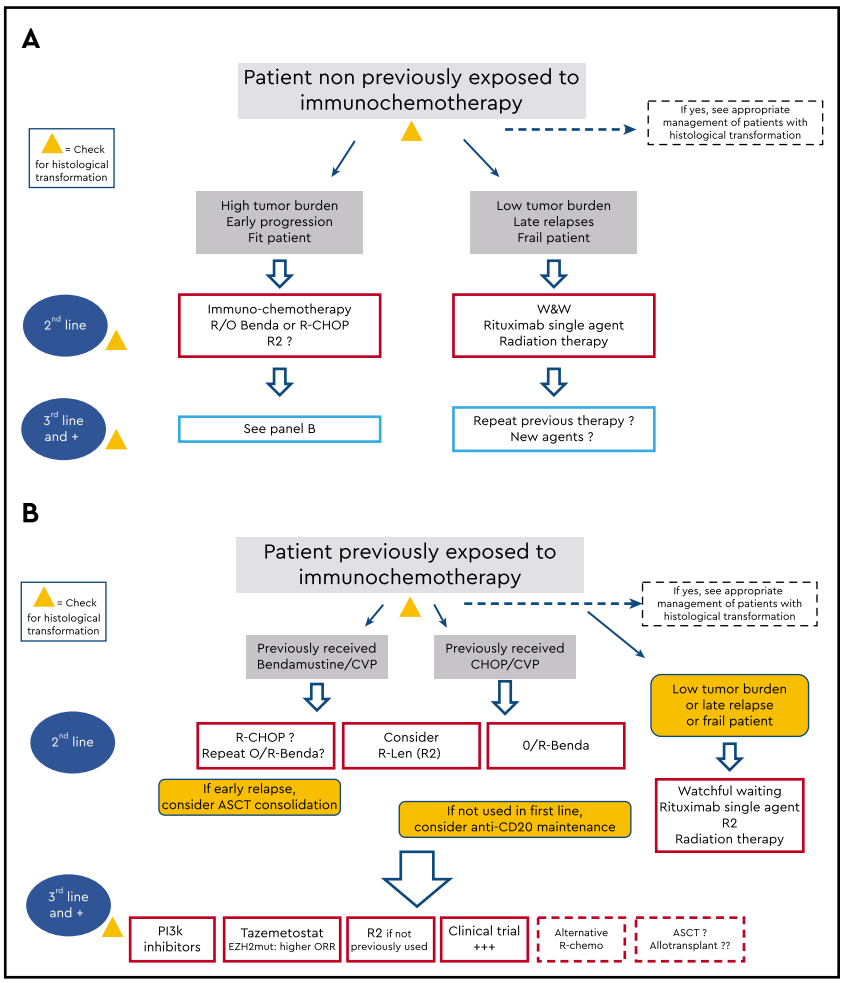

Figure 1. Schematic indications of the potential sequence of treatment during the clinical course of patients with follicular lymphoma. (A) Treatment options for patients who did not receive immunochemotherapy in the first line. (B) Options for patients for whom immunochemotherapy failed. W&W, watchful waiting.

compared with rituximab-based combinations (5-year PFS, 70.5% vs 63.2%[15]), although its use was associated with more side effects (all manageable). Altogether, given the lack of an overall survival benefit associated with one of these options over the others, the choice of both the chemotherapy backbone and the anti-CD20 antibody for this first immunochemotherapy sequence, followed or not by anti-CD20 maintenance, should be made according to physician and patient preferences. However, this choice will have significant consequences for potential next sequences, as outlined below.

Is there an optimal therapeutic sequence in the second line?

For chemotherapy-naive patients

For patients treated with radiation therapy or rituximab single-agent therapy who need a new line of treatment, the next sequence consists of an immunochemotherapy regimen in most cases. At this stage, all of the regimens previously discussed as first lines are acceptable. The recently approved lenalidomide–rituximab (R2) combination is also an acceptable option.[16] For a few patients, either frail, elderly, or with a low tumor burden and very long response to rituximab single-agent therapy (>5 years), rituximab single-agent therapy can be repeated, or radiation therapy can be used with different modalities, including the 2- × 2-Gy symptomatic but efficient schedule.

After first-line immunochemotherapy

For patients who have received immunochemotherapy, it appears logical to propose an alternative cytotoxic regimen, usually one combined with an anti-CD20 antibody. In cases where CHOP or CVP was used as the chemotherapy backbone in the first line, bendamustine is a favorite choice. O-benda followed by obinutuzumab maintenance was approved for patients with disease refractory to rituximab, with a significant overall survival benefit (hazard ratio of 0.67 favoring this regimen over bendamustine alone).[17] Despite the limitations of this study (lack of rituximab in the control arm and anti-CD20 maintenance administered only in the experimental arm), it established this regimen as a favorite option for patients with rituximab-refractory disease who have previously received R-CVP or R-CHOP, and its use has been expanded beyond patients with refractory disease.

We lack solid data on the efficacy of an alternative chemotherapy after R- or O-benda as first-line therapy, including

Table 2. Key results of the AUGMENT study for patients with follicular lymphoma[15]

Treatment arm	Number of patients*	ORR	CRR	PFS, median (mo)	DOR, median (mo)	2-y OS
Rituximab single agent	148	55%	29%	14	22	87%
Lenalidomide–rituximab (R2)	147	80%	51%	39	37	93%
P		<.0001	.004	<.0001	<.0001	.02

CRR, complete response rate; DOR, duration of response; ORR, overall response rate; OS, overall survival.
*All detailed results are provided for patients with follicular lymphoma.

R-CHOP, which is frequently used.[3] Bendamustine can be used again but not without significant risks of cumulative hematological toxicities, and it should not be recommended if the interval between treatments is short (<1 or 2 years; an interval of 2 years was recommended in the GADOLIN study[18]). Of note, many regimens that combine cytotoxic agents and are used to treat aggressive B-cell malignancies, such as rituximab, dexamethasone, high-dose cytarabine, and cisplatin/oxaliplatin,[19] can also be effective in the setting of disease relapse.[3] There are no data regarding the optimal anti-CD20 antibody for patients who have received obinutuzumab as first-line therapy.

R2 was recently approved for patients whose disease was not refractory to rituximab, after demonstration of its superiority to rituximab single-agent therapy, with a significant increase in PFS (39 months vs 14 months) and excellent overall survival probability (93% at 2 years) (Table 2).[15] Side effects were essentially represented by infections, neutropenia (often of grade 3 to 4), and cutaneous rashes. Although long-term data are not yet available, this regimen, or its variant where obinutuzumab is replacing rituximab in combination with lenalidomide (eg, as reported in the GALEN study[20]) have an acceptable tolerability and a fixed treatment duration, and they are attractive options in this setting.

Should responding patients receive any form of consolidation in the second line?

In the mid-2000s, the EORTC study indicated significant outcome improvement when rituximab maintenance was administered after CHOP or R-CHOP for patients who were previously rituximab naive.[21] Results of other studies supported the benefit of rituximab maintenance for patients with relapsed or refractory disease, and a meta-analysis showed a significant benefit in overall survival.[22] However, in the setting of anti-CD20 maintenance administered during first-line therapy, data regarding the risk/benefit ratio of another maintenance consolidation are lacking.

Table 3. Key results of the different currently available PI3K inhibitors

Drug	Disease characteristics	Number of patients (total/follicular)	ORR	CRR	PFS, median (mo)	DOR, median (mo)	2-y OS	Most common grade 3-4 adverse events (present in ≥5% of patients)*
Idelalisib[27,28]	Double refractory to rituximab and alkylating agents	72/125	66%†	14%†	11 (11†)	12 (11†)	70%†	Neutropenia (27%) ALT elevation (13%) Diarrhea (13%) Pneumonia (7%) Thrombocytopenia (6%)
Duvelisib[14]	Double refractory to rituximab and alkylating agents	129/83	42%†	1%†	10	10	~60%‡	Neutropenia (25%) Diarrhea (15%) Anemia (15%) Thrombocytopenia (12%) Febrile neutropenia (9%) Lipase increased (7%) ALT elevation (5%) Pneumonia (5%) Colitis (5%)
Copanlisib[29]	Relapsed or refractory after 2 lines of therapy	142/104	59%†	20%†	13	14	69% augment	Hyperglycemia (40%) Hypertension (24%) Neutropenia (24%) Pneumonia (11%) Diarrhea (9%) Anemia (5%) Thrombocytopenia (5%)

ALT, alanine transaminase; CRR, complete response rate; DOR, duration of response; ORR, overall response rate; OS, overall survival.
*Collected on all patients analyzed in the study.
†Patients with follicular lymphoma only.
‡Estimated from OS curve.

Table 4. Key results of the pivotal tazemetostat study[30]

Patient cohort	Number of patients	ORR (by IRC)	CRR	PFS, median (mo)	DOR, median (mo)	Treatment-related adverse events (any grade) in ≥10% of patients	Treatment-related adverse events (grade 3-4) in ≥2% of patients
EZH2 mutated	45	69%	13%	14	11	Nausea (19%)	Anemia (2%)
EZH2 wild-type	54	35%	4%	11	13	Diarrhea (12%) Alopecia (14%) Asthenia (14%) Fatigue (12%)	Thrombocytopenia (3%) Leukopenia (3%)

CRR, complete response rate; DOR, duration of response; IRC, independent review committee; ORR, overall response rate.

Multiple studies have shown that autologous stem cell transplant (ASCT) can achieve durable responses for patients with relapsed or refractory follicular lymphoma, and this strategy has been widely used by some institutions.[23-25] A comparison based on registry and cohort data suggested that ASCT (as a consolidation after salvage therapy) improved survival for patients with early treatment failure.[26] However, data from the PRIMA study indicated that there was no benefit in overall survival with ASCT for patients without evidence of histological transformation.[13]

Early progression or early histological transformation?

Several years ago, attention was brought to patients treated with first-line immunochemotherapy (essentially consisting of R-CHOP) who had experienced disease progression within 24 months after diagnosis: Their risk of death was significantly higher than that of patients who did not have disease progression, with only 50% alive at 5 years.[27] Other observations extended these findings to patients for whom observation was adopted at presentation and who had disease progression within 12 months, and to patients treated with single-agent rituximab or radiation therapy.[14,28] However, when early failure after immunochemotherapy was considered, a variable but substantial proportion of these patients presented with histological transformation of their lymphoma, from 35% to >75%, with the higher rates observed after use of a bendamustine-containing regimen.[29,30] Details about the treatment of patients with transformed disease are provided in a companion paper of this series by S. Smith,[31] and outcomes after transformation remain dismal. However, few data are available regarding outcomes and prognoses of patients with early progression of disease without histological transformation. This lack of data supports the need to document early progression with a new biopsy, preferentially performed in the lesion with the highest standardized uptake value on PET-CT scan. At present, there is a trend to adopt a different therapeutic strategy (including consideration for ASCT) for patients with early failure after first-line therapy (having experienced a progression at 12 or 4 months), given the poorer outcomes among these patients,[32] but robust data are lacking in this area. Results of ongoing studies using targeted therapies for patients with relapsed or refractory disease suggest that several approaches are effective in this population.

For patient 1, rituximab single-agent therapy (rather than repeating radiation therapy, because 2 distinct sites of lymphoma involvement were present) would be a good option, but it is also acceptable to pursue surveillance for a couple of months or years before initiating therapy for patients who are adherent and accept this strategy.[33]

For patient 2, despite a biopsy in the largest and more metabolic (standardized uptake value of 13) abdominal lesion, histological transformation was not demonstrated (change of histological grading does not constitute a specific indication). Two options were discussed with the patient: a combination of O-benda for 6 cycles followed by 2 years of obinutuzumab or a shorter duration of second-line therapy (rituximab, dexamethasone, cytarabine, and oxaliplatin ×3) consolidated (if responsive to salvage therapy) with ASCT. The use of maintenance after ASCT with rituximab every 3 months was not adopted, because the patient had already received maintenance in the first line of therapy and because of the lack of robust data supporting a second sequence of maintenance.

For patient 3, R2, according to the AUGMENT study, was chosen. Other options, such as R-CVP or R-CHOP, were considered to be more toxic but could also have been proposed.

Managing chronicity

For patients receiving a third line of therapy or beyond, several new options are available, such as phosphoinositide 3-kinase (PI3K) inhibitors and, recently, the enhancer of zeste homolog 2 (EZH2) inhibitor tazemetostat.

Table 5. Early results with selected new therapies in development for follicular lymphoma

Studies	Number of patients	ORR	CRR	Main adverse events
Bispecific antibodies				Cytokine release syndrome and ICANS (essentially grade 1-2), cytopenias (20%-25% grade ≥3)
Mosunetuzumab[36]	82	63%	43%	
Glofitamab[37]	24	68%	50%	
Chimeric antigen receptor T cells	80	95%	81%	Cytokine release syndrome (7% grade ≥3), ICANS (15% grade ≥3), cytopenias
Axicabtagene ciloleucel[38]				

CRR, complete response rate; ICANS, immune effector cell–associated neurotoxicity syndrome; ORR, overall response rate.

The PI3K inhibitors

Three inhibitors of the PI3K pathway have been approved for use in the United States (only 1 in Europe) (Table 3). The first, idelalisib, is an oral agent specific to the δ isoform of this enzyme. It has proven efficacy for patients with double-refractory disease to rituximab and alkylating agents. However, the pattern of initial (transaminitis, neutropenia) and mid- or long-term (diarrhea, colitis, pneumonitis, opportunistic infections) side effects necessitates attentive clinical management.[34] Some patients still enjoy a prolonged benefit, and dosage adaptations can sometimes increase tolerability.[35] Duvelisib is also an oral agent that targets the δ and γ PI3K isoforms. With the usual limitations of cross-study comparisons, the response rate observed in the pivotal trial was slightly lower than that of idelalisib, and the tolerability was similar (liver enzyme elevations were less common).[36] Copanlisib is specific for the α and δ isoforms of PI3K, but it is administered intravenously for 3 weeks out of 4, with some precautions regarding the risk of hyperglycemia and hypertension; altogether, it has a tolerability profile a little different from that of the other options.[37] Overall, these agents are useful, but few patients have a complete response, and the median response duration rarely exceeds 1 year (Table 3).

Epigenetic targeting

The transcription factor EZH2 plays a key role in germinal center formation, and the gene presents an activating mutation in ~20% of patients with follicular lymphoma. Tazemetostat is the first-in-class inhibitor of this pathway, and this oral drug was found to be more active in *EZH2*-mutated cases than in unmutated cases, although the response duration was comparable between cohorts (Table 4).[38] The side effects are quite limited, essentially of grade 1 to 2, and include low-grade nonspecific digestive symptoms, alopecia, or fatigue. On the basis of these data, the drug was recently approved by the US Food and Drug Administration (FDA) "for adult patients with relapsed or refractory (R/R) follicular lymphoma (FL) whose tumors are positive for an *EZH2* mutation as detected by an FDA-approved test and who have received at least 2 prior systemic therapies, and for adult patients with R/R FL who have no satisfactory alternative treatment options."

Can we also consider reusing rituximab?

Single-agent rituximab in cases of late relapse, the use of a different immunochemotherapy regimen when the disease volume is substantial, and the R2 combination if not previously prescribed are all acceptable options. Of note, the concept of rituximab resistance was developed as a pathway for drug development and regulatory approval but is not well documented biologically, and patients who did not have a response to rituximab once may still have a response months or years later.[39,40]

Are autologous and allogeneic transplants viable options in 2020?

Although it is probably more efficient at the time of first relapse, ASCT can still be considered in later lines[41]; for patients who are fit and for whom a donor is available, allogeneic transplant (with a reduced-intensity conditioning regimen) can also be proposed and may offer a sustained duration of response in a substantial proportion of patients.[42]

Future developments

Of the different agents in development, it is worth mentioning that bispecific antibodies have achieved promising results for patients with follicular lymphoma.[43,44] Recently, preliminary results with chimeric antigen receptor T cells (axicabtagene ciloleucel) have been encouraging (Table 5). Therefore, offering patients the opportunity to participate in a clinical trial that is evaluating new agents, alone or in combination, should always be considered, given the multiplicity of active drugs evaluated in follicular lymphoma.[45]

Conclusions

Assuming that histological transformation has been ruled out, the prognosis of patients with relapsed or refractory follicular lymphoma remains uncertain but not necessarily dismal. Multiple treatment options are available, and it is often possible to alternate regimens or drugs that might differ in terms of side effects and duration in the hope of achieving treatment-free intervals. In a reasonable proportion of patients, this approach may control the recurrent disease for 1 or 2 decades and allow the patient to access new forms of therapy that could be more efficient.

Epilogue

Patient 1: Although the usual rituximab regimen consists of 4 weekly rituximab infusions, in this situation I usually advise completing therapy with 4 additional infusions administered every 2 months, which nearly doubles the time to progression.[46] The patient experienced 3 years without symptoms and without treatment. In 2014, a new progression occurred, and the patient received her first chemotherapy, 6 cycles of R-benda, with a complete response on PET-CT scan at the end of treatment. A new progression was observed in 2017, and the patient participated in a study evaluating obinutuzumab and lenalidomide with obinutuzumab maintenance. She had a complete response at the end of this treatment in 2019.

Patient 2: The patient opted for ASCT. He experienced a complete response lasting 6 years. Progression with a limited tumor burden occurred at that time, and the patient received rituximab single-agent therapy but had progression within 6 months of the end of therapy. He received idelalisib for 8 months, stopping because of poor tolerability. He remained with limited and asymptomatic disease for ~1 year and then started R-benda, which was interrupted after 4 cycles for persistent neutropenia, with a good partial response. He was recently treated with chimeric antigen receptor T cells in a clinical trial and had a new complete response.

Patient 3: The patient had a response to R2. She had limited disease progression 2 years later and is currently undergoing observation. She is a good candidate for other new agents (eg, PI3K or EZH2 inhibitors, bispecific antibodies) in the near future.

Conflict-of-interest disclosure

G.S. has received honoraria for consultancy, participation in advisory boards, and educational events from Abbvie, Allogene, Autolus, Beigene, Celgene, Genmab, Gilead, Janssen, Kite, Morphosys, Novartis, Roche, Servier, and Velosbio.

Off-label drug use

Obinutuzumab

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How do we sequence therapy for marginal zone lymphomas?

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Marginal zone lymphomas are indolent diseases. Overall survival rates are very good, but patients tend to relapse and may do so several times. The concept of treatment sequencing is therefore important and necessary to preserve adequate organ function and to avoid excessive toxicity, with the final goal of achieving long survival times. Systemic treatments and chemotherapy are considered to be an option in multiply relapsing disease, in cases that are in an advanced stage at presentation or relapse, and in cases where initial local treatments lack efficacy. Targeted agents and new drugs can provide chemotherapy-free alternatives in heavily pretreated patients.

LEARNING OBJECTIVES

- Apply the most correct frontline treatment in extranodal, nodal or splenic marginal zone lymphoma
- Manage disease relapse in the era of targeted agents and new drugs

Introduction

Marginal zone lymphomas (MZLs) are rare diseases with a heterogeneous clinical presentation: extranodal MZL (EMZL) of the mucosa-associated lymphoid tissue (MALT) is the most common, accounting for nearly 70% of all MZLs, with the possible involvement of any anatomic site. They are followed by splenic marginal zone lymphoma (SMZL), which represents roughly 20% of cases, and by nodal MZL (NMZL), the most infrequently occurring entity.[1,2]

Given their rarity, it is often difficult to conduct clinical trials specifically designed for patients with MZL. Existing trials of patients with MZL generally include diseases with different presentations (eg, MZL collectively or multiple-site EMZL), although there are some trials for gastric MZL, as it is the extranodal site most frequently involved in MALT lymphoma. Most data on treatment of these entities are extrapolated from umbrella trials involving patients with indolent B-cell lymphomas or from retrospective reviews. In most cases, current guidelines are based on expert recommendations rather than evidence-based statements, as there are few randomized trials.[3,4]

MZLs are indolent diseases, and patients generally have long survival times but show a tendency to relapse, perhaps several times, as relapses are generally treatable. A MALT lymphoma prognostic index, based on age ≥70 years, lactate dehydrogenase levels, and advanced stage, helps to stratify patients according to their event-free survival (EFS) at 5 years.[5] Patients with a high MALT prognostic index may also have early disease progression, which clearly correlates with impaired survival.[6]

Subsequent lines of treatment are needed, as disease activity is often symptomatic and impairs the patient's quality of life. For this reason, the concept of treatment sequencing is important; providing effective local control of the disease means achieving an acceptable EFS with no (or just a few) harmful side effects. Systemic treatments and chemotherapy are options in multiply relapsing disease, in advanced-stage disease at presentation or relapse, and in cases where initial local treatments have failed.[1-4]

This review condenses the recommendations offered by the major international guidelines applied in the treatment of MZL and our personal clinical practice and point of view.

Clinical case

A 65-year-old man with a 6-month history of dyspepsia received a diagnosis of histologically documented *Helicobacter pylori* (HP)–positive gastric MALT lymphoma, appearing as a small, ulcerated lesion of the antrum, in the context of global erythema of the gastric mucosa. No signs of active bleeding were detected. The patient was taking

acetylsalicylic acid because of a history of transient ischemic attack. He was also taking valsartan and alfuzosin. His peripheral blood counts were normal, and only a subclinical iron deficiency, without anemia, was identified. Disease staging was accomplished by (1) a total-body computed tomography (CT) scan, which showed no enlarged lymph nodes (either perigastric or distant), with slight thickening of the gastric walls appreciated; (2) endoscopy with ultrasonography to document the involvement of the muscularis propria (stage I; T2 N0 M0); and (3) bone marrow biopsy, which was negative for disease infiltration. He was prescribed a proton pump inhibitor and antibiotics, and his disease status was reevaluated 2 months after treatment. The ulceration had completely resolved, but random gastric biopsies documented persistent disease. He was then referred for gastric radiation therapy.

Treatment sequencing in EMZL

Initial treatment choices in gastric MALT lymphoma

Gastric MALT lymphoma is localized to the stomach and to its tributary nodes most of the time. Disease spread beyond the serous membrane and to adjacent tissue is also possible, as well as concomitant involvement of multiple extranodal sites. Thorough locoregional staging is achieved by endoscopy with ultrasonography, and it is important to rule out the presence of HP. If histopathology is negative, then a stool antigen test or urea breath test is recommended. Initial disease stage (according to the Lugano or Paris staging systems; Table 1) and HP status are the most important parameters considered in any subsequent treatment decisions.[7]

The European Society of Medical Oncology (ESMO) guidelines recommend that an HP eradication therapy be administered to all patients with HP^+ gastric MALT lymphomas, independent of stage at presentation or histologic grade.[3] The same guidelines also suggest antibiotic eradication therapy be given, even in cases of HP^- disease, although responses are less likely, as occasional disease regressions have been documented, and the HP test may return a false-negative result. National Comprehensive Cancer Network guidelines recommend instead that patients with localized disease (stages I and II_1, according to the Lugano staging system), meaning lymphoma confined within the gastric walls and perigastric lymph nodes, receive antibiotic therapy if HP^+ and, preferentially if lacking the t(11;18) translocation.[4] This translocation, found in 15% to 40% of patients with gastric MZL and leading to the juxtaposition of the *BIRC3* and *MALT1* genes, is a predictor of lack of tumor response to antibiotics.[8] Patients with HP^- initial-stage disease or with t(11;18), even if HP^+, should undergo involved-site radiation therapy (ISRT) or, as a second option, systemic treatment with rituximab. All other presentations of stage IIE disease or higher that indicate spread to more distant nodal structures or to adjacent nodes require a systemic approach if patients are symptomatic (Figure 1). Treatment approaches for localized disease are reviewed briefly below. Systemic treatments for advanced-stage disease or recurrent disease are discussed in "Chemoimmunotherapy in advanced or resistant MALT lymphomas."

Antibiotic therapy

Antibiotic therapy is based on the epidemiology of the infection in the patient's country of residence and should take into account locally expected antibiotic resistance patterns. The most common approach is based on 3 drugs: a proton pump inhibitor with clarithromycin, in combination with amoxicillin or metronidazole for 10 to 14 days.[9] The outcome of HP eradication must be confirmed by urea breath test or stool antigen test at least 6 weeks after eradication therapy and at least 2 weeks after withdrawal of the proton pump inhibitor. Response assessment is performed by repeated endoscopy with biopsy. In patients with endoscopic remission of the disease and persistent microscopic lymphoma on histology, it is reasonable to wait at least 12 months before starting a new line of treatment, unless the patient is symptomatic. In case of failure to achieve a meaningful response, a subsequent treatment with ISRT can be considered.[3,4] A follow-up endoscopy (with biopsy) is recommended every 3 months in patients with persistent histologic infiltration but no treatment indications, then every 3 to 6 months for 5 years, and then yearly (or when clinically indicated) in those who achieve a complete histologic remission. In case of persistent HP positivity, regardless of the histologic presence of lymphoma, treatment with a course of second-line, non–cross-resistant antibiotics may be attempted.

Radiation therapy

ISRT is the preferred technique in cases of localized disease after failure of antibiotic therapy or HP^- localized disease. The International Lymphoma Radiation Oncology Group recommends that abnormal lymph nodes (sometimes porta hepatis nodes

Table 1. Staging of gastric MALT lymphoma: a comparison of staging systems and anatomic correlations

Lugano staging system		TNM (or Paris) staging system	Disease extension
Stage I	I_1: confined to mucosa or submucosa	T1 N0 M0	Mucosal or submucosal layer
	I_2: confined to muscularis propria or serosa	T2 N0 M0	Muscularis propria
		T3 N0 M0	Serosa
Stage II	II_1: extending into abdomen with local nodal involvement	T1-3 N1 M0	Perigastric lymph nodes
	II_2: extending into abdomen with distant nodal involvement	T1-3 N2 M0	More distant regional lymph nodes
Stage IIE	Penetration of serosa to involve adjacent organs or tissues	T4 N0 M0	Adjacent structures
Stage IV	Disseminated extranodal involvement or concomitant supradiaphragmatic involvement	T1-4 N3 M0	Lymph nodes on both sides of the diaphragm
		T1-4 N0-3 M1	Bone marrow invasion, additional extranodal sites

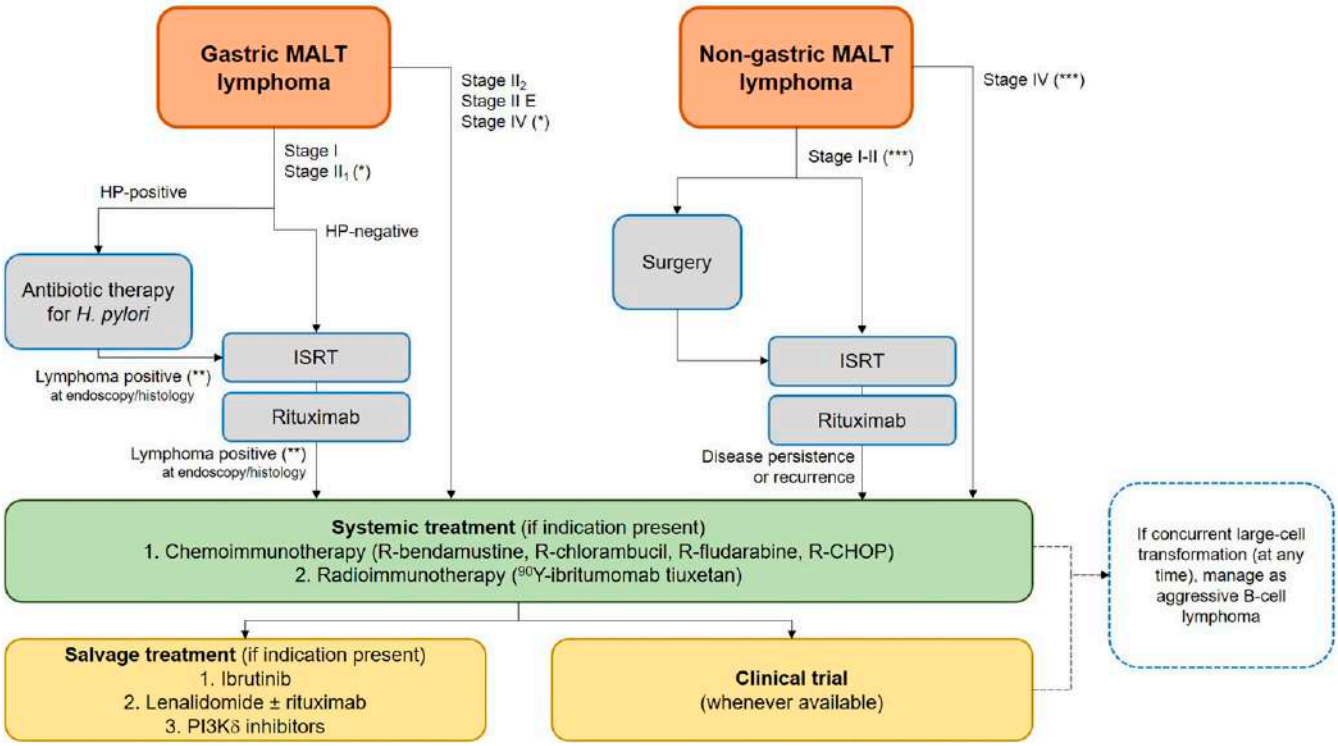

Figure 1. Treatment sequencing in gastric and nongastric MALT lymphoma. *Lugano (or corresponding Paris/TNM [tumor, node, metastasis]) staging system for gastric MZL. **Endoscopy performed during follow-up is always associated with multiple biopsies of gastric mucosa. A shift should be made to the next treatment step when the patient is symptomatic or in cases of overt progression or deeper invasion within gastric walls. Consider repeating antibiotic therapy if HP positivity is still detected. ***Ann Arbor staging system.

or para-aortic nodes, as well) be encompassed in the clinical target volume. Perigastric nodes are simply included in the treatment volume, although they are not pathologically confirmed to be involved with lymphoma.[10] Doses of 24 to 30 Gy to the stomach and perigastric nodes have been effective in local disease control in the long term, without significant toxic effects.[11-13]

Rituximab

The drug is recommended as a second-line treatment for those in whom antibiotic therapy has failed and who have contraindications to ISRT. The efficacy of 1 treatment per week for 4 consecutive weeks, at the standard dose of 375 mg/m^2, as a single agent for gastric and extragastric MALT lymphomas, is reported in retrospective studies.[14-16] The studies mainly include patients with extranodal MALT lymphoma in general, and primary gastric disease cases are just a proportion of the entire cohort of patients treated. The only study specifically considering gastric MALT lymphoma[15] enrolled 27 patients at any disease stage. In 55% of those, an initial antibiotic eradication therapy or surgery had failed, and 7% were treatment naive. In the remaining cases, chemotherapy was associated with antibiotic eradication therapy or surgery. Objective responses were recorded in 77% of the patients, with a histologic complete response (CR) in 46%, independent of the presence of the t(11;18) translocation. Relapses occurred in only 2 patients within the first 3 years of follow-up. The International Extranodal Lymphoma Study Group-19 (IELSG) trial, designed to evaluate the efficacy of rituximab+chlorambucil over chlorambucil alone in the treatment of patients with gastric MALT, in whom an initial antibiotic therapy has failed, was amended to include a third arm consisting of single-agent rituximab after the enrollment of the first 252 patients.[17] This trial and its results are described in detail in "Chemoimmunotherapy in advanced or resistant MALT lymphomas."

Surgery

The role of surgery has been questioned, as gastric MALT lymphoma is generally multifocal, thus requiring an extensive (total or subtotal) gastrectomy, usually severely impairing the quality of life. Gastrectomy should be considered as a first-line intervention in cases of life-threatening hemorrhage, gastric perforation, or pyloric stenosis.[18]

Initial treatment choices in nongastric MALT lymphomas

Nongastric (extragastric) MALT lymphomas can arise in any extralymphatic organ with an anatomically well-structured MALT (such as the gut, the nasopharynx, and the lung) and also at sites normally lacking lymphoid tissue, but with a (temporary) accumulation of B-lymphocytes as a response to chronic stimulation caused by an infection or an autoimmune process. Salivary glands, ocular adnexa, breasts, genitourinary organs, the skin, and the thyroid may be affected by MALT lymphoma in concomitance with some particular conditions, such as Sjögren syndrome or Hashimoto thyroiditis, or as a consequence of certain infections (eg, by *Chlamydophila psittaci* or *Borrelia*

burgdorferi). It is important to recognize, however, that, in most cases, the etiology is unknown.[2,19]

Given that this disease may arise at any site and without systemic manifestations, as symptoms are mainly related to the involvement of the affected organ (eg, neck lump or swelling in thyroid MALT lymphoma in a patient with autoimmune thyroiditis, skin nodules, unilateral proptosis or lacrimal gland swelling, or asymmetric parotid swelling), many patients seek a hematologist's advice after a histologically confirmed diagnosis has been obtained. This implies that, in many instances, surgical intervention, ranging from an excisional biopsy to the removal of an entire organ, if a nonhematologic malignancy is initially suspected, is the first step in the management of the disease.

Available guidelines indicate locoregional treatments such as surgery and radiation therapy as the mainstay of the initial management of localized disease (Ann Arbor stage I and II). The functional and anatomic peculiarities of each affected organ should be considered when choosing among different approaches and during treatment planning, to maximize efficacy and reduce immediate and long-term adverse events.[3,4,7,19] Because site-specific guidelines are lacking, our personal view of treatment sequencing in EMZL lymphomas is summarized in Figure 1 and Table 2.

Surgery

An initial surgical approach (always necessary for diagnosis), especially if the whole involved organ is removed, may be considered adequate for the treatment of localized EMZL at certain sites, such as lung, breast, thyroid, colon, and small bowel. Surgery alone cannot be considered a definitive therapeutic procedure if margins of resection are involved in disease, in which case, locoregional radiation therapy is strongly recommended. Rituximab monotherapy, discussed later, or systemic chemoimmunotherapy may also be an option.

Table 2. Site-specific extranodal MALT lymphoma treatment

Site	First-line, localized disease		First relapse	Advanced disease (bilateral or stage IV)	Notes
	First choice	Second choice			
Skin (single lesion)	Excision or punch; observe in case of negative margins	Radiotherapy (if margins are positive)	Rituximab	Rituximab or R-chemo	—
Skin (contiguous)	Radiotherapy	Rituximab	Rituximab or R-chemo	Rituximab or R-chemo	—
Skin (multiple)	Rituximab	None	R-chemo	Rituximab or R-chemo	—
Parotid	Parotidectomy; observe in case of negative margins	Rituximab (if residual tissue or positive margins)	R-chemo	Rituximab (if bilateral); R-chemo (if systemic)	Limit radiotherapy to reduce xerostomia (especially with Sjögren syndrome)
Orbit, lacrimal gland	Radiotherapy	Rituximab	Alternative first-line choice or R-chemo	Rituximab (if bilateral); R-chemo (if systemic)	—
Conjunctiva	Rituximab	Radiotherapy	Alternative first-line choice or R-chemo	Rituximab (if bilateral); R-chemo (if systemic)	Published experiences with intralesional rituximab
Thyroid	Thyroidectomy (total or partial)+R-chemo	None	R-chemo or targeted agents	R-chemo	Radiotherapy to be avoided to preserve residual thyroid function
Lung	Lob(ul)ectomy+ rituximab or rituximab only	None	R-chemo	R-chemo (if bilateral or systemic)	Radiotherapy to be avoided to reduce lung fibrosis; avoid extensive surgery
Stomach	Antibiotics (if HP-positive)	Radiotherapy (if HP-negative) or rituximab	Alternative first-line second choice or R-chemo	R-chemo	—
Small bowel	Surgical resection+rituximab	None	R-chemo	R-chemo (if multiple lesions detected on CT scan or systemic)	Radiotherapy to be limited
Kidney	Nephrectomy (total or partial)+rituximab	None	R-chemo	R-chemo	Use of radiotherapy: to be discussed
Breast	Nodulectomy+rituximab or rituximab only	None	R-chemo	R-chemo (if bilateral or systemic)	Use of radiotherapy: to be discussed for unilateral disease

First-line treatment choices for localized disease are provided, when appropriate, according to our personal experience. Treatment of first relapse may be based on the approach not previously chosen for first-line treatment (alternative choice). Stage IV (according to Ann Arbor) indicates any dissemination of the disease to any nodal site and/or marrow and/or more than one extranodal site (apart from the initial extranodal site). R-chemo, chemoimmunotherapy.

Radiation therapy

The use of external beam radiation therapy should be thoroughly evaluated, to avoid the irradiation of wide areas of the body and to reduce the incidence of significant morbidity (eg, radiation-induced sicca syndrome in patients with Sjögren syndrome and salivary gland MALT lymphoma and cataract and xerophthalmia in patients with ocular adnexal lymphoma). The dose of radiation varies by site of disease. When treating a salivary gland MZL, the entire parotid (or any of the other involved salivary glands) should be irradiated. The same is done when radiotherapy is chosen as the initial treatment of thyroid MZL. In the treatment of ocular adnexa lymphoma, the clinical target volume includes the entire bony orbit, along with definite or suspected extraorbital extensions. When the disease is limited to the conjunctiva, the clinical target volume includes the entire conjunctival sac and local extensions to the eyelid.[10]

Low-dose radiation therapy (4 Gy in 2 fractions) is used increasingly in the treatment of localized extranodal indolent lymphomas: although the progression-free survival (PFS) rate with 4 Gy is ~75% at 5 years, local PFS remains inferior to that obtained with 24 Gy.[20] Moreover, significantly more sites of irradiation responded to 24 Gy than did those in the 4-Gy group.

Rituximab

Two phase 2 trials describe the action of rituximab monotherapy in extragastric MALT lymphomas, regardless of stage.[14,16] As stated, both trials included patients with gastric and nongastric MALT: skin, salivary glands, lungs, and orbits were the most represented sites. Patients were eligible for enrollment if untreated or if a previous line of treatment had failed (antibiotics, chemotherapy, or radiation therapy). In both trials, rituximab was given at the standard dose of 375 mg/m^2 every week, for 4 consecutive weeks. Taken together, the overall response rate (ORR) varied between 67% and 73%, with CR achieved in 17% to 44% of cases. Comparable ORRs were obtained in patients with gastric and nongastric MALT lymphoma (64% vs 80%[14] and 67% vs 70%,[16] respectively), although neither trial was designed and powered to detect a significant difference. A trend toward better outcomes was seen in treatment-naive patients compared with the previously treated ones, as the median time to treatment failure was 22 months vs 12 months (P = .001) for each cohort, respectively.[14] Relapses were common, however: a proportion of patients between 36% and 75% experienced disease relapse after a median follow-up of 15 to 20 months. In their series, the investigators stated that patients were switched to either radiation or chemoimmunotherapy after relapse.[14]

Single-agent rituximab has also shown efficacy in treatment of cutaneous MZL, particularly in cases with multiple and noncontiguous lesions. In a retrospective study of primary cutaneous indolent B-cell lymphomas, all 5 patients with MZL obtained a response, with a CR in 4 cases.[21]

Back to the clinical case

Two years later, a follow-up CT scan showed the appearance of a solid and irregular nodular lesion of the right upper lobe. The patient was well and did not report any symptoms. No lymph nodes were detected at the hilar or mediastinal sites or at any other significant site. A positron-emission tomography scan showed positive uptake of the nodule, without any other pathologic finding. An adequate amount of tissue was collected via transbronchial fine-needle biopsy, and a diagnosis of bronchus-associated lymphoid tissue lymphoma was made. Endoscopy showed a macroscopically normal gastric mucosa, and random biopsies excluded the recurrence of marginal zone lymphoma.

What was the most suitable treatment strategy?

Systemic immunochemotherapy was prescribed, with rituximab+bendamustine given for 6 cycles. A CR was achieved at the end of the treatment program.

Chemoimmunotherapy in advanced or resistant MALT lymphomas

As a rule, systemic chemoimmunotherapy should be considered in cases of advanced-stage disease and in cases of persistent or recurrent lymphoma after treatment with radiotherapy or single-agent rituximab, if it occurs locally or spreads systemically.[3,4] Advanced-stage disease indicates lymphoma dissemination to adjacent organs and tissues (Lugano stage IIE for gastric MALT lymphoma), to distant lymph nodes (Lugano stage II$_2$ and Ann Arbor stage IV), bilaterally to an extranodal site (eg, lungs and parotid glands), or to the bone marrow or any other extranodal organ (Lugano and Ann Arbor stage IV). Chemoimmunotherapy should be initiated when a strict indication for treatment is present, as it is generally effective for any indolent lymphoma. Indications for systemic treatment include gastrointestinal bleeding, presence of systemic lymphoma-related symptoms, threatened end-organ function, bulky masses, and steady or rapid progression. If no indication is present, advanced disease should be managed conservatively, and close monitoring should be established.[4] Chemoimmunotherapy is also the mainstay of the management of disease transformation into aggressive B-cell lymphoma. In this case, specific drug combinations active in diffuse large B-cell lymphomas should be taken into account [eg, rituximab+cyclophosphamide, doxorubicin, vincristine, and prednisone (*R*-CHOP)].

Very few drugs have been directly tested in MZLs: most of the experience comes from trials that enrolled patients with indolent B-cell lymphomas in general. Alkylating agents (cyclophosphamide, chlorambucil, and bendamustine) or purine nucleoside analogues (fludarabine and cladribine) have shown efficacy in this context. An extensive review of chemotherapy-containing regimens in MALT lymphomas has been published.[22] Table 3 reports experiences in gastric MALT lymphoma.[23-26]

The IELSG-19 trial is to date the largest randomized trial to show the superiority of a rituximab-containing regimen over monotherapy in extranodal MALT lymphoma.[17] Initially designed as a phase 3 trial randomizing patients to rituximab+chlorambucil or chlorambucil alone in a 1:1 fashion, it was amended to include a third arm consisting of single-agent rituximab, and the enrollment ratio was changed at that point to 1:1:6. Patients with extranodal MALT lymphoma, either at diagnosis or after failure of a prior local therapy were eligible for the study. Of the patients eligible for final analysis (n = 401), 8% had received prior local therapy (surgery, radiation therapy, and antibiotics), 44% had advanced-stage disease (Ann Arbor stage III-IV), with nodal involvement in 35% of those cases and bone marrow involvement in 18%. Patients' clinical characteristics were all well-balanced between treatment arms. Chlorambucil was given daily at 6 mg/m^2 for 42 consecutive days (6 weeks),

Table 3. Chemotherapy and chemoimmunotherapy outcomes in gastric MALT lymphoma

Study	Patients	Early stage, %	Treatment	Outcomes, %
Hammel et al[23]	24	71	Cyclophosphamide or chlorambucil	75 CR
Avilés et al[24]	83	100	CHOP×3 + CVP×4	100 CR
Jäger et al[25]	19	100	Cladribine	100 CR
Salar et al[26]	21	64	Bendamustine+rituximab	94 CR; 6 PR
Zucca et al[17]	53	—	Rituximab+chlorambucil	91 CR
	57	—	Chlorambucil	61 CR
	61	—	Rituximab	67 CR

then resumed at the same dose for 2 weeks with 4-week intervals for up to 4 months (maintenance treatment, if at least a stable disease was obtained). Rituximab was given at a standard dose of 375 mg/m^2 on days 1, 8, 15, and 22, then on day 1 of each cycle for maintenance. Patients receiving single-agent rituximab did so on the schedule used for combination therapy. Treatment with the rituximab+chlorambucil combination produced better responses than chlorambucil alone (ORR, 95% vs 85%; CR rate, 79% vs 63%), as reported earlier.[27] Rituximab alone produced an outcome that was similar to chlorambucil alone (ORR 78%; CR rate 56%), with a 5-year EFS of 68%, 51%, and 50% for patients treated with the combination, with chlorambucil alone, or single-agent rituximab, respectively.[17] Despite the demonstrated superior efficacy of the combination regimen, longer overall survival (OS) rates were not appreciated, most likely because of effective salvage treatment options in this indolent disease. Although EFS was inferior with single-agent rituximab, the lack of OS differences among treatment arms reinforces its role in the initial treatment of extranodal MALT lymphomas, as its application may delay the toxic effects related to chemotherapy or radiotherapy.

The addition of rituximab to bendamustine (the latter given at a dose of 90 mg/m^2) produced significant results in a phase 2 prospective trial.[26] The trial enrolled 60 patients (57 evaluable) with untreated extranodal MALT lymphoma (any stage) in need of systemic treatment, patients with gastric MALT with active disease after HP-eradicating treatment, and patients with cutaneous MZL in whom initial local treatment had failed. Treatment was given in 4 cycles if the patient achieved an early CR after the third course or in 6 cycles if the patient was in partial response at midtreatment evaluation. The regimen produced an ORR of 100% with CR in 98% of the cases, with no significant differences according to the primary site of disease (gastric vs nongastric). As 75% of patients achieved a CR after only 3 cycles, treatment duration was 4 months in most instances. The 7-year EFS and PFS were 88% and 93%, respectively; outcomes were not significantly different according to disease localization.[28] Our institutional experience with rituximab and bendamustine in MALT and non-MALT MZLs yielded similar results.[29]

Fludarabine-containing regimens have also demonstrated their efficacy in the treatment of MZL, including extranodal MALT lymphomas with advanced-stage presentation and need for treatment. A retrospective experience from our group with the rituximab-fludarabine-mitoxantrone regimen in indolent nonfollicular lymphomas included 49 patients with MALT lymphoma: we recorded an ORR of 96%, with a CR rate of 90% and a disease-free survival rate at 10 years >90%.[30] In a homogeneous cohort of 17 patients with bronchus-associated lymphoid tissue lymphoma from our institution, treated with the rituximab-fludarabine-mitoxantrone regimen (10 patients) or with fludarabine-mitoxantrone (7 patients), the ORR was 100%, with a CR in 82% of the cases, with an OS of 100% and a PFS of 71% at 14 years. No comparisons could be made between rituximab-treated and untreated patients because of the small sample.[31] Importantly, however, fludarabine-based regimens have been increasingly abandoned in the treatment of indolent non-Hodgkin lymphoma, given the associated risk of secondary malignant disease. For this reason, their use should follow a careful risk-benefit evaluation.

Treatment sequencing in SMZL

Treatment should be established in symptomatic patients with SMZL with massive splenomegaly causing pain, early satiety, or cytopenia, defined as hemoglobin < 10.0 g/dL, platelets < 80 × 10^3/microL, or neutrophils < 1 × 10^3/microL. Asymptomatic patients may be followed clinically without intervention for many years, given that treatment does not influence survival.[3] Autoimmune manifestations, such as autoimmune hemolytic anemia or immune thrombocytopenia, are not infrequent in patients with SMZL and may complicate the course of the disease, even if proper treatment criteria are absent. They should be promptly diagnosed and specifically treated. Single-agent rituximab may be useful in this regard.[32] In patients with concomitant hepatitis C virus (HCV) infection who do not need immediate treatment, antiviral therapy should be considered, if feasible, and appropriate hepatology consultation is advised.[33,34] Rituximab and splenectomy are the recognized first-line treatment options in symptomatic patients. Chemoimmunotherapy is an option in case of first-line treatment failure (Figure 2).

Splenectomy

Surgery is traditionally considered the initial treatment for SMZL with massive and symptomatic splenomegaly and peripheral cytopenias. It rapidly corrects anemia, thrombocytopenia, and neutropenia, when it is related to hypersplenism, and removes the dominant focus of the disease.[35] Evidently, such management does not influence marrow infiltration or blood lymphocytosis. Postsplenectomy remissions are generally stably maintained for years, with PFS and OS rates of 50% to 60% and 70% to 80%, respectively, and patients can remain asymptomatic for very long periods, with a median time to the next treatment of 8 years.[36] Importantly, however, not all patients are deemed eligible for

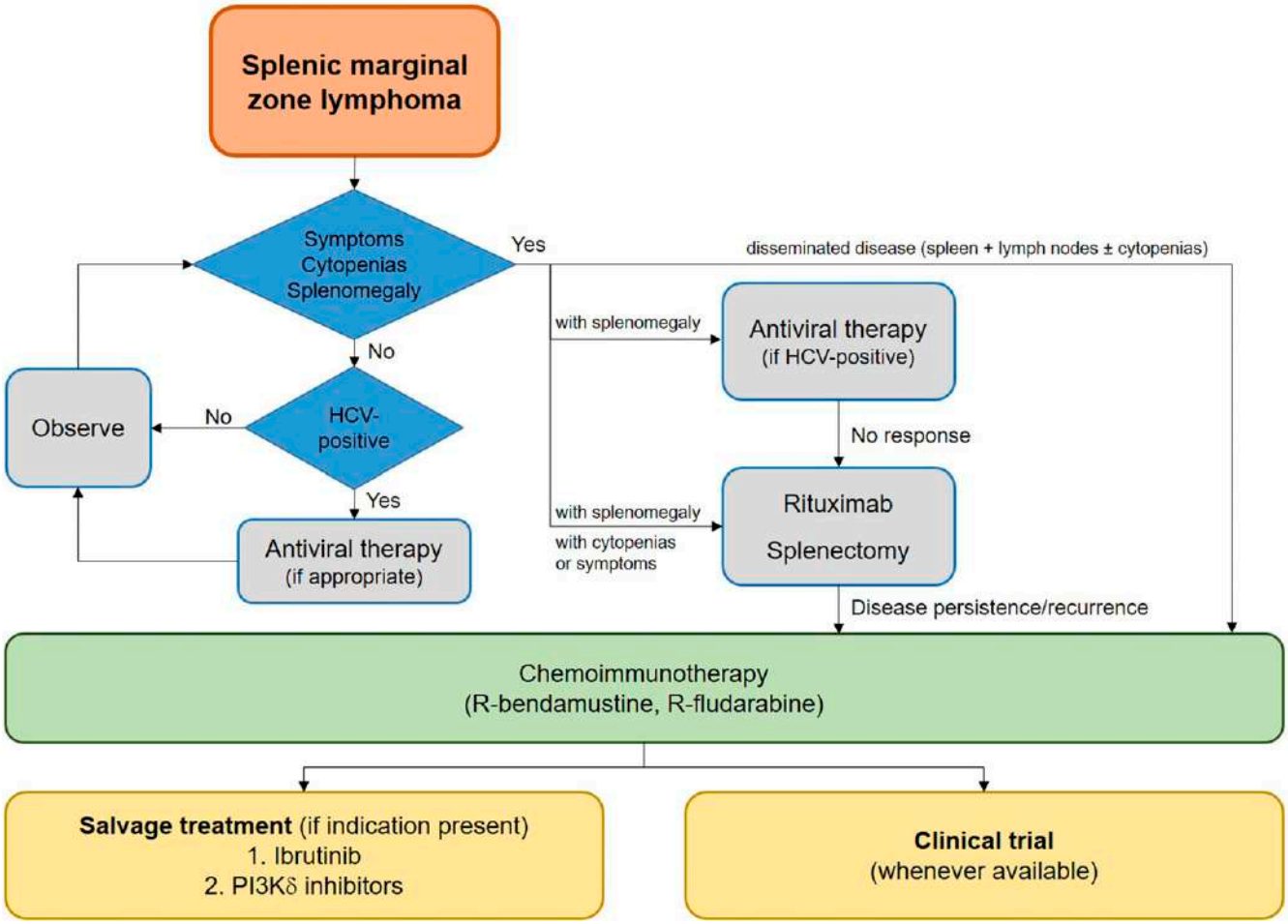

Figure 2. Treatment sequencing in splenic MZL. R, rituximab.

splenectomy, which is contraindicated in cases with disease dissemination to distant lymph nodes or other parenchymas, as well as those in which cytopenias are secondary to massive bone marrow infiltration and are not believed to be correctable by splenectomy alone. It must be noted that splenectomy is a major surgical procedure, with potential acute and late complications. Vaccinal prophylaxis against capsulated bacteria is always necessary.[37] Splenectomy also has the advantage of ruling out a possible histologic transformation, which can be suspected in cases with rapid spleen enlargement, elevation of lactate dehydrogenase, or appearance of systemic symptoms.

Rituximab

Rituximab, used as a single agent or combined with chemotherapy, is highly effective in this subgroup of patients and is preferred in comparison with splenectomy by some clinicians.[36,38-41] Single-agent rituximab (375 mg/m^2 weekly for 4-8 weeks) produces rapid responses, with an ORR of 88% to 100%, CR in nearly 45% to 90% of cases, and a 10-year PFS that may exceed 60%.[36] It also remains active in cases with disease relapse.

Chemoimmunotherapy

Chemoimmunotherapy regimens are based on rituximab combined with alkylating agents (cyclophosphamide or bendamustine), anthracyclines, or fludarabine.[36] This approach is particularly indicated in cases of disseminated disease at presentation with clinical symptoms, as well as in patients who are unresponsive to first-line therapy or have disease recurrence. Available data are from retrospective experiences involving a limited number of patients with the application of various combinations of drugs and treatment schedules. For this reason, comparison of published series is risky, and none of the described regimens can be deemed the gold standard. Reported ORRs are higher than 80%, with CR in more than half of the treated patients and long response durations, however with not negligible toxicity, especially infections. The BRISMA (Italian Lymphoma Foundation; FIL/IELSG-36) study has recently investigated the efficacy of the combination of rituximab+bendamustine in 56 SMZL patients with symptomatic disease, who were ineligible for splenectomy and had not responded to HCV-directed antiviral treatment.[42] Bendamustine was given at a dose of 90 mg/m^2 on days 1 and 2 in cycles of 28 days, for 6 cycles. The ORR rate was 91% and the CR rate was 73%, with 3-year PFS and OS of 90% and 96%, respectively. Toxicity was mostly hematologic, with grade 3-4 neutropenia recorded in 43% of patients, which compares favorably with a previous Italian experience with rituximab with cyclophosphamide, liposomal doxorubicin, vincristine and prednisone (R-COMP) in the same subset of patients.[43] Based on the results in B-cell indolent lymphomas,[44,45] we suggest

the rituximab+bendamustine combination be used as the frontline treatment in patients with disseminated disease (splenomegaly and distant nodal involvement) in need of therapy and as a salvage regimen after failure of single-agent rituximab or splenectomy.

Treatment sequencing in NMZL

Guidelines recommend that NMZL be treated as any other indolent non-Hodgkin lymphoma with lymph node involvement, according to the principles applied in follicular lymphoma.[3,4,46]

Rituximab and radiotherapy

In patients with limited-stage disease (stage I or contiguous stage II, according to Ann Arbor), ISRT or rituximab monotherapy (after surgical excision of the affected lymph node for diagnostic purposes) is often appropriate. Single-agent rituximab is also a valuable first-line option in cases of noncontiguous stage II disease; chemoimmunotherapy is a suitable alternative. Observation may also be adequate in these cases if the potential toxicity of a systemic approach outweighs the expected clinical benefit.

Chemoimmunotherapy

Chemoimmunotherapy is indicated in patients with symptomatic (high tumor burden), advanced-stage disease. In contrast, a watchful-waiting policy is adopted if the tumor burden is low, although the disease is disseminated. Rituximab+bendamustine,[44,45] rituximab+fludarabine,[30] and rituximab+cyclophosphamide, vincristine, prednisone±anthracyclines (R-CHOP/R-CVP) are the most widely used regimens. Rituximab maintenance after induction is optional, as there is no evidence from prospective studies of any benefit over observation only, if frontline therapy was successful. Chemoimmunotherapy is also indicated for disease progression or when radiotherapy or rituximab therapy fail. Lymph node biopsy is always recommended at disease relapse to rule out the possibility of histologic transformation.

Autologous stem cell transplantation

The role of autologous stem cell transplantation (ASCT) in patients with MZL was investigated in a retrospective study by the European Society for Blood and Marrow Transplantation, the Italian Lymphoma Foundation and the Italian Group for Bone Marrow Transplantation.[47] The study involved 199 patients with nontransformed NMZL (55 patients), MALT lymphoma (111 patients), and SMZL (33 patients), with a median age at transplantation of 56 years. The median number of prior therapies was 2; patients had chemosensitive disease at the time of conditioning, which was chemotherapy-based in 89% of the cases. A trend toward performing more ASCTs in non-MALT MZL has been noted in more recent years. After a median follow-up of 5 years, the 5-year cumulative incidence of relapse-progression and nonrelapse mortality was 38% and 9%, respectively. Five-year EFS and OS were 53% and 73%, respectively. The much higher OS compared with EFS indicates that patients experiencing ASCT failure can still be salvaged, especially in this era of new drugs. This observation is of importance, because it indicates that the role of ASCT in treatment sequencing for MZL needs further clarification. For this reason, we consider high-dose therapy and ASCT only for young patients with aggressive presentations and short duration of remission after standard immunochemotherapy regimens, and mainly for those with nodal disease.

Immunomodulators, targeted agents, and new drugs under development

Systemic approaches with targeted agents, used either as single agents or in combination with immunotherapy, represent a step forward in the optimization of treatment of patients for whom conventional immunochemotherapy is unsuitable and in cases of relapsed or refractory disease.[48] Enrollment of patients in clinical trials exploring new agents is always desirable when standardized treatment strategies are unavailable. A complete survey of agents being explored in clinical trials is beyond the scope of this article. We provide a review of the existing results with agents we usually apply in patients who experience multiple relapses and meet treatment requirements (Table 4). It is important to note that most of these agents are not formally approved worldwide, as data are generated from subgroup analyses or derived from monocentric experiences.

Radioimmunotherapy

The most significant experience with single-agent ^{90}Y-ibritumomab tiuxetan (^{90}Y-IT) radioimmunotherapy in relapsed or refractory EMZL is reported in a prospective Italian trial with 30 patients,[49] 17 affected by extragastric MALT lymphoma (including skin, orbit and conjunctiva, soft tissues, parotid, liver, and kidney) and 13 by HP$^-$ gastric MALT lymphoma, all of whom had undergone at least 1 treatment (including radiation or surgery). High response rates were reported (90%), with 23 (77%) patients achieving a CR. Only 2 CR patients had a relapse, whereas all the remaining ones maintained their status for at least 3 years.

Samaniego et al[50] and Lossos et al[51] investigated the activity of ^{90}Y-IT in untreated patients. Both studies reported high response rates (at least 90%), with median PFS durations exceeding 4 years. Similar results with ^{90}Y-IT have been reported recently by our group[52] in a series of both untreated and pretreated patients with EMZL: 94% of patients responded to radioimmunotherapy, with CR achieved in 63% of the cases; 45% of the treated patients were free of disease at 4 years.

The efficacy of radioimmunotherapy as consolidation after chemotherapy in untreated patients with indolent lymphoma, including 10 untreated MZL, was reported in 2008 by our group. ^{90}Y-IT was given after 6 cycles of fludarabine+mitoxantrone and 2 doses of rituximab (250 mg/m^2, 7 days before and immediately before the administration of ^{90}Y-IT). ^{90}Y-IT is appropriate for patients achieving at least a partial response after chemotherapy, with a bone marrow disease infiltration <25% of the cellularity and a platelet count ≥100 × 10^3/microL. All but 1 patient with MZLs successfully obtained a CR at the end of the treatment.[53]

Promising results with β-lutin, a murine anti-CD37 antibody conveying the β-emitter ^{177}Lu are now emerging with relapsed/refractory follicular lymphoma and patients with MZL, with responses in nearly 60% of the cases and CR in up to 28%.[54]

Lenalidomide, with or without rituximab

Kiesewetter et al reported initial data on single-agent lenalidomide, given at a dose of 25 mg/day for 21 consecutive days in patients with untreated or relapsed nongastric MALT lymphoma and HP$^-$ gastric MALT lymphoma, that were consistent with an ORR of 61% and a CR rate of 33%.[55] Responses were seen in both treatment-naive and pretreated patients, and conversions to better responses were documented with continuous therapy in nearly 40% of patients. The same group demonstrated the activity of lenalidomide (20 mg/d for 21 days) and rituximab

Table 4. Experiences with targeted and novel agents in marginal zone lymphomas

Study	Drug	Setting	Patients	ORR %	CR %	Median PFS, mo
Vanazzi et al[49]	^{90}Y-ibritumomab tiuxetan	MALT relapsed	30	90	77	Not reached*
Samaniego et al[50]	^{90}Y-ibritumomab tiuxetan	MALT de novo	11	100	—	Not reached
Lossos et al[51]	^{90}Y-ibritumomab tiuxetan	MZL de novo	16	88	56	47.6
Lolli et al [52]	^{90}Y-ibritumomab tiuxetan	MALT de novo+relapsed	16	94	63	37.3
Zinzani et al [53]	Chemotherapy+^{90}Y-ibritumomab tiuxetan	MZL de novo	10	90	90	—
Kiesewetter et al [55]	Lenalidomide	MALT de novo+relapsed	18	61	33	—
Kiesewetter et al [56]	Lenalidomide+rituximab	MALT de novo+relapsed	46	80	54	—
Becnel et al [59]	Lenalidomide+rituximab	MZL de novo	30	93	70	59.8
Noy et al [61]	Ibrutinib	MZL relapsed	63	48	3	14.2
Gopal et al [62]	Idelalisib	MZL relapsed	15	47	7	7.0
Flinn et al [63]	Duvelisib	MZL relapsed	18	39	—	—
Dreyling et al [64]	Copanlisib	MZL relapsed	23	70	9	—
Forero-Torres et al [65]	Parsaclisib	MZL relapsed	9	78	33	—
Zinzani et al [66]	Umbralisib	MZL relapsed	69	55	10	71%†

*Time-to-treatment failure.
†PFS percentage at 12 months.

(375 mg/m^2 on day 1 of each cycle for 6 cycles) in 46 patients with extranodal MALT lymphoma. One quarter of the patients had received prior systemic treatment, and nearly 40% had disseminated disease at presentation. The combination demonstrated an ORR of 80% and a CR rate of 54%, along with a favorable toxicity profile.[56] Based on their experience with lenalidomide in MZL, the investigators reported that at least half of the patients were relapse free at a median follow-up of 68 months, with a median PFS of 72 months. According to their experience, patients with primary extragastric disease had better outcomes than those with primary gastric MALT lymphoma. They found no differences in PFS according to disease stage or previous systemic therapy, although there were limitations related to the number of patients included in their series.[57] In the experience reported by the MD Anderson Cancer Center in 30 previously untreated advanced-stage MZL patients, lenalidomide+rituximab (same schedule as in Kiesewetter et al, with possible treatment extension of lenalidomide only for 6 more cycles if patients were responding) produced an ORR of 93%, with a CR rate of 70% and a median PFS of 60 months at a median follow-up of 75 months.[58,59] Although generated from small cohorts of patients and from subgroup analyses of larger trials involving patients with multiple indolent histologies, these data suggest that lenalidomide and rituximab may represent an attractive chemotherapy-free treatment to be offered to patients with MZL during either induction or salvage treatment.

The improved efficacy of the combination of lenalidomide+rituximab over rituximab+placebo has been confirmed in a phase 3 randomized trial with 358 patients with relapsed/refractory follicular lymphoma and marginal zone lymphoma, with a median PFS of 39.4 months vs 14.1 months for each treatment arm.[60]

Ibrutinib

The efficacy and safety of ibrutinib in relapsed and refractory MZL have been demonstrated in a phase 2 study involving 63 patients. Nearly half of them had extranodal measurable lymphoma lesions: 22% had SMZL, and 22% had NMZL. Ibrutinib dosing was 560 mg once daily until progression or unacceptable toxicity. In 60 evaluable patients, the ORR was 48%, with a CR in 3% of patients. At a median follow-up of 19.4 months, the median duration of response was not reached, and the median PFS was 14.2 months.[61] These data constituted the rationale for the United States Food and Drug Administration's approval of ibrutinib for the treatment of patients with MZL in need of therapy, in whom at least 1 prior anti-CD20 therapy has failed.

PI3K inhibitors

The blockade of the phosphatidyl-inositol 3-kinase (PI3K) pathway seems highly promising for the treatment of relapsed or refractory MZLs. Several drugs now commercially available all inhibit the PI3Kδ isoform with different selectivity.[62-66] Idelalisib, duvelisib, copanlisib, and, more recently, parsaclisib and umbralisib have all been tested in clinical trials enrolling patients with pretreated indolent lymphomas, mostly follicular lymphoma, among which patients with MZL represented a small subset. These agents are all effective, as objective responses are seen in a proportion of cases varying from 47% to 78%, but the achievement of a CR is infrequent (7% to 33%), sometimes with relevant toxicity in terms of infections, inflammatory adverse events, or metabolic alterations.

Back to the clinical case

After 18 months, multiple nodular lesions were detected in both lungs on high-resolution CT scan, all with pathologic uptake in a positron emission tomography scan. A transthoracic biopsy of the largest nodule confirmed the diagnosis of MALT lymphoma. No extrathoracic evidence of disease was found.

Which salvage treatment can be started?

The patient was started on ibrutinib, obtaining a partial response after 4 months. At this writing, treatment was still ongoing, without significant side effects.

Conclusions

MZLs are indolent diseases with long survival rates but a high tendency to relapse over time. Extranodal disease is frequently confined to a single organ or site, even in cases with recurrence. SMZL, if asymptomatic, may be followed up without treatment for years. NMZL is often disseminated at presentation and requires treatment if advanced-stage disease is accompanied by high tumor burden.

Sequential application of various treatments is the key to success in the treatment of MZL. On the one hand, this strategy is necessary to preserve adequate organ function and avoid excess toxicity, as systemic treatment is limited to advanced symptomatic or recurrent disease. On the other hand, this approach increases survival rates. New drugs and targeted agents offer treatment opportunities in highly pretreated individuals and in particular categories of patients, often limiting the need for repeated application of cytotoxic drugs.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

Off-label drug use

None disclosed.

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Transformed lymphoma: what should I do now?

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Although the majority of indolent lymphomas (focusing on follicular lymphoma [FL]) have a prolonged waxing and waning course, a portion of patients experience histologic transformation (HT) to either diffuse large B-cell lymphoma or a higher-grade morphology, often with acquisition of *MYC* and *BCL2* and/or *BCL6* rearrangements (high-grade B-cell lymphoma–double-hit lymphoma/triple-hit lymphoma). The overall incidence of HT and transformed follicular lymphoma (tFL) may be declining, but outcomes remain inferior to those in simple indolent lymphoma progression. Recent data suggest that the majority of HT cases occur in higher-risk patients with FL, and they occur early after initial chemoimmunotherapy, comprising the majority of patients with progression of disease within 24 months. This latter point emphasizes the need for a sufficient biopsy at relapse in FL. Treatment options depend on the prior therapy for the indolent component as well as the histology at relapse, but they generally follow several principles discussed in this article. Anthracycline-naïve patients have the best outcomes if there is HT, and responses to R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) are similar to those of patients with de novo diffuse large B-cell lymphoma. Patients with anthracycline exposure prior to transformation have the best outcomes with salvage chemotherapy and a consolidative autologous stem cell transplant. However, a major challenge is the management of patients with tFL who experience relapse early after bendamustine-based treatment, in whom the role of consolidative transplant after anthracycline-based treatment is unclear. In the past several years, cellular therapy has emerged as an important tool for some but not all patients with tFL. This review focuses on the nuances of managing tFL.

LEARNING OBJECTIVES

- Develop a treatment strategy for patients with transformed follicular lymphoma
- Describe the time to transformation for high-risk patients and the need for a biopsy for patients with early progressing follicular lymphoma

Clinical cases

Patient 1

Patient 1 was a 59-year-old man who initially presented with an acute abdomen while traveling. He was found to have appendicitis but also was noted to have multiple retroperitoneal lymph nodes. An inguinal lymph node biopsy revealed follicular lymphoma (FL) grade 1 to 2. Staging showed diffuse adenopathy (largest lymph node, 4.6 cm). He had mild anemia but a normal lactate dehydrogenase (LDH) level. He was treated with bendamustine and rituximab that required dose reduction due to cytopenias for cycles 5 and 6. Twenty-two months later, he had progressive adenopathy in the axillary region. A biopsy again showed FL, but it was now grade 3A. A bone marrow biopsy was performed, which showed a hypercellular marrow (70%) comprised largely of $CD20^+CD10^+BCL2^+$ large cells consistent with transformed follicular lymphoma (tFL) (Figure 1).

Patient 2

Patient 2 was a 61-year-old man who had presented with a right neck mass 8 years ago. A biopsy showed FL grade 1. He had undergone active observation until several months ago, when he noted right axillary swelling. He was evaluated in the clinic and was found to have an extensive mass in his right axilla and infiltrating the chest wall (Figure 2). A biopsy showed transformation to an aggressive lymphoma with a Ki67 of 95% and sheets of mitotically active intermediate to large cells with a high nucleus/cytoplasm ratio, $CD20^+CD10^+MYC^+BCL2^+$. Subsequent fluorescence in situ hybridization confirmed rearrangements of *MYC* and *BCL2*.

Patient 3

Patient 3 was a 78-year-old man with a 15-year history of FL. He had previously been treated with rituximab monotherapy on 2 occasions, with the last one ~10 years ago. He

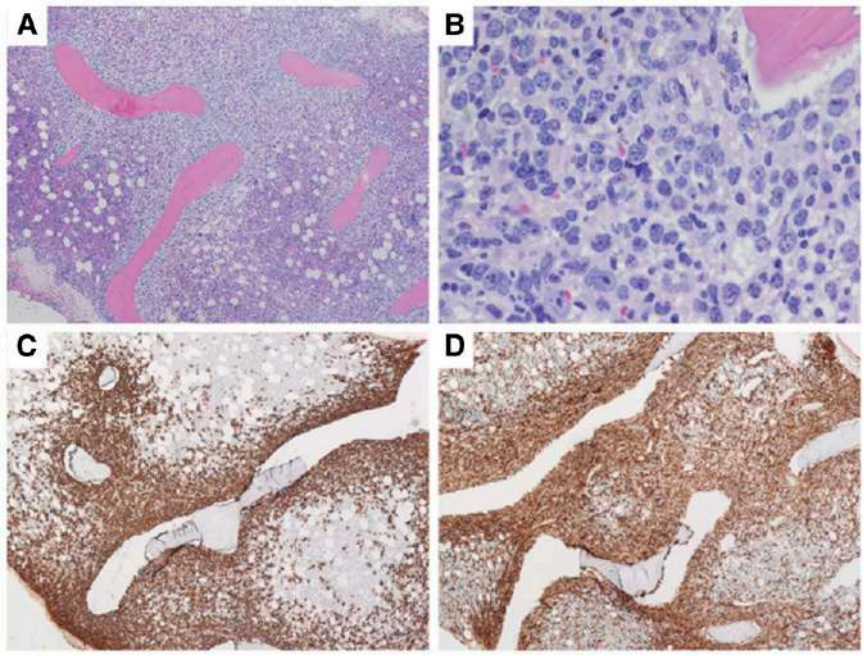

Figure 1. Bone marrow biopsy pathologic images of patient 1. (A) Low power view showing extensive paratrabecular aggregates of lymphoma cells; (B) higher power view showing large polylobate centroblasts that are CD20 positive (C) and CD10 positive (D). (Images courtesy of Dr. Girish Venkataraman, University of Chicago.)

is feeling well but presents to the clinic with a new right axillary mass measuring 6.4 × 3.3 cm. He is not sure how quickly this might have grown. Biopsy shows diffuse large B-cell lymphoma (DLBCL) involving >90% of the lymph node with a residual area of FL. Fluorescence in situ hybridization testing reveals no abnormalities.

Introduction

Indolent lymphomas, including FL, marginal zone lymphoma, lymphoplasmacytic lymphoma, and chronic lymphocytic leukemia/small lymphocytic lymphoma, have the potential to undergo histologic transformation (HT) into an aggressive disease with a mandatory change in treatment approach. The historic outlook for transformed lymphomas was quite poor, but current treatment paradigms can lead to prolonged survival, particularly if patients are minimally pretreated before the transforming event. Very few prospective trials have been dedicated solely to transformed lymphomas, and most decision making derives from subset analysis of trials on aggressive lymphoma. Furthermore, it is unclear if the preceding indolent histology affects outcome or should impact selection of therapies. Richter's transformation, the moniker for HT of chronic lymphocytic leukemia/small lymphocytic lymphoma to either an aggressive B-cell lymphoma or Hodgkin lymphoma, is the clearest example in which management of transformed disease may differ from that for aggressive lymphomas arising from a preceding FL or marginal zone lymphoma. Given the even more sparse data for nonfollicular HT, this review focuses on data impacting clinical decision making for tFL.

Incidence and diagnostic considerations in tFL

The incidence of transformation has been declining in the modern era, often attributed to better disease control of FL using rituximab and other anti-CD20 targeted agents.[1] However, it is important to note that transformation rates in high-risk patients remain elevated, and outcome measures, although improved, still lag behind those used for patients without HT. A recent French analysis found that more than half of FL-related deaths are due to HT.[2] In terms of incidence, the LymphoCare study, an observational FL dataset of >2600 patients, found that 14.3% of patients experience transformation with 6.8 years of median follow-up.[3] This study identified poor performance status, more than one extranodal site, elevated LDH, and B symptoms as being associated with a higher risk of transformation. The PRIMA trial identified several predictors of HT, including high Follicular Lymphoma International Prognostic Index (FLIPI) score (and its components of anemia and increased LDH), poor performance status, and presence of B symptoms without impact of depth of response to chemoimmunotherapy induction or delivery of maintenance rituximab.[4] Overall, 63% of patients with documented HT had a baseline FLIPI score of 3 to 5. Survival after transformation is inferior to that for progressive FL at 3.8 years (tFL) vs 6.4 years (FL) in the PRIMA trial. In addition to clinical factors, a number of genomic and biologic features may predict a higher risk of HT, including chromosome deletions and gains (del1q, del6q, +2, +3q, +5); loss of B2M; increased T-regulatory cells; or mutations in *TP53*, *PIM1*, *B2M*, and others (reviewed by Pasqualucci et al[5]). Whether newer FL prognostic indices such as the M7-FLIPI[6] are also predictive of HT risk remains unknown.

Data from the prerituximab era estimated a continuous 3% risk of HT per year over 15 years, with a median survival of 1.7 years after transformation.[7] In contrast, for patients with high tumor burden, many of whom also have high FLIPI scores, the time to transformation appears quite short. Furthermore, many patients with FL with progression of disease within 24 months (POD24)[8] actually have HT rather than simple FL progression. In the PRIMA trial, for example, more than half of transformations

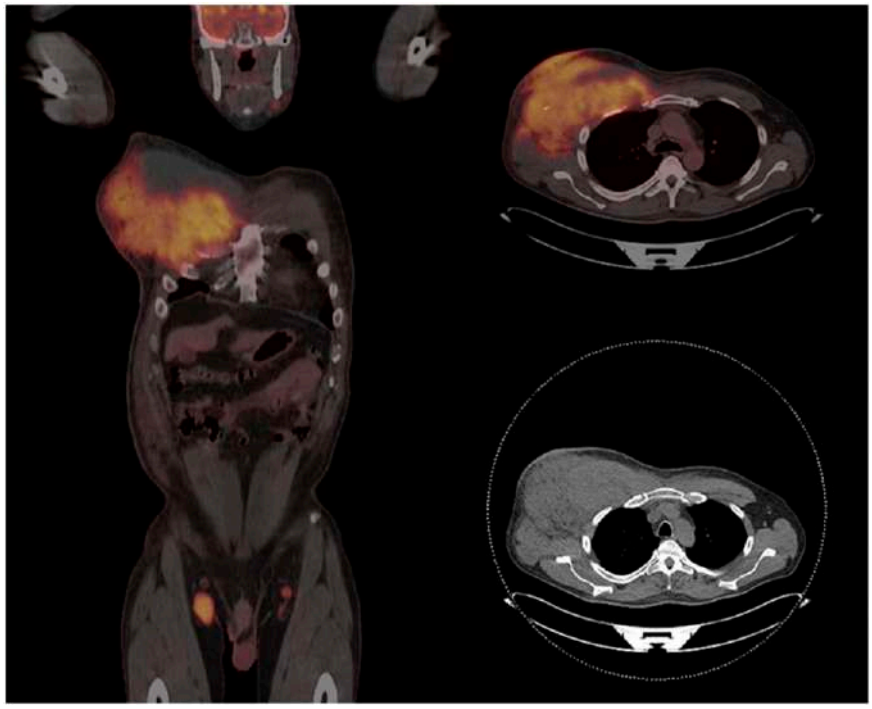

Figure 2. Patient with FL transforming to HGBL-DHL/THL, showing massive infiltration of the right chest wall and axillary adenopathy.

occurred in the first year of follow-up, with the median time to transformation being only 9.6 months, underscoring the need to perform biopsies in patients with early progression. A Canadian population-based study found that 76% of patients with FL progressing within 2 years had HT, with a median time to transformation of 8.4 months.[9] Two-year survival of patients with POD24 found to have HT was only 40%, emphasizing its dire prognosis. In these trials and others, it is highly possible that occult transformation was present at diagnosis.

Confirming HT requires a diagnostic biopsy of sufficient size. Functional imaging with 18-fluorodeoxyglucose–positron emission tomography can help guide the choice of biopsy site, based on the rationale that more highly proliferative lesions will have a higher standardized uptake value. However, this is somewhat controversial,[10] and occasionally a biopsy is neither safe nor feasible. In this case, supportive evidence for HT includes rapid progression of adenopathy associated with a significantly elevated LDH and symptomatic presentation. Another key diagnostic consideration is that transformation is not always DLBCL histology; because the vast majority of patients with FL already harbor the *t(14;18)* rearrangement, acquisition of *MYC* rearrangements [usually *t(8;14)*] will lead to a diagnosis of high-grade B-cell lymphoma (HGBL) with *MYC* and *BCL2* and/or *BCL6* rearrangements (high-grade B-cell lymphoma/double-hit lymphoma/triple-hit lymphoma [HGBL-DHL/THL]). In this case, treatment with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) is rarely sufficient, and more intensive regimens such as DA-EPOCH-R (etoposide phosphate, prednisone, vincristine sulfate, cyclophosphamide, doxorubicin hydrochloride [hydroxydaunorubicin], and rituximab) should be considered. The possibility of HT to a higher-grade process such as HGBL-DHL/THL makes an adequate biopsy even more critical in order to deliver the optimal treatment.

What is the best initial treatment of tFL, and should patients receive a consolidative autologous stem cell transplant?

The best initial treatment depends on the prior therapy for the underlying indolent lymphoma and the histology at the time of transformation (Figure 3). There are several clinical scenarios to consider: tFL in treatment-naïve patients with FL (including simultaneous diagnosis of FL/tFL), tFL after prior anthracycline-based chemoimmunotherapy, and tFL developing after prior therapy that did not include cytotoxic chemotherapy. An example of a scenario with almost no data is when HT occurs after prior bendamustine-based chemoimmunotherapy and whether to treat these patients similarly to anthracycline-exposed patients is unclear.

Initial approach for treatment-naïve or minimally pretreated patients with tFL

For patients who have never received treatment of FL or have only received nonchemotherapy approaches, management of tFL follows the same paradigm as de novo DLBCL or HGBL-DHL/THL, depending on the histology. A number of datasets show that tFL with no prior anthracycline exposure has a relatively good outcome similar to de novo DLBCL. A Mayo Clinic/Iowa study found equivalent event-free survival and overall survival (OS) among 109 patients with simultaneous presentation of FL and DLBCL when compared with patients with de novo DLBCL.[11] An Molecular Epidemiology Resource analysis showed 5-year OS rates of 66%, similar to de novo DLBCL.[1] A UK study of 87 patients with tFL reported a 5-year OS of 64% with R-CHOP–like therapy; importantly, the addition of autologous hematopoietic stem cell transplant (auto-HCT) for patients who were previously untreated for FL did not improve outcomes.[12] A Danish National Lymphoma Registry analysis evaluated 85 patients with transformed indolent lymphomas, all of whom had biopsy confirmation

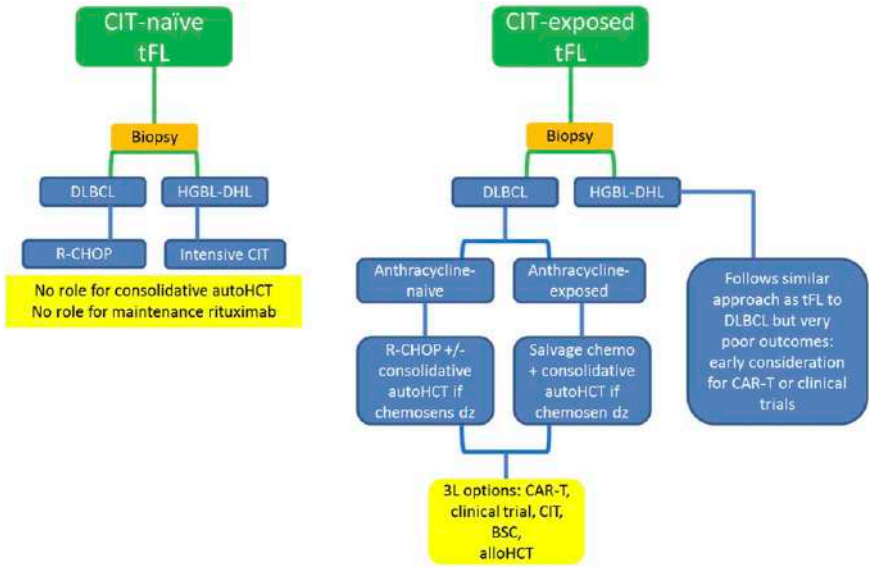

Figure 3. Graphic summary of how I treat tFL. CIT, chemoimmunotherapy; BSC, best supportive care; HCT, hematopoietic stem cell transplant.

of DLBCL in addition to the indolent component; the majority had tFL.[13] The authors found that consolidative auto-HCT had the greatest benefit in patients with "sequential transformed indolent lymphoma," where patients relapsed after prior treatment of their indolent lymphoma; in contrast, there was no significant benefit for patients presenting with simultaneous indolent and aggressive disease and no prior treatment. As might be expected, the magnitude of benefit was greatest in patients who were previously rituximab naïve. On the basis of these series, patients with tFL without prior chemotherapy for FL should be treated with anthracycline-based chemoimmunotherapy without a consolidative auto-HCT.

Maintenance rituximab in tFL

Given improved progression-free survival (PFS) and very prolonged response durations in FL, should maintenance rituximab be considered for patients with tFL? The preponderance of data suggests no benefit for tFL, similar to de novo DLBCL. For example, a Canadian registry analysis identified 107 patients with either discordant or composite lymphomas, of whom 55 received maintenance rituximab and 52 did not. With prolonged follow-up exceeding 7 years, there was no statistically significant difference in PFS, OS, or freedom from indolent progression.[14] Similarly, an MD Anderson Cancer Center identified 311 patients with treatment-naïve tFL treated with R-CHOP–like chemoimmunotherapy, of whom 50 received maintenance rituximab.[15] In a 1:2 propensity-based analysis, there were no PFS or OS advantages of maintenance rituximab. On the basis of these and other datasets, there is no indication for maintenance rituximab in tFL.

Initial approach for patients with tFL who have received prior chemoimmunotherapy

Patients who have received prior chemoimmunotherapy for FL and then experience a subsequent transformation are clinically challenging to manage and have poor outcomes. Prior anthracycline exposure appears to confer worse survival (21% vs 66%; $P < .001$) than in those without prior anthracycline exposure.[1] The National Comprehensive Cancer Network found that the 2-year OS was substantially worse (35% vs 100%; $P = .03$) for patients treated with chemotherapy (mainly anthracycline based) before HT.[16] A Spanish registry series reported that 5-year OS for patients treated with chemotherapy before HT was 55% (95% confidence interval, 38%-69%) vs 81% (95% confidence interval, 53%-93%; $P = .009$) for those who had not received prior chemotherapy.[17] The poor prognosis for patients with prior chemoimmunotherapy may be independent of prior anthracycline exposure; for example, patients with HT after prior bendamustine, rituximab (BR) have a 2-year OS of only 40%.[9] In this Canadian report, the authors reported that the main driver of POD24 was occult or early transformation.

There is sufficient evidence that patients with tFL previously treated with anthracycline-based chemoimmunotherapy for their underlying FL benefit from salvage chemotherapy and a consolidative autologous stem cell transplant if they have chemosensitive disease. A subset analysis of a Canadian intergroup study (NCIC CTG LY12) compared the outcomes of 87 patients with transformed lymphoma with outcomes of 429 patients with relapsed/refractory DLBCL; although many patient characteristics were similar (eg, time to relapse, percentage of refractory disease, median age), patients with transformed disease were more likely to have received more than one line of prior systemic therapy. Nevertheless, there was no difference in all outcome measures, including 4-year post-transplant event-free survival (45%), 4-year OS (~40%), and transplant rates.[18] The PRIMA trial described outcomes of 40 patients with documented HT at relapse. Seventeen (42%) of these patients underwent consolidative auto-HCT and had improved OS (not reached vs 1.7 years) compared with patients who either could not or did not undergo auto-HCT.[4]

A major unanswered question is whether patients who experience relapse after non–anthracycline-based chemoimmunotherapy such as bendamustine and rituximab should undergo consolidative auto-HCT. As mentioned above, the outcomes are poor, with 2-year OS only 40%.[9] None of the above discussion addresses this specific, increasingly common clinical dilemma. The author's personal perspective (acknowledging the

paucity of data) is that tFL in this setting occurs early and confers a poor prognosis; therefore, anthracycline-based induction and auto-HCT is favored, particularly if the tFL occurred within 2 years of BR.

Allogeneic hematopoietic stem cell transplant (allo-HCT) is also an option for patients with relapsed/refractory tFL. Several factors limit its widespread use, including higher nonrelapse mortality and major questions regarding the optimal timing of allo-HCT. One of the few side-by-side analyses, albeit in a registry and not a prospective trial, found no difference in PFS or OS between those treated with allo-HCT and those who received auto-HCT, but a significantly higher nonrelapse mortality of 23% vs 5%, respectively, was reported.[19] It should be noted that there are fundamental differences in patients selected for auto-HCT and those selected for allo-HCT, so these types of comparisons are difficult to interpret. According to the Center for International Blood and Marrow Transplant Research (https://www.cibmtr.org/ReferenceCenter), the number of allo-HCTs performed for lymphomas has declined in recent years as the introduction of cellular therapy has further challenged its role.

Is there a role for chimeric antigen receptor T-cell therapy in tFL?

The advent of cellular therapy has increased and improved options for patients with relapsed and refractory aggressive B-cell lymphomas, including tFL. To date, there are 2 US Food and Drug Administration–approved options, with a third agent close to approval. Anti-CD19 chimeric antigen receptor T-cell therapy (CAR-T) modifies autologous T cells to massively expand and become activated upon binding to target CD19 on the surface of B cells. All 3 compounds furthest along (axicabtagene ciloleucel [axi-cel], tisagenlecleucel [tisa-cel], and lisocabtagene maraleucel [liso-cel]) have included patients with tFL as part of development, but data separating outcomes of these patients from other patients with relapsed/refractory DLBCL are limited.

In the tisa-cel trial, 21 patients comprising 19% of the population proceeding to CAR-T had tFL.[20] The trial overall showed a 40% complete remission (CR) rate, and remission at 3 months predicted a 12-month remission of >80%. The axi-cel trial lumped patients with primary mediastinal B-cell lymphoma and tFL, with 19 patients in this group.[21] The overall outcomes showed an overall response rate of 82% with a CR rate of 58%; updated results with a median follow-up of 27 months showed a median duration of response of 11.1 months and a median OS >2 years.[22] "Real-world" follow-up of almost 300 patients included 76 patients with tFL. A direct comparison of DLBCL vs primary mediastinal B-cell lymphoma vs tFL showed no difference in the incidence of grade ≥3 cytokine release syndrome or neurotoxicity, best CR at 12 months (62% for tFL), PFS at 12 months (51% for tFL), or OS at 12 months (70% for tFL).[23]

A heartening finding of this and other "real-world" analyses is that many patients treated with commercial products did not meet the strict criteria of the clinical trials leading to US Food and Drug Administration approval, but they still had meaningful benefit. In particular, patients were older and had more comorbidities.

Overall, the activity and outcomes are exciting, and CAR-T should be considered for patients with tFL who have either experience relapse after an autologous stem cell transplant or have chemoresistant disease precluding a hematopoietic stem cell transplant. Optimal patient selection is an ongoing dialogue, but the ability to achieve durable remission in a portion of patients with refractory tFL is highly encouraging.

Are there options for patients with tFL who are not transplant candidates?

Despite the promise of the approaches outlined above, many patients are either ineligible for transplant or cellular therapy or experience relapse despite these modalities. Again, there are no trials specifically dedicated to tFL or other patients with transformed indolent lymphoma, and nearly all data are derived by culling subsets from trials designed for relapsed/refractory DLBCL. An interesting observation is that there may be a role for lenalidomide in tFL despite this being a germinal center–derived disease. In a small prospective trial, lenalidomide monotherapy had an overall response rate of 57% with a median duration of response of 12.8 months in relapsed and refractory tFL.[24] When combined with rituximab, a small prospective trial showed a response rate of ~50%, but durability was limited.[25] The addition of lenalidomide to tafasitamab appears active in transformed indolent lymphomas, with all patients responding; however, there were only 7 patients with transformed lymphomas included in this trial.[26] Other regimens approved for DLBCL either had very limited patients with tFL or excluded them entirely. For example, the recently approved regimen of polatuzumab-BR excluded patients with transformed lymphomas.[27] The median survival of patients with tFL in the relapsed/refractory setting is abysmal, and focused and biologically rational studies are needed.

Transformation to HGBL-DHL/THL

As discussed above, transformation of indolent lymphomas is not always to DLBCL, and acquisition of an *MYC* rearrangement in FL leads to DHL (or THL if *BCL6* is also rearranged). There are almost no data separating tFL in this context. If there is transformation of FL to HGBL-DHL/THL, R-CHOP is insufficient, and an intensified regimen such as DA-EPOCH-R or another therapy should be considered.[28,29] Extrapolating from retrospective series, there is no clear advantage to consolidative auto-HCT if patients have a metabolic CR after anthracycline-based treatment.[29] Very few patients who relapse after intensive frontline regimens are able to proceed to transplant, emphasizing the dire prognosis. In one series, only 11 of 55 patients with HGBL-DHL/THL were able to undergo transplant after salvage chemotherapy.[30] CAR-T trials have included patients with HGBL-DHL/THL but have not consistently described when disease has evolved from a lower-grade lymphoma; nevertheless, it is clear that CAR-T is effective in a portion of patients with HGBL-DHL/THL, and this would be an appropriate approach for patients with relapsed/refractory tFL and a high-grade histology.

Summary

Transformation of indolent lymphomas to an aggressive histology is a major event in patient management and forces a change in therapy. Although the overall incidence of transformation is declining, this remains a very high-risk disease and has a poor prognosis compared with the prognosis of patients without transformation. A biopsy at the time of suspected transformation is essential because there could be either a transformation to DLBCL or to a HGBL-DHL/THL. For patients without prior anthracycline exposure, R-CHOP or other anthracycline-based treatment is warranted. A major challenge is determining who benefits from consolidative auto-HCT, but the preponderance of data suggests this is best applied to patients who have HT after prior anthracycline treatment or early progression of disease with transformation after prior

chemoimmunotherapy. CAR-T has provided a new option for patients with transformed lymphomas. Overall, the outcomes of patients with transformed lymphomas, particularly if hematopoietic stem cell transplant or cellular therapy approaches are unavailable or ineffective, remain highly unsatisfactory. For now, enrollment in a clinical trial should be the highest priority.

Back to the clinical cases

Patient 1

Patient 1 was treated with R-CHOP, entered complete metabolic remission, and had complete clearance of disease from the marrow. Given that he had both early progression of FL (POD24) based on the axillary biopsy and tFL (bone marrow), we proceeded with auto-HCT consolidation.

Patient 2

Patient 2 has HGBL-DHL transformation of his FL. He is being treated with DA-EPOCH-R and has had an excellent early clinical response. There are no plans for consolidation if he enters CR.

Patient 3

Patient 3 has tFL with DLBCL histology with no history of prior chemoimmunotherapy. He was treated with 6 cycles of R-CHOP and has entered complete metabolic remission. There are no plans for consolidation.

Conflict-of-interest disclosure

The author has served as a consultant for Janssen, Bristol-Myers Squibb, Karyopharm, Genentech, TG Therapeutics, Celgene, Bayer, and Kite in the past 2 years. There is institutional funding for clinical trials from Epizyme, Genentech, Novartis, Celgene, Portola, Karyopharm, Acerta, Pharmacyclics, TG Therapeutics, and FortySeven.

Off-label drug use

None disclosed.

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Rituximab and eculizumab when treating nonmalignant hematologic disorders: infection risk, immunization recommendations, and antimicrobial prophylaxis needs

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Rituximab and eculizumab, monoclonal antibodies that deplete most B cells and activate the terminal complement, respectively, are used to treat nonmalignant hematologic disorders (NMHDs), sometimes with unfavorable effects on the immune system. Hypogammaglobulinemia and neutropenia have been reported with variable prevalence in patients treated with rituximab. Neutropenia is mild and transient, and serious infectious complications are uncommon, so treatment is not indicated. Hypogammaglobulinemia is of greater concern. There is a lack of agreement on a standardized definition, and pre- and posttreatment immunoglobulin (Ig) levels are not routinely obtained. The association among low Ig levels, infectious risk, and mortality and morbidity in this population is unclear. There are also no formal guidelines on indication, risk factors, and threshold level of IgG to prompt Ig replacement therapy (IgRT). Among patients with NMHD, preexisting or persistent hypogammaglobulinemia (PH) after treatment with rituximab has been linked to underlying primary immunodeficiency disorders; therefore, a high index of suspicion should be maintained, and immunologic and genetic evaluation should be considered. Overall, important strategies in managing patients who are receiving rituximab include routine monitoring of pre- and posttreatment IgG levels, immune reconstitution (eg, B-cell subsets), assessment of vaccination status and optimization before treatment, and individualized consideration for IgRT. Accordingly, we discuss immunizations. Eculizumab, most commonly used in the treatment of paroxysmal nocturnal hemoglobinuria and atypical hemolytic uremic syndrome, poses increased risk of meningococcal infections. To decrease the risk of infection, a meningococcal vaccination series is recommended before initiating therapy, and prophylactic antibiotics are preferred during the course of treatment.

LEARNING OBJECTIVES

- Get familiar with adverse effects and risk factors of anti-CD20 (rituximab)–depleting therapies in NMHDs
- Get familiar with adverse effects and risk factors of complement-inhibiting therapies (eculizumab, ravulizumab) in NMHDs

Introduction

Rituximab and eculizumab, monoclonal antibodies targeting CD20 and C5 complement, respectively, are off-label treatments for nonmalignant hematologic disorders (NMHDs), sometimes with unfavorable effects on the immune system. The increasing use of rituximab and eculizumab for a variety of conditions has given rise to important clinical questions regarding the best management practices for patients with NMHDs. Our discussion will focus on using these therapies to treat NMHDs. Specifically, we focus on the impact these treatments have on immunologic function and review the current understanding of infection risk, immunization recommendations, and antimicrobial prophylaxis needs of patients receiving these therapies. We highlight these clinical questions by discussing a patient case.

Clinical case

Our patient is a 16-year-old male diagnosed with acute warm autoimmune hemolytic anemia (AIHA) after he returned from a cruise with mild respiratory illness. He was

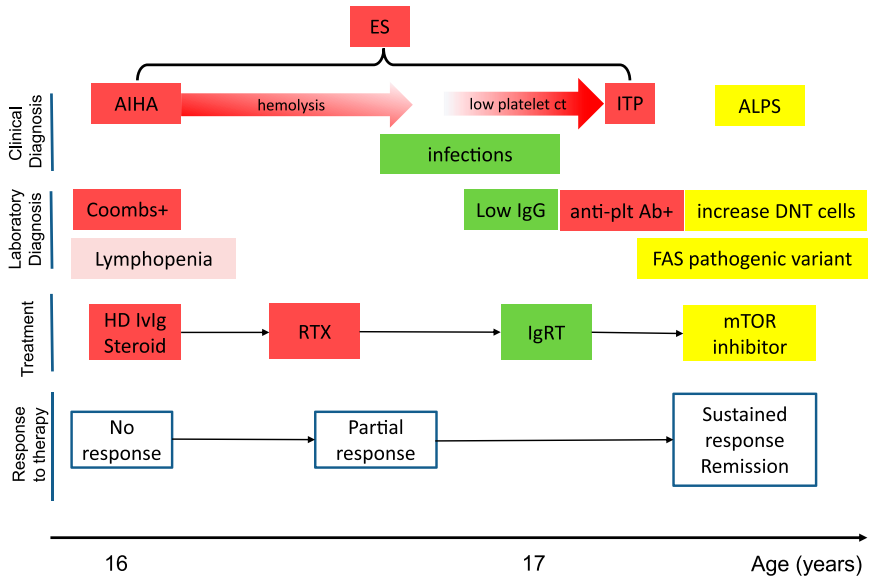

Figure 1. Diagnostic and treatment saga of a 16-year-old with autoimmune cytopenias. Diagnostic evaluation and steps of managements are color-coded (hematology in red, infection in green, and specific immune defect in yellow). AB, antibody; ALPS, autoimmune lymphoproliferative disease; ct, count; DNT, double negative T cell; HD, high dose; IvIg, intravenous Ig; plt, platelet; RTx, replacement therapy.

initially treated with high-dose steroids and intravenous immunoglobulins (Ig's), but he continued to have relapsing episodes of hemolysis. He was thus treated with a 4-dose course of rituximab and completely weaned off steroids; he partially responded with a low normal hemoglobin level and the absence of hemolysis. Complicating his clinical course was the presence of worsening infections, including hospitalization for pneumonia with respiratory distress. Basic immune status was monitored, and it revealed persistent moderate posttreatment hypogammaglobulinemia (lowest IgG level, 300 mg/dL), and pre- and post-rituximab lymphopenia. This prompted referral to the conjoint clinic with hematologists and immunologists where he underwent an extensive work-up that revealed a weak response to pneumococcal vaccination and increased double-negative TCRab$^+$ T cells. The primary immunodeficiency (PID) genetic panel revealed a pathogenic variant in the *FAS* gene, which has been associated with autoimmune lymphoproliferative syndrome. Checking his history more closely revealed an uncle who died of sepsis after splenectomy for chronic immune thrombocytopenia (ITP). Within 2 years of presenting with AIHA, he also developed ITP, now being classified as Evans syndrome (ES). Because he had persistent hypogammaglobulinemia (PH) with infections, Ig replacement therapy (IgRT) was initiated with good effect. ES responded to mTOR inhibitor therapy. While receiving IgRT, the patient could not receive routine immunizations except the yearly influenza vaccine (Figure 1). This case raises several important clinical questions for risk related to the use of rituximab in NMHD and the need for evaluation for underlying PID in selected cases. These considerations will be the focus of our discussion.

Implications of rituximab (anti-CD20) treatment

Rituximab is a B-cell–depleting therapy used to treat malignant and nonmalignant conditions across several specialties.[1] Rituximab works by binding the CD20 antigen expressed on most circulating B cells except plasma cells and results in B-cell destruction through complement-dependent cytotoxicity, antibody-dependent cellular cytotoxicity, and induction of apoptosis.[2,3] Figure 2 demonstrates the effect of rituximab and other B-cell–depleting therapies on the B-cell lineage. Rituximab was initially developed for treating non-Hodgkin lymphoma, but it has since gained approval by the US Food and Drug Administration (FDA) for treating chronic lymphocytic leukemia, rheumatoid arthritis, granulomatosis with polyangiitis, and microscopic polyangiitis in adults. The list of off-label uses is even longer and includes diseases such as nephrotic syndrome, acquired thrombotic thrombocytopenic purpura*P*, acquired factor VIII inhibitors, autoimmune cytopenias (AICs) including ITP, AIHA, or its combination (ES).[4-7]

PH secondary to rituximab

The prevalence of hypogammaglobulinemia secondary to rituximab for the treatment of NMHD is highly variable in recent reports (Table 1).[1,8-11] As an example, in the context of ITP, Levy et al[8] summarized the experience of 32 studies (prospective and retrospective) and showed a very low rate of hypogammaglobulinemia. Conversely, in a cohort of pediatric patients treated with rituximab for AIC, the prevalence of PH was 32%.[12] One contributing factor for this variability is the lack of agreement on a definition of hypogammaglobulinemia. Two studies of adult patients[8,10] defined hypogammaglobulinemia (IgG <500 mg/dL) differently: one study used <600 mg/dL[1] and another used a higher cutoff of <800 mg/dL with the highest incidence of hypogammaglobulinemia (8 [44%] of 18 patients),[9] as expected. Only 1 recent study reported outcomes on a solely pediatric cohort with a cutoff based on age-appropriate IgG level.[12] Other studies included pediatric patients, but the prevalence of hypogammaglobulinemia was not reported by age.

The duration of time with hypogammaglobulinemia after treatment with rituximab is of importance. Ottavio et al[12] defined

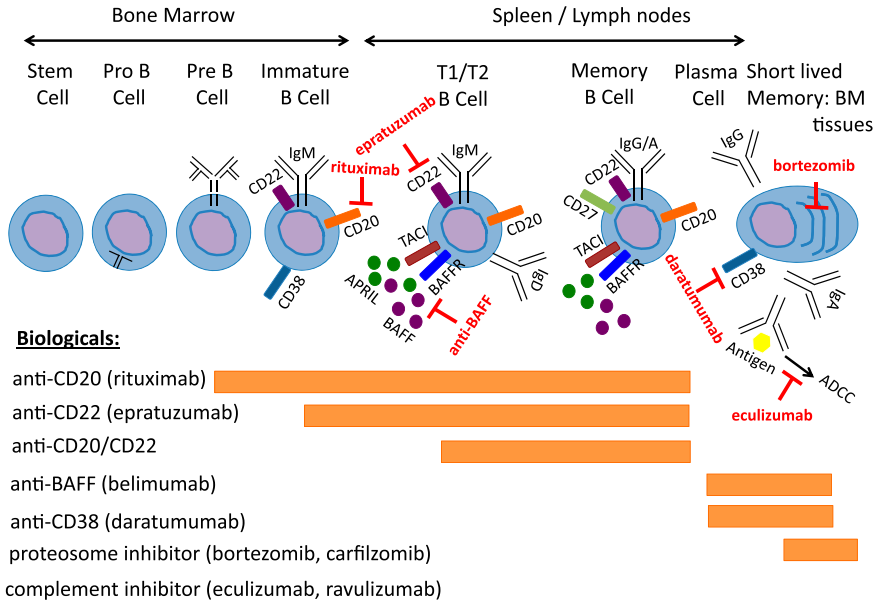

Figure 2. Biologicals targeting B-cell subsets or antibody-mediated immune response. A large selection of antibodies targeting B-cell epitopes, proteosomes, or the complement system are available for therapy in a variety of autoimmune diseases, including NMHDs. Our review focuses on rituximab (anti-CD20) and eculizumab (anti-C5). Shown are mechanisms of targeting B-cell pathology in the treatment of autoimmune and inflammatory diseases associated with PID. Monoclonal antibodies and mechanisms of action are highlighted. Adapted from Walter et al.[15]

PH as 2 standard deviations below the age-appropriate cutoff 12 months after the last dose of rituximab. Children with PH were more likely to have low or slow recovery of IgM and IgA levels and impaired B-cell immune reconstitution. Risk factors for PH included younger age (on average, age 4 years), diagnosis of AIHA/ES vs ITP, and lower IgA and IgM levels before therapy. In fact, a high fraction of patients with PH (53%) were diagnosed with an underlying PID. Curiously, pretreatment lymphocyte counts (T and B cells) and IgG level were not significantly lower among pediatric patients with PH. In this cohort, a history of autoimmune manifestations other than AIC was a risk factor; however, additional immunosuppressants were not associated with PH. Another pediatric study of 63 children with a variety of autoimmune conditions reported that 44% of patients developed hypogammaglobulinemia, and 61% evolved into PH for more than 6 months.[13]

Among adults with NMHD who received rituximab, there is a lack of in-depth studies regarding risk factors for developing PH. Conversely, studies in patients with malignancies and rheumatologic disease identify several risk factors for PH, including number of doses of rituximab, older age, use of chemotherapeutic agents, and low IgM at 12 months after rituximab.[14,15]

Without routine screening of pre- and posttreatment Ig levels, the detection of hypogammaglobulinemia is likely underreported. In a large cohort of nearly 4500 patients treated with rituximab (with a variety of disorders, including 340 with NMHD), the majority did not have their Ig levels checked before therapy was initiated (85%) or within 18 months after starting therapy (87.5%).[1] Of the 15% of patients for whom pretreatment Ig levels were available, nearly half had hypogammaglobulinemia before rituximab was initiated. Among patients with normal Ig levels before treatment, 19% went on to develop mild to severe hypogammaglobulinemia within 18 months of therapy initiation, which qualified them for a diagnosis of PH. Hypogammaglobulinemia also worsened for a portion of patients who had low Ig levels before treatment (23% of patients with mild hypogammaglobulinemia before treatment with rituximab evolved to a moderate or severe category after treatment with rituximab, whereas 21% of patients with moderate hypogammaglobulinemia before treatment with rituximab developed a severe category after treatment with rituximab).[1]

To summarize, the prevalence rates and risk factors for transient hypogammaglobulinemia and PH are variable and likely depend on the specific NMHD (eg, ITP vs ES), age of the patient, concomitant immunosuppressive therapy, and underlying PID. Standardizing the definition of hypogammaglobulinemia is essential to better understanding its prevalence and risk factors.

Risk of infection with hypogammaglobulinemia after treatment with rituximab and immunization strategies

When considering our patient, it was important to determine whether the observed hypogammaglobulinemia was associated with an increased risk of infections and whether he might benefit from IgRT. Much of the published literature discussing the risk of infections after treatment with rituximab includes cohorts of adult rheumatology and oncology patients.

Several studies have reported high rates of infections among patients with lymphoma who were treated with rituximab (16.7%-43%).[16-19] Infection rates were slightly lower among studies of rheumatologic patients (7%-31%).[19-23] Considering NMHD specifically, the infection rates seem to be lower than in rheumatologic or oncologic disorders (Table 1). Among 248 adult patients with ITP treated with rituximab, Deshayes et al[10] reported an overall infection rate of 24% and a severe infection rate of 8%. Severe infections included sepsis, pyelonephritis, pneumonia, and sinus and

Table 1. Studies describing prevalence of hypogammaglobulinemia secondary to rituximab and infections in patients with NMHDs

Study	Population	N	Definition of hypoG (IgG)	Prevalence of hypogG	Follow-up period	Prevalence of infection	Comments
Rao et al, 2009[11]	ITP, AIHA among adult and pediatric patients with ALPS	12		3 (25%) of 12	8 y		All were treated with IVIg; 1 additional patient had total absence of antibody response to polysaccharide vaccines for 4 years; 10 of 12 patients were age 18 years or younger
Levy et al, 2014[8]	ITP, adult and pediatric patients	189	<500 mg/dL	3 (1.6%) of 189	12 y	3 of 3 patients had recurrent severe infections	2 of 3 patients were later diagnosed with CVID
		192*		21 of 192*	16 (50%) of 32 of studies had >1 y of follow-up	5 of 192* patients had frequent minor infections	IgG levels, when tested, were normal before initiation of rituximab; 1 of 21 patients with hypoG was later diagnosed with CVID
Reboursiere et al, 2016[9]	ITP, mostly adult patients (2 patients younger than age 18 years)	35	<800 mg/dL; severe, <500 mg/dL	8 (44%) of 18; 1 severe	Median, 47 mo	No patients were known to have hypoG; 2 of 35 had pneumonia; 1 of 35 had recurrent herpes	3 patients had hypoG before initiation of rituximab and hypoG remained after treatment but did not become severe; 1 patient developed severe hypoG and had normal pre-treatment levels of IgG
Barmettler et al, 2018[1]	Hematologic disorder, adult patients	340	<600 mg/dL; mild: 400-599 mg/dL; moderate: 200-399 mg/dL; severe: <199 mg/dL	26 (7.6%) of 340; moderate or severe	18 mo	Infection rate in the 6 months before rituximab was 24.1% and after rituximab, it was 23.8% (P = .98)	33 (9.7%) of 340 patients received IgRT after treatment with rituximab
Deshayes et al, 2019[10]	ITP, adult patients	248	<500 mg/dL	5 (3.5%) of 142	5 y	59 (23.8%) of 248 patients; 21 (8.5%) had severe infection	142 (57%) of 248 patients had IgG levels; 1 patient with known hypoG developed infection
Ottaviano et al, 2020[12]	ITP, AIHA, ES; pediatric patients only, exclusion criteria was preexisting PID	53	IgG <2 SD for age	17 (32%) of 53	Mean, 30 mo	7 (12%) of 53 patients had infections requiring hospitalization; 8 (15%) of 53 had recurrent respiratory infections	9 (17%) of 53 patients were later diagnosed with PID

ALPS, autoimmune lymphoproliferative syndrome; CVID, common variable immune deficiency; hypoG: hypogammaglobulinemia; IVIg, intravenous Ig; SD, standard deviation.
*Pooled numbers from systematic review.

skin or soft tissue infections by *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Escherichia coli*, *Enterobacter cloacae*, and *Pneumocystis jirovecii*. There were no reported cases of progressive multifocal leukoencephalopathy. In comparison, Barmettler et al[1] reported no significant difference in infection rates before or after initiation of rituximab among adult patients with NMHD; however, there was a trend toward increase in the fraction of patients with severe infections (9% [at less than 12 months after rituximab initiation] vs 15% [at more than 12 months after rituximab initiation]). In the same study, patients had a higher risk of mortality if they had history of serious infections either before and/or after rituximab.[1]

In a pediatric cohort of 53 children treated for AIC, 15% developed recurrent respiratory infections and 12% required hospitalization for infections.[12] In a US-based national study of more than 2800 pediatric patients treated with rituximab, including 1057 with autoimmunity (359 with AIC [AIHA, ITP, ES] and 284 with PID), a high proportion of patients had at least 1 episode of infection during the 1-year study period (573 [54%] of 1057 in the autoimmunity group and 82 [32%] of 284 in the PID group). Although the PID group had fewer overall infections, they experienced more severe infections (eg, sepsis and herpes).[24]

Rituximab is often effective in treating Epstein-Barr virus infections because it resides in B cells. However, there have been reports of reactivation of other viruses, including hepatitis B virus (HBV), herpes simplex virus (HSV), and varicella-zoster virus (VZV), after initiation of rituximab treatment.[25] With the exception of HBV, evidence is lacking and there is no general recommendation to support the use of prophylactic acyclovir or valacyclovir to prevent HSV or VZV reactivation.[26] In addition, although it is rare, human polyomavirus 2 (commonly referred to as the JC virus or John Cunningham virus) and associated progressive multifocal leukoencephalopathy have been reported and are associated with high mortality rates. Providers should be aware of this potentially fatal infection, which presents with progressive neurologic deficits, which would be a reason for discontinuing rituximab.[27]

HVB reactivation. HVB reactivation has been reported as a serious complication in patients receiving rituximab.[26,28] The American Gastroenterological Association published guidelines on the prevention and treatment of HBV reactivation for patients receiving immunosuppressive treatment. According to their guidelines, they classified patients who are hepatitis B surface antigen (HBsAg)–positive/anti-hepatitis B core antibody (HBcAb)–positive or HBsAg-negative/anti-HBcAb–positive and who are receiving treatment with B-cell–depleting agents such as rituximab to be high risk with a greater than 10% risk of HBV reactivation. Thus, patients who are anticipating starting rituximab should be screened for HBV with HBsAg and anti-HBcAb. For patients who are HBsAg-positive or anti-HBcAb-positive with a positive viral load, antiviral prophylaxis is recommended for at least 12 months for patients receiving B-cell–depleting therapies. Sandherr et al[26] proposed using lamivudine for HBV prophylaxis in patients with low viral loads and short duration of immunosuppressive therapy. However, in patients with high viral loads (>2000 IU/mL) or anticipated longer duration of therapy (>12 months), an alternative agent such as entecavir or tenofovir are preferred because of the higher resistance rates associated with lamivudine.

Immunizations. Before rituximab treatment is initiated, immunization status should be assessed. Given that functional B cells are required to develop a robust immune response to vaccination, any pending immunizations should be administered before therapy is initiated. Patients should also be counseled that they might potentially be unresponsive to vaccines after they have been treated with rituximab.[25] Nazi et al[29] demonstrated that responsiveness to both pneumococcal polysaccharide vaccine and *Haemophilus influenzae* type b (Hib) conjugate vaccines was impaired for at least 6 months after treatment with rituximab. Additional consideration should be given to patients with NMHD who need to increase their therapy before splenectomy. These patients will be susceptible to encapsulated bacterial infections, and vaccination with polysaccharide and conjugate vaccines against *S pneumoniae*, *H influenzae*, and *Neisseria meningitidis* should be performed before the splenectomy. All patients should continue to receive their annual influenza vaccine. For patients who require IgRT to treat hypogammaglobulinemia, immunizations should be suspended until 6 months after completion of therapy.

Management of PH after treatment with rituximab

There are no formal guidelines and no agreement on the intervention threshold level of IgG or length of treatment for hypogammaglobulinemia before starting IgRT in post-rituximab hypogammaglobulinemia in patients with NMHD. In addition, there is no evidence supporting the use of prophylactic antibiotics for these patients, or any randomized trials that compare antibiotic prophylaxis vs IgRT to prevent infection.[30]

One study reported no correlation between IgRT and occurrence of serious infectious complications among hematology patients[1]; in contrast, the association for patients with cancer or rheumatologic diseases was significant.[1,15,22,31]

In some cases, the development of hypogammaglobulinemia after treatment with rituximab has led to the diagnosis of an underlying PID.[32] Certain autoimmune conditions such as ITP or AIHA may even precede the diagnosis of a PID, as reported among patients with common variable immune deficiency[33] and combined immunodeficiencies such as recombination-activating gene defects.[34,35] In fact, a recent national study from France has identified monogenic PID in 32 (40%) of 80 patients with ES (age 1.2-41 years).[36] In the Italian/United Kingdom pediatric cohort, 9 (17%) of 53 children (age 1-4 years) with AIC who had received treatment with rituximab were diagnosed with PID, even though previously diagnosed PID was an exclusion criteria. The children with PIDs were overrepresented in the PH group (53%).[36] Thus, a high level of suspicion for PID should be maintained for patients with ES and those pediatric patients with AIC who develop PH after treatment with rituximab.

Rituximab-associated neutropenia

Late-onset neutropenia has been reported as a benign complication of rituximab therapy, although the mechanism is poorly understood and the literature is limited. A recent study reported that 18% of 197 adult patients with NMHD developed neutropenia after treatment with rituximab (absolute neutrophil count [ANC] ≤1500 cells per mL), of which only 4% were severe (ANC ≤500 cells per mL).[37] Despite the presence of neutropenia, there were no episodes of febrile neutropenia and only 2 documented infections.[37] Patients who received ≥4 doses of rituximab or combination therapy with rituximab and another immunosuppressant were at greater risk of developing neutropenia and tended to develop a lower median ANC nadir (400 cells per mL).[37] These data correlate with a previous systematic review that reported an incidence of neutropenia ranging from 3% to 27% across studies. Onset of neutropenia was often delayed, but duration was limited (38-175 days from the last rituximab dose [duration of 5-77 days]).[38] Any associated infections were mild. It is likely that rechallenging with rituximab after an episode of neutropenia will result in additional episodes; however, the clinical significance of this is unclear.[37]

There is no evidence to support the routine use of granulocyte colony-stimulating factor or prophylactic antibiotics for patients with NMHD who develop neutropenia while they are receiving rituximab. Kanbayashi et al[39] reported an increased risk of infection with the use of granulocyte colony-stimulating factor in lymphoma patients who were receiving rituximab. Close monitoring for resolution is recommended, and additional interventions should be determined on a case-by-case basis.

Complement inhibitors and implications of treatment

Eculizumab and ravulizumab are monoclonal antibodies that target complement protein C5 and prevent the activation of the terminal complement complex C5b-9. Eculizumab is FDA-approved for managing several specific conditions, including paroxysmal nocturnal hemoglobinuria, atypical hemolytic uremic syndrome, and refractory generalized myasthenia gravis; however, off-label exploratory use has increased to 39 distinct indications, including AIHA.[40] Eculizumab requires frequent dosing (injections every 2 weeks), so ravulizumab was approved by the FDA in 2018 with a similar mechanism of action but a better pharmacologic profile allowing for maintenance dosing every 8 weeks.[41] In phase 3 trials, ravulizumab was noninferior to eculizumab in efficacy and safety and thus may be replacing eculizumab as first-line therapy for paroxysmal nocturnal hemoglobinuria because of its more convenient dosing schedule.[41-43] Patients receiving eculizumab are at 1000 to 2000 times

greater risk of invasive meningococcal infection compared with healthy individuals. Thus the Advisory Committee on Immunization Practices recommends that all patients receive the quadrivalent meningococcal conjugate vaccine and serogroup B meningococcal vaccine at least 2 weeks before initiating eculizumab.[44] Vaccination alone, however, may not be sufficient to prevent meningococcal infections, so the use of antibiotic prophylaxis with penicillin is recommended for the duration of eculizumab therapy.[45] Patients should be counseled to seek medical attention when signs or symptoms of meningococcal infection develop. Ravulizumab also carries a black box warning for serious meningococcal infection based on results of a phase 2 study in which 2 patients developed meningococcal infection.[46] Thus, we recommend that patients receiving ravulizumab also receive the same vaccinations and antibiotic prophylaxis as patients receiving eculizumab.

Summary and recommendations

As demonstrated in our case presentation and discussion, there are several important clinical considerations when planning to use rituximab in treating NMHD. Treatment-associated effects include susceptibility for infections and transient or persistent modulation of the immune system. Standardizing the definition of hypogammaglobulinemia, close follow-up of pretreatment immune status, and immune reconstitution (ie, Ig testing, B-cell subset monitoring) are important steps toward recognizing and treating complications and prompting further evaluation for underlying PID. We propose the following recommendations: (1) Assess the vaccination status for all patients and administer vaccinations for *S pneumoniae*, *H influenzae*, and *N meningitidis* if needed before initiating therapy. (2) Screen for HBV infection with HBsAg and anti-HBcAb before initiating therapy. Initiate antiviral prophylaxis for those who are positive. (3) Assess baseline immunologic function before initiating therapy with Ig levels and B-cell subsets. We recommend monitoring Ig levels and B-cell subsets regularly (eg, at 6-month intervals). Patients with preexisting hypogammaglobulinemia or those who develop frequent or severe infections may warrant more frequent monitoring. (4) For patients with prolonged hypogammaglobulinemia after rituximab therapy, we recommend further immunologic evaluation to determine whether there is an underlying PID, particularly in pediatric patients. (5) Consideration for patients with IgRT is unclear, but we recommend this intervention early when infections occur.

Patients who will be treated with complement inhibitors are at increased risk for invasive meningococcal infections. We thus recommend the following for patients being treated with eculizumab or ravulizumab: (1) Assess vaccination status for all patients and administer vaccinations for *N meningitidis* if needed before initiating treatment. (2) Use antibiotic prophylaxis with penicillin for the duration of therapy. (3) Educate patients on the signs and symptoms of meningococcal infections.

Conflict-of-interest disclosure

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Off-label drug use

None disclosed.

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Practical approach to monitoring and prevention of infectious complications associated with systemic corticosteroids, antimetabolites, cyclosporine, and cyclophosphamide in nonmalignant hematologic diseases

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Corticosteroids constitute a first-line therapy for adults and children suffering from nonmalignant immune-mediated hematologic diseases. However, high disease relapse rates during the tapering period or upon drug discontinuation result in long-term corticosteroid use that increases the risk of infection. This same concept applies to other immunosuppressive agents, such as antimetabolites, calcineurin inhibitors, and cyclophosphamide. Corticosteroids are associated with a length-of-treatment and dose-dependent risk for infection. Screening and antimicrobial prophylaxis against tuberculosis, hepatitis B, *Strongyloides stercoralis*, and *Pneumocystis jirovecii* pneumonia (PJP) might be indicated in patients who are scheduled to be on high-dose corticosteroids for >4 weeks (>30 mg of prednisone-equivalent dose [PEQ]) or in patients chronically treated (≥8 weeks of continuous or intermittent corticosteroid use) with moderate doses (≥15 to <30 mg PEQ). Antimetabolites (azathioprine, mycophenolate) increase the risk of progressive multifocal leukoencephalopathy (PML); however, other opportunistic infections and viral reactivation have also been reported. In case of new onset of neurological symptoms, PML needs to be considered, and an urgent neurology consultation should be obtained. Cyclophosphamide-induced myelosuppression can lead to serious infections related to neutropenia. PJP prophylaxis should be considered with combination therapy of cyclophosphamide and corticosteroids until a PEQ dose ≤ 5 mg/d is reached. Data on infectious risk when cyclosporine is used in patients with nonmalignant hematologic diseases are lacking. Discontinuation of any immunosuppressive agent during an episode of infection is recommended. In all patients, adherence to an age-based immunization schedule is appropriate.

LEARNING OBJECTIVES

- Identify which patients receiving corticosteroids and other immunosuppressive agents are at higher risk for infection based on a patient's individual characteristics and the immunosuppressive agent used
- Choose the optimal and evidence-based infection prevention strategy to mitigate infection complications in patients receiving corticosteroids and other immunosuppressive agents

Clinical case

A 66-year-old woman presented with 2 weeks of easy bruising and epistaxis. She had chronic obstructive pulmonary disease (COPD), mild cognitive impartment, and essential hypertension. Her platelet count was 7000 per microliter (normal range, 150 000-400 000 per microliter); given her symptomatology, she was hospitalized for expedited workup and management. Physical and laboratory examinations were negative for rheumatologic or infectious causes of her thrombocytopenia. She was diagnosed with immune thrombocytopenic purpura (ITP). Her medications on admission were salmeterol, fluticasone, hydrochlorothiazide, lisinopril, and amlodipine. She lived alone. Her daughter lived 2 hours away but visits every weekend because her mother tends to confuse her

medications. The patient is anxious about starting a new drug and the side effects that she might experience from it. She has had 3 hospitalizations for COPD exacerbation in the past 12 months; however, she had never been in the intensive care unit or been intubated. The medical team discussed a 4-day course of dexamethasone 40 mg once daily; however, the patient and her daughter argued against it given an episode of confusion the patient experienced while on dexamethasone during her last admission for COPD exacerbation. However, the patient stated that she has been on prednisone before and tolerated it well. The plan is now for IV immunoglobulin and prednisone taper over 4 to 8 weeks, starting at 1 mg/kg per day.

Introduction

The use of immunosuppressive therapies in the management of nonmalignant immune-mediated hematologic diseases, such as ITP, thrombotic thrombocytopenic purpura, autoimmune hemolytic anemia, antiphospholipid syndrome, and acquired coagulation factor deficiencies, lead to an increased risk for infections. Because these infectious complications can severely affect a patient's outcome, preventive strategies, such as patient counseling, immunization, infectious disease screening, and antimicrobial prophylaxis, are essential tools in minimizing this risk. Here, we review the data on infectious risk and infectious disease prevention in patients with nonmalignant immune-mediated hematologic diseases treated with various immunosuppressive agents (corticosteroids, antimetabolites, calcineurin inhibitors, and cyclophosphamide [CP]) (see the Visual Abstract). We also highlight areas in which limited evidence exists and discuss our clinical approach given the existent knowledge base. The risk for infections associated with surgical and functional asplenia, as well as with the use of monoclonal antibodies (rituximab and eculizumab), is discussed elsewhere in this volume.[63,64]

What is this patient's risk for infection?

The existing evidence regarding the risk of infection in patients with nonmalignant immune-mediated hematologic diseases treated with corticosteroids and other immunosuppressive agents is scarce. However, publications about patients with ITP or autoimmune rheumatologic disease undergoing immunosuppressive therapies report infection as a major cause of morbidity, treatment interruption, and/or discontinuation.[1,2] When appraising the patient's risk of infection, the interactions of various endogenous and exogenous risk factors have to be considered: (1) nonmalignant immune-mediated hematologic diseases are themselves chronic conditions driven by an already dysfunctional immune system; (2) a patient's individual characteristics, such as advanced age (>65 years old), presence of preexisting comorbidities, polypharmacy (ie, ≥5 medications used daily), and risk of drug-drug interaction; the patient's compliance with the immunosuppressive therapy and dose adjustments; compliance and accessibility to clinical and laboratory monitoring; and a patient's rapid response to suspected infections; (3) specific immunosuppressive agents have particular mechanisms of causing immunosuppression, leading to an increased risk for infection with certain pathogens (eg, antimetabolites and risk for progressive multifocal leukoencephalopathy [PML]); and (4) higher risk for infection is seen in combination immunosuppressive therapy as opposed to single-agent therapy (Table 1).[2-6] It is a challenge for the clinician to estimate the contribution of each of these risk factors to the overall infection risk because no study has addressed this clinical issue.

With this in mind, the patient in our vignette can be considered as being high risk for infection based on the following risk factors: age > 65 years, chronic lung disease with multiple hospitalizations, cognitive impairment, polypharmacy, a nonnegligible risk for low adherence to medication, clinical/laboratory follow-up, and rapid response to suspected infection given her social/living situation; and a history of corticosteroid-induced side effects that led the team to choose a longer course of lower-dose oral corticosteroid.

Patient education and general recommendations

Preventing infectious complications in immunocompromised hosts requires engagement of the patient, their family, and

Table 1. Factors associated with increased risk for infection

	Variable	Risk factors
Patient-related factors	Age/functional status	Older age (>65 y old), poor functional status (frail)
	Medical history	Preexisting comorbidities: chronic lung/liver disease, uncontrolled diabetes, severe malnutrition, IV drug use, hematologic cancer or any cancer on active chemotherapy or radiation treatment, chronic kidney disease on dialysis, asplenia, HIV/AIDS, primary immunodeficiencies, history of infections while on immunosuppressive therapies
	Socioeconomic status	Travel, high-cost burden, poor family/caregiver support, cognitive impairment.
Therapy- and disease-related factors	Regimen chosen	Combination immunosuppressive therapy, high-cost burden
	Length of treatment	Long-term/maintenance immunosuppressive therapy to maintain response
	Drug safety and side effect profile	Poor drug tolerability, polypharmacy (caveat: drug-drug interaction), strong need for laboratory/clinical monitoring while on treatment (eg, oral CP and risk for myelosuppression)
	Time to treatment	Shorter time from diagnosis to therapy (eg, patients with high disease burden) precluding appropriate immunization administration and/or infectious disease screening
	Dose response	Higher doses needed to achieve disease response
	Long-term efficacy	Frequent relapses, refractory disease

Data are from Portielje et al,[2] Ekstrand et al,[3] Bouwman et al,[4], Listing et al,[5] and Fox et al.[6]

caregivers. Patients and their close contacts must be empowered and enabled to perform self-care in a way that minimizes preventable harm. For example, the most cost-effective way to prevent transmission of infections is hand hygiene. Teaching patients to cleanse their hands and enabling family members to help them perform this simple task can decrease the load of pathogens responsible for infections.[7] All members of the household should avoid interaction with the patient when they are experiencing an infectious process.[7] Patients should always be advised to seek prompt medical attention during a febrile illness. Early management of animal bites is critical in immunocompromised patients.[8] For travelers, the use of tick and mosquito repellents, netting while sleeping, antimalarial prophylaxis when traveling to endemic areas, and the advice from an infectious disease physician are all recommended.[9]

All patients should adhere to their age-based immunization schedule as per the Advisory Committee on Immunization Practices guidelines, including annual viral influenza vaccine and the herpes zoster vaccine for patients 50 years and older; the recombinant herpes zoster vaccine (SHINGRIX) is preferred over the live attenuated vaccine (Zostavax), as per Centers for Disease Control and Prevention (CDC) guidelines.[10,11] The US Food and Drug Administration (FDA) advises that the administration of live or live attenuated vaccines is contraindicated in patients receiving immunosuppressive therapy (eg, corticosteroids ≥ 10 mg prednisone-equivalent dose [PEQ] daily or a cumulative dose > 700 mg PEQ in 3 months) and that vaccination should be deferred for ≥1 month after discontinuation of such therapy. Killed or inactivated vaccines and toxoids may be administered; however, the response to such vaccines cannot be predicted.[10] HIV status should be known in any patient with a nonmalignant immune-mediated hematologic disease.

Clinical challenges and best practices

Patients with nonmalignant immune-mediated hematologic diseases may not adequately respond to first-line therapy; often, no clear consensus exists as to when to stop first-line therapy and what the optimal second-line therapy should be after first-line treatment failure. This may lead to suboptimal management approaches, including prolonged exposure to treatments that may not be optimal for long-term use, such as corticosteroids, which may fail to address symptoms and burden of disease and worsen health-related quality of life.[12]

In this context, it is relevant that clinicians have a good understanding of available second-line treatments to ensure the best use of therapeutic options and to avoid prolonged use of corticosteroids. Overall familiarity of the clinician with the therapy and rapidity of response appears to play a decisive role in long-term management of patients with nonmalignant immune-mediated hematologic diseases.[13,14] The use of evidence-based practice guidance and guidelines and/or consulting a medical colleague expert in the field (eg, online tools such as "You Make the Call" by the American Society of Hematology) can be of help in challenging situations and can assist in minimizing complications and optimizing patient outcomes.

Corticosteroids

Corticosteroids exert a complex quantitative and qualitative immunosuppressive effect that induces cellular immunodeficiency and, consequently, increased patient susceptibility to infections. The three key corticosteroid effects leading to an altered immunologic response against pathogens are (1) impaired opsonization and phagocytic function increasing the risk for bacterial infections, (2) impaired T-cell migration and proliferation increasing the risk for mycobacterial, viral, and fungal infection, and (3) impaired eosinophilic proliferation with increased apoptosis, resulting in an increased risk for parasitic infection.[15]

Even though corticosteroids are commonly used in the management of various autoimmune diseases, little is known about an individual patient's risk for infection associated with such treatment. A population-based cohort study using general practice records in the United Kingdom compared all adults who had been prescribed corticosteroids with adults who had not been prescribed them.[16] Hazard ratios (HRs) were significantly higher among corticosteroid recipients vs nonrecipients for cutaneous cellulitis (2.21; 95% confidence interval [CI] .06-2.37), bloodstream infection (HR, 3.96; 95% CI, 3.19-4.93), local candidiasis (HR, 4.93; 95% CI, 4.60-5.29), and lower respiratory tract infections (HR, 5.42; 95% CI, 5.23-5.61) ($P < .001$ for all comparisons).[16]

Current conclusions concerning corticosteroid-related infection risk in nonmalignant immune-mediated hematologic diseases are largely derived from studies of patients with rheumatologic and inflammatory bowel diseases.[17-20] Studies in patients aged 66 years and older with rheumatoid arthritis (after adjusting for disease severity, use of other immunosuppressive agents, and comorbidities) found an increased risk for serious bacterial infections with PEQ as low as 5 mg for 1 week (odds ratio, 1.03-3.96), as well as a dose-dependent (ie, >20 mg PEQ daily) and a duration-dependent (ie, > 5 mg PEQ chronically) stepwise increase in the risk of serious bacterial infections (odds ratio, 2.0-7.57).[18,21,22] Likewise, in patients aged 66 years and older with inflammatory bowel disease, the use of corticosteroids alone resulted in an estimated fivefold relative risk for bacterial infections, a fourfold relative risk for other infections (eg, *Strongyloides* and tuberculosis), and 1.5-fold risk for viral infections.[20]

Factors associated with an increased risk for infection with systemic corticosteroids include age > 65 years; lower functional status; preexisting comorbidities, such as diabetes mellitus, lung disease (asthma, COPD), and malnutrition (low albumin); higher corticosteroids doses (≥20 mg PEQ daily), and longer duration of corticosteroid therapy (≥4-8 weeks).[16–18] Although the absolute individual risk of infectious complications from corticosteroid use remains fairly small, the burden is significant at a population level because of the high frequency of corticosteroid use. Thus, most practitioners eventually encounter these complications during their career.

Although numerous opportunistic infections (eg, aspergillosis, nontuberculous mycobacterial disease, candidiasis, cryptococcosis) have been reported with the use of systemic corticosteroids, this section will focus on those for which data are most solid and for which implementation of infection-prevention strategies has demonstrated a lessening of their appearance and/or minimization of further complications. A summary of these infection complications and preventive strategies is presented in Table 2.[17,23-28]

Pneumocystis jirovecii pneumonia

Pneumocystis jirovecii pneumonia (PJP) is 1 of the most common causes of opportunistic lung infections in immunocompromised patients.[29] The available evidence about the association between PJP and corticosteroids is basically derived from case

Table 2. Infectious complications and preventive strategies with the use of systemic corticosteroids

Pathogen	Risk factors for infection	Preventive strategy
PJP	A. Corticosteroid dose ≥ 30 mg PEQ daily given for ≥4 wk B. Corticosteroids ≥ 15 mg to <30 mg PEQ daily given for ≥8 wk uninterrupted or in intermittent doses C. Combination of medium-dose corticosteroids (ie, ≥15 mg to <30 mg PEQ daily) and CP (oral or IV pulses) D. Corticosteroids ≥ 10 mg PEQ daily and ≥2 of the following: advanced age > 65 y, coexisting lung disease (eg, COPD, lung fibrosis), use of immunotherapeutics (eg, rituximab, anti-TNF).	Antimicrobial prophylaxis: • For all patients in (A) through (D), PJP prophylaxis is indicated. • TMP/SMX, 1 single-strength tablet (80 mg of TMP and 400 mg of SMX) daily, or TMP/SMX, 1 double-strength tablet 3 times weekly. • If TMP/SMX intolerance or contraindicated, alternative therapies are atovaquone, dapsone, or once-monthly nebulized pentamidine. • For patients in (D), PJP prophylaxis should be continued until the corticosteroid dose is ≤5 mg PEQ daily.
HZ (shingles)	A. Advanced age > 60 y B. Corticosteroid dose > 7.5 mg to 10 mg PEQ C. History of recurrent shingles	Immunization: • RZV (ie, SHINGRIX) preferred over ZVL (ie, Zostavax) • Indicated in all adults aged ≥ 50 y, including those who received ZVL in the past; had chickenpox or do not recall whether they had chickenpox; had shingles, but not an active flare at the time of vaccination; and have chronic comorbidities (eg, chronic renal failure, diabetes mellitus, autoimmune diseases, COPD) • In adults aged ≥ 50 y anticipating immunosuppression or currently on immunosuppressive therapy, important considerations are to vaccinate ideally ≥4 wk before treatment; okay in patients taking low-dose immunosuppressive therapy (eg, <20 mg/d prednisone or equivalent, or using inhaled or topical steroids, azathioprine, mycophenolate mofetil); and okay in patients who have recovered from an immunocompromising illness • Adults aged < 50 y: ACIP does not have a recommendation to administer either zoster vaccine to people younger than 50 y. However, based on the available evidence, clinicians may choose to administer a vaccine off-label, if, in their clinical judgment, they think that the vaccine is indicated (eg, history of shingles). The patient should be informed that the use is off-label and that efficacy and safety of the vaccine have not been tested in people younger than 50 y. Antimicrobial prophylaxis: • No evidence outside of the transplant setting exists on the use of antiviral prophylaxis. However, it might be reasonable that patients with history of recurrent shingles or heavily treated with immunosuppressive agent should consider antiviral prophylaxis. Doses as low as 400 mg of acyclovir daily have shown to an effective strategy in immunocompromised patients.
TB reactivation	A. Corticosteroid dose < 15 mg PEQ daily has a 2.8-fold increased risk B. Corticosteroid dose > 15 mg PEQ daily has a 7.7-fold increased risk	TB screening testing: • Patients taking corticosteroids at a dose ≥ 10 mg PEQ daily for ≥4 wk should be screened for latent TB using tuberculin skin test or interferon-γ release assays; the latter is preferred in patients with altered T-cell function (eg, HIV/AIDS), history of BCG immunization, and ongoing corticosteroid therapy or other immunosuppressive agents • If positive test, refer to an infectious disease specialist
Disseminated SS hyperinfection syndrome	A. Major risk factor is provenance/travel history: tourists, military, and immigrant populations coming from high prevalence areas, such as Africa (Ghana, Zambia, Gabon, Sudan), Asia (Thailand, Cambodia), Central America (Guatemala), and South America (Peru, Venezuela, Brazil). B. There are no clear data on the dosage or duration of corticosteroid therapy that triggers the risk for severe strongyloidiasis.	SS screening testing: • Given the available data, any patient coming from a high-risk area and scheduled to start corticosteroids at a dose > 10-15 mg PEQ daily for ≥4 wk should be screened with stool sample for ova and parasites and serum IgG against SS. Antimicrobial prophylaxis: • Given the poor sensitivity and high cost of SS screening, empiric therapy with ivermectin represents a safe and cost-effective approach in patients at high-risk for severe strongyloidiasis (ie, people walking barefoot in endemic areas).
HBV reactivation	A. High-dose corticosteroids (>20 mg PEQ daily) for >4 wk B. Chronic (≥8 wk) medium-dose corticosteroids (10-20 mg PEQ daily)	HBV screening testing: • Patients in (A) and (B) need hepatitis B screening with anti-HBc and HBsAg. Results interpretation: • Patients in (A) or (B) with positive anti-HBc and positive HBsAg have a high risk for HBV reactivation (≥10% risk for reactivation). • Patients in (A) with positive anti-HBc, but negative HBsAg, have a moderate risk for HBV reactivation (1-10% risk of reactivation). Antimicrobial prophylaxis: • Patients with high risk for HBV reactivation require antiviral prophylaxis. • For patients with moderate risk for HBV reactivation, 2 options are available: preemptive therapy guided by serial HBV DNA monitoring, with antiviral therapy initiated as soon as HBV DNA becomes detectable, and routine prophylactic antiviral therapy. • Entecavir or tenofovir is the preferred agent because of the low risk of resistance. • Infectious disease input is encouraged.

Data are from Youssef et al,[17] Cavallasca et al,[23] Dooling et al,[24] Yun et al,[25] Loomba and Liang,[26] Katsuyama et al,[27] and Center for Disease Control and Prevention.[28]

ACIP, Advisory Committee on Immunization Practices; anti-HBc, anti–hepatitis B core antibody; anti-TNF, anti–tumor necrosis factor inhibitors; BCG, bacillus Calmette-Guérin; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HZ, herpes zoster; IgG, immunoglobulin G; PJP, *P jirovecii* pneumonia; RZV, recombinant zoster vaccine; SMX, sulfamethoxazole; SS, *Strongyloides stercoralis*; TB, tuberculosis; TMP, trimethoprim; ZVL, zoster vaccine live.

series and single-center studies.[17,30,31] Although no guidelines exist regarding what dose and duration of corticosteroids are necessary to trigger PJP in patients with nonmalignant immune-mediated hematologic diseases, the estimated incidence rate of PJP in patients with rheumatologic diseases has been shown to vary depending on the dose of corticosteroids used. For instance, patients receiving low-dose corticosteroids (ie, ≤15 mg PEQ daily) have an estimated 1-year incidence of 0.1 per 100 person-years, whereas those on moderate- and high-dose corticosteroids have estimated incidences of 0.5 and 0.75 per 100 person-years, respectively.[30,31]

Based on the available data, a clinician should consider PJP prophylaxis in patients at higher incidence for PJP, such as those on (1) a corticosteroid dose ≥ 30 mg PEQ daily given for ≥4 weeks, (2) a corticosteroid dose ≥ 15 mg to <30 mg PEQ daily given for ≥8 weeks, either uninterrupted or in intermittent doses, (3) a combination of medium-dose corticosteroids (ie, ≥15 mg to <30 mg PEQ daily) and CP (oral or IV pulses), and (4) corticosteroids ≥ 10 mg PEQ daily and ≥2 of the following: age > 65 years, coexisting lung disease (eg, COPD, lung fibrosis), or use of immunotherapeutics (eg, rituximab, anti–tumor necrosis factor).[17,30,31] Because better therapies than long-term corticosteroids exist for the management of nonmalignant immune-mediated hematologic diseases, initiation of PJP prophylaxis at the time of diagnosis and initiation of corticosteroid treatment are typically not needed. However, a clinician should always assess a patient's length of exposure and dose-dependent disease response to corticosteroids at each clinic visit and consider PJP prophylaxis if longer-term corticosteroid therapy is being pursued.

PJP prophylaxis with trimethoprim (TMP)/sulfamethoxazole (SMX) can be prescribed as 1 daily single-strength tablet (80 mg of TMP, 400 mg of SMX) or 1 double-strength tablet 3 times weekly. For patients who exhibit intolerance or contraindication (eg, glomerular filtration rate < 15 mL/min) to TMP/SMX, alternative therapies are atovaquone (1500 mg daily), dapsone (100 mg daily), or nebulized pentamidine (300 mg once monthly).[17,31] No societal or other formal recommendation exists regarding the duration of prophylactic treatment. In our practice, we discontinue prophylaxis when the corticosteroid dose is <10 mg PEQ daily.

Herpes zoster

Herpes zoster (HZ) is caused by reactivation of latent varicella-zoster virus (VZV) in cranial nerve or dorsal root ganglia.[25] Although usually presenting as a painful vesicular rash with a dermatomal distribution, immunocompromised individuals can develop disseminated disease, with vesicles spreading beyond the affected dermatome and the potential to affect other organs producing pneumonia, encephalitis, hepatitis, and retinitis.[25] The incidence rate of HZ in healthy individuals has been reported to be 10.8 per 1000 person-years in people aged 60 to 69 years old and 6.7 per 1000 person-years in people aged 50 to 59 years.[32,33] The risk for HZ associated with autoimmune conditions has been estimated at ~1.5-fold to twofold higher than corresponding rates in healthy individuals, with a 2.37-fold increased risk for HZ in those receiving corticosteroids.[16,25] Although the incidence of disseminated VZV infection in patients with nonmalignant immune-mediated hematologic diseases is unknown, it has been reported that 10% to 40% of immunocompromised individuals suffering from HZ could develop disseminated disease.[34,35] Disseminated HZ infection has a 5% to 10% fatality rate.[25] Fulminant visceral disseminated VZV infection without skin involvement has been described in a patient with autoimmune hemolytic anemia.[36]

As previously discussed, all patients aged ≥50 years should receive HZ immunization. However, current guidelines do not address indications in immunocompromised patients with regard to the novel recombinant vaccine and indications outside of the approved age of ≥50 years.[10] Age > 60 years and systemic corticosteroid use > 7.5 to 10 mg PEQ daily have been associated with increased incidence and severity of HZ.[17] However, young adults with autoimmune diseases who are on immunosuppressive therapy are an important group also at high risk.[25] That said, and based on the available evidence, clinicians may choose to administer a vaccine off-label if, in their clinical judgment, the vaccine could be indicated (eg, young patient with history of shingles). The patient should be informed that the use is off-label and that efficacy and safety of the vaccine have not been tested in people younger than 50 years of age.

Finally, no evidence outside of the transplant setting exists on the use of antiviral prophylaxis; however, it might be reasonable to consider it in patients with history of shingles or patients heavily treated with immunosuppressive agents. Doses of oral acyclovir as low as 200 to 400 mg/d have shown effectiveness in preventing VZV reactivation in immunocompromised patients.[37,38] Antiviral therapy should be initiated in all immunocompromised patients with active HZ. Immunocompromised hosts with disseminated zoster should be hospitalized for IV therapy.

Tuberculosis reactivation

Patients with latent tuberculosis (TB) infection on corticosteroids are at risk for conversion to active disease. The limited data available regarding the risk of TB reactivation with systemic corticosteroids comes from patients with rheumatologic diseases. Patients treated with <15 mg vs >15 mg PEQ daily have a 2.8-fold and 7.7-fold increased risk for TB reactivation, respectively.[39] Those with TB reactivation are more likely to have received IV pulse-dose corticosteroids.[40]

The CDC recommends screening for latent TB infection in those who may need long-term immunosuppression (eg, ≥10 mg PEQ daily for >4 weeks).[28] Latent TB infection screening should be performed with a tuberculin skin test or serum interferon-γ release assays; the latter is recommended in patients with altered T-cell function (eg, HIV/AIDS), history of bacillus Calmette-Guérin immunization, or ongoing immunosuppressive therapy.[28] If a test is positive, the patient should be referred to an infectious disease specialist for appropriate management.

Strongyloides stercoralis infection

Strongyloides stercoralis (SS) is an intestinal nematode particular in its ability to produce chronic infection through cycles of autoinfection within the same host that can last for decades.[41] Disseminated strongyloidiasis, or hyperinfection syndrome, is a lethal condition in which the parasite spreads from the intestinal tract to different organs causing septicemia and multiorgan failure. Immunosuppressive states, such as those produced by systemic corticosteroid use, are the major risk factor.[41] A large systemic review on severe strongyloidiasis reported that 67% (163/244) of the cases occurred in patients on corticosteroid therapy, with a mortality rate of 62.7%; however, only 8% had

autoimmune diseases (rheumatoid arthritis and lupus), and the study was not able to report the cumulative dosage and the duration of the corticosteroid treatment.[42] In the United States, strongyloidiasis cases are seen in tourists, military, and immigrant populations coming from high-prevalence areas, such as Africa (Ghana, Zambia, Gabon, Sudan), Asia (Thailand, Cambodia), Central America (Guatemala), and South America (Peru, Venezuela, Brazil).[36]

Although no guidelines exist on the prevention of SS infection, given the available data, any patient coming from a high-risk area and scheduled to start a corticosteroid dose > 10 to 15 mg PEQ daily for ≥4 weeks should be screened with a stool sample for ova and parasites and serum immunoglobulin G against SS.[42] In our practice, given the poor sensitivity and high cost of SS screening, empiric therapy with ivermectin represents a safe and cost-effective approach in patients at high risk for strongyloidiasis (ie, those who have lived in areas of high incidence and endorse a history of walking outside barefoot). Infectious disease input in such patients is warranted.

Hepatitis B virus reactivation

Hepatitis B virus (HBV) reactivation is defined as a sudden and rapid increase in HBV DNA level by ≥100-fold in patients with previously detectable HBV DNA or the reappearance of HBV DNA viremia in individuals who did not have viremia before the initiation of immunosuppressive or biological therapies.[26] The timing of the onset and symptomatology of HBV reactivation is variable and depends on the host's immunity, underlying disease, and the type of immunosuppressive therapy used.[26] HBV reactivation may occur as early as 2 weeks from immunosuppressive therapy initiation or up to a year after the cessation of immunosuppression. Symptoms vary from mild constitutional symptoms, jaundice, abdominal pain and nausea/vomiting to fulminant liver failure.[26] The risk of HBV reactivation can be divided broadly into high risk (rate of HBV reactivation ≥ 10%), moderate risk (1-10% rate), and low risk (<1% rate). This classification applies to those with positive anti–hepatitis B core antibody (anti-HBc) with a positive (or negative) hepatitis B surface antigen (HBsAg) and is based on the type of immunosuppressive therapy used. For instance, patients scheduled to receive chronic (≥8 weeks) medium-dose corticosteroids (10-20 mg PEQ daily) or high-dose corticosteroids (>20 mg PEQ daily) for ≥4 weeks are considered at high risk and should be screened for HBV.[43] HBV viral screening should consist of anti-HBc and HBsAg in serum.

HBV prophylaxis is recommended to those with positive anti-HBc and positive HBsAg receiving chronic medium-dose corticosteroids and to those with positive anti-HBc and positive HBsAg receiving high-dose corticosteroids for ≥4 weeks (Table 2).[26] On the other hand, patients with positive anti-HBc but negative HBsAg who are on high-dose corticosteroids are classified as having a moderate risk for reactivation and require careful monitoring.[26] Antiviral drugs with a high barrier to resistance (ie, entecavir or tenofovir) are recommended.[43] Treatment with antivirals should be continued for ≥6 months after discontinuation of corticosteroids.

Table 3. Infectious complications and preventive strategies with the use of AZA, MMF, cyclosporine, and CP

Drug	Associated infection	Preventive strategy
AZA/MMF	Recognized association: • Virus: JC virus, cytomegalovirus, VZV Reported cases: • Bacteria: *Listeria*, *Mycobacterium* spp. • Viral: BK virus • Fungi: *Cryptococcus*, *Aspergillus*, PJP • Parasite: *Toxoplasma*	Clinical evaluation: • In patients managed with antimetabolites and presenting with new-onset neurological symptoms such as hemiparesis, apathy, confusion, cognitive deficiencies, ataxia, blurry vision or loss of vision, severe otalgia or hearing loss, need evaluation for a neurotropic infection (eg, PML, HZ reactivation, toxoplasmosis, *Cryptococcus*). • Brain imaging and neurology consultation are recommended in those with neurologic symptoms. Immunization: • HZ immunization is recommended and as stated in Table 2.
Cyclosporine	Recognized association: • Virus: cytomegalovirus in transplanted patients Reported cases: • Bacteria: Gram-negative sepsis • Virus: Herpes simplex, VZV	• No evidence outside of the transplant setting exists on the use of preventive strategies to minimize opportunistic infections.
CP	Recognized association: • Infections associated with neutropenia (common bacterial infection) Reported cases: • Bacterial: TB • Fungal: PJP, *Aspergillus* • Parasitic: SS	Laboratory testing: • Routine blood cell counts. Therapy should not be administered to patients with an absolute neutrophil count ≤ 1500/μL and/or platelets < 50 000/μL. Antimicrobial prophylaxis: • Antimicrobial prophylaxis against bacterial, fungal, or viral infection might be considered in certain cases of neutropenia and at the discretion of the managing physician. • In case of neutropenic fever, antibiotic therapy is indicated, as well as consideration for growth factors, especially in patients considered to be at increased risk for neutropenia complications (eg, elderly patients). • PJP prophylaxis in patients treated with combination CP and moderate-dose corticosteroids (ie, ≥15 mg to <30 mg PEQ daily). PJP prophylaxis can be discontinued once PEQ ≤ 5 mg daily.

Data are from Gibson et al,[47] Prometheus Laboratories Inc.,[48] Roche Laboratories Inc.,[49] Kim and Perfect,[50] and Baxter.[51]
PML, progressive multifocal leukoencephalopathy.

Clinical case continued

The patient was started on prednisone, 60 mg by mouth daily, with a plan for a taper over 4 weeks. Prior to therapy, the patient underwent HIV testing, as well as screenings for latent TB infection with a serum interferon-γ release assay and HBV with serum anti-HBc and HBsAg. Tests results were negative. The patient stated that she had been vaccinated against shingles 2 years ago and was told it was with the new shingles vaccine. She was started on TMP/SMX double-strength tablets 3 times weekly for PJP prophylaxis. After 2 weeks on therapy, she had a robust response, with a platelet count of 225 000 per microliter. However, after 2 weeks of being on a corticosteroid taper, she returned to the clinic with 2 days of easy bruising while on 10 mg of prednisone. Her platelet count during that visit was 15 000 per microliter. Over the next year, she received rituximab with no response, as well as trials of eltrombopag and romiplostim that produced intermittent spikes in her platelet count but not a sustained response. Splenectomy could not be performed because of her poor lung function. She remained dependent on prednisone, 20 mg daily, to maintain a platelet count of 20 000 to 30 000 per microliter. Following the American Society of Hematology 2019 evidence-based ITP management recommendations that discourage the prolonged use of corticosteroids, a new regimen with mycophenolate mofetil (MMF), 500 mg by mouth twice daily, is planned.

Other immunosuppressive therapies

A range of immunosuppressive drugs (eg, azathioprine [AZA], MMF, cyclosporine, and CP), drug combinations, and dosing regimens are used to treat relapsed/refractory nonmalignant immune-mediated hematologic diseases, all of which are off-label for these disorders.[13,44-46] A summary of recommendations is presented in Table 3.[47-51]

Antimetabolites: AZA and MMF

Infectious complications reported with the use of antimetabolites are bacterial (common bacteria and atypical bacterial infections, including *Listeria monocytogenes*, *Mycobacterium* spp.)[52,53], fungal (*Cryptococcus*, *Aspergillus*, *Mucor*, PJP)[47-49], parasitic (*Toxoplasma*)[54], and viral reactivation (disseminated HZ, JC virus, polyomavirus-associated nephropathy-BK virus infection).[48,49] Although AZA and MMF carry a "black-box" warning for the development of progressive multifocal leukoencephalopathy (PML), an opportunistic demyelinating disease caused by the JC virus, the exact incidence rate attributed to these drugs is unknown.[48,49] However, the incidence rate of PML in patients with autoimmune diseases other than rheumatoid arthritis and lupus erythematous systemic who are on immunosuppressive therapy is estimated to be 2 per 100 000 people.[55] Hemiparesis, apathy, confusion, cognitive deficiencies, and ataxia are the most frequent clinical features observed in PML. Additionally, disseminated HZ infection has been reported in patients treated with MMF outside of the transplant setting.[56,57]

No specific recommendations are available for the prevention of opportunistic infections with the use of antimetabolites. The FDA recommends that a diagnosis of PML be considered in any patient treated with AZA or MMF presenting with new-onset neurological manifestations and to consider consultation with a neurologist as clinically indicated.[56,57] All immunosuppressive drugs should be discontinued during an episode of infection.

Cyclosporine

Cyclosporine selectively impairs T-cell function, increasing a patient's risk for localized and/or generalized infections (viral, bacterial, fungal, or parasitic).[50] Evidence on the risk of infection in nonmalignant immune-mediated hematologic patients is lacking. A study of cyclosporine in psoriatic patients reported a low risk for viral reactivation compared with transplanted patients.[58] However, no head-to-head comparison on the safety of cyclosporine vs other immunosuppressant (eg, antimetabolites) has been reported. Correspondingly, no recommendations exist on the prevention of opportunistic infections in patients with nonmalignant immune-mediated hematologic disease treated with cyclosporine.

Cyclophosphamide

CP is an alkylating agent that is capable of inducing DNA single-strand breaks, thus preventing cells from dividing. CP is typically administered IV in pulses or orally as a continuous treatment.[13] A common side effect is myelosuppression with leukopenia and neutropenia, which may lead to serious and sometimes fatal infections, including bacterial, fungal, viral, protozoal, and parasitic infections. Bacterial pneumonia is a common infection (up to 30% of infections); however, fatal cases are uncommon. Serious infections have been reported with CP in patients receiving concomitant corticosteroids.[23,51,59] No difference in the risk for infection has been found in patients receiving IV vs oral CP.[60]

The FDA recommends routine blood cell counts in patients treated with CP. CP should not be administered to patients with absolute neutrophil count ≤ 1500 per microliter and/or platelet count < 50 000 per microliter. Antimicrobial prophylaxis might be considered in certain cases of neutropenia and at the discretion of the managing physician. In case of neutropenic fever, antibiotic therapy is indicated, as well as consideration for growth factors, especially in patients considered to be at increased risk for neutropenia complications (eg, patients aged ≥66 years). Additionally, we recommend PJP prophylaxis in patients treated with CP and moderate-dose corticosteroids (ie, ≥15 mg to <30 mg PEQ daily). PJP prophylaxis can be discontinued once PEQ is ≤5 mg daily.[59]

Clinical case continued

The patient's platelet count improved after 3 months on MMF monotherapy. At this time, plan of care include monitoring for any new-onset neurological manifestations (hemiparesis, apathy, confusion, cognitive deficiencies, ataxia, blurry vision or loss of vision, severe otalgia, or hearing loss), as well as any signs of viral reactivation, such as skin manifestations from shingles.

COVID-19 and immunosuppressive therapy

The effect of the COVID-19 pandemic on medical care for conditions such as nonmalignant hematologic diseases is difficult to quantify. There is limited evidence regarding the use of immunosuppressive therapy (eg, corticosteroids) and the theoretical risk of increasing susceptibility to COVID-19 infection. Similarly, the role of corticosteroids in mitigating COVID-19 hyperinflammatory syndrome remains controversial.

As of 30 June 2020, there are no data on the risk of COVID-19 infection and its consequences on clinical outcomes in patients with nonmalignant immune-mediated hematologic diseases who are undergoing immunosuppressive therapy with corticosteroids, antimetabolites, cyclosporine, and CP. The COVID-19

Global Rheumatology Alliance Provider Registry houses data on >1400 COVID-19 patients with inflammatory rheumatologic diseases; preliminary data were released for 600 SARS-Cov-2+ patients.[61] The use of ≥10 mg PEQ was associated with a more severe COVID-19 disease course and increased risk for hospitalization (odds ratio [OR], 2.05; 95% CI, 1.06-3.96; P = .03) compared with lower corticosteroid doses (OR, 1.03; 95% CI, 0.64-1.66; P = .91). Conversely, the use of disease-modifying antirheumatic drugs was associated with a lower hospitalization rate (OR, 0.46; 95% CI, 0.22-0.93; P = .03). Age > 65 years (OR, 2.56; 95% CI, 1.62-4.04; P < .01) and common comorbidities, such as hypertension and cardiovascular disease (OR, 1.86; 95% CI, 1.23-2.81; P < .01), lung disease (OR, 2.48; 95% CI, 1.55-3.98; P < .01), diabetes (OR, 2.61; 95% CI, 1.39-4.88; P < .01), and renal disease (OR, 3.02; 95% CI, 1.21-7.54; P = .02), were also linked to an increased risk for hospitalization.[61]

On 8 June 2020, the RECOVERY (Randomized Evaluation of COVid-19 thERapY) trial, an established randomized clinical trial to test a range of potential treatments for COVID-19, reported preliminary data on 2104 patients randomized to receive dexamethasone, 6 mg once per day (by mouth or by IV injection) for 10 days, vs 4321 patients randomized to usual care alone.[62] Dexamethasone was associated with a lower death rate in ventilated patients (29% vs 40.7%; rate ratio [RR], 0.65; 95% CI, 0.48-0.88; P = .0003), as well as a lower death rate in patients receiving oxygen only without mechanical ventilation (21.5% vs 25%; RR, 0.80; 95% CI, 0.67-0.96; P = .0021). There was no benefit among patients who did not require respiratory support (17% vs 13.2%; RR, 1.22; 95% CI, 0.86-1.75; P = .14).

Acknowledging the limitations of existing data and the fact that empirical decision making might be necessary under the current pandemic, the following are some recommendations to consider when evaluating patients undergoing immunosuppressive therapy for a nonmalignant hematologic disease: (1) patients on low-dose corticosteroids (ie, <10 mg PEQ daily) might not need modification of their current regimen if their hematologic disease is controlled; (2) in patients on higher doses of corticosteroids (ie, >10 mg PEQ daily), consideration of a more effective second-line therapy might allow for tapering and, possibly, discontinuation of the corticosteroid; and (3) in newly diagnosed patients with a nonmalignant hematologic disease or for those experiencing disease relapse but known to be responsive to corticosteroids, a short course of high-dose corticosteroid therapy (1-5 days) might be reasonable to treat the acute episode. In all cases, an approach based on individual patient factors (eg, urgency of need for immunosuppressive therapy, patient comorbidities, measures to minimize exposure to SARS-Cov-2 infection) is highly encouraged. Lastly, prospective studies are needed to better understand the impact of COVID-19 on the management of patients with nonmalignant hematologic diseases.

Conclusions

The use of immunosuppressive therapy in the management of nonmalignant immune-mediated hematologic diseases carries a risk for infection. Although the absolute risk for the individual patient remains small, the burden of such complications at a population level can be significant because of the frequent use of immunosuppressive agents in clinical practice. Patient education, immunization, laboratory screening, and antimicrobial prophylaxis can diminish the risk. Adherence to these preventive strategies is a key element to prevent infectious complications.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

Off-label drug use

None disclosed.

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Preventing infections in children and adults with asplenia

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An estimated 1 million people in the United States have functional or anatomic asplenia or hyposplenia. Infectious complications due to encapsulated organisms such as *Streptococcus pneumoniae*, *Neisseria meningitidis*, and *Haemophilus influenzae* can lead to fulminant sepsis and death, particularly in young children, in the period shortly after splenectomy, and in immunocompromised patients. Patients with asplenia are also at risk for less common infections due to *Capnocytophaga*, *Babesia*, and malaria. Antibiotic prophylaxis, vaccines, and patient and family education are the mainstays of prevention in these at-risk patients. Recommendations for antibiotic prophylaxis typically target high-risk periods, such as 1 to 3 years after splenectomy, children ≤5 years of age, or patients with concomitant immunocompromise. However, the risk for sepsis is lifelong, with infections occurring as late as 40 years after splenectomy. Currently available vaccines recommended for patients with asplenia include pneumococcal vaccines (13-valent pneumococcal conjugate vaccine followed by the 23-valent pneumococcal polysaccharide vaccine), meningococcal vaccines (meningococcal conjugate vaccines for serogroups A, C, Y and W-135 and serogroup B meningococcal vaccines), *H. influenzae* type b vaccines, and inactivated influenza vaccines. Ongoing booster doses are also recommended for pneumococcal and meningococcal vaccines to maintain protection. Despite the availability of prevention tools, adherence is often a challenge. Dedicated teams or clinics focused on patient education and monitoring have demonstrated substantial improvements in vaccine coverage rates for individuals with asplenia and reduced risk of infection. Future efforts to monitor the quality of care in patients with asplenia may be important to bridge the know–do gap in this high-risk population.

LEARNING OBJECTIVES
- Describe infection risks associated with asplenia
- Understand the latest recommendations for immunizing children and adults with asplenia

Introduction

Asplenia or hyposplenia occurs when there is a loss of function of the spleen that may be either anatomic or functional in nature. Anatomic asplenia is most commonly due to surgical removal secondary to trauma or for therapeutic reasons (eg, immune thrombocytopenic purpura [ITP], autoimmune hemolytic anemia, hereditary spherocytosis) or autoinfarction in sickle cell disease, and it is rarely due to congenital asplenia syndromes (eg, isolated congenital asplenia, heterotaxy syndromes). Functional asplenia or hyposplenism can be secondary to hematological diseases (eg, sickle cell disease, hemoglobinopathies), oncologic conditions (eg, chronic graft-versus-host disease after allogeneic hematopoietic stem cell transplant), immunological reasons (eg, antiphospholipid syndrome, severe celiac disease, autoimmune diseases), or untreated HIV infection.[1,2] The spleen functions as a critical organ of the reticuloendothelial system, serving as a filter for senescent blood cells and opsonized bacteria. Its role in preventing infections includes the ability to trigger innate and adaptive immune responses to pathogens, including encapsulated bacteria.

Currently, an estimated 1 million individuals in the United States are asplenic or hyposplenic, with ~100 000 cases being due to sickle cell disease.[3,4] With growing recognition of the harms associated with asplenia, indications for splenectomy have evolved over the past 2 decades.[5] National data from the Agency for Healthcare Research and Quality Healthcare Cost and Utilization Project between 1993 and 2014 indicate a modest decline in the number of splenectomies performed each year from ~30 000/y to 22 000/y (Figure 1).[6] The downward trend

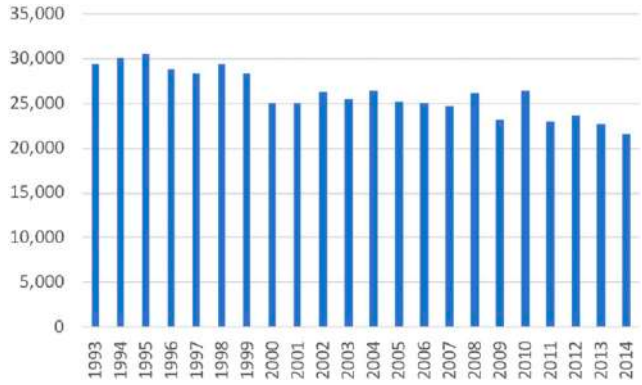

Figure 1. Total number of US hospitalizations associated with total splenectomy, 1993 to 2014.[6]

may be due in part to advances in therapies such as the use of rituximab, immunosuppressive agents, or thrombopoietin receptor agonists for hemolytic anemia and ITP. Modest variability by country is noted regarding indications for splenectomy for medical conditions, with the United States and Italy performing splenectomies for ITP and lymphoma more commonly than other countries where spherocytosis and sickle cell disease are predominant indications for splenectomy.[7] Aside from trauma, indications for splenectomy have been more commonly attributed to hematologic or oncologic indications.

Infectious complications associated with asplenia or hyposplenia

Asplenia can lead to infectious and noninfectious complications, including infection, thrombosis, and pulmonary hypertension.[8] We focus on the critical role of the spleen in infection, which is a major and potentially preventable complication of asplenia. The spleen contains 2 types of tissues with different functions. The white pulp is rich in T-cell lymphocytes, macrophages, and naïve B-cell lymphocytes. Antigen-presenting cells can enter the white pulp and activate T cells, which in turn activate naïve B cells and differentiate into plasma cells that generate immunoglobulin M antibodies followed by immunoglobulin G antibodies. B cells can also serve as antigen-presenting cells, as well as support phagocytic function to help opsonize encapsulated bacteria. The B-cell immune response is critical in the defense against encapsulated organisms. The red pulp is rich in macrophages and is responsible for filtering older, damaged red blood cells as well as phagocytosing opsonized bacteria. Because of its role in removing damaged erythrocytes, the spleen also plays an important role in the defense against intraerythrocytic parasitic infections such as malaria and *Babesia*.

The most feared complication of asplenia is overwhelming infection, due to either functional or anatomic asplenia, and is associated with mortality rates as high as 50% in the absence of prevention strategies such as vaccines and antibiotic prophylaxis. Pneumonia and meningitis may be more likely to occur; purpura fulminans episodes are also often associated with asplenic or hyposplenic states.[9] The incidence of sepsis after splenectomy in children is ~1.8 to 3 per 100 person-years, with children aged <3 years having the highest risk for infection.[10] In contrast, the risk for infection is generally higher in adults, particularly in adults aged ≥60 years (11 to 14 per 100 person-years).[11] The age-dependent risk may reflect a combination of indications for splenectomy and the risk of exposure to pathogens such as *Streptococcus pneumoniae*. Patients with thalassemia, sickle cell disease, or malignancy as indications for splenectomy may portend a higher future risk of sepsis or

Table 1. Antibiotic prophylaxis for patients with asplenia

Routine prophylaxis*	
<3 y	PCN VK 125 mg twice daily (or amoxicillin 10 mg/kg by mouth twice daily)
≥3 y	PCN VK 250 mg twice daily
Adults	PCN VK 250 mg by mouth twice daily (or amoxicillin 500 mg by mouth twice daily)
Adults with PCN allergy	Cephalexin 250 mg by mouth twice daily
	Azithromycin 250 mg by mouth once daily
Emergency antibiotics before ED arrival	
Child	Amoxicillin-clavulanate 45 mg/kg by mouth twice daily (maximum 875 mg per dose)
Child with PCN allergy	Cefdinir 7 mg/kg by mouth twice daily (max 300 mg per dose)
	Levofloxacin 10 mg/kg by mouth twice daily (max 375 mg per dose)
Adult	Amoxicillin-clavulanate 875 mg/125 mg by mouth twice daily
Adult with PCN allergy	Cefdinir 300 mg by mouth twice daily
	Levofloxacin 750 mg by mouth once daily
	Moxifloxacin 400 mg by mouth once daily
Preprocedural prophylaxis (for sinus surgery or airway procedure)	
Child	Amoxicillin 50 mg/kg 1 h before procedure (max 2 gram)
Adult	Amoxicillin 2 g 30-60 min before procedure

ED, emergency department; PCN VK, penicillin V potassium.
*Duration of routine prophylaxis depends on age, time since splenectomy, degree of immunocompromise, or prior episode of sepsis.

Table 2. ACIP recommendations for pneumococcal vaccines in children and adults with asplenia or hyposplenia[49,50]

Age at first dose	Timing of first dose PCV13	Total doses of PCV13	Interval between PCV13 doses	Timing of first dose of PPSV23	Total doses of PPSV23	Interval between PPSV23 doses
No prior PCV13 or PPSV23						
8 wk	—	4 doses	2-mo, 2-mo, 6-mo intervals	—	—	—
6-18 y	—	1 dose	—	>8 wk after PCV13 dose	2 doses	5 y
19-64 y	—	1 dose	—	>8 wk after PCV13 dose	2 doses	5 y
≥65 y*	—	1 dose	—	>8 wk after PCV13 dose	1 dose	—
Any PCV13; no prior PPSV23						
2-5 y + <3 prior PCV13 doses	≥8 wk after prior PCV13 dose	2 doses	8-wk interval	>8 wk after prior PCV13 dose	2 doses	5 y
2-5 y + 3 prior PCV13 doses	≥8 wk after prior PCV13 dose	1 dose	—	>8 wk after prior PCV13 dose	2 doses	5 y
6-18 y	—	—	—	>8 wk after prior PCV13 dose	2 doses	5 y
19-64 y	—	1 dose	—	>8 wk after PCV13 dose	2 doses	5 y
≥65 y*	—	1 dose	—	>8 wk after PCV13 dose	1 dose	—
Any PPSV23; no prior PCV13						
6-18 y	≥8 wk after prior PPSV23 dose	1 dose	—	>8 wk after PCV13 dose	1 dose	5 y
19-64 y	≥1 y after prior PPSV23 dose	1 dose	—	>8 wk after PCV13 dose	1 dose	5 y
≥65 y*	≥1 y after prior PPSV23 dose	1 dose	—	>8 wk after PCV13 dose	1 dose	5 y

*For adults aged ≥65 y, routine pneumococcal vaccination is recommended. For patients with asplenia or hyposplenia, consider PCV13 before PPSV23 administration. Only 1 dose of PPSV23 is recommended for adults aged 65 y with immunocompromising conditions.

overwhelming post-splenectomy infection.[12,13] In addition, the incidence of sepsis after splenectomy is substantially higher in the first 1 to 3 years after splenectomy, though infection can occur as late as 50 years after the procedure.[10,13,14] Partial splenectomy has been used as an alternative to total splenectomy, particularly for hematologic disorders, in order to reduce the burden of significant hemolysis or thrombocytopenia while salvaging splenic immune function.[15] The benefits of partial splenectomy are likely greatest in subpopulations with the highest risk of infection (eg, young age, lack of alternative management strategies, risk of exposure based on geographic location or occupation). However, patients with partial splenectomy may subsequently require a total splenectomy if the hematologic response is not sufficient. Currently, there are limited data available to determine whether partial or total splenectomy substantially changes the benefit–risk balance in patients with hematologic conditions, and it remains a shared decision based on context, values, and preferences.[16]

Infections in patients with asplenia or hyposplenia are most commonly due to encapsulated bacteria, including pneumococcal, meningococcal, and *Haemophilus influenzae* infections. *Capnocytophaga canimorsus* secondary to dog bites is also reported as a cause of sepsis.[17] Due to the role of the spleen in clearing damaged or infected erythrocytes, severe infections due to intracellular pathogens such as *Babesia*, *Bartonella*, and *Plasmodia* species have also been described in the literature.[18-21]

Management of patients with asplenia with fever

The most common presentation of infection in patients with asplenia is fever. These patients are at risk for rapid and fulminant progression due to encapsulated organisms.[14] Although the risk is lifelong, the risk may be particularly high for certain patients based on the indication for splenectomy, age at time of splenectomy, interval since splenectomy, risk of exposure to encapsulated organisms (ie, travel, age, low population vaccine coverage rates), underlying comorbidities, immunocompromise, and prior episode of sepsis.[10,22-25] Early detection and management of suspected infections is critical in the management of patients with asplenia. Early empiric treatment with antibiotics with any seemingly minor signs of infection, with specific recommendations for an emergency oral antibiotic supply on hand, should be given at the first sign of infection (Table 1). Patients with fever should subsequently seek emergency department care immediately after taking oral antibiotics for further evaluation, diagnostic testing, and administration of intravenous antibiotics. In general, ceftriaxone serves as the backbone of treatment, and some experts also

Table 3. ACIP recommendations for meningococcal ACWY and meningococcal B vaccines in children and adults with asplenia or hyposplenia[33,34,37,38,51]

Age at first dose	Timing of first dose of meningococcal vaccine	Total doses of meningococcal vaccine	Interval between meningococcal vaccines	Revaccination
MenACWY-CRM (MENVEO)				
8 wk	—	4-dose series	2-mo, 2-mo, 6-mo intervals	Revaccinate 3 y after primary series; additional boosters every 5 y if risk remains
7-23 mo	—	2-dose series	12-wk interval and second dose at ≥12 mo of age	Revaccinate 3 y after primary series; additional boosters every 5 y if risk remains
≥24 mo	—	2-dose series	8-wk interval	Revaccinate 3 y after primary series if most recent dose given before age 7 y; additional boosters every 5 y if risk remains
MenACWY-D (Menactra)				
9-23 mo	Do not administer, because of immune interference with PCV13			
≥24 mo	Administer >4 wk after completion of PCV13 series	2-dose series	8-wk interval	Revaccinate 3 y after primary series; additional boosters every 5 y if risk remains
MenB-4C (BEXSERO)*				
≥10 y	—	2-dose series	1-mo interval	Revaccinate 1 y after primary series and revaccinate every 2-3 y if risk remains†
MenB-FHbp (Trumenba)*				
≥10 y	—	3-dose series	1-mo, 4-mo intervals	Revaccinate 1 y after primary series and revaccinate every 2-3 y if risk remains†

*Use same product for all doses in a series
†MenB booster doses now recommended for person ≥ 10 y with asplenia as of June 2019.

recommend the addition of intravenous vancomycin, depending on local antimicrobial susceptibility patterns and clinical presentation. For patients with cephalosporin allergy, fluoroquinolones or carbapenems may be used as an alternative, although resistance patterns should be monitored for common pathogens. Patients may also be admitted pending diagnostic evaluation.

Prevention: antibiotic prophylaxis

Daily antibiotic prophylaxis is typically recommended after splenectomy or for conditions such as sickle cell disease in which functional asplenia or autosplenectomy occurs on the basis of trials that demonstrated a 50% to 63% reduction in pneumococcal infection among patients with sickle cell disease receiving penicillin prophylaxis in children aged ≤5 years.[26] Common regimens are listed in Table 1. However, there is little consensus on the duration of use. Lifelong prophylaxis is often recommended for patients with asplenia who previously experienced an episode of sepsis or who remain immunocompromised. In nonimmunocompromised populations, US and Australian guidelines recommend prophylaxis for 1 to 3 years after splenectomy or until a certain age threshold (ie, >5 years).[22,27] In the United Kingdom, antibiotic prophylaxis is recommended for children aged <16 years, adults aged >50 years, and patients with an inadequate serologic response to pneumococcal vaccination.[28] Of note, serologic testing is not routinely recommended for patients with asplenia. Rather, clinicians should consider whether patients are considered immunodeficient due to underlying disease or treatment and should ensure that on-time

Table 4. ACIP recommendations for Hib vaccines in children and adults with asplenia or hyposplenia[37,38,52]

Age at first dose	Timing of first dose of Hib	Total doses of Hib	Interval between Hib doses
ActHIB, HIBERIX, Pentacel			
2 mo	—	4 doses	2-mo, 2-mo, 6-mo intervals
PedvaxHIB			
2 mo	—	3 doses	2-mo, 8-mo intervals
Any licensed, age-appropriate Hib vaccine			
12-59 mo; ≤1 dose before age 12 mo	>8 wk after prior dose	2 doses	8-wk interval
12-59 mo; ≥2 doses before age 12 mo	>8 wk after prior dose	1 dose	—
≥5 y; no prior Hib doses	—	1 dose	—

vaccination and continued antibiotic prophylaxis are administered. Additional investigation regarding the optimal duration of antibiotic therapy is warranted, given the epidemiologic shifts in colonization and infection due to newer vaccines.

Prevention: vaccines

Perhaps the most effective prevention method for patients with asplenia or hyposplenia is to vaccinate, ideally >2 weeks before a planned splenectomy. In addition to routine childhood and adult vaccines, the Advisory Committee on Immunization Practices (ACIP) of the Centers for Disease Control and Prevention and the Infectious Diseases Society of America recommends pneumococcal, meningococcal, *H. influenzae* type b (Hib), and influenza vaccinations for this at-risk population. The vaccine schedule recommended by ACIP depends on vaccination history, age at the time of splenectomy, and whether the procedure was elective or emergent (Tables 2 through 4). Conjugate vaccines are preferred for priming the immune response, particularly for patients with asplenia, given the higher opsonophagocytic activity geometric mean antibody titers elicited by conjugate vs polysaccharide vaccines. In addition, polysaccharide vaccines are poorly immunogenic and may lead to hyporesponsiveness to subsequent doses.[29] If patients are unable to receive at least one dose of vaccine before splenectomy, administration of needed vaccines should be initiated ~2 weeks after splenectomy. We describe specific recommendations below for the United States; however, it should be noted that recommendations may vary by country.[30]

Pneumococcal vaccines: PCV13 and PPSV23

Two pneumococcal vaccines are currently licensed for use in the United States: the 13-valent pneumococcal conjugate vaccine (PCV13) and the 23-valent pneumococcal polysaccharide vaccines (PPSV23). The routine childhood immunization schedule recommends routine use of PCV13 for all children to reduce the risk of invasive pneumococcal disease, pneumonia, and otitis media.[31] For children with congenital asplenia, sickle cell disease, thalassemia, hereditary spherocytosis, or other inherited conditions resulting in functional asplenia, timely administration of pneumococcal conjugate vaccines is critical for disease prevention. In addition, due to the broader serotype coverage of PPSV23, booster doses of PPSV23 are also recommended after the PCV13 series. Among adults with functional or anatomic asplenia, PCV13 followed by PPSV23 is also recommended for use to reduce the risk of invasive pneumococcal disease. The sequence of administration is important for pneumococcal vaccines in order to optimize the immune response. Conjugate pneumococcal vaccines such as PCV13 should be administered before polysaccharide vaccines (PPSV23) whenever possible because patients who receive PPSV23 as the initial dose have lower antibody responses, shorter duration of immunity, and hyporesponsiveness to subsequent doses of either vaccine.[32]

Meningococcal vaccines: meningococcal conjugate vaccines for serogroups A, C, Y, and W-135 and serogroup B meningococcal vaccines

Vaccination with age- and formulation-appropriate meningococcal vaccines is recommended for individuals at increased risk for meningococcal disease. There are 4 licensed meningococcal vaccines currently available in the United States: 2 meningococcal conjugate vaccines for serogroups A, C, Y, and W-135 (MenACWY) and 2 serogroup B meningococcal vaccines (MenB). In healthy adolescents, ACIP routinely recommends MenACWY at 11 to 12 years of age and a booster at 16 years of age. MenB is also available for healthy teens and young adults 16 to 23 years of age under a shared decision-making recommendation. However, given the increased risk for severe morbidity and mortality attributable to meningococcal disease in children and adults with asplenia, both types of meningococcal vaccines (MenACWY and MenB) are specifically recommended for use in this at-risk population.

MenACWY-CRM (MENVEO) can be administered to children as young as 8 weeks of age and is recommended for use in children with functional or anatomic asplenia. Infants are recommended to receive a 4-dose series; children aged 7-23 months receive a 2-dose series with the second dose given at ≥12 months of age.[33] MenACWY-D (Menactra) may be administered only to children aged ≥2 years due to interference with the immune response to PCV13 in children aged <2 years. The choice of MenACWY will depend on the age of the patient and vaccine availability, and either series is considered acceptable for protection against serogroups A, C, Y, and W-135. Because neither vaccine provides protection against serogroup B disease, however, ACIP also recommends MenB vaccination of children and adults with asplenia. MenB-4C vaccine (BEXSERO) protects against serogroup B meningococcal disease and is recommended for use as a 2-dose series for at-risk persons aged ≥10 years.[34] Immunogenicity is similar for children with functional or anatomic asplenia when compared with healthy children.[35] Given the absence of impact of MenB-4C on carriage, vaccination will remain a critical component of protection because reliance on herd immunity is less likely.[36] MenB-FHbp vaccine (Trumenba) is recommended for use as a 3-dose series for persons at increased risk for serogroup B meningococcal disease aged ≥10 years. In contrast, healthy adolescents and young adults who are not at increased risk may receive a 2-dose series of MenB-FHbp, though for individuals with asplenia, a 3-dose series is strongly preferred to enhance protection in the short term. Either vaccine may be administered concomitantly with MenACWY vaccines, though the same MenB product is recommended for the entire series (ie, they are not interchangeable). Since June 2019, booster doses of MenB are also now recommended for those with asplenia 1 year after completion of a MenB primary series, followed by MenB booster doses every 2 to 3 years thereafter for as long as increased risk remains.[37,38]

Hib and other vaccines

The incidence of Hib disease has declined precipitously since the introduction of Hib conjugate vaccine in the 1990s, and vaccination remains a mainstay of disease prevention in healthy and immunocompromised children. Given the risk of invasive disease due to *H. influenzae* and evidence for immunogenicity after vaccination in children with asplenia, Hib vaccine continues to be strongly recommended in this population (see Table 4). In addition, annual influenza vaccination of the patient and all household members is considered an important strategy for prevention in patients with asplenia or hyposplenia due to the risk posed by subsequent bacterial coinfection, such as pneumococcal pneumonia. Any age-appropriate, licensed, inactivated influenza vaccines can be administered yearly to children and adults aged ≥6 months.[39] Live-attenuated influenza vaccines are not recommended for patients with asplenia, given the availability of inactivated influenza vaccines. For immunocompetent adults

aged ≥50 years, recombinant zoster vaccine (RZV) is routinely recommended as a 2-dose schedule and is preferred over the live attenuated zoster vaccine.[38] Although asplenia does not increase the risk for herpes zoster virus or postherpetic neuralgia, other associated immunocompromising conditions may increase an individual's risk for disease. All patients with asplenia aged ≥50 years should receive RZV as a routinely recommended vaccine. ACIP is currently considering use of RZV in immunocompromised adult patients.

Prevention: patient and family education

Patients and families should receive education about the risks associated with asplenia and hyposplenia. Early recognition of signs and symptoms (fever, chills, rigors, vomiting, diarrhea) of infection and immediate referral for care are critical. Symptoms may be quite mild initially but may rapidly progress to fulminant sepsis, shock, and death. Antibiotics should be prescribed to have on hand for empiric treatment before seeking care in an emergency department (see Table 1). Patients are also recommended to wear a medical alert bracelet or carry a medical alert card that indicates the presence of asplenia and the need for prompt antimicrobial therapy, allergies to medications, and emergency contact information. If additional space is available, information about vaccination history (pneumococcal, MenACWY, MenB, Hib) and current medications (eg, antibiotic prophylaxis) may also be included. When prophylaxis is stopped, benefit–risk assessment and discussion with the patient should include the risks of continued antibiotic prophylaxis, availability of established medical care, local epidemiology of antibiotic-resistant bacteria, and reinforcement of early recognition and detection of infection in patients with asplenia.

Additional considerations for patients and families is ensuring they are aware of the need for preprocedural prophylaxis for sinus or respiratory tract procedures (eg, functional endoscopic sinus surgery, bronchoscopy). Dog bites or wounds licked by dogs are also indications for presumptive antimicrobial treatment, even in the absence of symptoms. Travelers should be counseled on the risks associated with tick-borne illnesses such as babesiosis and mosquito-borne illnesses such as malaria. The risk for infectious complications is lifelong in patients with asplenia, which reinforces the importance of taking preventive measures and ensuring that emergency antibiotics are available when traveling. Furthermore, all patients should receive education about the importance of vaccines in reducing the risk of sepsis and other preventable infectious diseases. Important sources of information about vaccines are provided by the Centers for Disease Control and Prevention (https://www.cdc.gov/vaccines/hcp/acip-recs/general-recs/sources.html), and specific patient education for adults with asplenia is provided by the Immunization Action Coalition (https://www.immunize.org/catg.d/p4047.pdf).

Quality of care for patients with asplenia

Multiple studies have demonstrated that best practice recommendations are challenging to implement.[40-42] Vaccination rates are suboptimal (as low as 6%); adherence to antibiotic use may be low; and patient and family education and support may be sporadic in nature.[43] One of the largest barriers to care reported by physicians is the lack of clarity about which physician is responsible for the management of patients with asplenia (eg, primary care, surgeons, hematologists, oncologists).[44] Engagement of subspecialists in vaccine delivery is critical to protect patients with asplenia from infectious complications, particularly for adult patients who may not routinely receive care from a primary care physician. Furthermore, the timing and importance of vaccines may be most effectively conveyed by subspecialists who care for patients with complex or chronic illnesses. Dedicated teams or services for patients with asplenia are recommended to substantially improve adherence to preventive measures, including vaccination rates and antibiotic prophylaxis, and reduce occurrence of severe sepsis.[24,45,46]

Asplenia and coronavirus disease 2019 infection

In a large cohort of 17 million adult patients in England, there were 27 917 adults diagnosed with asplenia and 40 coronavirus disease 2019 (COVID-19)-associated deaths (0.14%).[47] Similar to other reported studies, older age, male sex, non-White ethnic groups, obesity, diabetes, other comorbid conditions (respiratory, cardiovascular, renal, hepatic, neurologic disease), and immunosuppression (eg, hematologic malignancy, organ transplant) were associated with a higher hazard of death. Patients with asplenia had a mildly elevated (hazard ratio, 1.3), but not statistically significant, risk in multivariate models. Clinical characteristics of COVID-19 in patients with hemoglobinopathies appear to be similar in nature to those described for the overall population, considering the presence of comorbidities (ie, obesity, diabetes, chronic respiratory or cardiovascular disease, immunocompromise), though direct comparisons are not yet available.[48] Until a safe and effective COVID-19 vaccine is recommended for use, patients with asplenia should follow guidance to minimize exposure to the virus through social distancing, masking, hand hygiene, and cleaning and disinfection.

Conclusions

Recommendations for patients with functional or anatomic asplenia include antibiotic prophylaxis, vaccination, and patient and family education to ensure prevention of infections and timely management of febrile illnesses. Adherence to best practices can be challenging without targeted efforts to provide care for this population and track key process measures such as vaccination rates. Given the lifelong risk associated with infection in these patients, future efforts should focus on improving the quality of care delivered to children and adults with asplenia.

Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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Managing toxicities of Bruton tyrosine kinase inhibitors

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Inhibition of Bruton's tyrosine kinase (BTK) has revolutionized the treatment landscape for patients with chronic lymphocytic leukemia (CLL). By targeting this critical kinase in proximal B-cell receptor signaling, BTK inhibitors (BTKis) impair cell proliferation, migration, and activation of NF-κB. Clinically, because indefinite inhibition is a mainstay of therapy, there is an extended period of exposure in which adverse effects can develop. Given the impressive efficacy and activity of BTKis in the treatment of patients with CLL, appropriate management of treatment-emergent adverse events (AEs) is of paramount importance. Here we review the BTKi landscape and present the available toxicity and safety data for each agent. The long-term toxicity profile of ibrutinib, a first-in-class inhibitor, is well characterized and includes a clinically significant incidence of cardiac arrhythmias, bleeding, infection, diarrhea, arthralgias, and hypertension. Acalabrutinib, the initial second-generation BTKi to earn approval from the US Food and Drug Administration, demonstrates improved kinase selectivity for BTK, with commonly observed adverse reactions including infection, headache, and diarrhea. Mediated by both on-target inhibition of BTK and variable off-target inhibition of other kinases including interleukin-2–inducible T-cell kinase (ITK), tyrosine-protein kinase (TEC), and endothelial growth factor receptor (EGFR), the toxicity profile of BTKis is closely linked to their pattern of kinase binding. Other emerging BTKis include second-generation agents with variable degrees of kinase selectivity and third-generation agents that exhibit reversible noncovalent binding to BTK. We also highlight critical considerations for the prevention and monitoring of AEs and offer practical management strategies for treatment-emergent toxicities.

LEARNING OBJECTIVES

- Increase familiarity with treatment-emergent toxicities commonly observed with BTKis, calling attention to their incidence, mechanism (where known), and appropriate clinical management
- Review the landscape of currently approved and in-development BTKis in CLL, highlighting differences in the kinase-binding and toxicity profiles of agents in this class

Introduction

Inhibition of Bruton's tyrosine kinase (BTK) has revolutionized the treatment landscape for patients with chronic lymphocytic leukemia (CLL). By targeting BTK, a critical kinase in proximal B-cell receptor (BCR) signaling, this class of small molecule inhibitors impairs BCR signaling and activation of NF-KB and inhibits cell proliferation and migration.[1,2] Clinically, because indefinite inhibition is a mainstay of therapy, there is an extended period of exposure in which adverse effects can develop, often leading to discontinuation after several years. Mediated by both on-target inhibition of BTK and variable off-target inhibition of other kinases including interleukin-2–inducible T-cell kinase (ITK), tyrosine-protein kinase (TEC), and endothelial growth factor receptor (EGFR), the toxicity profile of BTK inhibitors (BTKis) is closely linked to their pattern of kinase binding.

With 8 years of follow-up data since its initial pivotal study,[3] the long-term toxicity profile of ibrutinib (a first-in-class, irreversible inhibitor of BTK) is well characterized. It includes cardiac arrhythmias, bleeding, infection, diarrhea, arthralgias, and hypertension.[4-8] Acalabrutinib, the initial second-generation BTKi to earn approval from the US Food and Drug Administration (FDA), demonstrates improved kinase selectivity for BTK, with commonly observed adverse reactions including infection, headache, and diarrhea.[9-12] Other emerging BTKis include second-generation agents with variable degrees of kinase selectivity[13-16] and third-generation agents that exhibit reversible noncovalent binding to BTK.[17-20] At present, the relevant differences in the toxicity profiles of individual BTKis are challenging to discern based on limited preliminary data that consist primarily of

nonrandomized studies with limited direct comparisons. Notably, experience with ibrutinib has demonstrated that patient-specific risk factors (eg, age, comorbidities) can also influence the likelihood of adverse effects and that significant variation exists between rates of adverse effects documented in initial pivotal clinical trial studies[6] and subsequent real-world analyses of these agents, where AEs were responsible for 63% and 50% of discontinuations in the frontline and relapsed or refractory (R/R) settings, respectively.[21] To maximize the safety and long-term tolerability of BTKis, it is crucial to carefully consider each patient's clinical history before therapy initiation and to pay careful attention to patient-reported signs and symptoms observed during therapy.

Herein we review the BTKi landscape, present the available toxicity and safety data for each agent, and highlight critical considerations for the management of treatment-related toxicities in patients with CLL on BTKis.

Clinical case

A 68-year-old man without significant comorbidities received a diagnosis of CLL 10 years ago. He was previously treated with fludarabine, cyclophosphamide, and rituximab, achieving a complete response to therapy. After a multiyear period of observation, he has developed progressive fatigue and dyspnea on exertion over the past 6 months. His physical examination is notable for diffuse adenopathy (3 to 4 cm) and a spleen palpable 4 cm below the costal margin (white blood cell count 160,000/μL; 95% lymphocytes; hemoglobin 10 g/dL, platelets 95,000/μL; fluorescence in situ hybridization del(17p), del(11q); TP53 sequencing mutated; immunoglobulin heavy chain variable gene VH1-69, unmutated). The need for therapy was discussed with the patient.

Overview of the BTKi landscape

Irreversible inhibitors

Irreversible BTKis act via covalent binding to a cysteine residue at position 481, found in the adenosine triphosphate–binding pocket of BTK. At the time of this writing, two BTKis are FDA approved for use in CLL, ibrutinib and acalabrutinib, with multiple other agents under active investigation (Table 1). Clinical experience with long-term follow-up is greatest with ibrutinib, which has been extensively studied as a standard of care option for patients with CLL, demonstrating greater efficacy than comparator therapies in registration trials in both the frontline (RESONATE-2, FDA approval in 2016) and R/R settings (RESONATE, FDA approval in 2014) and in patients with deletion 17p. As a first-generation inhibitor, ibrutinib irreversibly inhibits ≥10 other kinases, with a half-maximal inhibitory concentration of <11 nmol/L.[22] Acalabrutinib is more selective for BTK than ibrutinib and notably demonstrates less inhibition of related kinases with a conserved cysteine residue; of these, relative inhibition of ITK and EGFR is less than that of TEC.[23,24] Initial studies demonstrated 97% BTK occupancy (the percentage of total BTK bound by drug),[9] with recent work highlighting more potent inhibition of the BCR and NF-KB pathways at 100 mg twice-daily dosing compared with daily administration.[24] The drug has been subsequently studied in phase 3 trials in both the treatment-naive (as monotherapy and in combination with obinutuzumab, ELEVATE-TN)[10] and R/R setting (single-agent, ASCEND),[12] earning FDA approval in 2019.

Multiple irreversible BTKis are at an earlier development stage and are under active investigation. Zanubrutinib (formerly BGB-3111) is an irreversible inhibitor with greater selectivity for BTK than for ITK. Extended follow-up from a phase 1/2 study was presented at ASH 2019,[25] and the drug is being compared with ibrutinib in the phase 3 setting (ALPINE).[15] It also gained FDA approval in 2019 in the treatment of patients with mantle cell lymphoma who have received ≥1 prior therapy. Tirabrutinib (formerly ONO/GS-4059) has a high degree of selectivity for BTK compared with the other TEC family kinases. It has been studied in the phase 1 setting as monotherapy for R/R patients[26] and in combination with spleen tyrosine kinase (SYK) and phosphoinositol 3-kinase (PI3K) inhibitors.[16]

Reversible inhibitors

Mutations in the C481 binding site are known to confer clinical resistance to irreversible BTK inhibition, contributing to ~65% of ibrutinib failures due to CLL progression (~40% with mutations in *BTK* alone and an additional ~25% with co-occurring mutation in *PLCG2*).[27-29] Reversible third-generation BTKis act independently of covalent binding to C481 and can achieve target inhibition of both wild-type and C481S-mutated BTK. Therefore, they theoretically offer the potential to overcome resistance mediated by this mutation. Several agents in this class are in development. Vecabrutinib (formerly SNS-062) is a selective, reversible inhibitor that was initially evaluated in a phase 1b trial in patients with CLL who received ≥2 previous regimens and progressed on therapy with an irreversible BTKi.[30] Based on a recent press release, its further development in CLL appears to be halted.[31] LOXO-305 is a highly selective noncovalent BTKi with minimal off-target kinase inhibition and binding to BTK C481S, with a half-maximal inhibitory concentration of 1.42 nM.[19] Preliminary data from 13 patients enrolled in a first-in-human phase 1 trial were presented at ASH 2019.[32] Fenebrutinib (formerly GDC-0853) was assessed for safety, tolerability, and pharmacokinetics in a cohort of 24 patients with B-cell malignancy and was generally well tolerated by 14 patients with R/R CLL.[33] In contrast to other noncovalent inhibitors, ARQ_531 maintains downregulation of the BCR pathway in the case of C481S *BTK* or autoactivating *PLCγ2* mutations by concurrently inhibiting additional kinases, including LYN and the Src family kinase MEK1.[20] Final results of a phase 1 trial demonstrated that the drug was well tolerated at 65 mg daily dosing in a cohort that included 26 patients with CLL or small lymphocytic lymphoma (SLL).[34]

The patient was initiated on ibrutinib 420 mg/day (monotherapy). His initial response was notable for a partial response with lymphocytosis (white blood cell count 180,000/μL; 79% lymphocytes; hemoglobin 12 g/dL; platelets 105,000/μL). His symptoms improved, with resolution of splenomegaly and lymphadenopathy. However, after 6 months on ibrutinib therapy, he now reports migratory arthralgias and fatigue that are impairing his activities of daily living.

AEs necessitating specialized management

Rates of discontinuation

Here we consider the incidence, mechanisms of action, and management of AEs that arise throughout therapy with BTKis, focusing on the 2 currently approved agents (Table 2). On the whole, AEs lead to clinically significant rates of discontinuation or dosage reduction seen in landmark clinical studies of ibrutinib (eg, 12% in RESONATE at initial publication,[4] 16% at final follow-up[35]), and discontinuation rates appear to be lower with acalabrutinib (eg, 9% to 11% at shorter 28.3-month follow-up).[10] Integrated analysis of multiple ibrutinib studies reveals that

Table 1. BTK inhibitors currently approved and under development

Compound	Indication	Stage of development	Clinical study	Mechanism	TEC family kinase inhibition half-maximal inhibitory concentration (nm)		
					BTK	ITK	TEC
Ibrutinib[22]	Newly diagnosed and R/R	Approved	RESONATE2[5] RESONATE[4]	Irreversible C481 binding	0.5	10.7	78
Acalabrutinib[9]	Newly diagnosed and R/R	Approved	ELEVATE-TN[10] ASCEND[12]	Irreversible C481 binding	5.1	>1,000	93
Zanubrutinib[13] (BGB-3111)	In development	3 (approved in mantle cell lymphoma)	SEQUOIA[14] ALPINE[15]	Irreversible C481 binding	0.22	30	1.9
Vecabrutinib[17] (SNS-062)	Early development	1b (CLL/B-NHL) Halted in CLL	NCT03037645	Noncovalent reversible	3	4	14
LOXO-305[19]	Early development	1/2 (CLL/B-NHL)	NCT03740529	Noncovalent reversible	3.15	>5,000	1,234
Fenebrutinib[18] (GDC-0853)	Early development	1 (CLL/B-NHL)	NCT01991184	Noncovalent reversible	0.91	>1,000	>1,000
ARQ-531[20]	Early development	1 (CLL/B-NHL)	NCT03162536	Noncovalent reversible	4.23	>10,000	5.8

NHL, non-Hodgkin lymphoma.

AEs contribute to a 14% rate of dosage reduction and rates of discontinuation of therapy of 10% in year 1, 5% in year 2, and 6% in year 3.[6] Outside the context of clinical trials, real-world analysis demonstrates an even more significant impact of AEs on treatment cessation (eg, contributing to half of an overall 42% discontinuation rate during early treatment),[21] highlighting the need for careful management of treatment-emergent toxicities.

Cardiac arrhythmia: atrial fibrillation

CLL is a disease of older adults, with a median age of 72 years at diagnosis. Beyond the rates of atrial fibrillation (AF) present in the general population of similar age (1% to 2%),[36] there is a higher rate of AF in treatment-naive patients with CLL (6.1% prevalence at diagnosis, with a subsequent incidence of 1% per year).[37] An early signal suggesting an association between AF and BTK inhibition was observed in 7% to 10% of patients in initial randomized trials of ibrutinib[4,5] (Table 3). This association persisted in extended follow-up and pooled analyses of multiple studies. For example, in a pooled safety analysis of 4 randomized trials of ibrutinib in CLL/SLL and mantle cell lymphoma,[38] new-onset AF was reported in 6% of patients on ibrutinib and 2% of patients treated with comparator agents; grade ≥3 AF occurred in 3% and <1% of these patients, respectively. The prevalence of AF associated with ibrutinib was greatest in the first 3 months on therapy (median time of onset 2.8 months), with late events (onset at month 18 or later) occurring in a minority (1%) of patients. More recent randomized comparisons of ibrutinib with chemoimmunotherapy (CIT) highlight the relevance of patient age to the risk of AF. In younger patients, the E1912 study[39,40] (median age 58) reported an incidence of grade ≥3 AF in 2.9% of patients on ibrutinib (vs 0% on CIT). In contrast, the Alliance 041202 study[41] (median age 71) reported a 9% incidence of AF on ibrutinib monotherapy (vs 3% for CIT). Compared with ibrutinib, acalabrutinib has a lower rate of AF in several series. For example, with a median follow-up of 41 months, AF was observed in 7% of R/R patients (3% grade ≥3) receiving acalabrutinib monotherapy.[11] Although the mechanism of BTKi-related AF remains unclear, inhibition of PI3K signaling—a critical regulator of cardiac protection under stress that is regulated by BTK and TEC—has been implicated.[42] Although the kinase binding profile of agents in earlier development may a priori be thought to affect their risk of AF, an exact estimate of incidence is difficult to determine because of smaller study populations.

The management of BTKi-related AF begins with a baseline clinical risk assessment of cardiovascular risk factors before initiating therapy. For patients with a long history of poorly controlled AF, we favor consideration of other treatment modalities (eg, BCL2 inhibition). Once AF arises, an interdisciplinary effort should be made to manage this complication along with the patient's underlying CLL while balancing the individualized risk of bleeding with that of stroke. Clinical risk assessments, such as the CHA_2DS_2-VASc and HAS-BLED scoring systems, can guide this approach. For patients with limited risk factors (CHA_2DS_2-VASc 0-1), most clinicians favor continuing BTKi therapy.[43,44] For patients with ≥2 risk factors, multiple strategies have been reported on, with limited data to clearly favor one approach over another. Management is therefore clinician dependent, with some authors advocating discontinuing BTKi and initiating anticoagulation.[44] In contrast, others prefer to hold drugs only temporarily and reinitiate therapy once control of AF is achieved.[43] Pharmacologically, it is crucial to keep in mind potential interactions between BTKis and AF-directed therapy or anticoagulation. In terms of the former, beta-blockade is often preferred as the first choice over CYP3A4 inhibitors (eg, verapamil and diltiazem) or P-glycoprotein substrates (amiodarone). Considering the latter, we favor expert management strategies for anticoagulation with either low-dose apixaban[43] (2.5 mg twice daily given CYP3A4 interaction) or enoxaparin[44] (at regular doses in patients with a platelet count >50,000/μL). We note that the pathophysiology of bleeding on ibrutinib is

Table 2. Management of selected adverse events

Adverse event	Management recommendations
Atrial fibrillation	• Obtain a baseline clinical risk assessment of cardiovascular risk factors before initiating therapy. • New AF: Interdisciplinary risk–benefit assessment. CHA2DS2-VASc 0-1, most clinicians favor continuing BTKi therapy; ≥2, consider temporary drug hold until AF control or discontinuation. • Consider beta-blockade, often preferred as the first choice over CYP3A4 inhibitors (eg, verapamil and diltiazem) or P-glycoprotein substrates (amiodarone), which interact with BTKis. • Anticoagulation strategies include either low-dose apixaban (2.5 mg twice daily given CYP3A4 interaction) or enoxaparin (at regular doses in patients with a platelet count >50,000/μL). Where possible, avoid combination with vitamin K antagonists.
Ventricular arrhythmia	• Obtain a detailed cardiac history and baseline electrocardiogram for all patients; reserve echocardiogram for patients with significant cardiac history or risk factors. • Instruct patients to remain vigilant for potential early warning signs of ventricular arrhythmia and immediately investigate incident lightheadedness, palpitations, or syncope.
Bleeding risk	• Commonly encountered bruising seen with BTKis does not confer an increased risk of major hemorrhage and does not necessitate cessation of therapy. • When possible, send patients for necessary procedures before starting therapy. • Hold BTKis for either 3 days (minor procedure) or 7 days (major procedure) both before and after invasive procedures because of increased periprocedural bleeding risk. • For minor bleeding, holding BTKi results in the resolution of bleeding tendency in 2-3 days. For severe bleeds, transfuse platelets as appropriate to overcome clinical bleeding, regardless of platelet count. • Encourage patients with bleeding to abstain from over-the-counter supplements that may exacerbate bleeding risk, such as vitamin E or fish oil. • Consider treatment options other than BTKi when dual antiplatelet therapy is indicated.
Infection	• Obtain a complete workup with an appropriate index of suspicion for opportunistic infections such as *Aspergillus fumigatus* and PJP. • In the case of severe infection, hold BTKi until a definitive diagnosis is determined and restart after the start of clinical improvement, except in the case of fungal infections. • Provide clinically indicated vaccinations (eg, against influenza and pneumococcus) of patients before treatment initiation. • Consider PJP prophylaxis for patients deemed at high risk of infection (eg, R/R or heavily pretreated patients) or patients with a prior history of infection.
Hypertension	• Optimize pharmacotherapy for control of baseline hypertension before treatment initiation. • Routinely monitor and begin appropriate medical therapy for incident hypertension in conjunction with the patient's primary care provider.
Diarrhea	• Most BTKi-related diarrhea can be managed with supportive care, antimotility agents, and evening dosing of ibrutinib to mitigate symptoms. • Consider temporary drug holds in the case of grade ≥3 diarrhea.
Fatigue, arthralgia, and myalgia	• Avoid dosage reductions for fatigue early in the course of therapy. Search for other potential causes of fatigue when observed later in the treatment course; consider drug holiday or dosage reduction only when severe and truly drug related. • Rule out other causes of arthralgia. When grade 1-2, we favor observation and supportive care. Consider dosage reduction when symptoms affect ADLs, with dose holds for grade ≥3 AEs (affecting self-care ADLs) and rechallenge at lower doses if resolution of symptoms. • Arthralgias can be adjunctively treated with pharmacotherapy, although evidence is anecdotal. Approaches include magnesium supplementation and quinine-containing tonic water. Severe arthralgias demonstrate a variable response to short-course steroids and anti-inflammatory agents.
Cytopenias	• Treatment-emergent flare of autoimmune cytopenias can be managed with short-course corticosteroids or CD20 monoclonal antibody treatment, and most patients can continue BTKi therapy.
Dermatologic manifestations	• BTKi-related skin manifestations are often responsive to corticosteroids or dose holds. • Textural changes in hair or nails can be treated with biotin supplementation and the application of nail oil.
Headache	• Acalabrutinib-associated headache resolves with extended treatment and is often responsive to caffeine.

Table 3. Frequency of adverse events in landmark studies of currently approved BTKis

	Ibrutinib		Acalabrutinib	
	RESONATE2[5]	RESONATE[83,84]	ELEVATE-TN[10]	ASCEND[12]
	TN n = 135	RR n = 195	TN n = 179	RR n = 154
Adverse events	f/u = 18.4 mo	f/u = 19 mo	f/u = 28.3 mo	f/u = 22 mo
Atrial fibrillation				
All grades	14 (10)	13 (7)	7 (3.6)	9 (6)
Grade ≥ 3	6 (4)	7 (4)	NR	2 (1)
Bleeding				
All grades	9 (7)	NR	70 (39)	44 (29)
Grade ≥ 3	8 (6)	4 (2)	4 (2)	4 (3)
Hypertension				
All grades	18 (14)	NR	9 (5)	7 (5)
Grade ≥ 3	5 (4)	8 (4)	4 (2)	4 (3)
Arthralgia				
All grades	27 (20)	36 (19)	28 (16)	23 (15)*
Grade ≥3	3 (2)	NR	1 (0.6)	2 (1)*
Infection				
All grades	NR	NR	116 (65)	97 (63)
Grade ≥3	21 (23)	59 (30)	25 (14)	30 (20)
Diarrhea				
All grades	57 (42)	105 (54)	62 (35)	30 (20)
Grade ≥3	5 (4)	9 (5)	1 (0.6)	3 (2)

f/u, follow-up; NR, not reported in original publication; RR, relapsed or refractory; TN, treatment-naive.
*Reported numbers reflect 16.1-month follow-up.[85]

complex and that this risk, both during BTK inhibition alone and when coadministered with anticoagulation, is greatest early in the BTKi treatment course.[45] Because early phase 1 clinical trials of ibrutinib included fatal subdural hematoma on warfarin and subsequent BTKi trials excluded these patients,[4] we generally avoid the combination of BTKi and vitamin K antagonists. When considering anticoagulation, we place extra emphasis on the instruction, recommended to all our patients on BTKis, to abstain from over-the-counter supplements that may exacerbate bleeding risk, such as vitamin E or fish oil.[45]

Ventricular arrhythmia

With longer-term follow-up of patients receiving ibrutinib, rare unexplained cases of incident ventricular arrhythmias and sudden cardiac death have emerged.[46] Independent analysis has confirmed this association in data from US-based[46,47] and international registries.[48] For example, in a global study of cardiovascular adverse drug reactions, ibrutinib was associated with a higher reporting of ventricular arrhythmias (odds ratio 4.7; 95% confidence interval, 3.7-5.9).[48] An increased rate of sudden death was observed in pooled data from ~1,000 patients participating in randomized trials of ibrutinib, with 788 events found per 100,000 person-years, more than the 200 to 400 events seen in 65-year-olds in the general population.[47] QT_c prolongation does not appear to be a likely contributor, because ibrutinib has been associated with a reduced QT duration.[48] For now, clinicians should maintain a high index of suspicion when encountering cardiac symptoms (eg, syncope, palpitations, light-headedness) in patients on all BTKis.

Bleeding risk

Ibrutinib is associated predominantly with minor bleeding (grade ≤2, low-grade ecchymoses and petechiae) in up to two-thirds of patients. Major bleeding (grade ≥3, necessitating transfusion or hospitalization) occurs less frequently in 2% to 9% of patients.[4,5,7] An integrated analysis of 15 ibrutinib studies (n = 1,768 patients) demonstrated a higher proportion of major bleeding on ibrutinib compared with comparator therapy (4.4% vs 2.8%). Nevertheless, this difference did not persist after adjustment for more prolonged exposure with ibrutinib (incidence of 3.2 vs 3.1 per 1,000 person-months).[38] Interestingly, a higher risk of major bleeding was observed with the use of anticoagulants or antiplatelet agents in both the ibrutinib-treated and comparator populations. In contrast with ibrutinib, in the ELEVATE-TN trial, only 2% major bleeding was seen on acalabrutinib monotherapy, with minor bleeding observed in 37% of patients.[10] In the relapsed setting, major bleeding was observed in 5% of patients in long-term follow-up of phase 2 data and 1% of patients in ASCEND.[11]

Although the mechanism of pathogenesis is complex and incompletely understood, both on- and off-target kinase

inhibition are implicated. In platelets, BTK and other related Tec family kinases play an important role in platelet aggregation mediated via the collagen receptor glycoprotein VI. Patients with CLL not on BTKi therapy exhibit less robust platelet aggregation than healthy controls[45,49,50] and greater impairment in collagen-induced aggregation responses on ibrutinib.[45,49] More recent work has also implicated ibrutinib-mediated shedding of GPIb-IX and integrin αIIbβ3.[51]

Our approach to managing bleeding risk on BTKis is as follows. Bruising, commonly seen with BTKis, does not confer an increased risk of major hemorrhage and does not warrant cessation of therapy. BTKis should be held for either 3 days (minor procedure) or 7 days (major procedure) both before and after invasive procedures because of the increased periprocedural bleeding risk. When possible, we recommend completing necessary surgical procedures before starting therapy. For patients who have already been on long-term ibrutinib, there is evidence that interruptions to therapy are less impactful later in the treatment course. For minor bleeding, holding ibrutinib results in the resolution of bleeding tendency in 2 to 3 days. For severe bleeds, we advocate providing platelet transfusions as appropriate to overcome clinical bleeding, regardless of platelet count. When considering anticoagulation, we follow the strategy outlined in the section on AF, above. We also consider alternative CLL therapy, if available, for patients needing dual antiplatelet therapy. Although second- and third-generation agents may ultimately confer less risk for all bleeding-related AEs, we currently manage bleeding risk agnostic to the particular BTKi agent used.

Infection

Patients receiving BTK inhibitors are immunocompromised and are at risk of infectious complications despite receiving effective therapy. Infection (of any grade) occurs in >50% of patients on BTKis (Table 3), particularly during the early period after starting treatment, and R/R patients are at greater risk. In real-world analysis, 11% of R/R patients needed treatment discontinuation because of infectious complications, with a median time to therapy cessation of 6 months.[21] Of all infectious complications, pneumonia was the most common, observed in an integrated analysis of landmark ibrutinib studies in 12% of patients (grade ≥3 infection).[6]

These data suggest that in many instances, infectious complications seen on BTKis may be largely attributable to the biology of the disease itself. Yet aside from CLL-related immune dysfunction, changes on BTKi therapy, including inhibition of ITK[52] and impairment of macrophages,[53,54] have also been implicated in the pathogenesis of susceptibility to infection. Ibrutinib has also been associated with multiple functional defects in neutrophil function (decreased reactive oxygen species production).[55] In a recent cross-trial comparison, similar immunologic changes during BTK inhibition were observed with both ibrutinib and acalabrutinib; both were associated with a sustained increase in serum immunoglobulin A, with patients exhibiting greater levels of this immunoglobulin at lower risk of infectious complications.[56]

Recent series have also highlighted the prevalence of opportunistic infections, including *Aspergillus fumigatus* and *Pneumocystis jirovecii* (PJP), in patients with CLL on BTKis.[57-60] A single-institution report of 566 patients (44.9% of whom received prophylaxis) documented no cases of PJP.[57] Another center reported no PJP infections in 130 patients receiving prophylaxis, with a 2.4% prevalence of PJP in 85 patients receiving BTKi monotherapy without prophylaxis.[58] In view of limited data, at present, current international workshop on CLL guidelines do not specifically address the role of PJP prophylaxis,[61] and practices vary across institutions. In our practice, PJP prophylaxis should be considered in patients deemed at high risk of infection (eg, R/R or heavily pretreated patients) or patients with a known prior history of infection. Troublingly, *Aspergillus fumigatus* infection has been reported at a higher-than-expected rate in patients on BTKis.[57,59,62] Data suggest that the risk of aspergillosis is highest early in the treatment course[57] and is higher among patients receiving corticosteroids.[60]

When an infection occurs, management begins with a complete workup, with an appropriate index of suspicion for opportunistic infections. During acute infection, some experts advocate holding kinase inhibitors until a definitive diagnosis is determined,[43] whereas others hold specifically in the case of grade 4 infection.[44] Regardless of approach, pharmacotherapy can be reinitiated after the start of clinical improvement when deemed appropriate by the caring provider. Particular attention should be paid to drug interactions (eg, with CYP3A4 inhibitors voriconazole or posaconazole).

We favor the administration of indicated vaccines (eg, against influenza and pneumococcus) before treatment initiation. In addition, based on preliminary data, we also consider recombinant, adjuvanted varicella-zoster virus vaccine.[63] Additionally, we reserve intravenous immunoglobulin therapy for patients with recurrent infections and known hypogammaglobulinemia.

Finally, at the time of this writing, preliminary data are emerging regarding the use of BTKis in the setting of coronavirus disease 2019 (COVID-19). There is concern that patients with CLL may be at particularly high risk of infection or poor outcome given their average age and comorbidities and the profound immune changes seen with this disease. To this end, early in the pandemic professional societies (based on expert consensus) recommended limiting their exposure to the health care system and postponing the initiation of treatment where feasible. An international case series of patients with symptomatic COVID-19 (n = 198) revealed high mortality rates in both watch-and-wait and treated patients with CLL during hospital admission.[64] Importantly, in this study, therapy with BTKi at the time of hospitalization did not affect the overall case fatality rate. Because this is a rapidly developing story with more data forthcoming, we point the reader toward the latest international guidelines[65,66] for the most up-to-date consensus practices regarding CLL, BTK inhibition, and COVID-19.

Other adverse events

Hypertension

The most extended follow-up data currently available for ibrutinib (PCYC-1102) report grade ≥3 hypertension (≥160/100 mm Hg) in 28% of patients.[3] Multiple series have documented that the prevalence of hypertension (any grade) increases over time (eg, 11% year 1, 15% year 2; 20% year 3 in a pooled analysis). Strikingly, in a series of 562 patients with lymphoid malignancies treated on ibrutinib, the incidence of new hypertension was 71.6%, 13 times higher than in a comparable Framingham cohort. In this analysis, 80% of patients exhibited a 10–mm Hg increase from baseline, and more than 10% of patients demonstrated an

increase of ≥50 mm Hg.[67] By comparison, at a median follow-up of 53 months in ACE-CL-001, the rate of grade ≥3 hypertension on acalabrutinib was 11%, with minimal late increases in incidence.[68] Accordingly it remains unclear whether hypertension is a broad class effect of BTKi inhibition. Proposed mechanisms include PI3k/Akt inhibition, leading to downregulation of PI3K-p110alpha and downregulation of nitrous oxide production.[42,67] Management involves judicious optimization of baseline hypertension before treatment initiation, regular monitoring of blood pressure at clinic visits, and appropriate medical therapy for incident hypertension in collaboration with the patient's primary care provider.

Diarrhea

Diarrhea is the most commonly observed AE on BTKi therapy, occurring early in treatment (before month 6) with a predominantly self-limited course.[4,8,10] Although the prevailing view is that ibrutinib-related diarrhea is probably EGFR mediated, rates of diarrhea seen on acalabrutinib are similar to those with ibrutinib[11,24] (despite differences in EGFR binding). It is our practice to manage most BTKi-related diarrhea with supportive care, antimotility agents, and nighttime dosing of drug (in the case of ibrutinib) to mitigate symptoms. We also consider temporary drug holds in the case of grade ≥3 diarrhea.

Fatigue, arthralgia, and myalgia

Fatigue is a commonly reported symptom seen early in the course of BTK inhibition and is usually self-limited. It has been reported in 36% of patients on ibrutinib (in a pooled analysis, 0% to 3% grade 3)[6] and in 28% to 34% of patients on acalabrutinib (0% to 2% grade 3).[10,11,24] Given the propensity for preexisting disease-related fatigue and the close temporal link between symptom onset and treatment start, we do not typically advocate dose interruptions on account of this toxicity, particularly if it occurs early after initiation of therapy. If it persists or occurs significantly later during the course of therapy, then an evaluation to search for other potential causes of fatigue should be undertaken, and a drug holiday or dosage reduction should be considered to assess whether it is drug related.

Arthralgia and myalgia are seen in 11% to 36% of patients on ibrutinib, with higher rates compared with comparators in a pooled analysis.[4,5,38,69] Patients describe a dynamic pattern of migratory arthralgias that can be debilitating; arthralgias were associated with 42% of ibrutinib discontinuations in real-world data.[21] A recent case series focusing on this toxicity noted that the majority of arthralgias developed at 7 months after treatment initiation, and risk factors included female sex and treatment-naive status.[69] After ruling out other possible etiologies of arthralgia, these authors recommended observation for grade 1 to 2 arthralgia, with dosage reduction considered when symptoms affect activities of daily living (ADLs). Dose holds were recommended for grade ≥3 AEs (affecting self-care ADLs), with rechallenge at lower dosages if symptoms resolve. Evidence for specific pharmacotherapy has not been studied formally but is anecdotal, and approaches include magnesium supplementation and quinine-containing tonic water. Severe arthralgias demonstrate a variable response to short-course steroids (we recommend an abbreviated course at low dosages given the increased risk for fungal infection) and anti-inflammatory agents (which must be used cautiously given the potentially higher risk of bleeding on BTKis).

Cytopenias

CLL is associated with autoimmune cytopenias (AICs) which occur in ~4% to 10% of patients; autoimmune hemolytic anemia is most common (~7%), followed by immune thrombocytopenia (<1% to 2%), with pure red cell aplasia occurring less frequently.[70] Most AICs are thought to be caused by a variety of complex mechanisms of immune dysregulation that are a consequence of CLL itself. BTKis are known to decrease the need for immunosuppressive therapy in the setting of preexisting autoimmune cytopenias,[71] and true flare or de novo treatment-emergent AICs are uncommon in both the clinical trial and real-world settings. A case series of patients on clinical trials of ibrutinib at 2 major institutions reported 13 total patients who had AICs at treatment start, with the majority (9 patients) continuing on the drug despite experiencing an initial flare.[72] Similarly, in real-world data from the Mayo Clinic, of 161 patients, only 11 (6%) developed treatment-emergent AICs, occurring a median of 59 days after therapy. Of these, 7 patients were able to continue therapy after temporary dose hold or reduction.[73] In this literature, authors report benefit from early use of additional immunosuppressive therapies, which can often be discontinued upon count recovery. It is our practice to treat treatment-emergent AIC flare with the addition of short-course corticosteroids or anti-CD20 monoclonal antibody treatment and then resume treatment with BTKis. Other cytopenias including neutropenia can be seen[74] but rarely necessitate dose interruption or discontinuation, and in our experience, when encountered it usually responds to growth factor support.

Dermatologic manifestations

In addition to the nonpalpable asymptomatic petechial rash thought to result from ibrutinib-induced platelet dysfunction, at least two other forms of skin manifestations of BTKi therapy have been characterized: a palpable rash that is often pruritic, associated with EGFR inhibition and infiltration of inflammatory cells; and erythema nodosum.[75,76] Both manifestations are often responsive to corticosteroids or dose holds. Textural changes in hair and nails are also observed. An early series from the National Institutes of Health recognized a higher incidence of brittle fingernails or toenails with the formation of vertical nail ridges in two-thirds of patients treated with ibrutinib.[77] These manifestations appear gradually, with a median reported onset of 9 months, and do not represent a dose-limiting toxicity. They can be treated with biotin supplementation and the application of nail oil.

Headache

Headache is seen uniquely with the second-generation inhibitor acalabrutinib. In pivotal studies, nearly 70% of patients experienced grade 1 or 2 headaches, most often during the first cycles (particularly weeks 1 to 3).[10,11] Headache has been reported to resolve with extended treatment, and the administration of acetaminophen with or without caffeine is often effective for symptomatic relief.

The patient demonstrated clinical improvement with ibrutinib therapy but developed impairment in his ADLs later in the course of treatment. His dosage was reduced initially, and magnesium and quinine were tried, but he had persistent arthralgias that did not improve. His ibrutinib was discontinued, and changing therapy to an alternative BTKi or initiating therapy with a venetoclax-based regimen was discussed. His arthralgias

resolved off therapy, and he subsequently began treatment with acalabrutinib without recurrence of arthralgias.

Additional considerations

Impact of dose modification and temporary interruption of therapy on clinical outcomes

The influence of dose modification and temporary interruption on clinical outcomes remains an active area of interest in CLL. Biologically, BTK target occupancy, a pharmacodynamic measure of covalent binding, is associated with the degree of CLL tumor cell inhibition.[24] Although BTK target occupancy is frequently measured in the setting of clinical trials, no optimal absolute threshold has emerged, and measurement outside this setting is not routine. Several analyses have looked at the impact of dose interruptions and reductions on clinical outcomes. In an analysis of the RESONATE data, higher dose intensity during the first 8 weeks of therapy was associated with longer median progression-free survival (NR vs 6.9 months), and dose interruptions of ≥8 consecutive days (reported with 9 months of follow-up) were associated with a shorter median progression-free survival (10.8 months vs NR).[78] Host-related factors may be a confounder here, because in other analyses patients with early alterations tended to have poor performance status and had a higher likelihood of permanent early discontinuation.[79,80] The timing of these events also probably plays an important role. For example, a clinically indicated reduction in starting dose (eg, patients receiving concurrent strong or moderate CYP3A inhibitors should have their ibrutinib starting dose reduced to 140 mg or 280 mg, respectively) does not appear to be associated with differences in event-free survival or overall survival.[81]

In general, given that many early secondary effects are likely to decrease in severity with time, we recommend a strategy where low-grade toxicities are treated with expectant management and supportive care when possible. It is our practice to try to avoid dose reductions or cessation of therapy within the first 6 months of starting treatment. However, evidence suggests that clinically indicated dose interruptions (eg, to mitigate significant adverse events grade ≥3) are unlikely to facilitate the emergence of drug-resistant clones and do not appear to compromise long-term clinical outcomes.[80] For this reason, in this setting we do not hesitate to hold drugs where appropriate.

Sequential therapy and toxicity of emerging second- and third-generation BTKis

As data continue to accumulate on the use of novel BTKIs, we will gain greater insight into the extent to which classic BTK-associated toxicities are influenced by kinase binding properties. Idiosyncratic drug-specific toxicities (eg, acalabrutinib headache) may continue to appear with newer inhibitors. These agents may ultimately prove to be an attractive option for patients intolerant to ibrutinib because of off-target toxicities. Although current data are limited, in a nonrandomized study of patients (n = 33) receiving acalabrutinib after discontinuing ibrutinib due to "intolerance," of 61 AEs, 72% of ibrutinib-related AEs did not occur during acalabrutinib treatment, suggesting continued disease control with improved tolerability.[82] Randomized data evaluating the impact of restarting ibrutinib (at dose reduction) compared with switching to acalabrutinib are not currently available. Studies of newer agents in previously BTKi-intolerant patients (eg, zanubrutinib NCT04116437) are ongoing, as are head-to-head phase 3 trials (eg, ELEVATE-RR, NCT02477696, and ALPINE NCT03734016), which will provide us with direct comparison data.

Conclusion

A thorough clinical history encompassing patient-specific comorbidities is of the utmost importance when therapy is considered for patients with CLL. Patients should be appropriately informed about the risk of adverse effects when initiating therapy. Patients with arrhythmias or poorly controlled hypertension will probably do better with other treatment modalities (eg, BCL2 inhibition). As data continue to emerge on the use of BTKi in combination with other therapeutic agents, opportunities to treat patients with CLL with a fixed duration of treatment rather than indefinite therapy may reduce the potential for longer-term toxicities. Data from ongoing phase 3 trials evaluating BTKis in combination with other agents will help discern the potential for overlapping toxicities and better define the role of combinations in the management of CLL. Although there may be some reason to prefer later-generation tyrosine kinase inhibitors (particularly for patients at higher risk of cardiovascular AEs), inference from cross-trial comparisons is inherently limited. Ongoing randomized clinical trial data will provide a more direct comparison to assess for advantages in the toxicity profile of one agent over another. Given the impressive efficacy and activity of BTKis in the treatment of upfront and R/R CLL, it is vital that caregivers become as familiar as possible with the management of BTKi-emergent toxicities, because this class will probably remain a mainstay of treatment either as monotherapy or in combination with other agents for quite some time.

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None disclosed.

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Managing toxicities of phosphatidylinositol-3-kinase (PI3K) inhibitors

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Despite the proven effective approach to targeting the phosphatidylinositol-3-kinase (PI3K) pathway in B-cell malignancies, the approved PI3K inhibitors idelalisib and duvelisib have been less commonly selected for patients with chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), given the availability of other more tolerable agents. However, patients with CLL/SLL can experience a disease course that is multiply relapsed, refractory, or intolerant to treatment, and PI3K inhibitors can achieve meaningful responses. This article reviews the common early- and late-onset (considered immune-mediated) toxicities with PI3K inhibitors, including infections, hepatotoxicity, diarrhea and/or colitis, and pneumonitis. Data on pretreatment considerations, toxicity management, and drug rechallenge are presented. In addition, next-generation PI3K inhibitors and novel treatment approaches with PI3K inhibitors, including combinations, time-limited treatments, and intermittent dosing, are highlighted.

LEARNING OBJECTIVES

- Identify and manage common early and late onset toxicities that can occur in treatment of patients with CLL/SLL on PI3K inhibitors
- Discuss next generation PI3K inhibitors and other novel treatment approaches with PI3K Inhibitors in CLL/SLL

Clinical case

A 72-year-old man with chronic kidney disease (creatinine, 2.2 mg/dL) was referred for relapsed, high-risk chronic lymphocytic leukemia (CLL). He had been diagnosed with CLL 4 years prior (normal fluorescence in situ hybridization findings; *IGHV* mutational analysis not performed) and had received frontline treatment with bendamustine plus rituximab (BR). Eighteen months after BR, he had symptomatic relapse of his disease (52% deletion of 17p by fluorescence in situ hybridization; *IGHV* unmutated) and was started on ibrutinib with improved lymphadenopathy. After 5 months, he presented with a severe headache and was found to have a subdural hematoma; his platelet count at the time was 120 × 10^9 /L, and he denied receiving anticoagulation or other antiplatelet agents. He recovered fully and was followed off therapy for 6 months until progression with symptomatic bulky lymphadenopathy occurred. He declined participation in clinical trials, and, with lack of support to complete venetoclax dose escalation, he elected to start idelalisib plus rituximab. He has returned for a scheduled visit 6 weeks after idelalisib initiation. Upon examination, he is well appearing with reduced lymphadenopathy. His complete blood count demonstrates improvement in his hemoglobin and platelets, and he is not neutropenic. His creatinine is stable (2.0 mg/dL), and his alkaline phosphatase and bilirubin are within normal limits, but his aspartate aminotransferase (AST) is increased to 410 IU/L, and his alanine aminotransferase (ALT) is 520 IU/L.

Introduction

The phosphatidylinositol-3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway is a well-recognized biologic target in malignancy governing key oncogenic processes such as survival, proliferation, and migration.[1,2] Class I PI3Ks are activated by surface receptor tyrosine kinases, including the B-cell receptor (BCR) and chemokine receptors (CXCR4, CXCR5), implicated in the pathobiology of CLL/small lymphocytic lymphoma (SLL). There are 4 isoforms of class I PI3Ks according to the catalytic domain: α (p110α/PI3Kα), β (p110β/PI3Kβ), δ (p110δ/PI3Kδ), and γ (p110γ/PI3Kγ). The isoforms are differentially expressed, with γ and δ dominant in hematopoietic cells; PI3Kδ mediates BCR-driven proliferation and chemotaxis, and PI3Kγ is important in diverse immune processes, including T-cell function. Therefore, in addition to direct antitumor effects

Table 1. Efficacy in select phases 2 and 3 clinical trials of idelalisib and duvelisib in CLL/SLL

Trial	Phase 3, idelalisib + rituximab vs rituximab in R/R CLL[8,9]	Phase 3, idelalisib vs placebo + BR in R/R CLL[14]	Phase 3, idelalisib + ofatumumab vs ofatumumab in previously treated CLL[13]	Phase 3, acalabrutinib vs investigator's choice (BR or idelalisib + rituximab) in R/R CLL[10]	Phase 2, treatment-naïve older patients with CLL, idelalisib + rituximab[11]	Phase 2, treatment-naïve CLL with idelalisib + ofatumumab[12]	Phase 2, DYNAMO trial, double-refractory FL, SLL, MZL[22]	Phase 3, DUO trial, duvelisib vs ofatumumab in R/R CLL/SLL[21]
Population	R/R CLL	R/R CLL	R/R CLL	R/R CLL	Treatment-naïve older patients with CLL/SLL	Treatment-naïve patients with CLL	iNHL (FL, SLL, or MZL) double-refractory to rituximab + chemoimmunotherapy or radioimmunotherapy	R/R CLL/SLL
Treatment	Idelalisib 150 mg by mouth twice daily plus rituximab IV 375 mg/m^2 in week 0, day 1, and 500 mg/m^2 day 1 of weeks 2, 4, 6, 8, 12, 16, and 20 vs rituximab + placebo	Bendamustine 70 mg/m^2 IV on days 1 and 2 C1-6 plus rituximab: 375 mg/m^2 on C1D1, and 500 mg/m^2 D1C2–6 plus idelalisib 150 mg twice daily vs BR plus placebo	Ofatumumab 300 mg in week 1, day 1 followed by 2000 mg weekly for 7 wk, then every 4 wk for 16 wk vs idelalisib 150 mg by mouth twice daily plus ofatumumab on same week schedule as control group but at 1000 mg from week 2	Acalabrutinib 100 mg by mouth twice daily vs investigator's choice of idelalisib 150 mg by mouth twice daily plus rituximab 375 mg/m^2 on C1D1 and 500 mg/m^2 D1C2–6 or bendamustine 70 mg/m^2 IV on days 1 and 2 C1-6 plus rituximab	Idelalisib 150 mg by mouth twice daily plus rituximab 375 mg/m^2 IV weekly for 8 wk	Idelalisib 150 mg by mouth twice daily plus ofatumumab 300 mg C3D1 followed by 1000 mg weekly for 7 wk, then 100 mg every 4 wk for 16 wk (6 mo total)	Duvelisib 25 mg by mouth twice daily	Duvelisib 25 mg twice daily or ofatumumab IV for up to 12 doses
Number of patients	220	416	261	398	64	27	129	159
Primary endpoint	PFS	PFS	PFS	PFS	ORR	ORR	ORR	PFS
mPFS	Not reached at 12 mo for idelalisib + R vs 5.5 mo in placebo arm; P < .001; 20.3 mo (17.3—26.3 mo) at 18-mo follow-up	20.8 mo (16.6-26.4) for idelalisib arm vs 11.1 mo (8.9-11.1) in placebo arm (P < .0001) at 14-mo follow-up	16.3 mo (13.6-17.8) in idelalisib plus ofatumumab arm vs 8.0 mo (5.7-8.2) with ofatumumab (P < .0001)	Acalabrutinib monotherapy (PFS NR) vs investigator's choice (16.5 mo; hazard ratio, 0.31; P < .0001) at 16.1-mo follow-up	mPFS was not reached; PFS at 12, 18, and 24 mo was 92.9%, at 36 mo was 82% (64%-92%)	23 mo (18-36)	9.5 mo (8.1-11.8)	13.3 mo in duvelisib arm vs 9.9 mo in ofatumumab arm (P < .0001)
ORR (CRs) in PI3Ki arm	85.5% (1 patient with CR)	70% (1%)	75.3% (1 patient; <1%)	NR separately for idela + R vs BR in investigator's choice arm	96.9% (14.1%)	88.9% (1 patient with CR)	47.3% (1.6%) entire population SLL, 67.9% FL, 42.2%; MZL: 38.9%	73.8% (1 patient with CR)
OS	mOS was 40.6 mo (28.5-57.3) vs 34.6 mo (16.0-NR)	Not adequately powered to show OS benefit	mOS not reached and not different from control	mOS not reached	mOS not reached; at 36 mo, was 90% (82%-99%)	mOS not reached; at 36 mo, was 88% (68%-96%)	mOS was 28.9 mo (21.4-NE); 1-y OS estimate of 77%	mOS not reached in either arm, with 12-mo OS of 86% (0.65-1.50) for both treatment arms

C3D1, cycle 3, day 1; C1-6, cycles 1-6; C1D1, cycle 1, day 1; CR, complete response; D1C2-6, day 1 cycles 2-6; FL, follicular lymphoma; iNHL, indolent non-hodgkin lymphoma; mPFS, median progression free survival; mOS, median overall survival; MZL, marginal zone lymphoma; NR, not reached.

Table 2. **AEs in idelalisib and duvelisib clinical trials for CLL/SLL and indolent lymphomas**

	Phase 1, R/R CLL, single-agent idelalisib[7]	Phase 1, R/R MCL[44]	Phase 1, R/R MCL and follicular lymphoma, idelalisib, lenalidomide, rituximab[16]	Phase 1, R/R indolent lymphoma, single-agent idelalisib[45]	Phase 2, R/R classical Hodgkin lymphoma, single-agent idelalisib[46]	Phase 2, treatment-naïve older patients with CLL, idelalisib + rituximab[11]
Number of patients	54	40	11	64	25	64
AEs						
Colitis/diarrhea	29.6% Grade ≥3, 5.6%	40% Grade ≥3, 17.5%	38%	36% Grade ≥3, 9.4%	4%	64% Grade 3, ≥42%
Hepatotoxicity	33-18% Grade ≥3, ~2%	60% Grade ≥3, 20%	63% Grade ≥3, 18%	53% Grade ≥3, 23%	Grade ≥3, 16%	67% Grade ≥3, 23%
Infections	44% Grade ≥3, 20%	32.5% Grade ≥3, 10%	25%, All grade ≥3	36% Grade ≥3, 17.2%	16%	44% Grade ≥3, 25%
Cutaneous reactions	22%	22.5% Grade ≥3, 2.5%	63% Grade ≥3, 54%	25% Grade ≥3, 3.1%	8%	58% Grade ≥3, 13%
Pneumonitis					4%	3%, All grade ≥3
Dose reductions					36%	45%
Drug discontinuation due to AEs	13%	18%	73%	21%	8%	29.7%
	Phase 2, double-refractory indolent lymphoma, single-agent idelalisib[47]	**Phase 2, idelalisib + entospletinib in R/R CLL and NHL[17]**	**Phase 2, treatment-naïve CLL with idelalisib + ofatumumab[12]**	**Phase 3, idelalisib + rituximab vs rituximab in R/R CLL[8]**	**Phase 3, idelalisib vs placebo + BR in R/R CLL[14]**	**Phase 3: idelalisib + ofatumumab in previously treated CLL[13]**
Number of patients	125	66	24	220	416	261
AEs						
Colitis/diarrhea	43% Grade ≥3, 16%	29% Grade ≥3, 2%	46% Grade ≥3, 17%	19% Grade ≥3, 4%*	38% Grade ≥3, 9%	54% Grade ≥3, 23%
Hepatotoxicity	47% Grade ≥3, 13%	23%	79% Grade ≥3, 54%	35% Grade ≥3, 5%*	61% Grade ≥3, 21%	20% Grade ≥3, 13%
Infections	25% Grade ≥3, 7%	~18%		Grade ≥3, 13%*	32% Grade ≥3, 12.5%-16%	78% Grade ≥3, 22%
Cutaneous reactions	13% Grade ≥3, 2%	30% Grade ≥3, ~17%		10% Grade ≥3, 3%*	16% Grade ≥3, 3%	18% Grade ≥3, 1%
Pneumonitis		17%; study terminated early	13%	4%*	1.4%	6% Grade ≥3, 5%
Dose reductions	34%				13%	58%
Drug discontinuation due to AEs	20%		Study terminated early due to AEs	5%	27%	39%
	Phase 1, duvelisib monotherapy, R/R CLL[48]	**Phase 1, duvelisib monotherapy, treatment-naïve CLL[48]**	**Phase 1, duvelisib monotherapy, R/R CLL, iNHL, TCL[49]**	**Phase 1, duvelisib monotherapy, R/R TCL[50]**	**Phase 1, duvelisib monotherapy, R/R iNHL[51]**	**Phase 1, duvelisib + rituximab vs BR, CLL or iNHL[52]**
Number of patients	55	18	210	35	31	46
AEs						
Colitis/diarrhea	47.3% Grade ≥3, 9.1%	77.8% Grade ≥3, 22.2%	41.9% Grade ≥3, 11.4%	31%	54.8% Grade ≥3, 25.8%	37% Grade ≥3, 13%

MCL, mantle cell lymphoma; TCL, T-cell lymphoma.
*In longer follow-up (median, 18 mo),[9] prolonged exposure saw increases in any grade, grade 2, and grade ≥3 diarrhea (46.4%, 17.3%, and 16.4%, respectively); any grade and grade ≥3 colitis (10.9% and 8.2%, respectively); and any grade and grade ≥ 3 pneumonitis, respectively (10.0% and 6.4%). The incidence of elevated hepatic aminotransferases did not increase with time.

Table 2. (Continued)

	Phase 1, R/R CLL, single-agent idelalisib[7]	Phase 1, R/R MCL[44]	Phase 1, R/R MCL and follicular lymphoma, idelalisib, lenalidomide, rituximab[16]	Phase 1, R/R indolent lymphoma, single-agent idelalisib[45]	Phase 2, R/R classical Hodgkin lymphoma, single-agent idelalisib[46]	Phase 2, treatment-naïve older patients with CLL, idelalisib + rituximab[11]
Hepatotoxicity	30.9% Grade ≥3, 10.9%	33.3% Grade ≥3, 16.7%	38.6% Grade ≥3, 19.5%	57% Grade ≥3, 40%	58.1% Grade ≥3, 38.7%	21.7% Grade ≥3, 6.5%
Infections	>62% Grade ≥3, >23.6%	22%	29.5% Grade ≥3, 105	23% Grade ≥3, 17%	19.4%	34.7% Grade ≥3, 6.5%
Cutaneous reactions	18.2%	38.9% Grade ≥3, 5.6%	16.2% Grade ≥3, 5.2%	23% Grade ≥3, 17%	42% Grade ≥3, 6.5%	41.3% Grade ≥3, 19.6%
Pneumonitis	7%	11%	4%		6.5%	
Dose reductions						
Drug discontinuation due to AEs	36%	33%	~30%	37%	19%	23.9%
	Phase 2, DYNAMO trial, double-refractory FL, SLL, MZL[22]	**Phase 3 DUO trial, duvelisib vs ofatumumab, R/R CLL/SLL[21]**				
Number of patients	129	319				
AEs						
Colitis/diarrhea	56.6% Grade ≥3, 20.1	64% Grade ≥3, 27%				
Hepatotoxicity	14% Grade ≥3, 5.4%	Grade ≥3, 3%				
Infections	7.8% Grade ≥3, 5.4%	48% Grade ≥3				
Cutaneous reactions	18.6% Grade ≥3, 4.7%	10% Grade ≥3, 2%				
Pneumonitis	4.7%	3%				
Dose reductions	66%					
Drug discontinuation due to AEs	24%	35%				

MCL, mantle cell lymphoma; TCL, T-cell lymphoma.
*In longer follow-up (median, 18 mo),[9] prolonged exposure saw increases in any grade, grade 2, and grade ≥3 diarrhea (46.4%, 17.3%, and 16.4%, respectively); any grade and grade ≥3 colitis (10.9% and 8.2%, respectively); and any grade and grade ≥ 3 pneumonitis, respectively (10.0% and 6.4%). The incidence of elevated hepatic aminotransferases did not increase with time.

from inhibition of PI3K isoforms in CLL cells, inhibition appears to exert antitumor activity indirectly through interruptions within the CLL microenvironment.[3-5]

Idelalisib (formerly GS1101, CAL101) is a first-in-class oral PI3Kδ-specific inhibitor that demonstrated promising clinical efficacy in very high-risk relapsed/refractory (R/R) CLL/SLL. In 2014, idelalisib was approved with rituximab in patients with R/R CLL for whom rituximab monotherapy would be appropriate and in patients with SLL receiving ≥2 prior therapies. Duvelisib (formerly IPI-145), a dual oral PI3Kγ/δ inhibitor, was approved in 2018 for patients with R/R CLL/SLL receiving ≥2 prior therapies. Despite early efficacy results, PI3K inhibitor (PI3Ki) use has been limited largely by toxicities that can lead to permanent discontinuation and the availability of other drugs with tolerability that is more favorable.[6] Like many of the BCR pathway tyrosine kinase inhibitors, the ability to apply continuous administration of the PI3Ki, especially early in the treatment course, is necessary for long-term disease control.

In the frontline and R/R settings, randomized studies have demonstrated superiority of Bruton tyrosine kinase (BTK) inhibitors (BTKis; ibrutinib, acalabrutinib) and venetoclax (a BCL-2 inhibitor) over chemoimmunotherapy, with rapid incorporation into practice. However, CLL/SLL remains an incurable disease for most patients, leaving an unmet need. Given the effectiveness of targeting the PI3K pathway, efforts remain underway to consider best use of current agents and next-generation PI3Kis and/or alternative dosing regimens. Early recognition and

treatment of PI3Ki toxicities is imperative for safe and effective treatment with these agents.

Clinical efficacy and safety: idelalisib and duvelisib

The recommended 150-mg twice-daily dosing of idelalisib is derived from observations of a dose plateau in the pharmacodynamic target (pAkt) and treatment response, combined with recognition of grade 3+ treatment-emergent adverse events, including diarrhea/colitis, transaminitis, and pneumonitis.[7] Table 1 summarizes the efficacy results of approved PI3Kis idelalisib and duvelisib in phase 2 and 3 trials for CLL/SLL. The pivotal idelalisib phase 3, double-blind, placebo-controlled trial in R/R CLL randomized 220 patients in whom rituximab monotherapy would have been appropriate to receive rituximab plus idelalisib (idela + R) or rituximab (R) plus placebo. The primary endpoint of progression-free survival (PFS) was met (not reached [NR]) at 12 months for the idelalisib regimen vs 5.5 months in the placebo arm ($P < .001$), and the study was stopped early due to significant efficacy.[8] At a median of 18 months of follow-up, the benefit still held for patients treated with idela + R (followed by idelalisib monotherapy) with a median PFS of 20.3 months (95% confidence interval, 17.3-26.3); the median overall survival in the idela + R arm was 40.6 months (95% confidence interval, 28.5-57.3 months) vs 34.6 months (95% confidence interval, 16.0-NR) in the placebo group.[9]

In the first reporting, adverse events (AEs) were similar between the idelalisib- and placebo-treated patients, with a 40% incidence of serious AEs (vs 35% in the placebo group) and grade ≥3 AEs occurring in 56% (vs 48%), with only 9 patients (8%) receiving idelalisib discontinuing due to toxicity. However, initial follow-up was short (3.8 months, with only 35% of patients receiving idelalisib >6 months), and at 18-month follow-up, the longer exposure increased the incidence of all-grade and grade ≥3 diarrhea/colitis and pneumonitis, highlighting the risk for early- and late-onset toxicities with PI3Kis.

Initial studies in R/R CLL (Table 1) compared the PI3Kis with chemotherapy or anti-CD20 antibody, considered standard of care at the time; currently patients with R/R CLL have multiple targeted novel therapeutic options, including BTKis (ibrutinib, acalabrutinib). The recently reported ASCEND trial randomized patients with R/R CLL to acalabrutinib or investigator's choice (idela + R or BR; 77% received idela + R) and demonstrated significantly longer PFS at a median follow-up of 16.1 months with acalabrutinib monotherapy (PFS NR) vs investigator's choice (16.5 months; hazard ratio, 0.31; $P < .0001$).[10] Because the overall response rate (ORR) was similar between the acalabrutinib and investigator's choice arms, toxicity and early drug discontinuation may have contributed to the improved PFS with acalabrutinib; AEs led to discontinuation less frequently with acalabrutinib (11%) than with idela + R (47%), and at the time of the data cutoff, 80% continued on acalabrutinib vs only 32% remaining on idelalisib.

In the first frontline idelalisib study, previously untreated patients ≥65 years old (n = 64) received idela + R with an encouraging ORR (97%; 19% complete response), including in patients with high-risk *TP53* aberrations (n = 9; ORR, 100%; 33% complete response).[11] In treatment-naïve patients receiving idela + R, however, AEs were much higher than in patients with R/R disease.[9] In frontline treatment, AST and/or ALT elevations were observed in 67% of all-grade AEs (23% grade ≥3) vs AST elevations in patients with R/R disease in 36% of all-grade AEs (6% grade ≥3) or ALT elevations in patients with R/R disease in 46% of all-grade AEs (9% grade ≥3). Diarrhea and/or colitis in frontline treatment were observed in 64% of all-grade AEs (42% grade ≥ 3) vs diarrhea in patients with R/R disease of 46% of all-grade (16% grade ≥3) or colitis in patients with R/R disease of 11% of all-grade (8% grade ≥3). Similar higher serious AEs were seen in the frontline treatment in the phase 2 idelalisib + ofatumumab[12] trial, which led to early closure due to hepatotoxicity (79% with AST/ALT elevations and 54% grade ≥3 AEs).

Toxicities of idelalisib across trials for CLL and other non-Hodgkin lymphomas are detailed in Table 2. In the phases 2 and 3 trials of idelalisib in CLL, grade ≥3 hepatotoxicity occurred in 2% to 54% of patients, grade 2 or higher diarrhea occurred in 5% to 42% of patients, and pneumonitis occurred in 1% to 17% of patients; these were more frequent for idelalisib in the frontline treatment or in combination with drugs besides anti-CD20 immunotherapy.[8,11,13-17] In a phase 2 trial of idelalisib and the Syk inhibitor entospletinib for R/R CLL and non-Hodgkin lymphoma, grade ≥3 or higher pneumonitis occurred in 17% of patients (5 patients [8%] required mechanical ventilation and 2 patients died). The combination of idelalisib with lenalidomide appeared especially toxic in 2 Alliance trials for relapsed mantle cell lymphoma and follicular lymphoma, both of which closed early.[16]

Toxicities from idelalisib may be higher for patients treated outside of clinical trials. A cohort study compared outcomes of Medicare beneficiaries aged ≥65 years treated with idelalisib for R/R follicular lymphoma and R/R CLL with those of patients in clinical trials.[18] The Medicare patients with CLL outside of trials were older, had more comorbidities, and had a significantly shorter time on treatment (173 days vs 472 days; $P < .001$) and a significantly higher fatal infection rate (18.4 vs 9.8 per 100 person-years; $P = .04$). In this study, the use of *Pneumocystis jirovecii* pneumonia (PJP) prophylaxis, despite recommendations for use, was low (~33%), and dose reductions were significantly lower than in many trials. Several large multicenter retrospective studies also reported higher "real-world" discontinuation rates of idelalisib due to toxicities.[19,20]

Duvelisib demonstrated efficacy in high-risk R/R CLL in the phase 3 DUO trial randomizing patients (n = 319) 1:1 to duvelisib 25 mg twice daily vs ofatumumab. At a median follow-up of 22.4 months, a significant improvement in the primary endpoint of independent review committee–assessed PFS (13.3 months vs 9.9 months; $P < .0001$) was observed, including in patients with *TP53* aberrations (n = 31; $P = .0002$); ORR was also significantly higher in the duvelisib arm (74% vs 45%; $P < .0001$).[21]

The toxicity profile of duvelisib in clinical trials has been comparable to idelalisib in the R/R CLL/SLL population. In DUO, grade 3+ AEs occurred in 87% of duvelisib-treated patients (vs 48% in ofatumumab-treated patients), with the most common grade 3+ AEs being neutropenia (30%), diarrhea (15%), and pneumonia (14%). Grade ≥3 AEs of special interest included colitis (12%), AST and/or ALT increase (3% each), and pneumonitis (3%). It is notable that in this trial, discontinuation due to AEs was higher than discontinuation due to disease progression (35% vs 22%).[21,22]

Across phases 1 to 3 trials of idelalisib and duvelisib (Table 2), ~5% to 73% of patients discontinued drug due to AEs, and toxicity was the most common reason for drug discontinuation in many studies.[23] Correlatives support an immune-mediated mechanism for many of the severe toxicities observed with

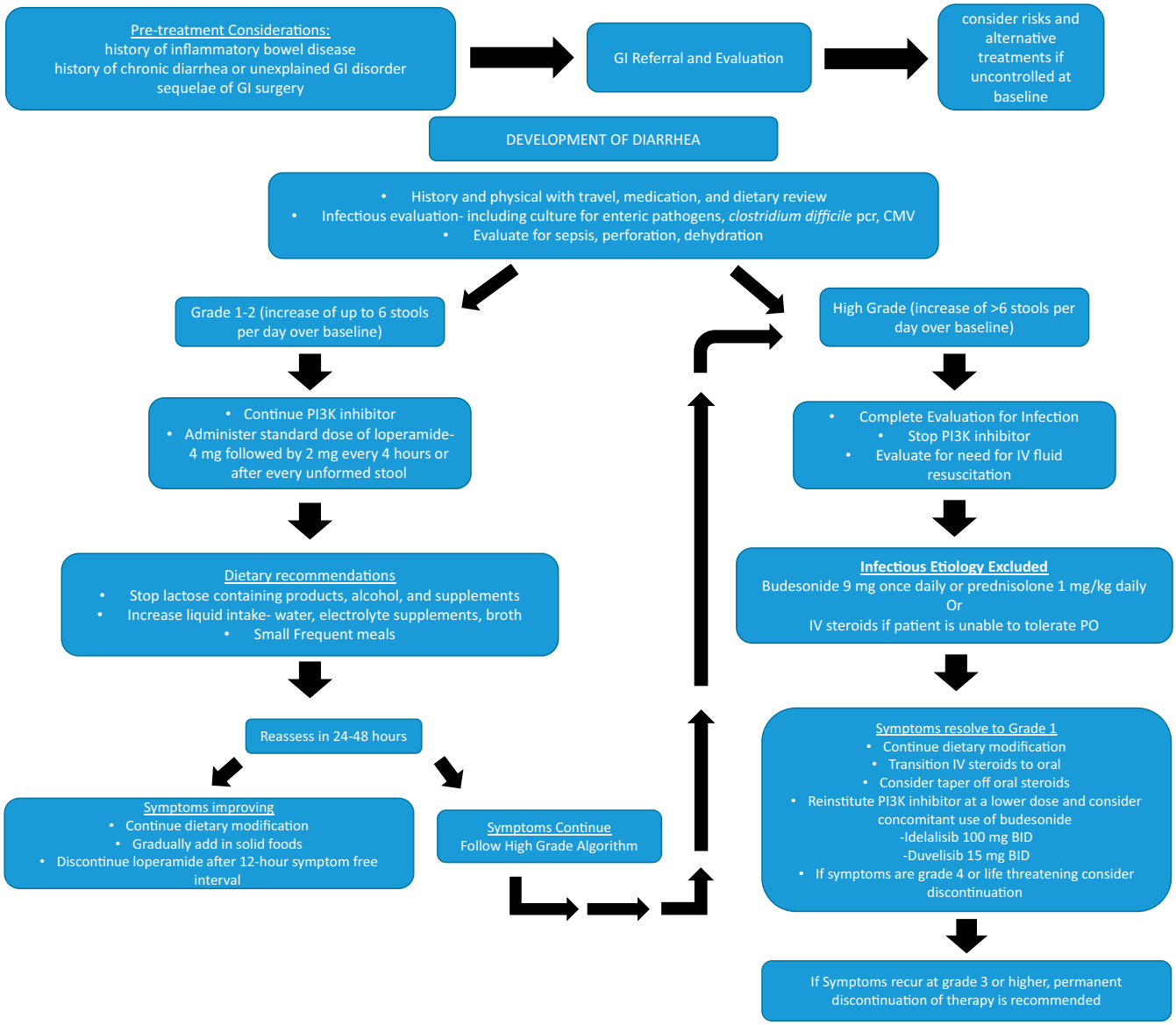

Figure 1. Management of diarrhea and/or colitis.[25-34]

idelalisib and duvelisib. In the phase 2 frontline idela + R trial,[11] there was an infiltration of T lymphocytes in colonic biopsies performed for persistent diarrhea, and in the frontline idelalisib + ofatumumab trial, lymphoid aggregates and increased activated CD3+ T cells were present in liver biopsies of patients with persistent transaminitis.[12] In addition, elevated proinflammatory cytokines/chemokines were higher in frontline idelalisib patients with hepatotoxicity[12] and in idelalisib plus entospletinib patients with pneumonitis.[17] Idelalisib can preferentially inhibit regulatory T cells (Tregs) important for self-tolerance, leading to unchecked T-effector cells.[24,25] Both CD4+ and CD8+ T-cell numbers remain less than half of pretreatment levels for several years after chemoimmunotherapy. The increased number and function of the T-cell repertoire in treatment-naïve patients with CLL may explain the increased immune-related toxicity with PI3Kis in this setting.[12,26]

Management of common toxicities for idelalisib and duvelisib

Idelalisib carries a black box warning for hepatotoxicity, severe diarrhea/colitis, pneumonitis, infection, and intestinal perforation.[27] Similarly, duvelisib has a black box warning for infections, diarrhea/colitis, cutaneous reactions, and pneumonitis.[28] Here, we review recommendations for the management of the most common and severe toxicities observed in patients treated with the approved PI3Kis in CLL: idelalisib and duvelisib. These recommendations incorporate product labels[27,28] and prior expert guidelines, safety reports, and special considerations within the National Comprehensive Cancer Network guidelines.[29-35] Where data are lacking or evolving, our approach to practical management in these scenarios is also included. Treatment with PI3Kis in clinical trials should always follow protocol-specific grading and management.

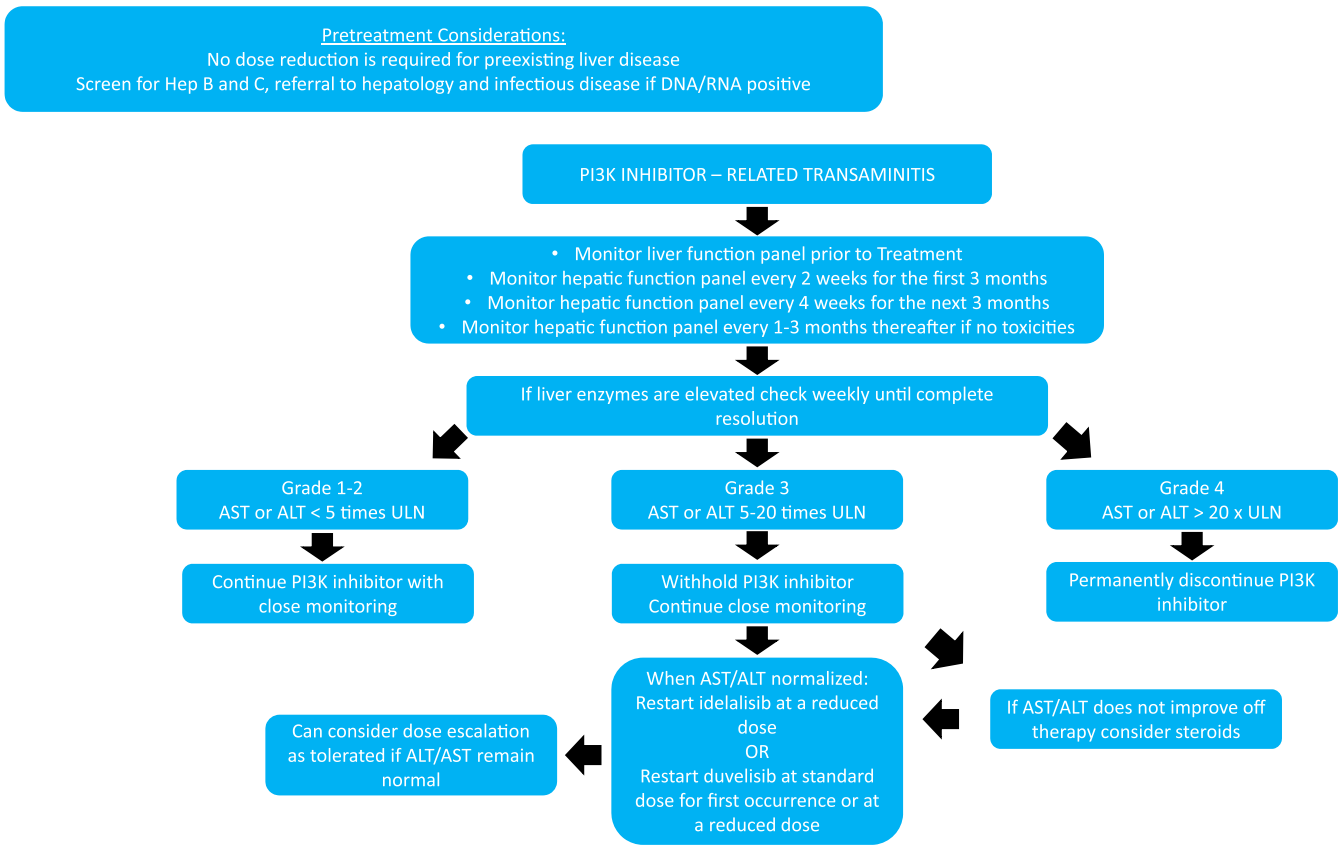

Figure 2. Management of hepatotoxicity.[25-34]

Neutropenia and infections

Neutropenia is common during the first months of PI3Ki use (~50% any grade; grade 3 or 4 in >20%). Though several idelalisib trials employed growth factor support for grade 4 neutropenia, interruption or discontinuation due to neutropenia was rare.[29] Monitoring with complete blood count is recommended every 2 weeks for the first 3 months of PI3Ki treatment and weekly if grade ≥3 neutropenia occurs.

Severe and fatal infections, including PJP and cytomegalovirus (CMV), have been observed, mainly in patients without prophylaxis. Therefore, patients should receive sulfamethoxazole/trimethoprim (preferred agent) or equivalent PJP prophylaxis until 2 to 6 months after cessation of the PI3Ki; an alternative approach is to use a quantitative measure of $CD4^+$ count >200/μL to account for differences in recovery after treatment. Because sulfamethoxazole/trimethoprim can be associated with myelosuppression, patients with refractory cytopenias while receiving PI3Kis should consider alternative PJP prophylaxis (options and National Comprehensive Cancer Network guidelines). CMV monitoring at baseline and approximately monthly while receiving PI3Kis should be employed, with consultation with an infectious disease specialist for treatment if CMV RNA is >100 000 copies or rising over several measurements. Acyclovir or an equivalent is often recommended for viral prophylaxis, given cases of severe cutaneous or systemic varicella zoster virus reaction. Baseline HIV, hepatitis B virus, and hepatitis C virus status is important, given the risk for severe infections and hepatotoxicity while receiving PI3Kis; if the patient has a positive test result, gastrointestinal and infectious disease specialist consultation should occur before starting the PI3Ki.

Diarrhea/colitis

Diarrhea is a common AE with use of PI3Kis, occurring in 4% to 77% across all grades (5% to 42% grade ≥3) and more commonly in treatment-naïve patients (Figure 1, Table 2). Colitis was defined separately from diarrhea in some trials when evidence of inflammation was seen on mucosal biopsies; however, biopsy was not required, and often the terms were reported together or overlapping. Two distinct entities of diarrhea/colitis occurring during PI3Ki use are recognized. The first, usually within the first 8 weeks, is often responsive to supportive care and antimotility agents. In contrast, late-onset (median time, ~7 months) diarrhea/colitis is typically more severe and believed to be immune mediated.[29] In all incidences, development of diarrhea while using a PI3Ki should be evaluated urgently with close follow-up until improvement. Though some consider recommendations for diarrhea/colitis management on the basis of traditional toxicity grading severity (ie, grade 1 or 2 or grade ≥3 by Common Terminology Criteria for Adverse Events criteria),[36] grade 2 late-onset diarrhea not responding to antimotility agents after 36 to 48 hours is significant and should be managed as a higher-grade event with withholding of the PI3Ki while completing workup and supportive care. For grade 4 or life-threatening diarrhea, the PI3Ki should be discontinued permanently. Though rechallenge (including overlapping with steroids) can be successful in select cases of grade 3 or grade 2 AEs of late onset/refractory to antimotility agents, we include a full discussion

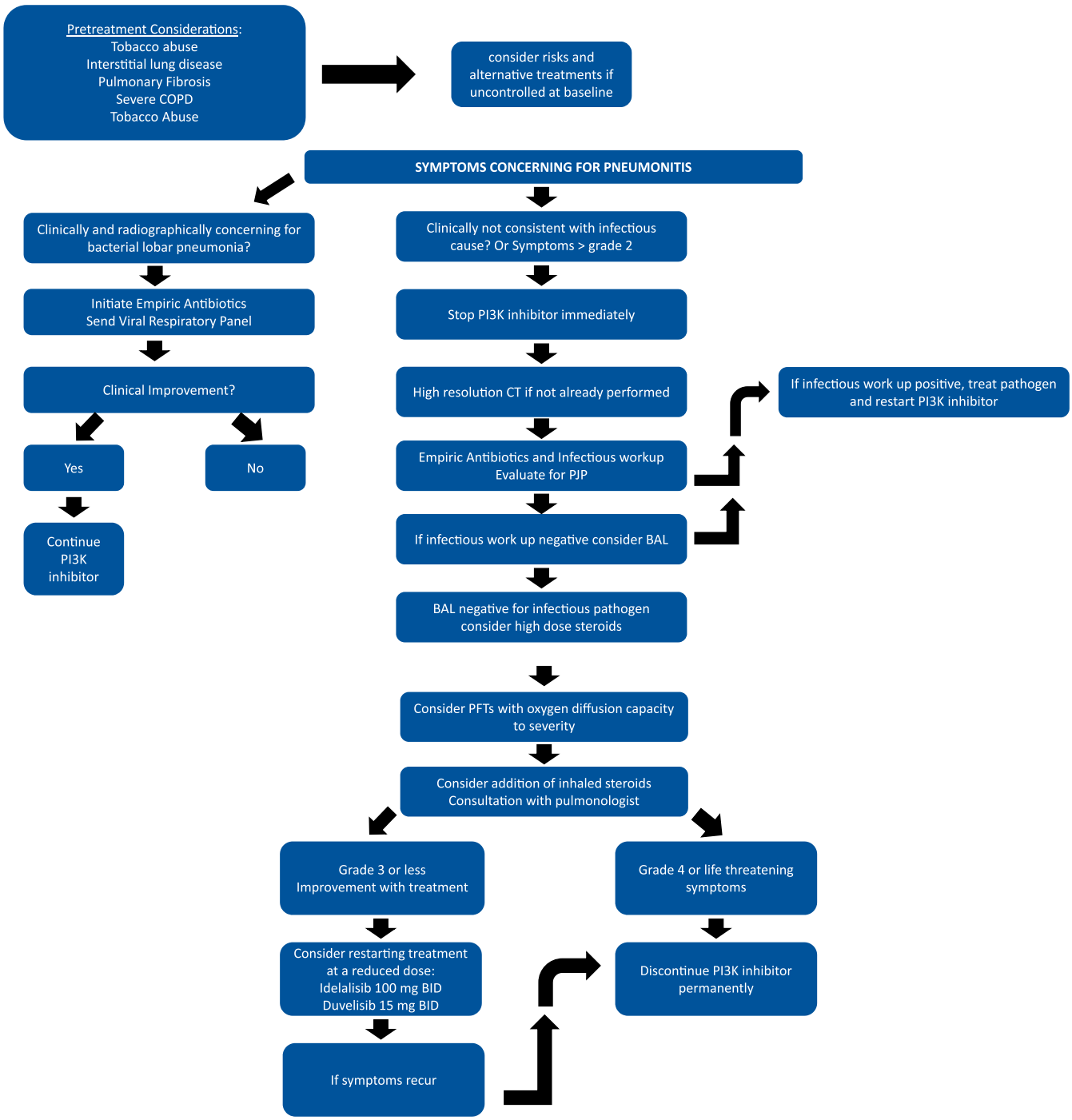

Figure 3. Management of respiratory complaints and suspected pneumonitis.[25-34]

of alternative treatment options, including clinical trials, before rechallenge in these cases, given the risk of diarrhea/colitis recurrence.

Hepatotoxicity

Hepatotoxicity, characterized by a hepatocellular type of injury (elevated AST or ALT rather than elevated bilirubin or alkaline phosphatase), is among the most commonly reported PI3Ki AEs; it was noted in 14% to 70% of patients with grade ≥3 AEs in 3% to 40% of patients across all trials (Figure 2, Table 2) and was more common in treatment-naïve patients. Unlike PI3Ki-associated diarrhea and pneumonitis, which can increase in incidence with longer drug exposure, hepatotoxicity is most often seen during the first 12 weeks with grade ≥3 AE occurrence, plateauing by 20 weeks in the phase 3 R/R disease idela + R trial.[9]

Given this risk and timing, hepatic function testing is recommended every 2 weeks during the first 3 months, then monthly for 3 months, and then every 1 to 3 months thereafter, depending on any toxicities that develop. A monitoring and treatment algorithm for hepatotoxicity based on AST/ALT elevation over the upper limit

Table 3. Currently approved and select investigational PI3Kis in CLL and other hematologic malignancies

Drug	PI3K isoform selectivity	Status	Approved dosing
Idelalisib	PI3Kδ	FDA approved for patients with R/R CLL for whom rituximab monotherapy is appropriate and in patients with SLL or FL after ≥2 prior therapies	150 mg by mouth twice daily
Duvelisib	PI3Kγ/δ	FDA approved for R/R CLL, SLL, and FL after ≥2 prior therapies	25 mg by mouth twice daily
Copanlisib	PI3Kα/δ	FDA approved for relapsed follicular lymphoma after ≥2 prior therapies	60 mg IV on days 1, 8, and 15 of a 28-d treatment cycle
Umbralisib	PI3Kδ	Investigational	Not applicable
MEI-401	PI3Kδ	Investigational	Not applicable
Parsaclisib	PI3Kδ	Investigational	Not applicable

FDA, U.S. Food and Drug Administration.

of normal is outlined in Figure 2. Severe and fatal cases of hepatotoxicity have occurred, but most AST/ALT increases resolve with withholding of the PI3K and supportive care.

Pneumonitis

Noninfectious, likely immune-mediated pneumonitis characterized by acute/subacute cough, dyspnea, and/or fever (similar to reports of hypersensitivity pneumonitis with mammalian target of rapamycin inhibitors)[29] occurred in 1.4% to 17% PI3Ki-treated patients, with some cases being fatal (Figure 3). A chest x-ray can show bilateral infiltrates, and a computed tomographic scan can demonstrate diffuse ground-glass opacities; alveolar consolidations; and, in some cases, pleural effusions. Given that these findings are nonspecific and that infectious complications are common with a PI3Ki, patients should receive empiric antibiotics and appropriate infectious evaluation (considering bacterial, viral, and opportunistic infections, including PJP), with pneumonitis as a diagnosis of exclusion. High-dose corticosteroids while withholding the PI3Ki may be helpful in severe cases.[29,37]

Return to the clinical case

The patient developed grade 3 hepatotoxicity (Figure 2; AST/ALT, 5 to 20 times the upper limit of normal), and the idelalisib was withheld. His AST/ALT trended toward normal over the course of 2 weeks without intervention, and idelalisib was successfully re-initiated at a reduced 100-mg twice-daily dose. Eight months later,

Table 4. Select clinical trials of PI3K in CLL/SLL or other hematologic malignancies with focus on next-generation agents, novel–novel combinations, and/or alternative dosing

PI3Ki	Phase (ClinicalTrials.gov identifier)	Population	Dosing/combination	Notes
Duvelisib	Phase 1 (NCT03534323)	R/R CLL	Duvelisib plus venetoclax	Fixed duration; dosing can be stopped if reaching MRD negativity at 1 y
Duvelisib	Phase 2 (NCT03961672)	R/R CLL	Duvelisib is administered at standard dosing during a 3-mo induction followed by twice-weekly maintenance	Intermittent dosing after induction continuous cycles
Umbralisib	Phase 3 UNITY (NCT02612311)	Frontline CLL	Umbralisib + ublituximab vs obinutuzumab + chlorambucil	Press release for meeting primary endpoint
Umbralisib	Phase 1 (NCT02268851)	R/R CLL and MCL	Umbralisib and ibrutinib	First clinical data on safety of doublet BTKi and PI3Kδi[53]
Umbralisib	Phase 1 ((NCT02006485)	Frontline and R/R CLL/SLL and B-cell NHL	Umbralisib, ublituximab, and ibrutinib	Triplet therapy combination safety data for anti-CD20 plus BTKi and PI3Kδi[54]
Umbralisib	Phase 2 (NCT04016805)	Patients with CLL currently on ibrutinib or venetoclax	Umbralisib and ublituximab	Addition of PI3Ki combination to increase MRD rates with addition of umbralisib and ublituximab to ibrutinib or venetoclax
Umbralisib	Phase 2 (NCT03801525)	Frontline CLL	Ublituximab, umbralisib, and venetoclax	Frontline triplet with limited treatment duration
ME-401	Phase 1		ME-401 as monotherapy or in combination with R	Alternate dosing strategy (continuous → intermittent dosing) effective with reduced toxicities[43]
ME-401	Phase 1 (NCT02914938)	R/R CLL/SLL or B-cell NHL	ME-401 alone or in combination with rituximab or zanubrutinib in R/R CLL/SLL or other	

MRD, minimal residual disease defined as by less than one CLL cell in the peripheral blood or bone marrow per 10,000 leukocytes ($<10^{-4}$)

however, he presented after 2 days of profuse diarrhea (8 to 10 stools per day), requiring hospitalization. Despite his improvement to grade 1 while withholding idelalisib plus providing supportive care and oral budesonide, his diarrhea recurred on rechallenge, and idelalisib was permanently discontinued (Figure 1).

Patients stopping PI3Ki for toxicity can be sequenced successfully to other targeted therapies, including ibrutinib or venetoclax.[20,38] In this case, the patient had already experienced severe ibrutinib toxicity (subdural hemorrhage) that would raise concern about returning to BTKi treatment; venetoclax remained an approved therapeutic option. Thirty-six patients treated in a trial with venetoclax after idelalisib had a 67% ORR and a 12-month PFS of 79%.[39] However, patients do not always require next-line therapy immediately after treatment is discontinued for intolerance and should be followed until International Workshop on Chronic Lymphocytic Leukemia treatment indications are present.[40]

The patient was monitored for several months after cessation of idelalisib until he required treatment and was started on venetoclax therapy. With his tumor lysis syndrome risk and reduced renal function, he met criteria for hospitalization during the venetoclax dose escalation.

Moving forward: future directions for PI3Kis in CLL/SLL

Next-generation PI3Kis (Table 3) may have improved tolerability through different off-target effects and/or employing alternative dosing. Umbralisib (TGR-1202), a selective PI3Kδ inhibitor, demonstrated favorable safety and ORR in patients with CLL/SLL in a phase 1 trial for R/R hematologic malignancies (n = 20; 50% objective response by International Workshop on Chronic Lymphocytic Leukemia criteria) and led to a randomized phase 3 frontline CLL/SLL study of umbralisib plus the anti-CD20 antibody ublituximab vs obinutuzumab plus chlorambucil. A press release announced meeting the primary endpoint (PFS), but data were pending at the time of publication of the present article. Preclinical work hypothesizes that CK1ε, inhibited also by umbralisib, may prevent depletion of Tregs and limit immune toxicities. In the phase 2 trial of umbralisib, which included 49 patients with previous intolerance to idelalisib or a BTKi, 58% of patients received umbralisib longer than the original tyrosine kinase inhibitor (median follow-up, 15.7 months) with an estimated median PFS of 23.5 months; only 6 patients discontinued due to umbralisib AEs in a population defined entirely by prior drug intolerance.[41]

ME-401 is a selective PI3Kδ inhibitor with longer PI3Kδ occupancy.[42] Considering the pharmacokinetics/pharmacodynamics and observed toxicities in the phase 1 dose escalation studies, intermittent dosing after continuous induction has demonstrated promising reduction in observed immune-mediated toxicities, potentially through allowing Treg recovery (grade ≥3 in 34% with continuous dosing vs 12% with continuous to intermittent dosing strategy) while maintaining efficacy.[43]

CLL/SLL remains an incurable disease for most patients, despite recent targeted therapy approvals, leaving an unmet need for patients with resistance, intolerance, and/or comorbidities that complicate available treatment options. Given the effectiveness of targeting the PI3K pathway, efforts remain critical to consider best use of current and next-generation PI3Kis in development and alternative PI3Ki regimens, including novel–novel combinations, fixed duration, and intermittent dosing to improve toxicities (Table 4). Even as these strategies are studied, early recognition of and intervention for PI3Ki toxicities remains crucial to mitigate risks and maintain meaningful disease control without compromising quality of life.

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Off-label drug use

None disclosed.

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Preventing and monitoring for tumor lysis syndrome and other toxicities of venetoclax during treatment of chronic lymphocytic leukemia

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Recent developments in the management of chronic lymphocytic leukemia (CLL) have moved the standard of care away from chemoimmunotherapy to targeted agents such as oral kinase inhibitors or BCL-2 antagonists, alone or in combination with anti-CD20 antibodies. Two different treatment approaches have evolved: continuous, indefinite treatment and, more recently, fixed-duration combination treatment. With venetoclax-based treatment, there is a requirement to follow the established guidelines for close monitoring during initiation and ramp up, to reduce the risk of tumor lysis syndrome. The patient's risk should be assessed before the initiation of venetoclax. Appropriate management strategies should be used, including uricosuric agents, hydration, and routine laboratory monitoring, per guidelines. With early identification, immediate management, and dose adjustments, we suggest that tumor lysis syndrome and other toxicities, such as neutropenia and infections, with venetoclax-based treatment can be dealt with successfully.

LEARNING OBJECTIVES

- Understand the clinical advances, opportunities and challenges associated with venetoclax therapy for patients with CLL
- Learn about the recommendations on how to prevent and monitor for tumor lysis syndrome and other toxicities of venetoclax

Introduction

Because of the availability of numerous therapies for patients with chronic lymphocytic leukemia (CLL), it is important to develop a tailored treatment strategy for the individual patient that considers balance of efficacy, toxicity, and the patient's preference.[1] Two different approaches can be considered: continuous treatment with Bruton's tyrosine kinase (BTK) inhibitors until disease progression or fixed-duration combination treatment with venetoclax and obinutuzumab. Despite the remarkable progress that has been made with these novel targeted therapies, neither is considered curative.[2,3,4] Moreover, it is important to note that each approach has a distinctive toxicity profile. In addition, hematological toxicities such as neutropenia and thrombocytopenia and also infections are often similar in frequency and severity when compared with chemoimmunotherapy.[5-9] Although tumor lysis syndrome (TLS) has not been a frequent complication in the management of indolent lymphoma,[10] early trials of venetoclax in patients with relapsed/refractory CLL reported a few cases of TLS, some of them fatal.[11] Based on these early observations, subsequent trials have implemented various measures of monitoring and mitigation to control venetoclax-associated TLS. With the drug now approved and widely available for routine clinical use, various procedures have been recommended to avoid or treat TLS in patients with CLL.[12-14] With venetoclax increasingly becoming the backbone of many different combination regimens for CLL, a solid understanding of the best ways to mitigate toxicities is increasingly important to the practicing hematologist. We summarize the current evidence with regard to preventing and monitoring TLS and other toxicities related to venetoclax. Ultimately, we propose specific recommendations for the management of venetoclax-based therapy, to tailor prophylaxis and mitigate risk for patients with CLL. Particular emphasis will be on the discussion of toxicity data and risk reduction strategies of venetoclax-based therapies of recently published clinical trials that have defined the prevalent standard of care and the ongoing trials that may influence the next generation of treatment options.

Clinical case

A 75-year-old female patient with a diagnosis of CLL was referred to our cancer center evaluation of her treatment. The patient had been diagnosed with stage Binet A/Rai I CLL 5 years ago with mild lymphocytosis of 12 × 10^9/L.

Initiating frontline therapy

To date, there is no evidence of a potential benefit of early intervention for asymptomatic CLL.[15-17] Therapy initiation should be postponed until active disease, defined according to International Workshop on CLL (iwCLL) guidelines, is observed.[18] Clinical trials evaluating the early use of novel inhibitors are currently ongoing, but so far, neither of these includes the BCL-2 inhibitor venetoclax or provides evidence that alters the current "watch and wait" standard of care.[16]

Clinical case (continued)

During the most recent watch-and-wait visits, an increasing lymphocyte count up to 80 × 10^9/L, hemoglobin of 8.5 × 10^3/L, and a platelet count of 70 × 10^9/L were observed. Moreover, the patient reported fatigue that impaired her mobility and well-being. Based on the symptom burden and cytopenias with stage Binet C/Rai IV disease, the need for leukemia treatment was discussed with patient.

Biologic and clinical factors guiding individualized treatment

At present, a tailored treatment approach requires knowledge of the patient's condition including the following parameters[19]: (1) the clinical stage, (2) the presence of *TP53* mutation and/or deletion, (3) the fitness (ie, coexisting conditions, such as cardiac conditions, or renal dysfunctions) of the patient, (4) the immunoglobulin heavy chain variable (IGHV) mutational status, and (5) the symptoms of CLL. The selection of the appropriate treatment paradigm (continuous indefinite vs fixed-duration treatment) follows these characteristics, because advanced age and poor performance status, among other factors, confer the highest risk of increased toxicity and intolerance.

Clinical case (continued)

A molecular and cytogenetic workup revealed unmutated IGHV gene status and TP53 wild-type and 13q deletions. The patient had several coexisting conditions, including hypertension (well controlled with ramipril and amlodipine), type 2 diabetes (treated with metformin and insulin replacement therapy), and chronic kidney disease (grade 2 with creatinine clearance of 75 mL/min).

How we choose a frontline therapy

On the basis of the factors described, we suggest the following algorithm for choosing a frontline therapy.[20] We seek to simplify the increasing number of treatment options and to tailor an individualized therapy. Discussion of toxicities and duration of therapy with patients is important and may aid in the decision of whether to treat with venetoclax and obinutuzumab or BTK inhibitors. Currently, to our knowledge, no data are available on a direct comparison.

Clinical case (continued)

Two treatment options were evaluated for the patient: ibrutinib as a continuous, but highly effective option, with a good chance of disease control over several years, and a fixed-duration option with a combination of venetoclax and obinutuzumab for 12 cycles, which required 8 infusions of an antibody and oral intake of a tablet for 12 cycles. Possible drug-specific toxicities were taken into account, including worsening of existing arterial hypertension with ibrutinib and worsening of renal function if TLS occurred with venetoclax treatment. The patient was in favor of a limited-duration treatment, and after careful consideration and receiving formal consent from the patient, we initiated treatment with venetoclax and obinutuzumab, according to the CLL14 protocol.

Venetoclax therapy for CLL

Venetoclax is a BH3-mimetic compound that selectively antagonizes BCL-2 and induces apoptosis of CLL cells. Its efficacy as a monotherapy has been described in patients with relapsed/refractory CLL, including those with del(17p).[11,21] Venetoclax received initial approval in 2016 on the basis of a phase 2 trial evaluating patients with relapsed/refractory disease with del(17p).[22] Subsequently, venetoclax was approved in combination with rituximab: the phase 3 MURANO trial showed improved progression-free survival, compared with chemoimmunotherapy with bendamustine and rituximab in patients with relapsed/refractory disease.[23,24] For first-line therapy, the phase 3 CLL14 trial evaluated fixed-duration venetoclax and obinutuzumab in patients with previously untreated CLL and coexisting medical conditions compared with chlorambucil and obinutuzumab. The results demonstrated the superiority of the fixed-duration treatment regimen of venetoclax and obinutuzumab over chlorambucil and obinutuzumab.[2] On the basis of these results, the combination of venetoclax and obinutuzumab was approved for the first-line treatment of patients with previously untreated CLL. The results influenced the choice of first-line therapy by establishing fixed-duration treatment as an alternative option to continuous, indefinite treatment with the BTK inhibitor. Most recently, longer follow-up confirmed a sustained benefit of fixed-duration venetoclax and obinutuzumab.[25]

Combinations of venetoclax and BTK inhibitors in clinical trials

With the favorable outcome of BTK inhibitors and BCL-2 inhibitors in patients with CLL, current trials are evaluating the combination of the 2 oral agents. The first data reported support high efficacy rates and manageable toxicity, although longer follow-up is warranted.[26-28] Ongoing clinical trials that are addressing the question of combination vs single-agent targeted strategies are outlined in Table 1.

Tumor lysis syndrome

The Common Terminology Criteria for Adverse Events (version 5.0) define TLS as a disorder characterized by metabolic abnormalities that result from spontaneous or therapy-induced lysis of tumor cells. Diagnostic criteria by Howard et al have identified variables, such as hyperkalemia, hyperphosphatemia, hyperuricemia, and hypocalcemia, to define laboratory indications of TLS without clinical symptoms.[29] First laboratory signs usually occur 6 to 24 h after treatment is initiated.[14,30] Clinical TLS is defined by clinical manifestations, most commonly renal, cardiac, or neuromuscular symptoms induced by worsening of the aforementioned metabolic and electrolyte in laboratory test results.[31] The risk of TLS is a continuum based on multiple predisposing factors, including coexisting conditions. Patients with

Table 1. Selection of ongoing/planned trials for venetoclax-based therapy in previously untreated CLL

Active disease	Study ID	Experimental agent(s) and comparator	N	Status	Trial registration
	FLAIR	Fludarabine-cyclophosphamide-rituximab vs ibrutinib vs ibrutinib-venetoclax	1516	Recruiting	ISRCTN01844152
	CLL13	Fludarabine-cyclophosphamide-rituximab/bendamustine-rituximab vs venetoclax-rituximab vs venetoclax-obinutuzumab vs obinutuzumab-ibrutinib-venetoclax	920	Recruitment completed	NCT02950051
	National Cancer Institute (ECOG 9161)	Ibrutinib-obinutuzumab vs obinutuzumab-ibrutinib-venetoclax in untreated younger patients	720	Recruiting	NCT03701282
	National Cancer Institute (Alliance 041702)	Ibrutinib-obinutuzumab vs obinutuzumab-ibrutinib-venetoclax in untreated older patients	454	Recruiting	NCT03737981
	CLL17	Ibrutinib vs venetoclax-obinutuzumab vs ibrutinib-venetoclax	920	In preparation	EudraCT 2019-003854-99

All are phase 3, multicenter, open-label trials. No results have been submitted.
Active disease, according to iwCLL criteria (ref. 19); ECOG, Eastern Cooperative Oncology Group.

high tumor burden (eg, any lymph node with a diameter ≥5 cm or a high absolute lymphocyte count [ALC] of >25 × 10⁹/L are at higher risk when initiating venetoclax.[32] Reduced renal function (creatinine clearance, <80 mL/min) and concomitant medications such as CYP3A4 inhibitors further increase the risk.

Tumor lysis syndrome associated with venetoclax-based therapy

Venetoclax with its high antitumor activity, achieves deep remissions by potently inducing apoptosis and thereby increasing the risk of TLS. In early phase 1 trials with venetoclax [11,33] there were 2 fatal cases associated with TLS: one in a patient treated with a starting dose higher than the currently recommended 20 mg and the other in a patient whose dose was escalated to 1200 mg. Currently, a target daily dose of 400 mg is recommended.[11,33] Consequently, initiation, escalation, and monitoring of venetoclax treatment were amended, and requirements for safe management were implemented. As a result of adherence to guidelines on the prevention of tumor lysis, the incidence of ~1.1% to 3.8% for laboratory-confirmed TLS within clinical trials, with no cases of clinical manifestation after venetoclax initiation is considered low.[2,24,26,27] Of note, monotherapy with obinutuzumab has been reported to be associated with an incidence of TLS of 4.8% in a phase 1/2 trial.[34] Reports on patients treated with venetoclax outside of clinical trials are heterogenous, with 1 large retrospective analysis of 297 patients reporting an incidence of TLS of 5.7%,[35] in contrast to a recent analysis of 48 patients with an incidence of 13%.[36]

Risk stratification of TLS associated with venetoclax-based therapy

The tumor mass burden varies from patient to patient, and several risk stratification and mitigation procedures have been implemented in the previously mentioned clinical trials. In general, the key parameters to estimate tumor mass are ALC and lymph node size (Table 2). Although physical examination and ultrasonography can provide a first impression of the lymph node mass, intraabdominal and intrathoracic lymph nodes cannot be safely assessed. Therefore, a CT or MRI scan of the neck, chest, abdomen, and pelvis is generally recommended. In addition, because most patients with CLL are >65 years of age and have coexisting conditions such as renal impairment, renal

Table 2. TLS risk categories and prophylactic measures for venetoclax-based treatment in CLL

Assessments before treatment	TLS risk category	Risk parameters	Mitigation measures		
			Prophylactic medication	Hydration	Hospitalization
Tumor burden assessment CT scan Lymphocyte count	Low	All lymph nodes <5 cm AND ALC <25 × 10⁹/L	2-3 d before venetoclax intake: allopurinol In cases of elevated uric acid: rasburicase	Oral hydration (1.5-2 L/d), starting 2 d before dose ramp up.	Outpatient, check TLS parameters and creatinine clearance at least 6 to 8 h and 24 h after each ramp up step
Blood chemistry Potassium Phosphate Calcium Uric acid	Medium	Any lymph node 5-10 cm OR ALC ≥ 25 × 10⁹/L		Oral hydration or consider IV hydration	Outpatient, check TLS parameters and creatinine clearance at least 6 to 8 h and 24 h after each ramp up step OR inpatient, in case of preexisting abnormalities or relevant coexisting conditions (creatinine clearance <80 mL/min)
Renal function Creatinine clearance	High	Any lymph node ≥10 cm OR Any lymph node ≥ 5 cm AND ALC ≥ 25 × 10⁹/L		Oral hydration AND intravenous hydration	Admission to an inpatient or day hospital to ensure sufficient IV hydration and TLS monitoring

function must be taken into account. Patients with creatinine clearance <80 mL/min are at risk for developing TLS.[37]

Clinical case (continued)

Before the patient started therapy, the risk for TLS was carefully assessed. Because of the lymphocyte count increasing to >25 × 10^9/L, the nonpalpable lymphadenopathy (which was confirmed via CT scan), and the mildly impaired renal function with a glomerular filtration rate <80 mL/min, an intermediate risk for development of TLS was assessed. The patient was treated in an outpatient setting, although admission for better monitoring generally can be discussed at the discretion of the treating physician in similar settings.

Debulking strategies for preventing TLS associated with venetoclax-based therapy

Because the risk of developing TLS is highest when treatment is initiated, when the overall tumor mass is highest, a further approach to mitigating TLS may be debulking. Pharmacological debulking strategies are commonly used in aggressive lymphoma to improve the tolerability and safety of first treatment cycles with chemoimmunotherapy.[38] Similar approaches are being tested in CLL: chemotherapy,[39-40] BTK inhibitors,[26-28] and anti-CD20 antibodies[2,41,42] have been shown to decrease the overall tumor burden and thereby reduce the individual risk for TLS.

Venetoclax in combination with chemotherapy

Chemoimmunotherapy can be an effective way to reduce the bulk of CLL before initiating venetoclax. Fludarabine-based and bendamustine-based regimens can be given as 1 to 3 cycles of standard-dose treatment before starting to increase the venetoclax dose. For instance, in the phase 2 CLL2-BAG trial, patients received sequential debulking treatment with 2 cycles of bendamustine followed by obinutuzumab and venetoclax.[43] Although this strategy has indeed been shown to reduce TLS risk, the few chemotherapy cycles add toxicity,[43] and therefore a careful risk-benefit evaluation should be made on an individual basis.

Venetoclax in combination with anti-CD20 antibodies

An early phase 1b/2 trial of venetoclax in combination with obinutuzumab evaluated a schedule with venetoclax followed by obinutuzumab and a schedule with obinutuzumab followed by venetoclax.[41] Based on the overall risk profile in this trial and the phase 3 CLL14 trial,[2] obinutuzumab was administered with 100 mg on day 1 and 900 mg on day 2 (or 1000 mg on day 1), 1000 mg on day 8, and 1000 mg on day 15 in the first treatment cycle before venetoclax ramp up. This treatment regimen allowed for an effective reduction of the ALC, which decreased the overall risk of TLS. The CLL14 trial reported that 3 patients developed laboratory-confirmed TLS, which was associated with obinutuzumab before exposure to venetoclax.[44] It is therefore also recommended to watch out for laboratory signs of TLS after the first obinutuzumab infusions, particularly in patients with higher disease bulks.

Venetoclax in combination with BTK inhibitors

To establish an all oral venetoclax-based combination therapy, current clinical trials are evaluating the combination of BTK inhibitors with venetoclax. These trials initiated treatment with single-agent ibrutinib for 2 to 3 months before starting the venetoclax ramp up. This protocol allowed for an effective reduction of risk of TLS and led to an overall low incidence of TLS (1%-4%).[27,28,45]

Risk reassessment after debulking strategies

As the risk for TLS may change after debulking with anti-CD20 monoclonal antibodies or BTK inhibitor, a reassessment of risk could be considered based on lymphocyte count and physical examination. Further radiological examination could be performed when clinically indicated.

Clinical case (continued)

The patient had initial uric acid level of 12 mg/dL and was given allopurinol 5 days before the first infusion of obinutuzumab and then 1 infusion of 7.5 mg rasburicase on the day of obinutuzumab infusion. The first obinutuzumab dose was split into to 100 mg on the first day and 900 mg on the second day. She was advised to drink 1 to 2 L of fluids at home before the obinutuzumab infusion and received 1 L of crystalloid fluids together with the first obinutuzumab infusion. To avoid an infusion-related reaction, a triple combination of an H_1 and H_2 blocker, together with 100 mg prednisolone, together with 1000 mg oral acetaminophen were administered before the first obinutuzumab infusion. The obinutuzumab infusion was administered 3 times over 3 wk. Afterward, the ALC had dropped to 10 × 10^9/L. Venetoclax was initiated, with 20 mg given on day 21 of the first cycle, followed by a weekly dose ramp up of 50, 100, 200, and 400 mg. Around each dose escalation, electrolytes (potassium, calcium, and phosphate), uric acid, and creatinine clearance were checked before and 8 hours after dose administration to detect any signs of TLS. No electrolyte shifts or decline in creatinine clearance was observed.

Other toxicities associated with venetoclax

Venetoclax treatment is associated with common hematological toxicities, including grade 3 to 4 neutropenia in ~40% of patients receiving single-agent venetoclax.[22] This adverse event becomes more frequent in combination with anti-CD20 antibodies, where grade 3 to 4 neutropenia frequencies of up to 60% have been observed,[2,24] or in combination with BTK inhibitors (up to 70%)[26,27] The rates of febrile neutropenia are usually low (3%-5%).[2,22,24,33] Specific guidance has been provided to react to a decrease in neutrophil count by use of granulocyte colony stimulating factor (GCSF), dose interruptions, or dose reduction.[37] Apart from hematological toxicities, serious infections, including cases of sepsis with fatal outcome, have been reported.[2] As the rate of opportunistic infections, such as pneumocystic jiroveci pneumonia, is very low with venetoclax, no specific antimicrobial prophylaxis is currently recommended.[46] Hence, similar to any management of treatment of CLL, due diligence and timely action are necessary when patients develop signs of infection during treatment with venetoclax. In addition, gastrointestinal side effects have been reported with venetoclax monotherapy and venetoclax combination therapy, such as mild diarrhea and nausea in up to 40% of patients.[22,24,25,33] After exclusion of possible infectious causes, these conditions are usually treated with supportive measures, such as loperamide or temporary dose reductions.[37]

Clinical case (continued)

At 200 mg of venetoclax, the patient developed grade 2 neutropenia without fever; GCSF was administered daily and the ramp up was continued to 400 mg after the neutrophil count had improved. GCSF was discontinued after 4 days, and the neutrophil count remained stable. The patient completed 6 cycles of obinutuzumab and 12 cycles overall of venetoclax and showed

complete remission with undetectable minimal residual disease at final restaging.

Current challenges associated with venetoclax and outlook

The current challenge is to identify the best treatment strategy to achieve the long-term control of CLL with minimal toxicity and optimal quality of life. Within clinical trials, potential long-term toxicities including the incidence of second primary malignancies need to be continuously monitored. To investigate these questions, further clinical trials are currently being conducted or are about to open for recruitment (Table 1). The phase 3 FLAIR trial is investigating ibrutinib monotherapy vs the combination of ibrutinib plus rituximab vs ibrutinib and venetoclax vs fludarabine, cyclophosphamide, and rituximab and will show data of a randomized comparison with ibrutinib and venetoclax (Table 1). Although CLL14 enrolled older patients with coexisting conditions, one may be comfortable in extrapolating the results of the trial to younger, fit patients. In addition, for these patients, the CLL13 trial will determine whether a fixed-duration treatment of obinutuzumab and venetoclax or obinutuzumab and venetoclax and ibrutinib is superior to chemoimmunotherapy. In particular, the safety data from this trial will elucidate the extent of drug-related toxicity resulting from regimens with different combination partners of venetoclax (Table 1). The triple combination is also studied in 2 US trials compared with the combination of ibrutinib and obinutuzumab (Table 1). Moreover, in the near future, a direct comparison will be conducted within the CLL17 trial, to study the 2 different treatment approaches of continuous treatment with ibrutinib and fixed-duration combination treatment with venetoclax and obinutuzumab or venetoclax and ibrutinib (Table 1). Ultimately, such trials will improve the understanding of these regimens for treatment of CLL, including drug-related toxicities, discontinuations, and quality-of-life parameters.

Conflict-of-interest disclosure

K.F. has received honoraria from AbbVie and F. Hoffmann-La Roche and travel grants from F. Hoffmann-La Roche. O.A.-S. discloses other financial relationships with AbbVie, F. Hoffmann-La Roche, Gilead, and Janssen. M.H. has received research funding and honoraria and serves on the speaker's bureau for F. Hoffmann-La Roche and AbbVie.

Off-label drug use

None disclosed.

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Management of AL amyloidosis in 2020

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In amyloid light chain (AL) amyloidosis, a small B-cell clone, most commonly a plasma cell clone, produces monoclonal light chains that exert organ toxicity and deposit in tissue in the form of amyloid fibrils. Organ involvement determines the clinical manifestations, but symptoms are usually recognized late. Patients with disease diagnosed at advanced stages, particularly when heart involvement is present, are at high risk of death within a few months. However, symptoms are always preceded by a detectable monoclonal gammopathy and by elevated biomarkers of organ involvement, and hematologists can screen subjects who have known monoclonal gammopathy for amyloid organ dysfunction and damage, allowing for a pre-symptomatic diagnosis. Discriminating patients with other forms of amyloidosis is difficult but necessary, and tissue typing with adequate technology available at referral centers, is mandatory to confirm AL amyloidosis. Treatment targets the underlying clone and should be risk adapted to rapidly administer the most effective therapy patients can safely tolerate. In approximately one-fifth of patients, autologous stem cell transplantation can be considered up front or after bortezomib-based conditioning. Bortezomib can improve the depth of response after transplantation and is the backbone of treatment of patients who are not eligible for transplantation. The daratumumab+bortezomib combination is emerging as a novel standard of care in AL amyloidosis. Treatment should be aimed at achieving early and profound hematologic response and organ response in the long term. Close monitoring of hematologic response is vital to shifting nonresponders to rescue treatments. Patients with relapsed/refractory disease are generally treated with immune-modulatory drugs, but daratumumab is also an effective option.

LEARNING OBJECTIVES

- Learn how to timely and correctly diagnose light chain amyloidosis
- Learn how to use currently available and novel regimens to treat light chain amyloidosis based on accurate risk and response assessment

Clinical case

A 65-year-old man with a history of hypertension developed worsening exertional dyspnea over the course of 6 months. During the previous 6 months he had progressively reduced and eventually discontinued his angiotensin-converting enzyme inhibitors because of "resolution" of hypertension. His cardiologist suspected amyloid heart involvement based on an echocardiography and recommended cardiac magnetic resonance (CMR) imaging, which showed late gadolinium enhancement. ^{99m}Tc-hydroxymethylene-diphosphonate scintigraphy revealed cardiac uptake. A diagnosis of transthyretin (ATTR) amyloidosis was presumed. The patient was referred to a medical geneticist to rule out hereditary amyloidoses and to a hematologist to rule out light chain (AL) amyloidosis. Genetic testing for hereditary ATTR amyloidosis was negative. Immunofixation revealed κ Bence Jones protein. The patient was then referred to our center for amyloid typing and presented with New York Heart Association class III (NYHA class III) heart failure and postural hypotension. The κ-free light chain (FLC) concentration was 206 mg/L (ratio [FLCR], 10.3, and differential FLC [dFLC], 186 mg/L); bone marrow plasma cell (PC) infiltrate was 12% without chromosomal abnormalities; blood count, calcium, and liver function test results were normal; estimated glomerular filtration rate (eGFR) was 48 mL/min; proteinuria was 2.8 g per 24 hours, predominantly albumin; *N*-terminal pronatriuretic peptide type-B (NT-proBNP) was 10 625 ng/L (upper reference limit [url], 227 ng/L); and cardiac troponin I (cTnI) was 124 ng/L (url, 44 ng/L). A computed tomographic (CT) scan showed no bone lesions. Abdominal fat aspirate showed amyloid deposits typed as AL κ by immunoelectron microscopy (IEM). A diagnosis of AL amyloidosis with cardiac (stage IIIb) and renal (stage II) involvement was established.

The patient received attenuated treatment with cyclophosphamide, bortezomib, and dexamethasone in subintensive care. Treatment was associated with fluid retention. Nevertheless, he received the second cycle as an outpatient. After 2 cycles, very good partial response (VGPR) was reached (dFLC, 11 mg/L; FLCR, 2.1; and persistence of κ Bence Jones protein), with improvement of markers of cardiac (NT-proBNP, 7225 ng/L) and renal (proteinuria, 1.7 g per 24 hours) involvement. Two more cycles were administered that were accompanied by fluid retention but did not improve hematologic (dFLC, 9 mg/L; FLCR, 2.0; and persistence of κ Bence Jones protein) and organ (NT-proBNP, 6792 ng/L; proteinuria, 1.5 g per 24 hours) response. Heart failure improved (NYHA class II), and treatment was discontinued based on the patient's preference. Follow-up testing was scheduled every 3 months. After 15 months, markers of organ involvement were stable (NT-proBNP, 7471 ng/L, proteinuria, 1.4 g per 24 hours), but FLC increased (dFLC, 98 mg/L; FLCR, 5.9). The patient was treated with daratumumab, bortezomib, and dexamethasone. After 1 week, VGPR was reestablished (dFLC, 8 mg/L; FLCR, 1.8; and persistence of κ Bence Jones protein), and after 4 months, complete response (CR) was attained (dFLC, 3 mg/L; FLCR. 1.2; and negative serum and urine immunofixation) with cardiac response (NT-proBNP, 2809 ng/L). Twelve months later, CR had been maintained, and minimal residual disease (MRD) was not detectable by next-generation flow cytometry.

Introduction

In systemic AL amyloidosis a PC clone, or, less frequently, a lymphoplasmacytic or marginal zone lymphoma, produces a toxic LC that causes organ dysfunction and damage and forms amyloid fibrils in tissues. In contrast, localized deposition of LCs causes nodules to develop in the skin and in the respiratory, urinary, and gastrointestinal tracts, with local symptoms and a benign course that usually is managed with local treatment.[1] In systemic AL amyloidosis, the PC clone is usually small (median infiltrate, 10%), and presents t(11;14) and gain 1(q21) in ~50% and 20% of clones, respectively, whereas high-risk aberrations are uncommon.[2,3] Patients whose PC clones harbor t(11;14) have a worse outcome with bortezomib and immunomodulatory drugs (IMiDs), whereas gain 1(q21) is associated with poorer results with oral melphalan.[3-5]

Heart involvement is the major determinant of survival. Preclinical models and clinical observation of rapid cardiac improvement after a decline in LC concentration disclosed a direct cardiotoxic effect of the circulating precursor.[6,7] The severity of organ involvement is assessed with biomarkers combined in accurate staging systems (Table 1).[8-12] Survival also depends on hematologic response (HR), because LCs are the agents directly causing organ dysfunction. If the disease is not treated promptly and effectively, organ dysfunction progresses and eventually leads to death. Recent trials of immunotherapies targeting the amyloid deposits (the anti-fibril antibody NEOD001 and the combination of the amyloid P component [which targets small-molecule miridesap], and dezamizumab) failed. Only one anti-amyloid fibril antibody CAEL-101 is still under evaluation (www.clinicaltrials.gov #NCT04304144). Current treatments target the underlying clone and are aimed at suppressing the production of LCs to restore organ function and extend survival. Advanced organ involvement, particularly cardiac, is associated with

Table 1. Staging systems for AL amyloidosis

Staging system	Markers and thresholds	Stages	Outcomes*
Cardiac (NT-proBNP based)	NT-proBNP >332 ng/L cTnT >0.035 ng/mL (or cTnI >0.01 ng/mL)	I. No markers above the cutoff II. One marker above the cutoff IIIa. Both markers above the cutoff and NT-proBNP <8500 ng/L IIIb. Both markers above the cutoff and NT-proBNP ≥8500 ng/L	I. Median survival not reached, 57% with 10-y survival II. Median survival 67 mo IIIa. Median survival 15 mo IIIb. Median survival 4 mo
Cardiac (BNP based)	BNP >81 ng/L cTnI >0.1 ng/mL	I. No markers above the cutoff II. One marker above the cutoff IIIa. Both markers above the cutoff and BNP <700 ng/L IIIb. Both markers above the cutoff and BNP ≥700 ng/L	I. Median survival 151 mo, 57% with 10-y survival II. Median survival 53 mo III. Median survival 13 mo IV. Median survival 4 mo
Revised Mayo Clinic	NT-proBNP >1800 ng/L cTnT >0.025 ng/mL dFLC >180 mg/L	I. 0 markers above the cutoff II. 1 marker above the cutoff III. 2 markers above the cutoff IV. 3 markers above the cutoff	I. Median survival not reached, 57% with 10-y survival II. Median survival 69 mo III. Median survival 16 mo IV. Median survival 6 mo
Renal	eGFR <50 mL/min per 1.73 m^2 proteinuria >5 g per 24 h	I. Both eGFR above and proteinuria below the cutoffs II. Either eGFR below or proteinuria above the cutoffs III. Both eGFR below and proteinuria above the cutoffs	I. 1% risk of dialysis at 2 y II. 12% risk of dialysis at 2 y III. 48% risk of dialysis at 2 y

dFLC, difference between involved (amyloidogenic) and uninvolved circulating free light chain.
*Observed in 1378 patients with AL amyloidosis newly diagnosed at the Pavia Amyloidosis Research and Treatment Center from 2004 through 2018.

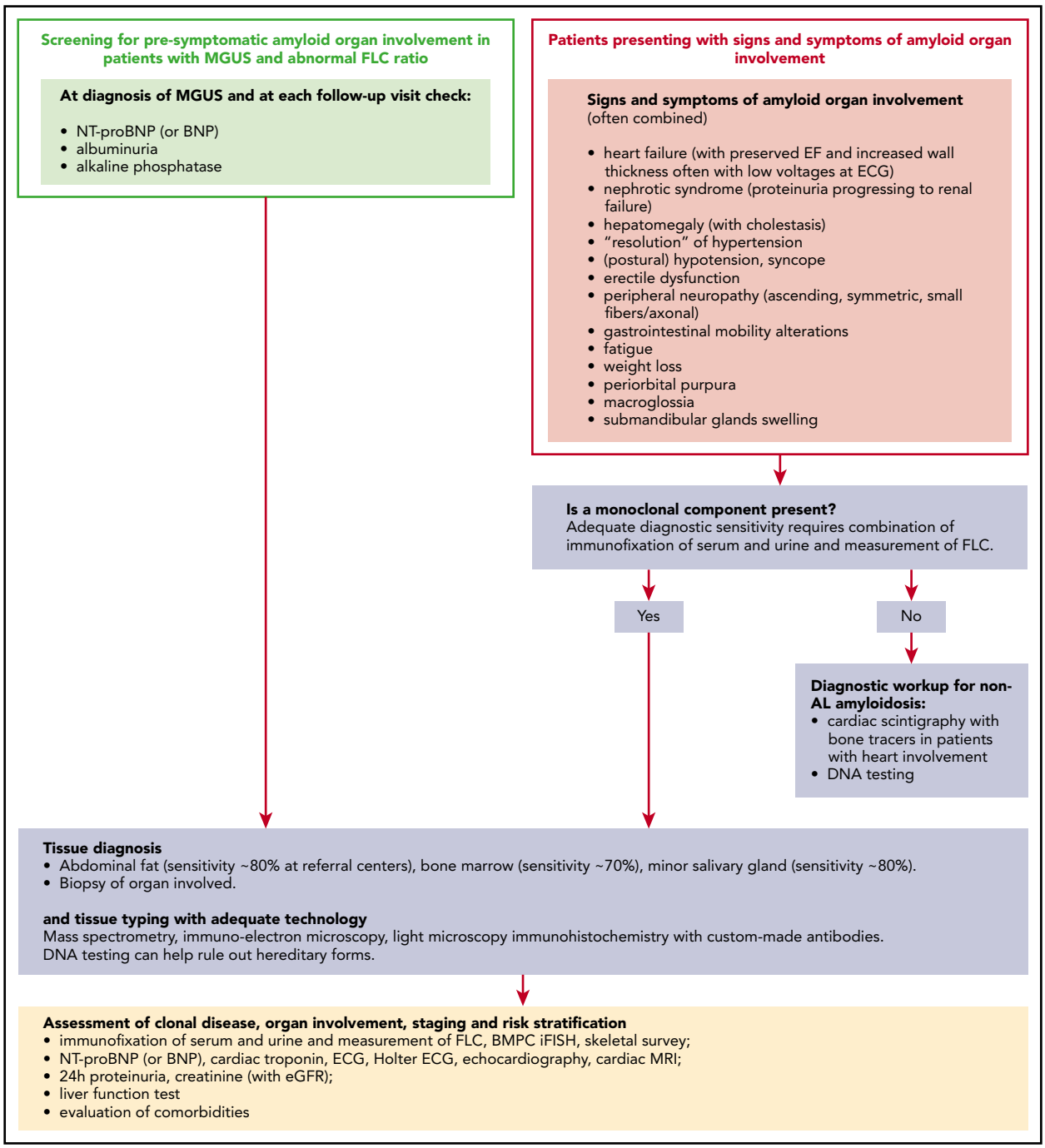

Figure 1. Presenting features and diagnostic algorithm for AL amyloidosis. Amyloidosis can be suspected if elevated biomarkers of organ involvement are detected during follow-up of patients with MGUS or if suggestive symptoms arise. The first scenario is ideal and enables early presymptomatic diagnosis. Based on relative rates of progression, appropriate screening programs should detect 1 patient with MGUS progressing to AL amyloidosis for every 7 to 10 who develop multiple myeloma. In patients with MGUS in whom elevated NT-proBNP or BNP is found, cardiac magnetic resonance imaging can be used as a higher specificity confirmatory test. If symptoms of systemic amyloidosis arise in a patient in whom a preexisting monoclonal gammopathy is not known, the first step should be searching for a monoclonal component, particularly if heart involvement is suspected, so as not to delay diagnosis. Only the combination of immunofixation of both serum and urine and FLC measurement grant adequate diagnostic sensitivity to detect amyloidogenic monoclonal proteins. MS-based methods are under investigation. Patients with suspect cardiac amyloidosis without monoclonal components can have an attempted nonbiopsy diagnosis of ATTR amyloidosis with cardiac scintigraphy with bone tracers. Validated tracers are ^{99m}Tc-diphosphono-propanodicarboxylic acid, ^{99m}Tc-pyrophosphate, and ^{99m}Tc-hydroxymethylene diphosphonate. DNA analysis is necessary to differentiate between hereditary and wild-type ATTR amyloidosis and to rule out other rarer hereditary forms. All other patients require a tissue diagnosis. Amyloid deposits can be found in abdominal fat, minor salivary

Table 2. Most common forms of systemic amyloidosis

Amyloid type	Precursor protein	Major organ involvement					
		Heart (bone tracer uptake)*	Kidney	Liver	PNS	ANS	ST
AL amyloidosis (acquired)	Immunoglobulin light chain	+++ (usually absent, can be intense)	+++	++	+	+	++
ATTRv amyloidosis (hereditary)	Mutated transthyretin	+++ (usually intense, can be absent in some variants)	—	—	+++	+++	—
ATTRwt amyloidosis (acquired)	Wild-type transthyretin	+++ (usually intense)	—	—	—	—	+
ApoAI amyloidosis (hereditary)	Mutated apolipoprotein AI	+ (present)	+	+++	—	—	—
AA amyloidosis (acquired)	Serum amyloid A protein	+	+++	+	—	+	—
ALECT2 (acquired)	Leukocyte chemotactic factor 2	—	+++	+	—	—	—

In AL amyloidosis, soft tissue involvement can manifest as macroglossia, shoulder pad sign, raccoon eyes, carpal tunnel syndrome, synovial enlargement, and firm, enlarged lymph nodes. In ATTRwt, carpal tunnel and lumbar stenosis are frequently reported.
ANS, autonomic nervous system; PNS, peripheral neuropathic involvement; ST, soft tissue; ATTRv, transthyretin amyloidosis variants; AA, serum amyloid A; ApoAI, apolipoprotein AI; ALECT2, leukocyte chemotactic factor 2 amyloidosis.
*Bone tracers validated for the detection of cardiac amyloidosis are ^{99m}Tc-diphosphono-propanodicarboxylic acid, ^{99m}Tc-pyrophosphate, and ^{99m}Tc-hydroxymethylene diphosphonate; +++, ≥50%; ++, 10%-30%; +, ≤10%; —, rare or not involved.

early death and causes extreme frailty limiting the delivery of effective therapy. In recent years, advancements in biomarker-based risk stratification and monitoring of response and novel anti-PC agents has improved outcomes. Early and correct diagnosis is the prerequisite to beneficial use of these tools.

Diagnostic workup

The clinical presentation of AL amyloidosis depends on organ involvement, and it is protean and deceitful. Symptoms are often misinterpreted and recognized late. When they appear, organ involvement is often irreversible. However, cardiac and renal amyloidosis can be detected by NT-proBNP and albuminuria before overt heart failure and nephrotic syndrome arise. Moreover, a monoclonal component can be found at least 4 years before diagnosis.[13] Therefore, hematologists can intercept, diagnose, and treat patients during the presymptomatic stage, by including biomarkers of organ involvement in the monitoring panel of subjects with monoclonal gammopathy of undetermined significance (MGUS).[14] Recent findings suggest that CMR can detect very early cardiac involvement[15]; hence, CMR may be used as a confirmatory test in subjects with MGUS in whom elevated NT-proBNP is found. However, the cost effectiveness of this approach is unknown. A diagnostic flowchart for AL amyloidosis is reported in Figure 1.

A tissue biopsy is always necessary to establish the diagnosis of AL amyloidosis. Notably, other types of systemic amyloidosis can have clinical presentations that overlap that of AL amyloidosis (Table 2). Of these, wild-type transthyretin (ATTRwt) amyloidosis, is the most common. Effective treatments are now available for wild-type and hereditary ATTR amyloidosis, but correct amyloid typing is mandatory. The other, rarer types should not be disregarded. In contrast to AL amyloidosis, the diagnosis of ATTRwt amyloidosis requires tissue typing only in patients in whom a monoclonal component is detected. In the absence of a monoclonal component, a nonbiopsy diagnosis of ATTRwt amyloidosis is possible based on cardiac uptake of bisphosphonate scintigraphy tracers.[16] Patients with cardiac AL amyloidosis usually have no or modest uptake; however, patients may have intense uptake. Moreover, one-fourth to one-third of patients with ATTRwt have a monoclonal component. AL amyloidosis is by far the most rapidly progressing type of cardiac amyloidosis and is the one that benefits most from early initiation of effective therapy. Thus, the first step in the diagnostic workup of cardiac amyloidosis should be searching for monoclonal components. In the present clinical case, a substantial diagnostic delay resulted from deferred testing for PC dyscrasia.

Figure 1 (continued) glands, and bone marrow, and most patients can be spared biopsy of the involved organ. However, if amyloidosis is deemed probable, for a prompt start of treatment, organ biopsy should not be deferred. Amyloid deposits are recognized as nonbranching fibrils of 7 to 10 nm in width, detected by light microscopy with green birefringence under polarized light after staining with Congo red or by electron microscopy. The diagnostic sensitivity of abdominal fat aspirate combined with bone marrow or minor salivary gland biopsy is ~90% at referral centers, but the recognition of amyloid deposits is affected by the experience of the pathologist. With a few exceptions (eg, patients with a monoclonal component and periorbital purpura and/or macroglossia, or combination of amyloid heart and renal involvement with albuminuria), the clinical presentation of AL amyloidosis cannot reliably be differentiated from that of other types of systemic amyloidosis. Thus, amyloid tissue typing with adequate technology is mandatory. Standard light microscopy immunohistochemistry does perform satisfactorily, and patients should be referred to specialized centers for typing with adequate technology (immunohistochemistry with custom-made antibodies, IEM, or MS). Accurate clonal studies, biomarker-based staging, and assessment of comorbidities are necessary to design the therapeutic strategy. MRI, magnetic resonance imaging.

Table 3. Validated criteria for HR and organ response

Category	Criteria
HR	CR (all of the following criteria must be met): • Serum and urine immunofixation negative for monoclonal protein • Normalized free light chain ratio
	VGPR reduction of dFLC below 40 mg/L
	PR 50% reduction of dFLC
Organ response	Kidney: a 30% reduction in 24-h urine protein excretion or a drop of proteinuria below 0.5 g per 24 h in the absence of progressive renal insufficiency, defined as a decrease in eGFR to 25% over baseline.
	Heart (NT-proBNP based): reduction of NT-proBNP of 30% and >300 ng/L over the starting value. Baseline NT-proBNP has to be ≥650 ng/L to be measurable.
	Heart (BNP based): reduction of BNP of 30% and >50 ng/L over the starting value. Baseline BNP has to be ≥150 ng/L to be measurable.
CHOR model	Patients are classified in 2 CHOR groups according to a score based on the HR and organ response criteria • Score for HR: CR, 0; VGPR, 1; PR, 2; no response, 3 • Score for organ response: response in all organs involved, 0; response in at least 1 but not all the organs involved, 1; no organ response, 2.
	CHOR group 1, score 0-3
	CHOR group 2, score 4-5

CHOR, composite hematologic/organ response model; PR, partial response.

Once the diagnosis of AL amyloidosis is established, the characteristics of the underlying clone and the extent and severity of organ involvement should be studied (Figure 1). This information is essential for designing the therapeutic strategy.

Up-front therapy

Treatment should be risk adapted, considering the severity of organ involvement, characteristics of the clone, and comorbidities and seeking to deliver the most rapid and effective therapy patients can safely tolerate. Delicate up-front therapy can sometimes trigger early improvement of organ dysfunction, allowing for subsequent, more aggressive treatment. Early and profound reductions of the amyloid LC are associated with the greatest chance of organ improvement, and prolongation of progression-free (PFS) and overall (OS) survival.[17-19] Changes in biomarkers can be used to assess response to therapy according to validated criteria (Table 3).[12,17,20,21] The optimal end point of therapy is still a matter of debate. Achievement of organ response can indicate that the amyloid LC level has fallen below the concentration needed to sustain organ dysfunction. Organ response can parallel HR, as it did in the patient described herein, but it is sometimes delayed. For this reason, assessment of treatment efficacy and a decision to shift to rescue regimens should be based on early HR assessment (3 months after autologous stem cell transplantation [ASCT], 1 to 2 months after nontransplantation therapies). Achievement of organ response and profound clonal response should be the long-term goal of therapy. Individual frailty, age, bone marrow PC infiltration, residual organ dysfunction, and treatment tolerability should be considered to decide whether CR should be pursued. Novel definitions of deep HR are being investigated.[18,19] The relatively low sensitivity and imprecision of available FLC assays demand novel technologies, such as mass spectrometry (MS)–based detection of monoclonal components[22] and assessment of MRD[23] that await validation in AL amyloidosis.

Accurate risk stratification is crucial in designing the treatment strategy (Figure 2). Approximately 20% of patients with newly diagnosed disease are candidates for ASCT, and more can become eligible after effective up-front therapy. Moreover, pretransplantation induction therapy independently improves PFS.[24] In a large series, 84% of patients attained HR after ASCT (VGPR, 33%; CR, 39%),[25] and the CR rate can increase to ~60% (~40% MRD^{-}) with posttransplantation bortezomib-based treatment.[26] OS of patients who reach CR with ASCT is >50% at 15 years.[27]

Most patients with AL amyloidosis are not eligible for ASCT. Oral melphalan + dexamethasone (MDex) has been a standard of care for many years in these subjects. Currently, bortezomib is the backbone of up-front treatment regimens and is combined with MDex (BMDex) or with cyclophosphamide and dexamethasone (CyBorD). A phase 3 trial in intermediate-risk patients (#NCT01277016) showed that BMDex induces a significantly higher HR rate (81% vs 57%; CR, 23% vs 20%; VGPR 42% vs 20%) than MDex, with prolonged OS.[28] Cardiac and renal responses were observed in 38% and 44% of cases with BMDex and in 28% and 43% of cases with MDex, respectively.[28] In a large, unselected, retrospective series, overall HR rate in response to CyBorD was 65% (CR, 25%; VGPR, 24%), with cardiac response in 33% of patients and renal response in 15%.[18] A phase 3 trial comparing CyBorD with CyBorD+subcutaneous daratumumab has been completed (#NCT03201965). The uncontrolled safety assessment performed in a portion of the study showed a remarkable 96% overall HR rate.[29] The randomized trial preliminary results indicate that addition of daratumumab results in significantly higher hematologic (92% vs 77%; CR/VGPR, 79% vs 42%), cardiac (42% vs 22%), and renal (54% vs 27%) response rates across cardiac and renal stages and independent of t(11;14) and prolonged PFS.[30]

Approximately 20% of patients have advanced (stage IIIb) cardiac involvement at diagnosis. Treatment of these patients remains an unmet need. However, if a profound response is reached within 1 month, OS can improve, even in these subjects.[31] Ideally, these patients need a very rapidly acting, safe regimen. The safety profile and rapidity of action of subcutaneous daratumumab make

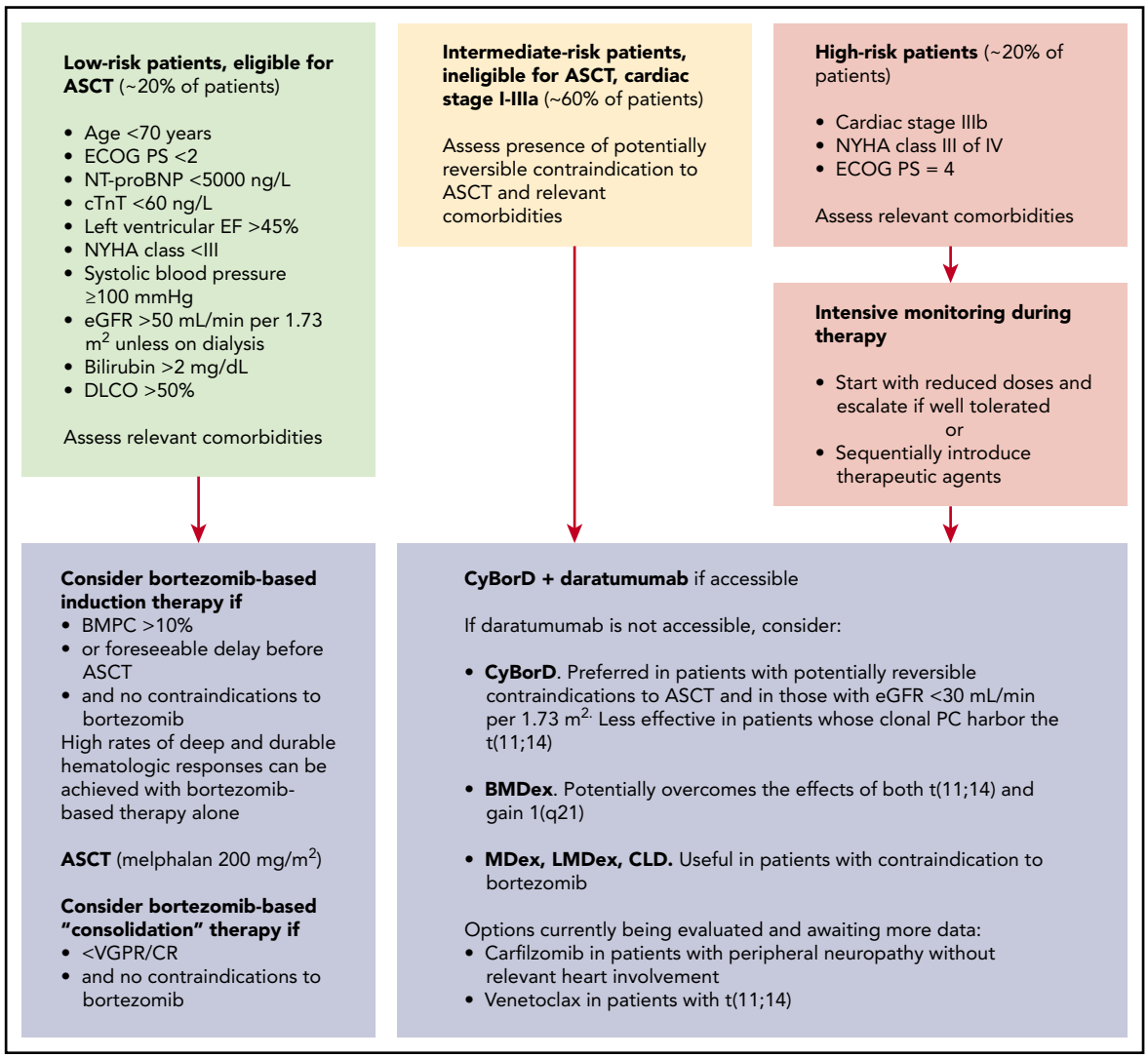

Figure 2. Treatment strategy for patients with newly diagnosed AL amyloidosis. The design of the treatment strategy requires accurate risk stratification. In the past, transplant-related mortality related to advanced amyloid organ involvement was very high. Refinement of selection criteria with the inclusion of cardiac biomarkers resulted in a significant improvement in tolerability of ASCT. Subjects who are not transplantation eligible at diagnosis, may become suitable transplantation candidates if they attain organ response after up-front therapy. Melphalan dose adjustment to extend ASCT eligibility does not decrease toxicity but negatively impacts response rate and should be discouraged. Pretransplant therapy with bortezomib-based regimens is beneficial in patients with a bone marrow PC infiltrate >10% but can be considered in all patients to attain rapid reduction of the amyloid light chain, if harvesting procedures and ASCT scheduling can result in a relevant delay. Moreover, recent data indicate that induction therapy independently increases PFS. Importantly, bortezomib-based therapy alone can grant satisfactory (CR and/or organ response) and durable response in some patients who may then not proceed to ASCT. Posttransplantation therapy with bortezomib-based regimens increases CR rate and extends PFS in patients who attain less than VGPR after ASCT. Amyloidogenic PCs depend on proteasomes to survive the stress caused by toxic LCs, resulting in particular sensitivity to proteasome inhibition, and bortezomib is also the cornerstone of treatment of patients who are not eligible for ASCT. Combination of bortezomib-based regimens with daratumumab will most likely become novel standards of care based on the results of recent clinical trials. In current clinical practice, daratumumab is still not widely accessible, and CyBorD is preferred over BMDex in patients with renal failure and in those who may later become eligible for ASCT, whereas BMDex may overcome the negative effects of both t(11;14) and gain 1q21. Venetoclax is also an appealing option for patients with t(11;14), but few data are available so far. Relevant comorbidities include potential contraindications to bortezomib, such as peripheral neuropathy and pulmonary fibrosis. Oral MDex or immunomodulatory drug (IMiD)-based regimens are valuable alternatives for subjects with contraindications to bortezomib. Carfilzomib can also be considered in patients with peripheral neuropathy, carefully balancing potential cardiac toxicity. Patients with non-PC clones should be treated with regimens specifically targeting the underlying amyloid clone.

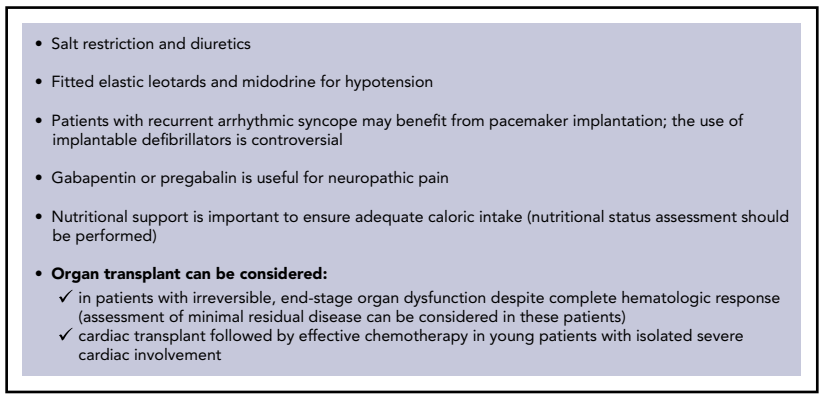

Figure 3. Supportive therapy in AL amyloidosis. Supportive measures have a fundamental role in the management of AL amyloidosis, with the goal of improving quality of life, relieving symptoms, and sustaining organ function while anti-PC therapy is delivered and takes effect. The mainstay of supportive treatment is diuretic therapy. However, in amyloidosis, cardiac function is preload dependent, and it is important to avoid reduction of intravascular volume. Angiotensin-converting enzyme inhibitors are generally poorly tolerated because of hypotension: they should be used at the lowest possible dose and discontinued if not well tolerated. Transplantation of the organs involved by amyloidosis may render patients with advanced disease eligible for aggressive specific treatment. The main concerns with organ transplantation are occurrence of amyloidosis in the graft and progression in other organs. However, the availability of effective anti-PC treatments allows for consideration of heart transplantation followed by effective chemotherapy in young patients with isolated severe cardiac involvement. Patients who have advanced, irreversible organ damage, despite achievement of complete HR, can also be considered for transplantation of the organs involved. However, early reports still awaiting confirmation suggest that patients who fail to attain organ response despite having achieved complete HR may have persistent minimal residual clonal disease. In these subjects, further chemotherapy, if deliverable, may lead to minimal residual disease negativity and improvement of organ dysfunction. Implantation of left ventricular assist devices is technically feasible for patients with severe heart failure caused by advanced cardiac amyloidosis, but the possible benefit is unclear.

this agent appealing in this setting, and a phase 2 trial is under way (#NCT04131309). Supportive therapy is vital to sustaining organ function while chemotherapy is delivered (Figure 3).

Treatment of relapsed/refractory disease

Patients who do not attain satisfactory response should be shifted to second-line treatment as early as possible. So far, there is no evidence to support maintenance therapy in responders, who should be closely followed. However, there is no consensus on when treatment should be started at relapse. We lack validated hematologic progression criteria, and the definition of PFS varies in different studies. Organ progression criteria predict shorter patient and renal survival and such progression should not be awaited before starting rescue therapy.[12,17] In general, organ progression is preceded by FLC increases, which can be small and should not be disregarded.[32] Other factors to be considered are FLC level and severity of organ involvement at diagnosis, as well as the quality of response to previous treatment.

The mainstay of rescue therapy is IMiDs that can overcome resistance to alkylating agents and proteasome inhibitors, with an OS benefit in responders. In a pooled analysis of patients enrolled in 2 phase 2 clinical trials of lenalidomide and 1 of

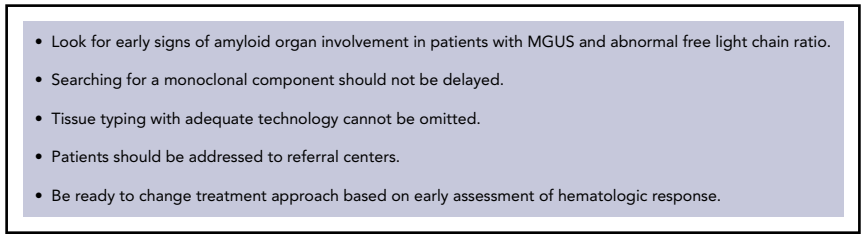

Figure 4. Common pitfalls in the management of patients with AL amyloidosis. A presymptomatic biomarker-based diagnosis is possible in patients at risk (subjects with MGUS and abnormal FLC ratio). Treating patients at early stages facilitates the access to effective therapies and can improve survival. The diagnostic pathways for AL and non-AL amyloidosis are different and the choice depends on the presence or absence of a monoclonal component. AL amyloidosis progresses more rapidly, but available treatments can rapidly reverse the course of the disease. The diagnosis of AL amyloidosis should not be delayed. Positive cardiac scintigraphy with bone tracers is not enough to establish a diagnosis of ATTR amyloidosis in a patient with a monoclonal component. Uncharacterized amyloid deposits on a tissue biopsy in a patient with a monoclonal component are not enough to establish a diagnosis of AL amyloidosis. Prespecified treatment duration and/or number of cycles should be avoided in AL amyloidosis. The goal is rapid and deep HR and if it is not reached, rescue therapy is needed. Organ response can sometimes be delayed.

pomalidomide, 39% of patients achieved VGPR or CR.[33] In a real-world European study of pomalidomide, overall HR rate was 44% (CR, 3%; VGPR, 23%).[34] Treatment with IMiDs interferes with cardiac response assessment, being associated with NT-proBNP increase, and worsening renal failure can be observed in patients with proteinuria. A phase 3 study compared the oral proteasome inhibitor ixazomib with physician's best choice (lenalidomide in 57% of patients) in relapsed/refractory AL amyloidosis.[35] The study failed to meet its primary end point, an improvement in overall HR rate (53% vs 51%), but CR rate was higher (26% vs 18%) and PFS longer in ixazomib-treated patients. Ixazomib can be safely combined with lenalidomide and dexamethasone in an all-oral regimen.[36]

Non-IMiD–based rescue has recently been evaluated. A phase 2 study reported a 57% HR rate (CR 11%) with bendamustine+ dexamethasone.[37] In the past few months, 2 phase 2 trials and numerous retrospective series have addressed the efficacy of daratumumab-based regimens in relapsed/refractory disease. Sanchorawala et al observed a remarkable 90% response rate (CR 41%) in a phase 2 single-agent trial of daratumumab.[38] Cardiac and renal response rates were also high (50% and 67%, respectively).[38] In a European trial, 55% of patients responded to daratumumab (CR 8%), with lower cardiac (25%) and renal (31%) response rates.[39] Notably, in this trial, >50% of patients had not achieved VGPR or better with previous lines of therapy.[39] Close monitoring revealed that most HRs occurred after the first daratumumab infusion.[38,39] The largest retrospective study included 168 patients treated with daratumumab alone or combined with bortezomib.[40] There was no significant difference in outcome with the 2 regimens, and overall HR rate was ~65% (CR/VGPR ~50%).[40] Interestingly, shorter PFS was observed in patients with nephrotic syndrome.[40] Combination of daratumumab with IMiDs is particularly interesting in relapsed/refractory disease, and several clinical trials are under way.

Conclusion

Despite late recognition of symptoms and delayed referral to a specialized center, the patient described herein benefited from sequential treatment with powerful and rapidly acting regimens guided by biomarker-based risk stratification and monitoring. General practitioners, cardiologists, and nephrologists should be able to recognize symptoms early and to suggest appropriate testing (first, search for a monoclonal component) that can direct patients to the diagnostic pathways of AL or non-AL amyloidosis. Hematologists are in the unique position of recognizing and treating presymptomatic patients. Common pitfalls in the management of patients with AL amyloidosis are reported in Figure 4.

After many years without positive controlled studies, in the past few months, 3 phase 3 trials have been published.[28,30,35] Daratumumab combined with bortezomib emerged as a new standard of care. Yet, therapeutic innovation left old questions unanswered and opened new ones. A standard of care for high-risk patients is lacking, and the role of maintenance must be clarified, as well as the positioning of ASCT in the new scenario. A validated definition of hematologic progression would be useful in patient care and in the design of future trials. Further advancements in anti-PC therapy is likely to be based on depth of response, and validation of MS-based detection of monoclonal components and of MRD assessment is warranted to establish optimal goals of therapy. Newer anti-PC approaches, including CAR-T cells and antibody drug conjugates are being considered. Exploration of additional treatment targets, such as interference with LC toxicity by LC stabilizers or doxycycline, which may also target amyloid deposits and is currently undergoing testing in a randomized trial (#NCT03474458), should not be abandoned.

Conflict-of-interest disclosure

G.P. has received honoraria and serves on the advisory board of Janssen-Cilag and has received travel grants from Celgene. P.M. has received honoraria as a speaker for Pfizer and Janssen-Cilag and travel support from Celgene. G.M. reports no competing financial interests.

Off-label drug use

None disclosed.

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Management of Waldenström macroglobulinemia in 2020

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The management of Waldenström macroglobulinemia (WM) has evolved tremendously with recent genomic discoveries that correlate with clinical presentation and could help to tailor treatment approaches. The current diagnosis of WM requires clinicopathological criteria, including bone marrow involvement by lymphoplasmacytic lymphoma cells, a serum immunoglobulin M (IgM) monoclonal paraprotein, and presence of the *MYD88 L265P* mutation. Once the diagnosis is established, the relationship between the patient's symptoms and WM should be carefully investigated, because therapy should be reserved for symptomatic patients. Bone marrow involvement and serum levels of IgM, albumin, and β2-microglobulin can be used to estimate the time until treatment initiation. The treatment of WM patients should be highly personalized, and the patient's clinical presentation, comorbidities, genomic profile, and preferences, as well as toxicity of the treatment regimens, should be taken into account. Alkylating agents (bendamustine, cyclophosphamide), proteasome inhibitors (bortezomib, carfilzomib, ixazomib), anti-CD20 monoclonal antibodies (rituximab, ofatumumab), and Bruton tyrosine kinase (BTK) inhibitors (ibrutinib, acalabrutinib, zanubrutinib) are safe and highly effective treatment options in patients with WM. Because novel covalent and noncovalent BTK inhibitors (tirabrutinib, vecabrutinib, LOXO-305, ARQ-531), BCL2 antagonists (venetoclax), and CXCR4-targeting agents (ulocuplumab, mavorixafor) are undergoing clinical development in WM, the future of WM therapy certainly appears bright and hopeful.

LEARNING OBJECTIVES

- Describe in detail the criteria for establishing the diagnosis of WM, as well as indications to treat
- Review current and upcoming treatment options for patients with symptomatic WM, focusing on the impact of genomic-driven therapies

Clinical case

A 66-year-old asymptomatic man underwent a routine physical examination and was found to have a high serum protein level. Serum protein electrophoresis detected an immunoglobulin M (IgM) κ monoclonal paraprotein. Complete blood count and renal and hepatic function tests were normal. The patient was referred to a hematologist/oncologist for further workup. Serum IgM level was 3500 mg/dL, serum albumin level was 4 g/dL, and serum β2-microglobulin level was 2.5 mg/L. A bone marrow biopsy was performed and showed 40% involvement by κ-restricted lymphocytes and lymphoplasmacytoid cells with positive CD20 and CD38 expression and negative CD5 and CD10 expression, consistent with lymphoplasmacytic lymphoma (LPL). The *MYD88 L265P* mutation was detected by polymerase chain restriction assay. *CXCR4* mutations were not evaluated. Computed tomography (CT) scans of the chest, abdomen, and pelvis showed no evidence of lymphadenopathy or organomegaly. A funduscopic examination did not show evidence of hyperviscosity-related changes.

Initial management

The first step in the management of Waldenström macroglobulinemia (WM) is to properly establish the diagnosis. Based on criteria from the Second International Workshop for Waldenström macroglobulinemia (IWWM), a bone marrow lymphoplasmacytic infiltrate of any level and an IgM monoclonal paraprotein of any size are required for WM diagnosis.[1] LPL typically has an intertrabecular pattern of bone marrow infiltration, and the immunophenotype is characterized by positive expression of surface IgM, CD19, CD20, CD22 (dim), CD25, and CD27 and negative expression of CD5, CD10, CD23, and CD103.[2] Approximately 5% of patients with LPL will secrete a different protein than

IgM and are not considered to have WM. However, the clinical features of non-IgM LPL are similar to WM, although non-IgM LPL patients are less likely to develop neuropathy or hyperviscosity and also have similar outcomes.[3] Therefore, the management of non-IgM LPL should follow the guidelines for WM. The *MYD88 L265P* mutation is detected in >90% of WM patients.[4-7] On the other hand, *MYD88* mutations are detected in 5% to 10% of patients with chronic lymphocytic leukemia (CLL) or marginal zone lymphoma, and no *MYD88* mutations have been detected in multiple myeloma. Non-L265P *MYD88* mutations have been described in WM patients, and testing requires sequencing of the entire MYD88 gene.[8] In this case, with an elevated serum IgM level, a lymphoplasmacytic infiltrate of the bone marrow, and presence of the *MYD88 L265P* mutation, the diagnosis of WM is confirmed.

The second step in the management of WM patients is to establish a relationship between the patient's symptoms, if any, and the underlying disease.[9] Asymptomatic or minimally symptomatic WM patients should not be treated. Reasons behind this recommendation include disease incurability, prolonged survival of patients, and toxicity and promotion of resistance associated with therapy. Common indications to treat WM patients include symptomatic anemia, lymphadenopathy, hyperviscosity, or neuropathy.[10] Symptomatic cryoglobulinemia, cold agglutinin disease, renal dysfunction, amyloidosis, pleural effusions, and central nervous system involvement are uncommon indications to treat. In our case, the patient is asymptomatic, not anemic, and without evidence of extramedullary disease or hyperviscosity. Therefore, treatment is not indicated. In these situations, the risk of progression to symptomatic disease should be estimated.[11] Given the patient's serum IgM level, percentage of bone marrow involvement, and serum albumin and β2-microglobulin levels, the patient would fall into an intermediate-risk category, with an estimated median time to symptomatic disease ~5 years. Monitoring without intervention is a reasonable approach. Patients in this setting can be seen every 3 months for clinical evaluations, including symptom reporting, physical examination, and laboratory studies, such as complete blood counts, comprehensive metabolic panel, and serum immunoglobulin levels. Yearly funduscopic examinations are recommended in all WM patients with serum IgM levels ≥ 3000 mg/dL, because the risk of developing symptomatic hyperviscosity appeared to be negligible at lower levels.[12]

Clinical case (continued)

The patient was clinically evaluated every 3 months and underwent yearly funduscopic examinations. Three years later, the patient presented with recurrent nosebleeds and progressive fatigue affecting his activities. Hemoglobin was 9.2 g/dL, platelets were 115 000 per microliter, and serum IgM level was 5500 mg/dL. There was no evidence of hemolysis or iron, cobalamin, or folate deficiency. Hepatitis B, hepatitis C, and HIV testing was negative. Funduscopic examination revealed engorgement of retinal vessels and scattered retinal microhemorrhages bilaterally. A bone marrow biopsy showed 80% involvement by LPL. *MYD88 L265P* was detected by polymerase chain reaction, and *CXCR4 T318fs* (frameshift) was detected by next-generation sequencing assays. CT scans of the chest, abdomen, and pelvis showed generalized lymphadenopathy, with maximum diameter of 3 cm, without hepatosplenomegaly.

Frontline treatment approach

At this time, the patient meets the criteria for treatment initiation, given his symptomatic anemia and evidence of hyperviscosity, according to the guidelines by the Second IWWM.[10] Because other causes of anemia and thrombocytopenia have been ruled out, we can assume that the patient's cytopenias are related to WM. Given the symptoms of hyperviscosity, prompt initiation of plasmapheresis is appropriate to prevent potential thrombotic and/or hemorrhagic complications.[13] Plasmapheresis, however, does not constitute definitive treatment of active WM and should be used as a transition toward primary therapy.[13] In this setting, screening tests for acquired von Willebrand disease (vWD), such as von Willebrand antigen, ristocetin cofactor, and factor VIII levels, should be performed. Patients with high serum IgM levels and *CXCR4* mutations had a higher incidence of acquired vWD,[14] which increases the risk of bleeding complications with surgical procedures. The levels of vWD markers typically improve with decreasing serum IgM levels on therapy.

There are several primary therapy options for patients with active symptomatic WM, and the safety and efficacy profiles of selected regimens are shown in Table 1. All patients with WM should be considered for clinical trials, whenever appropriate.[15] A suggested treatment algorithm for treatment-naive WM patients is shown in Figure 1. In this case, a treatment regimen associated with a rapid decrease in serum IgM levels would be preferred. Single-agent rituximab is less effective in WM patients with serum IgM levels ≥ 4000 mg/dL, and the median time to response ranges between 3 and 6 months.[16] Also, 40% to 50% of WM patients exposed to single-agent rituximab can experience an IgM flare, which can induce rapid increases in serum IgM ranging from 25% to 300% and could worsen hyperviscosity symptoms.[17] In this setting, alkylating agents or proteasome inhibitors in combination with rituximab, as well as ibrutinib with and without rituximab, are reasonable options.

A careful and thorough discussion between practitioners and patients should take place on the positive and negative aspects of each treatment option. All of the regimens mentioned above are associated with high overall and major response rates. Therapy selection in WM patients should be personalized, taking into account the patient's symptoms, comorbidities, genomic profile, preferences, and insurance coverage, as well as the safety profile of the regimen. *MYD88* wild-type and *CXCR4* mutated status have been associated with lower efficacy rates with ibrutinib monotherapy.[8,18] *CXCR4* mutations do not seem to impact progression-free survival (PFS) on alkylator-based or proteasome inhibitor–based regimens.[19,20] Alkylators are associated with a 1% to 2% risk for myeloid neoplasms, bortezomib is associated with a 20% to 25% risk for peripheral neuropathy, and ibrutinib is associated with a 5% to 10% risk for atrial fibrillation.[21-23] The administration of these agents also differs; bendamustine is administered IV and bortezomib is given subcutaneously and both are of finite duration, whereas ibrutinib is an oral agent of indefinite duration.

The role of maintenance rituximab therapy after induction chemoimmunotherapy in WM patients continues to evolve. Several retrospective studies have suggested a deepening of response, as well as PFS and overall survival benefits in WM patients treated with maintenance rituximab vs observation after rituximab-containing regimens.[24-26] However, preliminary data from the MAINTAIN study, presented at the 2019 American

Table 1. Selected treatment regimens for patients with WM

Study	Agent	N (TN/RR)	ORR, %	MRR, %	VGPR, %	PFS	Adverse events
Dimopoulos et al[44]	Cyclophosphamide, D, R	72 (72/0)	83	74	7	Median: 35 mo	Cytopenias, infections, myeloid neoplasms
Rummel et al[45]	Bendamustine, R	19 (19/0)	NR	NR	NR	Median: 69.5 mo	
	R-CHOP	22 (22/0)	NR	NR	NR	Median: 28 mo	
Rummel et al[27]	Bendamustine, R	257 (257/0)	92	88	4	Median: 65 mo	
Treon et al[22]	Bortezomib (twice weekly), D, R	23 (23/0)	96	83	22	Median: 66 mo	Neuropathy, neutropenia, infections
Dimopoulos et al[46]	Bortezomib (weekly), D, R	59 (59/0)	85	58	10	Median: 42 mo	
Treon et al[47]	Carfilzomib, D, R	31 (31/0)	87	68	35	Median: 44 mo	Hyperglycemia, hyperlipasemia
Castillo et al[48]	Ixazomib, D, R	26 (26/0)	96	77	15	Median: NR at 22 mo	Infections, hyperglycemia
Treon et al[28,29]	Ibrutinib	63 (0/63)	91	81	16	5 y: 54%	Cytopenias, bleeding, arrhythmias, hypertension
Treon et al[36]	Ibrutinib	30 (30/0)	100	83	20	18 mo: 92%	
Dimopoulos et al[34]	Ibrutinib, R	75 (34/41)	93	73	26	30 mo: 82%	
Owen et al[37]	Acalabrutinib	106 (14/92)	93	78	8 (IWWM6) 29 (IWWM3)	24 mo: 90% (TN); 82% (RR)	

D, dexamethasone; MRR, major response rate; NR, not reported; R, rituximab; R-CHOP, rituximab, cyclophosphamide, doxorubucin, vincristine, and prednisone; RR, relapsed/refractory; TN, treatment naive.

Society of Hematology Annual Meeting, did not find any PFS or overall survival benefit from maintenance rituximab vs observation after attaining a partial response or better to bendamustine and rituximab.[27] It is important to note that patients who attained a minor response after induction were not randomized and that patients older than 65 years or with high-risk disease, based on the International Prognostic Scoring System for WM, seemed to have derived survival benefit from maintenance therapy.

Clinical case (continued)

The patient went on to receive 6 cycles of bendamustine and rituximab. At the end of therapy, the patient's blood counts normalized, his lymphadenopathy resolved, and his serum IgM level was 1400 mg/dL, consistent with a partial response. His symptoms also resolved, and the patient was monitored every 3 months. Three years later, the patient presented with progressive fatigue and symptomatic anemia. His hemoglobin level was 9.7 g/dL, his platelet count was 110 000 per microliter, and his serum IgM level was 3400 mg/dL. Funduscopic examination did not show changes associated with hyperviscosity. CT scans did not show any evidence of lymphadenopathy or organomegaly. A bone marrow aspiration and biopsy showed 80% involvement by LPL, without evidence of dysplasia. *MYD88 L265P* and a frameshift *CXCR4* mutation were detected.

Treatment options in the relapsed setting

Current treatment options for patients with previously treated WM are highly effective. Selected regimens in this setting are shown in Table 1. A suggested treatment algorithm for previously treated WM patients is shown in Figure 2. As with primary therapy, a personalized approach should be followed when selecting treatments for patients with relapsed WM. Given prior exposure to alkylating agents, Bruton tyrosine kinase (BTK) inhibitors are reasonable in this setting, because they have been associated with response rates well over 90% and median PFS in excess of 5 years.[28,29] In the pivotal phase 2 study of 63 relapsed WM patients, ibrutinib monotherapy, at a dose of 420 mg by mouth every day, was associated with high overall response rate (ORR), major response, and very good partial response (VGPR) rate, with an estimated 2-year PFS of 69%. These results paved the way for the US Food and Drug Administration approval of ibrutinib in symptomatic WM patients in April of 2015. Long-term data from this study were presented at the 2019 Lugano Conference[29] and showed deepening of major response and VGPR, with a 5-year PFS rate of 54%. Patients with *MYD88* mutation and without *CXCR4* mutations had higher ORR, major response rate, and 5-year PFS to ibrutinib monotherapy than patients with *MYD88* and *CXCR4* mutations. Although *CXCR4* mutations adversely impact depth and duration of response to ibrutinib, patients with frameshift *CXCR4* mutations (rather than nonsense mutations) seem to derive similar benefits from ibrutinib therapy as do patients without *CXCR4* mutations.[18]

However, one must be aware of specific side effects associated with ibrutinib therapy. Early side effects include rash, diarrhea, abdominal bloating, and nausea, which improve and resolve within a few weeks on therapy in most patients. Long-term side effects include bleeding, arrhythmia, and withdrawal symptoms. Ibrutinib affects platelet aggregation and adhesion,[30] increasing the risk of bleeding with surgical procedures, and it should be held temporarily for a few days before and after each procedure to minimize bleeding risk. Ibrutinib has also

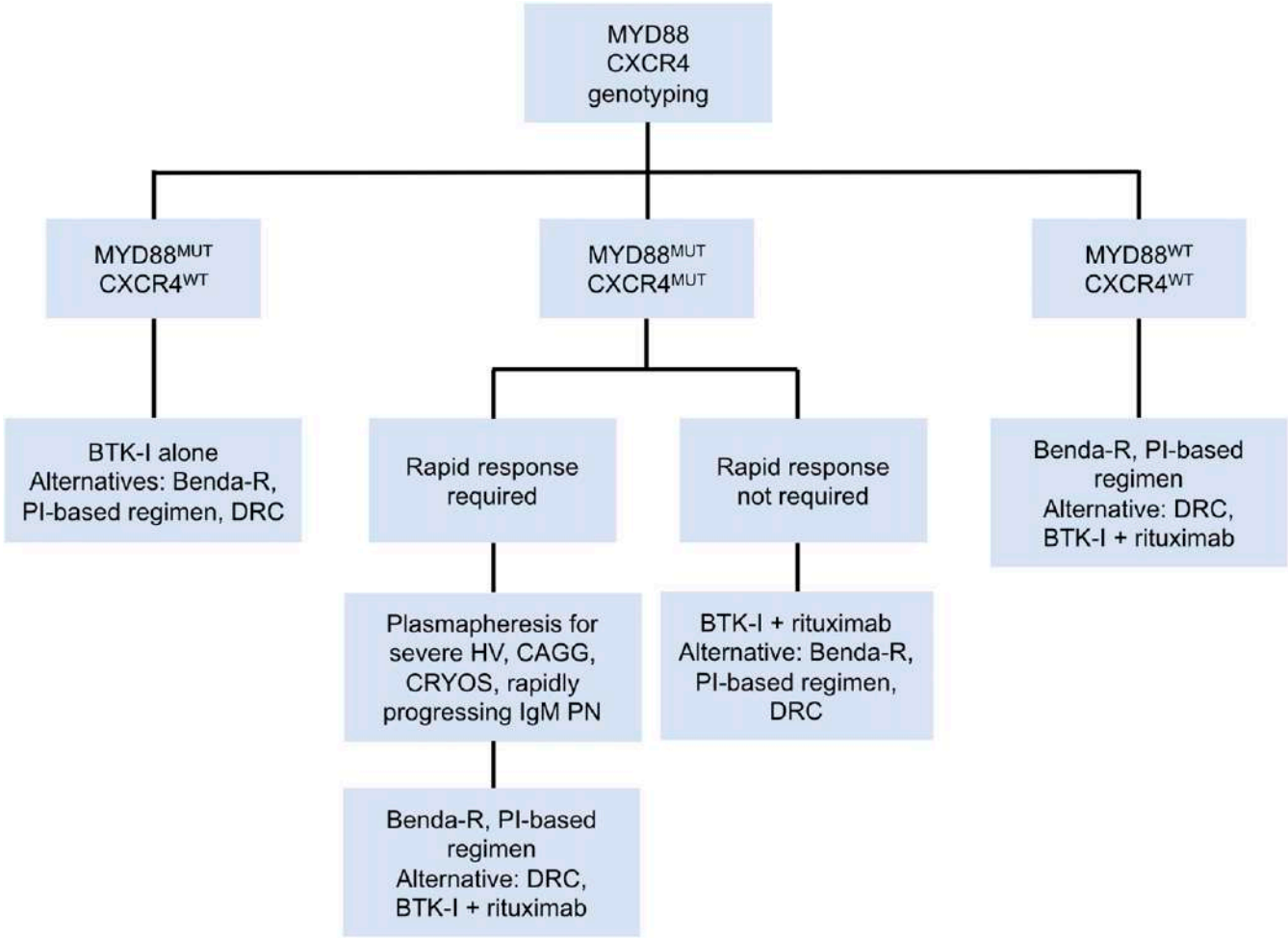

Figure 1. Genomic-based treatment algorithm for symptomatic treatment-naive patients with WM. Benda-R, bendamustine and rituximab; BTK-I, Bruton tyrosine kinase inhibitor; CAGG, cold agglutinin disease; CRYOS, cryoglobulins; DRC, dexamethasone, rituximab, cyclophosphamide; HV, hyperviscosity; PI, proteasome inhibitor; PN, progressive neuropathy. Adapted with permission from Treon et al.[49]

been associated with an increased risk for arrhythmia, especially atrial fibrillation.[31] Βετα-blockers, anticoagulants, antiarrhythmics, and/or cardiac ablation can be used, if necessary, under the care of a cardiologist with experience with this complication. An algorithm for the management of ibrutinib-related atrial fibrillation has been published.[32] About 20% of WM patients who discontinue ibrutinib temporarily might experience withdrawal symptoms, such as fever, night sweats, and fatigue, which could be managed with low doses of steroids during the hold.[33] An increase in serum IgM levels can also be seen during holds and should not be considered disease progression, because serum IgM levels decrease promptly after restarting ibrutinib.

A multicenter randomized phase 3 study (INNOVATE) evaluated the combination of ibrutinib and rituximab vs placebo and rituximab in 150 patients with WM.[34] The combination of ibrutinib and rituximab was associated with a higher ORR (92% vs 47%) and major response rate (72% vs 32%), as well as higher 30-month PFS (82% vs 28%), compared with placebo and rituximab. There were higher rates of atrial fibrillation, hypertension, and serious respiratory infections and lower rates of infusion-related reactions and IgM flare in patients who received ibrutinib and rituximab compared with placebo and rituximab. Based on these results, the US Food and Drug Administration approved the combination of ibrutinib and rituximab for symptomatic WM in August of 2018. The results of that study do not address whether the combination of ibrutinib and rituximab is superior to ibrutinib monotherapy in WM patients, and it is unlikely that a study addressing that question in WM patients will ever be done. In a randomized study of patients with CLL, the combination of ibrutinib and rituximab was not associated with superior response rates or longer PFS compared with ibrutinib alone,[35] making the addition of rituximab to ibrutinib of unclear long-term benefit in CLL. On the other hand, the combination of ibrutinib and rituximab induced major responses in 54% of the 16 WM patients without *MYD88* mutation, whereas the major response rate to ibrutinib alone was 0% in 5 WM patients without *MYD88* mutation.[8] In patients with *CXCR4* mutations, the median time to major response with the combination of ibrutinib and rituximab was 3 months, whereas prior studies had reported a median time to response of 6 to 7 months with ibrutinib monotherapy.[29,36] It is unclear whether the addition of rituximab to ibrutinib would benefit all patients. The combination of

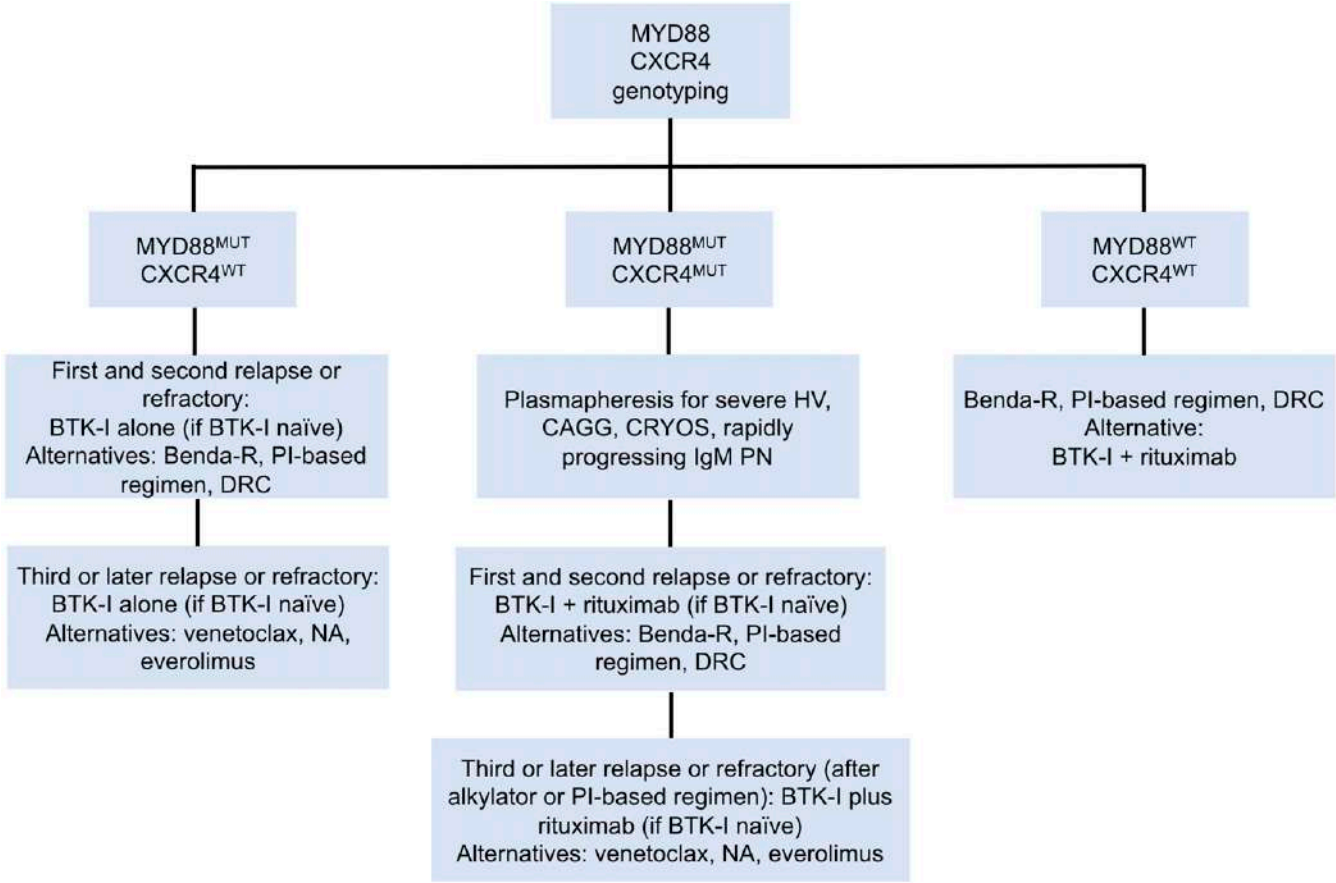

Figure 2. Genomic-based treatment algorithm for symptomatic, previously treated, or refractory patients with WM. Benda-R, bendamustine and rituximab; BTK-I, BTK inhibitor; CAGG, cold agglutinin disease; CRYOS, cryoglobulins; DRC, dexamethasone, rituximab, cyclophosphamide; HV, hyperviscosity; NA, nucleoside analogs; PI, proteasome inhibitor; PN, progressive neuropathy. Adapted with permission from Treon et al.[49]

ibrutinib and rituximab can be considered in WM patients with *CXCR4* mutations or in *MYD88* wild-type patients.

Clinical case (continued)

The patient was started on ibrutinib, 420 mg by mouth every day. Within 3 months of therapy, the patient's hemoglobin normalized, and his serum IgM level decreased to 320 mg/dL, consistent with a VGPR to therapy. The patient remains on ibrutinib monotherapy.

Future treatment options

Despite the depth of response attained by the patient within the first 3 months of therapy, one could expect progression of disease at some point in the future. Therefore, additional research is needed to identify novel treatment options. Selected ongoing clinical trials are shown in Table 2.

Ibrutinib is being evaluated in combination with chemoimmunotherapy, proteasome inhibitors, BCL2 inhibitors, and anti-CD38 antibodies. Acalabrutinib, zanubrutinib, and tirabrutinib are covalent BTK inhibitors also being studied in WM patients. A large multicenter phase 2 study evaluated acalabrutinib in 106 WM patients and reported an ORR of 93%, major response of 80%, and 2-year PFS rate of 80% to 90%.[37] Most common grade ≥3 adverse events included neutropenia and lower respiratory tract infections. The rate of atrial fibrillation was 5%. A phase 1/2 prospective study evaluated zanubrutinib in 77 WM patients.[38] Zanubrutinib was associated with an ORR of 92%, major response rate of 82%, VGPR rate of 41%, and 24-month PFS rate of 82%. Adverse events of bruising/bleeding and atrial fibrillation (5%) were observed. A randomized phase 3 study evaluating zanubrutinib (Arm A) vs ibrutinib (Arm B) in symptomatic WM patients (ASPEN) has completed accrual.[39] At 19 months of follow-up, VGPR rates for zanubrutinib and ibrutinib were 28% and 19%, respectively, and 12-month PFS rates were 90% and 87%, respectively. There were lower rates of atrial fibrillation, diarrhea, and bleeding, but higher rates of neutropenia, with zanubrutinib. Preliminary results of ASPEN Arm C showed that zanubrutinib induced responses in patients without *MYD88* mutations, with an ORR of 77%, major response of 54%, and VGPR rate of 15%.[40] Tirabrutinib was evaluated in 27 patients with WM.[41] ORR was 94% and 100%, and major response rates were 78% and 89% in treatment-naive and previously treated patients, respectively. Rash was reported in 41% of patients. The acquisition of BTK mutations has been associated with resistance to covalent BTK inhibitors in patients with WM.[42] Second-generation noncovalent BTK inhibitors (eg, vecabrutinib, LOXO-305, ARQ-531) are being investigated in WM patients. A multicenter prospective phase 2 clinical trial evaluating a 2-year course of

Table 2. Selected ongoing clinical trials with novel agents in patients with WM

ClinicalTrials.Gov ID	Agents	Mechanism of action	Phase
NCT04263480	Ibrutinib Carfilzomib	BTK inhibitor Proteasome inhibitor	3
	Ibrutinib	BTK inhibitor	
NCT04061512	Ibrutinib Rituximab	BTK inhibitor Anti-CD20 mAb	2/3
	Cyclophosphamide Rituximab Dexamethasone	Alkylating agent Anti-CD20 mAb Steroid	
NCT03506373	Ibrutinib Ixazomib	BTK inhibitor Proteasome inhibitor	2
NCT03620903	Ibrutinib Bortezomib Rituximab	BTK inhibitor Proteasome inhibitor Anti-CD20 mAb	2
NCT04273139	Ibrutinib Venetoclax	BTK inhibitor BCL2 antagonist	2
NCT03679624	Ibrutinib Daratumumab	BTK inhibitor Anti-CD38 mAb	2
NCT03187262	Daratumumab	Anti-CD38 mAb	2
NCT03630042	Pembrolizumab Rituximab	Anti-PD1 mAb Anti-CD20 mAb	2
NCT02962401	Idelalisib Obinutuzumab	PI3K inhibitor Anti-CD20 mAb	2
NCT03364231	Umbralisib	PI3K inhibitor	2
NCT03225716	Ibrutinib Ulocuplumab	BTK inhibitor Anti-CXCR4 mAb	1/2
NCT02457559	Tirabrutinib	BTK inhibitor	1/2
NCT03037645	Vecabrutinib	BTK inhibitor	1/2
NCT03162536	ARQ-351	BTK inhibitor	1/2
NCT03740529	LOXO-305	BTK inhibitor	1/2
NCT04274738	Ibrutinib Mavorixafor	BTK inhibitor CXCR4 antagonist	1
NCT04115059	Dasatinib	HCK inhibitor	Pilot

HCK, hematopoietic cell kinase; mAb, monoclonal antibody; PD1, programmed cell death protein 1; PI3K, phosphatidylinositol-3 kinase.

venetoclax in 30 previously treated WM patients has completed accrual.[43] Preliminary results showed ORR, major response rate, and VGPR rate of 90%, 83%, and 20%, respectively, and 18-month PFS rate of 82%. Grade ≥3 adverse events included neutropenia, anemia, and diarrhea. Studies evaluating CXCR4-targeting agents, such as ulocuplumab (monoclonal antibody) and mavorixafor (small molecule), are ongoing.

In conclusion, there have been a series of advances in the diagnosis and management of WM in recent years. Rational genomic-driven treatment options are increasing in number, and it is hoped that they will translate into deeper and more durable responses, as well as lower toxicity rates.

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Conflict-of-interest disclosure

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Off-label drug use

None disclosed.

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Monoclonal gammopathies of clinical significance

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"Monoclonal gammopathy of clinical significance" (MGCS) is the term used to describe nonmalignant monoclonal gammopathies causing important disease. MGCS is the differential diagnosis for any patient presenting with what appears to be a monoclonal gammopathy of undetermined significance but is also experiencing other unexplained symptoms. Broadly, these conditions can be separated into symptoms and signs referable to the nerves, the kidneys, and the skin. The first step in making these diagnoses is to consider them. With a particular condition in mind, the next step is to order those tests that can help confirm or dismiss a particular diagnosis. Nearly all of the renal and dermatologic conditions are diagnosed by renal and skin biopsies, respectively. The importance of a highly competent renal pathologist and dermatopathologist cannot be underestimated. Biopsy is less specific for the neuropathic conditions. Because several of the MGCSs are syndromes, recognizing other manifestations is also key. Treatment recommendations for many of these conditions are anecdotal because of their rarity, but for several of the conditions, IV immunoglobulin, rituximab, and plasma cell–directed therapy are the best options.

LEARNING OBJECTIVES
- Recognize the differential diagnosis for a monoclonal gammopathy with peripheral neuropathy, skin abnormalities, or renal disease
- Diagnose MGCS

Clinical case

Approximately 18 months before diagnosis and at age 67, a man began experiencing anorexia, nausea, vomiting, and weight loss. By 12 months before diagnosis, he had lost 25 kg. An extensive nondiagnostic evaluation was performed, including thyrotropin, antineutrophil cytoplasmic autoantibodies, antinuclear antibodies, endoscopies, gastric emptying studies, and positron emission tomography–computed tomography. Seven months before diagnosis, an immunoglobulin Aλ monoclonal gammopathy was found. Over the ensuing months, this patient developed sensorimotor peripheral neuropathy, progressive muscle weakness, volume overload, and pseudogout. He was admitted to our hospital, where a diagnosis was made. In addition to the preceding presentations, he had malnutrition and anasarca (Table 1). His Eastern Cooperative Oncology Group performance status was 4.

Introduction

The term "monoclonal gammopathy of clinical significance" (MGCS) was coined subsequent to monoclonal gammopathy of renal significance (MGRS) when it became increasingly apparent that a term was required for a patient with a small B-cell clone and small monoclonal proteins that were causing serious and even life-threatening disease (Figure 1A).[1,2] Monoclonal gammopathy of "undetermined significance" (MGUS) is a misnomer for these conditions. Some patients with MGCS have sufficient clonal burden to satisfy the definition of smoldering multiple myeloma or smoldering Waldenstrom macroglobulinemia. In short, MGCS is a monoclonal gammopathy featuring two main characteristics: a quiescent underlying clone and symptoms that are related to the monoclonal immunoglobulin or to the clone itself by mechanisms other than the tumor burden. The MGCSs are best divided into different systems that are affected, the most common of which are kidney, nerve, and skin, recognizing that in some cases there is overlap due to a systemic, multiorgan presentation and/or course.

Neurologic MGCS

The major MGCS considerations for a patient with neuropathy include (Figure 2) amyloid light-chain (AL) amyloidosis, POEMS syndrome (polyradiculoneuropathy, organomegaly, endocrinopathy, monoclonal plasma cell disorder, and skin changes), cryoglobulinemia, CANOMAD (chronic ataxic neuropathy, ophthalmoplegia, immunoglobulin M [IgM] paraprotein, cold agglutinins, and disialosyl antibodies), and DADS-M (distal acquired demyelinating

Table 1. Baseline characteristics, interpretation, and resolution for patient case

Characteristics	At diagnosis	Comment	After therapy
Demyelinating PN		Classic for POEMS syndrome	Walks with AFOs; still foot drop
Nausea, vomiting, anorexia		Due to adrenal insufficiency	Promptly resolved
Hb, g/dL	8.6	Due to adrenal insufficiency, hypothyroidism and chronic disease; unusual to have cytopenias in POEMS	Normalized
Platelets, $\times 10^9$/L	109		
Albumin, g/dL	2.3		
Creatinine, g/dL	2.3	Hypovolemia, diuretic use	
M spike	IgA λ	IFE positive	IFE positive
IgA, mg/dL	536	Elevated	Normal range
κ-FLC, mg/dL	8.26	Acute renal insufficiency and POEMS syndrome: polyclonal FLC elevation common.	
λ FLC, mg/dL	13.2		
FLC ratio	0.626		
TSH/T4, IU/L, ng/dL	9.5/0.4	Hypothyroidism	Replaced
Cortisol	3.3	Hypoadrenalism	
Plasma VEGF, pg/mL	320	ULN 86 pg/mL: consistent with POEMS	Normalized
IL-6, pg/mL	62.9	Bed sores due to chronic debility	
Urine 24-h protein, mg	381	AKI versus POEMS	
BMPC, %	10 (λ)	On the high side for POEMS syndrome	<5%, but still clonal
RVSP, mmHg	64	Moderate pulmonary hypertension	Normalized
DLCO	Normal		Normal
CT skeletal survey	Bones negative, but ascites, effusions, anasarca	>85% patients with bone lesions	Resolved
ECOG PS	4		0-1

AFOs, ankle foot orthotics; AKI, acute kidney injury; BMPC, bone marrow plasma cells; CT, computed tomography; DLCO, diffusion capacity of carbon monoxide; ECOG, Eastern Cooperative Oncology Group; FLC, immunoglobulin free light chains; Hb, hemoglobin; IFE, immunofixation; IgA, immunoglobulin A; IL-6, interleukin-6; POEMS, polyradiculoneuropathy, organomegaly, endocrinopathy, monoclonal plasma cell disorder, and skin changes; PN, peripheral neuropathy; PS, performance status; RVSP, right ventricular systolic pressure; T4, thyroxine; TSH, thyrotropin; ULN, upper limit of normal; VEGF, vascular endothelial growth factor.

symmetric neuropathy with M protein [formerly known as "MGUS-associated peripheral neuropathy"]). The first three are diseases with multiple systemic manifestations, whereas the last two are primarily in the nervous system only.

POEMS syndrome

POEMS syndrome is the acronym for polyradiculoneuropathy, organomegaly, endocrinopathy, monoclonal plasma cell disorder, and skin changes.[3] It is a rare condition with a prevalence of 3 per 1 million. Median age at presentation is in the 50s, and there are slightly more men than women affected. Symptoms and signs refer to the acronym as well as features covered by another acronym, PEST, which includes papilledema, extravascular volume overload, sclerotic bone lesions (Figure 1B), thrombocytosis, and erythrocytosis. Other elements not covered in either of the acronyms are elevated vascular endothelial growth factor, pulmonary hypertension, reduced diffusion capacity of carbon monoxide, and arterial and venous thromboembolisms (Table 2). The dominant symptom in this disease is a progressive length-dependent ascending sensorimotor demyelinating peripheral neuropathy.

Adverse risk factors in this disease are age, pleural effusion, reduced estimated glomerular filtration rate, pulmonary hypertension, coexisting Castleman disease, and lack of complete hematologic response to plasma cell–directed therapy.[4] Autologous stem cell transplant (ASCT) is a favored therapy, but lenalidomide and dexamethasone are also active (Table 3).[5,6] Data associated with use of proteasome inhibitors and daratumumab are emerging. Overall survival in patients with POEMS syndrome is excellent with plasma cell–directed therapy, with estimated 10-year survivorship at 79%.

DADS-M

IgM monoclonal gammopathy accounts for ~60% of neuropathies associated with monoclonal gammopathy.[7] Patients are more often male and in their 50s to 80s. They present with a distal, demyelinating symmetric neuropathy. Sensory ataxia is the most common sign. The diagnosis is one of exclusion. Even in the presence of a monoclonal gammopathy, other explanations, such as inherited neuropathies, diabetes, alcoholism, and drug use, should be ruled out.

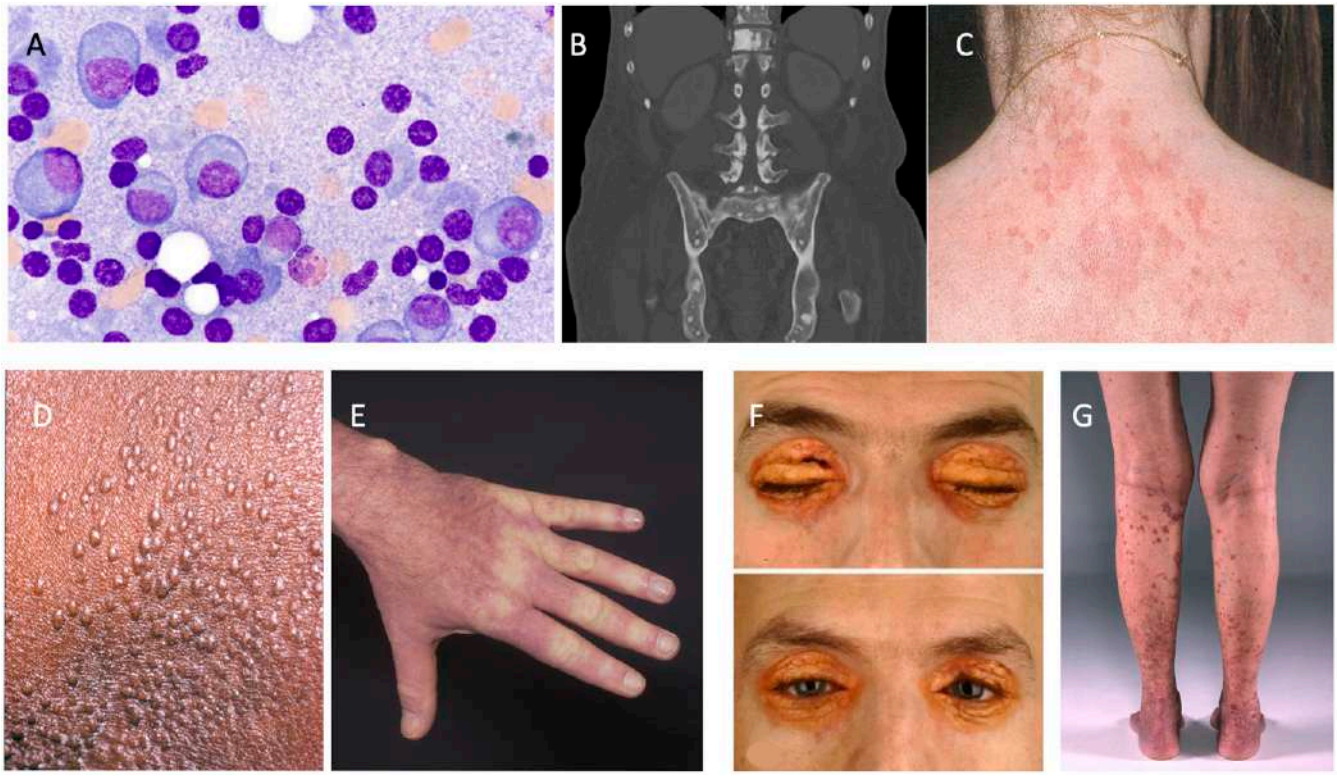

Figure 1. Part of the monoclonal gammopathy of clinical significance spectrum. (A) Bone marrow plasma cells (original magnification, 100×). (B) Osteosclerotic lesions of POEMS syndrome (polyradiculoneuropathy, organomegaly, endocrinopathy, monoclonal plasma cell disorder, and skin changes). (C) Schnitzler syndrome. (D and E) Scleromyxedema. (F) Necrobiotic xanthogranuloma. (G) Cryoglobulinemia vasculitis.

Pathologic studies have identified demyelination and widened myelin lamellae with IgM deposits in the widened lamellae of myelin fibers and myelin debris contained in Schwann cells and macrophages. Despite the fact that these M proteins may bind to myelin-associated glycoprotein (MAG) or other gangliosides, anti-MAG antibodies are not specific for peripheral neuropathy, and reduction in anti-MAG antibody titers with rituximab or other anti-CD20 antibodies has not correlated with clinical improvement. Treatments include IV immunoglobulin (IVIG) and rituximab.

CANOMAD

CANOMAD is a rare condition characterized by a chronic neuropathy with sensory ataxia and IgM disialosyl antibodies.[8] Patients may or may not have motor weakness involving oculomotor and bulbar muscles or cold agglutinins. The most frequently targeted gangliosides are CD1b, GD3, GT1b, and GQ1b. Both axonal and demyelinating patterns have been recognized. The most effective therapies are IVIG, rituximab, and plasmapheresis.

Sporadic late-onset nemaline myopathy

Sporadic late-onset nemaline myopathy is a rare muscle disease that can be associated with a monoclonal protein or HIV infection.[9] It is not a neuropathy, but it does cause significant motor dysfunction. On biopsy, muscle fibers accumulate nemaline rods, and there is no associated inflammation. Patients present with predominantly proximal or axial muscle weakness, including respiratory muscle weakness. Treatment strategies include IVIG and plasma cell–directed therapies, including ASCT.

Our patient had a symmetrical, ascending, demyelinating, sensorimotor peripheral neuropathy, along with systemic signs. The multisystemic nature of his disease made CANOMAD and DADS-M unlikely. Although AL amyloidosis and cryoglobulinemia are multisystemic diseases, the former is associated with a small-fiber neuropathy and the latter with an axonal neuropathy due to vasculitis.

Monoclonal gammopathy of renal significance

MGRS is a group of disorders in which a monoclonal immunoglobulin secreted by a nonmalignant or premalignant B-cell or plasma cell clone causes renal damage. As shown in Figure 2, several conditions are renal only, whereas others potentially have systemic features. All MGRS diagnoses without other systemic features are made by a renal pathologist (Figure 2). With the exception of C3 glomerulopathy with monoclonal gammopathy and thrombotic microangiopathy, the other MGRSs are broken into "nonorganized monoclonal immunoglobulin deposits" and "organized" monoclonal immunoglobulin deposits.[10] The nonorganized deposits include monoclonal immunoglobulin deposition disease and proliferative glomerulonephritis with monoclonal immune deposits. In contrast, the organized deposits are categorized as (1) fibrillar deposits, which include AL amyloidosis and monoclonal fibrillary glomerulonephritis; (2) microtubular deposits, which include immunotactoid glomerulonephritis and cryoglobulinemia glomerulonephritis; and (3) inclusions or

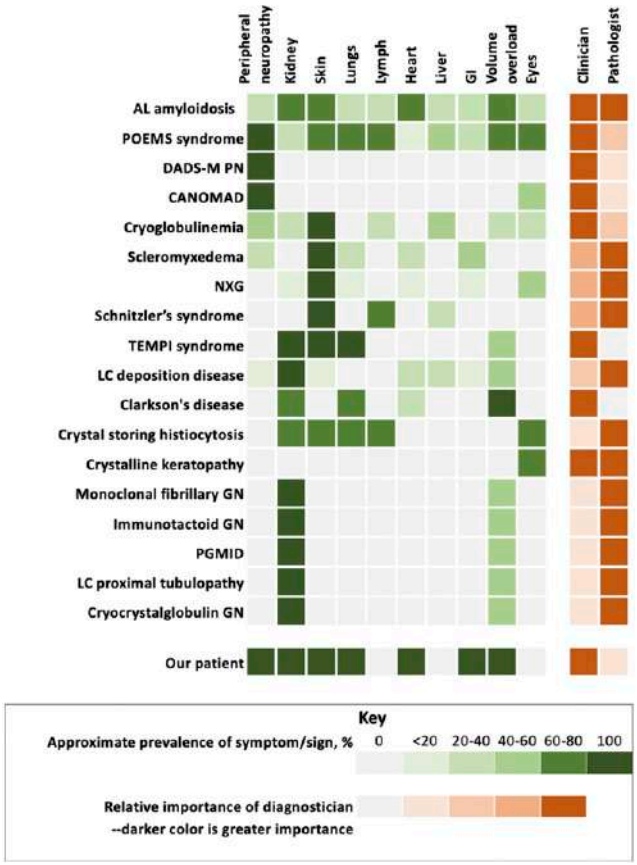

Figure 2. Monoclonal gammopathy of clinical significance and organ system involvement. CANOMAD, chronic ataxic neuropathy, ophthalmoplegia, immunoglobulin M paraprotein, cold agglutinins, and disialosyl antibodies; DADS-M-PN, distal acquired demyelinating symmetric neuropathy with M protein; GN, glomerulonephritis; LC, light chain; NXG, necrobiotic xanthogranuloma; PGMID, proliferative glomerulonephritis with monoclonal immune deposition; POEMS, polyradiculoneuropathy, organomegaly, endocrinopathy, monoclonal protein, skin changes.

crystalline deposits, which include light chain proximal tubulopathy, crystal-storing histiocytosis, and cryocrystalglobulin glomerulonephritis.

This classification allows for a uniform vocabulary among nephrologists, renal pathologists, and hematologists such that diagnostic algorithms can be constructed and natural history and therapeutic interventions can be analyzed (Table 3). Although renal transplant can be considered in these patients, there is risk for recurrence.[11] Which diseases respond best to which plasma cell or lymphoproliferative disease–directed therapies is still a work in progress. The most data exist on light chain deposition disease (LCDD), so that topic is described next.

LCDD

LCDD shares similarities with AL amyloidosis in that both are immunoglobulin deposition diseases. Both can rarely involve immunoglobulin heavy chains as well. LCDD is less common than AL amyloidosis, less likely to involve other organs, and more often is due to a κ-restricted light chain (immunoglobulin kappa variable 4 [IGKV4]) and to present with impaired creatinine clearance. The median plasmacytosis in the bone marrow is ~10%.[12,13] Immunofluorescence of the kidney biopsy reveals linear deposits of the involved monoclonal immunoglobulin along tubular basement membranes and along glomerular basement membranes. Electron microscopy shows that these deposits have a granular appearance.

Treatment is similar to that used for patients with AL, and survival tends to be better because it is unusual for patients with LCCD to have cardiac involvement. Although our patient had reduced renal function and mild proteinuria, no renal biopsy was performed. The renal injury was presumed to be due to hypotension and exuberant application of diuretics. Significant renal disease is rare in POEMS syndrome, but numerous pathologic changes have been reported, including membranoproliferative glomerulonephritis–like lesions, microangiopathic lesions, mesangiolytic lesions, and even immunotactoid lesions.

Cutaneous MGCSs

Cutaneous MGCSs include Schnitzler syndrome, scleromyxedema, necrobiotic xanthogranuloma (NXG), TEMPI syndrome (telangiectasias, elevated erythropoietin and erythropoiesis, monoclonal gammopathy, perinephric fluid, intrapulmonary shunting), cryoglobulinemia, systemic capillary leak syndrome (SCLS), and POEMS syndrome.

Schnitzler syndrome

Schnitzler syndrome is characterized primarily by chronic urticaria (Figure 1C) and the presence of an IgM monoclonal gammopathy. Interleukin (IL)-1β plays a critical role in in the disease. Aberrant NLRP3 inflammasome signaling and cytokine pathway dysregulation also play a role. Schnitzler syndrome can rarely be associated with an IgG monoclonal gammopathy. Other features and diagnostic criteria are shown in Figure 2 and Table 2.[14-16] Although the presence of a dermal neutrophilic infiltrate on skin biopsy became a minor criterion with the Strasbourg revision, such a biopsy finding is nonspecific, and its relative importance in the diagnostic criteria has been questioned. Therapy with anakinra, an anti–IL-1 antibody, is quite effective (Table 3). More novel anti–IL-1 antibodies, such as rilonacept and canakinumab, have also been effective.[17] Following C-reactive protein can be helpful in monitoring this disease.

Scleromyxedema

Scleromyxedema is characterized by generalized papular and sclerodermoid cutaneous eruptions (Figure 1D-E) and is typically associated with an IgG monoclonal gammopathy.[18] Extracutaneous involvement can include the nervous system, joints, gastrointestinal system, and heart (Figure 2). The infiltrates are composed of mucin. How the monoclonal protein induces fibroblast proliferation is not well understood. The science behind the pathology is gradually emerging through skin transcriptome analyses and the study of peripheral blood immune cells. Transforming growth factor-β is overexpressed. Other proteins, including collagen Ia and several interferon-inducible proteins, are also overexpressed.[19,20] Diagnostic criteria are shown in Table 2. IVIG is considered first-line therapy, with plasma cell–directed

Table 2. Diagnostic criteria for five selected syndromes

POEMS syndrome*[3]	Schnitzler syndrome†‡[16]	Necrobiotic xanthogranuloma§[21]	Scleromyxedema[18]	TEMPI syndrome[23]
Mandatory major criteria	Obligate criteria	Major criteria	1. Generalized papular and sclerodermoid eruption	Major criteria
1. Polyneuropathy (typically demyelinating) 2. Monoclonal plasma cell-proliferative disorder (almost always λ)	1. Chronic urticarial rash 2. Monoclonal IgM or IgG	1. Cutaneous papules, plaques, and/or nodules, most often yellow or orange in color 2. Histopathological features demonstrating palisading granulomas with lymphoplasmacytic infiltrate and zones of necrobiosis. Variably present cholesterol clefts and/or giant cells	2. Evidence of monoclonal gammopathy 3. Microscopic triad associating dermal mucin deposition, thickened collagen, and fibroblast proliferation or an interstitial granuloma annulare–like pattern 4. Absence of thyroid disease	1. Telangiectasias 2. Monoclonal gammopathy 3. Elevated erythropoietin and erythrocytosis 4. Perinephric fluid
Major criteria 3. Castleman disease‖ 4. Sclerotic bone lesions 5. Vascular endothelial growth factor elevation **Minor criteria** 6. Organomegaly (splenomegaly, hepatomegaly, or lymphadenopathy) 7. Extravascular volume overload (edema, pleural effusion, or ascites) 8. Endocrinopathy‡‡ (adrenal, thyroid, pituitary, gonadal, parathyroid, pancreatic) 9. Skin changes (hyperpigmentation, hypertrichosis, glomeruloid hemangiomas, plethora, lipodystrophy, acrocyanosis, flushing, white nails) 10. Papilledema 11. Thrombocytosis/polycythemia[a] Other: clubbing, weight loss, hyperhidrosis, pulmonary hypertension/restrictive lung disease, thrombotic diatheses, diarrhea, low vitamin B_{12}	**Minor criteria** 3. Recurrent fever¶ 4. Objective findings of abnormal bone remodeling with or without bone pain# 5. A neutrophilic dermal infiltrate on skin biopsy** 6. Leukocytosis and/or elevated CRP††	**Minor criteria** 3. Periorbital distribution of cutaneous lesions 4. Paraproteinemia, most often IgG-λ, plasma cell dyscrasia, and/or other associated lymphoproliferative disorder		**Minor criteria** 5. Intrapulmonary shunting 6. Other: venous thrombosis

CRP, C-reactive protein; IgG-λ, immunoglobulin G-λ; POEMS, polyneuropathy, organomegaly, endocrinopathy, M protein, skin changes; TEMPI, telangiectasias, elevated erythropoietin and erythropoiesis, monoclonal gammopathy, perinephric fluid, intrapulmonary shunting.
*POEMS syndrome diagnosis is confirmed when both of the mandatory major criteria, 1 of the 3 other major criteria, and 1 of the 6 minor criteria are present.
†Definite diagnosis of Schnitzler syndrome: if IgM, both obligate criteria and at least 2 minor criteria; if IgG, both obligate criteria and 3 minor criteria.
‡Probable diagnosis of Schnitzler syndrome: if IgM, both obligate criteria and 1 minor criteria; if IgG, both obligate criteria and 2 minor criteria.
§For necrobiotic xanthogranuloma diagnosis, both major criteria and at least 1 minor criterion, applicable only in the absence of foreign body, infection, or other identifiable cause.
‖There is a Castleman disease variant of POEMS syndrome that occurs without evidence of a clonal plasma cell disorder that is not accounted for in this table. This entity should be considered separately.
¶Must be >38°C and otherwise unexplained. Occurs usually—but not obligatory—together with the skin rash.
#As assessed by bone scintigraphy, magnetic resonance imaging, or elevation of bone alkaline phosphatase.
**Corresponds usually to the entity described as "neutrophilic urticarial dermatosis," absence of fibrinoid necrosis, and significant dermal edema.
††Neutrophils >10 000/mm^3 and/or CRP >30 mg/L.
‡‡Because of the high prevalence of diabetes mellitus and thyroid abnormalities, this diagnosis alone is not sufficient to meet this minor criterion.
[a]Approximately 50% of patients will have bone marrow changes that distinguish it from a typical monoclonal gammopathy of undetermined significance or myeloma bone marrow. Anemia and/or thrombocytopenia are distinctively unusual in this syndrome unless Castleman disease is present.

therapy (eg, lenalidomide or bortezomib) added if no response is achieved or more severe disease develops (Table 3).[20]

NXG

NXG is a non–Langerhans cell histiocytosis typically associated with monoclonal proteins attributable to plasma cell disorders or lymphoproliferative disorders. The mean age of presentation is 62 years, with a slight predominance of women.[21] The classic presentation is yellow-to-orange papules, plaques, and/or nodules involving the eyelids (Figure 1F). Cutaneous lesions may also be found on other locations of the face, the trunk, and the extremities. NXG plaques can occasionally be pruritic and also painful if they ulcerate. Extracutaneous involvement includes the eye, heart, gastrointestinal tract, liver, and lung but is relatively rare (Figure 2). A French group has suggested an association between monoclonal gammopathy and both hyperlipidemic and non-hyperlipidemic xanthomatosis and suggested that the association can be strengthened if complement levels, especially C4, are low.[22]

On biopsy of NXG, palisading granulomas with nonclonal lymphoplasmacytic infiltrate and zones of necrobiosis are seen. Cholesterol clefts and large bizarre foreign body giant cells are also classic. The pathogenesis of the disease is unknown, but it has been speculated that there is a monoclonal protein–lipoprotein interaction. Diagnostic criteria have been proposed (Table 2). The leading differential diagnosis is necrobiosis lipoidica, which is a necrotizing skin condition that can occur in patients with diabetes mellitus or rheumatoid arthritis. In contrast to monoclonal gammopathy–associated hyperlipidemic and nonhyperlipidemic xanthomatosis, CD163-positive foam cells and Touton giant cells are seen but typically without necrobiosis. Treatment with IVIG is one of the most

Table 3. Treatments

Conditions	Therapy
POEMS syndrome	First line: most experience with ASCT and lenalidomide/dexamethasone First line: if only 1-2 bone lesions and negative bone marrow, radiation
DADS-M-PN	First line: IVIG Second line: rituximab
CANOMAD	First line: IVIG or plasmapheresis Second line: rituximab
SLOMN	First line: IVIG Second line: plasma cell–directed therapy
Light chain deposition disease	First line: most experience with ASCT and bortezomib/dexamethasone
PGMID	First line: rituximab Second line: bortezomib/dexamethasone
Monoclonal fibrillary glomerulonephritis	First line: unknown
Immunotactoid GN	First line: rituximab or clone directed therapy
Inclusions/crystalline deposits	First line: may consider clone-directed therapy
Cryoglobulinemia	First line: treat underlying cause (eg, HCV, CTD, PCD); for severe cases, plasmapheresis, high-dose methylprednisolone, and/or cyclophosphamide may be considered Second line: rituximab
Scleromyxedema	First line: IVIG Second line: add lenalidomide or bortezomib
Necrobiotica xanthogranuloma	First line: IVIG Second line: may consider clone-directed therapy
Schnitzler syndrome	First line: anti–IL-1 monoclonal therapeutics Second line: Waldenstrom macroglobulinemia therapy
TEMPI syndrome	First line: plasma cell–directed therapy
Clarkson disease	Prophylactic IVIG
Crystal-storing histiocytosis[29]	First line: observation Second line: may consider clone-directed therapy
Monoclonal gammopathy keratopathy	No treatment required

These treatments are based on case series and not on high levels of evidence.
ASCT, autologous stem cell transplant; CANOMAD, chronic ataxic neuropathy, ophthalmoplegia, immunoglobulin M paraprotein, cold agglutinins, and disialosyl antibodies; CTD, connective tissue disease; DADS-M-PN, distal, acquired, demyelinating, symmetric neuropathy with M protein; GN, glomerulonephritis; HCV, hepatitis C virus; IL-1, interleukin-1; IVIG, intravenous immunoglobulin; PCD, plasma cell disorder; PGMID, proliferative glomerulonephritis with monoclonal immune deposition; POEMS, polyradiculoneuropathy, organomegaly, endocrinopathy, monoclonal protein, skin changes; SLOMN, sporadic late-onset nemaline myopathy.

promising therapies, but limited success has also been reported with plasma cell–directed therapies, intralesional triamcinolone, and antimalarials.

TEMPI syndrome

TEMPI syndrome is a rare acquired disorder characterized by the features that comprise the acronym: telangiectasias, elevated erythropoietin and erythrocytosis, monoclonal gammopathy, perinephric fluid collections, and intrapulmonary shunting.[23] The underlying pathophysiology is not understood, but it is clear that plasma cell–directed therapy reverses the clinical manifestations.

Telangiectasias involving the face and upper body and erythrocytosis comprise the most common presentation. Unlike the erythrocytosis of polycythemia rubra vera and of POEMS syndrome, patients with TEMPI syndrome have a high erythropoietin level.[24] These patients develop progressive hypoxia. The pulmonary shunting is not evident on high-resolution computed tomography of the chest and is best demonstrated by ^{99m}Tc macroaggregated albumin scintigraphy. The perinephric fluid collections have the same electrolyte composition as serum. Proposed diagnostic criteria are shown in Table 2. Unlike POEMS syndrome, there is no bias in clonality for λ-restricted clones, and there are no features of a myeloproliferative neoplasm.

Plasma cell–directed therapy appears to be useful, specifically bortezomib, daratumumab, lenalidomide, and high-dose melphalan. All of the features can improve upon achievement of complete hematologic response (Table 3).

Cryoglobulinemia

Cryoglobulinemia is a multisystem disease that can affect almost any organ system, but cutaneous manifestations are almost always present (Figure 1G). Type I cryoglobulins arise from clonal plasma cell proliferative disorders or lymphoproliferative disorders. Type II and type III cryoglobulins may be related to plasma cell disorders or lymphoporliferative disorders but are more often due to infections such as hepatitis C virus and connective tissue disorders.[25]

Manifestations and disease severity are quite variable. Type I cryoglobulins more often cause occlusive symptoms due to occlusion of capillary lumina, and vasculitis is uncommon.[25] Patients report cold-induced skin symptoms, including purpura, livedo, and cold urticaria. Ulceration can occur. Less than one-third of patients will have renal involvement, but up to 50% may have peripheral neuropathy. In contrast, in type II/III cryoglobulinemia or mixed cryoglobulinemia, small-vessel vasculitis is the major mechanism driving morbidity. Skin symptoms, including purpura, occur in the vast majority of patients; arthralgia is also very common in peripheral neuropathy followed by renal involvement.

The aim of treatment of cryoglobulinemia is to treat the underlying cause. For patients with hepatitis C virus, who comprise the majority of type II and III cases, sustained virologic responses can be achieved in >50% with antiviral therapy. For patients not responding to antiviral therapy, rituximab and other immunosuppressants can play an important role in treating vasculitis. For disease driven by autoimmune disease, rituximab and corticosteroids are the best first-line options. Plasmapheresis can be used in patients with severe end organ damage and/or refractory disease.[26] Other disease modifiers, including corticosteroids and cyclophosphamide, can also play a role in therapy, especially in patients with severe end organ damage.

Idiopathic SCLS (Clarkson disease)

This devastating disease was first described in 1960. SCLS is characterized capillary leak resulting in sudden-onset shock and anasarca caused by plasma extravasation (up to 70% of total plasma volume). The diagnostic triad is composed of the "3 Hs," which occur in the absence of secondary causes of these findings:

Table 4. Selected relevant references

Conditions	References
Neurologic	
POEMS syndrome	3-6
Distal acquired demyelinating symmetric neuropathy with monoclonal protein (DADS-M)	7
Chronic ataxic neuropathy, ophthalmoplegia, IgM paraprotein, cold agglutinins, and disialosyl antibodies (CANOMAD)	8
Sporadic late-onset nemaline myopathy (SLOMN)	9
Renal[10]	
AL amyloidosis	30
Monoclonal immunoglobulin deposition disease	12,13
Proliferative glomerulonephritis with monoclonal immunoglobulin deposition (PGMID) Monoclonal fibrillary glomerulonephritis	11,31
Monoclonal fibrillary glomerulonephritis	32
Cryoglobulinemia	25,26
Immunotactoid glomerulonephritis	33
Acquired Fanconi syndrome and other light chain proximal tubulopathies	34
Crystal-storing histiocytosis	29
C3 glomerulonephritis	35
Monoclonal gammopathy–associated thrombotic microangiopathy	36
Dermatologic	
Schnitzler syndrome	14-17
Scleromyxedema	18-20
Necrobiotica xanthogranuloma	21,22
Hyperlipidemic and nonhyperlipidemic xanthomatosis	22
TEMPI syndrome	23
Acquired cutis laxa	37
Neutrophilic dermatosis	38
Clarkson disease (systemic capillary leak syndrome)	27,28
Macroglobulinosis	39
Other	
Monoclonal gammopathy keratopathy	40
Acquired C1 inhibitor deficiency	41
Acquired von Willebrand disease	42
Cold agglutinin disease	43

A single category was chosen for systemic diseases. See Figure 2, which demonstrates multiorgan involvement.
AL, amyloid light chain; POEMS, polyradiculoneuropathy, organomegaly, endocrinopathy, monoclonal protein, skin changes; TEMPI, telangiectasias, elevated erythropoietin and erythropoiesis, monoclonal gammopathy, perinephric fluid, intrapulmonary shunting.

hypotension, hemoconcentration, and hypoalbuminemia. Sixty-eight percent of adult patients with SCLS have monoclonal proteins, most commonly IgG-κ. Details of the limited understanding of disease mechanisms of the vascular endothelial hyperpermeability of SCLS can be found in a review published elsewhere.[27]

The differential diagnosis for an acute attack includes sepsis, anaphylaxis, and hereditary angioedema. Treatment at the time of an acute attack is supportive with fluid resuscitation until flare subsides, which typically occurs over the course of a few days. Empiric prophylaxis with IVIG is recommended because it has been demonstrated that there are fewer attacks in those patients managed as such.[28]

POEMS syndrome

Our patient had POEMS syndrome evolving over the course of nearly 18 months. His first symptoms were those of adrenal insufficiency and peripheral neuropathy. His characteristics and his course are summarized in Figure 2 and Table 1. His case exemplifies the importance of an extensive review of systems in patients with a monoclonal protein and other unexplained conditions. A focused review of systems prompts additional testing from scans to disease-oriented blood work and/or biopsies. By the time he was diagnosed, our patient had a performance score of 4 and was bedridden with bedsores. With adrenal and thyroid replacement and plasma cell clone-directed therapy, his life has returned to nearly normal, though he does still use ankle foot orthotics. Had the syndrome not been considered, he would have died. Five years later, he continues to enjoy life.

In summary, MGCSs are a constellation of diseases associated with clonal—but not malignant–B cells or plasma cells that produce monoclonal proteins and pathology through diverse, often ill-defined mechanisms. Similar end organ damage can occur in the context of malignant plasma cell or B-cell clonal disorders, but these are no longer MGCSs, but rather the cancer with associated disease (eg, myeloma with associated cryoglobulinemia). The most commonly affected organs among patients with MGCS are the kidney, nerve, and skin. A thorough discussion of all of the MGCSs is beyond the scope of this article, but Table 4 highlights some of the most recent and relevant references pertaining to MGCS. Some MGCSs predominantly affect only one organ, and others are systemic diseases affecting multiple organ systems. In order to help patients, these diagnoses and the severity of the symptoms must be considered so that appropriate therapy may be instituted. Conversely, not every patient with a monoclonal gammopathy and an unexplained symptom or sign has MGCS, given the high prevalence of true MGUS, especially with advancing age. In many instances, the appropriate therapy is clone-directed therapy, even though there is no malignancy; in others, data indicate that therapies such as IVIG, rituximab, or anti–IL-1 antibodies are most appropriate. The treating physician should strive to assign causality before proceeding with clone-directed therapy.

Conflict-of-interest disclosure

A.D. has received research funding from Celgene, Millennium, Pfizer, and Alnylam; has received a travel grant from Pfizer; and has served on an advisory board for Janssen.

Off-label drug use

None disclosed.

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A patient with Gaucher disease and plasma cell dyscrasia: bidirectional impact

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Patients with Gaucher disease (GD), a rare autosomal recessive glycosphingolipid storage disease, commonly present to hematologists with unexplained splenomegaly, thrombocytopenia, anemia, and bone symptoms. Patients with GD may develop other manifestations, such as autoimmune thrombocytopenia, monoclonal gammopathy, multiple myeloma, or, even more rarely, other hematological malignancies; sometimes they are first diagnosed during an assessment of those disorders. Although the diagnosis and management of patients with GD have significantly evolved over the last 30 years, some patients remain poor responders to GD-specific therapy, needing novel and investigational therapies. Ideally, patients with GD, like patients with other rare diseases, should be managed by a multidisciplinary team expert with the diverse clinical manifestations and potential GD-related or -unrelated comorbidities. The hematology community should be knowledgeable regarding the presentation and the variety of hematologic complications and comorbidities associated with Gaucher disease.

LEARNING OBJECTIVES

- Recognize the clinical features and methods of diagnosis of Gaucher disease (GD) while avoiding pitfalls
- Review current and new therapies for GD
- Learn that plasma cell dyscrasias are potential comorbidities in GD

Introduction

Gaucher disease (GD) is an autosomal recessive glycosphingolipid storage disease caused by mutations of the lysosomal enzyme glucocerebrosidase gene (*GBA1*), leading to the accumulation of the substrate glucocerebroside in the cells of the macrophage–monocyte system. It is 1 of the 2 most common lysosomal storage disorders and has an estimated frequency of 1:50 000 to 1:100 000 in the general population, but with a much higher prevalence (~1:850) in the Ashkenazi Jewish population. More than 860 different mutations in *GBA1* have been identified, explaining, in part, the great phenotypic heterogeneity that is a hallmark of GD. Early diagnosis is important for timely initiation of specific therapy before the development of irreversible, mainly skeletal complications and for prenatal diagnosis in subsequent pregnancies.[1]

Most presenting features are hematological, as are some of the associated diseases (comorbidities) that develop in patients with GD at a higher incidence than in the general population, including immune thrombocytopenia, plasma cell dyscrasias, and other hematological malignancies, making it all the more important for hematologists to be familiar with this disease even if uncommon. Table 1 lists the main comorbidities among patients with GD.

Case report part 1: the pre–enzyme replacement therapy era

In 1974, a 19-year-old Ashkenazi Jewish woman was referred for hematological consultation for evaluation of menorrhagia and easy bruising. She had experienced epistaxis and blue markings since childhood, as well as a protuberant abdomen, but her parents were told that these were common in childhood. She had no history of bone pain or fractures. Her physical examination revealed a healthy-looking woman with multiple ecchymoses and marked splenomegaly. Bone marrow aspiration led to the diagnosis of GD based on the demonstration of engorged macrophages with cytoplasmic striations typical of "Gaucher cells" (Figure 1). With this benign diagnosis, no further workup or follow-up was recommended.

In the years before the introduction of enzyme replacement therapy (ERT) in 1991, the management of GD was purely symptomatic, including analgesics, splenectomy, and orthopedic surgery. Splenectomy was performed in severely

Table 1. Comorbidities among patients with Gaucher disease

GD related	ERT related	SRT related
Amyloidosis **Cholelithiasis** Celiac disease Hashimoto thyroiditis Hepatocellular carcinoma Immune thrombocytopenia Liver cirrhosis Lymphomas **MGUS** **Multiple myeloma** Myelodysplastic syndrome **Parkinson disease** Portal hypertension Pulmonary hypertension **Recurrent abortions** Uveitis Vitamin B_{12} deficiency To be determined: gout, hypothyroidism, vitamin D deficiency	*Class effect*: ASIA/reactive arthritis Diabetes mellitus type 2 Hyperlipidemia Metabolic syndrome NAFLD/NASH Polyneuropathy Pulmonary hypertension Weight gain *Drug-specific*: Allergy/hypersensitivity	*Class effect*: GI symptoms (eg, diarrhea, heartburn) Headache *Drug specific*: Cardiotoxicity Cognitive impairment Drug–drug interaction NSVT Peripheral neuropathy Tremor Weight loss

ASIA, autoimmune syndrome induced by adjuvant; bold, most common GD-related comorbidities; ERT, enzyme replacement therapy; GD, Gaucher disease; GI, gastrointestinal; MGUS, monoclonal gammopathy of undetermined significance; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NSVT, nonsustained ventricular tachycardia; SRT, substrate reduction therapy.

symptomatic patients, frequently children with hypersplenism (the majority had thrombocytopenia with a bleeding diathesis, anemia, or uncommonly leukopenia), growth retardation, and even cachexia. However, because splenectomy removed the main storage organ, these surgeries often resulted in worsening of the hepatic and bony involvement, causing massive hepatomegaly (with or without liver function abnormalities) or cirrhosis, in addition to bone crises with pathological fractures and osteonecrosis. When this became recognized, splenectomies were deferred as long as possible for severely affected patients and avoided for milder patients.

The delayed diagnosis at age 19, about a decade after the onset of the first symptoms, is typical for GD, as it is for many rare disorders. In 2017, Mehta et al[2] reported delays of 4 to 10 years in the United States and 0.6 to 26 years in the United Kingdom from first symptoms to diagnosis. In the case of our patient, who had a relatively mild clinical course, perhaps no harm was done. When she was diagnosed, in the premolecular era, carriers could not be accurately identified; genetic counseling was limited; and many Ashkenazi Jewish patients were advised to marry outside the Ashkenazi community.

Case report part 2: 24 years later, the era of GD-specific therapies

The patient presented at our Gaucher unit in 1998 at the age of 43 years and was married with 6 children. She was referred by the preoperative screening clinic (before repair of an umbilical hernia), where she was noted to have marked hepatosplenomegaly, pancytopenia, and abdominal ultrasonographic findings of multiple splenic lesions, suspicious for malignancy (Figure 2).

The diagnosis of GD was confirmed by reduced glucocerebrosidase activity and homozygosity of the N370S mutation in *GBA1* (new nomenclature c.1226A>G; p.N409S). The patient's family history was remarkable for 4 children with GD and a mother with Parkinson disease (PD), diagnosed at age 64.

Although recognized for almost 140 years, the natural history of untreated GD has not been fully appreciated. Many mildly affected and even asymptomatic patients receive lifelong ERT because their physicians erroneously assume that, without therapy, the disease will invariably deteriorate. However, GD progression (even in those with moderate to severe phenotypes) occurs during childhood, adolescence, and early adulthood with a tendency to stabilize later in life. In the majority of the mild or asymptomatic patients, GD remains unchanged for decades.[3] Our moderately affected patient has remained stable

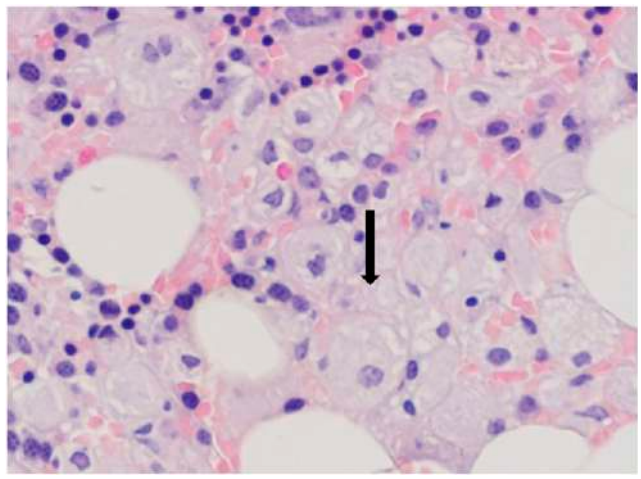

Figure 1. Bone marrow trephine biopsy showing typical "Gaucher cells" (arrow).

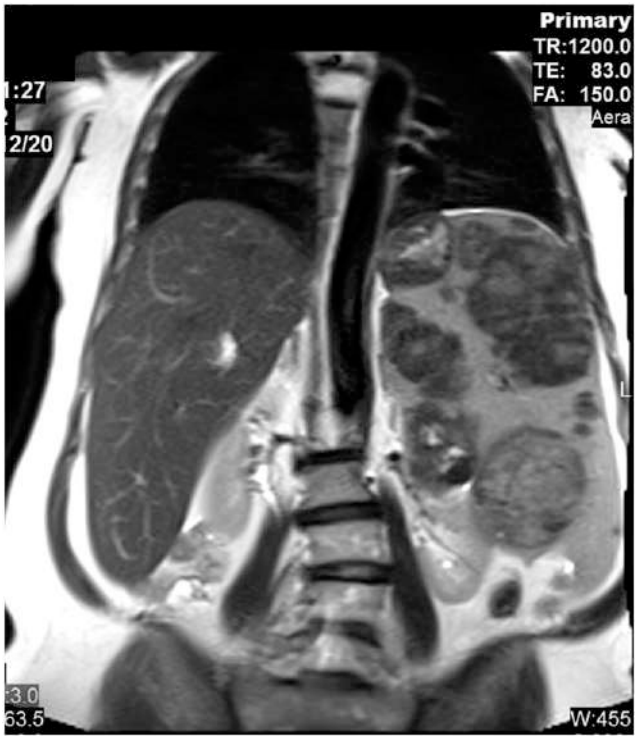

Figure 2. Abdominal magnetic resonance imaging showing hepatosplenomegaly with prominent intrasplenic lesions.

for more than 2 decades. Intrasplenic (and less commonly intrahepatic) lesions—hypoechogenic, hyperechogenic, or mixed—are typical in GD, but for the inexperienced radiologist, they may mimic hematological malignancies, as in our patient.[4]

The gold standard for GD diagnosis is the demonstration of reduced β-glucocerebrosidase activity combined with whole *GBA1* sequencing (to avoid pitfalls). Testing for specific biomarkers, such as chitotriosidase or glucosylsphingosine (LysoGb1), is helpful for diagnosis and for monitoring disease progression (in the untreated) and therapeutic response (for the treated patients).[1] All 3 tests are conveniently done today as dry blood spots on filter papers. The key laboratory findings are delineated in Table 2.

Although the N370S mutation, also known as the "common Jewish mutation," is often associated with a mild or asymptomatic phenotype, various factors may negatively influence the clinical course of patients with this genotype, who may have more severe manifestations, including bony complications.[5] With a 1:17 prevalence of this mutation in the Ashkenazi population and no premarital screening, our patient married an N370S carrier, and GD was diagnosed in 4 of her 6 children. They demonstrated the phenotypic diversity associated with this genotype: Her older son developed osteonecrosis of the hip and sacroiliac joints; one daughter had long-term thrombocytopenia before ERT; and the remaining 2 children are asymptomatic.

Another interesting aspect is her mother's PD. PD is by far the most common GD-related comorbidity, and a family history of PD is described in ~25% of patients. An increased risk for PD is also found among GD carriers with an increased risk of 3- to 15-fold relative to the general population, according to the severity of the mutation.[6] On the basis of the assumptive underlying pathophysiology (ie, "loss of function" [haploid insufficiency] related to excess of the substrate glucocerebroside in the dopaminergic neurons versus "gain of function," the impact of the misfolded mutant glucocerebrosidase on the aggregation of α-synuclein), there are several clinical trials in *GBA1*-related PD using substrate reduction therapy (SRT) (venglustat), in vivo adeno-associated virus 9–based gene therapy, or pharmacological chaperones (PCs).[7,8] A family history of PD in a patient presenting with splenomegaly and/or thrombocytopenia should suggest GD in the differential diagnosis.

Case report part 3: poor response to specific therapies

With marked splenomegaly, severe thrombocytopenia (<50 × 10^9/L), and anemia, the patient fulfilled the criteria for ERT but was reluctant to receive IV imiglucerase (the only ERT available in 1998) and elected to join the clinical trial of a then-novel oral SRT (miglustat). After 12 months on miglustat, which she tolerated, she had no significant improvements in any of the key disease features, and she chose to remain untreated for the next 7 years.

In 2007, she joined the pivotal trial of taliglucerase alfa, the first plant cell–derived human recombinant ERT. Again, she experienced no adverse effects but no efficacy either. She remained off therapy for 4 more years before starting velaglucerase alfa, which she received for 6 years, still with no improvement despite increased dosages from 15 to 60 U/kg every other week.

The first ERT has revolutionized the management of patients with GD and opened the door for the development of many other treatment modalities for rare diseases in general. It was harvested from a huge volume of human placentas and in 1994 was replaced by the human recombinant imiglucerase, which

Table 2. Key laboratory findings

Parameter	Description
Diagnosis	β-Glucosidase enzyme level, *GBA1* mutations
Biomarkers	
Nonspecific	Elevated ferritin, acid phosphatase, and ACE
	Reduced HDL cholesterol
Specific	Elevated glucosylsphingosine (Lyso-Gb1), CCL18, and chitotriosidase
Hematology	
Cytopenia	Thrombocytopenia, anemia (less common), leukopenia (rare)
Coagulation	Low factor XI, coagulation abnormalities (less common), platelet dysfunction (adhesion, aggregation), abnormal thrombin generation
Gammopathies	Polyclonal, monoclonal
	Free light chain abnormalities, serum immunofixation for detection of monoclonal immunoglobulins
Biochemistry	Abnormal liver function tests
	Reduced vitamin B_{12}, folic acid, vitamin D

ACE, angiotensin-converting enzyme; CCL18, C-C motif chemokine ligand 18; HDL, high-density lipoprotein.

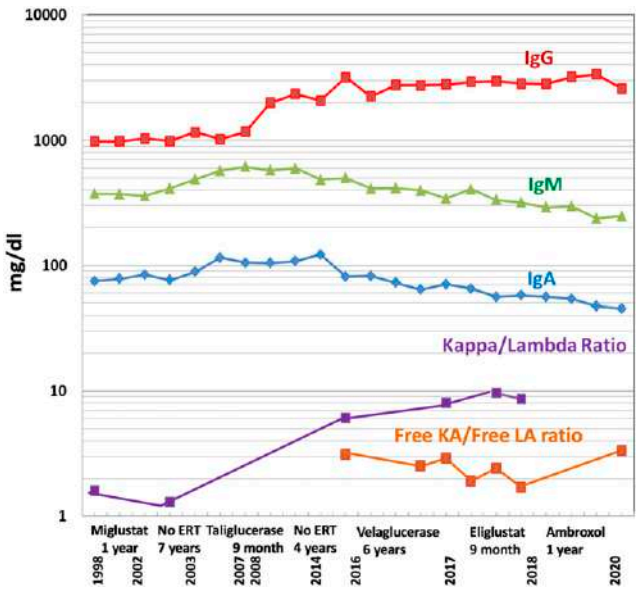

Figure 3. Immunoglobulins and free light chain ratios over time, showing increased IgG levels from 2013 with a gradual decrease of IgM and IgA and a stable free light chain ratio despite various GD-specific therapies.

was until 2010 the only ERT available worldwide. Due to its prohibitive cost, many countries other than the United States have defined criteria for treatment reimbursement, typically covering the more severely affected adult patients as well as symptomatic children. In Israel, patients who fulfill the government criteria usually receive a lower dosage than patients in the United States.[9] Our patient joined the seminal clinical trial of the oral SRT miglustat (the iminosugar *N*-butyldeoxynojirimycin).[10] There are different motivations for patients to join a clinical trial, particularly for rare diseases; these include seeking a cure, improving available treatments, personal gain, or scientific curiosity. The key motivation of our patient to participate in clinical trials (3 so far) was altruism, helping to advance medical knowledge and the opportunity to improve the health of others.

Two observations are relevant to her clinical course in the era of choices.[11] First, patients with GD often maintain stability even when they experience significant disease manifestations, whether they are untreated or nonresponders. Therefore, "maintaining stability" should not be considered a positive endpoint in future clinical trials.[12] Second, massive focal lesions within an enlarged spleen predict a poor platelet and splenic response to ERT[13] and probably to any specific therapy. Thus, these findings may represent a rare indication for splenectomy even nowadays, which can be performed by hand-assisted laparoscopy even for huge spleens.[14]

Case report part 4: GD and monoclonal gammopathy of undetermined significance

During the follow-up period, a gradual increase in quantitative serum immunoglobulin G (IgG) was noted but did not raise concerns, given the relatively high prevalence of polyclonal gammopathy in GD.[15] However, in 2017, a monoclonal gammopathy IgGκ was confirmed by immunofixation, with a mildly elevated free κ/λ ratio of 3.1 and serum IgG of 27.9 mg/dL (Figure 3). The patient's platelet count was 20 × 10^9/L, and her hemoglobin was 9.2 g/dL. Her renal function, serum albumin, lactate dehydrogenase, calcium, phosphorus, and C-reactive protein levels were within normal limits, and her β_2-microglobulin concentration was 3.72 mg/L.

Trephine bone marrow biopsy showed massive infiltration by Gaucher cells; all lineages were present; and 10% infiltration by plasma cells expressing monoclonal κ-light chains was present. These findings were summarized by the pathologist as compatible with GD and multiple myeloma (Figure 4). Flow cytometry and positron emission tomography/computed tomography were nondiagnostic; the finding of a myeloma fluorescence in situ hybridization panel was negative; and the overall picture did not fulfill the diagnostic criteria for myeloma. Erythropoietin and zoledronic acid were added as supportive therapies, and eliglustat was added as GD-specific therapy. The patient received 9 months of eliglustat 84 mg twice daily (as a cytochrome P450 2D6 [CYP2D6] extensive metabolizer), again without effect. There was no significant change in the patient's immunoglobulin profile thereafter.

The association between GD and monoclonal gammopathy of undetermined significance (MGUS) was first reported in 1968.

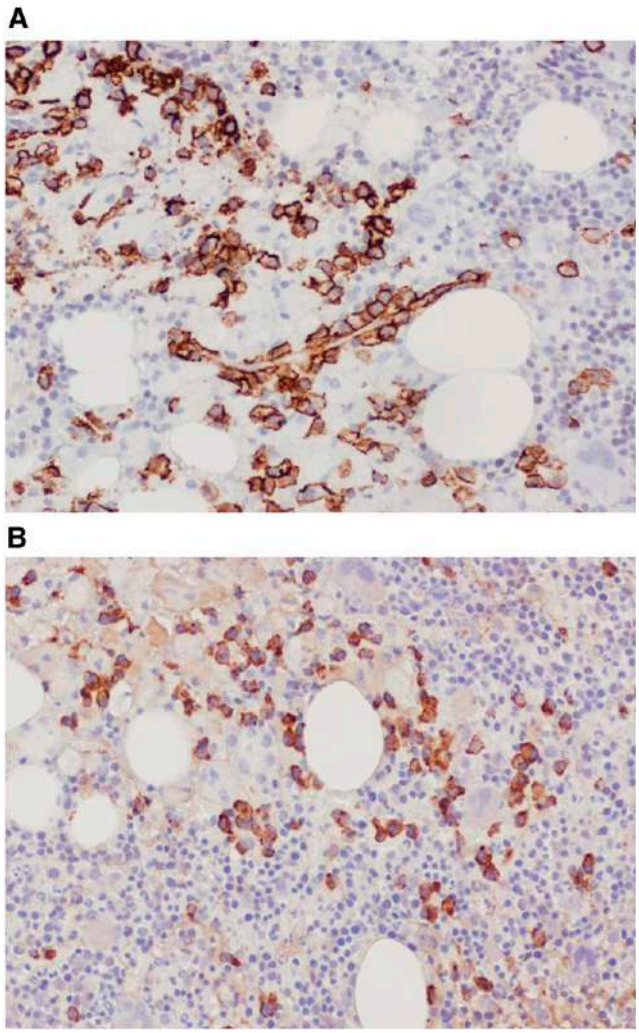

Figure 4. (A) Bone marrow trephine biopsy showing CD138 staining of plasma cells. (B) Bone marrow trephine biopsy showing κ-light chain staining.

In the 1980s, it was suggested that the production of polyclonal and monoclonal gammopathies, including MGUS and myeloma, may be due to chronic stimulation of the immune system secondary to inflammatory cytokines, particularly interleukin-6, derived from the storage cells.[16] Multiple myeloma is recognized as a GD-related comorbidity,[17] and hence, immunoglobulins are included in the laboratory tests done during follow-up visits of our adult patients.[18] Estimations of the prevalence of MGUS range between 1% and 39% of all adult patients with GD, and the magnitude of the actual myeloma risk is between 5- and 50-fold greater than in the general population.[19] Recently, there has been a heated scientific debate regarding whether the monoclonal antibodies, and accordingly the underlying mechanism of GD-related myeloma, are specifically directed at macrophage CD1d-presented glycosphingolipid antigens[20] or at saposin C.[21]

Although no therapy has been proved to ameliorate the paraproteinemia in GD, on the basis of anecdotal reports showing antimyeloma effects of eliglustat in a mouse model,[20,22] eliglustat was attempted unsuccessfully in our patient. It is also unknown if early administration of any GD-specific therapy can prevent MGUS or myeloma or modify the clinical course or response to myeloma therapy in GD. Because glycosphingolipids may impact the immune system in opposing directions,[23] it could be speculated that in some patients with GD, the accumulated glycosphingolipids may promote mainly immune tolerance and thereby paradoxically be associated with a milder course of disease or a lesser risk of progression from MGUS to myeloma.

Case report part 5: novel therapeutic approaches for GD

Currently, after another year of no GD-specific therapy, the patient joined investigator-initiated research evaluating high-dose ambroxol as a glucocerebrosidase PC (ClinicalTrials.gov identifier NCT03950050), so far with no response. The 12 months are going to end in the northern summer of 2020, and a decision needs to be made regarding the next step.

Although it is very unusual for a patient with GD type 1 to fail to respond to all conventional, registered therapies, consideration of alternative and novel modalities becomes relevant. The option of splenectomy has come up repeatedly because it is likely to end most of her key disease abnormalities. Although uncommon in the era of ERT/SRT, there are several cases of nonresponders or patients unable to receive ERT (mainly due to allergies or neutralizing antibodies) or SRT (CYP2D6 ultrarapid metabolizers)[24] whose disease parameters and quality of life improved dramatically after splenectomy. With regard to quality of life, a greater emphasis is given nowadays to disease-specific patient-reported outcome measures, which are also required by regulatory agencies in the assessment of new drugs, including for rare diseases.[25] In a first study of GD-specific patient-reported outcome in a relatively large cohort of adult patients, we have recently reported that despite the known association between GD and cancer, mainly myeloma, almost two-thirds of patients reported a lack of concern about this risk.[26]

The use of PC is based on the recognition that beyond a glycolipid storage disease, GD is also a protein-misfolding disorder,[27] and the proof of concept was reported in 2013.[28] Unfortunately, because ambroxol is inexpensive and exists in different generic formulations, a formal pharmaceutical company–sponsored clinical trial has not been performed. After encouraging data from IIR in Japan in patients with neuronopathic GD,[29] we have launched an open-label clinical trial of ambroxol in patients with suboptimal response to ERT as well as for untreated patients with one of the following 3 abnormalities: thrombocytopenia, elevated LysoGb1, or reduced lumbar spine bone density. Our patient will soon complete the high-dose ambroxol IIR (600 mg/day), but again with no apparent improvement, so the next steps are under discussion.

Apart from splenectomy, there are several new clinical trials that are under consideration for our patient. First is arimoclomol, an investigational heat shock protein amplifier.[30] Results from a 6-month phase 2 dose-finding study (NCT03746587) have just been announced, and when or if a phase 3 trial opens, it could be an interesting oral option (the preferable mode of administration for patients with GD).[31] Second is gene therapy; there are currently 3 companies applying for phase 1/2 clinical trials for either ex vivo or in vivo GD trials. Although our patient might not fulfill the inclusion and exclusion criteria of the early trials (particularly due to her age), the various risks associated with these novel approaches are quite significant.

It seems that our patient will continue to refuse splenectomy, will not be enthusiastic or able to join the forthcoming gene therapy trials, and hence will remain untreated, as patients with GD did in the past, before the advent of specific therapies, and we hope that she will continue to maintain an uncomplicated stable status.

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Conflict-of-interest disclosure

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Off-label drug use

Discussion of off-table drug use: ambroxol.

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Histiocytic disorders: insights into novel biology and implications for therapy of Langerhans cell histiocytosis and Erdheim-Chester disease

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Langerhans cell histiocytosis (LCH) and Erdheim-Chester disease (ECD) are caused by mutations of the MAPK pathway, most often *BRAFV*^{600E}, in myeloid dendritic cells that lead to some overlapping and other unique presentations of the two diseases. LCH occurs in both children and adults, but ECD is primarily found in the latter. The challenges in diagnosing these conditions relates to the rarity of the conditions and that they mimic diseases that are more widely understood, such as certain rashes; bone, lung, and renal diseases; and other malignancies. The histopathology of LCH is definitive, but not so for ECD. Treatment with BRAF and MEK inhibitors has become one of the important advances in the care of these patients.

LEARNING OBJECTIVES

- Have a better understanding of the pathophysiology of Langerhans cell histiocytosis (LCH) and Erdheim-Chester disease (ECD)
- Be aware of new therapies for LCH and ECD

LCH case report

An ear, nose, and throat surgeon has referred a 37-year-old woman with a recent history of chronic otitis externa and a mass in her right external auditory canal. The mass was biopsied and was found to be Langerhans cell histiocytosis (LCH). She also has a history of chronic ulcerative lesions of the vulva. The *BRAFV*^{600E} mutation was found in the tumor tissue (Table 1).

What is important in her past history? What evaluations should be done? What are the treatment options?

This patient represents the chronic course of LCH for many patients, although only 10% of my adult patients with LCH had the disease in childhood. She also is a good example of the many sites involved with LCH and the need to ask specific questions in history taking (Table 2).

What is the pathophysiology of LCH? In 2010, two important discoveries overturned decades of mystery about the disease. Although it was known to be a clonal proliferation of histiocytes, the conundrum of "immunologic disease" vs malignancy had persisted. Gene expression array experiments proved that the pathologic histiocytes of LCH were myeloid dendritic cells that originated in the bone marrow, not from the normal cutaneous dendritic cells known as "Langerhans cells."[1] The most highly upregulated genes were those of early myeloid cell development and osteopontin. Osteopontin attracts T cells to a lesion. Also, in 2010, the groundbreaking discovery of the BRAF mutation in >50% of biopsies of patients with LCH was published.[2] Although the remaining patients did not have the BRAF mutation, all patients with LCH had elevated phosphorylated extracellular signal-regulated kinase (phospho-ERK), meaning that there was some abnormality of the MAPK or associated pathways in every patient with LCH. Subsequent publications showed the BRAF mutation in ≥ 67% of patients with LCH. BRAF-negative patients often have MAP2K mutations, BRAF internal deletions, BRAF rearrangements, and concurrent kinase or other mutations.[3-6] Approximately 15% of patients with LCH have no known mutation causing the elevated phospho-ERK. The BRAF mutation proved to be the perfect "bar code" for identifying the LCH cell of origin. Analysis of bone marrow from patients with LCH and in vitro growth of stem cells revealed the BRAF mutation was in CD34$^+$ stem cells, CD11c$^+$ myeloid dendritic cells, CD68$^+$ macrophages, and in rare cases CD3$^+$ T cells. Ultimately, this work led to the concept that LCH is an "inflammatory myeloid neoplasm." In a mouse model of LCH, upregulation of *BCL21*, which codes for BCLXL, inhibits apoptosis, so the pathologic CD207$^+$ cells accumulate in the lesion. Activation of the MAPK

Table 1. Past medical history

Age	Clinical findings
Birth	Extensive rash, resolves over 1 y
12 y	Stopped growing
14 y	Growth hormone deficiency diagnosed, frequent otitis externa
20 y	Vulvar lesions; biopsy proves LCH, also axillary rash; never had menarche; hypogonadism
23 y	Diabetes insipidus
25 y	Treated with radiotherapy to pituitary, vinblastine/prednisone
26 y	Recurrent skin LCH; reinduction with vinblastine/prednisone, then 6MP/Mtx for 1 y
32 y	Chronic bone pain, bone scan suggesting lesions, biopsy inconclusive
33 y	Hormone supplementation required for pregnancy

6MP/Mtx, 6-mercaptopurine and methotrexate.

pathway leads to loss of CCR7 expression, preventing the pathologic cells from migrating out of a lesion.[7]

Why do patients with LCH have such varied presentations? Why do some with single bone lesions or limited skin lesions resolve spontaneously? Why are some patients cured with a single course of treatment, but others have multiple relapses? The answers to these questions seems to lie in the maturation stage of the myeloid dendritic cell when the BRAF (or other) mutations occurs and whether a patient has the BRAF mutation.

An important clinical division of patients with LCH is whether they have involvement of the liver, spleen, and bone marrow. Patients with those organs involved are classified as "high risk" because in the early Histiocyte Society clinical trials, it was shown that they had the highest risk of a fatal outcome. "Low-risk" patients included those with any other single or multiple non–high-risk organ system affected.[8]

Bone marrow specimens from high-risk patients had the BRAF mutation in $CD34^+$ stem cells, which could spread to an organ system, but especially the spleen or liver, and remain in the bone marrow.[3] It is hypothesized that a BRAF mutation of slightly more mature myeloid dendritic cells would allow circulation to single or multiple organ systems but exclude the high-risk organs. A tissue-resident myeloid dendritic cell with mutated BRAF could account for LCH in a single organ system and limited extent of cutaneous involvement.

If a biopsy of a patient with LCH is $BRAF^{V600E+}$, what does that tell us about prognosis? A study of 100 patients with LCH found that those with the mutation were more likely to relapse and more likely to have neurodegenerative syndrome if $BRAF^+$ cells were present in the peripheral blood at the time there were no obvious LCH lesions elsewhere.[3] There was no clear indication that BRAF mutation in the biopsy differentiated high- and low-risk patients, although circulating $BRAF^+$ cells were almost universally found in the high-risk cases. Subsequently, a larger study reported that a $BRAF^+$ biopsy had a significant association with being a high-risk patient, relapsing, and developing diabetes insipidus and the neurodegenerative syndrome.[9] Evaluation of brain specimens from patients with the neurodegenerative syndrome revealed a surprising finding of $CD1a^+/CD207^+/BRAF^+$ cells in the cerebral blood vessels that migrated into brain tissue and morphed into microglia-like cells staining with $CD14^+$, $CD33^+$, $CD163^+$, $P2RY12^-/BRAF^+$.[10] Molecular analysis of tissue from the cerebellum and pons, with the most dramatic magnetic resonance imaging (MRI) changes, showed high levels of $BRAF^+$ cells and the osteopontin protein. Brain regions with no MRI abnormalities had essentially no mutated cells or osteopontin.

The LCH case I reviewed highlights many of these comments: multiple organ systems involved and multiple relapses with poor response to vinblastine/prednisone.[8] How should she be evaluated now? We perform positron emission tomography (PET) in all patients with new or recurrent LCH to best define sites of disease.[11] PET also provides the best indicator of response to therapy. Given the otic involvement, I would perform computed tomography (CT) of the skull to evaluate her mastoids and temporal bones. Patients with diabetes insipidus have a 50% chance of developing infiltrations of the cerebellum, pons, and basal ganglia with LCH cells. About 5% to 10% of these patients will have neurologic deficits, including ataxia, dysmetria, dysarthria, and mental status changes.[12] Therefore, MRI of the brain is indicated to define masses of the pituitary and T2-weighted fluid-attenuated inversion recovery imaging abnormalities of the areas I indicated above. Patients with long-standing neurodegenerative symptoms can have abnormalities of the globus pallidus, mesotemporal lobes, and rarely the frontal lobe.[13]

What are the treatment options for our patient? Vinblastine/prednisone therapy has failed twice. This is not my regimen of choice, because, in a retrospective series at my institution, we found that neuropathy and dislike of steroids were frequent in our adult patients.[14] Likewise, the outcomes were poor, with a 30% progression-free survival at 2 years. Other publications have suggested better outcomes, but careful analysis of the data confirms that progression-free survival is not better than 30% to 40%.[15] My initial treatment choice for adults with multisystem disease is usually cytarabine 100 mg/m^2 for 5 days monthly for 1 year. Our retrospective series revealed 80% progression-free survival at 2 years. Obviously, this should be studied prospectively, but that is unlikely, given the limited patient numbers and other therapy options I discuss further. Cladribine and clofarabine are also used for patients with multisystem LCH.[16]

This patient's radiographic examinations revealed no bone lesions and no pituitary or cerebellar abnormalities. She had a soft tissue mass plus skin involvement of the external auditory canal and vulva. I started her on hydroxyurea 500 mg twice daily.[17] After 6 months of treatment, all lesions resolved, and we plan to

Table 2. The "seven deadly sins of Langerhans cell histiocytosis"

1. Chronic rashes? Especially seborrheic/scaly or papular rashes for months at any age
2. Bone pain? Lytic lesions? Mastoiditis?
3. Oral lesions? Buccal, gingival, or lingual ulcers, gingivitis, unusual dental decay/loss?
4. Otitis externa, hearing loss?
5. Dyspnea, chest pain?
6. Chronic diarrhea/abdominal pain?
7. Polydipsia/polyuria or other endocrine symptoms?

continue the hydroxyurea for another 6 months, then stop. Hydroxyurea alone or with small doses of oral methotrexate has been a successful treatment of the majority of patients with cutaneous LCH I see. Alternatives include oral methotrexate with or without 6-mercaptopurine, thalidomide, or lenolidomide.[18,19] Cytarabine was not chosen for this patient, because she had only cutaneous lesions with no bone involvement or evidence of pituitary mass. If I had been treating the patient when she was 23 years old, when she developed diabetes insipidus, then I would have treated her with cytarabine. She did not have circulating *BRAF*$^{V600E+}$ cells in her blood, so dabrafenib would not have been chosen. Even if she had had these mutated cells, I would have used hydroxyurea first for economic reasons and because of the fact that the inhibitors never "kill the clone."

BRAF/MEK inhibitor treatments have been used with increasing frequency in patients with relapsed disease and as initial therapy for those with the neurodegenerative syndrome.[20,21] A majority of patients will experience relapse when taken off treatment.

Erdheim-Chester disease

Case report

A nephrologist calls you about a 55-year-old man in renal failure who has some strange tumor around his kidneys and proximal ureters. The biopsy shows lipid-laden macrophages that do not have the atypia of lymphoma cells and stain with anti-CD68 but not CD1a antibodies. The referring physician is mystified because his pathologist said the biopsy was consistent with xanthogranuloma or Erdheim-Chester disease (ECD).[22]

When you first visit with the patient, you are struck by the exuberant xanthelasmas under his eyes and the history of increasing pain in his distal femurs and proximal tibias for years. In the past year, he has developed chronic headaches and cognitive impairment. You order PET/CT and find the following:

- Marked uptake in sclerotic lesions of both distal femurs;
- Circumferential coating of the aorta; and
- Rind around the kidney ("hairy kidney") and hydronephrosis.

The patient's brain MRI shows cerebral and cerebellar atrophy with T2 abnormalities in the cerebellar white matter (Table 3).

Table 3. Erdheim-Chester disease diagnostic criteria[23]

Clinical or morphological
1. Symmetric diaphyseal and metaphyseal osteosclerosis in the legs; best seen by PET scan
2. Other typical ECD findings: perirenal infiltration or periaortic sheathing (CT), right atrial pseudotumor (MRI), or physical examination findings of xanthelasma, exophthalmos, or osteosclerosis of facial sinuses
Histopathological
1. Foamy or lipid-laden histiocytes, fibrosis, and sometimes Touton giant cells
2. Staining for CD68 or CD163 and negative for CD1a
Molecular
1. *BRAF*^{V600E} mutation
2. Other activating mutations of the MAPK pathway: *KRAS*, *NRAS*, *MaP2K1*, *ARAF*, *MAP3KI*, and others
3. Gene fusion activating the MAPK pathway or
4. Activating mutation in *CSF1R*

ECD pathophysiology

The biology of ECD and LCH is remarkably similar, and, in fact, sometimes patients develop both diseases. This is because both diseases derive from a myeloid dendritic cell. In the case of ECD, the developmental pathway is more toward the macrophage lineage. Similar to LCH, 80% of patients have mutations in the RAS/RAF/MEK/ERK cellular signaling pathway.[6] Less frequent mutations include *ALK*, *NTRK1*, *KRAS*, *NRAS*, and *PIK3C*. Patients with ECD with the BRAF mutation are more likely to have right atrial pseudotumors, cardiac and aortic infiltrations, and pericardial and central nervous system (CNS) involvement.[24-26] The proliferation rate as judged by Ki67 staining is low. It is not surprising that a subset of patients have both LCH and ECD, with ECD following or coincident with the LCH diagnosis, given the common origin of these myeloid dendritic cell disorders.[27] Up to 10% of patients with ECD or mixed histiocytosis have myeloid neoplasms, including chronic myeloid leukemia and myeloproliferative and myelodysplastic syndromes.[28] There is a robust inflammatory response in ECD leading to elevated C-reactive protein in 80% and elevated interferon-γ, interleukin-1 (IL-1)/IL1-RA, IL-6, IL-12, monocyte chemoattractant protein-1, and chemokine ligand 18. The latter has been associated with an exuberant fibroblastic response.[29,30]

Our patient manifested several of the classic criteria of ECD. Bone involvement occurs in 80% to 95% of patients but is symptomatic in slightly more than one-third. It can be detected by plain radiography, CT, MRI bone scan, or PET scan, usually in the distal femurs and proximal tibias.[31] Cardiovascular findings include the "coated aorta" in 40%, which is asymptomatic and not associated with dilatation, dissection, or aneurysm.[24] Less than 25% have coronary artery infiltration leading to stenosis and myocardial infarction. Pericardial infiltrates may cause effusions, tamponade, and death. Right atrial pseudotumors are found in slightly over one-third of patients by MRI. Nearly 50% have decreased right ventricular/atrioventricular anatomy, and >60% have decreased right atrial closure.

Pulmonary ECD results in an interstitial lung pattern, interlobular septal thickening, and rarely nodules and ground-glass opacities in one-third to one-half of patients.[32] Few patients have pulmonary symptoms.

Hydronephrosis from sheathing of the proximal ureter by the tissue that surrounds the kidney (hairy kidney sign) and retroperitoneal fibrosis are frequent findings.[33] Like LCH, ECD can cause diabetes insipidus in nearly one-fourth of patients and frequent anterior pituitary deficiencies of which growth hormone deficiency and hyperprolactinemia or low follicle-stimulating hormone and luteinizing hormone levels are found.[34] Unlike patients with LCH, males with ECD may have infiltration of the adrenal glands and testicles.[35]

CNS damage from ECD occurs in over one-third of patients, frequently in conjunction with xanthelasma and diabetes insipidus. MRI findings are reminiscent of LCH with tumor masses and neurodegenerative findings.[36]

Pegylated α-interferon has been considered the primary therapy for patients with ECD who have bone, skin, renal,

and/or sheathing of the aorta.[37] Alternatives include anakinra,[38] infliximab,[39] and sirolimus + steroids.[40]

The outcomes for patients with ECD have dramatically improved since the discovery of mutations in the MAP2K pathway and use of specific drugs inhibiting the effects of these specific mutations.[41] Some experts recommend reserving the inhibitor therapies for patients with cardiac and CNS involvement and using pegylated interferon as the first intervention.[37] Given that nearly 90% of patients with ECD responded to MEK inhibition by cobimetinib, it is likely that more patients will be treated with this class of drugs.[21] Principal side effects include cutaneous and cardiac rhythm abnormalities. The majority of patients experience relapse when taken off any inhibitor treatment, so long-term treatment is required.38 We have now reached a consequential level of understanding of both LCH and ECD. It is fascinating that the biology and therapy of two diseases that formerly seemed so distinct are now woven together. It is critical that a biopsy be promptly performed to distinguish LCH and ECD from lymphomas; Rosai-Dorfman disease; and other, more common conditions.

Conflict-of-interest disclosure

K.M. is on the medical advisory board of Sobi.

Off-label drug use

None disclosed.

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Updates on the diagnosis and management of the most common hereditary porphyrias: AIP and EPP

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The porphyrias are a family of metabolic disorders caused by defects in the activity of one of the enzymes in the heme biosynthetic pathway. Acute intermittent porphyria (AIP), caused by autosomal dominant mutations in the gene encoding hydroxymethylbilane synthase, can lead to hepatocyte overaccumulation and systemic distribution of the proximal porphyrin precursors, 5-aminolevulinic acid (ALA) and porphobilinogen (PBG). ALA and PBG are toxic to neurons and extrahepatic tissue and cause the neurovisceral clinical manifestations of AIP. Management of AIP includes awareness and avoidance of triggering factors, infusions of hemin for severe acute attacks, and, if indicated for chronic suppressive therapy, maintenance treatment with hemin or givosiran, a small interfering RNA molecule that antagonizes ALA synthase 1 transcripts. Erythropoietic protoporphyria (EPP) is most commonly caused by autosomal recessive mutations in the gene encoding ferrochelatase (FECH), the heme pathway terminal enzyme. FECH deficiency leads to erythrocyte overaccumulation and high plasma levels of lipophilic protoporphyrins that photoactivate in the skin, causing burning pain and erythema. Protoporphyrins excreted in the bile can cause gallstones, cholestasis, fibrosis, and ultimately liver failure. Management of EPP includes skin protection and afamelanotide, an α-melanocyte stimulating hormone analog that increases melanin pigment and reduces photoactivation. Liver transplantation may be necessary for severe EPP-induced liver complications. Because AIP and EPP arise from defects in the heme biosynthetic pathway, hematologists are often consulted to evaluate and manage suspected or proven porphyrias. A working knowledge of these disorders increases our confidence and effectiveness as consultants and medical providers.

LEARNING OBJECTIVES

- Understand the pathobiological basis of acute intermittent porphyria (AIP) and erythropoietic protoporphyria (EPP), as related to genetic mutations affecting specific enzymes in the heme biosynthetic pathway and clinical manifestations caused by overaccumulation of harmful porphyrin precursors
- Diagnose AIP or EPP and appropriately screen at-risk family members by using and accurately interpreting biochemical assays and genetic confirmatory tests
- Manage AIP or EPP by using proper avoidance measures, effective pharmacologic interventions, and longitudinal supportive care, including monitoring and interventions for chronic end-organ complications

Why should hematologists know about porphyrias?

The porphyrias are a family of metabolic disorders caused by defects in the activity of one of the enzymes in the heme biosynthetic pathway leading to overaccumulation and excretion of porphyrin precursors in hepatocytes or erythroid cells, leading to extrahepatic or extramedullary cellular, tissue, and end-organ injury. The heme biosynthetic pathway involves 8 enzymatic steps and 7 committed intermediate porphyrin precursors that result in a protoporphyrin ring that incorporates iron to form the heme molecule. Hemoglobin and myoglobin use heme moieties to bind oxygen, and a number of other enzymes contain heme prosthetic groups that react with molecular oxygen and participate in electron transfer reactions.

As clinical hematologists we understand that elevated free protoporphyrin and zinc protoporphyrin are useful biomarkers of iron deficiency and lead poisoning. We might remember that lead inhibits ferrochelatase (FECH), the enzyme that catalyzes the insertion of ferrous iron into protoporphyrin IX (PPIX), thereby allowing PPIX to incorporate zinc ions. We also learned that X-linked sideroblastic anemia is caused by mutations in the 5-aminolevulinate

CASE VIGNETTE 1

A 24-year-old woman is referred by her primary care provider for recommendations after recently being diagnosed with porphyria. Since age 16, she has had sudden-onset episodes of severe abdominal, chest, or pelvic pain associated with burning skin, migratory numbness, and mental "spacing out." These episodes, which last anywhere from 3 hours to 7 days, may be spontaneous or triggered by prolonged sun exposure, spicy foods, chemical fumes, or menses. Numerous evaluations over the years have failed to identify a clear diagnosis, and elimination diets were only transiently beneficial. After the patient learned that her cousin had acute intermittent porphyria (AIP), she purchased a direct-to-consumer genetic test kit and subsequently submitted the raw genomic data to a third-party online service for more detailed interpretation. That report identified a single-nucleotide polymorphism in the hydroxymethylbilane synthase (*HMBS*) gene that was determined to be of unclear clinical consequence. The patient's diagnosis of porphyria was based on this genetic interpretation and a 24-hour urine quantitative porphyrin assay, collected during an acute abdominal pain episode, that revealed isolated, 2-fold elevation in coproporphyrin. Urinary levels of all other porphyrin precursors, including porphobilinogen (PBG), were normal. She reports almost immediate resolution of her symptoms after receiving an infusion of hemin during her most recent acute abdominal pain episode.

synthase 2 gene (*ALAS2*), which encodes the erythroid-specific isoform of the enzyme that initiates porphyrin production.

Thus, our foundational knowledge of heme production and function provides insight into the pathobiological features of the 2 most common congenital porphyrias: AIP and erythropoietic protoporphyria (EPP). Because hematologists are often consulted to evaluate and treat patients with suspected or proven porphyrias, understanding these disorders increases our confidence as consultants and our skills as medical providers.

A brief primer on relevant steps in the heme biosynthetic pathway

Erythroid precursors produce roughly three quarters of total body heme, and hepatocytes produce much of the rest.[1] Heme biosynthesis begins with production of 5-aminolevulinic acid (ALA). This step is catalyzed by 5-aminolevulinate synthase 2 (ALAS2) in erythroid cells and by 5-aminolevulinate synthase 1 (ALAS1) in the liver and other tissues. ALA production is the major rate-limiting step, and, importantly, ALAS1 and ALAS2 are differentially regulated. Hepatic ALAS1 is activated by cytochrome P450 inducers such as smoking, alcohol consumption, calorie deprivation, numerous pharmacologic agents, and female hormones, particularly progesterone. Heme and glucose decrease the production and stability of ALAS1, and exogenous heme (in the form of pharmaceutical hemin) is a strong negative feedback inhibitor of porphyrin synthesis in the liver (Figure 1). By comparison, ALAS2 production is downmodulated during erythroid maturation via the GATA1 transcription factor. Post-transcriptional regulation of ALAS2 is primarily through an iron-responsive translational control element in the *ALAS2* messenger RNA. At the terminal end of heme biosynthesis is FECH, an iron–sulfur cluster protein that is controlled by a number of transcription factors that are active during erythropoiesis. Iron deficiency and impaired iron–sulfur cluster biogenesis reduce FECH activity and heme formation.

Classification and characterization of porphyrias

Acute hepatic porphyrias

Four disorders are associated with high systemic levels of ALA, leading to neurovisceral clinical manifestations and end-organ complications.[1,2] Acute symptomatic attacks are triggered by physiological or exogenous "porphyrinogenic" factors that upregulate ALAS1 and flood the heme biosynthetic cascade with porphyrin precursors. AIP is an autosomal dominant (AD) disorder with a high estimated frequency in Whites, at roughly 1 in 2000, but with low penetrance (1%-10%) for symptomatic disease.[1,2] AIP is caused by mutations in the hydroxymethylbilane synthase gene (*HMBS*), which encodes HMBS, the third enzyme of the heme biosynthetic pathway (also known as PBG deaminase). Defective HMBS can lead to accumulation of the proximal porphyrin precursors, ALA and PBG, that circulate systemically and saturate extrahepatic tissues to cause the various neurovisceral clinical manifestations of AIP (Figure 1).

Variegate porphyria (VP) is caused by AD mutations in protoporphyrinogen oxidase (PPOX). VP is less common than AIP except in South Africa, where 3 in 1000 Whites of Dutch ancestry are affected. This higher prevalence is related to a founder effect of immigrants from the 17th century. Of note, the incidence of VP in the Netherlands is similar to that in other European countries. Hereditary coproporphyria (HCP), caused by AD mutations in coproporphyrinogen oxidase (CPOX), affects roughly 1 to 2 in 1,000,000 Whites. Because the enzyme deficiencies with HCP and VP occur in the distal end of the heme biosynthetic pathway, the highest porphyrin precursor levels in the urine and feces are coproporphyrin and protoporphyrin, respectively (Figure 1). Different from AIP, the "downstream" lipophilic porphyrin intermediates that build up with HCP and VP deposit in the skin, where solar photoactivation can cause blistering and altered hair growth. ALA dehydratase (ALAD) porphyria is an extremely rare and severe acute hepatic porphyria caused by autosomal recessive (AR) mutations in the *ALAD* gene, which encodes the second enzyme in the heme biosynthetic pathway.

Photocutaneous porphyrias

Four disorders are associated with predominant skin signs and symptoms, with or without end-organ complications. The most common inherited disorder, affecting roughly 0.5 to 2.7 in 100,000 White children, is EPP.[2] The majority of symptomatic EPP cases are caused by AR inheritance of a loss-of-function *FECH* mutation on 1 allele, with a common low expression *FECH* genetic variant on the other allele, resulting in FECH deficiency (<30%) (Table 1). Reduced FECH leads to excess red cell and plasma protoporphyrins that photoactivate in the skin, causing severe burning, erythema, and swelling. Protoporphyrins excreted in the bile can lead to cholestasis, gallstones, fibrosis,

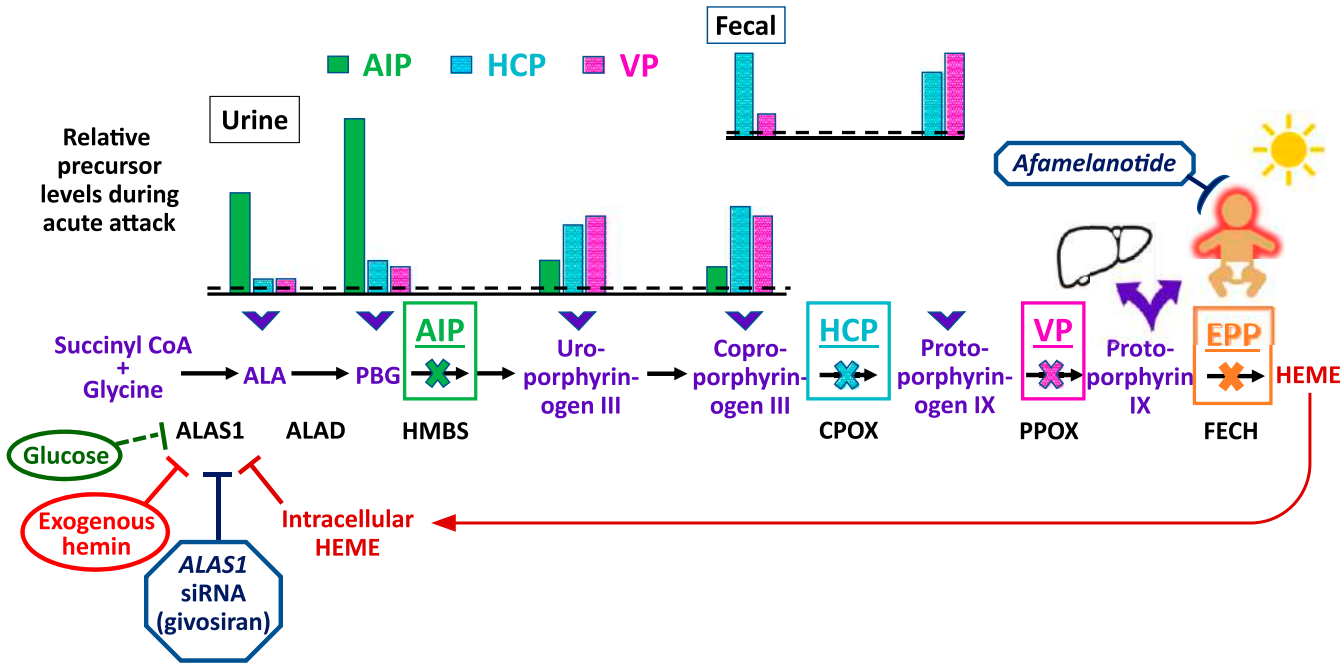

Figure 1. Schematic of the heme biosynthetic pathway with clinical correlates of three autosomal dominant AHPs: AIP, HCP, and VP; and autosomal recessive EPP. The 8 enzymatic steps (represented by arrows) and the key enzymes (protein abbreviations under arrows) highlight the pathobiological consequences of enzyme insufficiency. In patients with an AHP, induction of ALAS1 activity leads to buildup of the neurotoxic porphyrin precursors ALA and PBG, which distribute into tissues and cause neurovisceral signs and symptoms. The bar charts above the pathway depict the relative elevations of proximal and distal porphyrin precursors that are excreted into urine and feces with AIP, HCP, or VP during an acute symptomatic attack (dotted line represents relative 4-fold elevations above the upper ranges of normal). Relative fecal levels of coproporphyrin and protoporphyrin differentiate HCP from VP. Physiological inhibitors of ALAS1 include glucose and intracellular heme. Pharmacologic inhibitors include exogenous hemin and a hepatocyte-directed, small interfering RNA drug, givosiran, which targets the *ALAS1* transcript. A fourth, extremely rare AHP is caused by ALAD deficiency. FECH mutations lead to erythrocyte buildup and leakage of PPIX. Lipophilic PPIX deposits in the skin, where it causes painful photosensitivity. Afamelanotide, which stimulates melanin production, can protect against photoactivation for patients with EPP. Biliary excretion of PPIX can lead to chronic cholestatic liver injury.

and liver failure.[2] A less common inherited protoporphyria, X-linked protoporphyria (XLP), is caused by a gain-of-function mutation in the *ALAS2* gene, resulting in high PPIX levels and a clinical syndrome identical to EPP.

Porphyria cutanea tarda is caused by acquired inhibition (in 80% of cases) or AD inherited deficiency of the uroporphyrinogen decarboxylase enzyme (the fifth step in the heme biosynthetic pathway). Suppression of uroporphyrinogen decarboxylase activity to <20% leads to skin deposition of uroporphyrin and heptacarboxyporphyrin, with resultant fragility, blistering, ulceration, and bullae. Congenital erythropoietic porphyria is caused by mutations in uroporphyrinogen-III synthase, which catalyzes the fourth step in the heme biosynthetic pathway.

Role of DNA testing for patients with suspected porphyria

Significant recent progress has been made in identifying pathogenic allele variants in heme synthesis genes that correlate with biochemical abnormalities and clinical phenotypes.[3] Public databases, such as the Human Gene Mutation Database (www.hgmd.cf.ac.uk), provide catalogs of variant allele frequencies based on whole exome and genome sequencing. However, absence of important unpublished variants and inclusion of unvalidated variants limit their usefulness for porphyrias. For these reasons, the International Porphyria Molecular Diagnostic Collaborative was formed to classify and validate disease-specific genetic alterations for acute hepatic porphyrias (AHPs).[4]

Recently, direct-to-consumer (DTC) genetic testing has become a highly popular method to trace ancestry, assess disease risk, and inform wellness practices. People can also acquire their raw genotypic data from the DTC vendor for a web-based third-party company to query publicly available databases to identify additional genetic variants. The pitfalls with this practice include inadequate coding sequence coverage for many genes, technical errors, false positives, and misinterpretation.[5] This issue is particularly relevant for people who believe they have an acute hepatic porphyria based on erroneous interpretation of DTC raw single-nucleotide polymorphism data,[4] despite the lack of biochemical evidence and other clinical parameters, as illustrated in Case Vignette 1.

Genetic testing is not part of first-line evaluation for a patient with suspected porphyrias (Figure 2). For AHPs, genetic testing is important after biochemical characterization to confirm the diagnosis and identify the relevant pathogenic variant in the index case. Recommendations about DNA testing and the use of mutation analyses for confirmation and family screening have

Table 1. Clinicopathologic features of the 2 congenital porphyrias most relevant to the hematology consultant

	AIP	EPP and XLP
Affected gene	*HMBS*	*FECH* (EPP)
		ALAS2 (XLP; 2%–10% cases)
		CLPX (1 case)
Prevalence of disease-causing mutation*	1/2000	*FECH* c.315-48T>C in 31%–43% (Southeast Asia and Japan), 10% (Europe), <3% (Africa)
Prevalence of symptomatic disease*	0.5–10/100,000	0.5–2.7/100,000
Tissue origin	Liver	Erythroid precursors
Pathologic porphyrin precursors	ALA	Protoporphyrins
	PBG	

*Western Europeans and European Americans.[1]

been published by the Porphyrias Consortium of the National Institutes of Health's Rare Diseases Clinical Research Network[6] and are available online (https://www.rarediseasesnetwork.org/cms/porphyrias). Not all sequence variants have been validated as pathogenic, and a small number of pathogenic mutations are not detected by gene sequencing; therefore, the biochemical profile of porphyrin precursors in urine, feces, and blood remains the standard for diagnosis.[7] Once a pathogenic mutation is identified, targeted gene testing can be used to screen all first-degree relatives.

Clinicopathological features of AIP

Insights into the pathobiology of AIP have come from studies of patients who underwent orthotopic liver transplantation. These cases confirmed the liver as the source of PBG and ALA, the tissue-toxic porphyrin precursors. They also identified

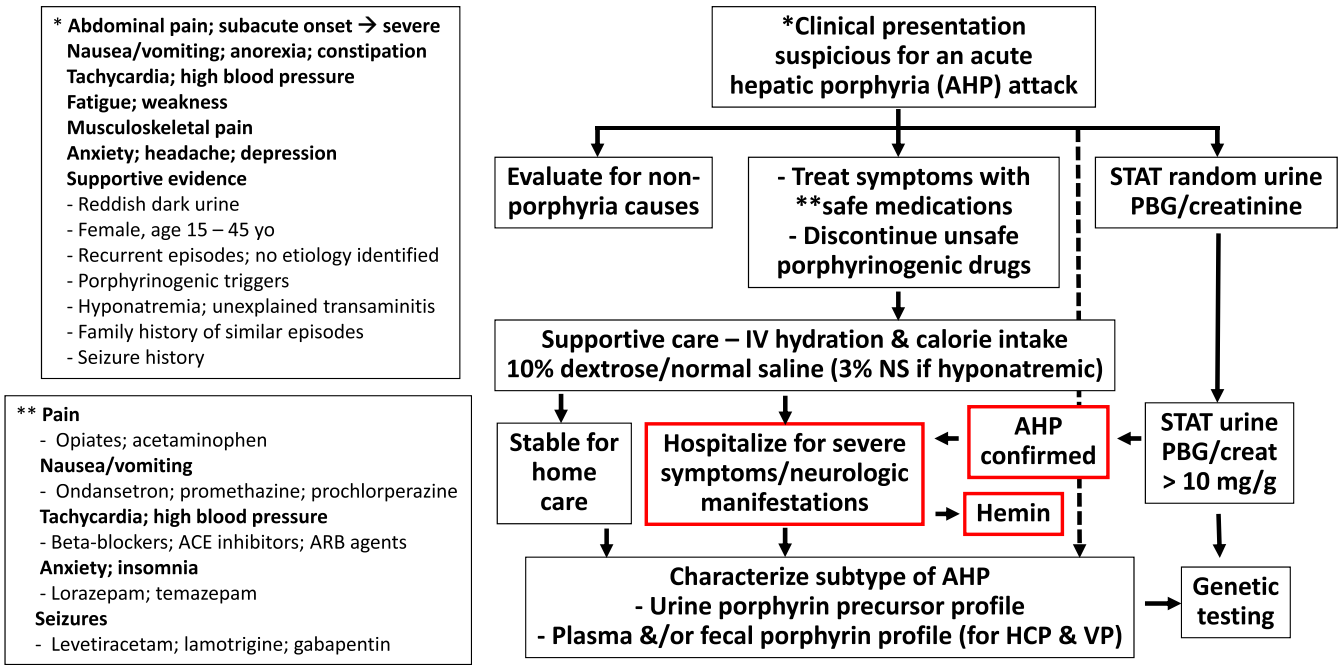

Figure 2. Diagnostic and intervention algorithm for patients presenting with signs and symptoms suspicious for an AHP attack. The neurovisceral manifestations of an attack with AIP are similar to those with HCP and VP. HCP and VP may also have a history of blistering skin rash on sun-exposed areas. A rapid spot urine quantitative assay for PBG can identify pathological accumulation and hyperexcretion of PBG and, by inference, ALA, the neurotoxic precursors that cause acute signs and symptoms. A urine PBG/creatinine ratio >10 mg/g is a sensitive and specific indicator of an AHP. Importantly, asymptomatic patients with AHP (particularly AIP) can have basal high urine PBG levels; therefore, it is always important to evaluate for alternative etiologies and porphyrinogenic triggers of acute signs and symptoms (eg, infection). Additional studies of urine, stool, and blood are needed to fully characterize the AHP subtype biochemically. Genetic testing is not appropriate for initial screening but is used to confirm the diagnosis based on biochemical testing results and can be very helpful for family screening. ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blocker.

CASE VIGNETTE 2

You are consulted on a 20-year-old woman admitted from the emergency department with a suspected acute porphyria attack. Since the birth of her first child 1 year ago she has had increasingly severe and more frequent episodes of gradual-onset abdominal pain with nausea, constipation, and occasional vomiting. The episodes occur at least monthly, usually before her menstrual cycle, and have also been associated with respiratory infections and heavy alcohol intake. Recent episodes have been more severe and prolonged, lasting 3 to 4 days, and associated with musculoskeletal pain, mental fogginess, distal paresthesia, headache, anxiety, and insomnia. Previous evaluations for gallstones, pancreatitis, appendicitis, and endometriosis have been negative. The symptom pattern has not improved with elimination diets, antacids, antiemetics, or hormonal manipulation with oral contraceptives. The patient takes trazodone for sleep and depression. She smokes tobacco and uses edible cannabis products. She reports that her 48-year-old mother was recently diagnosed with porphyria of unknown type. On presentation, she was afebrile, with blood pressure 160/100 mm Hg, pulse 100 bpm, and epigastric abdominal pain rated at 10 out of 10 severity. Abdominal examination revealed hypoactive bowel sounds with generalized tenderness on palpation but without rebound, guarding, organomegaly, or mass. Neurologic examination was notable for 4 out of 5 strength in the proximal arm muscles bilaterally. Laboratory data revealed a serum sodium of 128 mEq/L with otherwise normal electrolytes, renal function tests, and complete blood count. Liver function tests revealed mild elevations in alanine aminotransferase and aspartate aminotransferase, with normal amylase and lipase. A random urine semiquantitative assay for PBG was significantly elevated at 35 mg/L (normal 0–2.3 mg/L) with urine creatinine 1.7 g/L. Urine toxicology screen was positive for cannabinoids. The patient received an intravenous infusion of 10% dextrose overnight and hydromorphone for pain. Your examination confirms the abdominal and neurological findings noted in the ED without new deficits.

biomarkers of inflammation in liver explants along with elevated activity of heme oxygenase 1, which converts heme to biliverdin.[8,9] A murine model of heterozygous AIP recapitulates these liver changes but also implicates hemin infusions in mediating inflammation and oxidative stress.[9]

The mechanisms responsible for acute and chronic neuronal and tissue injury with AIP are incompletely defined. ALA alone is sufficient to cause neurovisceral clinical manifestations, as exemplified by patients with rare ALAD porphyria, which is not associated with high PBG levels. A mouse model of severe homozygous dominant AIP suggests that ALA and PBG alter central nervous system (CNS) myelination and mediate neurotoxicity.[10] At the cellular level, ALA affects the binding affinity of γ-amino butyric acid in neurons and compromises mitochondrial integrity and bioenergetics. ALA also causes vasoconstriction, presumably through an impaired oxidative stress response.[11]

Hyponatremia commonly accompanies AIP symptomatic attacks, and kidney disease develops in >50% of patients with symptomatic AIP.[12] Patients with AIP also have increased risks of hepatocellular carcinoma and cholangiocarcinoma, which, hypothetically, may result from chronic inflammation, exogenous heme, and iron-mediated oxidative injury.[13]

Clinical presentation and chronic complications of AIP

The cardinal symptoms of AIP attacks are listed in Table 2. The "classic triad" of severe abdominal pain, peripheral neuropathy, and central or autonomic nervous system manifestations are variable and nonspecific and carry a broad differential diagnosis. Thus, diagnosis can be delayed for many years, and many patients are misdiagnosed or receive unhelpful treatments.[7,14] Abdominal pain typically builds gradually over hours to days and is often diffuse and associated with nausea, vomiting, constipation, or diarrhea.[14-16] Sensory neuropathy may be manifested as paresthesia, dysesthesia, hyporeflexia, and musculoskeletal pain in the extremities or trunk. Motor neuropathy often starts with proximal muscle weakness in the upper extremities and may progress to the distal and lower extremities. Respiratory muscle compromise can be life-threatening. Autonomic dysfunction is responsible for tachycardia, hypertension, diaphoresis, fever and chills, and bladder and gut dysmotility. CNS manifestations include insomnia, anxiety, depressed mood, dysphoria, confusion, delirium, seizure, and coma.

Historical and clinical features that support AIP as the cause of acute neurovisceral signs and symptoms are listed in Figure 2. Roughly 50% of women with symptomatic AIP report severe premenstrual symptoms, 42% experience exacerbations with oral contraceptive pills (OCPs), and 17% worsen during pregnancy.[14] Patients with symptomatic VP and HCP present with similar acute neurovisceral manifestations as patients with AIP.[15] A differentiating feature of VP and HCP (if present) is solar-activated skin pain and blisters with chronic pigmented areas and scars on sun-exposed areas.

Roughly two-thirds or more of patients with early adult-onset symptomatic AIP who experience recurrent attacks ≥2 times yearly develop by their fourth decade of life chronic disease complications with signs and symptoms that persist between acute episodes[14-16] (Table 2). Quality-of-life parameters are also severely affected, with at least one-half of these patients reporting daily difficulties with pain, discomfort, anxiety, or depression. A significant portion are unable to perform usual activities or continue full-time work; and many experience economic hardship.[15,16]

Diagnosis of AIP

Patients with unexplained neurovisceral signs and symptoms suggestive of AIP should be questioned about hormonal, nutritional, or pharmacological triggering factors and family history supportive of the diagnosis. A publicly available database of porphyrinogenic drugs (https://porphyriafoundation.org/drugdatabase/) should be consulted to determine a possible causal association. Screening laboratory studies may reveal hyponatremia, mild transaminitis, red or brown urine (not related to hemoglobin or bilirubin), or leukocytosis without identifiable infectious, gastrointestinal, hepatobiliary, pancreatic, renal, or

Table 2. Acute and chronic conditions with AIP

A. Acute signs and symptoms during AIP attack	Frequency[14,15]	
Abdominal pain	74%–92%	
Dark urine	81%	
Nausea and vomiting	73%–85%	
Insomnia, fatigue, weakness	70%–80%	
Musculoskeletal and other pain	72%	
Constipation	60%–70%	
Anxiety, depression, headache, trouble concentrating	50%–60%	
Hypertension, tachycardia, diaphoresis, numbness, tremulousness	40%–60%	
Fever, chills	18%–33%	
Diarrhea, heartburn, dysphoria	20%–30%	
Seizures	9	
B. Chronic conditions and symptoms between acute attacks in patients with long-standing, recurrent AIP episodes	**Prevalence in symptomatic AIP[14-16]**	**Prevalence in asymptomatic *HMBS* carriers[16]**
Pain syndrome, neuropathy	43%–100%	17%–30%
Psychiatric symptoms	22%–82%	19%
Hypertension	43%–73%	26%
Chronic kidney disease	29%–64%	13%
Seizures	9%–46%	0%
Dark urine	5%	–
Insomnia, fatigue, weakness	10%–20%	–
Nausea	20%	–
Constipation	10%	–
Hepatocellular carcinoma	1%–9%	1.9%

A, incidence of symptoms and signs during an acute neurovisceral attack. B, prevalence of chronic signs and symptoms between attacks among more severely affected patients with AIP in the fourth decade of life and with multiple yearly attacks, compared with asymptomatic HMBS mutation carriers. Adapted from references 14-16.

gynecologic cause. Because people with undiagnosed AIP may have multiple previous ED visits or take narcotics for unexplained severe pain episodes, they may be labeled as "drug seeking," and urine toxicology screening studies are often obtained during initial evaluation. Such studies can be helpful to identify prescribed medications and nonprescribed substances of abuse.

The first-line screening test for a symptomatic patient with clinical manifestations suggestive of a neurovisceral porphyria attack is a quantitative assessment of PBG in a light-protected random urine sample. Urine creatinine should also be obtained to calculate a PBG/creatinine ratio. This will account for a possible spuriously low PBG result (ie, false negative) secondary to dilute urine from vigorous hydration. A substantially elevated random urine PBG/creatinine of >10 mg/g (>5 μmol/mmol) implicates an AHP (ie, either AIP, VP, or HCP) as the cause of the neurovisceral signs and symptoms.[6,7] In the absence of a urine creatinine level, a spot urine PBG value >4 times the upper limit of normal (eg, >9.2 mg/L if the upper limit of normal is 2.3 mg/L) is significantly sensitive and specific to rule in a diagnosis of a neurovisceral porphyria. By comparison, a random urine PBG/creatinine level that is normal or only slightly elevated is strong evidence that neurovisceral porphyria is *not* the cause of the patient's acute presenting signs and symptoms. Importantly, some patients with AIP persistently excrete high levels of PBG into the urine during asymptomatic periods between acute attacks. Therefore, nonporphyria etiologies (eg, gastroenteritis, peptic ulcer disease, biliary colic, pancreatitis, appendicitis, nephrolithiasis, urinary tract infection, pelvic inflammatory disease) should be considered, particularly if the presenting signs and symptoms are atypical.

To confirm the diagnosis and define the type of AHP for a patient with acute symptoms and a high urine PBG/creatinine ratio, full quantitative biochemical porphyrin precursor analyses should be performed on a properly collected (in appropriate buffer) urine sample at the time of an acute symptomatic episode, along with fecal and plasma porphyrin levels. The relative elevations of ALA, PBG, and more distal porphyrins provide clues to the etiology. Given the pitfalls of successfully collecting, handling, and processing a 24-hour urine sample for porphyria assessment, many centers have moved away from this previous approach. In AIP, ALA and PBG are markedly elevated, with lower relative increases in uroporphyrin, coproporphyrin, and protoporphyrin (Figure 1). Alternatively, marked elevations of the "downstream" porphyrins, with lower increases in ALA and PBG, suggest VP or HCP. The fecal and plasma porphyrin profiles

can differentiate VP (with high protoporphyrin) from HCP (with high coproporphyrin).

Because full biochemical testing can be confounded by improperly collected 24-hour urine (ie, lacking the appropriate buffer, not light protected, or prolonged storage at room temperature) or collection after hemin infusion, repeat testing should be performed when in doubt. Ideally, this is done during an acute attack with typical signs and symptoms. False positive interpretation may also occur when the assay reveals <4-fold elevation of distal porphyrins, particularly an isolated mild increase in coproporphyrin alone.[7] For suspected AIP, erythrocyte HMBS (PBG deaminase) enzyme activity assay is available; however, borderline normal to low levels may be seen in healthy people and patients with AIP. Thus, the HMBS activity does not add to biochemical characterization and genetic confirmation of AIP. Patients who seek consultation because of infrequent or remote neurovisceral symptoms with or without a family history could be screened with spot urine, fecal, and plasma porphyrin profiles. However, negative results mandate repeat testing during a symptomatic attack.

DNA testing for disease-causing mutations in *HMBS*, *CPOX*, and *PPOX* is not recommended for front-line screening.[6,7] However, genetic screening may be considered in selected cases with a confirmed family history or personal history strongly suggestive of a neurovisceral porphyria.[7] Genetic testing is otherwise indicated to identify the specific disease-causing mutations in biochemically confirmed symptomatic patients and for screening at-risk relatives of the index case.[6,7]

Management and monitoring of AIP

Management of acute signs and symptoms of a patient with known or highly suspected AIP involves recognizing the neurovisceral manifestations (and not overlooking other etiologies), treating precipitating factors (eg, occult infection), withdrawing porphyrinogenic medications, carbohydrate loading, symptom control, and supportive care including safe medications. Vomiting patients need intravenous hydration and may need correction of hyponatremia. Infusion of 10% dextrose, to deliver 300 to 500 g of glucose, may abort an early, mild attack; however, this is often not effective for more severe episodes and could worsen hyponatremia. For clinical complications severe enough to necessitate aggressive support in the clinic or ED, and with any new neurological deficit, intravenous infusion of hemin is indicated.[1,2,6,7] Hemin should be reconstituted in 25% albumin and administered by slow infusion (eg, 60 minutes via peripheral intravenous line) to minimize coagulopathy and phlebitis.[7] Patients who need frequent infusions of hemin benefit from placement of a semipermanent central venous catheter. To optimally suppress ALAS1 activity, 3 to 4 mg/kg/day for 4 days is needed. Although serial urine PBG/creatinine determinations during a course of hemin can reflect a biochemical response, clinical manifestations often do not correlate with absolute levels of urine PBG excretion. This is especially true for patients who chronically excrete high levels of urine PBG between episodes.

After nonporphyria etiologies of pain have been ruled out, escalating doses of opioids and anxiolytics can be carefully titrated while the patient is closely monitored for response and CNS side effects. Antiemetics and β-adrenergic blocking agents are useful for nausea and autonomic complications of tachycardia and hypertension. Levetiracetam and lamotrigine are safe antiseizure medications.

Longitudinal management of recurrent AIP episodes requires awareness and avoidance of factors that precipitate attacks, maintenance of a well-balanced diet with adequate carbohydrates (60%–70% total calories), a healthy lifestyle (eg, avoiding smoking and alcohol intake), and surveillance with management of the vascular and end-organ complications (Table 2). Some patients with frequent acute attacks benefit from scheduled maintenance infusions of hemin. These may be given up to twice weekly but require careful monitoring for benefit and tolerance.[1,6,7] Complications include the need for central venous access, iron overload (which can be managed with phlebotomy), and a concern (based on animal models) that hemin may induce hepatic inflammation and potentially exacerbate clinical attacks.[1,7,9]

Women with symptomatic AIP and frequent cyclic neurovisceral attacks triggered by the luteal phase of the menstrual cycle may benefit from a low-dose combination oral contraceptive pill. However, this approach may worsen attacks in half of women and therefore must be carefully monitored. Standard-dose OCPs, progestin-only agents, implants, and intrauterine devices should be avoided. Alternatives include monthly preemptive infusions of hemin or ovarian suppression with a gonadotropin-releasing hormone agonist combined with a low-dose estrogen supplement to avoid menopausal symptoms and bone loss. Fertility is not impaired by AIP. Pregnancy exacerbates the frequency and severity of neurovisceral attacks in 15% to 20% of women. Hemin infusions can be safely given during pregnancy, when indicated, and are usually well tolerated. Ideally, a pregnant patient with AIP is co-managed by a high-risk obstetrician and, at the time of delivery, an anesthesiologist who is familiar with AIP and the contraindicated medications, potential complications, and interventions.

Chronic pain syndromes and neuropathy pose particular challenges. More severely affected patients benefit from co-management with a pain specialist. Multiple interventional approaches may be needed, including narcotics, gabapentin, antidepressants, and cognitive behavioral therapy. Psychiatric manifestations, including anxiety and depression, along with insomnia and fatigue significantly reduce quality of life and adversely affect family and social interactions.[17] Safe antidepressants, anxiolytics, and other psychotropic agents may be used judiciously but must be closely monitored. Social service support is often needed because of the significant negative impact of recurrent AIP attacks on functional status and employability.[16,17] For patients ≥50 years of age, liver ultrasounds and α-fetoprotein determinations are recommended every 6 months to monitor for hepatocellular carcinoma.[6]

Givosiran, a novel, liver-specific small interfering RNA molecule directed against *ALAS1* messenger RNA, was approved in 2019 to treat patients with acute hepatic porphyrias and recurrent symptomatic attacks. Administered as a monthly subcutaneous injection, givosiran potently reduces *ALAS1* transcripts and ALAS1 protein, thereby preventing overproduction of ALA and PBG during steady state and porphyrinogenic triggers. In the phase 3 pivotal trial, patients with AIP and a history of ≥2 symptomatic attacks in the preceding 6 months who received givosiran experienced a 74% reduction in the mean annualized rate of porphyria attacks and a 77% reduction in the mean annualized number of days of hemin, compared with patients who

CASE VIGNETTE 2: FOLLOW-UP

The high spot urine PBG/creatinine level was interpreted as diagnostic of AHP. The patient was aggressively treated with daily hemin infusions at 4 mg/kg/day for 4 days, and spot urine PBG/creatinine levels decreased significantly but did not normalize. Her symptoms improved, and she was stable for discharge after day 4 hemin infusion. Urine collected during the first hospital day revealed ALA and PBG levels 12- to 15 times above the upper limit of normal and uroporphyrin, coproporphyrin, and protoporphyrin levels 5- to 8 times above normal. Genetic testing revealed a mutation in *HMBS* (c.517C>T; p.R173W) that has been identified as a common pathogenic mutation in numerous unrelated probands. Family genetic studies confirmed inheritance from the patient's mother. The patient subsequently needed monthly preemptive infusions of hemin to prevent cyclic AIP attacks, and she is under consideration for treatment with givosiran.

received placebo.[18] In an extension phase of the trial, biochemical benefit was reportedly sustained out to 26 months. Givosiran can cause injection site reactions, nausea, and elevations in creatinine, amylase, lipase, and liver function tests, but was generally well tolerated. Importantly, givosiran interacts with CYP1A2 and CYP2D6 substrate drugs, and it is also a high-cost drug. Therefore, the optimal indications and value-based use of this agent for symptomatic patients with AIP remain to be defined.

Asymptomatic patients who carry a pathogenic *HMBS* mutation (identified through family screening) must be counseled to avoid porphyrinogenic triggers, particularly the broad list of medications and progestins that activate ALAS1 (https://porphyriafoundation.org/drugdatabase/). Formal recommendations for latent cases (ie, carriers with normal basal PBG excretion) and asymptomatic high excreters (ie, those with baseline urine ALA or PBG levels >4 times above normal) have been published.[6] These include awareness of triggering factors, vigilance for signs and complications, healthy lifestyle practices, and a balanced diet without prolonged fasting or crash dieting. Because chronic high levels of ALA and PBG can induce end-organ damage in the kidneys and liver (with increased risk of hepatocellular carcinoma), asymptomatic high excreters should undergo thorough annual evaluations, including liver ultrasound and α-fetoprotein assessment when >50 years of age.[6]

Clinicopathological features of protoporphyria

EPP manifests with phototoxicity that happens when protoporphyrins in the superficial skin vasculature absorb light radiation and give off energy to form reactive oxygen species, resulting in lipid peroxidation, complement activation, and inflammation of the dermal capillaries.[19] Almost 40% of patients with EPP report that a correct diagnosis required a mean of 12 years and evaluations by ≥5 physicians[20] despite a similar percentage of patients presenting symptoms in the first year of life.[21] Therefore, pediatricians should suspect EPP in children with unexplained allergic reactions, often labeled as "allergy to the sun." A typical episode begins with burning and tingling sensation on skin exposed to the sun, developing into pain, swelling, and sometimes erythema. Pain can last ≤7 days and is not alleviated by medications, including opioids. Scarring, hyperpigmentation, hypopigmentation, and vesicles are uncommon, although repeated sun exposure leads to skin thickening and hyperkeratosis.[19] Phototoxicity may be triggered by artificial fluorescent light and sunlight through windows, because UVA and visible light are not filtered. Patients eventually develop a sun avoidance behavior that affects their quality of life and social interactions.

Mild iron deficiency anemia occurs in 37% to 44% of patients. The etiology is unclear, but it may result from iron-mediated stimulation of ALAS2 production and excessive PPIX triggering inflammation that can block iron absorption.[22] Excess protoporphyrins are excreted into the bile, accumulate in the liver, and cause cholestatic hepatitis, with severe disease in 2% to 5% of cases. Clinical hepatobiliary manifestations and gallstones usually develop after age 30. Table 3 compares the clinical presentations of EPP and XLP.

Diagnosis of protoporphyria

The investigation of suspected protoporphyria should include measurements of total, metal-free, and zinc erythrocyte protoporphyrins (ePPs).[23] FECH deficiency with EPP leads to high ePP levels that are predominantly metal-free ePP (85% to 100%), with normal zinc ePP. By comparison, XLP, with preserved FECH and increased ALAS2 activity, leads to elevations in both

CASE VIGNETTE 3

A 25-year-old woman seeks a second opinion on a diagnosis of porphyria. As a child, she lived in Hawaii and struggled with a "severe skin allergy" not responsive to antihistamines. By age 5, she was able to tell her parents that she had pain upon exposure to sunlight, and they asked her pediatrician test her for EPP. She was found to have "elevated blood porphyrins," but no genetic testing was performed at the time. The family then decided to move to Oregon to help her shelter from the sun. During high school, she took oral β-carotene for 6 months, but she disliked the yellow skin discoloration and still could not withstand more than her usual 20 minutes of sun exposure without burning, swelling, and redness on her cheeks and hands that lasted for 2 to 3 days. She was recently offered a job at a prestigious company in southern California, but she is concerned that she cannot tolerate the sunnier environment. She has read on the Internet that there is a new treatment for EPP and is wondering whether it would be helpful.

Table 3. Clinical presentation of EPP and X-XLP

Characteristic	EPP	Male patients with XLP	Female patients with XLP
Male	103 (50.5%)	10/10 (100%)	–
Female	101 (49.5%)	–	12/12 (100%)
Presentation			
Age of onset, y (SD)	4.1 (3.0)	2.7 (2.4)	11.6 (11.4)
Symptoms before age 12	188/204 (92.1%)	10/10 (100%)	5/12 (42%)
Cutaneous manifestations			
Time to symptoms <10 min	52/204 (25.5%)	2/10 (20%)	2/12 (17%)
Time to symptoms <30 min	116/204 (56.8%)	10/10 (100%)	6/12 (50%)
Burning	186/204 (91.2%)	9/10 (90%)	NR
Tingling	160/204 (78.4%)	10/10 (100%)	NR
Itching	159/204 (77.9%)	8/10 (80%)	NR
Swelling	176/204 (86.3%)	10/10 (100%)	NR
Redness	97/204 (47.5%)	4/10 (40%)	NR
Sensitivity to fluorescent light	48/204 (23.5%)	4/10 (40%)	2/12 (17%)
Extracutaneous manifestations			
Abnormal aminotransferases	19/140 (13.6%)	3/8 (37.5%)	2/9 (22%)
Gallstones	45/204 (22.1%)	4/10 (40%)	4/12 (33%)
Age at diagnosis of gallstones, y	30.4 (11.3)	43 (10.7)	24.8 (6.7)
Anemia	95/204 (46.6%)	3/10 (30%)	9/12 (75%)

Adapted from Balwani et al.[21]

metal-free (50% to 85%) and zinc ePP. Plasma protoporphyrins are also elevated, with a characteristic fluorescent peak at 632 to 634 nm. Notably, urinary protoporphyrins are normal. Of note, standard laboratories may not be able to provide reliable protoporphyrin levels; therefore, testing by a specialized laboratory is strongly recommended. Genetic testing establishes the diagnosis of EPP in the presence of two *FECH* gene mutations in *trans*, often including the hypomorphic variant c.315-48T>C (Table 1). Missense mutations are associated with lower ePP levels, longer tolerance to sun exposure, and a lower risk of hepatic complications. *ALAS2* gain-of-function mutations confirm XLP. Next-generation sequencing has the potential to identify novel pathogenic variants among the 5% of patients with biochemical EPP who do not have mutations in either gene.[24]

Management of protoporphyria

Sun avoidance is the mainstay of management of phototoxicity. Sunscreen containing titanium or zinc oxide can block UVA and visible light but is cosmetically unpleasant. Tinted windows and use of protective clothing are helpful. The use of light filters

Table 4. Recommendations for monitoring patients with EPP

Condition	Recommendation	Periodicity
Gallstones or cholestasis	Erythrocyte protoporphyrins	Every year
	Liver function tests (aspartate aminotransferase, alanine aminotransferase, bilirubin)	Every year
	Abdominal imaging (ultrasound or computed tomography)	Every year; consider shorter intervals for patients with erythrocyte protoporphyrins >2000 µg/dL
Low bone mineral density	Serum vitamin D	Every year
	Alkaline phosphatase	Every year
	Bone mineral density (dual-energy X-ray absorptiometry scan)	Baseline, then every year if treating osteopenia or osteoporosis; repeat every 3–5 y if normal scans
Iron deficiency anemia	Complete blood count	Every year
	Ferritin and transferrin saturation	Every year
Skin hyperpigmentation or melanocytic nevi	Full body skin exam	Every 6 mo if on afamelanotide

CASE VIGNETTE 3: FOLLOW-UP

Elevated total erythrocyte protoporphyrins (1155 µg/dL) with normal zinc protoporphyrins (55 µg/dL, reference value <60 µg/dL) yielded 95% of metal-free protoporphyrins, supporting the diagnosis of EPP. Genetic testing revealed coinheritance of the common variant *FECH* c.315-48T>C in *trans* with *FECH* c.1217G>A p. C406Y, a missense mutation. Liver function tests and a baseline abdominal ultrasound were normal. Complete blood counts showed iron deficiency anemia with hemoglobin 10.7 g/dL and ferritin 12 µg/L. Vitamin D level was low. Because the patient was asymptomatic, iron was not prescribed, but vitamin D supplements and a dual-energy X-ray absorptiometry scan were recommended. The patient was referred to a local specialist to start afamelanotide implants every 60 days. She will have full-body skin exams every 6 months and annual evaluations to monitor anemia, bone mineral density, and hepatobiliary status.

(eg, yellow 61011 filters) during prolonged surgery or procedures can prevent skin and internal organ burns from fluorescent light exposure,[25] particularly during liver transplant for cholestatic disease.

Oral β-carotene has been reported to benefit ≤40% of patients when used at therapeutic dosages that cause yellowish skin discoloration.[21] However, multiple well-designed studies have failed to demonstrate efficacy. *N*-acetylcysteine, cysteine, vitamin C,[26] and isoniazid[27] have been tried without success.

Afamelanotide, an α-melanocyte–stimulating hormone analog delivered by a subcutaneous implant, was approved by the US Food and Drug Administration in 2019 for the treatment of EPP. It binds to the melanocortin 1 receptor and increases the production of eumelanin, providing photoprotection and antioxidant defense in melanocytes. In 2 randomized, placebo-controlled trials, afamelanotide prolonged pain-free time, decreased phototoxic reactions, and improved quality of life.[28] The main side effects are nausea and application site reactions. Importantly, hyperpigmentation and increase in melanocytic nevi require full body skin examinations twice yearly. The maximum recommended regimen of 4 implants per year with an interval of 60 days represents a limitation for patients living in sunnier regions. Another stimulator of melanin production, MT-7117, is orally administered and now moving to phase 3 trials (NCT04402489).

Iron deficiency anemia with EPP is usually mild. Because iron can increase ALAS2 activity and worsen PPIX accumulation, iron supplementation should be prescribed only for patients with symptoms of iron deficiency. By comparison, preserved ferrochelatase activity in XLP facilitates iron incorporation into PPIX, and iron supplementation decreased photosensitivity in 7 of 8 female patients with XLP.[21]

Bone health is also a concern. Vitamin D deficiency, secondary to lack of sun exposure, affects 43% to 63% of patients with EPP, with elevated alkaline phosphatase and decreased bone mineral density.[29] Physical activity should be encouraged, and smoking and excessive alcohol intake should be avoided.

Progressive hepatopathy with EPP has been managed with ursodeoxycholic acid, cholestyramine, and activated charcoal.[1,2] Temporary use of red cell or plasma exchange with hemin infusions in cholestatic hepatitis is controversial.[30] Liver transplantation has been performed in >50 published cases.[31] However, a definitive cure can be achieved only with hematopoietic cell transplantation. It is usually considered after liver transplant for younger patients, for older patients with recurrent disease affecting the liver allograft, for progressive liver disease, or after reversal of liver failure without advanced liver fibrosis.[32] A promising approach using gene therapy targeting *ALAS2* in EPP erythroid precursors is under development.[33]

A summary of recommendations for monitoring of patients with EPP is shown in Table 4.

Conflict-of-interest disclosure

No conflict of interest declared.

Off-label drug use

None disclosed.

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Existing agents, novel agents, or transplantation for high-risk MDS

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The decision algorithm for treatment of advanced myelodysplastic syndrome (MDS) (intermediate- to very high-risk by the revised International Prognostic Scoring System [IPSS-R]) is complex. Often, the appropriate choice is unknown and not currently addressed by available clinical evidence. Although allogeneic hematopoietic cell transplantation (alloHCT) is curative for some patients with MDS, there is a concurrent high risk of mortality and morbidity. Alternatively, although hypomethylating agents (HMAs) have low toxicity, they are not thought to be curative, with a median increase in overall survival of only 9 months. Initial attempts to improve outcomes with HMAs through addition of novel agents failed, but there is hope that newer combination strategies will improve outcomes. Challenging clinical questions include who should be considered for alloHCT, appropriate timing and preparation for alloHCT, and appropriate therapeutic choices for patients who are not candidates for alloHCT. Given the interplay between alloHCT and non-alloHCT approaches, a unified coordinated approach is optimal for patients with advanced MDS. When possible, patients with advanced MDS should be encouraged to enroll into clinical trials that include alloHCT and non-alloHCT approaches.

LEARNING OBJECTIVES
- Identify which patients with MDS should be considered for alloHCT
- Recognize that HMA failure has poor prognostic implications
- Optimize non-alloHCT therapy choices for MDS, including hypomethylating agents and enrollment in clinical trials

Introduction

Although allogeneic hematopoietic stem cell transplantation (alloHCT) is the only cure for myelodysplastic syndrome (MDS), it carries risk for treatment-related mortality (TRM) and morbidity. The major alternative option is hypomethylating agents (HMAs), which have a lower TRM and less morbidity, but they are not curative and offer limited improvement in overall survival (OS). Despite several failed initial attempts to improve outcomes with HMA combination strategies, there is much hope with newer targeted therapies. For example, oral administration of HMA allows increased exposure duration, potentially improving outcomes. Regardless of initial therapeutic choice, relapse after HMA and alloHCT is associated with short survival and a lack of available treatment options. Enrollment in clinical trials should be strongly supported by clinicians, MDS support networks, and medical societies because it remains the only way to improve historical outcomes. The treatment of MDS requires a unified approach incorporating cellular-based therapies, HMA combination approaches, and novel targeted agents.

Who should be considered for alloHCT?

AlloHCT for patients with advanced MDS shows a 5-year OS of 58%, 39%, and 23% for patients with intermediate-, high-, and very high-risk disease, respectively, by the revised International Prognostic Scoring System (IPSS-R). Relapse is the most common cause of failure after alloHCT. Multiple retrospective studies compared outcomes with alloHCT versus non-alloHCT approaches.[2-4] These studies show increased OS for patients with advanced MDS (intermediate-, high-, and very high-risk MDS by the IPSS-R) who undergo alloHCT. They are limited by the retrospective nature, leading to selection bias and the foundation of the Markov decision models, which assume a stochastic approach.

Recently, 2 large multicenter studies have evaluated alloHCT for patients with MDS prospectively.[5,6] The French trial enrolled 162 patients aged 50 to 70 years with advanced MDS into a prospective biological assignment trial. Patients with matched related donors (MRDs) or matched unrelated donors (MUDs) were planned to undergo alloHCT; there was no plan for patients to receive cord blood or

haploidentical donor alloHCT.[5] There were 54 patients with an MRD, 58 patients with an MUD, and 50 patients with no donor. The majority of patients received HMAs in both the donor (71%) and no-donor (88%) groups. Outcomes were assessed based on intent to treat, with 72% of patients in the donor group receiving alloHCT and 22% of patients in the no donor group receiving alternative donor alloHCT. The patients in the no-donor group who ultimately underwent alloHCT were censored at time of alloHCT. With a median follow-up of 43 months, the OS was 27% in the no-donor group and 63% in the donor group. Four-year survival was 24% in the no-donor group and 37% in the donor group, which was significantly different (P = .02) (Figure 1). Of note, the survival curves did not separate until 2 years after enrollment (landmark analysis 3 months after enrollment to account for donor search). Thus, the benefit of alloHCT for patients with an expected survival of <2 years without alloHCT would be questionable. A similar study through the Bone Marrow Transplant Clinical Trials Network (BMT-CTN) 1102 has completed accrual, but final results have not been released.[6]

The definition of risk in the context of alloHCT for MDS is mutable. BMT-CTN 1102 defined risk based solely on IPSS with intermediate-2 (int-2) or high risk. In contrast, the French trial defined the alloHCT population of interest by IPSS int-2, high, intermediate-1 (int-1) with poor-risk cytogenetics, low-risk patients with severe thrombocytopenia, and patients with chronic myelomonocytic leukemia (CMML) and ≥2 of the following: splenomegaly, thrombocytopenia, or leukocytosis. Patients with severe thrombocytopenia or neutropenia who might otherwise be in lower-risk categories should be considered for alloHCT because of the life-threatening complications of cytopenias and the lack of ability to offer continued transfusion support. Patients whose HMA treatment fails have a median OS of 5.6 months and 17 months for high/int-2 and low/int-1 risk MDS, respectively.[7,8] Both of these retrospective studies showed

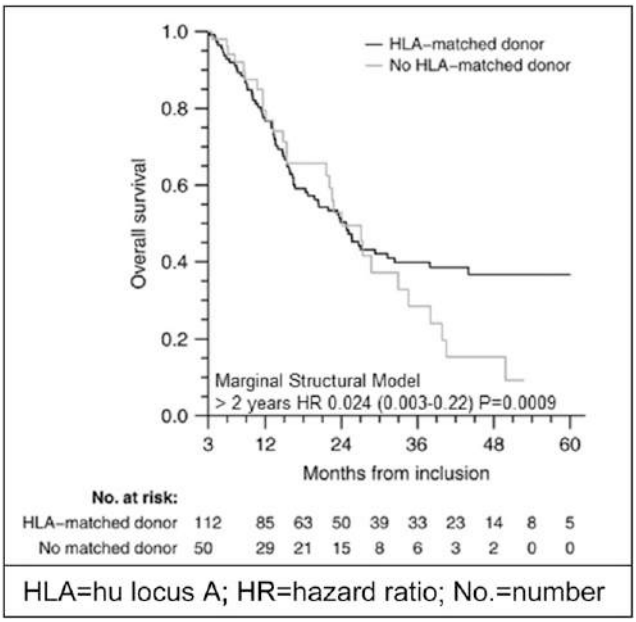

Figure 1. OS based on donor availability from a prospective trial by Société Française de Greffe de Moelle et de Thérapie Cellulaire and Groupe Francophone des Myélodysplasie: intent-to-treat analysis.[5]

greater OS for patients who received alloHCT compared with conventional care after HMA failed. This is a highly selective population of patients, and no prospective trials have specifically addressed outcomes for patients with HMA failure who subsequently undergo alloHCT. However, a retrospective analysis from the Fred Hutchinson Cancer Research Center demonstrated that the 3-year relapse-free survival after alloHCT was 23.8% for patients with MDS for whom HMA therapy failed but was 42% for patients with MDS for whom HMA therapy succeeded (hazard ratio, 1.88; 95% confidence interval, 1.19-2.95; P < .01).[9] Although outcomes after failure of HMA might be better with alloHCT than with non-alloHCT approaches, these results suggest that it is better to proceed with alloHCT while patients are responding to HMA rather than wait for failure. Additional disease-specific risk factors would include the type and total number of underlying mutational abnormalities (Figure 2).[10,11] Younger patients with high-risk mutations (as shown in Figure 2) should be considered for alloHCT even if otherwise considered to be at low risk because of the poor outcomes with standard treatments. High-risk mutations are also associated with inferior outcomes after alloHCT, primarily because of higher rates of relapse.[12-15] Reported relapse rates as high as 80% after alloHCT in patients with TP53 may argue against using this modality. However, the published outcomes are poor regardless of chosen intervention, and alloHCT remains the single most potent antimyeloid therapy available. Furthermore, alloHCT is not a static field; there are constant attempts to improve outcomes, especially for patients who are at extremely high risk of relapse.

Most patients with advanced MDS do not undergo alloHCT. A single-center study indicated that 65% of transplant-eligible patients with MDS are referred and only 33% underwent alloHCT.[16] Multiple factors underlie this difference, including advanced age, comorbidities, and lack of donor availability. Although age alone is not a contraindication for alloHCT, it is certainly a consideration. A prospective observational study compared the outcomes of patients with MDS after alloHCT based on age (55 to 64 vs ≥65 years).[17] A total of 688 patients aged ≥65 years and 592 patients aged 55 to 64 years underwent alloHCT. The median age in the older age group was 68 (range, 65-79) years. With a median follow-up of 47 months, the 3-year TRM/OS was 28%/37% and 25%/42% for patients aged ≥65 and 55 to 64 years, respectively (Figure 3). There was no significant difference in OS between the 2 cohorts, as measured by multivariate analysis adjusted for excess risk for mortality. Although there is no strict age cutoff for alloHCT, I generally recommend alternative approaches in patients aged ≥75 years. Multiple studies have demonstrated that the HCT comorbidity index (HCT-CI) predicts TRM.[18] Patients with HCT-CI of ≥4 are considered for alternative approaches exclusive of alloHCT.

One factor that has changed recently is donor availability. A retrospective analysis was performed of 228 patients with MDS who underwent haploidentical HCT; 102 received post-HCT cyclophosphamide for graft-versus-host disease prophylaxis.[19] With a median follow-up of 18 months, the patients who received post-HCT cyclophosphamide had a 3-year OS and TRM of 38% and 41%, respectively. A similar retrospective analysis with cord blood transplantation for 176 patients with MDS showed a 3-year OS of 31% and 3-year TRM of 40%.[20] Based on current donor algorithms, which are inclusive of haploidentical, cord blood, and mismatched unrelated donors, most patients who are considered candidates for alloHCT have identifiable donors.

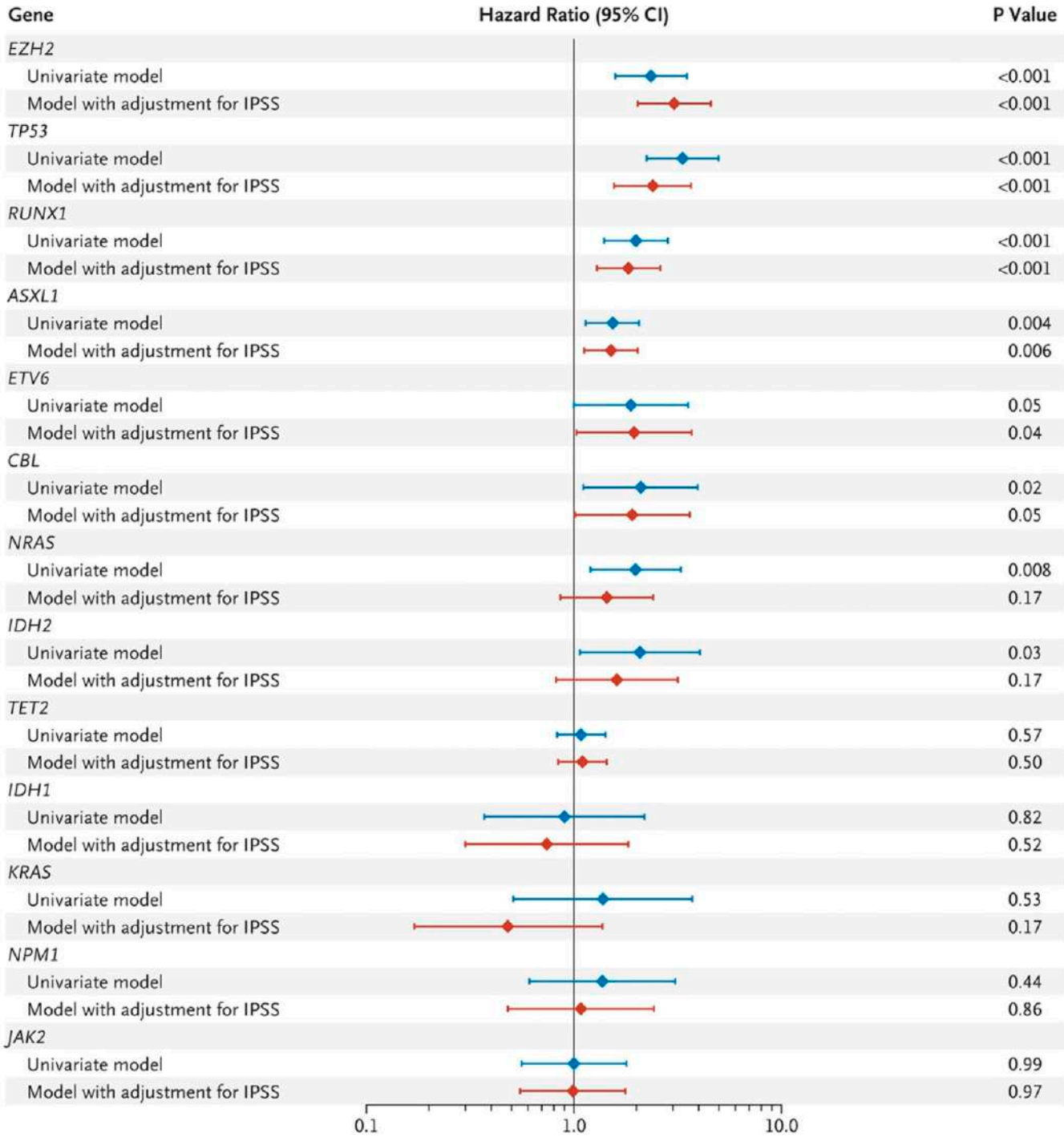

Figure 2. Hazard ratio for mortality, according to presence or absence of mutation.[10]

Case 1

Patient 1 is a 52-year-old man who developed easy bruising with epistaxis. A complete blood count with differential shows a total white blood cell count of 8,200, hemoglobin 10 g/dL, platelet count 29,000, 8% peripheral myeloid blasts, and an absolute neutrophil count of 4,800. Bone marrow aspirate and biopsy shows 10% blasts by morphology, increased nucleated red blood cells with megaloblastoid maturation, and left-shifted myeloid maturation. The diagnosis is MDS-EB2. Standard karyotype shows multiple chromosomal abnormalities with a complex monosomal karyotype including monosomies 5, 13, 18, and 20, trisomy 8, and deletion 4q. Genotype sequencing shows mutation with TP53 (p.V143M, NM_000546.5:c.427G>A) and PDGFRA (p.P581S, NM_006206.4:c.1741C>T). IPSS-R score is 8, very high risk; HCT-CI score is 0.

Case 2

Patient 2 is a 77-year-old man who presents with dizziness and shortness of breath that is worse with exertion. A complete

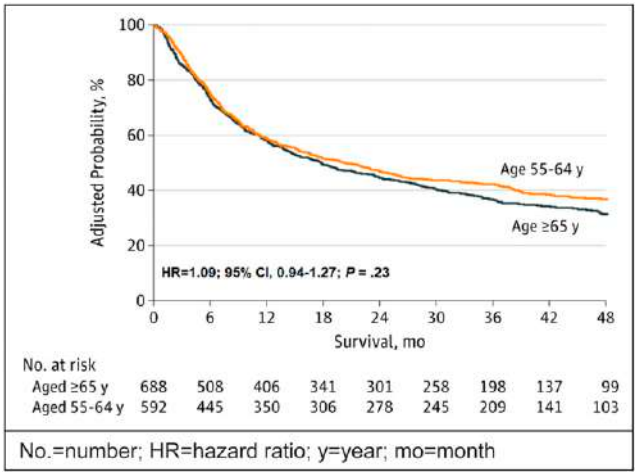

Figure 3. OS by age after alloHCT.[17]

blood count with differential shows a white blood cell count of 1,400, hemoglobin 7.9 g/dL, platelet count 41,000, absolute neutrophil count 210, and no peripheral blasts. He receives a transfusion of 2 U packed red blood cells. A bone marrow aspirate and biopsy demonstrate 6% myeloid blasts by morphology; myeloid and erythroid lineages show megaloblastoid changes. Standard karyotype shows multiple chromosomal changes with trisomy 1, 2, 6, 11, 14, 15, 22, del5q in 11 metaphases; 2 metaphases with a normal male karyotype; and a separate clone with sole del5q in 7 metaphases. Mutational analysis reveals TP53 (p.Cys176Trp, NM_000546.5:c.528C>G). IPSS-R is 9, very high risk. He has multiple comorbidities including coronary vascular disease with stent placement and current atrial fibrillation, morbid obesity, and type 2 diabetes mellitus; HCT-CI = 4.

Cytoreductive therapy before alloHCT

Retrospective studies giving intensive chemotherapy (IC) to patients with advanced MDS before alloHCT showed minimal or no benefit.[21-23] All are hampered by selection bias and inclusion bias, including patients who ultimately underwent alloHCT. Randomized clinical trials (RCTs) comparing IC with no IC before alloHCT for patients with advanced MDS have failed. Many physicians were unwilling to randomly assign patients with ≥10% bone marrow myeloblasts to proceed directly to alloHCT despite the absence of evidence showing benefit due to the known higher post-alloHCT relapse rates. Retrospective studies comparing IC and HMA as a pre-alloHCT debulking strategy showed similar long-term OS after alloHCT.[24-26] A phase 2 RCT (NCT01812252) comparing IC with HMA before alloHCT is currently enrolling patients.

Case 1

Patient 1 enrolls in an RCT comparing IC with HMA as a pre-alloHCT debulking strategy. He achieves complete remission after treatment with 3 cycles of azacitidine (aza) and venetoclax but with minimal identifiable disease (MID) detected by high-resolution flow cytometry, with 0.6% abnormal myeloid blasts. Standard karyotype was normal, but fluorescence in situ hybridization testing showed 11.3% of interphase cells with deletion 5q. The patient undergoes an MUD alloHCT with a 4-day busulfan conditioning regimen, with plans for post-HCT maintenance APR-246 (mutant p53 reactivating small molecule) on a clinical trial.

Intensive chemotherapy

Unless patients are candidates for alloHCT, I generally do not recommended IC for patients with advanced MDS. Although there is an expected complete remission rate of 62%, the duration is ~1 year without consolidative alloHCT, and IC has significant morbidity and mortality.[27] The role for IC in the setting of pre-alloHCT cytoreduction would be through the potential elimination of MID. An ongoing multicenter phase 2 trial is evaluating CPX-351 as a pre-alloHCT debulking strategy for patients with advanced MDS who are candidates for alloHCT (NCT03572764).

Hypomethylating agents

HMAs remain the only treatment approved by the US Food and Drug Administration for patients with advanced MDS; however, the benefit for most patients remains marginal. Two RCTs with decitabine (dec) showed no significant OS benefit with dec compared with best supportive care (BSC); however, significantly improved responses and decreased rates of acute myeloid leukemia (AML) transformation were reported.[28,29] Two RCTs (CALGB and AZA001) compared aza with BSC or conventional care regimens (IC, low-dose cytarabine, or BSC) and showed significantly increased leukemia-free survival and OS, respectively.[30,31] On average, the median OS was significantly higher by 9 months with aza. The median response time with aza is 3 months, with 96% of responders doing so by 6 cycles. With dec, the median response time is 1.7 months, with most patients who do respond responding by 4 months. Many patients with MDS are undertreated with HMA therapy because treatment is stopped prematurely or significant dose reductions are performed. I recommend that patients who are treated with aza receive ≥6 cycles and that patients treated with dec receive ≥4 cycles unless there is clear evidence of progression. I do not delay or dose reduce cycles for cytopenias unless there is concurrent evidence of infection.

Given the modest results seen with HMAs, there are ongoing attempts to improve outcomes. These efforts are hindered by a lack of understanding of the mechanism of action and appropriate dosing. Oral administration may lead to increased activity through prolonged exposure. CC-486, an oral formulation of aza,

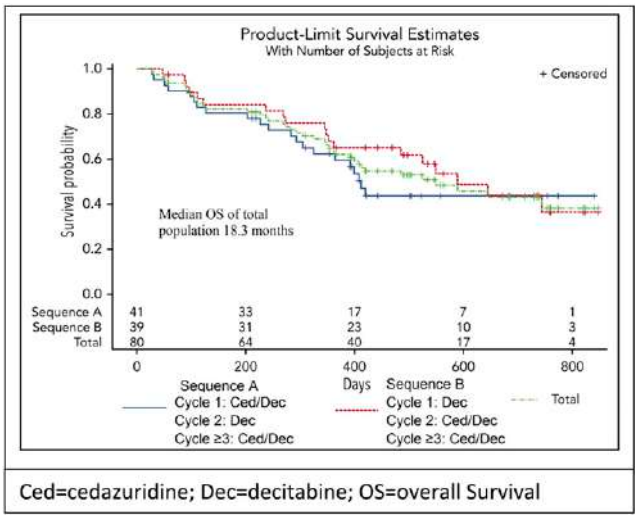

Figure 4. OS by randomized sequence of ced/dec vs dec and in the total population. Modified from Garcia-Manero et al[33] with permission.

has adequate absorption and an acceptable side effect profile and is effective for patients with MDS.[32] Ongoing trials are evaluating CC-486 as post-alloHCT maintenance (NCT04173533) or as treatment of anemia for patients with low-risk MDS (NCT01566695). Oral HMA may be inactivated by cytidine deaminase (CDA) in the gastrointestinal tract. Cedazuridine (ced) is an oral CDA inhibitor shown to increase HMA exposure after oral administration. A phase 2 RCT with oral ced/dec and intravenous dec for patients with advanced MDS or CMML indicates similar pharmacokinetic profiles and similar OS (Figure 4).[33] Based on the results, the US Food and Drug Administration has granted priority review of ced/dec. Given the marginal results with HMAs, patients who are not candidates for alloHCT should be encouraged to enroll into clinical trials evaluating HMA combination therapy or novel agents.

Hypomethylating therapy combination therapy

Multiple attempts to improve outcomes with HMA combination therapy have largely failed. Although numerous phase 2 trials have shown potential increased response rates with novel agents, none to date have increased efficacy in an RCT.[34-37] Many agents chosen for addition to HMAs increase hematopoietic toxicity, leading to cycle delay and dose decrease, thereby limiting the efficacy of HMAs. The design of clinical trials to improve HMA outcomes is complicated by a lack of knowledge about the mechanism of action and appropriate dosing. Current RCTs evaluating combination HMA therapy are summarized in Table 1.

Case 2 continued

Patient 2 is enrolled into a phase 3 multicenter RCT comparing aza with aza + APR-246 (NCT03745716) and randomly assigned to the aza-alone arm. He has received 9 cycles of aza. Most recent bone marrow analysis shows complete remission but with MID, with 0.9% abnormal myeloblasts detected by flow. He has not needed transfusion support in 6 months.

Treatment after failure of HMAs

Guadecitabine is a next-generation HMA with deoxyguanosine added to dec, which limits the activity of CDA. This prolongs the half-life of dec and decreases peak plasma exposure, leading to potentially higher response rates and lower toxicity even for patients who are refractory or resistant to HMAs. A phase 2 trial conducted in the United States with guadecitabine showed an overall response rate of 43% and a median OS of 1 year, with a

Table 1. RCTs with HMA combination therapy as initial therapy for MDS

Trial	Design	Disease	Investigational agent	Study arms	Patients	Primary endpoint	Anticipated study completion date
STIMULUS MDS1	Phase 2 double-blind	Intermediate- to very high-risk MDS	MBG 433: anti-TIM-3 antibody ICPI	MBG 453 + HMA PBO + HMA	60 60	CR and PFS	14-Aug-23
PANTHER	Phase 3 open label	Intermediate- to very high-risk MDS	Pevonedistat: selective NEDD8 inhibitor induces DNA repair	Pevonedistat + aza Aza	227 227	EFS	31-Mar-23
A18-15331	Phase 3 open label	Intermediate- to very high-risk MDS	APR-246: stabilizes mutated p53	APR-246 + aza Aza	77 77	CR	1-Nov-20
HOVON 156 AML	Phase 3 open label	AML + MDS-EB2 FLT-3+	Mido and Gilt: FLT-3 inhibitor	IC + midostaurin IC + gilteritinib	384 384	EFS	1-May-23
VERONA	Phase 3 double-blind	Intermediate- to very high-risk MDS	Venetoclax: BCL-2 inhibitor	Venetoclax + aza PBO + aza	250 250	CR and OS	26-Jan-25
ENHANCE	Phase 3 double-blind	Intermediate- to very high-risk MDS	Magrolimab: anti-CD47 antibody; ICPI	Magrolimab + aza PBO + aza	90 90	CR	1-Jul-25
	Multiphase trial 1-3	MDS, AML, CMML, MDS-MPN	ASTX030: ced/aza deaminase inhibitor	Sequencing trial subcutaneous aza vs ced/aza	245	PK	1-Apr-23
LEAP	Phase 2/3 open label	MDS-EB2, AML	Midostaurin: FLT3 inhibitor nivolumab: ICPI	Midostaurin + aza Nivolumab + aza Dec + LDAC Aza	16,70	OS	1-Aug-23
HO-155	Phase 2 open label	MDS-EB, AML	Mido: FLT3 inhibitor	Midostaurin + dec Dec	70 70	CR	1-Mar-26
Cusatuzumab combination	Phase 2 open label	Intermediate- to very high-risk MDS, CMML	Cusatuzumab: anti-CD 70 antibody	Cusatuzumab + aza Aza	75 75	ORR	18-Jul-22
	Phase 3	Intermediate- to very high-risk MDS	Rigosertib: Ras kinase inhibitor	Rigosertib + aza Aza	Unknown Unknown	ORR	Unknown

CR, complete remission; EFS, event-free survival; Gilt, gilteritinib; IC, induction chemotherapy; ICPI, immune checkpoint inhibitor; LDAC, low-dose ara-c; Mido, midostaurin; MPN, myeloproliferative neoplasm; ORR, overall response rate; PBO, placebo; PFS, progression-free survival; PK, pharmacokinetics.

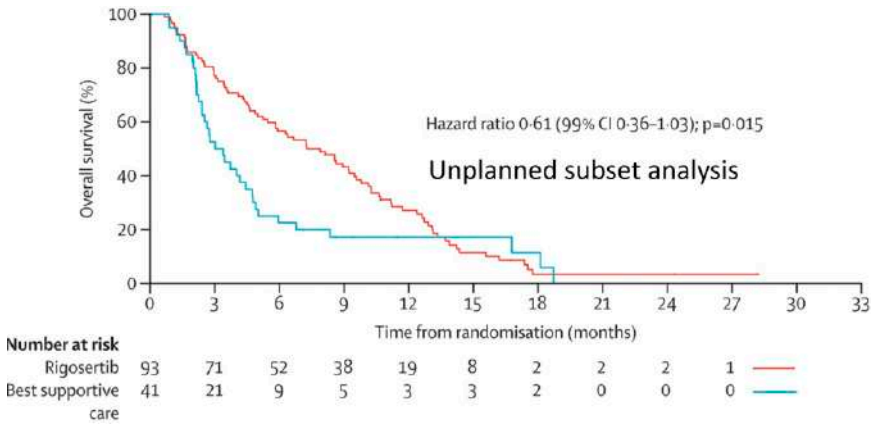

Figure 5. OS in patients with very high-risk -MDS as measured by the IPSS-R.[40]

2-year survival rate of 25% (14%-38%) for patients for whom HMA failed.[38] A similar trial conducted in France showed a median OS of 7.1 months, with a 1-year survival of 33%.[39] A phase 3 RCT (NCT020907359) comparing guadecitabine with conventional care regimens has completed accrual and is in follow-up. Rigosertib, a small-molecule RAS activation inhibitor, has been compared with BSC in a phase 3 RCT for patients for whom HMA failed.[40] There was no significant difference in OS; however, a subset analysis of patients with very high-risk disease showed an OS benefit with rigosertib (Figure 5). A subsequent phase 3 trial (NCT02562443) in a restricted population of patients with MDS has completed accrual but not follow-up. An RCT using oral rigosertib with or without aza for upfront therapy for patients with advanced MDS is planned. Given the dearth of effective treatment options in patients for whom HMA has failed, enrollment into clinical trials in both the non-alloHCT and alloHCT settings should be encouraged.

Conclusions

There are currently 347 clinical trials for MDS listed on clinicaltrials.gov that are actively accruing patients. It is likely that most will fail to complete accrual. To date no RCT with HMA combination therapy has shown improved efficacy. A collaborative effort by investigators, patients, MDS support networks, and medical societies will be needed to move the field forward.

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Conflict-of-interest disclosure

B.L.S. is a consultant for BMS, Celgene, Incyte, Agios, and Alexion.

Off-label drug use

None disclosed.

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Patient stratification in myelodysplastic syndromes: how a puzzle may become a map

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Heterogeneity is the disease-defining epithet of myelodysplastic syndromes (MDS), a clonal disorder of hematopoietic stem and progenitor cells. During the last decade, significant progress has been made to better understand the diversity of clinical, molecular, cellular, and immunological factors that are bound to the prognosis and outcomes of patients with MDS. Despite the rapid generation of all of this biological information, how to implement it has fallen short. Redefining clinical tools to use this new information remains a challenge. The holistic integration of novel, high-impact individual risk parameters such as patient-reported outcomes or mutational and immunological data into conventional risk stratification systems may further refine patient subgroups, improve predictive power for survival, and provide a next-generation classification and prognosis system for patients with MDS. Dichotomic treatment strategies in patients with MDS according to their patient and disease profiles highlight the importance of precise risk stratification, which may be complemented by the definition of granular cohorts of patients with myeloid neoplasms and a druggable target (ie, *IDH1/2* mutations) across conventional blast thresholds.

LEARNING OBJECTIVES

- Understand the advantages and limitations of current MDS prognostic scoring systems in patient risk stratification
- Gain insight into a potential next-generation classification and prognosis system for patients with MDS

Clinical case 1

A general practitioner referred a 63-year-old man after detecting moderate anemia during a routine peripheral blood analysis. Moreover, the patient had been experiencing moderate fatigue (grade 2[1]) since acquiring a viral infection 10 weeks ago. His laboratory values consisted of hemoglobin 8.5 g/dL, absolute neutrophil count 2.5 × 10^9/L, platelet count 250 × 10^9/L, serum erythropoietin 148 U/L, and normal liver and kidney values. The results of his cytomegalovirus, Epstein-Barr virus, human herpesvirus 6, and parvovirus B19 polymerase chain reaction diagnostic tests were negative, and so far, he had not received packed red blood cell (pRBC) transfusions.

Subsequent bone marrow aspiration revealed isolated erythroid dysplasia in >20% of cell lines, 24% ring sideroblasts (RSs), and 3% marrow blasts, consistent with the diagnosis of myelodysplastic syndrome (MDS) with RSs and single-lineage dysplasia (MDS-RS-SLD). Karyotype analysis confirmed trisomy 19, and molecular studies of the bone marrow aspirate detected *TET2* (35% variant allele frequency [VAF]), *DNMT3A* (VAF, 25%), and *SF3B1* (VAF, 22%) mutations.

Brief overview of MDSs

MDSs are very heterogeneous clonal disorders of hematopoietic stem and progenitor cells and are usually suspected if a (mostly elderly) patient presents with unexplained cytopenia in routine peripheral blood analysis.[2-4] The extent of clinical presentation can vary significantly; the spectrum ranges from a mild disease course with minimal or no intervention needed to patients with multiple treatment failures and early progression to acute myeloid leukemia (AML). The highly variable clinical course of patients with MDS represents a challenge, not only with regard to individual prognosis assessment but also in terms of the decision about appropriate but still limited treatment regimens.[5-7]

Clinical case 1 (continued)

The diagnosis of MDS-RS-SLD is confirmed, but what's next?

The manual count of bone marrow blasts, 3% in our patient's case, is fundamental for risk assessment. It is important to note that the percentage of blast counts matters with regard to prognosis, even below the 5% threshold.

Therefore, bone marrow smears should be assessed by an experienced hematopathologist. In addition, cytogenetic analysis helps in predicting risk and selecting the right individual treatment strategy. Because of the highly variable prognosis of patients with MDS, prognostic systems allowing risk stratification and subsequent therapeutic decision are of utmost importance. On the basis of the International Prognostic Scoring System (IPSS),[8] the patient has a score of 0.5 (intermediate 1 risk), whereas with the revised IPSS (IPSS-R),[9] the patient's score is 4.0 (intermediate).

After initial diagnosis, the patient underwent first-line therapy with an erythropoiesis-stimulating agent (ESA). Nevertheless, after 12 months, his anemia worsened, and he required RBC transfusions (RBC transfusion dependent [RBC-TD]) every 1 to 2 months. Thus, after ESA failure and recent U.S. Food and Drug Administration and European Medicines Agency (EMA) approval of luspatercept, the patient is now eligible for luspatercept treatment.[10,11]

Current classification of risk and prognosis

MDS prognostic scoring systems have been the focus of research for many years.[12] The most commonly used include the IPSS,[8] revised IPSS (IPSS-R),[9] World Health Organization (WHO) Prognostic Scoring System,[13] and MD Anderson Prognostic Scoring System.[4,12,14,15]

Since 1997, the IPSS[8] has been a widely accepted standard for assessing prognosis and stratification of primary untreated adult patients with MDS. The IPSS includes covariates for prognostic discrimination such as the number of cytopenias at initial diagnosis, the percentage of bone marrow blasts, and the number of cytogenetic abnormalities (Tables 1 and 2),[8] which distinguish four prognostic categories (low-, intermediate 1–, intermediate 2–, and high-risk disease) with significant differences in overall survival (OS) and rate of AML transformation.[8,14] Until today, this simple and highly reproducible prognostic scoring system has been of essential importance and has been the basis for a number of MDS-specific drug approvals within the last 2 decades.[12,14,16] However, several limitations of the IPSS became evident, most likely due to the fact that only little weight is given to the diversity of cytogenetic changes and to the extent of cytopenias in mainly patients with RBC-TD anemia.[17]

To refine the IPSS, multiple statistically weighted clinical and genetic features were integrated to generate a new prognostic categorization model.[9,17] Since 2012, the IPSS-R (Tables 3 and 4) has been the standard tool to assess the risk of disease progression and death of patients newly diagnosed with MDS. The model captures additional and more precise prognostic elements (eg, chromosomal abnormalities, percentage blast count, severity of cytopenia) and defines five rather than four major prognostic categories (very low, low, intermediate, high, and very high risk).[9]

Table 1. IPSS[8]

	Score				
	0	**0.5**	**1**	**1.5**	**2.0**
Medullary blasts, %	0-4	5-10	—	11-20	21-29
Number of cytopenias*	0-1	2-3	—	—	—
Cytogenetic risk group†	Low	Intermediate	High	—	—

†Low risk = normal karyotype, 5q-, 20q-, -Y; intermediate risk = all other aberrations; High risk = complex karyotype (≥3 anomalies), chromosome 7 anomalies.
*Platelets <100 000/μL; hemoglobin <10 g/dL, absolute neutrophil count <1 800/μL.

Table 2. IPSS prognostic risk categories[8]

Score	Risk groups
0	Low risk
0.5-1	Intermediate risk 1
1.5-2	Intermediate risk 2
≥2.5	High risk

Chronic myelomonocytic leukemia is a myelodysplastic/myeloproliferative neoplasm with a highly variable clinical course and prognosis. The chronic myelomonocytic leukemia–specific prognostic scoring system (CPSS) groups patients into risk categories by accounting for cytogenetic abnormalities, disease subtype according to French-American-British and WHO classifications, and RBC transfusion dependency. The CPSS stratifies patients into 4 different risk groups with significantly different survival and risk of AML evolution.[18,19] Recently, the CPSS model was updated to include molecular abnormalities, including *ASXL1*, *RUNX1*, *NRAS*, and *SETBP1* mutations.[19]

In patients with lower-risk (LR) MDS (IPSS-R very low to intermediate risk up to 3.5 points[20]), therapy is aimed mainly at improving cytopenia(s) to prevent complications such as bleeding and severe infections, decreasing transfusion burden, and improving quality of life.[2] Conversely, in patients with higher-risk (HR) MDS (IPSS-R intermediate risk above 3.5 points,[20] high, or very high risk), a more aggressive treatment strategy, including hypomethylating agents (HMAs) or allogeneic hematopoietic stem cell transplant (allo-HCT) with the aims of delaying disease progression, improving survival rates, and potentially curing the disease, should be initiated.[2,5] These dichotomic treatment strategies in patients with LR- vs HR-MDS highlight the importance of precise risk stratification at initial diagnosis. Because age did not affect AML transformation risk, the patient's age is not formally included in the IPSS or IPSS-R, and individual survival prediction based on age and risk status is realized by the age-adjusted IPSS-R (IPSS-RA) formula: ([Age in years − 70] × (0.05 − [IPSS-R risk score × 0.005]).[9,21]

Our 63-year-old patient in clinical case 1 has an age-adjusted IPSS-RA score of 3.79 (intermediate). We recommend adjustment by age especially in patients with MDS younger than 50 years old, when a potentially curative allo-HCT is considered a possible treatment option.

In some cases, adding age to IPSS-R could potentially upstage patients in the intermediate-risk group to HR disease (IPSS-RA score, >3.5). Patients with an intermediate-risk IPSS-R score represent a group with a highly divergent clinical outcome due to widely variable disease courses.[22] Age ≥66 years, peripheral blood blasts ≥2%, and history of RBC transfusion have been identified as additional stratification factors for this challenging subgroup of patients with MDS.[22] These factors, all of them associated with inferior survival, enable the classification of patients with IPSS-R intermediate-risk MDS into 2 prognostic subgroups (intermediate-favorable vs intermediate-adverse) with significant divergent outcomes.[22] In addition, outcomes of IPSS-R intermediate-risk patients and those with confirmed

Table 3. IPSS-R[9]

	Score						
	0	0.5	1	1.5	2	3	4
Cytogenetic group*	Very good	—	Good	—	Intermediate	Poor	Very poor
Medullary blasts, %	≤2	—	>2 to <5	—	5-10	>10	—
Hemoglobin	≥10	—	8 to <10	<8	—	—	—
Platelets	≥100	50 to <100	<50	—	—	—	—
ANC	≥0.8	<0.8	—	—	—	—	—

ANC, absolute neutrophil count.
*Very good = del(11q), -Y; good = normal karyotype, del(20q), del(5q), del(12p), double including del(5q); intermediate = +8, del(7q), i(17q), +19, any other single or double independent clone; poor = −7, inv(3)/t(3q)/del(3q), double including −7/del(7q), complex: 3 abnormalities; very poor = complex >3 abnormalities.

SF3B1 mutation may be underestimated because of the favorable prognosis of *SF3B1*-positive patients.[23] However, other molecular data, such as *TP53* and *EZH2*, can upstage patients from intermediate-risk into HR category.[24,25]

Previous analyses demonstrated that comorbidities also had a significant independent impact on survival.[26] In fact, the addition of comorbidity scores such as the MDS-specific comorbidity index[27] or the newly developed MDS-specific frailty index[28] to prognostic systems such as IPSS and IPSS-R can further enhance their prognostic value.[26,29,30] Nevertheless, their standardized incorporation into daily workup has not become reality in many centers.

Apart from comorbidities, fatigue is an important marker in individual risk assessment. Independent from current standard risk classification systems, self-reported fatigue severity is a negative prognostic factor for survival.[31] A recent international observational study compared the ability of IPSS and IPSS-R to capture baseline fatigue burden in patients with newly diagnosed MDS.[32] After stratifying patients according to IPSS score, there was a lack of sensitivity in capturing the burden of fatigue across its four risk categories.[32] Contrarily, the IPSS-R determined clearly distinct subgroups with regard to burden of fatigue and thus provided a better stratification of patients related to fatigue severity, possibly enhancing patient management in clinical practice.[32] Because fatigue is a readout of several factors, including age, inflammation, pain, emotional distress, sleep disturbance, anemia, and diminished activity level, its regular assessment is recommended in clinical decision making by completing fatigue questionnaires at baseline and during the course of therapy.[31,32]

Clinical case 2

Another 63-year-old man also presented initially with moderate anemia (hemoglobin, 7.9 g/dL) but normal absolute neutrophil and platelet counts. His serum erythropoietin level was 122 U/L, and he had not received RBC transfusions. A subsequent bone marrow assessment confirmed the diagnosis of MDS-RS-SLD with 2% bone marrow blasts and 21% RSs. Cytogenetic analysis showed trisomy 19, and molecular studies of the bone marrow aspirate confirmed high-risk somatic mutations, including *ASXL1* (VAF, 28%), *ETV6* (VAF, 32%), and *U2AF1* (VAF, 22%). Similar to the patient in clinical case 1, this patient has an IPSS score of 0.5 (intermediate 1 risk), but an IPSS-R score of 3.5 (intermediate). In accordance with the current treatment guidelines, the patient underwent first-line therapy with an ESA. Nevertheless, after 10 weeks of ESA treatment, his hemoglobin levels dropped from 7.9 g/dL initially to 6.2 g/dL, and he became heavily RBC transfusion dependent (6 to 8 RBCs units/month). A subsequent bone marrow diagnostic test demonstrated slightly rising blast counts (4% bone marrow blasts) with 32% RSs. Similar to the patient in clinical case 1, he is now eligible for luspatercept treatment after ESA failure.

Does the molecular coat fit? The impact of *SF3B1* mutations

The compendium of common MDS-associated somatic mutations has extensive implications in patient care and prognosis,[33] but single (or even cluster) gene mutations are not incorporated into current prognostic scoring systems.[4,14] Our patient in clinical case 1 has an IPSS score of 0.5 (intermediate 1 risk; LR-MDS) and an IPSS-R score of 4.0 (intermediate; HR-MDS). The discrepancy between IPSS and IPSS-R can result in a potential therapeutic dilemma. In such a clinical case, the individual molecular profile can be crucial in terms of treatment decision and with regard to the question whether allo-HCT should be considered.

The molecular abnormalities described in clinical cases 1 and 2 demonstrate in an exemplary way how the patient's individual prognosis depends on the predominant mutations. The 2 clinical

Table 4. IPSS-R prognostic risk categories and clinical outcomes[9]

Score	Risk groups	Median survival, y	Median time to 25% AML evolution, y
0-1.5	Very low risk	8.8	Not reached
1.5-3	Low	5.3	10.8
>3-4.5	Intermediate	3.0	3.2
4.5-6	High	1.6	1.4
>6	Very high	0.8	0.73

cases display similarities in laboratory values, bone marrow blast counts, karyotypes, and risk classification scores (Table 5), but the patient in clinical case 1 exhibits somatic mutations associated with a rather favorable outcome (*TET2*, *DNMT3A*, *SF3B1*),[23,34] whereas those in the patient in clinical case 2 are linked to an adverse prognosis (*ASXL1*, *ETV6*, *U2AF1*).[34,35]

This molecular discrepancy in both cases is associated with completely different individual risks of disease progression and survival. It becomes clear that the currently available prognostic scoring systems are not sufficient to derive personalized and precise therapeutic decisions in the absence of data on molecular abnormalities. Thus, the addition of molecular data to the IPSS-R will be essential to create a personalized, dynamic risk prediction model with the ability to make individual treatment recommendations (Figure 1, Table 6). The development of such a new molecular IPSS-R system is currently in progress.

If we go back to our clinical cases 1 and 2, it can be concluded that both patients display RSs ≥15%, but only the patient in clinical case 1 carries a mutation of *SF3B1*. In MDS with RSs, *SF3B1* mutations define a homogeneous subgroup with isolated erythroid dysplasia and favorable prognosis.[34] Patients with MDS with RSs and wild-type *SF3B1* (clinical case 2) are characterized mainly by multilineage dysplasia and an unfavorable prognosis.[34]

Within the phase 2 PACE-MDS study, luspatercept (ACE-536), an ActRIIB ligand trap fusion protein, was investigated in anemic patients with LR-MDS.[11] Among patients treated with luspatercept, 29 (69%) of 42 RS-positive patients achieved erythroid hematologic improvement vs only 3 (43%) of 7 RS-negative patients. Among *SF3B1*-positive patients, 77% (24 of 31) achieved erythroid hematologic improvement compared with 40% (6 of 15) of *SF3B1*-negative patients.[11] Within the subsequent placebo-controlled phase 3 MEDALIST trial, the safety and efficacy of luspatercept in TD patients with LR-MDS and RSs (with either ≥15% RSs or ≥5% RSs if *SF3B1*-positive), who were either refractory to or unlikely to respond to ESA, were confirmed.[10] Of 153 patients receiving luspatercept, 93% had an *SF3B1* mutation, and 38% achieved the primary endpoint of RBC transfusion independence for 8 weeks or longer compared with 13% receiving placebo ($P < .001$).[10] Interestingly, the percentages of patients with a response to luspatercept treatment were similar, regardless of *SF3B1* allelic burden and the total number of baseline somatic mutations.[10]

After the recent U.S. Food and Drug Administration and European Medicines Agency approvals of luspatercept, both of our patients are now eligible to receive luspatercept treatment. However, because of the existing HR mutational profile (*ASXL1*, *ETV6*, *U2AF1*), including the absence of *SF3B1* mutation in the patient in clinical case 2, an intensified surveillance strategy to detect disease progression early should be considered.

Table 5. Comparison of clinical variables at initial diagnosis

	Clinical case 1	Clinical case 2
Hb level	8.5 g/dL	7.9 g/dL
PLT level	250 × 10⁹/L	192 × 10⁹/L
ANC count	2.5 × 10⁹/L	2.7 × 10⁹/L
BM blast count	3%	2%
Cytogenetic analysis	+19	+19
Molecular analysis	*TET2* (VAF, 35%)	*ASXL1* (VAF, 28%)
	DNMT3A (VAF, 25%)	*ETV6* (VAF, 32%)
	SF3B1 (VAF, 22%)	*U2AF1* (VAF, 22%)
MDS WHO subtype	MDS-RS-SLD	MDS-RS-SLD
IPSS	0.5 (intermediate 1)	0.5 (intermediate 1)
IPSS-R	4.0 (intermediate)	3.5 (intermediate)

ANC, absolute neutrophil count; BM, bone marrow; Hb, hemoglobin; PLT, platelet.

Are there other potential treatment options available after ESA failure?

When restricted to primary endpoint responders RBC- transfusion independent (RBC-TI, ≥8 weeks) with an RBC transfusion burden similar to that in the luspatercept trial (≥2 units/8 weeks), the response rate of lenalidomide was almost comparable to that of luspatercept[36] (26.9% vs 38%, respectively) in a phase 3, randomized, placebo-controlled study in LR-MDS non-del(5q) patients ineligible for or refractory to ESA treatment[36]. However, lenalidomide is not registered for that indication, and grade 3 or 4 neutropenia and thrombocytopenia were reported in 61.9% and 35.6% of lenalidomide-treated patients, respectively.[36] In a randomized phase 3 trial comparing lenalidomide monotherapy with lenalidomide plus ESA in ESA-resistant, RBC-TD (≥4 units/8 weeks) patients with LR-MDS, the overall rate of RBC-TI ≥8 weeks was 13.8% vs 24.2% in the lenalidomide vs lenalidomide plus ESA arms, respectively.[37]

Moreover, in a phase 2 study evaluating azacitidine with or without ESA in patients with ESA-resistant RBC-TD (≥4 units/8 weeks) LR-MDS, RBC-TI ≥8 weeks was achieved in 14.3% of patients receiving azacitidine plus ESA and in 16.3% receiving azacitidine monotherapy.[38] These results were consistent with those of the Nordic MDS group, who found that RBC-TI was achieved in 20% of cases in a similar patient population (LR-MDS with RBC-TD ≥4 units/8 weeks and ESA resistance) treated with azacitidine monotherapy, but response duration was short at <6 months in most patients.[39] In these 2 azacitidine trials, specific results for patients with RS after ESA failure were not available. Therefore, treatment with luspatercept remains our preferred option in our 2 clinical cases.

Incorporation of molecular data: curse or blessing?

Generally, the prognostic significance of somatic mutations in patients with MDS is connected to other risk factors, including karyotype, blast number, and cytopenias (Table 6), which are captured by existing clinical risk scoring systems such as the IPSS-R. Genome sequencing of 104 genes in a study of 944 patients with MDS showed that 25 of 48 mutations were associated with reduced OS, including *RUNX1*, *ASXL1*, *NPM1*, *EZH2*, *TP53*, *PRPF8*, *LUC7L2*, *NRAS*, *KRAS*, *FLT3*, *PTPN11*, *NF1*, *LAMB4*, *GATA2*, *SMC1A*, and *STAG2*.[33,40] Again, only *SF3B1*^{mut} status was associated with improved OS[40]; even after adjustment for IPSS-R risk groups, the mutation was strongly associated with a favorable impact on OS in patients with <5% bone marrow blasts.[13,16,33] Contrarily, *SRSF2*, *ASXL1*, and *U2AF1* mutations had an independent negative impact on OS in patients with blast percentages <5%, but not in patients with higher blast percentages (clinical case 2).[16,33] Thus, the prognostic significance of MDS-associated mutations should always be considered with regard to the individual bone marrow blast count. Interestingly, the same pattern has been described for the impact of RBC-TD, where its negative impact was rather moderate in MDS with excess blasts.[13]

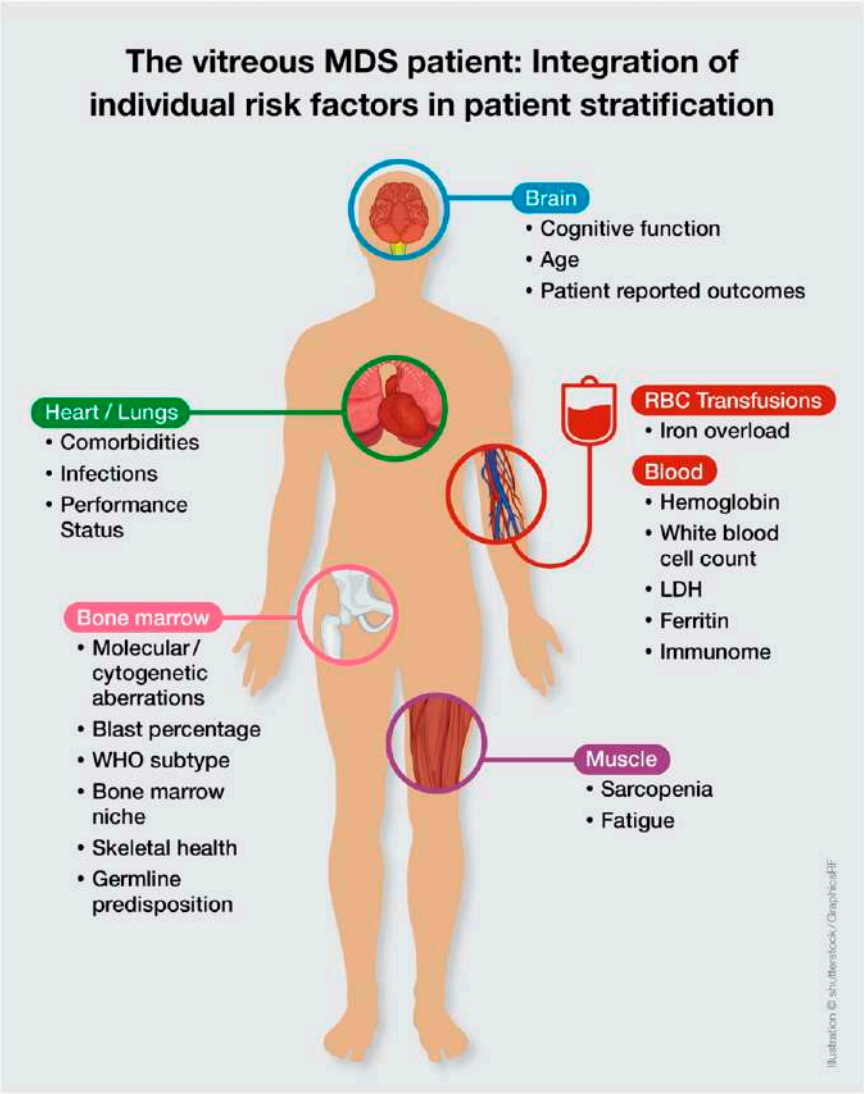

Figure 1. The vitreous MDS patient: integration of individual risk factors in patient stratification.

In a comprehensive genetic analysis with the aim to identify an association between somatic mutations and clinical variables (OS, severity of cytopenia, proportion of blasts), *TP53*, *EZH2*, *ETV6*, *RUNX1*, or *ASXL1* mutations were associated with an OS similar to that of patients in the next-higher IPSS risk group.[41] Mutations of *IDH2* were associated with shorter OS, whereas *IDH1* mutations were only slightly associated with reduced OS, demonstrating the diverse influence of these strongly related genes.[33]

Although there is broad consensus on the impact of mutations on prognosis, often there is no clinical consequence, given the paucity of available treatment options. This is a currently unmet medical need that requires novel therapeutic approaches.

Clinical case 3

A 68-year-old woman with known MDS with isolated del(5q) according to WHO 2016 criteria[42] and confirmed *TET2* (VAF, 25%) and *DNMT3A* (VAF, 18%) somatic mutations has been receiving lenalidomide treatment for the past 25 months because of RBC-TD. Eight weeks ago, bone marrow diagnostic tests confirmed sustained complete cytogenetic remission, but she now presents with new RBC-TD anemia (hemoglobin, 6.5 g/dL) and worsening thrombocytopenia (platelet count, 48 × 10⁹/L). A subsequent bone marrow assessment showed a rising blast count of 12%. In the setting of suspected disease progression or treatment failure, we recommend repetition of next-generation sequencing testing. Molecular studies revealed a new *TP53* mutation (VAF, 30%) with multihit allelic state and slightly rising VAFs for *TET2* (VAF, 38%) and *DNMT3A* (VAF, 32%) mutations. Furthermore, cytogenetic analysis revealed reemergence of del(5q) plus a 17p deletion in 8% of metaphases.

How does *TP53* matter?

First, the presence of a *TP53* mutation (in ~10% to 15% of MDS cases) significantly affects the prognosis of patients with MDS.[43] *TP53* mutations are selectively enriched in cases with complex karyotype (~70%)[44] or therapy-related myeloid neoplasms, and the mutation independently predicts poor OS even after correcting for clinical variables.[45] Especially patients with *TP53* mutations and complex karyotypes represent a group with an extremely poor prognosis and an OS <6 months.

Table 6. Key factors for the development of a potential personalized medicine approach[60]

Variable	Entity	Grading	Potential clinical consequence
Performance status	ECOG 0-1	Good	Standard therapy including allo-HCT
	ECOG >1	Poor	Supportive care or low-intensity therapy
EPO level	<200 U/L	Low	Treatment with ESA in case of anemia
	>200 U/L	High	No ESA (limited response to ESA)
Ferritin level	>1500 ng/mL	High	Treatment with iron chelation*
Genetics	del(5q)		Targeted treatment with lenalidomide*
	Normal karyotype, 20q-, -Y: and absence of poor-risk molecular abnormalities	Good risk	Standard therapy or supportive care only
	All other aberrations, including complex karyotype, or poor-risk molecular abnormalities	Poor risk	Intensified surveillance strategy, allo-HCT, clinical trial
Druggable molecular targets		*SF3B1* mutation	Treatment with luspatercept*
		TP53 mutation	Treatment with *TP53* modulators
		TP53 WT	Treatment with Nutlins
		IDH1 mutation	Treatment with *IDH1* inhibitors
		IDH2 mutation	Treatment with *IDH2* inhibitors
		Spliceosome mutations	Treatment with spliceosome modulators
Prognostic scoring systems (eg, IPSS-R)	IPSS-R score ≤3.5	Good risk	Standard therapy or supportive care only
	IPSS score >3.5	Poor risk	Hypomethylating agents,* allo-HCT
Inflammatory signature		Yes	Anti-inflammatory treatment
		No	Standard therapy

ECOG, Eastern Cooperative Oncology Group; EPO, erythropoietin; WT, wild type.
*FDA approved

Moreover, *TP53* mutational burden seems to matter with regard to clinical outcome and stratifies distinct prognostic groups independently of clinical prognostic scoring systems.[46] In a retrospective study of 219 patients with MDS, patients with a *TP53* VAF >40% had a median OS of 124 days; the same OS was not reached in patients with VAF <20% ($P < .01$).[46] In patients with LR-MDS, evaluation of OS determined a *TP53* VAF threshold of 6% as an optimal cutoff for patient stratification.[47] No significant impact on PFS or OS was observed in patients with LR-MDS with a VAF <6%, who remained stable for long periods without progression.[47]

Bernard et al[48] recently published data about the important prognostic role of *TP53* allelic state. In a cohort of 3324 patients with MDS, 490 *TP53* mutations in 380 patients (11% of the cohort) were characterized. Analysis revealed a segregation of *TP53* into 2 states: a monoallelic state (one wild-type allele remaining) was detected in 33% of patients with *TP53* mutations, and a multihit state (*TP53* altered multiple times) was shown in 67% of patients with *TP53* mutations.[48] Interestingly, the allelic state of *TP53* was associated with clinical presentation and patient outcome. Monoallelic *TP53* state was linked to a more favorable disease, including less severe cytopenias, lower bone marrow blast counts (4% vs 9%), and enrichment in LR-MDS subtypes compared with patients with multihit *TP53* state.[48] Moreover, patients with monoallelic *TP53* state had rather similar survival rates compared with patients with wild-type *TP53* in accordance with their IPSS-R stratification.[48]

On the contrary, multihit allelic *TP53* state including a del17p, as in our patient in clinical case 3, was associated with complex karyotypes, worse OS, adverse prognostic subgroups independent of the IPSS-R, and higher rates of AML progression.[48] Thus, *TP53* allelic state is critical in disease monitoring and represents an important prognostic stratification factor in MDS. Thus, *TP53* allelic state characterization and the mutational burden evaluation must be considered as part of the MDS diagnostic workflow.

The potential consequence in our clinical case 3 is to consider allo-HCT, although *TP53* state also translates to a dismal outcome with a higher relapse rate.[43,49,50] Therefore, novel alternative therapeutic strategies such as APR-246, a p53 reactivator, are needed and currently under clinical investigation (Table 6). Preliminary results of a previous study in HMA-naïve patients with *TP53* mutations and HR-MDS and AML showed that the combination of APR-246 plus azacitidine has promising clinical efficacy (complete remission rate of 56%) with deep molecular remission in all patients with a complete response.[51]

Is every high-risk patient the same?

The principal aims of treatment in HR-MDS are modifying the natural course of disease, limiting disease progression, and improving outcome.[2] Initial stratification should primarily involve assessment of whether a patient is eligible for an intensified therapeutic approach including allo-HCT (Table 6). In patients with HR-MDS who have only mild, asymptomatic cytopenia, the treatment decision

may be delayed in the absence of poor-risk mutations, and an intensified surveillance strategy could be considered.

Although the IPSS/R system was developed mainly to determine the prognostic risk in patients with newly diagnosed MDS, its value in predicting post-transplant outcome has been confirmed in several studies.[52] Although already implemented in the existing scoring systems, karyotype abnormalities alone represent a significant risk factor for relapse, as do certain molecular abnormalities.[43] Although not every complex karyotype is associated with an extremely poor prognosis, the presence of a *TP53* mutation adds significantly to the adverse prognosis of these patients with HR-MDS. Thus, routine testing of *TP53* and possible AML-defining mutations in patients with high-risk disease and complex karyotypes should be implemented.[53]

Interestingly, retrospective data in patients with HR-MDS and patients with AML treated with a 10-day decitabine regimen suggest that patients with *TP53* mutations had higher response rates than patients with other mutations.[54] Although data stating that *TET2* mutational status may be a predictor of HMA treatment response exist, higher response rates did not translate into a survival benefit for these patients, and confirming prospective data are missing.[55]

A very smart and elegant new method will be the application of targeted and personalized drugs against specific molecular structures in MDS; here, the presence of druggable targets (Table 6) such as *TP53* or *IDH1/2* can be predictive of treatment response. However, targeted therapies such as APR-246, IDH1/2, or spliceosome inhibitors are just beginning to emerge. In fact, these targets may pave the way for holistic approaches covering the entire spectrum of myeloid neoplasms (Table 6).[53]

Integration of the "immunome" into personal risk stratification: a possible breakthrough?

In MDS, several mutations affecting the epigenetic modifiers (eg, *TET2*) or RNA splicing factors (eg, *U2AF1*) have been linked to NLRP3 inflammasome activation and enhanced innate immune signaling.[56] Inflammatory cytokines are increased in the serum and bone marrow of these patients, and high cytokine levels correlate with a worse prognostic outcome.[57] Consequently, our patient in clinical case 3 not only is exhibiting somatic mutations with higher risk for early disease progression (eg, *TP53*) but also displays mutations with a known high immunogenic impact (*TET2*, *DNMT3A*).

Inflammation within the bone marrow microenvironment in association with aberrant cellular immune responses evolving during MDS disease progression has independent prognostic value.[58,59] Consequently, in addition to the incorporation of molecular data into current prognostic scoring systems, the inclusion of specific immunological data ("immunome") could possibly further refine risk stratification in the future.[58] Thus, a new immunologically based patient stratification model aiming to identify patients with prominent "autoinflammatory" features could be the next important step toward the development of personalized risk prediction models in MDS.[58] A comprehensive clarification of the dysregulated immune pathways will enable patient stratification and, as a consequence, the development of new targeted drugs[58] (Table 6).

Conclusion and future directions

Overall, increasing evidence exists that current MDS prognostic scoring systems are only an approximation in personal risk stratification. Thus, putting the puzzle together will require the integration of complex molecular and immunological interactions with clinical variables with the aim of establishing optimal personalized risk stratification models (Figure 1). Nevertheless, this also requires further expansion of our therapeutic armamentarium.

Conflict-of-interest disclosure

U.P. has received honoraria from Celgene/Jazz Pharmaceuticals; has served in a consulting or advisory role for Celgene/Jazz Pharmaceuticals; has received research funding from Amgen (institutional), Janssen (institutional), Novartis (institutional), BerGenBio (institutional), and Celgene (institutional); has received reimbursement for travel, accommodations, and expenses from Celgene. A.S.K. has received honoraria from Novartis and reimbursement for travel, accommodations, and expenses from Celgene, Novartis, Takeda, and Jazz Pharmaceuticals.

Off-label drug use

None disclosed.

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Therapy for lower-risk MDS

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Lower-risk myelodysplastic syndromes (MDS) are characterized by the presence of dysplasia, low bone marrow blast percentage, low number and depth of cytopenia(s), and relatively good-risk karyotpic and molecular abnormalities. A score of ≤3.5 on the Revised International Prognostic Scoring System classifies patients as lower-risk MDS. Information from a mutational profile of the MDS at time of diagnosis (and over serial time points) can be reassuring for predicted behavior of lower-risk MDS compared with one expected to progress more rapidly (higher-risk MDS). Supportive care continues to be the crux of treatment, although the options to reduce transfusion needs have improved in 2020. Erythropoiesis stimulating agents, lenalidomide, and luspatercept address the most frequent (and symptomatic) cytopenia (anemia) and are started only when patients are transfusion dependent. Patients can derive long-term benefits (years) from these approaches but will often progress to higher-risk MDS. Interestingly, some patients with lower-risk MDS can present with an isolated thrombocytopenia for which thrombopoietin receptor analogs such as romiplostim and eltrombopag are options (as long as blast counts are low). The presence of pancytopenia and or intensifying and unremitting clinical symptoms are often treated with hypomethylating agents or (anti–thymocyte globulin if hypocellular MDS is of concern). Targeted therapies are emerging for small subsets of MDS patients with specific somatic mutations (ie, *TP53*, *IDH1/2*, *FLT3*), although currently, there are no approved, mutation-directed medications to treat MDS.

LEARNING OBJECTIVES

- Review the treatment paradigm and agents for lower risk MDS, with attention to differing treatment options for transfusion dependent anemia, isolated thrombocytopenia, and pancytopenia
- Describe the expected clinical outcomes for patients diagnosed with lower-risk MDS
- Describe the impact of somatic mutations on diagnosis of lower-risk MDS (ie; $SF3B1^{mut}$ vs $TP53^{mut}$) and identify novel therapies under investigation for a targeted precision medicine approach

Clinical case

A 66-year-old man with history of relapsing polychondritis on hydroxychloroquine presented with an isolated macrocytic anemia (hemoglobin, 12.1 g/dL) and minimal symptoms. Flow cytometry for large granular lymphocytic leukemia and paroxysmal nocturnal hemoglobinuria were negative. Other factors were ruled out as contributors to the anemia (hypothyroidism, nutritional deficiencies, infection). Serum erythropoietin (sEPO) level was 77 mIU/mL. A bone marrow biopsy (BMbx) demonstrated a hypercellular marrow (80%) with tri-lineage hematopoiesis, granulocytic hyperplasia, dysmegakaryopoiesis, and 0% blasts consistent with low-grade myelodysplastic syndrome (MDS). Cytogenetic testing revealed 46,XY karyotype. Based on these data, the calculated International Prognostic Scoring System (IPSS) score was 0.5 (low risk), and the IPSS revised (IPSS-R) score was 1 (very low risk), both consistent with a lower-risk MDS. Clinical monitoring was recommended. Approximately 2 years later, he had an acute non-ST elevation myocardial infarction, and he presented to clinic 5 months later. A complete blood count showed worsening anemia (hemoglobin, 8.6 g/dL) and new thrombocytopenia (platelets, 50 000/mL). Repeat BMbx is the same except for 95% cellularity and 1% blasts. NGS testing shows an *EZH2* mutation. He is started on the erythropoiesis-stimulating agent (ESA) darbopoetin-alfa (DARBO) at 500 µg every 3 weeks with a meaningful hematologic response.

Introduction

MDS is a heterogenous group of clonal myeloid neoplasms most often characterized by a hypercellular marrow, ineffective hematopoiesis with ≥10% dysplasia in a single cell line, cytopenias, and a risk of progression to acute myeloid leukemia (AML).[1] The incidence of MDS in the United States is around 21 000 new cases annually. More than 80% of MDS

patients are over 60 years of age,[2] and older patients can have a poor long-term overall survival (OS) because of lack of curative therapies (other than hematopoietic stem cell transplant [HSCT], which is limited to a minority of patients). About two-thirds of all MDS patients will present with lower-risk (LR-MDS) disease with minor clinical symptoms and mild cytopenias, and a rare number of patients can have a symptom burden that is out of proportion to their minimally affected laboratory parameters. Notably, for some with LR-MDS, their symptoms can contribute to a decreased quality of life despite their lower-risk scoring, and about 25% of LR-MDS patients will die within 2 years. This clinical heterogeneity has long been recognized.

For all MDS patients, existing scoring systems are used to help identify the risk of MDS progression to AML and to aid in a physician's treatment recommendations. The IPSS is based on karyotype, bone marrow blast percentage, and number of cytopenias.[3] IPSS has since been adopted in clinical trials and subsequently revised in 2012 (IPSS-R), where additional refinement of the specific details on karyotype grouping, degree of cytopenias, and blast counts was made.[4] These scoring systems allow for risk stratification into either lower risk (LR) or higher risk. Therapeutic options are guided by these 2 categories and further distinguished by patient-specific characteristics such as age, comorbidities, performance status, and an individual's goals of care. Nonetheless, these scoring systems currently remain suboptimal because exact attribution and impact on MDS prognosis from the presence of somatic mutational data still remain unclear except for a handful of select genes. For example, LR-MDS patients with a somatic mutation of any of the genes *TP53*, *EZH2*, *ASXL1*, *RUNX1*, or *SRSF2* have a decreased survival than predicted by IPSS.[5,6] Bejar et al[5] also reported that the presence of *EZH2* mutation in combination with the use of LR-IPSS could identify 29% of patients with LR-MDS with a worse prognosis. *SF3B1* mutations are associated with a longer OS than calculated by IPSS-R.[6,7] Clinical management of the same IPSS-R score is distinct for the aforementioned cases with differing somatic mutational profiles (ie; $TP53^{mut}$ vs $SF3B1^{mut}$) but as of yet are not formally incorporated to universally adapted scoring systems.[8] Importantly, the mere presence of a mutation is not a substitute for the pathologic diagnosis of MDS (ie; requiring the presence of >10% dysplasia) and should not be used as the sole indication for treatment decisions. Mutations in some non-MDS genes indicate the presence of neoplasms that can mimic MDS. These include *CALR* mutations associated with primary myelofibrosis, *C3F3R* mutations with atypical chronic myelogenous leukemia and chronic neutrophilic leukemia, and *STAT3* mutations with large granular lymphocytic leukemia.

The therapeutic algorithm (Figure 1) for symptomatic LR-MDS is limited to supportive care with transfusions, growth factors, and the 4 US Food and Drug Administration (FDA)-approved drugs in MDS: lenalidomide (LEN), hypomethylating agents (azacitidine or decitabine), and luspatercept. HSCT is the only curative option, but most MDS patients are ineligible because of comorbidities. For those patients that have mild cytopenias with minimal symptoms, watchful observation is appropriate. Early intervention with current modalities has not shown a mortality benefit or impact on clonal evolution in LR-MDS, supporting this strategy.

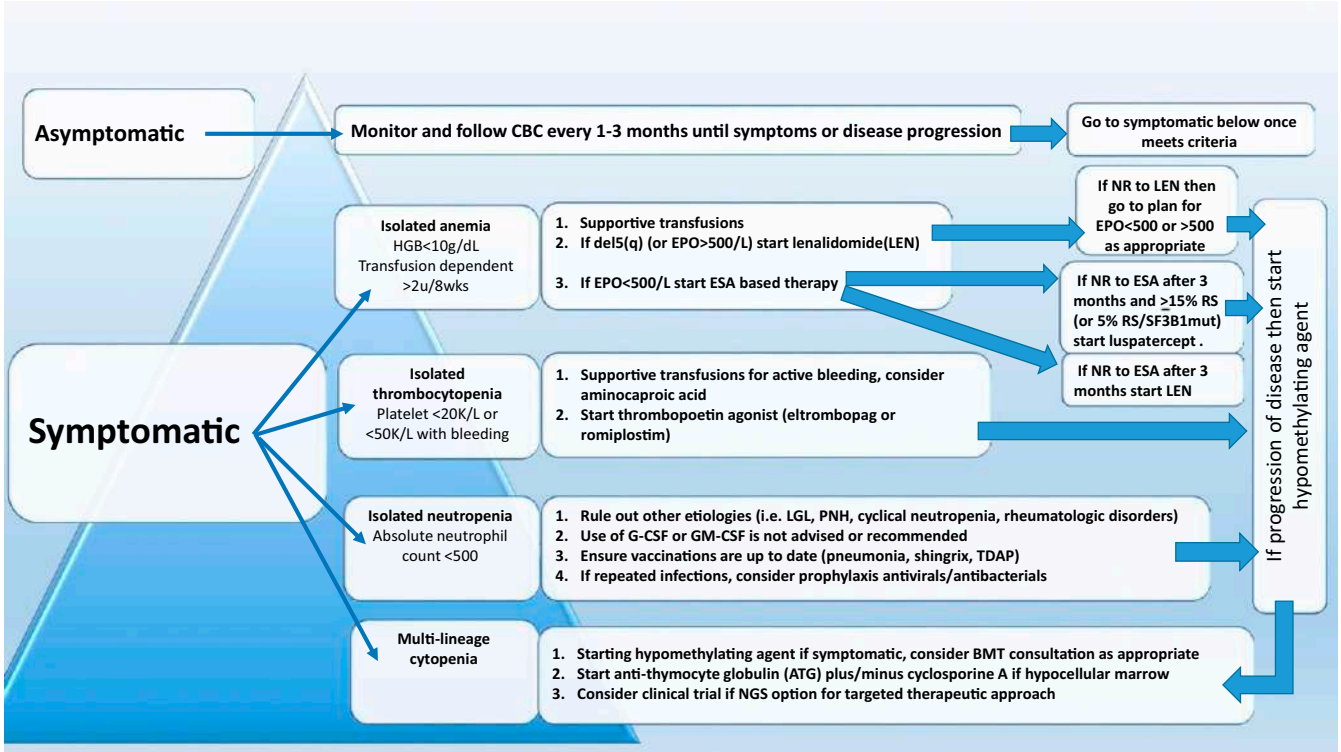

Figure 1. Treatment algorithm for lower-risk MDS (IPSS score ≤ 1 or IPSS-R score ≤ 3.5).

Treatment of anemia

Anemia is the most common symptom in LR-MDS and is present in almost 90% of the cases. With a median age at diagnosis of 71 years, MDS patients can be severely impacted by chronically low levels of hemoglobin, and this can lead to worsening cardiopulmonary function, increased falls, and significant cognitive decline. As such, treatment of anemia is essential for overall health and quality of life. Approach to management is initially focused on packed red blood cell (PRBC) transfusions. However, transfusion-dependent (TD) MDS patients are at higher risk of iron overload and transfusion reactions and report a decreased quality of life. Thoughtful decisions regarding the choice of transfusion support vs initiation of ESA therapy are important, because no evidence supports that early ESA-based therapy improves survival.

A central part of MDS is the ineffective erythropoiesis that contributes to the development of anemia and is characterized by abnormal maturation and differentiation of erythroid progenitors and increased destruction of abnormal erythroblasts.[9] The combination of increased proliferation and reduced differentiation results in a net increase of erythroid progenitors and is associated with a stress response that increases EPO levels and signals for preservation of these progenitors in the bone marrow. Unfortunately, there is an uncoupling of proliferation, maturation, differentiation, and resultant increase in cell death, leading to a reduction in functional red blood cells.[10] ESAs (recombinant EPO and DARBO) are the first-line agents used for anemia in LR-MDS patients having sEPO levels ≤ 500 U/L and low transfusion burden (Figure 1). The target hemoglobin range for LR-MDS patients is 10 to 12 g/dL with ESA treatment or to achieve an increase in hemoglobin level by ≥1.5 g/dL or a decrease in PRBC transfusion requirements by 4 PRBC transfusions over a period of 8 weeks (Table 1).[11] Notably, revised International Working Group (IWG) criteria for assessment of hematologic response have been proposed and are inclusive of a requirement for duration of hematologic improvement to last 16 weeks (vs 8 weeks) among other recommendations.[12] In general, overall response rates are 20% to 40% with a general duration of response (DOR) of between 18 and 24 months. Using the validated Nordic scoring system, LR-MDS patients with sEPO < 100 U/L and a transfusion requirement of <2 units of red blood cells (RBCs) have >70% probability of responding to ESA therapy (Figure 2).[13] In a phase 3 randomized trial comparing EPO vs best supportive care (BSC), erythroid response rates (RRs) were 36% vs 9.6% at the initial treatment step, which was further increased to 47% in the EPO arm by adding granulocyte colony-stimulating factor and increasing EPO dose in nonresponders.[14] Most responding patients had sEPO levels < 200 U/L, and EPO therapy was not associated with OS. In a subsequent phase 3 study of LR-MDS patients with a low transfusion burden, therapy with EPO led to 32% erythroid RR.[15] All responses occurred in patients with sEPO < 200 U/L; therefore, approval of ESA use in the European Union was based on this sEPO threshold. In the United States, ESAs are approved for management of chronic anemia, although not specifically for MDS.

DARBO has an increased sialylated carbohydrate content that prolongs its half-life and possibly in vivo efficacy. In a phase 2 study of LR-MDS patients with sEPO < 500 U/L, 12-week treatment with DARBO 300 μg/wk resulted in 71% erythroid RR.[16] However, in a phase 3 placebo-controlled study of DARBO 500 μg every 3 weeks, RR was lower at 14.7% vs 0% in the placebo group.[17] This was likely because of an ineffective dose interval, because RR increased to 34.7% when dose frequency was increased. In an international pooled analysis of 1698 LR-MDS patients treated with ESAs, most responses occurred within 3 months, with a median DOR of 17 months.[18] The effect was dose dependent, with EPO 60 000 U/wk and DARBO 300 μg/wk being superior to lower doses. The addition of granulocyte colony-stimulating factor may rescue responses in 10% to 20% of cases, particularly in the presence of ring-sideroblasts (RS).[19]

Key point

ESA therapy often represents the first step of management for transfusion-dependent LR-MDS patients, with overall response rates of 20% to 40% and an 18- to 24-month duration of response.

MDS progenitors exhibit increased SMAD2/3 signaling that contributes to ineffective erythropoiesis by inhibiting RBC maturation.[20] Luspatercept (ACE-536) is a novel recombinant fusion protein, composed of modified activin receptor type IIB linked to the Fc domain of human immunoglobulin. It binds select transforming growth factor β superfamily ligands, decreasing SMAD2/3 signaling and enabling late-stage erythroblast differentiation. In a single-arm phase 2 dose-finding MDS study, 58 LR-MDS patients (hypomethylating agent [HMA] naïve) were treated with luspatercept. Among the patients treated with higher doses of luspatercept, erythroid RR was 63%, with 38% achieving transfusion independence (TI).[17] Although low sEPO concentration was predictive of increased response, 43% of patients with sEPO > 500 U/L were still able to attain erythroid response. Most notably, responses were more frequent among patients with *SF3B1* mutation compared with non-*SF3B1* (77% vs 40%). These findings led to the placebo-controlled phase 3 MEDALIST trial of luspatercept vs placebo in LR-MDS patients with either ≥15% RS or ≥5% RS with *SF3B1* mutation, who were TD with disease refractory to or unlikely to respond to ESAs.[21] At the dose levels of 1 to 1.75 mg/kg, erythroid RR during the first 24 weeks was 53% in the luspatercept arm vs 12% in the placebo arm. TI for ≥8 weeks was achieved in 37.9% vs 13.2%, respectively ($P < .0001$). The median DOR was 30.6 weeks in the luspatercept group. The MEDALIST study led to the US FDA approval of luspatercept for MDS with RS and *SF3B1* mutation in 2020. The ongoing COMMANDS trial (Efficacy and safety of luspatercept (ACE-536) versus epoetin alfa for the treatment of anemia due to IPSS-R very low, low or intermediate risk MDS in ESA naïve subjects who require red blood cell transfusions.) is a randomized trial comparing luspatercept vs ESA in the upfront setting for TD LR-MDS patients irrespective of *SF3B1* status (#NCT03682536). It is likely that combination therapies with luspatercept will be investigated in upcoming clinical trials (ie; with lenalidomide or oral hypomethylating agent).

Key point

Luspatercept was FDA approved in April 2020 for transfusion-dependent MDS with ring sideroblasts. It has been 15 years since the last FDA approval of a drug for the treatment of MDS. Luspatercept was not studied in MDS patients who had prior exposure to LEN or hypomethylating therapy.

The role of iron chelation

MDS patients with high RBC transfusion burden may accumulate excessive amounts of iron and have end-organ damage associated with secondary hemochromatosis. In a phase 2 placebo-controlled study investigating deferasirox in LR-MDS patients with

Table 1. Response criteria for hematologic improvement for MDS patients undergoing therapy

Item	Suggested modified IWG 2018 criteria	IWG 2006 criteria
Baseline criteria Definition of TB categories	3 groups NTD = (0 RBC in 16 wks) LTB = (3-7 RBC in 16 wks in at least 2 TRSFN episodes, max 3 in 8 wks) HTB = (≥8 RBC in 16 wks, ≥ 4 in 8 wks)	2 groups TD = (at least 4U of RBC with 8 wks for Hb < 9 g/dL) TID = (<4U of RBC with 8 wks for Hb <9 g/dL)
Pretreatment RBC TRSFN policy	TRSFN policy for the individual pt prior to the therapy should be monitored on treatment. Baseline Hb level <10 g/dL as prerequisite for pts in need of therapy	TRSFN threshold of 9 g/dL, no exception for clinical indication. Baseline Hb level <11 g/dL as prerequisite for pts in need of therapy
Response evaluation criteria: HI-E		
NTD	At least 2 consecutive Hb measurements ≥ 1.5 g/dL for a period of min 8 wks in observation period of 16-24 wk compared with lowest mean of 2 Hb measurements (apart from any TRSFN) with 16 wks before treatment onset**	Hb increase by 1.5 g/dL and/or relevant reduction of U of RBC TRSFN by an absolute number of at least 4 RBC TRSFN/8 wks compared with the pretreatment transfusion number in the previous 8 wks; only RBC TRSFN given for a Hb of ≤9 g/dL pretreatment will count in the RBC TRSFN response evaluation
LTB	HI-E in LTB pts corresponds to TRSFN independence, defined by the absence of any TRSFN for at least 8 wks in an observation period of 16-24 wks with the same TRSFN policy compared with 16 wks prior to the treatment**	
HTB	Major response: Defined as TRSFN independent (TID) and requires the absence of any TRSFN over a period of min 8 wks in an observation period of 16-24 wks with the same transfusion policy compared with the 16 wks prior to treatment** Minor response: defined as a reduction by at least 50% of RBC over a min of 16 wks with the same transfusion policy compared with 16 wks prior to treatment	
Platelet response (pretreatment PLT <100 x 10^9/L)		
HI-P	No change from 2006 criteria except: 1. Evolution of bleeding symptoms is to be taken into account and 2. increments of platelets also for pts with pretreatment PLT >100 x 10^9 are to be reported	Absolute increase of 30 x 10^9/L for pts starting with >20x10^9/L platelets Increase from <20 x 10^9/L to >20 x 10^9/L and by at least 100%
Neutrophil response (pretreatment ANC<1.0 x10^9/L)		
HI-N	No change from 2006 criteria except: 1. Increments for neutrophils also for pts with pretreatment ANC count of >1.0 x 10^9/L are to be reported	At least 100% increase and absolute increase >0.5 x 10^9/L

Abbreviations are as follows: ANC = absolute neutrophil count, Hb = hemoglobin, HTB = high transfusion burden, min = minimum, NTD = not transfusion dependent, pt = patient, PLT = platelet count, TRSFN = transfusion, TB = transfusion burden, RBC = red blood cells, TD = transfusion dependent, TID = transfusion independent, LTB = low transfusion burden.
*Adapted from Cheson, et al[10] and Platzbecker, et al.[11]
**Only a response duration of at least 16 weeks, however, is considered clinically meaningful.

serum ferritin > 1000 ng/mL and transfusion history of 15 to 75 PRBC units, 225 patients were randomized, and iron chelation was associated with a 36.4% risk reduction in event-free survival.[22] However, important limitations to highlight in the TELESTO trial (MDS Event Free Survival with Iron Chelation Therapy Study, #NCT00940602) include a long period of enrollment, dramatic reduction in sample size, and a nonstandard definitions of events. Published literature on iron chelation in MDS management is limited by retrospective or single institution studies, which have a lack of statistical prowess. Although chelation can be considered for lower-risk MDS patients with a high transfusion burden along with evidence of end-organ damage from iron deposition, its use should be deliberate and individualized.

LR-MDS with del(5q)

Stemming from the idea of targeting dysregulated immune microenvironment in MDS, early studies investigated the immune-regulatory agent thalidomide in MDS, which resulted in significant toxicity but modest activity in LR-MDS patients.[23] This led to the phase 1 MDS-001 study investigating LEN in MDS, which showed manageable toxicity and increased activity in patients with del(5q).[24] The phase 2 MDS-003 study tested LEN in 148 TD del5(q) LR-MDS

Feature	Range	Points Assigned
Serum EPO (sEPO) (units/Liter)	<100	+2
	100 to 500	-1
	>500	-3
Transfusion PRBC (units/month)	<2	+2
	+ or >2	-2

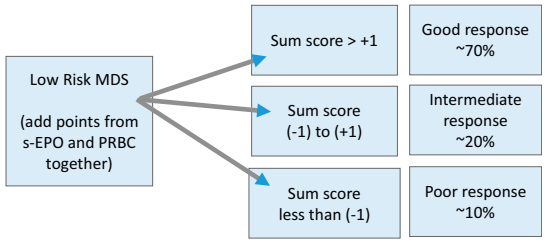

Figure 2. Scoring system for prediction of response to ESA-based therapy in MDS patients. Adapted from Hellström-Lindberg et al.[13]

patients. Erythroid RR (per IWG-2000) was 76%, TI was seen in 67%, and 75% experienced a cytogenetic response (50% complete and 25% partial cytogenetic remission).[25] Median time to response was 4.6 weeks, and median DOR was 2.2 years.[26] The MDS-003 study led to the FDA approval of LEN for del5(q) LR-MDS in 2005. The subsequent phase 3 placebo-controlled MDS-004 study confirmed these results with up to 56% of patients achieving TI for ≥26 weeks.[27] Across these studies, the most common side effect of LEN therapy is myelosuppression (50%-60% of patients), which is more pronounced during the first 3 months of therapy. Other less common side effects include rash, diarrhea, pruritus, venous thrombosis, and endocrine pathologies.

Key point

Therapy with LEN is the standard of care for transfusion-dependent del(5q) LR-MDS, with a transfusion independence rate of 67% and a 2- to 3-year duration of response. Patients with concomitant del(5q) and *TP53*-mutated disease have a decreased likelihood and duration of response.

Given the observed activity of LEN in some MDS patients without del(5q) in the phase 1 study, a phase 2 MDS-002 study was conducted in TD non-del(5q) LR-MDS patients.[28] Erythroid RR was 43%, and 26% of patients achieved TI after a median of 4.8 weeks, for which median DOR lasted 41 weeks. The confirmatory phase 3 MDS-005 study also reported 26% TI rate and suggested a more favorable response among patients with baseline sEPO level ≤500 U/L.[29] Preclinical data suggest that LEN can restore sensitivity to EPO in MDS cells by stabilizing lipid rafts that are enriched with signaling receptor complexes.[30,31] This was further explored in 2 recent phase 3 studies of ESA-refractory TD LR-MDS patients with non-del(5q). In the study by Toma et al,[32] combined therapy with LEN-EPO led to higher erythroid RR (39% vs 23%) and TI rate (24% vs 13%) vs LEN monotherapy. However, the DOR was not prolonged (18 vs 15 months, respectively), and the benefit of combination therapy was more prominent in patients with lower transfusion burden and favorable cytogenetics. The E2905 study also investigated this combination with a similar design and reported major erythroid RR of 28.3% in the LEN-EPO arm vs 11.5% LEN monotherapy.[33] Among 136 patients who completed 16 weeks of study treatment, RRs were 38.9% vs 15.6% (P = .004). Similar to the first study, the DOR doubled with LEN-EPO vs monotherapy (24 vs 13 months). Concerns regarding the lower than expected RR in the comparator arm (based on prior single arm and randomized trial data) have tempered excitement, although combined therapy can be considered to improve LEN response in non-del5(q) LR-MDS patients.

Key point

Transfusion independence rates are 25% in non-del5(q) transfusion-dependent LR-MDS patients treated with LEN and are associated with a less than 1-year median duration of response. Addition of EPO to Len should be considered and appears helpful to increase frequency of transfusion independence in those EPO-resistant patients with lower EPO level, low transfusion burden, and favorable cytogenetics.

Treatment of thrombocytopenia

Hemorrhage leads to death in 13% of patients with LR-MDS, directly caused by severe thrombocytopenia.[34] Intrinsic functional defects of dysplastic megakaryocytes increases bleeding risk, as does concurrent medications frequently used in elderly patients (aspirin, platelet inhibitors, ibuprofen, and others). Platelet transfusions and thrombopoietin-receptor agonists (TPO-RA) are first-line treatment options. Platelet transfusions are not durable, are highly immunogenic, and contribute to splenomegaly. Romiplostim was tested in a randomized phase 2 study of 250 LR-MDS patients treated with subcutaneous dosing vs placebo. Platelet RRs were 36.5% vs 3.6%, respectively, with the incidence of bleeding events and platelet transfusions significantly reduced in the romiplostim group vs placebo (relative risk = 0.71 and 0.35, respectively; P < .0001).[35] Although the trial was stopped because of concerns related to excess blasts and progression to AML in the romiplostim arm, 5-year follow-up data did not demonstrate an increased risk of AML or death.[36] The oral TPO-RA eltrombopag was also studied in a randomized placebo-controlled phase 2 study of LR-MDS patients.[37] Eltrombopag-treated patients had significantly lower bleeding events (14% vs 42%), and higher rate of platelet response (47% vs 3%) compared with placebo (odds ratio, 27.1; 95% confidence interval, 3.5-211.9; P < .0017). Median time to response was 2 weeks. In summary, TPO-RA therapy can improve thrombocytopenia and decrease bleeding in LR-MDS. However, transient elevations of circulating blasts were observed in ~10% of patients, for which close monitoring is recommended, as well as avoidance of TPO-RA use in MDS patients with excess blasts (>5%).

Key point

Romiplostim increased platelet counts and decreased bleeding events compared with placebo, when given 750 µg subcutaneously once a week. Eltrombopag 150 to 300 mg taken by mouth once daily increased platelet counts and decreased bleeding events compared with placebo in LR-MDS. These agents should be avoided when blast counts are >5% in LR-MDS.

Clinical case (continued)

Our patient experienced an increase in PRBC transfusion requirement at 19 months despite ESA therapy. ESA was stopped. He was reluctant to add LEN given the long-standing history of issues with rashes from relapsing polychondritis. BMbx was performed to evaluate disease status, and it showed 95% hypercellularity with 1% blasts and persistent EZH2 mutation. No

Table 2. Emerging therapies for LR-MDS

Agent	Mechanism of action	Route of administration	Suggested patient population	Single or combination	Response rate	Reference
Roxadustat	Hypoxia-inducible factor (HIF) inhibitor	Oral	LR-MDS (non-del5(q)) with low transfusion burden, sEPO ≤400 U/L	Phase 3 study ongoing ROXA vs Placebo	Dose finding cohort results: N=24. HI-E=54%, TI=38% after 28 wk of treatment. TI=78% at higher dose level 2.5mg/kg	46
Imetelstat	Telomerase inhibitor	Intravenous	LR-MDS (non del5(q))with high transfusion burden and ESA failure	Phase 2/3 iMERGE study	HI-E=68%, TI for 8 wk=42%, TI for >24 wk=29%, CR=13% and CRi=10%. No PR. High rates of myelosuppression	47,48
H3B-8800	Spliceosomal inhibitor: synthetic lethality	Oral	LR-MDS with spliceosome mutations	Phase 1 dose escalation study	14% HI, no CR/PR PD studies demonstrated dose dependent splicing modulation	49
APR-246	*TP53* modifier	Intravenous	Treatment naïve HR-MDS/AML with *TP53* mutation	Combined with HMA	RR=75-87%, CR=55% CR in phase 2 when combined with HMA	50,51
Ivosidenib	IDH1 inhibitor	Oral	R/R MDS with *IDH1* mutation (N=12)	Phase 1 single agent	CR=5/12 and RR=11/12	52
FT-2102	IDH1 inhibitor	Oral	MDS and AML (N=36)	Phase 1/2 single agent and + HMA	CR/CRi 38% single agent (N=16) CR=27% combo with HMA	53
Enasidenib	IDH2 inhibitor	Oral	R/R MDS with *IDH2* mutation (N=17)	Single or combined	1/17=CR and 10/17=Response	54

CR = complete remission, CRi = complete remission with incomplete count recovery, ESA = erythroid stimulating agent, HI-E = erythroid hematological improvement, HMA = hypomethylating agent, LR = lower risk, PR = partial response, R/R = relapsed/refractory, RR = response rate, sEPO = serum erythropoetin level, TI = transfusion independence.

RSs were appreciated. Cytopenias progressed to high severity (platelets < 20,000/mL) along with a high transfusion burden for PRBCs. Discussion for next options included azacitidine, clinical trial, and/or HSCT. Given his, young age, profound cytopenias, and high transfusion burden, he was referred for HSCT consultation to initiate typing.

Treatment of multiple and/or refractory cytopenias

Because growth factors have limited efficacy in LR-MDS, patients who are failed by the aforementioned first-line agents are often considered for anti–T-cell immunosuppressive therapy (IST) or HMA therapy. For some patients with pancytopenia, the clinical picture can parallel a bone marrow failure phenotype such as acquired aplastic anemia with a hypocellular marrow (hMDS). Selection of patients who are likely to respond to IST has been challenging because studies are inconsistent about potential predictors of response. Some predictors include younger age, hypocellular marrow, blasts < 5%, normal karyotype, HLA-DR positivity, and short duration of TD.[38] A phase 3 trial comparing horse anti–thymocyte globulin (hATG) plus oral cyclosporine (CSA) vs BSC in MDS reported 29% RR with hATG (vs 9% in BSC; $P = .02$), with a median DOR of 16.4 months.[39] In this study, hMDS patients had a much higher RR at 50%, whereas no significant OS or AML-free survival difference was found between the arms. A phase 2 study of single-agent rabbit anti–thymocyte globulin also showed clinical activity with 33% hematologic improvement rate and median DOR of 8.2 months.[40] In a large retrospective analysis of 207 MDS patients treated with IST, hATG plus CSA was more effective than rabbit anti–thymocyte globulin, and the highest rate of RBC TI was achieved in patients with hMDS.[41] Taken together, the use of IST in hMDS is limited, but hATG plus CSA should be considered. As previously mentioned, younger age can be a predictor of response to IST, so this IST-based therapy should also be considered in younger patients who have LR-MDS without clinical response to ESA-based approaches.

HMA therapy can be used for LR-MDS refractory to first-line therapies. Dose-reduced regimens (ie, 5 days of azacitidine [AZA] 75 mg/m^2 per day or 3 days of 50 mg/m^2 per day decitabine [DAC]) have been tested in LR-MDS. Two phase 2 trials investigating 5-day AZA combined with EPO in TD LR-MDS after ESA failure reported 20% to 25% erythroid RR and 15% to 20% TI rate.[42,43] The limited efficacy observed in these studies is likely because of enrollment of purely anemic patients with significant transfusion burden. Another randomized phase 2 study compared 3-day AZA vs 3-day DAC therapy in LR-MDS and reported superior overall RR (70% vs 49%, $P = .03$), cytogenetic remission rate (61% vs 25%, $P = .02$), and OS benefit (20 vs 13 months, $P = .1$) for the DAC arm.[44] However, the difference was likely because of the underdosing in the comparator AZA arm, and a phase 2 study comparing 5 days of AZA vs 3 days of DAC in LR-MDS is ongoing (#NCT01720225). Moreover, most patients enrolled in this study were ESA naïve, which likely accounted for the higher HMA RR compared with other HMA studies done in LR-MDS after ESA failure.

Emerging strategies for management of MDS

A number of emerging therapies with promising RRs are in development and summarized in Table 2. In particular, targeted therapies are of keen interest, especially if toxicities are able to be managed despite combination with other agents.

Clinical case (continued)

The decision was made for treatment with AZA given the transfusion-dependent anemia and progressive thrombocytopenia despite blasts <5%. Absolute neutrophil count was preserved. In conjunction with the HSCT team, recommendations to proceed with HSCT after 2 cycles of AZA was established.

Role of HSCT in LR-MDS

Fit patients with lower-risk IPSS-R with poor-risk genetic features, profound cytopenias, and high transfusion burden are candidates for HSCT.[45] Given our enhanced ability to profile MDS at the time of diagnosis (and clonal evolution), we are able to better identify those LR-MDS patients that are at higher risk for progression and allow for earlier curative intent–based therapies.

Conclusions

LR-MDS patients have a notably long survival, and the deliberate selection and sequencing of therapies will lead to an optimal risk/benefit ratio. We continue to identify clonal subpopulations of MDS for which targeted agents can prove useful and potentially restore normal hematopoiesis for some duration. It is likely that these approaches will require insight regarding clonal regression/evolution and the microenvironment in which they are housed. The genetic and biologic heterogeneity of MDS provides significant challenges in developing new clinical therapeutics, which has also been hampered by the lack of good preclinical in vivo models. Nonetheless, the addition of luspatercept to the therapeutic armamentarium for treatment of LR-MDS was encouraging after more than a decade of silence, and excitement regarding novel agents (or combinations of agents) is brightening the horizon.

Conflict-of interest disclosure

H.E.C. receives research funding for an investigator-initiated clinical trial from Celgene; has been on advisory boards for Celgene, Agios, BMS, Daiichi, Jazz, Stemline, and Novartis; has given talks for Agios, Celgene, Agios, Jazz, Novartis, and Stemline; and is on independent review committees for Abbvie, ASTEX, and Takeda. C.S. declares no competing financial interests.

Off-label drug use

None disclosed.

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Applied genomics in MPN presentation

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Polycythemia vera, essential thrombocytosis (ET), and primary myelofibrosis (PMF) are grouped together as myeloproliferative neoplasms (MPNs) because of shared clinical, pathologic, and molecular features. The 2005 discovery of the driver mutation *JAK2*V617F, found in more than 70% of individuals with MPNs and 98% of those with PV, has transformed the diagnosis and management of MPNs. Although PV is the most common phenotype associated with *JAK2*V617F, roughly 60% of individuals with ET or PMF also have the mutation, and *JAK2*V617F is now recognized as a common lesion in clonal hematopoiesis (CH). *JAK2*V617F+ CH and MPN are indolent disorders that evolve over time, with transitions to different disease phases, transformation to bone marrow failure or leukemia, and high thrombosis rates. Genomic assessment has taken center stage as an important tool to define disease phenotype, disease burden, prognosis, and even thrombosis risk of MPNs. Genomics has also unveiled the causes and factors that modify the risk of acquiring and expanding CH and MPNs and points to new pathways for targeted therapies to treat and ultimately prevent them. Genomic assessment of patients with MPNs, like other cancers, enables the clinician to capitalize on large population data sets to inform the individual patient of risk, identify treatment, and improve outcomes.

LEARNING OBJECTIVES

- Understand the the role of JAK2V617F mutational burden in MPN presentation
- Understand the thrombosis risk associated with JAK2V617F mutation burden

Clinical case

A 65-year-old computer engineer presented to a vascular medicine specialist for evaluation and management of left lower extremity deep venous thrombosis (DVT) after a traumatic ankle fracture in 2018. In 2014, he developed a pulmonary embolism on postoperative day 2 after a radical prostatectomy for early-stage prostate cancer and was treated for 6 months with warfarin. His vascular medicine specialist noted thrombocytosis, not only at the time of the DVT, but also for several years prior; submitted a *JAK2*V617F mutation assay that was positive with a variant allele fraction (VAF) of 49%; and referred him to a hematologist (Table 1). A bone marrow biopsy in 2020 showed hypercellular marrow with panmyelosis, compatible with a myeloproliferative neoplasm (MPN) with normal cytogenetics, and a next-generation sequencing panel revealed the *JAK2*V617F mutation with a variant allele fraction (VAF) of 63% and a *TET2* frameshift mutation with a VAF 30%. The patient fulfilled the 2016 World Health Organization (WHO) criteria for the diagnosis of polycythemia vera (PV). Phlebotomy and cytoreduction were initiated,[1] and systemic anticoagulation was continued.

Introduction

PV, essential thrombocytosis (ET), and primary myelofibrosis (PMF) are grouped together as MPNs because of shared clinical, pathologic, and molecular features. The 2005 discovery of the driver mutation *JAK2*V617F, found in more than 70% of individuals with MPNs and 98% of individuals with PV, has transformed the diagnosis and management of PV.[2] Although PV is the most common phenotype associated with *JAK2*V617F, roughly 60% of individuals with ET or PMF also have the mutation. MPNs are indolent disorders that evolve over time, with transitions to different disease phases, transformation to bone marrow failure or leukemia, and high thrombosis rates. Both quantitative and qualitative genomic assessments are important tools in defining disease phenotype, disease burden, prognosis, and even thrombosis risk in MPN. This case and discussion will highlight the impact of the JAK2V617F clonal burden on disease phenotype and review factors that determine progression from JAK2V617F clonal hematopoiesis (CH) to MPN.

Table 1. Serial laboratory values in the 7 years before 2020 MPN diagnosis

	Reference range	2013	2014*	2017	2018†	2020
WBC count	4.50-11.00 X 10^9/L	6.27	8.0	7.34	8.12	8.96
RBC count	4.50-5.90 X 10^6/uL	5.14	5.12	5.29	5.52	**6.19**
Hemoglobin	13.9-16.3 g/dL	15.6	15.2	15.9	**16.4**	**18.1**
Hematocrit	41.0%-53.0%	46.5	44.0	47.3	51.3	**56.4**
Mean corpuscular volume	80-100 fL	88	87	89	90	91
RBC distribution width	11.5%-14.5%	13.4	14.5	**15.0**	**15.3**	**16.5**
Platelet count	150-350 X 10^9/L	**438**	**521**	**467**	**468**	**589**
JAK2V617F VAF	<0.8%	—	—	—	**49**	**61**

RBC, red blood cell; WBC, white blood cell.
*Postoperative pulmonary embolism.
†DVT; bold indicates values outside of the reference range.

Mutations and clonal burden

Our patient had acquired *JAK2*V617F, the most prevalent driver mutation in MPNs. JAK2 transmits cytokine-mediated growth signals in blood stem cells and progenitors, enabling the bone marrow factory to produce the millions of red cells, white cells, and platelets required on a daily basis.[3,4] The acquired mutation *JAK2*V617F transmits an excessive growth signal, that induces a blood stem cell with the mutation to overproduce red cells, white cells, and platelets. Mutation of *JAK2* is highly associated with acquired uniparental disomy of the chromosome 9, p region, that contains *JAK2* and results in homozygosity of *JAK2*V617F.[5] Both the JAK2V617F mutation and the mitotic recombination events occur at the hemopoietic stem cell (HSC) level, so that individual HSCs may have 1 or 2 copies of the mutation, and within a single individual, HSCs with heterozygous and homozygous mutations and unaffected HSCs may coexist.[6-9] The emergence of a dominant homozygous *JAK2*V617F clone is a feature of many patients with PV compared with ET, suggesting that additional genetic or epigenetic events facilitate the expansion of *JAK2*V617F homozygous clones in PV.[10] Clonal heterogeneity of *JAK2*V617F in the HSC compartment results in quantitative measures of *JAK2*V617F that range from as low as a fraction of a percent to 100% (Figure 1).

Both mutation (*JAK2*V617F, *CALR*, or *MPL*) and clonal burden have an influence on clinical presentation and outcome in the MPN. *JAK2*V617F allele burden, or variant allele fraction (VAF) at the very lowest end subtends a phenotype of clonal hematopoiesis of indeterminate potential or ET, whereas on the higher end, it subtends phenotypes of PV or post-PV myelofibrosis (PMF; Figure 1).[8,11-13] High *JAK2*V617F clonal burdens associate with higher white cell counts, risk of myelofibrosis transformation, and risk of splenomegaly.[6] Not long after the 2005 *JAK2*V617F discovery, many studies observed a higher risk of thrombotic events in *JAK2*V617F$^+$, compared with *JAK2*V617F$^-$ ET and MF.[7,14] *JAK2*V617F is an independent predictor of thrombosis in ET, whether compared with JAK2$^-$, or, after the discovery of mutations in the calreticulin gene (*CALR*), in ET, when compared with *CALR* mutation–positive patients.[14,15] Categorical mutation status is now incorporated in the International Prognostic Score for Thrombosis in Essential Thrombocythemia (IPSET) assessment.[14,16,17] In 2020, the specific effect of *JAK2*V617F MPNs on thrombosis risk was refined by the analysis of thrombosis in 1537 patients in China with *JAK2*V617F MPNs. The risk of thrombosis in patients with PV was significantly higher at diagnosis than in those with ET or PMF, and, in patients with PV, a *JAK2*V617F VAF greater than 50% was an independent risk factor for thrombosis, especially arterial thrombosis. In patients with PV, the incidence of thrombosis in patients with a *JAK2*V617F VAF ≥50% was 4.6 times higher than that in patients with a VAF <50%.[18]

Our patient had sustained thrombotic events years before MPN diagnosis, and his blood counts suggested that he had had a latent process for many years (Table 1). Thromboses that occur before, at presentation, or during the course of a previously diagnosed MPN have been described since the initial descriptions of these types of blood cancers.[19] Recent large retrospective case series have established that most events occur at or during the years preceding MPN diagnosis; that arterial events outnumber venous events in the older population, whereas venous events outnumber arterial events in the younger MPN population; and that female sex is associated with unusual sites

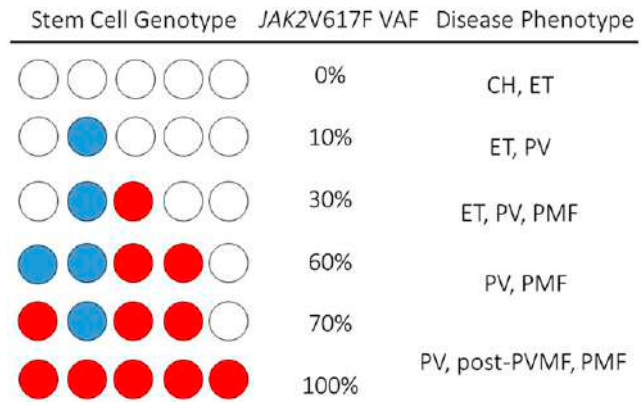


Figure 1. Schematic of hypothetical permutation and combination single-cell genotypes, VAFs, and associated clinical disease phenotypes in *JAK2*V617F$^+$ MPN. *JAK2*V617F can be present in a single cell as a single copy (heterozygote, blue circle) or as a double copy (homozygote, red circle) in a single-cell genome. In a single individual, multiple stem cell clones may have varied *JAK2*V617F genotypes, and a measured JAK2V617F may range from very low levels to 100% (center column). Clinical phenotypes associated with the *JAK2*V617F VAF are represented in the right column.

of venous thrombotic events, including splanchnic vein thromboses and cerebral sinus thrombosis.[18,20-25] Hultcrantz and colleagues examined thrombotic events in 9 429 Swedish patients with MPNs diagnosed between 1987 and 2009 and in 35 820 matched population controls, allowing for risk estimation at the time of diagnosis and thereafter. Both arterial and venous thrombotic events occurred more frequently around the time of MPN diagnosis, but the risk of occurrence of additional events persisted throughout the lifetimes of the patients with MPN. Both advanced age and male sex added to the cumulative risk of occurrence of both arterial and venous events in patients with MPN, but the thrombotic risk was higher in the MPN group, regardless of age or sex, as compared with the non-MPN control population.[24] In our patient, it is likely that the *JAK2*V617F mutation was evolving long before diagnosis of MPN and was exposing the patient to venous thrombosis risk at the time of cancer surgery. Indeed, mathematical modeling of *JAK2*V617F suggests that the mutation may be acquired up to 24 years before diagnosis of MPN.[26]

Clonal hematopoiesis and clonal evolution

Our patient had the *JAK2*V617F mutation, the most common mutation in the MPNs, but also the fifth most common lesion associated with clonal hematopoiesis (CH).[27,28] CH is defined as an expansion of blood stem cell clones that bear advantageous acquired somatic mutations of myeloid malignancy genes. Not long after its discovery in MPN, investigations into non-MPN populations for *JAK2*V617F$^+$ CH (*JAK2*V617F CH) revealed associations with both elevated blood counts and thrombosis risk, even at very low *JAK2*V617F VAFs and without fulfilling the criteria for an MPN diagnosis. Three large general population studies from China and Denmark found that low *JAK2*V617F VAFs were present in 0.1% to 0.2% of the general population[29-31] (Figure 2). Surprisingly, despite the absence of overt MPN, there were significantly higher platelet counts, white cell counts, and hemoglobin concentrations in individuals with *JAK2*V617F CH than in the controls and higher rates of both arterial and venous thrombotic events. Subsequent studies of CH demonstrated associations with arterial thrombotic events, and when segregated by specific mutated gene, *JAK2*V617F CH was associated with the highest relative risk of arterial events.[28,32]

In 2019, Cordua and coworkers provided further insight into associations of *JAK2*V617F CH and CALR-mutation–positive CH (CALR CH) in 19 958 participants in the Danish General Suburban Population Study.[33] Owing to a more sensitive assay, the researchers found a higher prevalence of *JAK2*V617F CH (3%) than that in prior studies (Figure 3). *CALR* CH was detected, but *JAK2*V617F CH was nearly 20-fold more prevalent. In MPN, the prevalence of *JAK2*V617F, although higher than that of the CALR mutation, was only fourfold more common than *CALR* mutation prevalence, a consistent finding across large MPN cohorts.[34-36] Blood counts and thrombosis risk of the 645 CH$^+$ individuals were significantly different than that of the CH$^-$ individuals. The 32 CALR-mutation CH individuals had higher platelet counts, whereas the 613 *JAK2*V617F CH individuals had significantly higher total white cell, neutrophil, red cell, and platelet counts, and a higher odds ratio of the occurrence of thrombotic events (Figure 3). Blood counts were significantly different, even when stratified by very low *JAK2*V617F VAF (<1%), and there was a dose-response relationship of CH <1%, compared with CH ≥1% to 2% in both blood counts and risk of venous thrombosis. In that study, *JAK2*V617F was detected in 613 of the 19 958 (3%) population, whereas only 14 of the 613 (2.3%) individuals with JAK2V617F had an MPN diagnosis.[33] It is not known whether all individuals with JAK2V617F CH evolve to JAK2V617F$^+$ MPN. Other large population studies of JAK2V67F CH that have had the opportunity to examine the change in allele burden over time and evolution from JAK2V617F CH to JAK2V617F MPN have found increases in VAF and evolution to an overt MPN in a substantial proportion of patients with CH.[13,29] Thus, identifying factors that expand *JAK2*V617F CH to *JAK2*V617F MPN is important for understanding disease evolution, and recognizing the potential for *JAK2*V617F CH to be a hidden force behind elevated blood counts and thrombosis risk is important in the non-MPN population. Our patient had blood counts and VTE risk in line with those associated with *JAK2*V617F CH 7 years before the MPN diagnosis was made.

Causes of CH and CH expansion to MPN

The acquisition of *JAK2*V617F CH, the expansion of *JAK2*V617F CH into MPN, and venous and arterial thromboses share smoking, older age, and a chronic inflammatory state as risk factors.[29,37,38] Inflammation drives thrombotic risk by increased white cell and platelet counts, related to activation of clotting factors, endothelial cells, platelets, and white cells and to activation of hypoxia-sensing signaling pathways.[39] Thromboinflammation is a concept that is particularly operational in cancer, aging, sickle cell anemia, and infection, and links inflammatory pathways to thrombotic risk.[40-43] *JAK2*V617F is uniquely connected to inflammation, both proximally and distally: inflammatory states may predispose to acquiring *JAK2*V617F CH, and *JAK2*V617F MPN signaling also drives inflammatory pathways.[44,45] For example,

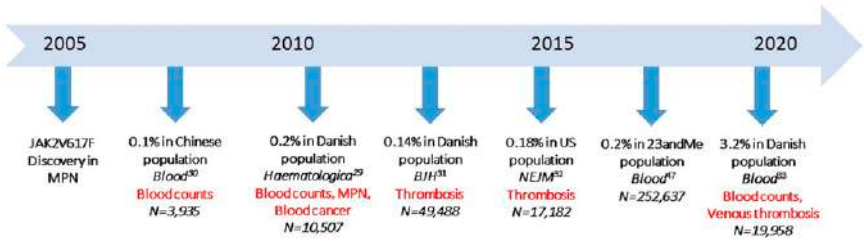


Figure 2. Evaluation of *JAK2*V617F CH in non-MPN populations. Since 2007, more than 350 000 individuals from general population studies have been examined for *JAK2*V617F CH. Published reports of 353 707 unique individuals, *JAK2*V617F CH prevalence, and clinical associations are listed on the timeline. Significant differences in blood counts, cancer risk, and thrombosis risk between *JAK2*V617F CH populations and controls are indicated in red.

Pedersen et al analyzed 107 969 individuals from the Copenhagen Population Study, a cohort in which 352 individuals developed MPN and in which a subcohort of 49 143 of the study individuals had *JAK2*V6717F testing, as reported by Nielsen et al.[13,44,46] Pedersen et al used a Mendelian randomization approach to test the role, in the acquisition of either MPN or *JAK2*V617F positivity, of a common loss-of-function IL-6 receptor variant associated with impaired IL-6 receptor signaling and reduced inflammation. Their results demonstrate that age- and sex-adjusted risk of acquiring any MPN is 40% lower among carriers of the loss-of-function IL-6 receptor variant. This relative risk reduction was even more prominent when examined in the *JAK2*V617F tested subcohort, further substantiating the concept that acquisition of *JAK2*V617F CH and *JAK2*V617F MPN subtend the same risk factors.[31,44,47] The JAK2 46/1 haplotype, which is highly associated with the development of JAK2V617F^{+} MPN, is also associated with the acquisition of *JAK2*V617F CH, suggesting that *JAK2*V617F CH is a precursor to *JAK2*V617F MPN.[31,47-49] The *JAK2*V617F mutation burden, measured prospectively in individuals with CH, is estimated to increase by roughly 0.5% per year and much more variably in individuals with *JAK2*V617F MPN, where disease type, age, sex, and other mutational burdens influence clonal burden and expansion.[12,13,26] Serial measures of *JAK2*V617F in our patient demonstrate that *JAK2*V617F VAF increased significantly as clinical disease evolved from thrombocytosis to PV (Table 1).

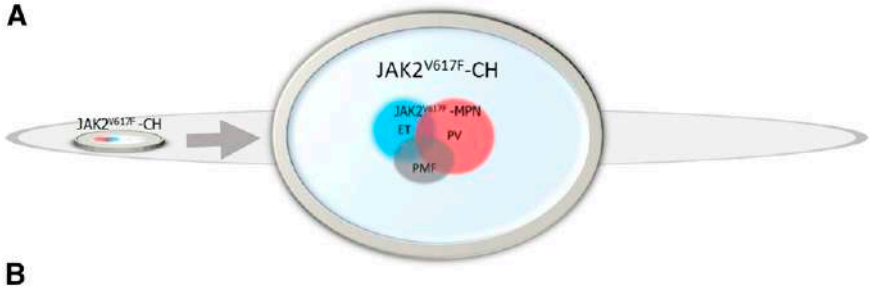
Mutations in myeloid cancer genes are common in the normal ageing population.[27,28,50-53] Although several myeloid gene mutations can be responsible for CH, coronary artery disease is most closely associated with mutations in *DNMT3A*, *TET2*, *ASXL1*, and *JAK2*.[28,32,54] Individuals with mutations in *DNMT3A*, *TET2*, or *ASXL1* had a 1.7- to 2.0-fold increase in coronary artery disease, whereas individuals with *JAK2*V617F CH had a 12.1-fold increase coronary artery disease.[32] The degree of elevation of the VAF of the mutated gene was also related to risk of coronary artery disease. Conversely, individuals with *JAK2*V617F MPN harbor the most common CH lesions that are associated with vascular disease, including *TET2*, *DNMT3A*, and *ASXL1*.[35,55,56]

A growing number of studies have indicated that loss-of-function mutations in *TET2* or *DNMT3A* lead to the development of a generalized inflammatory state that may contribute to clonal expansion and thrombotic risk. Low-density lipoprotein receptor–deficient mice, prone to develop atherosclerosis, that were genetically engineered to lack *TET2* in marrow cells acquired atherosclerotic plaques with increased size and an increased number of macrophages in the aortic walls.[32] The macrophages from *TET2*-deficient animals generated increased levels of cytokines and chemokines and promoted IL-1β transcription.[57] Individuals with *TET2* CH have been shown to have significantly elevated plasma levels of IL-8. Taken together, the results of these studies highlight the interplay between inflammation, clonal expansion, and thrombotic risk and provide a rationale for targeting inflammation to reduce acquisition of CH, expansion of CH, and vascular risk in both MPN and CH populations.[38,58,59]

Clinical case follow-up

Our patient had both *JAK2*V617F and a *TET2* frameshift mutation, with VAFs of 63% and 30%, respectively. Although we could not determine clinically which lesion came first, the mutation order of *TET2* and *JAK2* has been studied in PV and has important clinical and biological associations.[60] Patients who acquired the *JAK2*V617F mutation first were younger and had higher thrombosis rates than those who acquired the *TET2* mutation first. Mechanistically, the *TET2* mutation may alter the transcriptional impact of a subsequent *JAK2*V617F mutation in an HSC and may mitigate proliferation compared with an HSC with *JAK2*V617F

A



B

	Non-mutated	JAK2^{V617F} CHIP <1%	JAK2^{V617F} CHIP 1-2%	JAK2^{V617F} MPN >2%
N (%)	19313 (96.9%)	507 (2.5%)	92 (.46%)	16 (.08%)
WBC (109/L	7.3	7.5*	8.7*	11.0
Hg (gm/dL)	14.0	14.2	14.3*	13.5
Platelet (109/L	250	264*	361*	370
Odds ratio VTE	-	-	2.8	6.0
Odds ratio ICVD	-	-	-	4.6

Figure 3. Frequency of *JAK2*V17F in the general population and in associated laboratory and clinical phenotypes. Data are from the Danish Suburban Population Survey.[33] (A) *JAK2*V617F was identified in 3% (615/19958) of the general population, represented as the small chip within the larger oval. The arrow indicates an enlargement of this chip and shows that *JAK2*V617F MPN comprised only 2.6% of the total *JAK2*V617F CH population, with *JAK2*V617F MPN phenotypes indicated as overlapping colored circles. (B) Average blood counts and associated venous thromboembolism (VTE) and ischemic cerebrovascular disease (ICVD) from the non-mutated (*CALR*-negative and *JAK2*V617F-negative) and *JAK2*V617F-positive populations stratified by *JAK2*V617F VAF). Asterisks indicate significantly different values compared with the nonmutated population (*P < .05). WBC, white blood cell.

and normal *TET2* functions.[60] Our patient's median event-free survival is estimated to be 15 years, based on a personalized risk calculator.[56] The *TET2* lesion slightly shortens his estimated event-free survival, and in this sense, may indicate higher inflammatory risk that shortens survival via thrombotic events or genomic instability and acquisition and expansion of additional detrimental lesions. National Comprehensive Cancer Network (NCCN) guidelines have identified age >60 and/or history of thrombosis as the first point in their treatment algorithm and recommends treatment with hydroxyurea (HU) as the first-line therapy for older, high-risk patients with PV.[61] NCCN guidelines have incorporated the concept of treatment intolerance or resistance into the risk profile of PV. Patients with PV who are resistant or intolerant of HU are at an even increased risk of thrombotic events or disease progression, and in the case of HU resistance, the guidelines recommend alternative therapies.[62] Although not yet realized, genomics has the potential to predict treatment resistance. Genomics may move patients proactively to therapies with a higher probability of disease control without having to demonstrate treatment failure or may identify contexts in which targeted treatments induce both hematologic and molecular remission.

Conclusions

Genomics has informed diagnostics, prognostics, and treatment of MPN; has unveiled the causes and factors that modify risk of acquiring and expanding CH and MPN; and has pointed to new pathways for targeted therapies to treat and ultimately prevent CH and MPN. The progress in MPN genomics reflects the promise of 21st century medicine: molecularly defined entities, refined risk assessment, and opportunities for targeted therapy. The molecular pathogenesis of our patient's presentation and disease are now understood, and his thrombosis risk and symptom burden can be specifically addressed and monitored with ongoing genomic assessment. Thus, genomic assessment of patients with MPN enables clinicians to capitalize on large data sets to inform the individual of risk, identify therapies, and improve outcomes.[63]

Conflict-of-interest disclosure

A.R.M. has served as a consultant to PharmaEssentia. H.K. declares no competing financial interests.

Off-label drug use

None disclosed.

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Genomics of MPN progression

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The Philadelphia chromosome–negative (Ph⁻) myeloproliferative neoplasms (MPNs) are a heterogenous group of hematopoietic stem cell diseases characterized by activated JAK/STAT signaling and a variable propensity toward myelofibrotic and leukemic transformation. Acquisition of somatic mutations in addition to the canonical *JAK2*, *MPL*, and *CALR* mutations found in MPNs is an important catalyst in the clonal evolution and progression of these disorders. In recent years, our increasing understanding of the molecular landscape of Ph⁻ MPNs has generated important prognostic information that informs our approach to risk stratification and therapeutic decision-making. This review will focus on the critical impact of genomics on our approach to management of advanced Ph⁻ MPNs.

LEARNING OBJECTIVES

- Recognize the acquisition of somatic mutations as a catalyst for the clonal evolution and progression of MPNs
- Understand the incorporation of both clinical and molecular risk scores into the management of Ph-negative MPNs, including consideration for allo-HCT

Introduction

The classic Philadelphia chromosome–negative (Ph⁻) myeloproliferative neoplasms (MPNs) are clonal hematopoietic stem cell disorders typified by proliferation of myeloid lineage cell lines and include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).[1] These disorders are characterized by constitutive activation of the JAK/STAT-signaling pathway and the majority of cases will harbor canonical mutations in *JAK2*, *CALR*, or *MPL*.[2,3] In recent years, advances in next-generation sequencing (NGS) methodologies have led to the identification of other mutations that are associated with clonal evolution and progression in Ph⁻ MPNs. This review will focus on the practical ways in which this knowledge is informing our approach to risk stratification and therapeutic decision-making, including the timing of allogeneic stem cell transplantation.

Clinical case part 1

A 60-year-old man with PV presented with complaints of night sweats, a 10-kilogram weight loss, early satiety, and abdominal fullness over the preceding 6 months. He was still working full-time but was no longer able to exercise due to complaints of significant fatigue. His original diagnosis was made 15 years prior when he was concurrently diagnosed with a deep vein thrombosis. His disease had been controlled with hydroxyurea and he had also been on low-dose aspirin after completing a course of therapeutic anticoagulation. His physical examination revealed palpable splenomegaly 15 cm below the costophrenic angle. The complete blood count showed a hemoglobin of 11 g/dL, platelet count of 165 × 10^3/μL, and white blood cell count (WBC) of 15 × 10^3/μL (70% neutrophils, 20% lymphocytes, 3% monocytes, 3% myelocytes, 2% metamyelocytes, 1% basophils, 1% eosinophils). Peripheral blood smear was notable for leukoerythroblastosis and dacrocytes. A bone marrow biopsy was performed and the morphologic findings were consistent with progression to myelofibrosis (MF). Bone marrow blasts were not increased. Reticulin stain revealed 2+ marrow fibrosis. The bone marrow cytogenetic analysis demonstrated a normal male karyotype. NGS analysis revealed the following pathogenic variants: an *ASXL1* Q910Tfs*14 mutation, an *SRSF2* R94dup mutation, and a *TP53* R248W mutation in addition to the previously noted *JAK2* V617F mutation.

Clonal evolution of MPNs and prognostic impact of comutations

Myelofibrotic transformation is recognized as an advanced presentation of Ph⁻ MPNs and is associated with a negative impact on survival. MF can arise de novo (PMF) or secondary to underlying PV or ET (post-PV/ET MF), with a largely indistinguishable clinical presentation. There is

significant heterogeneity in outcomes in MF with survival ranging from <2 years to more than a decade. This has spurred the development of conventional prognostic scoring models over time, including the International Prognostic Scoring System (IPSS) and the Dynamic IPSS (DIPSS), which are largely derived from clinical variables. The DIPSS-plus model also incorporates clonal cytogenetic abnormalities (Table 1).[4-6]

Over the last decade and a half, considerable progress has been made in identifying the genotypic drivers that facilitate clonal expansion and clonal dominance in the Ph⁻ MPNs, and characterizing their intersection with aging, clonal hematopoiesis, epigenetic events, host genetic, and environmental factors in modulating the MPN phenotype and impacting prognosis.[7-9] For example, early on, there was a particular focus on the role of *JAK2*V617F in disease progression and a positive correlation was established between the *JAK2* allelic burden and progression from PV to MF.[10] More recent studies have demonstrated that beyond the canonical mutations in *JAK2*, *MPL*, and *CALR*, mutations in myeloid malignancy driver genes, including those involved in DNA methylation (*IDH1/2*, *TET2*, *DNMT3A*), chromatin modification (*ASXL1*, *EZH2*), RNA splicing (*U2AF1*, *SF3B1*, *SRSF2*), and DNA repair (*TP53*, *PPMID*) are implicated in the pathogenesis of Ph⁻ MPNs. In general, patients with a sole canonical MPN mutation were at low risk of disease progression; additional somatic mutations, however, were associated with a higher risk of an MF phenotype and/or transformation to acute myeloid leukemia (AML) and inferior overall survival (OS).[11] In PMF, the presence of mutations in *ASXL1*, *EZH2*, *SRSF2*, *U2AF1*, and *IDH1/2* portends a high risk for shortened OS or leukemia-free survival.[12,13] The number of detrimental mutations was inversely correlated with OS, consistent with the aforementioned impact of multiple mutations with clonal evolution. By contrast, the presence of a mutation in exon 9 of *CALR* was noted to be associated with improved OS independent of other mutations and conventional risk-scoring systems.[12,14]

Given the critical impact that the mutational profile has on disease progression and outcomes in MF, an ongoing challenge in recent times has been development of risk-stratification models that are more comprehensive and that reflect our current understanding of the impact of genomics on prognosis and its interdigitation with clinical risk factors. Such models might provide greater nuance when considering the potential merits of pursuing potentially curative but relatively high-risk therapeutic strategies such as allogeneic hematopoietic stem cell transplantation (allo-HCT). Accordingly, newer molecularly inspired prognostic models have been developed with the goal of integrating clinical, cytogenetic, and mutational data to refine risk stratification (Table 2). The Mutation-Enhanced International Prognostic Score System (MIPSS70), MIPSS70+, MIPSS70+ 2.0, and Genetically Inspired Prognostic Scoring System (GIPSS) were developed from multicenter cohorts of PMF patients <70 years of age with a primary objective of refining risk to aid decision-making with regard to allo-HCT, but they have also been validated in adults over the age of 70 years with PMF.[15-17] The Myelofibrosis Secondary to PV and ET-Prognostic Model (MYSEC-PM) was specifically developed for those with post-PV/ET MF.[18] The primary strength of these molecularly based models in comparison with clinically based models is the refinement in risk stratification based on knowledge of cytogenetic and molecular profile. For example, 46% of patients in the MIPSS70 high-risk category were upgraded from lower-risk IPSS categories, and 14% of those in the high-risk IPSS category were considered intermediate risk by MIPSS70.[15] Ultimately, there is a critical need for prospective validation of current models that integrate clinical and genomic information with a view to identifying optimal ways to incorporate these into therapeutic decision-making in MF.

Therapeutic implications of genomics in MF

JAK inhibition

Given the aberrant activation of the JAK/STAT-signaling pathway in MPNs, JAK inhibition has become an important strategy in the management of MPNs over the past decade. Ruxolitinib, an orally bioavailable inhibitor of JAK1 and JAK2, is US Food and Drug Administration (FDA) approved for the treatment of MF, based upon data from the COMFORT-1 and COMFORT-2 trials.[19,20]

Table 1. Clinical prognostic indices in PMF

Prognostic Index/Reference	Prognostic factors	Outcomes	Notes
IPSS[4]	Age >65 y old Constitutional symptoms Hgb <10 g/dL WBC >25 × 10^3/μL Circulating blasts >1%	Median OS, mo Low risk: 135 Int-1 risk: 95 Int-2 risk: 48 High risk: 27	Designed for PMF population Calculated using values at diagnosis
DIPSS[5]	Age >65 y old Constitutional symptoms Hgb <10 g/dL WBC >25 × 10^3/μL Circulating blasts >1%	Median OS, y Low risk: not reached Int-1 risk: 14.2 Int-2 risk: 4 High risk: 1.5	Designed for PMF population Can be recalculated throughout clinical course
DIPSS+[6]	Age >65 y old Constitutional symptoms Hgb <10 g/dL WBC >25 × 10^3/μL Circulating blasts >1% Platelet <100 × 10^3/μL Transfusion dependence Unfavorable karyotype*	Median OS, mo Low risk: 180 Int-1 risk: 80 Int-2 risk: 35 High risk: 16	Designed for PMF Incorporates additional prognostic factors to DIPSS

Hgb, hemoglobin; Int, intermediate; OS, overall survival.
*Unfavorable karyotype: complex karyotype or sole or 2 abnormalities that include 8, 7/7q−, i(17q), 5/5q−, 12p−, inv(3), or 11q23 rearrangement.

Table 2. Genomic prognostic indices in MF

Prognostic Index/ Reference	Prognostic factors	Outcomes	Notes
MIPSS70[15]	Hgb <10 g/dL WBC >25 × 10^3/μL Platelets <100 × 10^3/μL Circulating blasts ≥2% Constitutional symptoms Bone marrow fibrosis grade ≥2 No. of HMR mutations* Absence of type 1/like *CALR* mutation	Median OS, y Low risk: 27.7 Int risk: 7.1 High risk: 2.3	Designed for PMF patients ≤70 y old
MIPSS70+[15]	Hgb <10 g/dL WBC >25 × 10^3/μL Platelets <100 × 10^3/μL Circulating blasts ≥2% Constitutional symptoms No. of HMR mutations* Absence of type 1/like *CALR* mutation Unfavorable karyotype†	Median OS, y Low risk: 20 Int risk: 6.3 High risk: 3.9 Very high risk: 1.7	Designed for PMF patients ≤70 y old
MIPSS70+ v2.0[16]	Severe or moderate anemia‡ Circulating blasts ≥2% Constitutional symptoms No. of HMR mutations§ Absence of type 1/like *CALR* mutation VHR or unfavorable karyotype‖	Median OS, y Very low risk: not reached Low risk: 16.4 Int risk: 7.7 High risk: 4.1 Very high risk: 1.8	Designed for PMF patients ≤70 y old Assigns more points for severe anemia compared with moderate anemia Assigns more points for VHR karyotype compared with unfavorable karyotype
GIPSS[17]	VHR or unfavorable karyotype‖ Absence of type 1/like *CALR* mutation *ASXL1* mutation *SRSF2* mutation *U2AF1* Q157 mutation	Median OS, y Low risk: 26.4 Int-1 risk: 8 Int-2 risk: 4.2 High risk: 2	Designed for PMF Assigned greater points for unfavorable karyotype compared with very high-risk karyotype
MYSEC-PM[18]	Age at diagnosis of SMF Hgb <11 g/dL Platelet <150 × 10^3/μL Circulating blasts ≥3% *CALR* unmutated Constitutional symptoms	Median OS, y Low risk: not reached Int-1 risk: 9.3 Int-2 risk: 4.4 High risk: 2	Designed for SMF

GIPSS, Genetically Inspired Prognostic Scoring System; HMR, high molecular risk; MIPSS, Mutation-Enhanced International Prognostic Score System; MYSEC-PM, Myelofibrosis Secondary to PV and ET-Prognostic Model; SMF, secondary myelofibrosis; VHR, very high risk. See Table 1 for expansion of other abbreviations.
*HMR mutations include *ASXL1*, *SRSF2*, *EZH2*, *IDH1*, and *IDH2*.
†Unfavorable karyotype defined as any abnormal karyotype other than normal karyotype or sole abnormalities of 20q2, 13q2, +9, chromosome 1 translocation/duplication, 2Y, or sex chromosome abnormality other than 2Y.
‡Moderate anemia is defined by hemoglobin levels of 8 to 9.9 g/dL in women and 9 to 10.9 g/dL in men; severe anemia is defined by hemoglobin levels of 8 g/dL in women and 9 g/dL in men.
§HMR mutations in MIPSS70+ v2.0 include *ASXL1*, *SRSF2*, *EZH2*, *IDH1*, *IDH2*, and *U2AF1* Q157.
‖VHR karyotype defined as single/multiple abnormalities of −7, i(17q), inv(3)/ 3q21, 12p−/12p11.2, 11q−/11q23, or other autosomal trisomies not including +8/+9 (eg, +21, +19).

The agent is relatively well tolerated. Myelosuppression can, however, be dose limiting, particularly in patients with baseline cytopenias. Infectious complications requiring dose adjustment are less common and include respiratory tract infections, urinary tract infections, and herpes zoster infection. Of note, each of these infectious complications was seen at similar rates in placebo-treated patients with the exception of zoster.[21] Isolated cases of serious opportunistic infections such as progressive multifocal leukoencephalopathy and viral reactivation have been reported in the literature, but whether there is a definitive link to ruxolitinib, particularly because these infections were not noted in long-term follow-up of COMFORT-1 or COMFORT-2, remains unclear.[20-22]

Although ruxolitinib has been shown to be very effective at reducing spleen volume and controlling the constitutional symptoms associated with MPNs, it has minimal impact on the underlying malignant clone. Mutant *JAK2* variant allele frequency (VAF) was only modestly reduced in ruxolitinib-treated patients enrolled on COMFORT-1 and COMFORT-2,[19,20] and the rate of progression to leukemia was not significantly changed. Longer follow-up of COMFORT-1 demonstrated that the median duration of response to ruxolitinib is 3 years, and >70% of patients are off therapy by 5 years.[21] Mutational complexity negatively impacted the likelihood of response and patients with 2 or fewer mutations had a ninefold greater likelihood of response to ruxolitinib compared

with patients with 3 or more mutations. In addition, patients with 3 or more mutations had a shorter time to treatment discontinuation, and poor OS.[23]

Currently, allo-HCT is the only treatment option with curative potential for MF. Given the potential risks associated with allo-HCT, however, an ongoing question in transplant-eligible patients is the choice of JAK inhibition as the initial treatment of choice and delaying transplantation vs proceeding as soon as feasible to an allo-HCT.

Optimal timing of allo-HCT

Given the heterogeneous clinical course seen in chronic-phase MPNs, the decision to pursue allo-HCT should be carefully considered and discussed with patients. As discussed in further detail in recent comprehensive reviews, factors to consider in addition to risk stratification of disease include age, comorbidities, and donor options.[24,25] A number of large retrospective series have analyzed allo-HCT outcomes based upon clinical risk score, some of which are highlighted in Table 3.[26-28] These have demonstrated that clinical risk scores such as DIPSS predict outcome even after allo-HCT. In addition, when the relative risk of death after allo-HCT for MF was compared with a cohort treated without transplant, the data suggest that the benefit of allo-HCT is most evident in intermediate-2 and high-risk patients and that low-risk patients should have transplant deferred. These findings hold up even in the JAK-inhibitor era when outcomes following allo-HCT were compared with non-transplant therapies that included JAK inhibition.[28] The decision to pursue allo-HCT in intermediate-1 risk disease is less well defined and could be potentially impacted by the mutational profile.

The impact of genomics on outcomes?

Given the accumulating body of evidence that underscores the prognostic impact of specific somatic mutations in MPNs, incorporation of these data may help to identify candidates that serve to gain the most from transplant, including candidates with high-risk mutations who may otherwise have been categorized as having low/intermediate-1 disease by conventional risk-stratification models. Multiple retrospective analyses have been carried out to try and ascertain the impact of mutational status on outcome of MF patients undergoing allo-HCT. Regarding canonical MPN mutations (*JAK2*, *CALR*, and *MPL*), Panagiota et al found that triple-negative (those lacking mutations in *JAK2*, *CALR*, and *MPL*) MF patients undergoing transplant had inferior OS whereas *CALR*-mutated patients had improved OS.[29] Of note, this may have been reflective of underlying disease biology given the favorable prognosis associated with type 1 *CALR* mutations even in the absence of allogeneic stem cell transplantation. More recent analyses have gone beyond evaluating the canonical MPN mutations and their prognostic impact in the allo-HCT population, to analyze the impact of additional mutations (Table 4).[30-33] Kröger et al reported that having a *CALR*

Table 3. Outcomes of allo-HCT in MF stratified by DIPSS

Lead author/ Reference	Disease and no. of patients	1-y outcomes	5-y outcomes	Impact of clinical risk score	Notes
Scott 2012[26]	170 MF patients who underwent allo-HCT	OS = 74% RFS = 68% NRM = 26%	OS = 57% RFS = 57% NRM = 34%	HR for OM across risk scores: Low: 1 Int-1: 1.97 Int-2: 3.15* High: 4.11*	Single-center, JAKi-naive population
Kröger 2015[27]	190 PMF patients who underwent allo-HCT 238 PMF patients who underwent non-tx strategies	OS for allo-HCT† Low = 100% Int-1 = 78% Int-2 = 82% High-risk = 65% OS for nontransplant† Low = 98% Int-1 = 97% Int-2 = 77% High = 67%	OS for allo-HCT† Low-risk = 69% Int-1 = 52% Int-2 = 50% High-risk = 32% OS for non-tx† Low = 95% Int-1 = 77% Int-2 = 41% High = 11%	RR of death after allo-HCT vs non-tx: Low = 5.6* Int-1 = 1.6 Int-2 = 0.55* High = 0.37*	2 multicenter databases, JAKi-naive population
Gowin 2020[28]	551 MF patients who underwent allo-HCT 1337 MF patients who underwent non-tx strategies	Not reported	OS for allo-HCT† Low = 73% Int-1 = 73% Int-2/high = 44% OS for non-tx† Low = 82% Int-1 = 57% Int-2/High = 32%	HR of 1-y OM in non-tx vs allo-HCT: Low = 0.16* Int-1 = 0.26* Int-2/high = 0.39* HR of OM beyond 1 y in non-tx vs allo-HCT: Low = 1.38 Int-1 = 2.64* Int-2/high = 2.55*	Multicenter, both JAKi-naive and JAKi-treated patients Impact of allo-HCT vs non-tx on survival was consistent whether patients received ruxolitinib or not

HR, hazard ratio; non-tx, nontransplant; JAKi, JAK inhibitor; NRM, nonrelapse mortality; OM, overall mortality; RFS, relapse-free survival; RR, relative risk. See Table 1 for expansion of other abbreviations.
*Statistically significant.
†Stratified by DIPSS.

mutation was significantly associated with improved OS whereas having an *IDH2* or *ASXL1* mutation was significantly associated with inferior relapse-free survival (RFS).[30] Stevens et al retrospectively evaluated a cohort of MF patients who underwent allo-HCT and demonstrated that the presence of 3 or more mutations in addition to a *JAK2* or *CALR* mutation was associated with inferior outcomes regardless of DIPSS+ score. Of note, 75% of the patients in this cohort underwent a myeloablative regimen.[31] In contrast, Tamari et al demonstrated that a *U2AF1* mutation was associated with inferior OS and RFS whereas a *DNMT3A* mutation was associated with inferior RFS. Mutational complexity did not impact outcomes and MIPSS70 high-risk patients did not have inferior outcomes when compared with intermediate-risk patients. Interestingly, patients who received a myeloablative conditioning (MAC) regimen, which in this cohort was generally paired with a T-cell–depleted allograft, had a superior outcome to those who underwent a reduced-intensity conditioning (RIC) regimen.[32] Ali et al reported on a cohort exclusively treated with a RIC regimen; they demonstrated that a *CBL* mutation was associated with inferior OS and DFS while a *U2AF1* mutation was associated with increased non-relapse mortality. In addition, the high-risk MIPSS70 group had inferior OS and DFS when compared with the intermediate-risk group. Similarly, the MIPSS70+ v2.0 very high-risk group had worse OS and DFS when compared with the high-risk group.[33]

The conflicting data derived from these various analyses may have arisen from the relatively small sample size in the various cohorts, particularly with regard to the less common mutations, as well as the heterogeneity of conditioning regimens used. This suggests that larger cohorts of patients who are more uniformly treated need to be analyzed to more definitely answer the question of the impact of specific mutations on outcome of patients with MF undergoing allo-HCT. Ideally, as has been demonstrated in the context of other myeloid neoplasms,[34,35] the optimal choice of conditioning regimen (MAC vs RIC) also deserves prospective study, especially in a fit, younger cohort of MF patients. However, these patients are significantly underrepresented in clinical practice. Given the predictive impact of measurable residual disease by molecular monitoring on relapse incidence,[36] novel targeted maintenance strategies to eradicate measurable residual disease post–allo-HCT will be necessary to improve long-term outcomes in patients with MF, the majority of whom are older and will continue to be offered RIC regimens. The role of JAK inhibition as a posttransplant maintenance strategy is also unclear as there are limited data to currently guide clinicians in this setting.[37]

Clinical case part 2

The patient is classified as intermediate-1 risk by DIPSS/DIPSS+ and high risk by MIPSS70/MIPSS70+ 2.0. He is transitioned from hydroxyurea to ruxolitinib due to his significant splenomegaly and constitutional symptoms and is simultaneously referred for transplant evaluation given his MIPSS70 high-risk disease. He ultimately decides against pursuing allo-HCT despite being

Table 4. Genomic impact of outcomes in MF patients undergoing allo-HCT

Lead author/ reference	Disease and no. of patients	No. of genes tested	Conditioning regimen	Survival data	Notes
Kröger 2017[30]	169 MF patients who underwent allo-HCT	16	MAC: 2% RIC: 98%	5-y PFS = 48% 5-y OS = 52%	*CALR* mutation associated with improved OS *IDH2* mutation associated with inferior RFS *ASXL1* mutation associated with inferior RFS
Tamari 2019[32]	101 MF patients who underwent allo-HCT	585	MAC: 18% RIC: 82%	5-y RFS = 51% 5-y OS = 52%	*U2AF1* mutation associated with inferior OS and RFS *DNMT3A* mutation associated with inferior RFS ≥3 somatic mutations not associated with worse OS compared with ≤2 somatic mutations MAC associated with improved OS High-risk MIPSS70 not associated with inferior OS compared with intermediate-risk MIPSS70
Ali 2019[33]	110 MF patients who underwent allo-HCT	72	RIC: 100%	5-y PFS = 60% 5-y OS = 65%	*CBL* mutation associated with inferior OS and DFS *U2AF1* mutation associated with increased NRM MIPSS70 high-risk group with worse OS and DFS compared with intermediate-risk group MIPSS70+ v2.0 very high-risk group with worse OS and DFS when compared with high-risk group.
Stevens 2020[31]	55 MF patients who underwent allo-HCT	54	MAC: 75% RIC: 25%	10-y OS in DIPSS+ low/Int-1 risk = 82% 10-y OS in DIPSS+ Int-2/high risk = 50% 10-y PFS in DIPSS+ low/Int-1 risk = 82% 10-y PFS in DIPSS+ Int-2/high risk = 46%	≥3 somatic mutations in addition to *JAK2* or *CALR2* mutation associated with worse PFS in comparison with ≤2 mutations regardless of DIPSS+ score

DFS, disease-free survival; MAC, myeloablative conditioning; PFS, progression-free survival; RIC, reduced-intensity conditioning. See Tables 1 and 3 for expansion of other abbreviations.

counseled about the negative implications of his high-risk molecular risk profile, both for OS and leukemia-free survival. He achieves significant clinical improvement with regard to splenomegaly and constitutional symptoms with ruxolitinib, but 18 months later, he presents to the clinic with complaints of worsening fatigue and weight loss. Physical examination is notable for worsening splenomegaly. Complete blood count is notable for a hemoglobin of 8.2 g/dL, platelet count of 96 × 10^3/μL, and a leukocyte count of 17 × 10^3/μL (51% neutrophils, 15% lymphocytes, 3% monocytes, 3% myelocytes, 2% metamyelocytes, 2% basophils, 2% eosinophils, 25% blasts). Repeat bone marrow biopsy demonstrates grade 3+ reticulin fibrosis and 26% blasts, consistent with a blast-phase (BP) MPN (MPN-BP). Cytogenetics demonstrate a monosomy 7. Repeat NGS demonstrates development of an *IDH2* mutation and an additional mutation in *TP53*. The previously noted mutations in *JAK2*, *ASXL1*, *SRSF2*, and *TP53* persist.

Genomics of AP and BP MPNs

The development of additional somatic mutations can drive MPNs toward leukemic transformation.[11,38] The incidence of progression to BP, operationally defined as 20% or more blasts in the peripheral blood and/or marrow, varies based upon the underlying MPN. An analysis of 826 patients at the Mayo Clinic demonstrated a 20-year incidence of BP of 3.8% for ET, 6.8% for PV, and 14.2% for PMF.[39] Historically long term survival of PMF patients

- We obtain comprehensive clinical, histopathologic, cytogenetic and molecular evaluation using a next generation sequencing panel encompassing myeloid malignancy associated genes
- We risk stratify using conventional models including DIPSS/DIPSS+ and molecularly inspired models including MIPPS70/MIPPS70+
- For asymptomatic low/intermediate-1 risk disease by DIPSS/DIPSS+, we observe. If high/very high risk by MIPSS70/MIPSS70+, we refer early for transplant evaluation, and monitor closely for any evidence of progression
- For symptomatic intermediate-1 risk disease by DIPSS/DIPSS+, we employ JAK inhibitor-based therapy. If high/very high risk by MIPSS70/MIPSS70+, we refer early for transplant evaluation, and monitor closely for risk of progression
- For intermediate-2/high risk disease by DIPSS/DIPSS+, we recommend allogeneic hematopoietic cell transplant evaluation, and commence JAK inhibitor- based therapy simultaneously if patient is symptomatic or has significant splenomegaly. If high/very high risk by MIPSS70/MIPSS70+, we strongly recommend transplant evaluation, and recommend proceeding with transplantation as soon as feasible if patient is transplant-eligible
- We strongly consider clinical trial enrollment for all patients with advanced MPNs who require therapy especially those who are cytopenic, or those who are intolerant and/or refractory to JAK inhibitors; these patients represent a significant area of unmet need
- For patients with MPN- accelerated/blast phase disease, we strongly encourage clinical trial enrollment whenever feasible and transplant evaluation
    - We recommend IDH inhibitor-based therapy for those who are *IDH1/2* mutated
    - For *TP53* mutated disease, in the absence of a clinical trial option, we recommend consideration of hypomethylating agent-based therapy especially if less fit

Figure 1. Our approach to risk stratification and management of advanced-phase MPNs.

Table 5. Combination therapies with JAK inhibitors

JAK inhibitor	Added drug	Mechanism of action of added drug	Patient population	Trial no.
Ruxolitinib	Navitoclax	BCL2/BCLXL/BCLw inhibitor	MF	NCT03222609
Ruxolitinib	Umbralisib	PI3Kδ inhibitor	PV, MF	NCT02493530
Ruxolitinib	Decitabine	Hypomethylating agent	AP and BP MPNs	NCT02076191
Ruxolitinib	Enasidenib	IDH2 inhibitor	AP and BP MPNs with an *IDH2* mutation	NCT04281498
Ruxolitinib	CPI-0610	BET inhibitor	MF	NCT02158858
Ruxolitinib	KRT-232	MDM2 inhibitor	*TP53* wild-type MF with previous ruxolitinib exposure	NCT04485260
Fedratinib	Luspatercept	ActRII ligand trap	MF with previous ruxolitinib exposure	NCT03755518

with 5% to 9% circulating blasts, accelerated-phase (AP) MPN with 10% to 19% blasts (MPN-AP), and MPN-BP has been quite poor regardless of treatment approach including intensive induction chemotherapy or hypomethylating agent therapy.[40-43] Early-phase trials evaluating hypomethylating agents in combination with ruxolitinib in patients with MPN-AP/BP have demonstrated limited impact on the natural history of disease, with median OS in the 7- to 8-month range.[44,45] In addition, patients who develop MPN-BP after receiving treatment with ruxolitinib have incredibly poor outcomes. In an analysis of 589 MF patients treated with ruxolitinib, 11% of patients developed BP at median follow-up of 3 years from initiation of ruxolitinib (range, 0.1-7.6 years). Median survival was 2 months upon progression to BP. Although canonical MPN mutation status was not identified as a risk factor for progression to BP, patients with intermediate-2/high-risk disease by DIPSS or MYSEC-PM were at higher risk of progression to BP.[46]

The mutational spectrum of MPN-BP is distinct from that of de novo AML. Mutations in genes such as *FLT3*, *NPM1*, and *CEBPA*, which are typical of AML de novo, are rare in MPN-BP. By contrast, MPN-BP is enriched for mutations in epigenetic modifiers, DNA repair, and spliceosomal genes.[40,47,48] The evolutionary pathway to MPN-BP is complex with evidence supporting evolution from a *JAK2* V617F–mutated clone, clonal expansion of a pre-*JAK2* V617F ancestral clone, or in some cases biclonal disease at the outset. The emerging information on the molecular drivers of blast transformation coupled with the increasing availability of targeted therapies has the potential to inform a molecularly based approach to treatment of these disorders.[42,49] A number of therapies specific to underlying somatic mutations are either FDA approved or in clinical investigation in myeloid malignancies. For example, the IDH1 inhibitor ivosidenib and IDH2 inhibitor enasidenib are both approved for the treatment of *IDH1*- or *IDH2*-mutated AML in the first- and second-line settings, respectively. *IDH1/2* mutations predict a short leukemia-free survival in MF and are significantly enriched in MPN-BP. There are emerging retrospective data to suggest that *IDH*-mutated patients with MPN-AP/BP can have relatively durable responses with IDH inhibitor–based treatment strategies.[50] For *TP53*-mutated patients, hypomethylating agent–based strategies are often used especially in less fit individuals due to the very low likelihood of success from an intensive approach from a risk/benefit perspective. *TP53* loss of heterozygosity is often associated with the progression to MPN-BP, and outcomes are particularly inferior in this subset even after allo-HCT.[51] Preliminary reports from early-phase trials utilizing APR-246, a p53 modulator, and magrolimab, an anti-CD47 monoclonal antibody, have shown promising efficacy in *TP53*-mutated AML/myelodysplastic syndrome.[52,53] There is a paucity of data with these novel agents in MPNs and such strategies merit investigation in *TP53*-mutated MPN AP/BP.

Allogeneic stem cell transplant is the only known cure for MPN AP/BP, but the overall cure rate is low. The optimal depth of remission in MPN-BP prior to proceeding with allo-HCT has not yet been defined.[42] Recent reports suggest that the degree of

Table 6. Novel investigational agents

Drug	Mechanism of action	Patient population	Trial no.
Luspatercept	ActRII ligand trap	MF with Hgb <10 g/dL	NCT03194542
Sotatercept	ActRII ligand trap	MF with Hgb <10 g/dL	NCT01712308
Selumetinib	MEK inhibitor	High-risk myeloid neoplasms	NCT03326310
Imetelstat	Telomerase inhibitor	MF ET with at least 1 prior therapy MF with previous JAKi exposure	NCT01731951 NCT01243073 NCT02426086
Bomedemstat	LSD-1 inhibitor	MF with previous JAKi exposure ET or PV with at least 1 prior therapy ET previously treated with hydroxyurea ET with at least 1 prior therapy	NCT03136185 NCT04262141 NCT04081220 NCT04254978
KRT-232 (in combination with decitabine or low-dose cytarabine)	MDM2 inhibitor	*TP53* wild-type BP MPNs	NCT04113616
Venetoclax (in combination with decitabine)	BCL2 inhibitor	*TP53*-mutated BP MPNs	NCT03844815

See Tables 1 and 3 for expansion of abbreviations.

Table 7. Summary of prognostic mutations in MPNs

Notes	Frequencies in MPNs	References
Canonical MPN mutations		
CALR mutation		
Absence of type 1/like CALR mutation associated with inferior outcomes in PMF and SMF Presence of *CALR* mutation associated with improved OS in MPN patients undergoing allo-HCT	PMF: 20-25% ET: 20-25% MPN-AP/BP: 13%-20%	14, 18, 24, 29, 30, 40, 47, 48, 57
DNA methylation		
IDH1/2 mutation		
IDH1 associated with inferior LFS in PMF *IDH2* associated with inferior LFS in PV *IDH2* associated with inferior OS in ET *IDH2* associated with inferior LFS in PMF *IDH2* associated with inferior RFS in MPN patients undergoing allo-HCT	PV: 3% PMF: 6% ET: 9% MPN-AP/BP: 19%-26%	12, 24, 30, 40, 47, 48, 57
Chromatin modification		
ASXL1 mutation		
Associated with inferior OS in PV Associated with inferior OS and LFS in PMF Associated with inferior RFS in MPN patients undergoing allo-HCT	PV: 7% PMF: 30% ET: 2% MPN-AP/BP: 25%-47%	12, 14, 24, 40, 47, 48, 57
EZH2 mutation		
Associated with inferior OS and LFS in PMF	PV: 2% PMF: 5%-7% ET: 1% MPN-AP/BP: 7%-15%	12, 24, 40, 47, 48
Signaling		
SH2B3 mutation		
Associated with inferior OS in ET	PV: 5% ET: 2% MPN-AP/BP: 2%-11%	24, 40, 47, 48, 57
Splicing		
SRSF2 mutation		
Associated with inferior OS, LFS, and MFS in PV Associated with inferior OS and LFS in PMF	PV: 3% PMF: 9%-14% ET: 2% MPN-AP/BP: 13%-22%	12, 24, 40, 47, 48, 57
SF3B1 mutation		
Associated with inferior LFS and MFS in ET	PV: 10% PMF: 9%-14% ET: 5% MPN-AP/BP: 7%	24, 40, 47, 48, 57
U2AF1 mutation		
Associated with inferior MFS in ET Associated with inferior OS in PMF Associated with inferior OS and RFS in MPN patients undergoing allo-HCT	PV: 7% PMF: 5%-20% MPN-AP/BP: 5%	13, 24, 32, 40, 47, 48, 57
DNA repair		
TP53 mutation		
Associated with inferior LFS in ET	PV: 5% PMF: 5% ET: 6% MPN-AP/BP: 16%-36%	24, 40, 47, 48, 57

LFS, leukemia-free survival; MFS, myelofibrosis-free survival. See Tables 2 and 3 for expansion of other abbreviations.

disease control at time of allo-HCT may not have a significant prognostic impact.[51,54,55] This may be due in part to the fact that deep remissions pre-allo-HCT for MPN-BP are rare, and therefore the relapse rate is uniformly high. This underscores the need for prospective novel approaches focused on this patient population.

Clinical case part 3

Given the presence of an *IDH2* mutation, clinical trial options incorporating IDH2 inhibition were reviewed with the patient. He elected to participate in a clinical trial of enasidenib in combination with azacitidine and achieved a complete response of the leukemic component of his disease with histopathologic evidence of persistence of his chronic-phase MPN.[56] His hematologic parameters have improved and he has now been on enasidenib-based therapy for several months. Follow-up NGS analysis demonstrated persistence of the *JAK2* mutation, however, the *TP53*, *ASXL1*, *SRSF2*, and *IDH2* mutations are no longer detectable. He has now agreed to move forward with an allo-HCT.

Summary

Our approach to the management of advanced MPNs is summarized in Figure 1. The integration of clinical variables and mutational data into risk-stratification efforts is paving the way for personalized prognostic modeling to identify patients who are at particularly high risk of progression and may serve to benefit the most early on, from potentially curative but relatively high-risk interventions such as allo-HCT. Many patients will be deemed ineligible for allo-HCT, and treatment of such patients remains a significant area of unmet need. A number of novel therapeutic approaches are under investigation for advanced MF, including MPN AP/BP disease (Tables 5 and 6). There is a need for ongoing investigation to define the most relevant molecular drivers of disease progression and validation of these both in nonclinical models and in early phase trials, as credible therapeutic targets (Table 7). The ultimate hope is that these efforts will significantly impact the natural history of advanced-phase MPNs.

Conflict-of-interest disclosure

O.O. has served on the advisory boards of AbbVie, Celgene, Impact Biomedicines, and Novartis, and has received research support (paid to institution) from AbbVie, Aprea, Astex, Agios, Celgene, CTI Biopharma, Daiichi, Janssen, Kartos, and Oncotherapy Sciences. A.A.P. declares no competing financial interests.

Off-label drug use

None disclosed.

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Genomics of myelodysplastic syndrome/myeloproliferative neoplasm overlap syndromes

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Myelodysplastic syndrome (MDS)/myeloproliferative neoplasm (MPN) overlap syndromes are uniquely classified neoplasms occurring in both children and adults. This category consists of 5 neoplastic subtypes: chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), *BCR-ABL1*–negative atypical chronic myeloid leukemia (aCML), MDS/MPN-ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), and MDS/MPN-unclassifiable (U). Cytogenetic abnormalities and somatic copy number variations are uncommon; however, >90% patients harbor gene mutations. Although no single gene mutation is specific to a disease subtype, certain mutational signatures in the context of appropriate clinical and morphological features can be used to establish a diagnosis. In CMML, mutated coexpression of *TET2* and *SRSF2* results in clonal hematopoiesis skewed toward monocytosis, and the ensuing acquisition of driver mutations including *ASXL1*, *NRAS*, and *CBL* results in overt disease. MDS/MPN-RS-T demonstrates features of *SF3B1*-mutant MDS with ring sideroblasts (MDS-RS), with the development of thrombocytosis secondary to the acquisition of signaling mutations, most commonly *JAK2*V617F. JMML, the only pediatric entity, is a bona fide RASopathy, with germline and somatic mutations occurring in the oncogenic RAS pathway giving rise to disease. *BCR-ABL1*–negative aCML is characterized by dysplastic neutrophilia and is enriched in *SETBP1* and *ETNK1* mutations, whereas MDS/MPN-U is the least defined and lacks a characteristic mutational signature. Molecular profiling also provides prognostic information, with truncating *ASXL1* mutations being universally detrimental and germline *CBL* mutations in JMML showing spontaneous regression. Sequencing information in certain cases can help identify potential targeted therapies (*IDH1*, *IDH2*, and splicing mutations) and should be a mainstay in the diagnosis and management of these neoplasms.

LEARNING OBJECTIVES

- Define the landscape of cytogenetic and molecular abnormalities in patients with MDS/MPN overlap neoplasms including chronic myelomonocytic leukemia (CMML), juvenile myelomonocytic leukemia (JMML), *BCR-ABL1*–negative atypical chronic myeloid leukemia (aCML), MDS/MPN-ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), and MDS/MPN-unclassifiable (U)
- Characterize molecular signatures that can be used in the context of appropriate clinical and morphological features to help diagnose CMML, JMML, MDS/MPN-RS-T and *BCR-ABL1*–negative aCML
- Underscore the importance of molecular profiling in MDS/MPN overlap syndromes with regard to diagnosis, prognosis, and clinical therapeutics

Case

A 71-year-old man presents with a 6-month history of effort intolerance, weakness, intermittent drenching night sweats, and low-grade fevers. His last complete blood count 2 years ago had demonstrated mild thrombocytopenia. On examination his vital signs are stable. He his spleen is palpable 10 cm below the left costal margin. He has no hepatomegaly or lymphadenopathy. His past medical history is significant for hypertension controlled with lisinopril. His blood counts reveal hemoglobin of 9.6 g/dL, white blood cell count 15 × 10^9/L, absolute monocyte count 2.3 × 10^9/L, and platelet count 110 × 10^9/L. His blood smear did not have elevated blasts or promonocytes, but there were circulating metamyelocytes and myelocytes. A bone marrow biopsy was 90% cellular with megakaryocytic atypia and hyperplasia. Bone marrow blasts were estimated at 7%. The karyotype was normal, and next-generation sequencing identified

mutations involving *ASXL1*: c.1934dup; p.Gly646Trpfs*12 (20%), *TET2* c.1648C>T; p.Arg550* (41%), *SRSF2* c.284C>T; p.Pro95Leu (43%); and *NRAS*c.38G>A; p.Gly13Asp (46%) (variant allele frequency for each mutation added in parentheses).

What is the diagnosis, and how would you risk stratify this patient?

Introduction

Myelodysplastic syndrome (MDS)/myeloproliferative neoplasm (MPN) overlap syndromes are well-defined myeloid neoplasms characterized by overlapping features of MDS and MPN.[1] This uniquely classified entity consists of 4 adult-onset subtypes: chronic myelomonocytic leukemia (CMML), *BCR-ABL1*–negative atypical chronic myeloid leukemia (aCML), MDS/MPN-ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), and MDS/MPN-unclassifiable (MDS/MPN-U). There is also one pediatric subtype: juvenile myelomonocytic leukemia (JMML) (Table 1).[1] Although the classification of these neoplasms relies largely on clinical features and peripheral blood and bone marrow (BM) morphology, the incorporation of next-generation sequencing (NGS) techniques has helped in defining the molecular landscape and ability to diagnose, risk stratify, and plan appropriate treatment strategies. Among the subtypes, CMML is the most common, demonstrating marked clinical heterogeneity and an inherent tendency to transform to acute myeloid leukemia (AML).[2]

Whereas CMML and JMML are defined by the presence of clonal monocytosis, aCML presents with dysplastic neutrophilia, MDS/MPN-RS-T with anemia and thrombocytosis, and MDS/MPN-U with poorly defined overlapping features. Table 1 outlines the 2016 World Health Organization (WHO) criteria for the diagnosis of MDS/MPN overlap syndromes, including key associated genes and epidemiologic features (incidence, median age, and median overall survival [OS]).[1] Although cytogenetic abnormalities are seen in a small fraction of patients with overlap neoplasms, molecular aberrations occur in most (>90%). In this review, we highlight salient features with regard to the genetic landscape of MDS/MPN overlap neoplasms.

Molecular aberrations in MDS/MPN overlap neoplasms

To establish a diagnosis of an MDS/MPN overlap syndrome, molecularly defined neoplasms that present with similar or overlapping features must be ruled out.[1] These include *BCR-ABL1*–driven CML

Table 1. WHO diagnostic criteria, epidemiology, and gene mutations in MDS/MPN overlap neoplasms

MDS/MPN Overlap Neoplasms	WHO Diagnostic Criteria	Epidemiology	Somatic Gene Mutations (Frequency %)	
Diagnosis of MDS/MPN overlap syndromes must exclude Philadelphia chromosome *(BCR/ABL rearrangement), PDGFRA, PDGFRB, FGFR1, PCM1- JAK2* rearrangements and have PB and BM blast counts <20%				

JMML	**Clinical Features (mandatory):**
	• PB monocyte count ≥ 1 x 10^9/L
	• Splenomegaly
	Presence of 1+ Molecular Feature:
	• Somatic mutations in *PTPN11** or *KRAS** or *NRAS**
	• Clinical diagnosis of neurofibromatosis-1 or *NF1* mutation
	• Germ-line *CBL* mutation and loss of heterozygosity of CBL**
	If Molecular Feature is not present, the following criteria must be met:
	• Monosomy 7 or other chromosomal abnormality or at least 2 of the following:
	  • Myeloid or erythroid precursors on PB smear
	  • Hemoglobin F increased for age
	  • GM-CSF hypersensitivity in CFA;
	  • hyperphosphorylation of STAT5
		• Incidence: Rare; 0.67/million/yr (Cases with neurofibromatosis have 200-fold increased risk of JMML)
		• Median Age: 1.1-1.8 yrs
		• Median OS: 10-12 months; In those without HSCT = <12 months; cases that received HSCT = 5-yr OS rate is 64%, event free survival of 52%.The 5-yr cumulative incidence of relapse is 35%, while the 5-yr cumulative incidence of transplantation-related mortality is 13%
		• Rate of LT: Infrequent
			• *PTPN11** (38%)
			• *NRAS** (18%)
			• *KRAS** (14%)
			• *CBL* (12-18%)
			• *NF1* (5-10%)	

CMML	• Persistent PB monocyte count ≥ 1x10^9/L (≥3 months) • Dysplastic changes in one or more lineages, if no dysplasia then must include: • An acquired clonal cytogenetic or molecular genetic abnormality (*TET2*, *ASXL1*, *SRSF2*, and/or *SETBP1*) **Sub-types:** • CMML-0 (<2% PB blasts and <5% BM blasts) • CMML-1 (2-4% PB blasts and 5-9% BM blasts) • CMML-2 (5-19% PB blasts and 10-19% BM blasts and/or when any Auer rods are present).	• Incidence: Rare; 1 case/100000/yr • Median Age: 71-74 yrs • Median OS: CMML-1 (38 months), CMML-2 (24 months) • Rate of LT: 15-30%	• *TET2* (60%) • *SRSF2* (50%) • *ASXL1* (40%) • *NRAS* (4-16%) • *CBL* (10-22%) • *RUNX1* (15%) • *SETBP1* (15%)	• *KRAS* (7-18%) • ≤10%: *JAK2, SF3B1, U2AF1, EZH2, DNMT3A, PTPN11, ZRSR2, FLT3; NF1; IDH1/2*
MDS/MPN RS-T	• Platelet count ≥ 450 x 10^9/L • ≥ 15% ring sideroblasts in the bone marrow or > 5% with *SF3B1* mutation • Presence of megakaryocytic atypia resembling ET or MF	• Incidence: Rare; <1% of all MDS • Median Age: 71-75 yrs • Median OS: 5-7 yrs • Rate of LT: <5%	• *SF3B1* (90%) • *JAK2* (50%) • *TET2* (10%) • *DNMT3A* (13%)	• *SETBP1* (13%) • *ASXL1* (29%) • <10% of: *SRSF2, CBL*
aCML	• WBC ≥ 13x10^9/L with increased and dysplastic neutrophils • No or minimal absolute basophils and monocytosis • Hypercellular BM with granulocyte dysplasia (neutrophil precursors >10%)	• Incidence: Rare; not established • Median Age: >60 yrs • Median OS: 22 months • Rate of LT: 30-40%	• *ASXL1* (60%) • *SETBP1* (48%) • *N/KRAS* (35%) • *TET2* (30%)	• *EZH2* (13%) • <10% of: *ETNK1, CBL, FLT3, RUNX1, CEBPA*
MDS/MPN-U	• Features of MDS • Prominent myeloproliferative features, no preceding history of MPN or MDS and no recent cytotoxic or growth factor therapy • No isolated del(5q),	• Incidence: Rare; not established • Median Age: 70 yrs • Median OS: 12-28 months • Rate of LT: unknown	• *ASXL1* (50%) • *SRSF2* (37%) • *SETBP1* (21%) • *JAK2* (19%) • *N/KRAS* (15%)	• *TET2* (15%) • <10% of the following: *CBL*

WHO : World Health Organization (according to 2016 revision, *Blood 2016; 127:2391*); yr = year; JMML - Juvenile myelomonocytic leukemia; CMML = Chronic myelomonocytic leukemia; MDS/MPN-RS-T = Myelodysplastic syndrome myeloproliferative neoplasm with ring sideroblasts and thrombocytosis; aCML = Atypical chronic myeloid leukemia; MDS/MPN-U = Myelodysplastic syndrome myeloproliferative neoplasm unclassified; PB = peripheral blood; BM = bone marrow; OS = Overall survival; LT = Leukemic transformation; WBC = White blood cell count; HSCT = Hematopoietic Stem Cell Transplant

* Germ line mutations (indicative of Noonan syndrome) need to be excluded.
** Occasional cases may harbor heterozygous splice site mutations.

(especially the p190 variant), *PDGFRA*, *PDGFRB*, *FGFR1*, and *PCM1-JAK2* rearranged myeloid neoplasms.[1,3] In patients with overlap who present with monocytosis and eosinophilia, aberrations in *PDGFRA*, *PDGFRB*, *FGFR1*, and the *PCM1-JAK2* fusion should be assessed by fluorescence in situ hybridization or quantitative polymerase chain reaction studies. Of note, whereas the most common *PDGFRA* abnormality, the *FIP1L1-PDGFRA* fusion secondary to *CHIC2* deletion, is karyotypically occult, the *ETV6-PDGFRB* fusion and *FGFR1* rearrangements are regularly detected by conventional karyotyping.[4]

Chronic myelomonocytic leukemia

Gene mutations are seen in >90% of patients with CMML and most commonly involve *TET2* (60%), *SRSF2* (50%), *ASXL1* (40%), and the oncogenic RAS pathway (30%).[5-7] Additional genes mutated at lower frequencies include *SETBP1* (15%), *RUNX1* (15%), and *JAK2*V617F (10%), with *DNMT3A*, *IDH1*, *IDH2*, *STAG2*, *PHF6*, *CEBPA*, *ETNK1*, and *EZH2* occurring at < 5%.[2,8,9] Unlike in MPN and chronic neutrophilic leukemia (CNL), driver mutations in *MPL*, *CALR*, and *CSF3R* (CNL) are very infrequent, and if found they suggest an alternative diagnosis.[10] Similarly, leukemia-associated driver mutations including *NPM1* and *FLT3* are very uncommon and if present suggest AML in evolution.[11]

CMML is a disease of aging, resulting from the acquisition of clonal hematopoiesis–related mutations (*TET2*, *ASXL1*, and *SRSF2*), resulting in monocyte-biased hematopoiesis and disease progression secondary to acquisition of additional driver mutations along with cell-intrinsic and -extrinsic factors.[12] Among mutations seen in CMML, truncating (frameshift and nonsense) *ASXL1* mutations are universally deleterious, adversely affecting both OS and leukemia-free survival (LFS),[2,13,14] whereas *TET2* mutations are associated with favorable outcomes, especially in the absence of *ASXL1* mutations, with the *ASXL1*wt/*TET2*mt genotype predicting best survival rates.[10,15] In fact, this genotype is also most predictive of responses to hypomethylating agent therapy (HMA) in CMML.[10,15] Heterozygous splicing mutations (*SRSF2*, *SF3B1*, *U2AF1*, and *ZRSR2*) are common, with *SRSF2* (P95 hot spot) being most frequent, with no clear impact on survival.[16] Acquisition of oncogenic RAS pathway mutations (*NRAS*, *CBL*, *KRAS*, *PTPN11*, and *NF1*) drives a proliferative phenotype (MPN-CMML), with marked leukocytosis/monocytosis, pronounced constitutional symptoms, splenomegaly, and lower survival.[13] *RUNX1* and *SETBP1* mutations are seen in 15% of patients, with *RUNX1* mutations associated with severe thrombocytopenia, and both mutations negatively affect outcomes.[2,17] Of note, *TP53* mutations are uncommon in CMML (<1%) and if seen are usually present in the context of therapy-related CMML.[18]

Juvenile myelomonocytic leukemia

JMML is the only pediatric-onset neoplasm in this category and is considered a bona fide RASopathy, with germline and somatic mutation in the RAS/RAF/MEK/ERK pathway giving rise to disease.[19] Germline mutations associated with JMML include *NF1*, *RAS* mutations in the context of Noonan syndrome (*PTPN11, KRAS, NRAS, RIT1*), and *CBL*, with *CBL* mutant JMML often demonstrating spontaneous regression.[20,21] Somatic mutations that give rise to JMML include *PTPN11* (38%), *NRAS* (18%), *KRAS* (14%), *RRAS* and *RRAS2* (<10%). Unlike in CMML, mutations in epigenetic regulators including *ASXL1* and *SETBP1* and in signaling genes such as *JAK3* are late events and are often responsible for disease progression to AML.[19,22]

MDS/MPN-ring sideroblasts and thrombocytosis

This is a unique overlap neoplasm, most recently formally assigned to this category in the 2016 WHO classification, characterized largely by features of MDS with ring sideroblasts (MDS-RS) and concomitant thrombocytosis.[23] Unlike for other neoplasms in this category, the median OS is favorable at 5 to 7 years, with AML transformation rates of <5%.[23,24] The disease is defined by the specific presence of *SF3B1* (90%) and *JAK2*V617F (50%) mutations, and apart from BM RS it also demonstrates atypical megakaryocytes in the BM with peripheral blood thrombocytosis.[23,24] It is believed that *SF3B1*-mutant MDS-RS clonally evolves into MDS/MPN-RS-T, because of the acquisition of signaling mutations such as *JAK2*V617F. Of note, although *CALR* mutations have been documented in a small fraction of patients with JAK2/MPL wildtype MDS/MPN-RS-T, they tend to be infrequent (<5%).[24,25] Additional mutations seen in MDS/MPN-RS-T include *ASXL1* (29%), *DNMT3A* (13%), *SETBP1* (13%), and *TET2* (10%),[23,24] with the *ASXL1*wt/*SETBP1*wt genotype associated with better outcomes.[23,24]

BCR-ABL1–negative atypical CML

This is a rare overlap neoplasm characterized by dysplastic neutrophilia in the absence of monocytosis and basophilia.[1] Gene mutations encountered include *ASXL1* (28%), *TET2* (16%), *EZH2* (15%), *NRAS* (15%), *SETBP1* (12%), and *RUNX1* (12%), with *ETNK1* mutations seen in 10%.[26,27] Initial data ascribed *CSF3R* mutations to 30% of patients with aCML, but in our experience these mutations are uncommon in WHO-defined aCML and are more reflective of CNL.[28] aCML and CMML share overlapping mutational profiles largely differentiated by the frequencies of *NRAS*, *CBL*, *TET2*, *SRSF2*, and *ETNK1* mutations.[15,29]

MDS/MPN-U

This subtype consists of a conglomerate of poorly defined MDS/MPN overlap syndromes, not meeting criteria for other well-defined entities in this group[1]. Frequencies of gene mutations encountered include *ASXL1* (30% to 50%), *SRSF2* (23% to 37%), *SETBP1* (11% to 21%), *JAK2* (19% to 25%), *NRAS* (10% to 15%), and *TET2* (15% to 27%).[30,31] Less frequent occurrences of *TP53* and *CBL* mutations have also been documented, with a negative impact on survival.[30] Although MDS/MPN-U does not have a specific prognostic scoring system, 2 studies have shown that MDS-centered prognostic models such as the international prognostic scoring system can be used to risk stratify affected patients.[30,31]

Functional categories of mutated genes encountered in MDS/MPN overlap neoplasms

These categories are listed in Table 2 and illustrated in Figure 1.

Epigenetic regulator genes

Key altered epigenetic regulator genes include *TET2*, *ASXL1*, *EZH2*, *DNMT3A*, *IDH1*, and *IDH2*. *TET2* is a critical dioxygenase that helps convert 5-methylcytosine to 5-hydroxymethylcytosine and other oxidative metabolites, which regulate the state of DNA accessibility (methylation).[10] *TET2* is mutated in 60% of patients with CMML, and in the absence of *ASXL1* mutations it has a favorable prognostic impact. *ASXL1* regulates chromatin dynamics through its interaction with the polycomb group repressive

Table 2. Gene mutations and cytogenetic abnormalities seen in MDS/MPN overlap neoplasms

		Gene	Chr Position	JMML	CMML	aCML	MDS/MPN RS-T	MDS/MPN U	Mutation Info
GENE SPECIFIC ALTERATIONS	SIGNALING	*PTPN11*	12q24.13	38%	5%[a]	4%			• Somatic mutations usually involve exons 3, 4, and 13, but can be distributed throughout • In JMML, most common somatic mutations include codons: E76, A72, Q79, and D61
		NRAS	1p13.2	18%	4-16%[a]	16%		15%	• Hot spots include point mutations at codons: G12, G13, and Q61
		KRAS	12p12.1	14%	7-18%[a]				• Hot Spots include point mutations at codons: G12, G13, and Q61
		CBL	11q23.3	12-18%	10-22%[a]	0-10%	<4%[a]	>10%	• Mutations typically occur in the linker and RING finger domains (intron 7, exons 8 and 9) • In JMML, germline HET mutations lead to a Noonan syndrome-like phenotype, somatic mutations include LOH due to UPD with common change: Y732H • In CMML, can be associated with high allelic burden
		NF1	17q11.2	5-10%	1-7%[a]				• Mutations found throughout gene and consist of missense, frameshift, and nonsense • Hot spot: T676 (seen in AML) • In JMML, germline NF1 mutation with loss of second allele due to UPD; in some cases compound heterozygosity / somatic interstitial deletions have been seen
		JAK2	9p24.1		10%	4-8%	50%	19%	• *JAK2*V617F is most common and presence associated with higher hemoglobin/plt counts
		FLT3	13q12.2		<5%	<5%			• Internal tandem duplications in ITD (juxtamembrane region) /TKD mutations (activation loop)
	SPLICING	*SF3B1*	2q33.1		5-10%	8%	90%		• Hot spots include codon K700, and less frequent codons K666, H662, E622, and R625
		SRSF2	17q25.2		50%	12-40%		37%	• Hot spot is codon P95; small in/del are also reported involving this region
		U2AF1	21q22.3		5-15%	0-20%			• Hot spots include codons S34 and Q157
		ZRSR2	Xp22.2		8-10%	4%			• Somatic mutations occur throughout gene
	EPIGENETIC	*ASXL1*	20q11.21	rare	40%[a,b]	28%[b]	29%[a,b]	50%[a,b]	• Heterozygous nonsense/frameshift mutations most often along or upstream of exon 12, resulting in C-terminal truncation of the protein upstream of the PHD finger • Most frequent mutation is G646Wfs12
		TET2	4q24		60%[b]	16%[a,b]	10%[b]	15%[b]	• Somatic mutations occur throughout gene • Favorable outcome especially in ASXL1wt cases • In CMML, ASXL1wt/TET2mut genotype most responsive to HMA
		IDH1/2	IDH1: 2q34 IDH2: 15q26.1		<5%	<4%		5-10%	• Mutation hot spots include: (*IDH1*) R132, and (*IDH2*) R140 and R172 • Targeted therapy available
		EZH2	7q36.1		<5%	8-20%		10%	• Somatic mutations occur throughout gene
		DNMT3A	2p23.3		<5%[a,b]	rare[b]	13%[b]	4%[b]	• Mutation hot spot includes R882 • Other mutations can occur throughout gene
	TRAN.	*RUNX1*	21q22.12		15%[a]	12%		14%	• Mutations located throughout gene • Familial conditions present with germline mutations
	OTHER	*SETBP1*	18q12.3	rare	15%[a]	12%	13%[a]	21%	• Mutations frequently are located in SKI homologous region, codons: E858-I871 • Other mutations can occur throughout gene • In CMML, strongly overlap *ASXL1* mutations • Presence usually mutually exclusive with *JAK2* and *TET2* • In MDS/MPN-RS-T, *ASXL1wt/SETBP1wt* genotype associated with better survival
		ETNK1	12p12.1		<5%	10%		rare	• Hotspots are in kinase domain codons: H243-G245
		STAG2	Xq25		<5%	<4%			• Mutations can be located throughout gene • Splice site mutations are frequent

	JMML	CMML	aCML	MDS/MPN RS-T	MDS/MPN U
Abnormal karyotype	30%	~30%	30-40%	20%	50%
CHROMOSOMAL ALTERATIONS:	• Monosomy 7 (clusters with *KRAS*-mutant cases) • Deletion 7q	• -Y • Monosomy 7 and del7q • Trisomy 8 (+8) or +21 • Interstitial deletions of chr 20q, 11q, and 12p • 50% of cases may have large areas of UPD • Complex karyotypes	• Trisomy 8 • Monosomy7 • Deletion 12p • Less common are: idic(Xq), del(5q), t(6;8)(p23;q22), -9, del(11q), del(12q), del(15q), del(17p), t(17;20), and add(21q)	• Trisomy 8 • Less common are: complex/monosomal, del(5q31), del(7q), del(20q), inv(3)(q21q26.2)	• Trisomy 8 • Complex karyotype (15%) • Monosomy 7 (10%)

Key: [a] = Gene alterations which have been shown to have independent adverse prognostic impact on survival outcomes; [b] = Genes common for clonal hematopoiesis of indeterminate potential (CHIP)/age-related clonal hematopoiesis (ARCH); GOF = Gain of function mutation; CMML = ; JMML = ; aCML = ; MDS/MPN-RS-T = ; MDS/MPN-U = ; Chr = Chromsome; RING = Really interesting new gene domain; HET = heterozygous; LOH = loss of heterozygosity; UPD = Uniparental disomy; ITD = Internal tandem duplication domain; TKD = Tyrosine kinase domain; PHD = Pleckstrin homology domain; del = deletion; Diseases with nothing n the frequency column indicate that mutations are not typically seen, and they may be rare or acquired as disease progresses. TRAN. = Transcription Factor

complex proteins (PRC1 and PRC2).[32] It is believed that *ASXL1* mutations result in loss of PRC2-mediated H3K27 (histone 3 lysine 27) tri-methylation.[33] In addition, recent data suggest that *ASXL1* truncations confer enhanced activity on the ASXL1-BAP1 (BRCA associated protein 1) complex. Both of these pathways result in a global erasure of H2AK119Ub and depletion of H327Kme3, promoting dysregulated transcription.[34] EZH2 is a key catalytic component of PRC2, and loss-of-function mutations result in

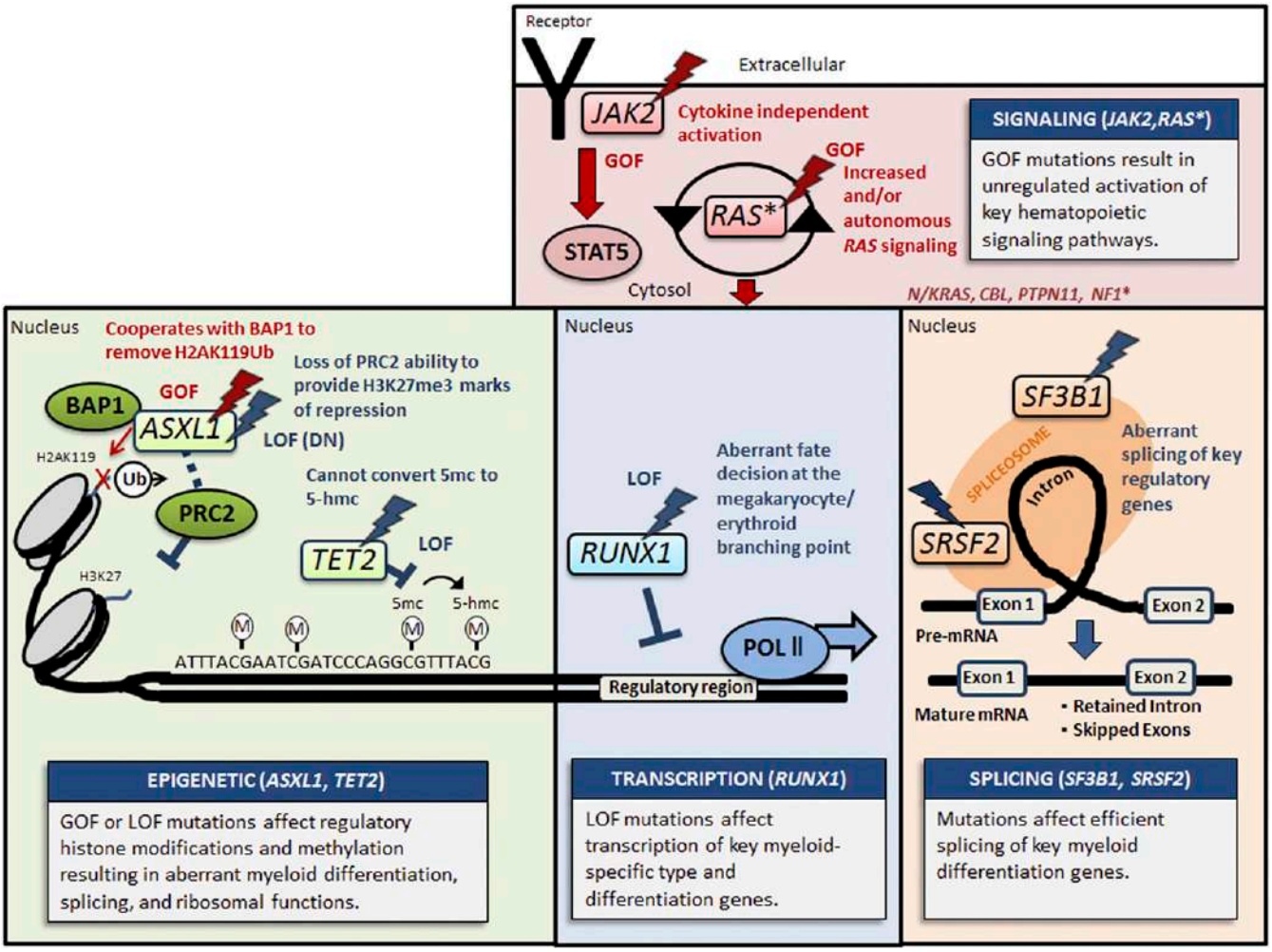

Figure 1. Mechanisms of key mutations in gene categories represented in MDS/MPN overlap syndromes. This figure illustrates the top represented mutated genes in each of four categories: signaling (pink), epigenetic (green), transcription (blue), and splicing (orange). Key mutated genes in each panel are highlighted by lightning, and the color red corresponds to gain-of-function (GOF) mutations, whereas the color blue denotes a loss-of-function (LOF) or dominant negative (DN) mutant effect. Other RAS pathway genes* include *NRAS*, *KRAS*, *CBL*, *PTPN11*, and *NF1*.

dysregulated chromatin dynamics. *EZH2* mutations are seen in aCML (15%) but are uncommon in CMML (<5%), where they often co-occur with *ASXL1* mutations and are associated with poor outcomes.[9,35] *DNMT3A* encodes for the DNA methyltransferase responsible for the conversion of cytosine to 5-methylcytosine. *DNMT3A* mutations are seen in <5% of patients with CMML and are associated with poor outcomes.[8] *IDH1* and *IDH2* are key components of oxidative phosphorylation, with mutant *IDH1/2* generating the oncometabolite 2-hydroxyglutarate (2-HG). 2-HG in turn suppresses *TET2* activity, mimicking a *TET2* mutant effect on methylation, with *IDH1/2* mutations being infrequent (<5%).[5] It is believed that because of the convergence of pathways (2-HG–mediated suppression of TET activity), *TET2* and *IDH1/2* mutations are largely mutually exclusive.

Splicing mutations

Spliceosome components are critical regulators of pre-mRNA splicing, with gene mutations involving *SRSF2*, *SF3B1*, *U2AF1*, and *ZRSR2* implicated in myeloid oncogenesis.[16] In CMML, *SRSF2* mutations are seen in 50% of patients, with no clear impact on survival, whereas *SF3B1* mutations are less common and phenotypically associated with BM RS.[16,36] *SF3B1* mutations are seen in 90% of patients with MDS/MPN-RS-T, often co-occurring with *JAK2*V617F.[24]

Signaling mutations

Aberrant signaling in overlap neoplasms involves mainly the oncogenic RAS pathway and is secondary to mutations involving *NRAS*, *KRAS*, *CBL*, *PTPN11*, and *NF1*.[37] In MPN-CMML, these mutations can be early clonal/dominant events and are associated with poor outcomes,[5] whereas they occur later and impose transformation risk in MDS-CMML. *JAK2* is the other common signaling mutation seen, with *JAK2*V617F identified in 50% of patients with MDS/MPN-RS-T and in 10% of patients with CMML.[38] *CSF3R*, *MPL*, *CALR*, and *FLT3* mutation are uncommon (<5%). Signaling mutations in MDS/MPN overlap neoplasms are associated with cytokine deregulation and inflammation. Mutations involving the JAK/STAT pathway (*JAK2/CALR/MPL*) and the oncogenic RAS pathway (*NRAS*, *KRAS*, *CBL*, *PTPN11*, and *NF1*) result in complex ligand-independent deregulation in cytokine

production and secretion. Cytokines significantly elevated in patients with CMML and signaling mutations include IL-10, CCL2/MCP-1, CD44, IL-1RA, and CXCL7, whereas lower IL-6 levels have been seen in *TET2*-mutant CMML.[39] Although granulocyte–macrophage colony-stimulating factor (GM-CSF) levels were not statistically different between patients with CMML and controls, GM-CSF hypersensitivity has been well documented in both JMML and RAS pathway mutant CMML patient samples.[40]

Transcription factors

RUNX1, a critical transcription factor gene, can be mutated in CMML (15%), MDS/MPN-U (14%), and aCML (12%).[17,30] *RUNX1* mutations are associated with lower platelet counts and a shortened LFS.[17] These mutations should be curated manually to ensure that they are not germline, given that RUNXI-FPD (familial platelet disorder) is associated with an inherent risk for myeloid neoplasms.[17] This is particularly relevant when *RUNX1* mutation variant allele frequencies are in the heterozygous range (40% to 60%). Based on a family history of thrombocytopenia and myeloid neoplasms, personal history of antecedent thrombocytopenia, and the clinical scenario (eg, choosing matched related donors), germline tissue (skin biopsy–derived fibroblast or hair follicle–derived DNA) assessments should be considered. In addition, RUNX1-FPD can result from gene deletions, which are often missed by amplicon based-NGS assays.[17] In these circumstances, copy number analysis can be carried out with array comparative genome hybridization assays.

Others

SETBP1 mutations are found in 15% of patients with CMML and aCML and are associated with inferior outcomes.[2,41] Various oncogenic mechanisms have been proposed, including binding to the SET region and interfering with methylation of lysine residues on histone tails. *TP53* is a critical tumor suppressor gene, and mutations are infrequent in MDS/MPN overlap syndromes. *STAG2* and *RAD21* are components of the cohesion complex, with mutations seen in <10% of patients, with no clear impact on outcomes.

Cytogenetic abnormalities in MDS/MPN overlap neoplasms

Clonal cytogenetic abnormalities are seen in ~30% of patients with CMML, with common alterations including trisomy 8 (+8), −Y, abnormalities of chromosome 7 (monosomy 7 and del7q), trisomy 21, and complex karyotypes.[42-44] The CMML-specific cytogenetic risk stratification (CPSS) system categorizes patients in three groups: high risk (+ 8, chromosome 7 abnormalities, or complex karyotype), intermediate risk (all except for those in the high- and low-risk categories), and low risk (normal karyotype or −Y), with 5-year OS of 4%, 26%, or 35%, respectively.[43] The Mayo–French cytogenetic risk stratification system was developed to refine this prognostication and has three distinct risk categories: high (complex and monosomal karyotypes), intermediate (all abnormalities not in the high- or low-risk groups), and low (normal, sole −Y, and sole der(3q)), with median survivals of 3 (hazard ratio, 8.1; 95% confidence interval, 4.6-14.2), 21 (hazard ratio, 1.7; 95% confidence interval, 1.2-2.3) and 41 months, respectively.[45]

Cytogenetic abnormalities in JMML are uncommon; monosomy 7 is the most common, with this abnormality clustering with *KRAS*-mutant JMML.[21] Although cytogenetic abnormalities are uncommon in MDS/MPN-RS-T (80% with normal karyotype), approximately 50% of patients with MDS/MPN-U have cytogenetic aberrations (+8 and complex karyotypes, 15% each, and monosomy 7, 10%).[23,30] Cytogenetic changes are seen in approximately 30% to 40% of patients with aCML, with +8 being most common.[26]

Integration of molecular and cytogenetic abnormalities for diagnosis, prognostication, and therapeutics of MDS/MPN overlap syndromes

Diagnosis

Although none of the aforementioned gene mutations or cytogenetic abnormalities are specific to a single MDS/MPN subtype, molecular signatures can be used in combination with clinical and morphological features to help establish a diagnosis (Figure 2). Data from clonal hematopoiesis and clonal architectural studies in CMML have shown that coexpression of *TET2* and *SRSF2* mutations result in clonal monocytosis, with the acquisition of subsequent driver mutations defining dysplastic (*RUNX1*, *SETBP1*, *DNTM3A*, *ASXL1*) or proliferative (*NRAS*, *KRAS*, *CBL*, *PTPN11*, *JAK2*) CMML subtypes.[46,47] Based on their frequency and co-occurrence, the presence of *ASXL1*, *TET2*, and *SRSF2* mutations in the presence of adult-onset sustained monocytosis (>3 months) can be used to establish a diagnosis of CMML.[1] As mentioned before, *MPL* and *CALR* mutations are uncommon in CMML, and their presence points toward a differential diagnosis of MPN with monocytosis.[38] In MDS/MPN-RS-T, there is acquisition of driver signaling mutations, most commonly *JAK2*V617F in the context of antecedent *SF3B1* mutant MDS-RS, giving rise to anemia and thrombocytosis.[48] *SF3B1* mutations correlate strongly with the presence of BM ring sideroblasts, and the presence of *JAK2/SF3B1* mutations with BM RS and thrombocytosis can be used to establish a diagnosis of MDS/MPN-RS-T.[36] The presence of germline or somatic RAS pathway mutations, in the context of early-onset monocytosis (infants and children), can be used to establish a diagnosis of JMML, whereas subsequent clonal hematopoiesis (*SETBP1*, *ASXL1*, and *JAK3*) is usually a marker of disease progression. Although aCML and MDS/MPN-U do not have classic molecular features, the relative enrichment of *SETBP1* and *ETNK1* mutations in aCML can be helpful in the presence of dysplastic neutrophilia.

Prognosis

Gene mutations have prognostic value in MDS/MPN overlap neoplasms. *ASXL1* mutations are universally detrimental across myeloid neoplasms and have a particularly poor outlook in CMML.[2,13,14] In CMML, these mutations have been incorporated into three molecularly integrated prognostic models: Mayo Molecular Model, CPSS-molecular, and the Groupe Francophone des Myelodysplasies model.[2,13,14] All three models effectively integrate clinical and molecular features and help risk stratify patients with regard to OS and LFS (Table 3). In addition to *ASXL1* mutations, the CPSS-molecular model includes *NRAS*, *RUNX1*, and *SETBP1* mutations and also incorporates clonal cytogenetic abnormalities (genetic score).[13] In JMML, the presence of germline mutations in *CBL* and *PTPN11* can be associated with spontaneous regressions, and the secondary acquisition of *SETBP1* and *JAK3* mutations is associated with disease progression and inferior OS.[19,22] In fact, in JMML, knowledge on specific nucleotide changes is informative, with somatic *NRAS* and *KRAS*G12S mutations being associated with better outcomes

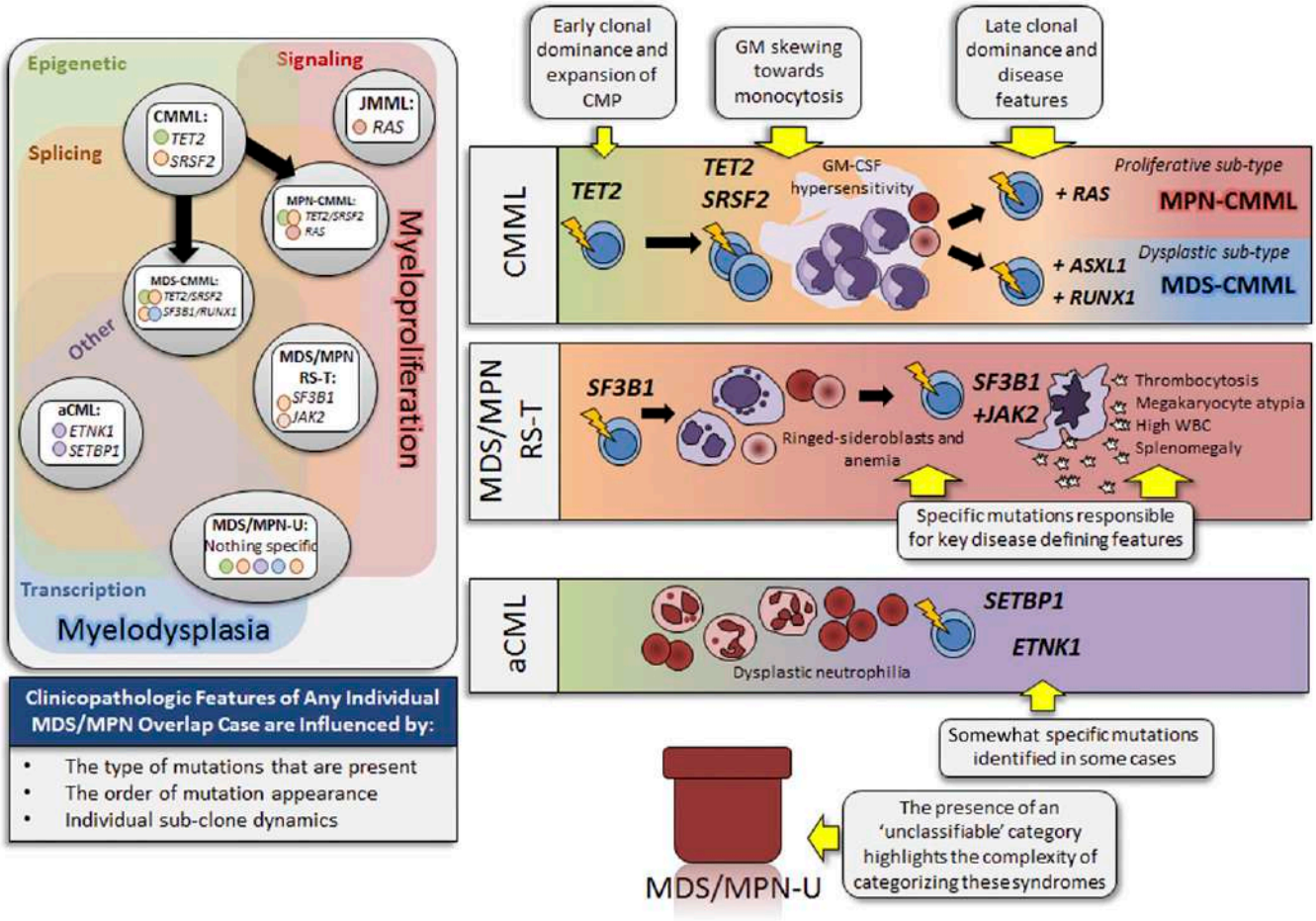

Figure 2. Clonal architecture and molecular signatures of MDS/MPN overlap syndromes. The panel on the left illustrates all 5 MDS/MPN overlap syndrome entities with corresponding specific mutational signatures. CMML has additional subcategories based on the relative enrichment of mutation types in proliferative (MPN-CMML) or dysplastic (MDS-CMML) CMML. Each entity is spatially placed according to mutation type in relation to myeloproliferative (on the right) and myelodysplastic (on bottom) features. The five mutated gene categories are represented in the left panel: epigenetic (green), signaling (pink), splicing (orange), other (purple), and transcription (blue). The panels on the right depict the influence of mutations on each MDS/MPN overlap subtype. aCML, atypical chronic myeloid leukemia; CMML, chronic myelomonocytic leukemia; CMP, common myeloid progenitor; GM, granulocytic-monocytic; JMML, juvenile myelomonocytic leukemia; MDS/MPN-RS-T, MDS/MPN-ring sideroblasts and thrombocytosis; MDS/MPN-U, MDS/MPN-unclassifiable.

than the typical G12D mutations.[49] In MDS/MPN-U we recently demonstrated the negative prognostic impact of *TP53* and *CBL* mutations, and the *ASXL1*mt/*SETBP1*mt genotype is associated with adverse outcomes in aCML.[26,30] Gene mutations are also predictive of allogenic hematopoietic cell transplantation (HCT) outcomes. In a molecularly annotated cohort of 52 CMML patients who underwent HCT, *NRAS* mutations were associated with higher relapse rates, whereas *ATRX* and *WT1* mutations were associated with relapse and an inferior OS.[50] This study also showed that higher mutational burdens (≥10) and mutations involving ≥4 epigenetic regulator genes were associated with poor outcomes.[50]

Clinical therapeutics

Currently, allogenic HCT remains the only curative option for higher-risk MDS/MPN overlap neoplasms, with HMA being used for HCT-ineligible patients. Although HMA epigenetically restores hematopoiesis in a subset of patients with CMML (30% to 40%), serial monitoring of somatic mutations has shown that they do not affect mutational allele burdens, with disease progression occurring in most.[51] Gene mutations that serve as therapeutic targets in myeloid neoplasms are uncommon in MDS/MPN overlap neoplasms. Effective targets such as mutations involving *IDH1*, *IDH2*, and *FLT3* are seen in <10% of patients,[8] and emerging targets such as *TP53* are even more uncommon (<5%). Given the ubiquitous nature of splicing mutations in these diseases, spliceosome component inhibitors in clinical trials are being eagerly watched. MEK inhibition in RAS mutant subtypes has not proven to be an effective strategy.[52] In CMML, the presence of the *ASXL1*wt/*TET2*mt genotype is best associated with responses to HMA,[10,53] whereas clonal RAS pathway mutations (MPN-CMML) are associated with resistance. Gene mutations affecting prognosis (*ASXL1*, *NRAS*, *RUNX1*, and *SETBP1*) in CMML also help with important decisions with regard to timing and the need for allogenic HCT.

Table 3. Genetically integrated prognostic models in MDS/MPN overlap neoplasms

CMML

Model		Risk Categories	Survival (m)	Risk Factors
CMML-Specific Cytogenetic Risk Stratification (CPSS)		Low risk	35% 5-yr OS	• Normal or isolated loss of Y
		Intermediate risk	26% 5-yr OS	• All others
		High risk	4% 5-yr OS	• Trisomy 8, chr 7 abnormalities, or complex karyotype
Mayo-French Cytogenetic Risk Stratification System		Low risk	41	• Normal, sole -Y, and sole der(3q)
		Intermediate risk	21	• All abnormalities not in low or high categories
		High risk	3	• Complex and monosomal karyotypes
Mayo Molecular Model (MMM)	MMM	Low risk (0 pts)	97	• AMC > $10x10^9$/L (2 pts)
		Intermediate-1 risk (≥ 2 pts)	59	• Presence of circulating IMCs (2 pts)
		Intermediate-2 risk (2.5-4.5 pts)	31	• Hemoglobin level < 10g/dL (2 pts)
		High risk (≥ 5 pts)	16	• *ASXL1* mutation (1.5 points) • Platelet count < $100x10^9$/L (1.5 pts)
Groupe Francophone de Myelodysplasies (GFM)	GFM	Low risk (0-4 pts)	65	• WBC > $15x10^9$/L (3 pts)
		Intermediate risk (5-7 pts)	28	• *ASXL1* mutations (2 pts) • Age > 65 years (2 pts)
		High risk (8-12 pts)	17	• Platelet count < $100x10^9$/L (2 pts) • Hemoglobin <10g/dL in females and <11g/dL in males (2 pts)

CMML-Specific Prognostic Scoring System (CPSS-Mol)

Genetic Risk* Score for CPSS

Points for Mutation Status	Unmut	Mut
ASXL1	0	1
NRAS	0	1
RUNX1	0	2
SETBP1	0	1

+

Points for Karyotype Status based on CPSS	Pts
Normal or -Y	0
Anything between	1
Trisomy 8, Monosomal, Complex	2

=

Genetic Risk* for CPSS mol Model	Pts
Low	0
Int-1	1
Int-2	2
High	≥3

CPSS-Mol

Risk Categories	Rate of AML	Survival (m)	CPSS-Mol Score	0 pts	1 pt	2 pts	3 pts
Low (0 pts)	0%	Not reached	• WHO Subtype:	CMML-1	CMML-2		
Intermediate-1 (1 pt)	8%	64	• FAB Sub-type:	MDS-CMML	MPN-CMML		
Intermediate-2 (2-3 pts)	24%	37	•Genetic Risk*:	Low	Inter-1	Inter-2	High
High (≥ 4pts)	52%	18	• RBC transfusion dependence:	No	Yes		

aCML

Model	Risk Categories	Survival (m)	Risk Factors
Mayo Prognostic Model for aCML	Low risk, (0-1 risk factors)	~18	• Age > 67 years • Hemoglobin < 10 g/dL • Presence of *TET2* mutations
	High risk, (≥ 2 risk factors)	~7	

MDS/MPN-RS-T

Model	Risk Categories	Survival (m)	Risk Factors
Mayo Prognostic Model for MDS/MPN-RS-T	Low risk, (0 pts)	80	• Abnormal karyotype (2 pts)
	Intermediate risk (1 pt)	42	• *ASXL1* or *SETBP1* (1 pt each)
	High risk, (≥ 2 pts)	11	• Hemoglobin < 10 g/dL (1 pt)

Key: Pts = Points; CMML = Chronic myelomonocytic leukemia; m = Month; Y = y-chromosome; der = Derivative chromosome; OS = Overall survival; AMC = Absolute monocyte count; IMCs = Immature myeloid cells; WHO = World Health Organization; WBC = White blood cell count; RBC = Red blood cells; CMML-MDS = Dysplastic CMML; CMML-MPN = Proliferative CMML; FAB = French-American-British; *Corresponds to CMML-Specific Cytogenetic Risk Stratification; Inter = Intermediate; depend. = Dependent; Rate of AML = Rate of Transformation (cumulative at 48 months)

Our patient is a 71-year-old man who presented with constitutional symptoms, splenomegaly, anemia, leukocytosis, monocytosis, and thrombocytopenia (Figure 3). His BM has features of dysplasia, and NGS testing has identified mutations involving *ASXL1*, *TET2*, *SRSF2*, and *NRAS*. These features suggest a diagnosis of CMML-1. According to the CPSS-molecular model, he fits into the intermediate-2 risk category, with an estimated median OS of 18 months and a 48% cumulative incidence of AML at 48 months.[13] According to the Mayo Molecular Model, he fits into the high-risk category, with a median OS of 16 months.[2] This patient will benefit from an allogenic transplant consult and will probably need pretransplant cytoreductive therapy with HMA.

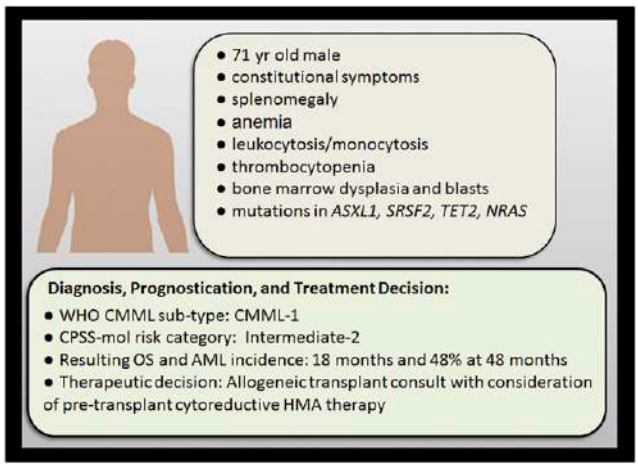

Figure 3. MDS/MPN overlap case study. Shown is the current clinical vignette with symptoms, laboratory results, diagnosis, and resulting prognostication. AML, acute myeloid leukemia; HMA, hypomethylating agent; OS, overall survival.

Conclusions

MDS/MPN overlap neoplasms are a well-defined group of myeloid neoplasms with unique molecular signatures. Mutations in *ASXL1*, *TET2*, and *SRSF2* are common in CMML, whereas the *SF3B1*/*JAK2V617F* genotype often defines the pathobiology of MDS/MPN-RS-T. JMML is a RAS-driven disease, with germline and somatic mutations in the RAS pathway accounting for most cases. aCML is enriched in *SETBP1* and *ETNK1* mutations, and MDS/MPN-U is the least defined in this group. Understanding the molecular landscape in overlap neoplasms is important, because it helps with establishing a diagnosis, helps with disease prognostication, and in certain cases allows selection of appropriate treatment strategies.

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Conflict-of-interest disclosure

M.M.P. has served on the advisory boards for Kura Oncology and Stemline Therapeutics. T.L.L. has no competing interests to declare.

Off-label drug use

None disclosed.

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EVIDENCE-BASED MINIREVIEW

Myelodysplastic syndrome/myeloproliferative neoplasm overlap syndromes: a focused review

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Myelodysplastic syndrome (MDS)/myeloproliferative neoplasm (MPN) overlap syndromes are unique myeloid neoplasms, with overlapping features of MDS and MPN. They consist of four adult onset entities including chronic myelomonocytic leukemia (CMML), MDS/MPN-ring sideroblasts-thrombocytosis (MDS/MPN-RS-T), *BCR-ABL1* negative atypical chronic myeloid leukemia (aCML) and MDS/MPN-unclassifiable (MDS/MPN-U); with juvenile myelomonocytic leukemia (JMML) being the only pediatric onset entity. Among these overlap neoplasms, CMML is the most frequent and is hallmarked by the presence of sustained peripheral blood monocytosis with recurrent mutations involving *TET2* (60%), *SRSF2* (50%) and *ASXL1* (40%); with RAS pathway mutations and *JAK2*V617F being relatively enriched in proliferative CMML subtypes (WBC ≥13 × 10^9/L). CMML usually presents in the 7th decade of life, with a male preponderance and is associated with a median overall survival of <36 months. Adverse prognosticators in CMML include increasing age, high WBC, presence of circulating immature myeloid cells, anemia, thrombocytopenia and truncating *ASXL1* mutations. While allogeneic stem cell transplantation remains the only curative option, given the late onset of this neoplasm and high frequency of comorbidities, most patients remain ineligible. Hypomethylating agents such as azacitidine, decitabine and oral decitabine/cedazuridine have been US FDA approved for the management of CMML, with overall response rates of 40-50% and complete remission rates of <20%. While these agents epigenetically restore hematopoiesis in a subset of responding patients, they do not impact mutational allele burdens and eventual disease progression to AML remains inevitable. Newer treatment modalities exploiting epigenetic, signaling and splicing abnormalities commonly seen in CMML are much needed.

LEARNING OBJECTIVES

- Understand that chronic myelomonocytic leukemia (CMML) is an overlap syndrome that has features of myelodysplastic syndromes (anemia, thrombocytopenia, bone marrow dysplasia) and myeloproliferative neoplasms (leukocytosis, monocytosis, splenomegaly, and constitutional symptoms)
- Understand that, although hypomethylating agents are able to epigenetically restore hematopoiesis in a subset of CMML patients, they fail to significantly alter mutational allele burdens and clonal transformation to acute myeloid leukemia

Clinical case

A 66-year-old male presents with progressive fatigue, unintentional weight loss, early satiety, and drenching night sweats. His complete blood count demonstrates a white blood cell (WBC) count of 44 × 10^9/L, 25% monocytes, circulating myelocytes/metamyelocytes, hemoglobin of 8.5 g/dL, and a platelet count of 110 × 10^9/L. On examination, he is found to have massive splenomegaly. A bone marrow (BM) biopsy was noted to be hypercellular (90%) and demonstrates megakaryocytic atypia, without megakaryocyte clusters and 10% BM blasts. The cytogenetics are normal (46, XY), *BCR-ABL1* polymerase chain reaction testing negative, and molecular genetics identify mutations involving *ASXL1, TET2, SRSF2*, and *NRAS*. He is diagnosed as having chronic myelomonocytic leukemia-2 (CMML-2). His only associated comorbidity is hypertension, which is well controlled on lisinopril. His echocardiogram reveals a normal ejection fraction, and his hepatic, renal, and pulmonary function assessments are within normal limits. For this patient, would frontline therapy with hypomethylating agents (HMAs) or allogeneic stem cell transplantation (HCT) be preferred?

Introduction

Myelodysplastic syndrome (MDS)/myeloproliferative neoplasm (MPN) overlap syndromes are unique myeloid neoplasms

Table 1. 2016 WHO diagnostic criteria for MDS/MPN overlap neoplasms

MPN/ MDS overlap subtypes	2016, WHO diagnostic criteria*	Frequency of somatic gene mutations	Median age at Dx, y	Median OS	Rate of LT
aCML	WBC count > 13 × l09/L with increased and dysplastic neutrophils. No or minimal absolute basophils and monocytosis. Hypercellular BM with granulocytic proliferation and dysplasia (neutrophil precursors greater than equal to 10%).	*ASXL1* (60%), *SETBP1* (48%), *N/KRAS* (35%), *TET2* (30%), *EZH2* (13%) and <10% for the following: *CSF3R, ETNK1, CBL, FLT3, RUNX1, CEBPA, IDH1/2*	>60	22 mo	30-40%
CMML	Persistent PB monocytosis ≥ 1 × 10⁹/L. Dysplasia in ≥1 lineage, if no dysplasia, then must include an acquired clonal cytogenetic or molecular genetic abnormality (*TET2*, *ASXL1*, *SRSF2*, and/or *SETBP1*).	*TET2* (60%), *SRSF2* (50%), *ASXL1* (40%), *NRAS* (15%), *CBL* (15%), *RUNX1* (15%), *SETBP1* (15%), *KRAS* (10%), *IDH1/2* (5-10%), and <10% for the following: *JAK2, SF3B1, U2AF1, EZH2, DNMT3A, PTPN11, ZRSR2, FLT3*	71-74	28-32 mo	15-30%
MDS/ MPN-RS-T	Platelet count ≥ 450 × 10⁹/L. 15% ring sideroblasts in the BM or >5% with *SF3B1* mutation. Presence of megakaryocytic atypia resembling ET or MF.	*SF3B1* (93%), *JAK2* (57%), *TET2* (25%), *DNMT3A* (15%), *ASXL1* (15%), and <10% for the following: *SRSF2*, *CBL*, *SETBP1*, *IDHl/2*	71-75	76 mo	1-2%
MDS/ MPN-U	Myeloid neoplasm with mixed MDS and MPN features, not meeting WHO criteria for other MDS/MPN overlap neoplasms, MDS or MPN.	*TET2* (30%), *RUNX1* (14%), *CBL* (11%), *EZH2* (10%), *N/KRAS* (10%), *SETBP1* (10%), and <10% for the following: *DNMT3A*, *CEBPA*, *IDH1/2*	70	12-28 mo	Unknown
JMML	PB monocyte count ≥ 1 × 109/L. Splenomegaly. Genetic features (must include 1 of the following): somatic mutation in *PTPN11*,† *KRAS*,† or *NRAS*†; diagnosis of neurofibroatosis-1 or *NF1* mutation; or germline *CBL* mutation and loss of heterozygosity of CBL. If no genetic features then, must have a clonal chromosomal abnormality or all of the following: GM-CSF hypersensitivity, hyperphosphorylation of STAT5, fetal hemoglobin increased for age, myeloid or erythroid precursors on PB smear	*PTPN11*† (38%), *NRAS*† (18%), *KRAS*† (14%), *CBL* (12-18%), *NF1* (5-10%)	1.4-2	10-12 mo	Infrequent

Dx, diagnosis; ET, essential thrombocythemia; GM-CSF, granulocyte-macrophage colony-stimulating factor; LT, Leukemic transformation; MF, myelofibrosis; OS, overall survival; STAT5- signal tansducer and activator of transcription 5.
*All MDS/MPN overlap subtypes are negative for BCR-ABL1 fusions, or rearrangements involving PDGFRA, PDGFRB, FGFR1 and PCM1-JAK2 and have <20% blasts in the PB and BM.[1]
†Germline mutations (indicative of Noonan syndrome) need to be excluded.

with overlapping features of MDS and MPN. According to the 2016 World Health Organization (WHO) classification, MDS/MPN overlap neoplasms consist of 4 adult-onset entities, including CMML, *BCR-ABL1*⁻ atypical chronic myeloid leukemia (aCML), MDS/MPN-ring sideroblasts and thrombocytosis (MDS/MPN-RS-T), and MDS/MPN-unclassifiable (MDS/MPN-U), along with 1 pediatric entity, juvenile myelomonocytic leukemia (JMML) (Table 1).[1] Among these, CMML is the most common (crude and age-standardized incidence ratios per 100 000 people in the United States are as follows: CMML, 0.6 (0.57-0.63); aCML, 0.06 (0.04-0.62); MDS/MPN-U, 0.07 (0.006-0.009); MDS/MPN-RS-T < 1% of new MDS cases; and JMML, 1.2 per 1 000 000 people)[2] and is a clonal stem cell disorder that is characterized by sustained peripheral blood (PB) monocytosis (≥1 × 10⁹/L and ≥10% of WBC differential) and an inherent tendency for transformation to acute myeloid leukemia (AML; 15-20% over 3-5 years).[3] The median age at diagnosis for CMML is 73 years, with a male preponderance.[3] Histologically, CMML can be classified as CMML-0 (<2% PB blasts and <5% bone marrow [BM] blasts), CMML-1 (2-4% PB blasts and/or 5-9% BM blasts), and CMML-2 (5-19% PB blasts and/or 10-19% BM blasts or when Auer rods are present). Clinically, and based on the presenting WBC count, CMML can be classified as MPN-CMML (WBC count ≥ 13 × 10⁹/L) or MDS-CMML (WBC count < 13 × 10⁹/L); the former has poor outcomes and higher rates of AML transformation.[1,3]

Diagnosis

CMML is diagnosed based on the presence of sustained PB monocytosis (>3 months and in the absence of reactive etiologies), the absence of molecular aberrations that can be associated with monocytosis (*PDGFRA*, *PDGFRB*, *PCM1-JAK2*, and *BCR-ABL1*), and <20% PB and BM blasts, with or without BM dysplasia.[1,3] In the absence of BM dysplasia, WHO criteria allow ascertainment of clonality by demonstration of clonal cytogenetic abnormalities or gene mutations involving *TET2*, *ASXL1*, *SRSF2*, and *SETBP1*.[1,3] Recently, PB flow cytometry demonstrating monocyte subset repartitioning has become a useful adjunct in CMML diagnosis, with most CMML patients demonstrating an expansion (>94%) of classical monocytes (MO1⁻CD14⁺/CD16⁻). This modality has also shown promise in distinguishing CMML from other causes of monocytosis, including

Table 2. Molecularly integrated CMML prognostic Scoring Systems

Mayo Molecular Model (MMM)			Groupe Francophone de Myelodysplasies (GFM)			CMML-Specific Prognostic Scoring System (CPSS-Mol)			
Risk Factors			**Risk Factors**			**Risk Factors**			
• Platelet count < $100x10^9$/L (1.5 pts) • Hemoglobin level < 10g/dL (2 pts) • ***ASXL1*** mutation (1.5 points) • Presence of circulating IMCs (2 pts) • AMC > $10x10^9$/L (2 pts)			• Platelet count < $100x10^9$/L (2 pts) • Hemoglobin <10g/dL females /<11g/dL males (2 pts) • ***ASXL1*** mutations (2 pts) • WBC > $15x10^9$/L (3 pts) • Age > 65 years (2 pts)			• WHO Sub-type [CMML-1 (0 pt)/CMML-2 (1 pt)] • FAB Sub-type [MDS-CMML (0 pt)/MPN-CMML (1 pt)] • RBC Transfusion dependence [No (0 pt)/Yes (1 pt)] • Genetic Risk* [Low (0 pts), Int-1 (1 pt), Int-2 (2 pts), High (≥3 pts)] *TOTAL of Molecular and Karyotype score: **Molecular:** No mutations (0 pt) ***ASXL1*** (1 pt) ***NRAS*** (1 pt) ***RUNX1*** (2 pts) **Karyotype:** Normal (0 pt) All other (1 pt) Trisomy 8/Monosomal, Complex (2 pts)			
Risk Category	**Pts**	**Survival (m)**	**Risk Category**	**Pts**	**Survival (m)**	**Risk Category**	**Pts**	**Survival (m)**	**Rate of AML**
Low	0	97	Low	0-4	65	Low	0	Not reached	0%
Intermediate-1	≥ 2	59	Intermediate	5-7	28	Intermediate-1	1	64	8%
Intermediate-2	2.5-4.5	31				Intermediate-2	2-3	37	24%
High	≥ 5	16	High	8-12	17	High	≥ 4	18	52%

AMC, absolute monocyte count; FAB, French-American-British; Int-1, intermediate-1; Int-2, intermediate-2; m, month; MDS-CMML, myelodysplastic syndrome-like CMML; MPN-CMML, myeloproliferative neoplasm-like CMML; pt/pts/Pts, point(s); RBC, red blood cell; rate of AML, rate of transformation (cumulative at 48 mo).

MPN with monocytosis. CMML patients usually have between 10 and 12 mutations per kilobase of DNA coding region, with the 3 most frequently mutated genes being *TET2* (60%), *SRSF2* (50%), and *ASXL1* (40%).[4] Cytogenetic abnormalities are seen in 20% to 30% of patients, with no abnormality being unique to CMML. Importantly, given the high frequency of *TET2* mutations in CMML and the reported convergence of mutant *TET2* and *IDH* pathways (2-hydroxyglutarate–mediated suppression of TET2 activity), *IDH1/2* mutations are infrequent in CMML (<10%).[4]

Pathobiology

CMML is a disease of ageing, with CMML-related driver mutations occurring in the context of age-related clonal hematopoiesis.[5] Mutations involving *TET2* (epigenetic) and *SRSF2* (splicing) often skew hematopoiesis toward monocytosis, with subsequent mutations in epigenetic regulators (*ASXL1*) or signal pathways (*NRAS*, *CBL*, *KRAS*, *PTPN11*, and *JAK2*) giving rise to disease. MPN-CMML is enriched in active RAS/MAPK signaling, with ~70% of patients demonstrating RAS pathway mutations; *NRAS* is the most common. RAS pathway mutations, along with epigenetic events, also play a role in CMML transformation to AML. The classical monocytes expanded in CMML produce an inflammatory milieu, resulting in elevated levels of tumor necrosis factor-α, interleukin-6, and interleukin-8, which can result in exaggerated leukemoid reactions and cytokine release syndromes.

Prognosis

Several prognostic models exist for CMML, with 3 contemporary models integrating the aforementioned molecular abnormalities: the Mayo molecular model (*ASXL1*), Groupe Francophone des Myélodysplasies model (*ASXL1*), and the CMML-specific prognostic scoring system-molecular model (*ASXL1*, *RUNX1*, *NRAS*, *SETBP1* and cytogenetic abnormalities) (Table 2).[3,6] Frameshift and nonsense mutations in *ASXL1* truncate the protein and are universally deleterious in CMML, with the *ASXL1*wt/*TET2*mt genotype having the best outcomes, including response to HMA therapy.[4] Clinical factors adversely impacting outcomes include anemia, advanced age, leukocytosis, monocytosis, circulating immature myeloid cells (IMCs), and thrombocytopenia.[3]

Management

Allogeneic HCT remains the only curative modality; however, eligibility includes <10% of patients because of their advanced age at presentation and comorbidities. HCT is also fraught with morbidity (eg, graft-versus-host disease) and mortality and tends to be poorly tolerated by older frail patients. However, the advent of reduced-intensity conditioning (RIC) and the use of alternate donor sources continue to expand eligibility and improve tolerability. In general, HCT outcomes have been suboptimal, with 5-year overall survival rates of 30% to 40% and transplant-related mortality rates of 20% to 30%.[3] In a large registry study (Center for International Blood and Marrow Transplant Research, N = 209), overall survival rates at 1, 3, and 5 years were 61%, 48%, and 41%, respectively, for CPSS low/intermediate-1–risk patients and were 38%, 32%, and 19%, respectively, for intermediate-2/high–risk patients.[7] In younger patients, retrospective studies have shown better outcomes, in particular with myeloablative conditioning.[8] Nevertheless, given the absence of prospective randomized studies, questions with regard to optimal timing for HCT in CMML, disease risk–based selection criteria (lower vs higher risk), and the role for pre-HCT therapies (HMAs) remain to be answered. In general, extrapolating from data available in patients with MDS and MPN, HCT in CMML is reserved for higher-risk patients with acceptable organ functions and appropriately matched donor options.

For HCT-ineligible patients, in addition to supportive care measures, HMAs (5-azacitidine and decitabine) are often used to

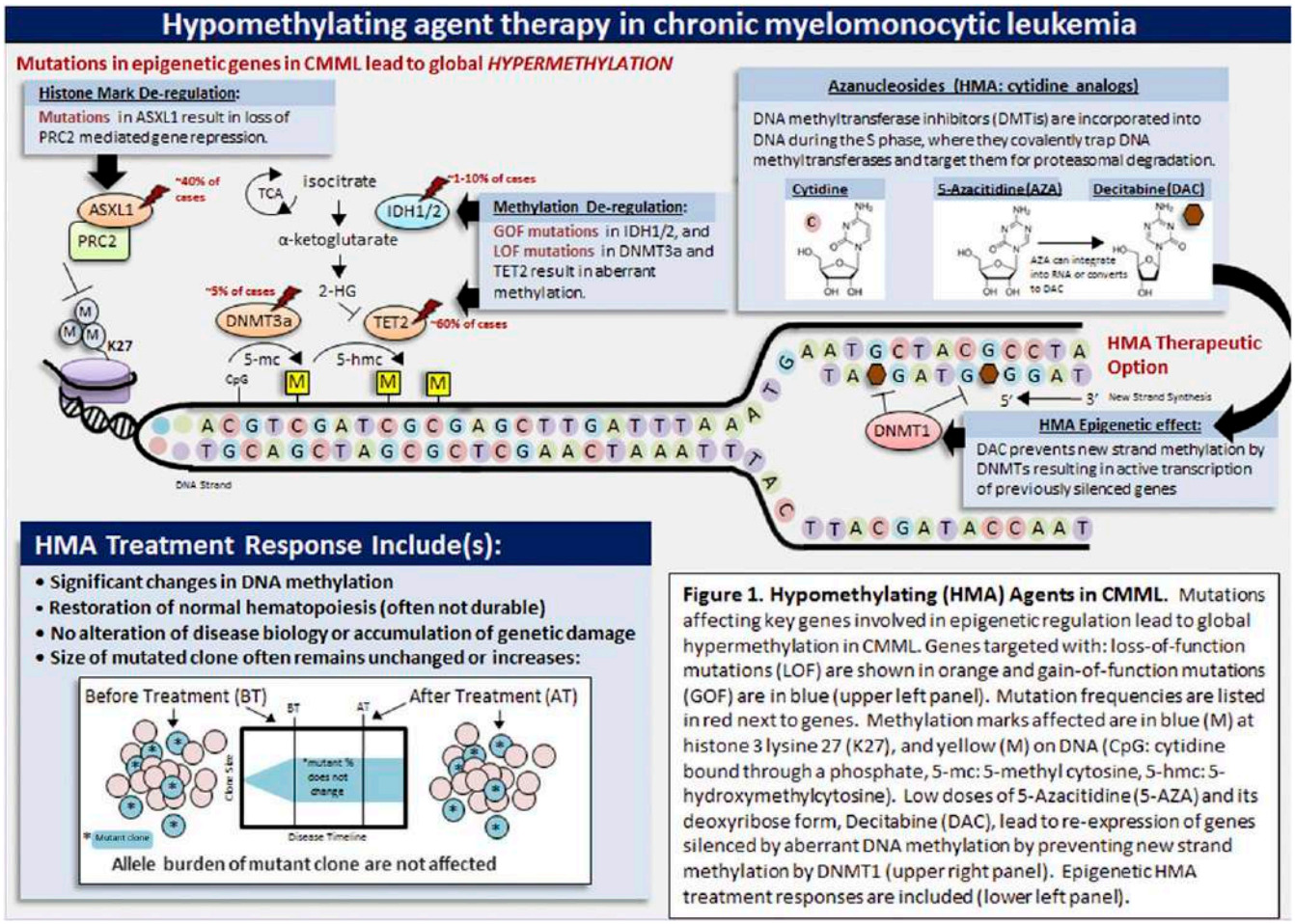

Figure 1. Hypomethylating (HMA) Agents in CMML. Mutations affecting key genes involved in epigenetic regulation lead to global hypermethylation in CMML. Genes targeted with: loss-of-function mutations (LOF) are shown in orange and gain-of-function mutations (GOF) are in blue (upper left panel). Mutation frequencies are listed in red next to genes. Methylation marks affected are in blue (M) at histone 3 lysine 27 (K27), and yellow (M) on DNA (CpG: cytidine bound through a phosphate, 5-mc: 5-methyl cytosine, 5-hmc: 5-hydroxymethylcytosine). Low doses of 5-Azacitidine (5-AZA) and its deoxyribose form, Decitabine (DAC), lead to re-expression of genes silenced by aberrant DNA methylation by preventing new strand methylation by DNMT1 (upper right panel). Epigenetic HMA treatment responses are included (lower left panel).

Figure 1. HMAs in CMML. Mutations affecting key genes involved in epigenetic regulation lead to global hypermethylation in CMML. Genes targeted with loss-of-function (LOF) mutations are shown in orange and gain-of-function mutations (GOF) are in blue (upper left panel). Mutation frequencies are listed in red next to genes. Methylation marks affected are in blue (M) at histone 3 lysine 27 (K27) and yellow (M) on DNA. Low doses of 5-azacitidine and its deoxyribose form, decitabine, lead to re-expression of genes silenced by aberrant DNA methylation by preventing new strand methylation by DNA methyltransferases (upper right panel). Epigenetic HMA treatment responses are included (lower left panel). CpG, cytidine bound through a phosphate; 5-hmc, 5-hydroxymethylcytosine; 5-mc, 5-methylcytosine.

manage disease-related features.[3] These drugs have been approved based on the inclusion of a small number of predominantly MDS CMML patients in MDS-predominant phase 3 clinical trials; there has not been any prospective phase 3 randomized study proving their efficacy in CMML. In general, HMAs act by inhibiting DNA methyltransferases, reducing genome-wide and sequence-specific methylation changes that contribute to neoplasia (Figure 1). HMAs are associated with an overall response rate of 40% to 50%, with true complete remission rates < 20%; with previously responding patients on therapy demonstrating AML transformation.[9] Elaborate sequencing studies have demonstrated that, although HMAs can epigenetically restore normal hematopoiesis in a subset of CMML patients, they fail to alter mutational allele burdens, even in responders, with disease evolution being inevitable in the vast majority (Figure 1).[10] In addition, smaller prospective studies and retrospective data have revealed that these drugs are particularly ineffective in MPN-CMML subtypes.[11] That being said, epigenetic restoration of hematopoiesis is useful in alleviating cytopenias and cytopenia-related symptoms, as well as in reducing the need for red blood cell and platelet transfusions. In addition, HMAs have a direct cytotoxic effect and can be used to reduce PB and BM blasts in patients with advanced disease.

The current patient is a 66-year-old male with high-risk CMML based on his BM blast percentage and the presence of high-risk features, including leukocytosis, monocytosis, circulating IMCs, anemia, thrombocytopenia, and the presence of *ASXL1* and *NRAS* mutations. Given his age, he is not a candidate for a myeloablative conditioning HCT and would need an RIC regimen, once an appropriate donor is identified. His HCT comorbidity index is acceptable (http://www.hctci.org/Home/Calculator); given the higher relapse rates with RIC HCT, he will benefit from disease control prior to HCT. In his case, HMA therapy with 4 to 6 cycles of 5-azacitidine was recommended, with the goal of reducing BM blasts to <5% and then proceeding with an RIC allogeneic HCT with an appropriately matched donor.

In summary, although advances in CMML biology have clearly been made, therapeutic options for affected patients remain limited. Although HMAs do have a role to play in ameliorating cytopenias and possibly as a bridge to allogeneic HCT, they do not alter mutational allele burdens, and disease progression remains

inevitable. In addition, their efficacy in MPN-CMML subtypes is limited. Better elucidation of genetic (RAS signaling and splicing) and epigenetic (*TET2* and *ASXL1*) events frequently seen in CMML and the development of rationally derived therapies targeting exposed vulnerabilities are much needed.

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Conflict-of-interest disclosure

M.M.P. has served on advisory boards for Kura Oncology and Stemline Therapeutics. T.L. declares no competing financial interests.

Off-label drug use

None disclosed.

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Inherited microcytic anemias

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Inherited microcytic anemias can be broadly classified into 3 subgroups: (1) defects in globin chains (hemoglobinopathies or thalassemias), (2) defects in heme synthesis, and (3) defects in iron availability or iron acquisition by the erythroid precursors. These conditions are characterized by a decreased availability of hemoglobin (Hb) components (globins, iron, and heme) that in turn causes a reduced Hb content in red cell precursors with subsequent delayed erythroid differentiation. Iron metabolism alterations remain central to the diagnosis of microcytic anemia, and, in general, the iron status has to be evaluated in cases of microcytosis. Besides the very common microcytic anemia due to acquired iron deficiency, a range of hereditary abnormalities that result in actual or functional iron deficiency are now being recognized. Atransferrinemia, DMT1 deficiency, ferroportin disease, and iron-refractory iron deficiency anemia are hereditary disorders due to iron metabolism abnormalities, some of which are associated with iron overload. Because causes of microcytosis other than iron deficiency should be considered, it is important to evaluate several other red blood cell and iron parameters in patients with a reduced mean corpuscular volume (MCV), including mean corpuscular hemoglobin, red blood cell distribution width, reticulocyte hemoglobin content, serum iron and serum ferritin levels, total iron-binding capacity, transferrin saturation, hemoglobin electrophoresis, and sometimes reticulocyte count. From the epidemiological perspective, hemoglobinopathies/thalassemias are the most common forms of hereditary microcytic anemia, ranging from inconsequential changes in MCV to severe anemia syndromes.

LEARNING OBJECTIVES

- Understand the criteria to define microcytic anemias and their differential diagnosis: acquired and congenital/hereditary
- Understand the roles of hepcidin and erythroferrone in iron abnormalities in hereditary microcytic anemias (IRIDA, atransferrinemia, and DMT1 deficiency)

Introduction and classification of microcytic anemias

Erythropoiesis is a complex and sophisticated process through which hematopoietic cells differentiate into erythroid progenitors and then to reticulocytes that become mature red blood cells (RBCs) in the peripheral blood. Approximately 2×10^{11} erythrocytes are released daily and replace the 2×10^{11} erythrocytes removed daily from the peripheral circulation mainly through the reticuloendothelial system, particularly the spleen. The late phase of erythropoiesis is characterized by ≥4 distinct events: (1) progressive reduction of cell volume, (2) condensation of chromatin, (3) synthesis of hemoglobin (Hb) and (4) organization of the red cell membrane.[1]

Microcytic anemia is the most common form of anemia, both in childhood and in adulthood. Microcytic anemias are highly heterogeneous, and they may be either acquired (mostly due to iron deficiency) or inherited. These latter forms may be present at or around birth; however, in the vast majority of cases, the clinical appearance is delayed, and the diagnosis is achieved during childhood.[2]

Microcytic anemia is defined as a reduced Hb synthesis associated with RBC mean corpuscular volume (MCV) <80 fL during adulthood.[2] Of note, MCV is lower in childhood than in adulthood, and a nutritional iron deficiency may account for the appearance of congenital microcytic anemia (present at or around birth) that can be misdiagnosed as an inherited form of microcytic anemia.[3]

Inherited microcytic anemias embrace a wide spectrum of conditions associated with different pathogenic mechanisms. Indeed, these conditions can be broadly classified into 3 subgroups: (1) defects in globin chains (hemoglobinopathies and thalassemias), (2) defects in heme synthesis (truly, protoporphyrin IX deficiency), and (3) defects in iron

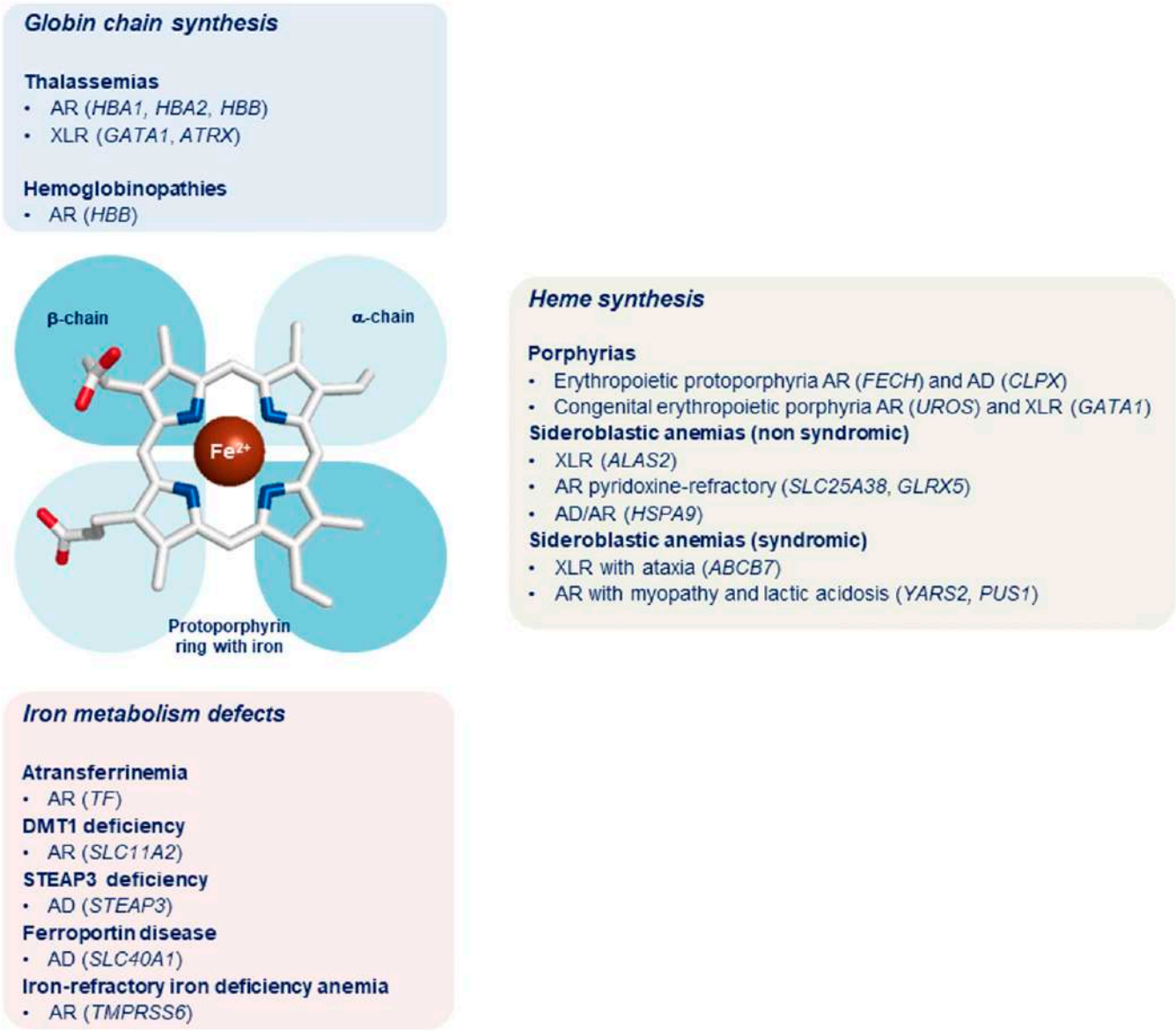

Figure 1. Classification of microcytic anemias. In the middle of the figure is a schematic representation of the prosthetic group of hemoglobin with a protoporphyrin ring (with iron) and the globin chains. In the top panel (light blue) is the classification of microcytic anemias caused by alterations of globin chain synthesis. In the right panel (gray) is the classification of microcytic anemias caused by impairment of heme synthesis. In the bottom panel (pink) is the classification of microcytic anemias caused by iron metabolism defects. AD, autosomal dominant inheritance; AR, autosomal recessive inheritance; XLR, X-linked recessive inheritance.

availability or iron acquisition by the erythroid precursors (Figure 1). These conditions are characterized by decreased availability of Hb components (globins, iron, and heme) that in turn causes a reduced Hb content in RBC precursors with subsequent delayed erythroid differentiation. As a main consequence, it has been hypothesized that erythroid precursors undergo an additional mitotic cycle to overcome the reduced Hb concentration, with subsequent production of erythrocytes with reduced cellular volume.[4] Moreover, the heme-regulated inhibitor, a key heme-binding protein that senses intracellular heme concentrations to balance globin protein synthesis with the amount of heme available for Hb production, plays a crucial role in iron/heme deficiency and β-thalassemia. Indeed, the repression of globin mRNA translation by heme-regulated inhibitor reduces proteotoxicity and permits the expression of ATF4 protein, which plays a pivotal role in terminal erythropoiesis by maintaining oxidative homeostasis and mitochondrial functions. Furthermore, ATF4 represses mammalian target of rapamycin complex 1 (mTORC1) signaling and provides a feedback mechanism to attenuate erythropoietin-mTORC1–stimulated ineffective erythropoiesis in iron deficiency anemia (IDA). Inhibition of mTORC1 also improves anemia and promotes erythroid differentiation of Foxo3$^{-/-}$, β-thalassemic, and mice with sickle cell disease.[5] On the contrary, in atransferrinemia, DMT1 deficiency, and ferroportin disease, microcytic anemia is associated with iron overload because the iron is not made available for erythropoiesis and accumulates in some organs.[6] This review focuses on congenital and hereditary

microcytic anemias due to impaired synthesis of globin chains (thalassemias) and heme or due to iron metabolism defects resulting in either iron deficiency or iron overload.[7]

Clinical cases

In this section, we describe 2 patients with hereditary microcytic anemia due to different genetic disorders. The first is a typical iron-refractory iron deficiency anemia (IRIDA) case; the second is a more difficult case with an original clinical suspicion of β-thalassemia subsequently diagnosed as pyruvate kinase deficiency (PKD).

Case 1

The patient in case 1 was an 8-year-old girl with consanguineous parents from Saudi Arabia. Her personal history was characterized by IDA unresponsive to oral iron and partially responsive to parenteral iron administration.[8] Her family history highlighted the presence of 2 siblings with a similar clinical picture. The recessive transmission was suggested by parents with normal hematological phenotype, the presence of affected sibling pairs, and consanguinity.

At the time of the diagnosis when the girl was 5 years old, she presented with severe microcytic hypochromic anemia with low serum iron and low transferrin saturation (Tsat). Her complete blood count results included Hb of 8.83 g/dL, MCV of 53.3 fL, mean corpuscular hemoglobin (MCH) of 15.9 pg, mean corpuscular hemoglobin concentration of 29.8 g/dL, red blood cell distribution width (RDW) of 19.6%, and platelet count of 526 × 10^3/μL. Her iron status was serum iron level of 20 μg/dL, ferritin concentration of 101 ng/mL, Tsat of 3.3%, and soluble transferrin receptor (sTfR) concentration of 5.6 mg/L. Her serum hepcidin levels were above the normal range (5.63 nM; normal range, 3-7 nM).

The finding of the girl's Hb electrophoresis was normal, and the result of the first-line genetic test for both the common α- and β-thalassemia mutations was negative. Given the girl's personal and familial history as well as the clinical and biochemical data of the proband, second-line genetic testing was performed with mutational screening of the *TMPRSS6* locus. The analysis showed the presence of a homozygous nonsense variant c.1796C>A, p.Ser561*. The analysis of the inheritance pattern confirmed the biallelic inheritance of the variant as well as the presence of the same genotype in the 2 siblings of the proband, both showing the same clinical picture.[8]

Case 2

The patient in case 2 was a 41-year-old man with nonconsanguineous parents from Naples, Italy. His personal history was characterized by anemia with splenomegaly (spleen measuring 18 cm) since childhood. He had been transfusion dependent from 2 years to 7 years of age. Moreover, he presented with numerous hemolytic crises following infectious episodes. His bone marrow biopsy showed erythroid hyperplasia and a moderate degree of dyserythropoiesis with the presence of binucleated erythroblasts. Target cells and several erythroblasts were present on his peripheral blood smear.

He had first been clinically diagnosed with hereditary spherocytosis, and he had been referred for splenectomy and cholecystectomy at 9 years of age. He was then referred to the Medical Genetics Unit at the Federico II University in Naples for infertility treatment. Indeed, he was diagnosed with hypogonadotropic hypogonadism. At the time of the consultation, he presented with chronic microcytic anemia with signs of hemolysis. His complete blood count results showed an RBC count of 3.20 × 10^6/μL, Hb concentration of 8.55 g/dL, hematocrit of 27.2%, MCV of 61.5 fL, MCH of 20.5 pg, mean corpuscular hemoglobin concentration of 31.2 g/dL, RDW of 19.4%, platelet count of 279 × 10^3/μL, and absolute reticulocyte count of 304 × 10^3/μL (9.5%). The hemolysis signs were total bilirubin of 7.6 mg/dL, unconjugated bilirubin of 6.2 mg/dL, lactate dehydrogenase of 1180 U/L, and undetectable haptoglobin. The patient's iron balance highlighted a markedly increased level of ferritin (1500 ng/dL).

Globin chain electrophoresis showed increased fetal Hb levels (5%) and increased HbA2 (6%). The patient's family history highlighted the presence of a first-degree cousin with β-thalassemia major.

Given the patient's personal and familial histories as well as the clinical and biochemical data of the proband, first-line genetic testing was performed with mutational analysis of the *HBB* locus. The analysis showed the presence of the common β0-39 mutation in heterozygous state, but no additional variants in the *HBB* gene were identified. Moreover, deletions/duplications of the *HBA* locus were excluded, too. Thus, second-line genetic testing was performed using a 71-gene custom panel for hereditary anemias.[9] The genomic analysis of the proband highlighted the presence of a well-known pathogenic variant, c.1456C>T, p.Arg486Trp, in the *PKLR* gene as homozygous. This genotype was compatible with the definitive diagnosis of PKD.

Summary

These 2 paradigmatic cases underline how genetic diagnosis is valuable not only for achieving a correct and conclusive diagnosis but also for guiding possible treatment of patients with anemia. This is mainly true for the treatment of patients with PKD, who require splenectomy for severe forms. Moreover, an allosteric activator of pyruvate kinase enzyme is now available that increases the enzymatic activity in patients with reduced PK enzyme activity.[10]

Challenges in the diagnosis of hereditary microcytic anemias

Iron metabolism alterations remain central in the diagnosis of microcytic anemia, and, in general, iron status must be evaluated in all cases of microcytosis. Recent years have seen enormous developments in the understanding of iron metabolism, and besides the very common microcytic anemia due to acquired iron deficiency, hereditary iron metabolism abnormalities should be considered in patients with unexplained microcytic anemias (Figure 1). Atransferrinemia (*TF* gene), DMT1 deficiency (*SLC11A2* gene), ferroportin disease (*SLC40A1* gene), and IRIDA (*TMPRSS6* gene) are now well-established hereditary disorders due to iron metabolism abnormalities, some of which are associated with iron overload.[6] Despite this progress, the diagnostic approach for iron metabolism abnormalities remains based on 3 historical tests: serum iron, transferrin (or total iron-binding capacity), and ferritin. Tsat (ie, Tsat is the ratio of serum iron/total iron-binding capacity), serum ferritin, and noninvasive magnetic resonance imaging measurements of liver and heart iron content are other parameters useful in the diagnosis of iron overload conditions.[11] The serum sTfR is another marker related to the expansion of erythropoiesis or iron deficiency. The level of hepcidin could be useful in the diagnosis of IRIDA and to decide the therapeutic option for iron supplementation (oral vs IV).[12] Tsat/log hepcidin ratio is a

parameter that further contributes to the diagnosis of IRIDA.[13] A new parameter that is going to enter the diagnostic field is the human serum erythroferrone concentration, which is now available only for research purposes.[14,15]

Other causes of microcytosis than iron deficiency should be considered. Thus, it is important to evaluate several other RBC and iron parameters in the presence of reduced MCV: MCH, RDW, reticulocyte Hb content, serum iron and serum ferritin levels, total iron-binding capacity, Tsat, Hb electrophoresis, and occasionally reticulocyte blood count. Each of these parameters, as well as the patient's clinical and family histories, should be taken into account when evaluating a case of microcytic anemia. The latter should be considered to identify transmission modalities and is useful to distinguish between acquired and genetic conditions. Three different steps could be considered for the diagnostic approach to hereditary microcytic anemia (Table 1). In the first evaluation step for a patient with microcytic anemia, the possible presence of a β-thalassemic trait must be excluded. Indeed, microcytosis associated with reduced MCH is the hallmark of β-thalassemia carriers, who usually have normal or very slightly elevated iron parameters and increased HbA2 detected by Hb electrophoresis (high-performance liquid chromatography). RDW is often increased in iron deficiency conditions, such as in DMT1 deficiency, thalassemia, and IRIDA, and it is normal or mildly raised in anemia of chronic disease.[16] In patients with IRIDA, the serum iron is low with normal/high serum ferritin, particularly after IV iron therapy has been initiated. Hepcidin is within normal range, but it is inappropriately high in a patient with iron deficiency. The serum iron level is high in the setting of hypotransferrinemia and DMT1 and STEAP3 defects as well as in sideroblastic anemia. Tsat is low in patients with IDA and IRIDA, whereas it is usually elevated in those with sideroblastic anemia. High serum ferritin in the presence of high serum iron and normal transferrin is typical of sideroblastic anemia.

Secondary investigations include erythrocyte zinc protoporphyrin (zinc protoporphyrin or zinc protoporphyrin/heme), plasma hepcidin, and plasma sTfR levels. Bone marrow examination is required to evaluate a potential diagnosis of sideroblastic anemia, although it is not entirely required in patients with a typical presentation and a supportive genetic testing result. Magnetic resonance imaging is generally reserved for monitoring iron in the liver and heart in patients with biochemical evidence of iron overload. High levels of sTfR are found in sideroblastic anemia and in patients with DMT1 and STEAP3 mutations and IRIDA, but not in those with hypotransferrinemia. The serum hepcidin levels are usually reduced in IDA, whereas in patients with IRIDA, hepcidin levels are high or normal.[17]

A third level of investigations comprises genetic testing, generally reserved for those cases without a satisfactory biochemical explanation. This analysis is used to define atypical forms of anemia and provides useful information for prognosis and treatment. As mentioned in the clinical cases, genetic

Table 1. Main features of microcytic anemias

	Acquired conditions		Inherited conditions									
	IDA	ACD	BT trait	IRIDA	AHMIO1	AHMIO2	EPP1/2	SIDBA1	SIDBA2	SIDBA3	ASAT	Hypotransferrinemia
Genetic features												
Causative gene(s)	—	—	*HBB*	*TMPRSS6*	*SLC11A2*	*STEAP3*	*FECH/CLPX*	*ALAS2*	*SLC25A38*	*GLRX5*	*ABCB7*	*TF*
Inheritance	—	—	AR	AR	AR	AD	AR/AD	XLR	AR	AR	XLR	AR
Hematological and biochemical features												
RBC	↓	↓	↑	↓↓	↓	↓	↓	↓	↓	↓	↓	↓
Hb	↓	↓	= or ↓	↓ or ↓↓	↓↓	↓↓	↓	↓	↓↓	↓↓↓ (age dependent)	↓	↓
MCV	↓	↓	↓	↓↓	↓↓↓	↓	↓↓	↓	↓	↓↓	↓	↓↓
RDW	=	= or ↑	= or ↑	=	=	↑	=	=	↑	=	=	=
Reticulocytes	↓	= or ↑	= or ↑	↓	↓	↓	↓	↓	↓	↓	↓	↓
Tsat	↓↓	=	=	↓↓ or ↓↓↓	↑↑	↑↑	↑	↑	↑	↑	↑	100%
Ferritin	= or ↓	=	=	= or ↓	↑	↑	=	=	=	=	=	=
FEP	= or ↑	=	=	↑↑	↑	=	↑↑↑	= or ↓	=	=	= or ↓	=
Iron administration												
Oral response	Yes	Unpredictable	No	No	No	—	No	No	—	No	No	No
Intravenous response	Yes	Unpredictable	No	Yes (not long lasting)	No	—	No	No	—	No	No	No
Suggested therapy	Oral iron supplement	Etiologic therapy (EPO, IV iron)	—	—	EPO	—	β-carotene	Vitamin B_6	—	Iron chelation	Vitamin B_6	Plasma; apotransferrin

ACD, anemia of chronic disease; AD, autosomal dominant; AHMIO, anemia, hypochromic microcytic, with iron overload; AR, autosomal recessive; ASAT, sideroblastic anemia with ataxia; BT, β-thalassemia; EPO, erythropoietin; EPP, erythropoietic protoporphyria; FEP, free erythrocyte porphyrin; SIDBA, sideroblastic anemia; XLR, X-linked recessive.

Table 2. New drugs for the treatment of microcytic anemia

Drug	Mechanism/effect	Preclinical data/clinical trial
Hepcidin analogs and mini-hepcidin	Replace endogenous hepcidin	Clinical trial
Anti-TMPRSS6 (ASO, siRNA)	Hepcidin inhibition	Clinical trial
FPN inhibitor VIT-2763	Blocking the hepcidin receptor	Clinical trial
Transferrin injection	Decreasing transferrin receptor/reducing iron uptake	Preclinical study in animal model
Luspatercept	Activin receptor IIB ligand trap/ameliorating anemia and reducing hepcidin inhibition	Clinical trial
Protoporphyrin IX	Reducing iron cycling by inhibiting heme oxygenase 1	Preclinical study in animal model
Anti–IL-6 and anti–IL-6R	Reducing the hepcidin signaling pathway	Preclinical study in animal model
Anti-BMP6 MoAb	Reducing the hepcidin signaling pathway	Clinical trial
BMP receptor inhibitors	Reducing the hepcidin signaling pathway	Preclinical study in animal model
Antihemojuvelin MoAb	Reduced hepcidin/correction of hypoferremia/correction of anemia	Preclinical study in animal model
Antihepcidin MoAb	Reduced hepcidin/correction of hypoferremia/correction of anemia	Preclinical study in animal model
Antihepcidin Spiegelmer	Reduced hepcidin/correction of hypoferremia/correction of anemia	Clinical trial
Antihepcidin anticalin	Reduced hepcidin/correction of hypoferremia/correction of anemia	Clinical trial
Antiferroportin MoAb GDP	Reduced hepcidin/correction of hypoferremia/correction of anemia	Preclinical study in animal model

ASO, antisense specific oligonucleotides; BMP, bone morphogenetic protein; FPN, ferroportin; GDP, guanosine 5′-diphosphate encapsulated in lipid vesicle; IL, interleukin; MoAb, monoclonal antibodies; siRNA, short interfering RNA; VIT-2763, small molecule oral ferroportin inhibitor.

testing can be performed by either single-gene or multigene analysis using next-generation sequencing approaches. The selection of the appropriate method is based on the evaluation of different criteria: genetic heterogeneity, phenotypic characterization, gene size, and prevalence of the disease. As demonstrated by case 2, next-generation sequencing–based genetic testing is a powerful strategy for those cases with overlapping phenotypes or polygenic conditions.

Therapy of microcytic anemias: old and new approaches

In most of the hereditary microcytic anemias, the treatment is only supportive. Regarding the most common acquired IDA, oral iron therapy is the first choice for most of the patients.[18] Numerous oral iron preparations exist, such as iron salts, ferrous sulfate, ferrous fumarate, and ferrous gluconate. If tolerated, this treatment should ameliorate the Hb levels within 2 to 3 weeks. An increase of Hb by 2 g/dL after 3 weeks of therapy is a commonly used criterion to define the patient's hematological response to oral iron.[19] For patients in whom oral iron therapy fails, the diagnosis of IRIDA becomes very suspicious, and, in such cases, IV iron is advisable. Several IV iron formulations are available and safe.[20]

On the contrary, for the iron overload microcytic anemias, the therapy aims to prevent the complications of iron accumulation in various organs and systems more than correcting the anemia, which can be mild/moderate. The treatment in such cases is based on iron chelation, and today there are 3 iron chelators in clinical practice: deferoxamine (due to the reduced half-life, it needs continuous subcutaneous infusion), deferiprone orally administered 3 times per day, and deferasirox that is orally administered in a single daily dose.[12,20]

Hepcidin, either in iron deficiency or in iron overload microcytic anemias, plays a major role; thus, currently, researchers are looking for new therapies able to modulate the hepcidin pathway. Indeed, hepcidin levels can help the response or resistance to oral iron administration, explaining part of iron refractoriness.[21] Hepcidin agonists (that increase hepcidin levels), hepcidin antagonists (inhibitors of hepcidin synthesis), hepcidin binders (that block hepcidin function), and compounds that interfere with hepcidin–ferroportin interaction are in preclinical and clinical studies (Table 2).[22,23] Hepcidin agonists can be used in anemias with ineffective erythropoiesis, such as β-thalassemia.[24] The hepcidin analogs include minihepcidins, inhibitors of hepcidin repressors such as anti-TMPRSS6 molecules, and compounds that block ferroportin activity (Table 2). The use of these compounds demonstrated improvements in both anemia and iron overload in preclinical thalassemia models.[25,26] The hepcidin agonists are currently used in phase I-II clinical trials. Moreover, drugs that improve erythroid maturation, such as the activin receptor IIB ligand trap, luspatercept, demonstrated their action not only by ameliorating anemia but also by reducing hepcidin inhibition.[26] Hepcidin antagonists can be useful to release sequestered iron in IRIDA. In this latter condition, the use of a humanized antibody against hemojuvelin to modulate the hepcidin pathway has been proposed (Table 2).[27]

Conflict-of-interest disclosure
The authors declare no competing financial interests.

Off-label drug use
None disclosed.

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Global look at nutritional and functional iron deficiency in infancy

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Iron-deficiency anemia (IDA) affects many infants in low- and middle-income countries (LMICs) and may impair cognitive development and adaptive immunity. Effective interventions to improve iron intakes for infants in LMICs are urgently needed. However, absorption of oral iron fortificants and supplements is low, usually <10%, and most of the iron passes into the colon unabsorbed. In randomized controlled trials, provision of iron to infants in LMICs adversely affects their gut microbiome and increases pathogenic *Escherichia coli*, gut inflammation, and diarrhea. To minimize these detrimental effects of iron, it is important to provide the lowest effective dosage and maximize fractional iron absorption. Prebiotic galacto-oligosaccharides and apo-lactoferrin may prove useful in iron formulations in LMICs because they increase absorption of fortificant iron and at the same time may mitigate the adverse effects of unabsorbed iron on the infant gut. Providing well-absorbed iron early in infancy may improve immune function. Recent data from a Kenyan birth cohort suggest IDA at the time of infant vaccination impairs the response to diphtheria, pertussis, and pneumococcus vaccines. A randomized trial follow-up study reported that providing iron to Kenyan infants at the time of measles vaccination increased antimeasles immunoglobulin G (IgG), seroconversion, and IgG avidity. Because IDA is so common among infants in LMICs and because the vaccine-preventable disease burden is so high, even if IDA only modestly reduces immunogenicity of vaccines, its prevention could have major benefits.

LEARNING OBJECTIVES

- Understand the adverse effects of iron fortification on the infant gut microbiome, gut inflammation, and diarrhea and potential approaches to mitigate these adverse effects of iron
- Understand the links between iron deficiency anemia, adaptive immunity, and infant vaccine response

Clinical case

A 10-week-old breastfeeding male infant presents to an outpatient clinic in southern rural Kenya for routine vaccination. The mother states that the infant, who was born with low birth weight, is irritable and not feeding well. The infant has had several bouts of fever and watery diarrhea over the past month. On clinical examination, he is afebrile, and chest auscultation is unremarkable. However, he is underweight, and his mucosae and conjunctiva are markedly pale. Malaria test is negative, but hemoglobin is 8.8 g/dL. What is the likely etiology for his anemia? What treatment will you provide? Is supplemental iron safe in this setting? Should iron-fortified foods be added to his diet? Will supplemental iron aggravate the infant's diarrheal episodes?

Introduction

Iron-deficiency anemia (IDA) affects >1 billion people worldwide and is one of the five leading global causes of years lived with disability.[1] IDA is particularly prevalent in low- and middle-income countries (LMICs), and in Africa, 62% of preschool children are anemic,[2] mainly because of iron deficiency. Infants in LMICs have particularly high rates of IDA during the weaning period because high iron needs for growth and erythropoiesis typically are not covered by the low amounts of iron present in breastmilk and plant-based complementary foods.[3] Defining iron status in very young infants is challenging because of rapid changes in iron metabolism during the first few months after birth.[4] IDA in infants and young children may impair cognitive development, and this impairment may be irreversible or only partially reversible by iron repletion.[3] Because IDA during infancy is common and can lower the intelligence quotient, it has serious health and economic costs and may hinder national development.[5] Moreover, recent findings have linked IDA to impairments in adaptive immunity and,

possibly, vaccine response.[6] Thus, effective, sustainable, and safe interventions to improve iron intakes in infants in LMICs are urgently needed.[3]

Iron fortification, the gut microbiome, and diarrhea

Dietary iron absorption is tightly regulated in humans because there is no active pathway for iron excretion.[7] Iron absorption from iron-fortified foods or iron supplements is generally low, typically <10%.[3] In LMICs, iron absorption is likely to be even lower, because common infections and inflammation increase plasma hepcidin, which reduces iron absorption.[7] Thus, the majority of oral iron passes unabsorbed into the colon, where the iron is available to gut microbes. Iron plays a key role in replication and virulence (eg, adhesion, invasion, and induction of virulence factors) of many enteric gram-negative bacteria (eg, *Salmonella*, pathogenic *Escherichia coli*).[8,9] In contrast, beneficial commensal gut bacteria, such as Bifidobacteriaceae and Lactobacillaceae, which provide an important barrier against colonization by enteropathogens, need very little or no iron.[10] Thus, an increase in unabsorbed dietary iron entering the colon may tip the balance toward growth of potential enteropathogens over important commensal "barrier" strains. Systemically, hepcidin-mediated iron sequestration withholds iron from invading microorganisms and plays a key role in innate immune responses to infection.[11] Similarly, hepcidin production by mucosal dendritic cells, independent of systemic iron or hepcidin levels, may sequester iron into colonic myeloid cells; failure to sequester iron locally results in dysbiosis, increases microbial translocation, and worsens gut inflammation.[12]

The World Health Organization recommends providing micronutrient powders (MNPs) containing 12.5 mg of iron to infants aged 6 to 23 months in areas where the anemia prevalence is high.[13] However, these iron-containing MNPs are not entirely safe for infants,[14] because they increase diarrhea risk. A systematic review reported a 15% increased risk for diarrhea (risk ratio 1.15; 95% confidence interval, 1.06-1.26) with iron dosages given at ≥80% of the recommended daily intake.[15] A review of potential mechanisms linking oral iron dosages to diarrhea suggested an increase in Enterobacteriaceae and pathogenic *E. coli* or a decrease in Bifidobacteriaceae may play a role.[16] Pathogenic *E. coli* is an important cause of diarrhea[17] and bacteremia[18] among African infants. Diarrhea is a leading cause of child mortality in LMICs; the World Health Organization estimates that diarrhea contributes to 19% of under-5 deaths globally.[19]

Recent randomized controlled trials assessed the impact of iron-containing MNPs on the infant gut microbiota, gut inflammation, and diarrhea in Kenyan infants.[20,21] Infants in the first trial (n = 115) consumed MNPs containing 2.5 or 12.5 mg iron or without iron daily for 4 months.[20] Iron deficiency was defined as a low serum ferritin (SF) or an elevated soluble transferrin receptor (sTfR) or erythrocyte zinc protoporphyrin; screening for hemoglobinopathies was not performed. There was a significant positive treatment effect of the MNP containing 12.5 mg iron on SF, sTfR, and zinc protoporphyrin (for all, $P < .05$) but no significant effect of the MNP containing 2.5 mg iron. At baseline, the gut microbiota of the infants harbored a high abundance of beneficial Bifidobacteriaceae (63%). At the same time, many were carrying potential pathogens: 65% were positive for enteropathogenic *E. coli*, 49% for enterotoxigenic *E. coli* producing heat-labile toxin, 57% for *Clostridium difficile*, and 22% for *Salmonella*. In the iron groups compared with the no-iron control groups, there was a significant increase in Enterobacteriaceae, particularly *Escherichia/Shigella* spp., the enterobacteria/bifidobacteria ratio, and *Clostridium* (for all, $P < .05$). Moreover, there were higher abundances of pathogenic *E. coli* at endpoint in the iron groups (6.0 ± 0.5 log gene copy number/g feces) compared with the no-iron groups (4.5 ± 0.5) ($P = .029$) (Figure 1). In addition, fecal calprotectin (a marker of gut inflammation) was significantly higher in the iron compared with the no-iron control group (Figure 2), and diarrheal rates were higher.[20] This study was the first to demonstrate that provision of iron to African infants adversely affects gut microbiome composition and increases gut inflammation.

In a subsequent 4-month randomized controlled trial in the same study area,[21] infants aged 6.5 to 9.5 months (n = 155) were randomly assigned to receive daily an MNP without iron (control group), the identical MNP but with 5 mg iron (Fe group), or the identical MNP as the Fe group but with 7.5 g galactooligosaccharides (GOS) (FeGOS group). Iron deficiency was defined as a low SF or an elevated sTfR; screening for hemoglobinopathies was not performed. At 4 months, compared with the control group, there was significant improvement in PF and sTfR in the Fe and FeGOS groups ($P < .001$ for both). GOS is a nondigestible prebiotic carbohydrate that enters the colon intact and selectively enhances the growth of beneficial commensal *Bifidobacterium* and *Lactobacillus*.[22] Prebiotics may protect from colonization and overgrowth of potential enteric pathogens by increasing colonization resistance, increasing production of short chain fatty acids, and decreasing colonic luminal pH.[22] The addition of GOS to the iron-containing MNP

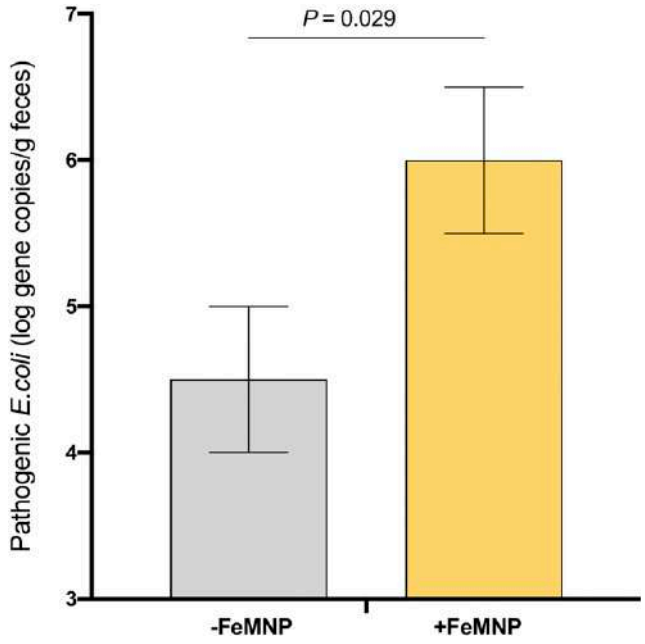

Figure 1. Abundance of pathogenic *E. coli* at endpoint, by group, in Kenyan infants (n = 115) receiving daily for 4 months an MNP containing either no iron (−FeMNP) or 2.5 mg or 12.5 mg iron (+FeMNP). Univariate general linear models with baseline values as covariates were used to estimate the intervention effect. Adapted from Jaeggi et al.[20]

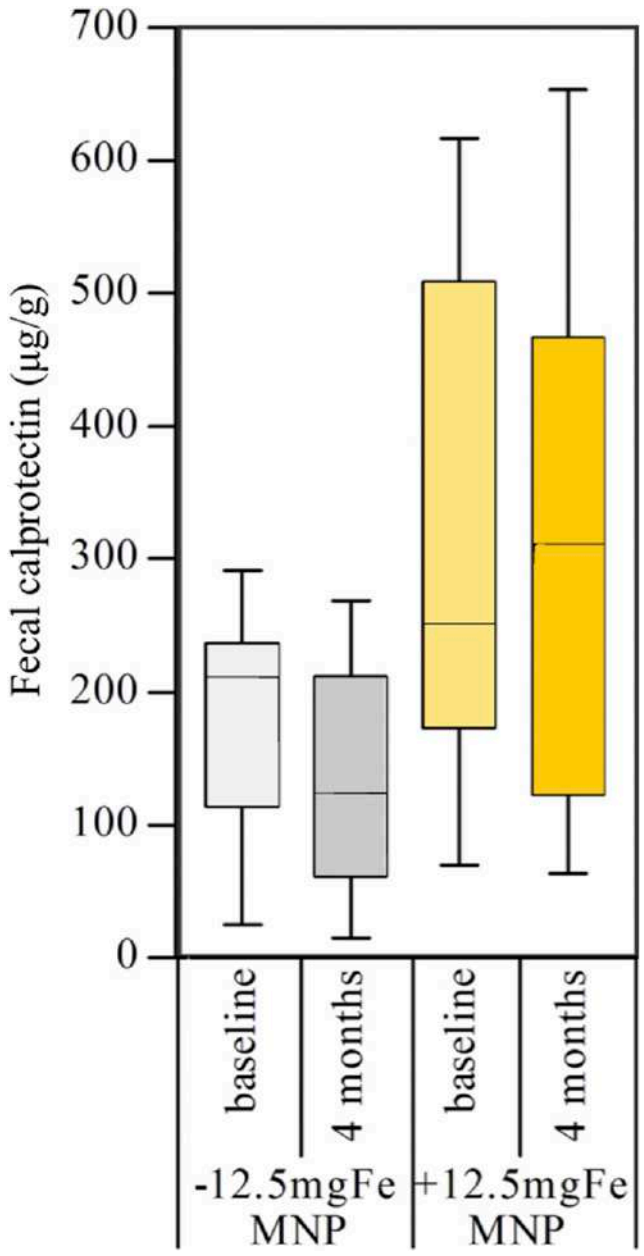

Figure 2. Fecal calprotectin levels at baseline and 4 months, by group, in Kenyan infants (n = 115) receiving daily for 4 months an MNP containing no iron (−12.5mgFeMNP) or containing 12.5 mg iron (+12.5mgFeMNP). At endpoint, fecal calprotectin was significantly higher in the +12.5mgFeMNP group than in the −12.5mgFeMNP group ($P = .008$). Differences were investigated via general linear models with baseline variables as covariates. Boxplots are shown with the 10th to 90th percentiles. Adapted from Jaeggi et al.[20]

mitigated most of the adverse effects of iron on the infant gut microbiota.[21] After 4 months of intervention, compared with the Fe group, in the FeGOS group there was a higher abundance of *Bifidobacterium* and *Lactobacillus* and lower abundance of Clostridiales (Figure 3). Remarkably, there were no significant differences in the abundances of *Bifidobacterium*, *Lactobacillus*, Enterobacteriaceae, Clostridiales, or Bacteroidetes between the control and FeGOS groups (Figure 3). After 3 weeks of intervention there were lower abundances of the sum of virulence and toxin genes of all pathogens in the FeGOS group compared with both the control group and the Fe group (Figure 4). Also, there were significantly lower abundances of the sum of virulence and toxin genes of pathogenic *E. coli* in the FeGOS group compared with the control group. At 4 months, plasma intestinal fatty acid–binding protein (a biomarker of enterocyte damage) was significantly higher in the Fe group than in the control group but was not higher in the FeGOS group when compared with the control group.[21] Results of a recent study of Swedish infants also suggest that iron-fortified formula and iron supplements may adversely affect the gut microbiome and that prebiotic GOS in infant formula may be protective.[23]

Maximizing iron absorption from iron supplements and fortificants in infancy

Because of the detrimental effects of unabsorbed iron on the gut microbiome of African infants,[19] it is important to provide the lowest effective dosage by maximizing fractional iron absorption. Recent data demonstrate that prebiotic GOS may increase iron absorption when added to iron-containing MNPs.[16] Kenyan infants (n = 50; aged 6–14 months) consumed maize porridge that was fortified with an MNP containing iron (5 mg) and GOS (7.5 g) or the same MNP without GOS each day for 3 weeks. Then all infants were provided an isotopically labeled maize porridge and MNP test meal containing iron either as a mixture of ferrous fumarate and sodium iron ethylenediaminetetraacetate or as ferrous sulfate. Iron absorption was measured as the erythrocyte incorporation of stable isotopes. GOS consumption by the infants significantly increased iron absorption by +62% from the MNP containing ferrous fumarate and sodium iron ethylenediaminetetraacetate.[16]

Lactoferrin is a glycosylated protein highly concentrated in human milk and can exist in an apo (iron-free) state or can bind two ferric ions with very high affinity, forming holo-lactoferrin.[24] Because lactoferrin binds iron with high affinity at the pH of the infant small and large intestine, it may be a bacteriostatic agent for withholding iron from enteropathogens, whose growth and virulence depend on an adequate iron supply.[24] Whether lactoferrin also binds iron to facilitate its absorption remains uncertain, but a lactoferrin receptor has been identified on enterocytes, which may mediate iron transport from lactoferrin into mucosal cells.[25] In a recent randomized crossover trial, Kenyan infants (n = 25, mean age 4 months) were fed multiple stable iron isotopes in ferrous sulfate–fortified test meals with and without bovine apo-lactoferrin, with iron absorption quantified by measurement of erythrocyte iron incorporation.[26] The addition of apo-lactoferrin significantly increased iron absorption by +56% (Figure 5). Iron absorption was also measured from intrinsically labeled holo-lactoferrin, and it was comparable to iron absorption from ferrous sulfate alone (Figure 5). Intervention studies of infants assessing the effect of supplemental lactoferrin on iron status are equivocal, but the use of lactoferrin in iron-fortified infant formula is associated with a reduced incidence and duration of diarrhea.[27] Supplemental lactoferrin has also demonstrated benefits on iron status in pregnant women.[28] Therefore, prebiotic GOS and bovine apo-lactoferrin may prove useful in iron formulations for infants in LMICs because they both increase absorption of iron and potentially mitigate the adverse effects of unabsorbed iron on the gut microbiome.

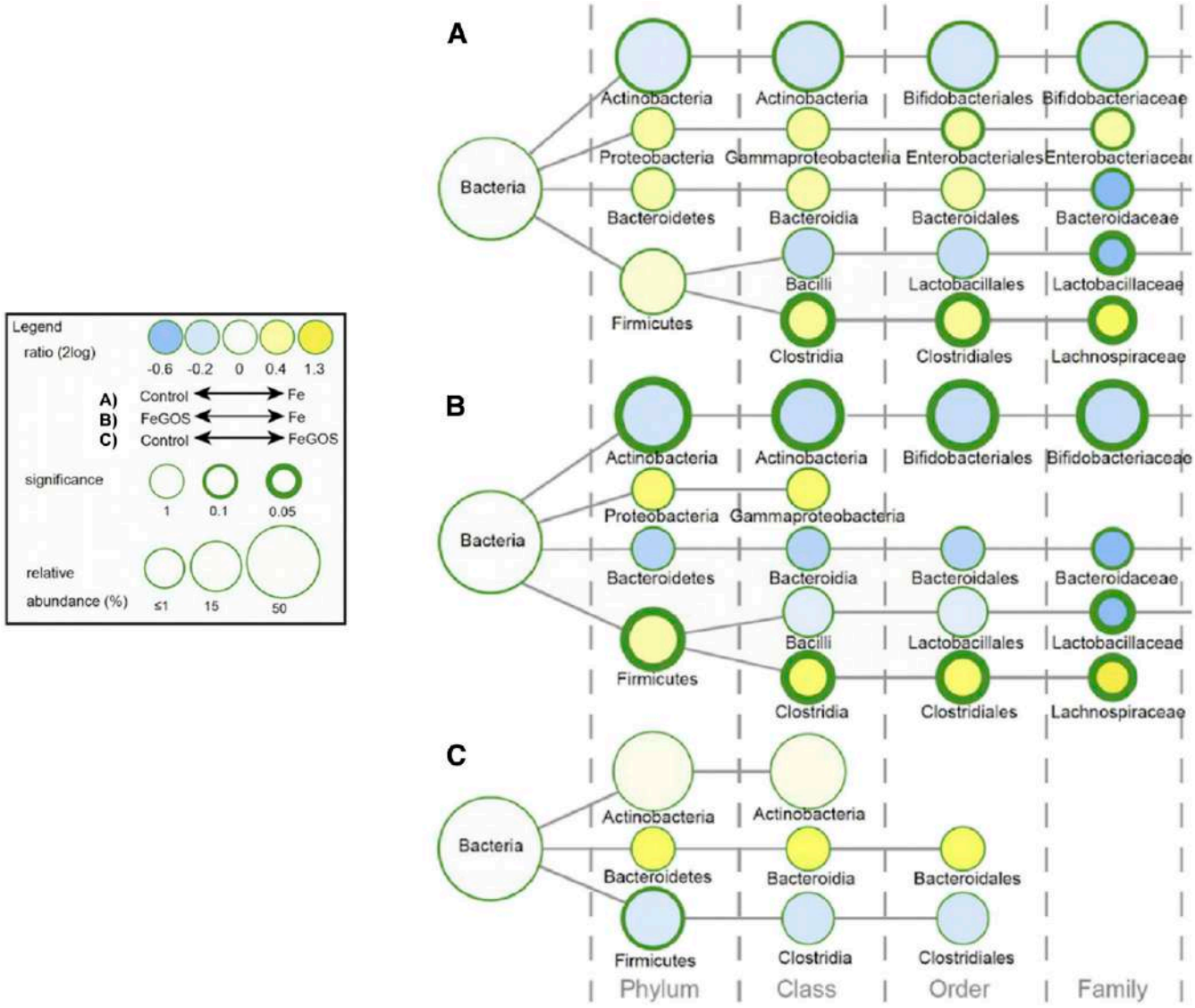

Figure 3. Differences in the gut microbiota composition among Kenyan infants (n = 155) after receiving daily for 4 months an MNP without iron (control group), with 5 mg of iron (Fe group), or with 5 mg iron and 7.5 g galacto-oligosaccharides (FeGOS group). Nodes represent taxa; edges link the different taxonomic levels. Node sizes correspond to the relative taxa abundance (%). The fold difference is calculated as the 2log of the ratio of the relative abundance between groups. Mann–Whitney U tests were used for statistical comparisons. Adapted from Paganini et al.[21]

Iron deficiency anemia, adaptive immunity, and infant vaccine response

Immunization programs in LMICs have achieved high coverage, yet 1 in 5 children worldwide are not fully protected, contributing to 1.5 million child deaths yearly from vaccine-preventable diseases.[29] Vaccines often underperform in LMICs.[30] For example, effectiveness of measles vaccine is generally <75% in Sub-Saharan Africa.[31] Why vaccines underperform in LMICs remains uncertain,[30] but new data suggest iron deficiency (ID) may play a role.[32,33]

Previous reviews[34] have suggested multiple mechanisms by which iron status might influence adaptive immunity. ID in mice attenuates T-cell–dependent and T-cell–independent antigen-specific antibody responses and impairs cyclin E1 induction and S-phase entry during B-cell proliferation.[32] In some animal and cell models, ID reduces the proportion of mature T cells and impairs T-cell activation and proliferation.[34] In humans, studies on ID and immune function show varying results depending on what aspect of immunity is measured and the severity of ID, age, and underlying nutritional status.[34] Iron uptake via transferrin receptor 1 is essential for lymphocyte development, and clinically, a homozygous mutation in transferrin receptor 1 causes severe immunodeficiency in children and reduced numbers of circulating memory B cells.[33] In vitro, this mutation prevented T- and B-cell proliferation, and addition of iron citrate in vitro rescued the proliferative defect.[33] Thus, adequate iron availability may be critical for adaptive immunity. If ID during infancy limits iron availability to responding lymphocytes,[32] this could impair response to vaccination. Recent studies showing impaired antigen-specific immune responses in

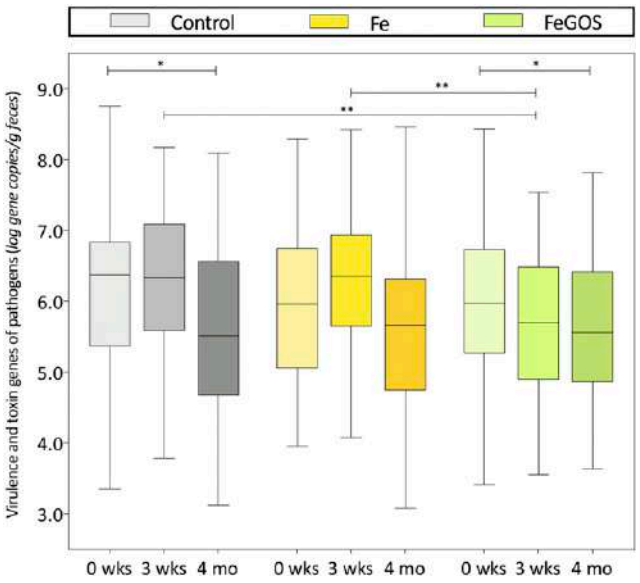

Figure 4. Abundances of the sum of virulence and toxin genes of 10 pathogens at baseline (0 weeks), 3 weeks, and 4 months, by group in Kenyan infants (n = 155) consuming daily an MNP containing either no iron (control), 5 mg of iron (Fe), or 5 mg of iron and 7.5 g of galacto-oligosaccharides (FeGOS). Significance is expressed as the *P* value of a Wilcoxon rank-sum test, **P* < .05, ***P* < .01. Boxes show the median and 25th and 75th percentiles; whiskers show the range. Adapted from Paganini et al.[21]

hypoferremic mice support this concept (Joe Frost et al, University of Oxford, written personal communication, June 25, 2020).

IDA is particularly common among infants age <1 year in Sub-Saharan Africa: In southern Kenya, 70% to 75% of infants are anemic at the time they receive their routine vaccinations.[35] Term infants born to iron-sufficient mothers should have adequate birth iron stores to cover iron requirements for the first 4 to 6 months.[36] However, in Sub-Saharan Africa, 46% of pregnant women have IDA,[37] reducing maternal–fetal iron transfer.[36] The umbilical cord is often clamped too early, and 15% to 25% of newborns have low birthweight.[38] These factors sharply reduce newborn iron stores: It is estimated that body iron is 40% to 50% lower in newborns who are low birthweight or whose mothers were anemic during pregnancy.[36] Low iron stores at birth, together with frequent infections increasing serum hepcidin[35] and diarrhea or intestinal parasites causing blood loss, result in many infants depleting their iron stores within 3 to 4 months after birth.

Two recent studies in southern coastal Kenya suggest that IDA during infancy impairs vaccine response.[6] A birth cohort study assessed whether anemia or ID at time of vaccination predicted vaccine response to 3-valent oral polio, diphtheria–tetanus–whole cell pertussis–*Haemophilus influenzae* type b vaccine, 10-valent pneumococcal conjugate vaccine, and measles vaccine. Primary outcomes were anti-vaccine immunoglobulin G (IgG) and seroconversion at age 24 weeks and 18 months. A total of 573 infants were enrolled at birth, and 303 completed the study.[6] More than half of infants were already anemic at age 10 weeks. With sex, birthweight, anthropometric indices (*z* scores for length and weight), and maternal antibodies controlled for, hemoglobin at time of vaccination was the strongest positive predictor of antidiphtheria and anti-pertussis-IgG at 24 weeks and 18 months, antipertussis filamentous hemagglutinin-IgG at 24 weeks, and anti–pneumococcus 19 IgG at 18 months (for all, *P* < .05). Anemia and serum transferrin receptor at time of vaccination were the strongest predictors of seroconversion against diphtheria and pneumococcus 19 at 18 months (for both, *P* < .05). In a randomized trial cohort follow-up,[6] infants (n = 155) received an MNP with 5 mg iron daily or an MNP without iron for 4 months starting at age 7.5 months and received measles vaccine at age 9 months. Vaccine response was measured at age 11.5 months and 4.5 years. Compared with infants who did not receive iron, those who received iron at time of vaccination had higher antimeasles IgG, seroconversion, and IgG avidity (for all, *P* < .05) at age 11.5 months (Figure 6).[6]

These are the first prospective data from Africa assessing the impact of anemia and ID at the time of vaccination on response to a range of pediatric vaccines. These data suggest that anemia or ID at the time of infant vaccination may impair the response to diphtheria, pertussis, and pneumococcus vaccines and that improving iron status may improve response to measles vaccine.[6] These findings must be confirmed in other prospective cohorts and larger intervention trials. Powerful emerging techniques combining mass cytometry and systems-level omics tools[39] may allow identification of the mechanisms underlying the effects of iron status on the infant immune system and response to vaccines. If confirmed, these findings argue strongly

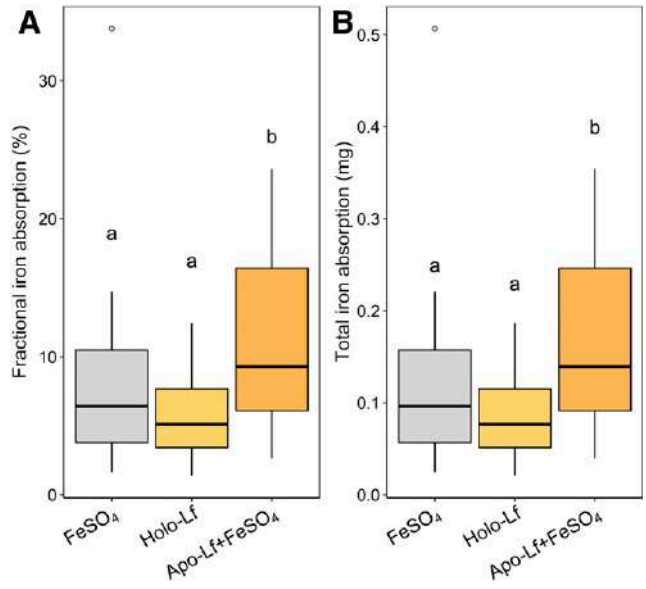

Figure 5. Fractional iron absorption (%) (A) and total iron absorption (mg) (B) in Kenyan infants (n = 25) from maize porridge containing a mixture of 1.5 mg iron as ^{54}Fe-labeled $FeSO_4$; as ^{58}Fe-labeled $FeSO_4$ + 1.41 g apo-lactoferrin; and as intrinsically ^{57}Fe-labeled holo-lactoferrin containing 1.41 g lactoferrin. The horizontal lines show the geometric means, and the whiskers extend from −SD to +SD. Data analyzed with repeated-measures analysis of covariance with Bonferroni post hoc analysis. Geometric means without a common letter differ, *P* < .05. $FeSO_4$, ferrous sulfate; Lf, lactoferrin. Adapted from Mikulic et al.[26]

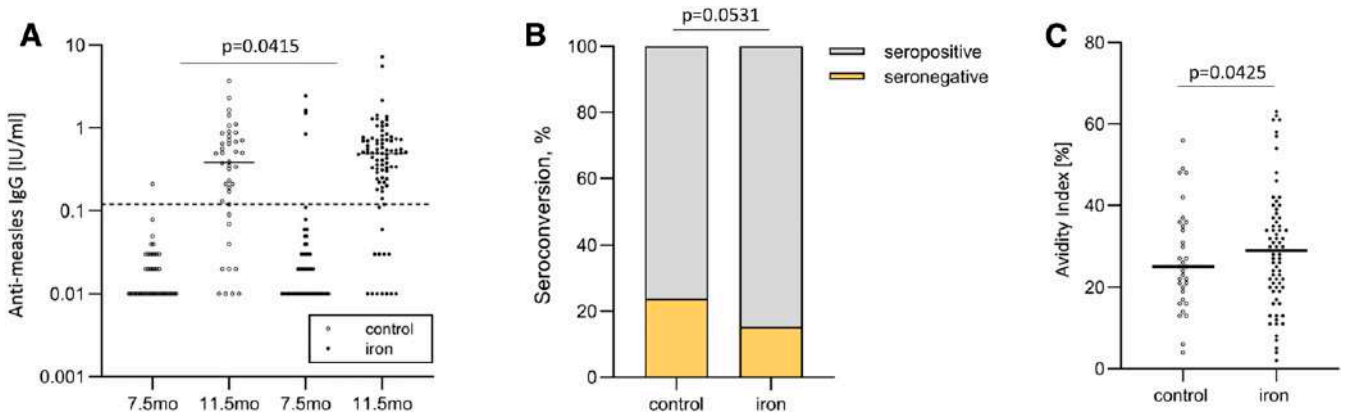

Figure 6. In a randomized controlled trial follow-up study of Kenyan infants (n = 155), antimeasles serum IgG concentrations, seroconversion, and IgG avidity in the control and iron groups. The iron group received 5 mg iron daily for 4 months at time of measles vaccination. (A) Anti-measles serum IgG concentrations at age 7.5 and 11.5 months (at baseline and end of intervention), (B) seroconversion at age 11.5 months (at end of intervention), and (C) IgG avidity at age 11.5 months (at end of intervention). Data analyzed with random intercept linear mixed effect models with Bonferroni corrected multiple comparisons. Adapted from Stoffel et al.[6]

for early detection of IDA in infancy and its correction with effective iron treatment, as well as ensuring adequate iron status in pregnant women. Because anemia is so common in African infants and because the vaccine-preventable disease burden is so high,[29] even if IDA only modestly reduces the immunogenicity of childhood vaccines, its prevention could have major benefits.

Conclusions

Returning to our clinical case, the likely etiology of anemia in this infant is iron deficiency due to several factors: low birthweight, resulting in low birth iron stores; exclusive breastfeeding, providing only very low levels of dietary iron; frequent diarrhea, resulting in increased gastrointestinal iron losses; and common infections, increasing serum hepcidin, which reduces iron absorption. According to protocols from the Kenyan Ministry of Health,[40] the infant should be treated with oral iron syrup at a dosage of 2 to 6 mg/kg body weight for 30 days, and exclusive breastfeeding should continue until age 6 months. Provided at time of vaccination, this additional iron may improve the infant's vaccine response. However, the mother should be told that the iron will probably darken the infant's stool, and it may cause diarrhea. If the latter occurs, the iron syrup should be discontinued and the infant be brought back to clinic for treatment. At age 6 months, if anemia persists, complementary foods rich in iron, such as eggs and green leafy vegetables, should be regularly fed to the infant. In addition, iron-fortified MNPs can be given to the infant; if possible, these should be given with a prebiotic to reduce adverse effects on the gut microbiome and risk for diarrhea.

Conflict-of-interest disclosure

No conflicts of interest.

Off-label drug use

None disclosed.

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Diagnosis and management of iron deficiency in chronic inflammatory conditions (CIC): is too little iron making your patient sick?

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While iron deficiency remains the most common cause of anemia worldwide, low iron stores are associated with symptoms regardless of the presence of typical microcytic, hypochromic anemia and may be hard to recognize in patients with concurrent inflammation. Diagnosing and treating iron deficiency become more of a challenge because markers of iron status are influenced by low-grade inflammation present in common conditions, such as chronic kidney disease, cirrhosis, or heart failure. Here I present a pragmatic way of interpreting diagnostic lab tests to help clinicians recognize patients who are most likely to benefit from iron supplementation, choose between oral and parenteral administration, and make personalized decisions when patients do not fit usual guidelines.

LEARNING OBJECTIVES

- Recognize chronic inflammatory conditions that affect the interpretation of laboratory markers of iron status
- Identify patients most likely to benefit from iron supplementation using ferritin and transferrin saturation
- Understand risks and benefits of oral and IV iron preparations

Clinical case

A 56-year-old woman was referred for evaluation of anemia. She had a medical history of rheumatoid arthritis treated with methotrexate, hypertension treated with lisinopril, type 2 diabetes mellitus treated with pioglitazone, nonalcoholic fatty liver disease, and stage 3 chronic kidney disease with an estimated creatinine clearance of 32 mL/min per 1.73 m^2. She reported progressive fatigue, dyspnea on exertion, and mental fogginess in the past 6 months. She had hemoglobin, 7.9 g/dL; hematocrit, 24%; mean corpuscular volume, 83 fL; and mean corpuscular hemoglobin, 29 pg, with reticulocytes at 2%. Additional laboratory results showed ferritin of 89 µg/L (reference range, 20-200 µg/L) and C-reactive protein (CRP) of 1.8 mg/L (reference value, <5 mg/L). Her rheumatologist was concerned that the patient's anemia was too severe to be explained by her autoimmune disease, which was under control, or by her comorbidities and requested a hematologist's opinion.

Distinguishing IDA from iron deficiency

Iron deficiency anemia (IDA) is the most common acquired anemia and should be the first consideration in a patient with unexplained anemia. The World Health Organization (WHO) defines anemia as hemoglobin <13 g/dL and <12 g/dL in adult men and nonpregnant women, respectively,[1] a well-known trigger for an investigation of ID. Low red cell mass occurs secondary to chronic reduction in iron availability, impairing the incorporation of the metal into the porphyrin ring to form heme, making hemoglobinization of erythroid precursors in the bone marrow (BM) incomplete.[2] In IDA, mature erythrocytes are typically hypochromic (with low mean corpuscular hemoglobin [MCH; <28 pg]) and microcytic (with low mean corpuscular volume [MCV; <80 fL]). A percentage of hypochromic red cells >6% and a reticulocyte hemoglobin equivalent (CHr or Ret-He) <29 pg, as provided by some modern cell counters, also supports iron-restricted erythropoiesis.

ID is defined as "a health-related condition in which iron availability is insufficient to meet the body's needs and which can be present with or without anemia,"[3(p1069)] and it is fundamentally recognized that IDA is simply the most advanced stage of ID. In ID, iron stores are progressively exhausted before red cell morphology of hemoglobin levels are affected, and patients may experience early

symptoms such as fatigue, reduced cognitive performance, and exercise intolerance. Overlap of ID and other disorders, such as chronic liver or kidney disease, may prevent the MCH and MCV from decreasing, and such indices also become unreliable for use in screening for ID in the presence of thalassemia trait, a frequent hereditary anemia.

We therefore recommend investigating ID in all patients with unexplained signs and symptoms of ID, regardless of the presence of anemia, low MCH, or low MCV, and in those patients with conditions that pose a higher risk for ID, either by increased iron loss (caused by chronic or recurrent bleeding and use of anticoagulants) or by reduced iron absorption (related to, eg, gastrointestinal [GI] disorders, surgical resections, or chronic use of proton pump inhibitors) (Table 1).

Ferritin in absolute and functional ID

Measuring ferritin levels is the recommended approach to initiating an investigation of ID, because serum ferritin correlates well with body iron determined by serial phlebotomies.[4] Ferritin is primarily an intracellular iron-binding protein with main functions of sequestering iron and ensuring correct incorporation into enzymes and hemoglobin, while preventing the generation of reactive species through Fenton chemistry.[5] Nevertheless, only a minute fraction circulates as serum ferritin, which is rather iron poor and is secreted by macrophages through a nonclassic lysosomal pathway.[6,7] Thus, the serum ferritin level should not be regarded as a direct measurement of iron stores.

WHO guidelines recommend a ferritin level <15 μg/L as a sign of absolute ID in adults,[8] although a cutoff of 30 μg/L is more often used because of its higher sensitivity (~92%) and high specificity (98%).[9] Unfortunately, its high accuracy is lost in the presence of inflammation. An acute-phase reaction is triggered by proinflammatory cytokines, particularly interleukin-6 (IL-6), IL-1, and tumor necrosis factor (TNF), in response to infection or tissue injury, making hepatocytes increase the synthesis of acute-phase proteins,[5] including ferritin and hepcidin. One of the functions of an acute-phase reaction is to prevent iron from being scavenged by pathogens. Hepcidin is a predominantly liver-derived regulator of iron trafficking. It binds to ferroportin, the only iron exporter found on the membrane of mammalian cells and reduces iron export, lowering iron in circulation. Hepcidin-mediated ferroportin blockade traps iron inside cells, such as hepatocytes and macrophages, which in turn produce ferritin to store iron safely. That mechanism underlies functional iron deficiency (FID; pathogenesis and management are reviewed elsewhere[10]). In absolute ID, mechanisms are activated to replenish iron: low hepcidin production keeps ferroportin on the membranes to facilitate iron absorption, and transferrin is upregulated to increase total iron binding capacity (TIBC) and transport of iron to the tissues. A comparison between absolute ID (Figure 1) and FID (Figure 2) shows that both have low serum iron and elevated ferritin, and low TIBC characterizes FID. Their opposing reactions to low and high intracellular iron render ferritin levels of limited help in distinguishing between isolated FID and the association between absolute ID and FID.[4] Other biomarkers, such as soluble transferrin receptor, the soluble transferrin receptor/log ferritin index, and hepcidin levels, have been regarded as improving the ability to detect absolute ID in combination with FID, but there is a lack of standardization and limited availability for broader use.[11]

Serum inflammatory markers in CICs

Despite their limitations, markers of inflammatory activity, such as erythrocyte sedimentation rate (ESR) and CRP levels have survived the test of time and are often used in clinical practice to help interpret ferritin levels, because ferritin is an acute-phase reactant. Overt inflammation with high ESR and CRP levels has usually been found in active autoimmune disorders (eg, Still's disease, rheumatoid arthritis, and inflammatory bowel disorders [IBDs]) and in chronic infections (eg, tuberculosis and chronic osteomyelitis). Nevertheless, ESR varies with hematocrit and is driven mostly by the production of fibrinogen and immunoglobulins, which last for several days in the circulation, whereas CRP is mainly produced by the liver in response to cytokines, particularly IL-6, and has a much shorter half-life; discrepancies between ESR and CRP are unsurprisingly common.[12] CRP >50 mg/L is frequent in bacterial infections, making it an excellent marker of acute inflammation, whereas the less-noted α-1-acid glycoprotein (AGP) increases later in the inflammatory process and is more suitable for confirming chronic inflammation.[13] Because ferritin increases >5 times in patients with CRP >80 mg/L than in those with CRP <10 mg/L,[14] studies have examined the

Table 1. Mechanisms contributing to ID in CICs

Condition	Reduced iron absorption	Increased iron loss
CKD	Anorexia/GI tract edema; frequent use of proton pump inhibitors; use of phosphate chelators; high hepcidin with blockade of duodenal absorption	Uremic platelet dysfunction; antiplatelet therapy and anticoagulation; blood loss from hemodialysis
HF	Anorexia/GI tract edema; high hepcidin with blockade of duodenal absorption	Antiplatelet therapy and anticoagulation
IBDs	High hepcidin with blockade of duodenal absorption; small bowel resection	Chronic diarrhea with high epithelial turnover; GI tract bleeding; use of corticosteroids
Obesity	High hepcidin due to adipose tissue inflammation; bariatric surgery	Increased uterine bleeding (when associated with polycystic ovarian syndrome)
Liver disease	Anorexia/GI tract edema; diarrhea caused by laxatives	Variceal bleeding; thrombocytopenia; coagulopathy
Rheumatologic disorders	High hepcidin with blockade of duodenal absorption	Use of corticosteroids and NSAIDs

NSAIDs, nonsteroidal anti-inflammatory drugs.

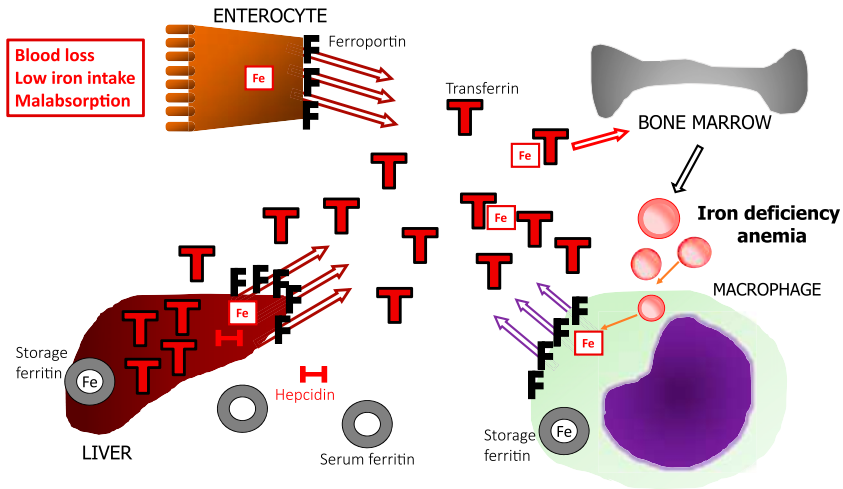

Figure 1. Schematic representation of the regulation of iron metabolism in absolute ID. Iron depletion occurs commonly and is related to associations among blood loss, low dietary iron intake, and malabsorption. Low iron decreases hepcidin (H) production, allowing for ferroportin (F) activity in duodenal enterocytes, to transfer iron (Fe) absorbed from the diet to transferrin (T), and mobilize iron stored in hepatocytes and macrophages. With progressive iron depletion, the intracellular store of ferritin (iron-rich) is depleted, and serum ferritin (iron-poor) release by macrophages decreases proportionately, along with a progressive decrease in circulating transferrin-bound iron. Low iron also upregulates hepatic production of transferrin, resulting in high TIBC, contributing to low TSAT. Lack of iron available to the BM eventually manifests as hypochromic, microcytic anemia.

possibility of correcting ferritin for inflammatory activity. The Biomarkers Reflecting Inflammation and Nutrition Determinants of Anemia (BRINDA) research group found that a regression correction of ferritin using CRP >5 mg/L and AGP >1 g/L increased the prevalence of ID by 3% to 7%, even in countries with a low burden of infection, such as the United States,[15,16] and a different regression correction using CRP and albumin increased the prevalence of ID from 7% to 24% in another study.[17] Therefore, in areas of widespread inflammation or infection, the 2020 WHO guidelines[18] strongly endorse the measurement of CRP and AGP, but make a conditional recommendation to use a ferritin threshold of 70 µg/L to define iron deficiency in patients with CRP >5 mg/L or AGP >1 g/L or to implement arithmetic or regression correction of ferritin levels based on those markers. The guideline may not apply to all patients with chronic inflammatory conditions (CICs), such as obesity, chronic

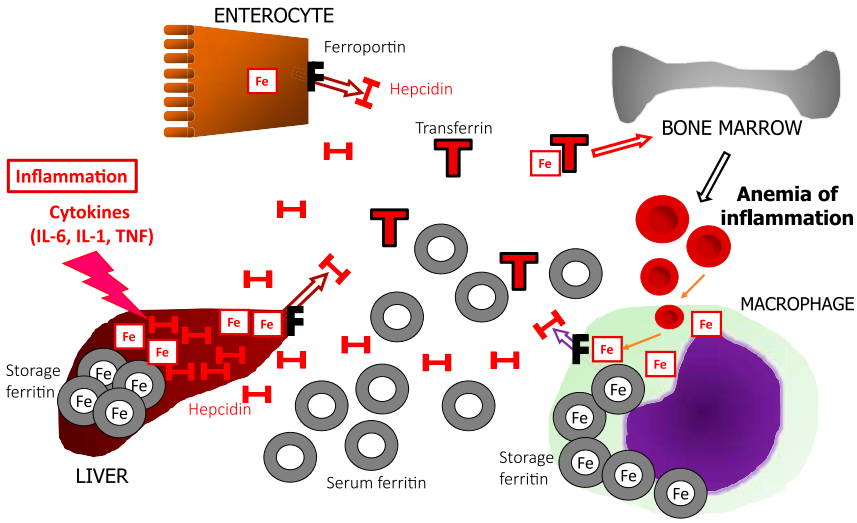

Figure 2. Schematic representation of the regulation of iron metabolism in FID in CICs. Inflammation with increased cytokine production causes upregulation of liver hepcidin (H), which binds to ferroportin (F). Enterocytes are prevented from exporting absorbed iron (Fe) to transferrin (T) in the bloodstream. In hepatocytes and macrophages, iron is also trapped intracellularly and is stored as iron-rich ferritin, whereas macrophages increase iron-poor serum ferritin in circulation. High intracellular iron also downregulates transferrin production, lowering TIBC. Iron release is so restricted that the decrease in serum iron still lowers TSAT despite low TIBC. Iron restriction eventually leads to the anemia of inflammation. Both ID and FID have hypoferremia but low TIBC, and high ferritin characterizes FID.

kidney disease (CKD), liver disease, and heart failure (HF), in whom an increase in CRP is frequently absent, or where AGP measurements are not routinely available. Low-grade inflammation in a CIC is enough to disrupt iron metabolism by increasing hepcidin, but does not necessarily correlate with inflammatory markers. Moreover, other mechanisms put patients with CICs at higher risk of ID and underscore the need to make a correct diagnosis despite interference in iron parameters (Table 1).

Ferritin in CIC: making the best of an imperfect tool

Various ferritin cutoff values have been recommended to help detect ID in different patient populations, such as in those with CKD, HF, and IBD.[3] There is a general consensus that the usual ferritin cutoff of 30 μg/L is inappropriate in the presence of a CIC but the recommended ferritin values range between 50 and 500 μg/L across guidelines.

A pragmatic way of understanding the implications of a certain ferritin threshold is to examine studies comparing ferritin levels with BM iron, the gold-standard test for determination of iron stores. Bone marrow iron deficiency (BMID) is ID confirmed by the absence of granules of hemosiderin in macrophages and erythroblasts and requires an invasive procedure to obtain an adequate BM sample stained with Prussian blue (or Perls' stain). Its indication in clinical practice by itself has become rare with the ease of the use of ferritin, but it may occasionally prove useful in patients who undergo BM sampling for other reasons.

A systematic review[19] examined 38 studies of BM iron in nonhealthy adults with rheumatoid arthritis, liver disease, hematologic disorders, and other CICs. They found 1023 people with confirmed BMID with mean ferritin between 33.6 and 158.3 μg/L, whereas individuals with detectable BM iron had a mean ferritin >171.6 μg/dL. In the CKD population, ferritin values vary more broadly. Ten deceased patients with dialytic CKD and BMID had ferritin values between 537 and 3994 μg/L; the researchers acknowledged that 4 of the patients had rare minute deposits of iron, but even assuming they would have the highest ferritin values, the maximum value of ferritin in a patient with BMID with dialytic CKD would be in the 1000 to 2000 μg/L range.[20] Another study found that 3 of 96 patients were receiving hemodialysis with BMID, with ferritins in the 100 to 1100 μg/L range.[21] More recent studies reported ferritin of 36 to 100 μg/L in HIV$^+$ patients with BMID, of whom half had a diagnosis of tuberculosis or Epstein-Barr viremia, and >25% had CMV viremia.[22] In HF, patients with true BMID were found to have ferritin levels ranging from 44 to 162 μg/L (interquartile range).[23] Except in patients with CKD and some with HF, patients with BMID in CICs appear to have a ferritin level rarely >200 μg/L.

Transferrin saturation in CIC: a helping hand

Transferrin saturation (TSAT) <6% in combination with low ferritin is diagnostic of ID, but in the presence of inflammation, a seemingly arbitrary TSAT <20% is often used to diagnose ID. Because there is a significant overlap in ferritin levels between samples with BMID and normal BM iron (range, 50-500 μg/L), TSAT helps identify patients who are more likely to benefit from iron supplementation. Normal TSAT of 20% to 45% is associated with adequate iron stores in most CICs, because hepcidin is expected to lower TSAT by blocking iron export. In patients with HF who undergo coronary artery bypass graft, TSAT <19.8% and serum iron <13 μmol/L were independently associated with mortality and were most accurate for BMID. In patients with HF, TSAT >20% essentially excluded the possibility of BMID, regardless of ferritin levels.[23] In patients with nondialysis CKD (ndCKD) who underwent BM evaluation, TSAT below 20% had only 50% sensitivity but 83% specificity to detect BMID, and the specificity for BMID improved to 98% if associated with a ferritin level <100 μg/L, with a reduction in sensitivity to 33%. TSAT <25% yielded maximum sensitivity of 71%.[24] In another study, BMID was identified in only 50% of patients with both TSAT <20% and ferritin <100 μg/L, but TSAT <20% alone had a sensitivity of 85% and specificity of 48%.[25] Those data suggest that underlying ID can still be considered in patients with CKD with TSAT of 20% to 25%, whereas for other CICs, TSAT <20% along with judicious evaluation of ferritin to diagnose ID seems appropriate.

Figure 3 shows TSAT and ferritin levels found in patients with different CICs, with and without BMID. BMID is found in patients with a ferritin range between 30 and 200 μg/dL and TSAT between 10% and 20%. Based on the available evidence from BMID studies, the map in Figure 4 has been designed to help estimate the adequacy of iron stores and to aid in interpreting ferritin and TSAT in patients with CIC. Patients with iron stores estimated to be low (in black and red) should be considered for iron supplementation. Yellow striped areas represent areas in which iron supplementation may be considered, depending on the CIC; only patients with CKD are likely to benefit from iron supplementation with ferritin <200 μg/L and TSAT of 20% to 25%, whereas patients with HF or CKD treated with erythropoiesis-stimulating agents (ESAs) and/or hemodialysis may be considered for iron supplementation if TSAT is <20% and ferritin is up to 500 μg/L. Patients in the green areas most likely have adequate stores and should not receive supplemental iron.

Back to the case

A complete iron panel showed low serum iron (54 mg/dL), normal TIBC (300 mg/dL), and low TSAT (18%). Despite a ferritin level of 89 μg/L (considered normal for healthy individuals), the presence of ferritin <200 μg/L, a TSAT <20% in the presence of several CICs (liver disease, controlled rheumatoid arthritis, and stage 3 ndCKD), and hypoproliferative normochromic, normocytic anemia supported a diagnosis of IDA. The patient underwent an upper endoscopy and colonoscopy, and a bleeding gastric ulcer was detected, for which omeprazole was prescribed. She asked whether she could take iron tablets or should receive "iron injections," which she had heard carry a risk for allergic reactions.

Management of ID in CICs

The treatment of absolute IDA has been extensively reviewed elsewhere,[26,27] but the mainstay of the recommendations for ID in patients with CIC must include investigating underlying causes and implementing appropriate iron supplementation.

Investigation of underlying causes of ID

Patients should always be investigated for blood loss, such as uterine and GI bleeding. Hematuria and epistaxis should be included in the inquiry because patients frequently fail to mention them. Vegetarianism or veganism should not be

Table 2. Characteristics and side effects of most commonly available oral iron supplements

Iron formulation (example US brand names) (dose per tablet/Fe dose)	Usual dose	Most common side effects (>1%)	Observations
Ferric citrate (Auryxia) (1000 mg/210 mg Fe)	2 tablets once daily (LR)	>20%: fecal discoloration, diarrhea; 10-20%: constipation, nausea; 1-10%: hyperkalemia, cough.	Phosphate binder, approved for use in ID in ndCKD.
Ferrous fumarate (Ferretts, Ferrimin, Hemocyte) (324 or 325 mg/106 mg Fe)	1 tablet every other day (>100 mg Fe per dose)	>10%: constipation, fecal discoloration, nausea, stomach cramps, vomiting; 1-10%: dental discoloration, diarrhea, urine discoloration	Cereals, dietary fiber, tea, coffee, eggs, and milk may decrease absorption.
Ferrous gluconate (Ferate) (240 mg/27 mg Fe; 324 mg/38 mg Fe)	3-4 tablets every other day (>100 mg Fe per dose)	>10%: constipation, fecal discoloration, nausea, stomach cramps, vomiting; 1-10%: dental discoloration, diarrhea, urine discoloration.	
Polysaccharide iron complex (EZFE, Ferrex, NovaFerrum) (50 mg Fe)	2 tablets every other day (>100 mg Fe per dose)	>10%: constipation, fecal discoloration, nausea, stomach cramps, vomiting; 1-10%: dental discoloration, diarrhea, urine discoloration.	
Ferrous sulfate (several) (324 or 325 mg/65 mg Fe)	2 tablets every other day (>100 mg Fe)	>50%: fecal discoloration, abdominal pain, nausea; 20-50%: constipation, vomiting, diarrhea.	
Ferric polymaltose (Maltofer; not available in the US) (357 or 370 mg/100 mg Fe)	1-3 tablets once daily (LR)	>10%: fecal discoloration; >1%: diarrhea, nausea, abdominal pain, constipation.	—
Heme iron polypeptide (Proferrin) (10.5-12 mg Fe)	1 tablet once daily (LR)	Incidence unknown: constipation, abdominal pain, diarrhea, muscle cramps.	—
Ferric maltol (Accrufer) (30 mg Fe)	1 tablet twice daily (LR)	1-10%: fecal discoloration, constipation, diarrhea, abdominal pain, nausea, vomiting.	Absorption may be decreased with food.

Fe, elemental iron; LR, label recommendation.

considered to cause ID, because compensatory upregulation of the absorption of nonheme iron occurs. In CICs, polypharmacy is the rule, and chronic use of some medications can predispose patients to GI bleeding (eg, corticosteroids, nonsteroidal anti-inflammatory drugs, aspirin, and anticoagulants), and use of other medications can impair iron absorption (eg, proton pump inhibitors and laxatives). In patients with chronic diarrhea, high epithelial turnover impairs absorption and mucosal inflammation can cause bleeding. Blood loss may also increase with frequent blood draws during an admission or in equipment circuits, in patients on hemodialysis, for example.

Iron supplementation: oral or IV?

The choice of route of administration of iron should take comorbidities and the patient's preference into consideration. Oral treatment is cost effective, easily available, and should always be considered. There is no specific iron-containing preparation recommended to treat ID (Table 2), and evidence in pure ID/IDA supports that a single minimum dose of 60 mg of elemental iron administered on alternate days can be adequate and maximize tolerability,[26,28,29] but studies in patients with CIC who are following such a regimen are lacking.

Parenteral iron is often used because numerous systematic reviews have identified the superiority of parenteral iron over oral iron for patients with IBD, HF, CKD, or perioperative anemia. Other indications for parenteral iron include GI tract resection (including bariatric surgery), prolonged use of inhibitors of iron absorption (eg, proton pump inhibitors), and GI intolerance to oral iron (reported in 30% to 70% of patients).

CIC cause hepcidin elevation and may preclude GI absorption. In the future, hepcidin measurement may help identify patients with significant blockade of duodenal iron absorption indicating upfront parenteral iron. Patients with several comorbidities may also prefer parenteral iron to avoid adding another pill to their routine.

A growing portfolio is currently available in the United States: low-molecular-weight iron dextran, iron sucrose, ferric gluconate, ferumoxytol, ferric carboxymaltose (FCM), and ferric derisomaltose (previously known as iron isomaltoside; Table 3). Low-molecular-weight iron deficiency, iron sucrose, and ferric gluconate may require several shorter infusions, whereas the remainder have become increasingly popular because of the lower number of visits required to administer high-dose infusions, despite the higher cost of the medication.

The route of administration in CICs may shift back to oral with the ongoing success of trials of novel iron formulations that have better absorption and tolerance, such as ferric citrate (a phosphate binder approved for use in ndCKD) and ferric maltol,

Table 3. IV iron preparations: test dose, dosage, side effects, and average wholesale pricing

Iron preparation	Test dose	Usual dosage for adults	Most common side effects	Estimated AWP (US dollars)	Observations[32]
Iron sucrose/ iron saccharate	Not recommended; If history of drug allergies, consider test dose with 25-mg IV, slow push.	1000 mg per treatment; 10 doses of 100 mg IV 2-5 min in consecutive hemodialysis sessions; 5 doses of 200 mg within 14 d or weekly, by IV 2-5 min or in 100 mL NS infusion for >15 min.	>20%: hypotension and muscle cramps in hemodialysis patients; 10-20%: nausea, headache, nasopharyngitis; 1-10%: hypotension, hypertension, edema, chest pain, dizziness, pruritus, GI symptoms, injection site reaction, myalgia, conjunctivitis, dyspnea, cough, fever.	$432.00 (1000 mg Venofer)	1-wk interval recommended before MRI.
LMW iron dextran	Recommended before the first dose; 25 mg (0.5 mL) IV push for 30 s; observe for 1 h.	1000 mg per treatment; 10 doses of 100 mg >2-min undiluted IV infusion daily; Single dose of 1000 mg 1-h IV infusion in 250 mL NS (off label).[35]	Incidence unknown: hypotension, flushing, headache, urticaria, GI symptoms, anaphylaxis, injection site reaction, myalgia, dyspnea, wheezing, fever.	$350.60 (1000 mg INFeD)	Not to be confused with high-molecular-weight dextran (discontinued); 4-wk interval recommended before MRI.
Ferric gluconate	Previously recommended, but currently not on label; If history of drug allergies, consider test dose with 25 mg (2 mL) in 50 mL NS for 60-min infusion.	1000 mg per treatment; 8 doses of 125 mg IV bolus undiluted for 10 min for patients in hemodialysis or 1-h infusion in 100 mL NS.	>20%: hypotension, vomiting, nausea, headache, diarrhea, injection site reaction, muscle cramps; 10-20%: dizziness, tachycardia, hypertension, dyspnea; 1-10%: chest pain, thrombosis, edema, pruritus, hyperkalemia, abdominal pain, pharyngitis, cough, fever; <1%: flushing, hypersensitivity reaction.	$610.40 (1000 mg Ferrlecit)	1-wk interval recommended before MRI.
Ferumoxytol	Not recommended	1020 mg per treatment; 2 doses of 510 mg, >15 min infusion in 50-250 mL NS or 5% dextrose, 3-8 d apart or single 1020-mg dose, 30-min infusion; Observation for 30 min after infusion recommended.	1-10%: hypotension, edema, chest pain, hypertension, dizziness, headache, pruritus, rash, diarrhea, nausea, constipation, vomiting, abdominal pain, hypersensitivity reaction, cough, dyspnea, fever.	$2571.42 (1020 mg of Feraheme)	12- (US) to 24- (Europe) wk interval recommended before MRI.
FCM	Not recommended	1500 mg per treatment; 2 doses of 750 mg undiluted IV at 100 mg/min, or in 250 mL NS 15-min infused 7 d apart; Observation recommended for 30 min after infusion.	>10%: hypophosphatemia (<2 mg/dL); 1% to 10%: hypertension, hypotension, flushing, skin discoloration at injection site, nausea, vomiting, abdominal pain, increased ALT, dizziness, headache; <1%: abdominal pain, anaphylaxis, diarrhea, increased GGT, injection site reaction, paresthesia, skin rash, sneezing.	$2735.10 (1500 mg of Injectafer)	Verification of phosphate levels is recommended for repeated infusions; 1-wk interval recommended before MRI.
Ferric derisomaltose	Not recommended	1000-mg single dose in 100-500 mL NS with a final concentration >1 mg iron/mL, infused over >20 min.	1-10%: hypophosphatemia, skin rash, nausea; <1%: asthma, severe hypersensitivity reaction.	$2957.10 USD (1000 mg of Monoferric)	Previously iron isomaltoside; verification of phosphate levels is recommended for repeated infusions; 4-wk interval recommended before MRI.

ALT, alanine transferase; AWP, average wholesale price (reported on UpToDate.com; last accessed 25 September 2020); GGT, γ-glutamyl transferase; LMW, low-molecular-weight; MRI, magnetic resonance imaging; NS, normal saline (0.9% sodium chloride); USD, US dollars.

or those that do not depend on ferroportin (eg, Sucrosomial iron) and are currently in clinical trials.[30]

Back to the case

IV iron was indicated because the use of a proton pump inhibitor precludes adequate oral iron absorption, and the patient's concerns about side effects of parenteral iron were addressed. She received an infusion of FCM uneventfully. A week later, she called the office to report that she was still feeling weak and wondered whether her anemia was getting worse. Her laboratory results showed that her hemoglobin had had a minor increase from 7.9 to 8.2 g/dL, but her phosphate levels were moderately decreased at

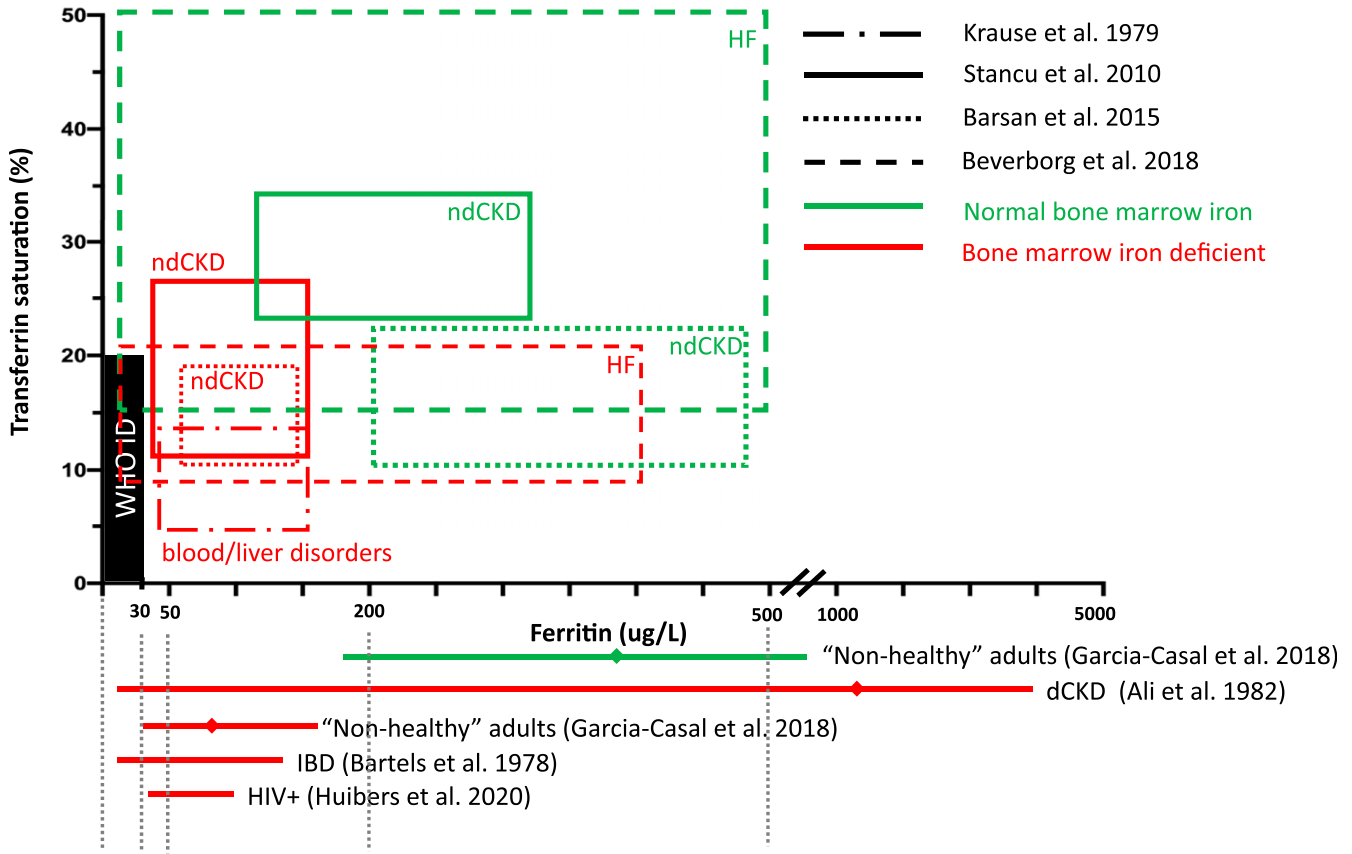

Figure 3. Ferritin and TSAT ranges reported by studies that evaluated BM iron in patients with CICs. Data include patients with HF, dialytic CKD or ndCKD, HIV infection, IBDs,[36] and data from a systematic review of 38 studies in nonhealthy patients, including blood disorders, liver conditions, rheumatoid arthritis, among others.[19] The area in red represents the thresholds for absolute ID recommended by WHO (ferritin >30 µg/L and TSAT >16%). Patients with BMID have ferritin <160 µg/L and TSAT <20%. TSAT 20% to 25% is still associated with BMID in CKD, and TSAT <20% may still predict BMID in patients with ferritin up to 500 µg/L with HF or CKD treated with ESAs, with or without hemodialysis. Studies that reported only ferritin levels are represented by red lines beneath the x-axis that encompass the range, and means are represented by diamonds situated on the lines.

1.6 mg/dL. She eventually completed her treatment with hemoglobin of 10.4 g/dL, ferritin of 359 µg/L, and TSAT of 35%.

Adverse events with IV iron supplementation and management

Before starting parenteral iron, patients should be informed about potential adverse events. Parenteral iron still enjoys the bad reputation of causing severe allergic reactions, mostly because of frequent reactions to high-molecular-weight iron dextran, which has been discontinued, but some manufacturers still recommend a test dose for some formulations (Table 3). The most common side effects of current IV iron formulations are hypotension, headache, injection site reactions, and GI symptoms. Skin discoloration from extravasation is also a possible complication and patients should be informed of that particular risk. FCM and ferric derisomaltose have been associated with the development of hypophosphatemia in 27% to 90% and 4% of treatments, respectively, attributable to an increase in fibroblast growth factor 23 with renal phosphate wasting. Hypophosphatemia is usually asymptomatic, but exacerbation of symptoms of anemia may be caused by lower levels of 2,3-diphosphoglycerate in erythrocytes, an increase in hemoglobin's affinity for oxygen, and limited oxygen delivery to the tissues.[31] Verifying phosphate levels is recommended in symptomatic patients, in those who require repeated infusions with those compounds, or in those at higher risk for low phosphate levels (eg, patients treated with renal replacement therapy, those with chronic diarrhea, and those who have undergone a parathyroidectomy secondary to end-stage renal disease), or in those on medications associated with low absorption or increased excretion of phosphate (antacids, phosphate binders, niacin, acetazolamide, imatinib, and sorafenib). Mild (1.8-2.5 mg/dL or 0.6-0.8 mmol/L) to moderate (1.0-1.8 mg/dL or 0.3-0.6 mmol/L) decreases in phosphate levels can be managed with dietary changes to increase ingestion of phosphate-rich food (eg, dairy, poultry) and/or oral potassium phosphate. Severe hypophosphatemia (below 1.0 mg/dL or 0.3 mmol/L) is exceedingly rare but requires parenteral phosphate infusion to prevent seizures and arrhythmias.

Iatrogenic iron overload is another concern in the absence of reliable ferritin levels. Infused iron is captured by Kupffer cells,

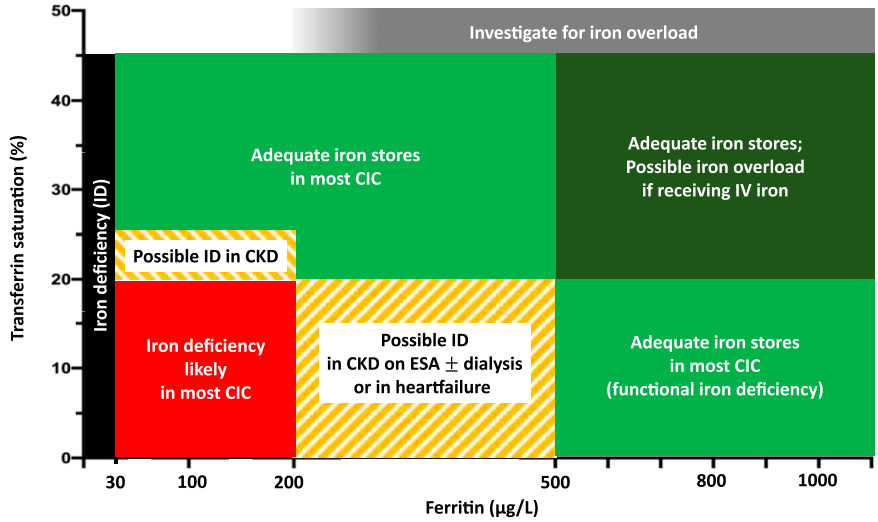

Figure 4. Assessment of iron stores using ferritin and TSAT in CICs. Ferritin <30 µg/L in the presence of TSAT <45% is indicative of absolute low iron stores (black). Most patients with CICs in association with true ID are found to have TSAT<20% and ferritin <200 µg/L (red). Patients in the yellow region may be considered for iron supplementation if TSAT is 20% to 25% in CKD, or if TSAT is <20% and ferritin is up to 500 µg/L in HF, if they are receiving dialysis, and/or if they are using ESAs. Adequate iron stores are expected in the green areas, but caution is recommended for patients in the dark green area (TSAT >20% and ferritin >500 µg/L) if they are receiving parenteral iron, because they may be at risk of iatrogenic iron overload.

which become overloaded and gradually shuttle the iron to hepatocytes. Liver iron overload has been diagnosed by MRI in up to 84% of patients with dialytic CKD and is associated with the infusion of more than 250 mg of iron per month.[32] Kidney Disease Improving Global Outcomes 2012 guidelines[33] warn against iron supplementation in patients with CKD with ferritin >500 µg/L, but MRIs have shown that patients with ferritin in that range may have significant iron overload. Recently, a large randomized clinical trial favored the use of a high-dose regimen of 400 mg/mo of iron to lower risk of death and nonfatal cardiovascular events in patients in hemodialysis within a 2-year time frame, but did not report incidence of liver iron overload, so concerns for late effects of excess iron remain.[34] If iatrogenic iron overload is suspected, MRI can be used, but different intervals for each iron formulation are recommended before MRI scans, to prevent interference with imaging (Table 3).[32] In patients on hemodialysis with confirmed iron overload, the discontinuation of iron infusions has been shown to correct it slowly over several months without the need for iron chelators.

Conclusion

CICs caused by CKD, HF, and other disorders make the diagnosis of ID more difficult, but knowledge of how ferritin and TSAT measurements behave in concurrent CICs and ID helps identify patients who are more likely to benefit from iron supplementation. Patients and physicians should discuss risks and benefits of oral and parenteral iron preparations to make personalized treatment decisions, especially when patients have multiple comorbidities and do not fit the available guidelines.

Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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Chimeric antigen receptor T cells for mature B-cell lymphoma and Burkitt lymphoma

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Chimeric antigen receptor (CAR) T-cell therapy has changed the landscape of immunotherapy for B-cell malignancies, including mature B-cell lymphomas. Although two CD19 CAR T-cell products have been commercially approved to treat relapsed/refractory B-cell lymphomas, outcomes in these patients remain inferior to those of patients with B-cell leukemia, regardless of therapy. Recent clinical studies and preclinical reports suggest that certain characteristics, such as the suppressive lymphoma tumor microenvironment and inferior endogenous T-cell fitness, may contribute to discrepant responses in these patients. In addition, these studies revealed that limited CAR T-cell persistence and tumor antigen escape, which also impact B-cell acute lymphoblastic leukemia, may play a more prominent role in lymphoma. Multiple promising strategies to overcome these barriers have advanced to clinical trials. In this review, we assess CAR T-cell therapies for pediatric relapsed/refractory mature B-cell lymphomas, potential obstacles diminishing antitumor activity and limiting CAR T-cell persistence, and current strategies to overcome these obstacles.

LEARNING OBJECTIVES

- Understand current chimeric antigen receptor T-cell therapy options for mature B-cell and Burkitt lymphomas in pediatric patients
- Describe challenges and optimization strategies specific to chimeric antigen receptor T-cell therapy for mature B-cell lymphomas

Introduction

T cells genetically modified to express chimeric antigen receptors (CARs) targeting CD19 have changed how practitioners salvage refractory B-cell acute lymphoblastic leukemia (B-ALL), including use of allogeneic hematopoietic stem cell transplant (allo-HSCT). The widespread expression of CD19 and accessibility of leukemic blasts make B-ALL an ideal target for CAR T cells. Several groups reported remarkable complete remission (CR) rates, up to 90%, in heavily pretreated patients with relapsed/refractory (r/r) disease receiving a single CD19.CAR T-cell infusion preceded by cyclophosphamide and fludarabine (Cy/Flu) lymphodepletion.[1-3] On the basis of these successes, in 2017, tisagenlecleucel (tisa-cel; Kymriah [FMC63 single-chain variable fragment, 4-1BBζ]; Novartis) was commercialized for refractory acute lymphoblastic leukemia (ALL) in second or greater relapse.

In adults with B-cell non-Hodgkin lymphoma (B-NHL), CD19.CAR T cells have induced durable responses,[4,5] leading to US Food and Drug Administration approval of axicabtagene ciloleucel (axi-cel; Yescarta [FMC63 single-chain variable fragment, CD28ζ]; Kite Pharma/Gilead Sciences) and later tisagenlecleucel. However, these therapies remain limited to investigational trials in pediatric B-cell lymphomas.

CAR T cells are an appealing therapy for B-cell lymphomas due to the widespread expression of targetable antigens, with CD19 being the most frequent. Still, despite the fact that CD19 is uniformly expressed on >95% of B-cell lymphomas/leukemias, response rates to CD19.CAR T cells remain lower for lymphomas. Results in adults with B-NHL have been variable, with CR rates ranging from 52% to 82%.[4-6] However, it remains largely unknown why mature CD19$^+$ malignancies appear to be less sensitive to CD19.CAR T cells than their less mature counterparts. Furthermore, Burkitt lymphoma (BL), a predominantly pediatric/young adult malignancy, is the least studied CD19-expressing malignancy in CAR T-cell trials, and results reported to date have varied. Using a patient scenario, we review obstacles that decrease responses to CAR T cells in pediatric mature B-cell lymphomas, as well as strategies under investigation to overcome them, before reviewing ongoing and upcoming clinical trials for this patient population.

Clinical case

A 16-year-old boy presented with a 2-week history of neck and right arm swelling and a large mediastinal mass seen upon imaging. Lymph node biopsy revealed diffuse large B-cell lymphoma (DLBCL) positive for CD19/CD20/BCL-2/BCL-6. After rituximab, cyclophosphamide, vincristine, prednisone, doxorubicin, and high-dose methotrexate, he achieved CR. Eight months after completing treatment, the mediastinal mass recurred. He was treated with 6 cycles of rituximab, ifosfamide, carboplatin, and etoposide, followed by radiation to the mediastinum (achieving CR), followed by high-dose chemotherapy and autologous HSCT. Eight months after HSCT, however, he experienced relapse. Now 18 years old with multiply relapsed disease, he received a commercially available CD19 CAR product after lymphodepletion. Following infusion, he developed grade 2 cytokine release syndrome (CRS) requiring tocilizumab and anakinra. Four weeks after CAR T-cell treatment, positron emission tomography–computed tomography (PET-CT) revealed a partial response.

Understanding the mechanisms of this patient's PR will inform his treatment. The residual PET avidity may represent disease, though activated T cells at disease sites could cause a "pseudoflare" effect. Ongoing research addresses whether patients with B-NHL have a slower response to CAR T cells than patients with ALL. Also, whether biopsy would be helpful in this situation remains to be seen. Several clinical studies incorporate checkpoint inhibition to improve CAR T-cell persistence and clinical responses, a strategy that might benefit this patient. Below, we explore in detail how the field is addressing these and other pressing questions to optimize CAR T cells in pediatric patients with mature B-cell lymphoma.

Pediatric mature B-cell lymphomas: pathophysiology and treatment

NHL is the fourth most common malignancy in children and adolescents[7] and encompasses a heterogeneous group of malignancies that originate from B or T lymphocytes and natural killer (NK) cells. Unlike adult NHL, which typically presents as low- or intermediate-grade disease, mature B-NHLs in children (eg, BL, DLBCL, and primary mediastinal large B-cell lymphoma) often present as aggressive, disseminated disease, sometimes with marrow and central nervous system involvement. BL is the most common aggressive pediatric B-cell lymphoma. Although BL and Burkitt-like NHL predominate among younger children, DLBCL occurs more commonly in adolescents. Despite aggressive phenotypes, most children with BL and DLBCL initially respond to treatment, with >90% 4-year event-free survival.[8] These tumors exhibit a mature phenotype, expressing surface immunoglobulin and B-cell markers CD19, CD20, and CD10.[7]

Adding the anti-CD20 monoclonal antibody rituximab to chemotherapy has improved outcomes in B-NHL.[9] However, in the 10% to 20% of patients who experience relapse or have refractory disease, objective response rates (ORRs) are only 20% to 30% after conventional salvage chemotherapy, and 5-year survival rates are dismal (10% to 30%, rising to ~30% to 50% in patients who qualify for subsequent transplant).[10] These statistics and impressive results of CD19.CAR T cells for pre–B-ALL have increased interest in CD19.CAR T cells for pediatric B-NHL.

CAR T cells for mature B-cell lymphomas

In adults (aged ≥18 years), two products—tisagenlecleucel and axi-cel—have been approved by the US Food and Drug Administration for patients with B-cell lymphoma in whom two previous lines of treatment have failed.

The ZUMA-1 phase 2 multicenter trial treated 81 patients with r/r DLBCL with axi-cel preceded by lymphodepletion, reporting an ORR of 82% and CR of 49%.[5] OS at 18 months was 52%. Results were similar after 2 years: ORR of 83% and CR of 58%.[11] Notably, patients did not receive "bridging chemotherapy" during CAR T-cell manufacture, which averaged 17 days, but only 1 patient died of disease progression before receiving CAR T cells.

In the JULIET phase 2 multicenter study, 93 patients with r/r DLBCL received tisagenlecleucel preceded by Cy/Flu.[4] Despite a disease burden similar to that in ZUMA-1, 92% of patients received bridging chemotherapy. CAR T cells were manufactured from fresh as opposed to cryopreserved (as in ZUMA-1) apheresis products. Median time from enrollment/apheresis to infusion was 54 days, and manufacture failure occurred in only 7% of patients.[6] ORR was 52%, and 40% of patients achieved CR. Overall relapse-free survival was ~65% at 12 months.

The TRANSCEND NHL-001 trial tested, in adults with DLBCL after Cy/Flu, a single dose of JCAR017 (lisocabtagene maraleucel [liso-cel]), a CD19-directed 4-1BB.CAR T-cell product with a defined 1:1 ratio of CD4:CD8 T cells in bulk. Bridging chemotherapy was used in 59% of patients. In long-term follow-up of the 255 evaluable patients with DLBCL treated, the ORR was 73% and CR was 53%, with a median duration of response of 13.3 months.[12]

Differences in CAR engineering and manufacturing among the trials may have contributed to the variation in response rates. Axi-cel includes a CD28 costimulatory domain, whereas tisa-cel and liso-cel use 4-1BB costimulatory domains. Axi-cel and liso-cel both include a CD28 transmembrane domain, whereas tisa-cel includes a CD8-α transmembrane domain. In addition to the CAR construct, the T-cell composition may have influenced outcomes because liso-cel used equal doses of CD4 and CD8 T cells, whereas axi-cel and tisa-cel used bulk T cells for CAR T-cell manufacture.

Furthermore, lengthy manufacture period and bridging chemotherapy may have contributed to the decreased ORR in JULIET compared with ZUMA-1. Although bridging chemotherapy can reduce the number of malignant cells each CAR T-cell must target, one may speculate that these chemotherapy regimens could add toxicity without controlling tumor growth in these heavily pretreated patients with refractory disease. Of note, the TRANSCEND trial also allowed bridging chemotherapy, although a smaller percentage of patients received it. Table 1 compares the efficacy of various CD19.CAR T-cell products in the treatment of B-ALL vs B-NHL, with consistently lower CR rates in the B-NHL group when using the same CD19.CAR T-cell product.

Ongoing CD19 CAR T-cell trials in pediatric mature B-cell lymphoma

There are 31 actively recruiting trials evaluating CD19.CAR T cells for pediatric and young adult patients with r/r lymphomas. Commercial CAR T-cell products approved for adult B-cell lymphoma and pediatric B-ALL are now being tested in pediatric patients with r/r B-NHL. A phase 2 multicenter study is testing tisagenlecleucel in patients ≤25 years old with CD19$^+$ r/r B-NHL in whom one or more therapies have failed, including HSCT (BIANCA, NCT03610724). As of November 2019, 8 patients were enrolled (4 with DLBCL, 3 with BL, 1 with gray zone lymphoma).[13] CAR T cells were manufactured for all 8 patients, and all received

Table 1. Efficacy of various CD19.CAR T-cell products in r/r B-ALL compared with B-NHL

CD19.CAR T cells in r/r B-ALL					CD19.CAR T cells in r/r B-NHL				
CAR T-cell product	Age (y)	No. of pts	Efficacy CR (MRD-neg CR)	Median duration of response	CAR T-cell product	Age (y)	No. of pts	Efficacy CR (PR)	Median duration of response
Tisagenlecleucel (Maude, 2018[41])	3-21	75	81% MRD-neg CR	Not reached	Tisagenlecleucel (Schuster et al, 2019[4])	22-76	93	40% (12%)	Not reached
Axicabtagene ciloleucel (Wierda, 2018[42])	18-69	35	78% MRD-neg CR	NP	Axicabtagene ciloleucel (Neelapu et al, 2017[5])	23-76	101	54% (28%)	8.1 mo
JCAR014 (Turtle, 2016[43])	20-73	29	93% (86%)	NP	JCAR014 (Turtle, 2016[44])	22-70	32	50% (22%)	NP
CD19.CD28ζ (Lee et al, 2015[2])	1-30	21	70% (60%)	NP	CD19.CD28ζ (Kochenderfer, 2017[45])	26-67	22	55% (18%)	12.5 mo

MRD-neg, minimal residual disease–negative, NP, not provided; PR, partial response.

bridging chemotherapy per investigator discretion. Five patients received tisagenlecleucel after Cy/Flu, and the safety profile was similar to that in B-ALL.

A phase 1/2 multicenter study is evaluating brexucabtagene autoleucel (KTE-X19, formerly KTE-C19) preceded by Cy/Flu for patients with r/r B-ALL or B-NHL aged ≤21 years in whom 2 or more lines of systemic therapy have failed, including HSCT (ZUMA-4, NCT02625480). JCAR017 (see above) after Cy/Flu is also being evaluated in a phase 1/2 study in patients with r/r B-NHL aged ≤25 years (NCT03743246). To date, no data have been reported regarding the B-NHL cohorts.

Although BL is aggressive, posing specific challenges for timely autologous CAR T-cell treatment, several cases of CD19. CAR T cells in BL have been reported. Avigdor et al[14] described a 32-year-old patient with refractory BL and innumerable fluorodeoxyglucose-avid nodal, intestinal, and skeletal lesions visualized by PET-CT. The patient received Cy/Flu followed by infusion of CD3ζ-CD28.CD19 CAR T cells. He developed grade 2 CRS and neurotoxicity and achieved CR 1 month after infusion. He underwent haploidentical HSCT 3 weeks later, but he died 9 days after HSCT of sinusoidal obstruction syndrome and sepsis.

Deciphering the role of disease- and patient-specific characteristics

Tackling the immunosuppressive TME

In order to be effective, CAR T cells must expand in vitro to suitable numbers, engage the intended antigen, proliferate and kill tumor cells, and persist to provide durable tumor control. Although these predictors are relevant across malignancies, lymphomas present several unique challenges. Unlike ALL, lymphomas have a physical barrier that CAR T cells must penetrate.[15] CAR T cells also must overcome immunosuppressive cells in the tumor microenvironment (TME) that protect lymphoma from immune attack.[15] CAR T cells may also lack chemokines (eg, CXCR4 and CXCR5) that mediate entry into secondary lymphoid tissues, making them more likely to migrate into the circulation.[16]

The programmed cell death protein 1 (PD-1)/programmed death-ligand 1 (PD-L1) pathway in particular is an important component of the TME because it regulates immune responses by inhibiting T-cell cytokine production and cell-cycle progression.[17] Thus, the expression of PD-L1 on tumor and immunosuppressive cells can prevent CAR T cells from infiltrating the tumor. Checkpoint blockade targeting the PD-1–PD-L1 axis can reverse T-cell exhaustion in peripheral and intratumoral T cells.[18] Single-agent PD-1 inhibitors have been studied in the treatment of adult and pediatric r/r B-NHL, with select subtypes gaining more therapeutic benefit than others. On the whole, B-NHLs have been much less responsive to PD-1 inhibition than Hodgkin lymphoma.[19] To address the heterogeneity of NHL, combination therapy has been attempted, with case studies reporting antitumor response when PD-1 inhibition is combined with CAR T cells for r/r DLBCL.[20,21] One study treated 11 patients with r/r B-NHL with 4-1BB-CD3ζ CD19.CAR T cells followed by one 3 mg/kg nivolumab dose (anti–PD-1 antibody) 3 days later. Nine of 11 patients developed grade ≤3 CRS, and 1 developed transient neurotoxicity. With ORR and CR of 82% and 46%, respectively,[21] combination therapy only slightly improved response rates compared with previous studies of CD19.CAR T cells alone. Larger trials will need to determine if combination therapy improves outcomes in this population.

In another trial, investigators combined PD-L1 inhibitor atezolizumab and axi-cel (ZUMA-6) in 12 patients with refractory DLBCL with an ORR of 92% (CR, 58%).[22] Humanized PD-1/PD-L1 antibodies (eg, pembrolizumab and durvalumab) are also being studied with CD19.CAR T cells in B-NHL (NCT03310619, NCT03630159). As these strategies become more popular, in addition to determining whether PD-1/PD-L1 blockade enhances CAR T-cell efficacy, we must also consider the risk of T-cell "overactivation" and increased toxicity.

Other checkpoint receptors and inhibitory immune cells in the TME, including tumor-associated macrophages (TAMs) with an M2 phenotype, can decrease the efficacy of CAR T cells. TAMs minimize inflammation and immune surveillance, allowing tumor proliferation.[23] Increased TAMs, which often express PD-L1/PD-L2, indicate poor prognosis in DLBCL.[24] One proposed mechanism by which TAMs enable DLBCL to escape immune surveillance is by polarizing M1 TAMs to M2 TAMs, enabling them to engage PD-1 receptors on intratumoral T cells, further suppressing the antilymphoma response. Figure 1 illustrates proposed mechanisms to target the lymphoma TME to improve CAR T-cell efficacy. On the basis of these and similar studies, several groups, including ours, have generated immune effectors to simultaneously or separately target tumor antigens and the TME.[25,26]

Targets beyond CD19

As in B-ALL, CD19-directed immunotherapies result in "antigen escape" and CD19-negative relapse in up to 20% of patients with B-NHL.[1,27] On the basis of these risks and the success of the anti-CD20 monoclonal antibody rituximab in B-NHL, groups successfully developed CAR T cells targeting CD20.[28,29] A phase 1 trial tested 4-1BB.CD20.CAR T cells in adult patients with r/r DLBCL. Four of 6 evaluable patients achieved CR by 1 month; however, 3 of 4 patients eventually relapsed, with only 1 achieving continued CR.[28] Although promising, larger clinical trials need to be done to determine the efficacy of CD20 as a target for CAR T-cell therapy in B-NHL.

Other relevant targets, some of which have been studied in B-cell leukemias, include CD22, CD37, and the κ-light chain.[30,31] Early results of a phase 1 trial investigating CD22.4-1BB/CD3ζ CAR T cells after Cy/Flu in children/young adults with r/r B-ALL showed 44% of patients attained minimal residual disease–negative remission. Although CR rates were lower than those achieved with similar CD19.CAR T cells, CD19.CAR T cells had previously failed in several patients and/or these patients experienced CD19 relapse.[30] Though promising, there are no results yet from using CD22.CAR T cells in B-NHL. Table 2 shows clinical trials for CAR T cells targeting other antigens enrolling patients with r/r B-NHL.

Although patients with B-ALL whose disease progresses or relapses after receiving one CAR product are often enrolled in a trial targeting another antigen, this practice is rarely planned. Nevertheless, a small subcohort of pediatric patients with r/r BL was enrolled in a trial investigating sequential second-generation CD19, CD20, and CD22 4-1BB CAR T cells.[32] Three of 5 patients with BL achieved CR by day 77 after infusion of CD19.CAR T cells following Cy/Flu. One patient showed a transient response at day 30 followed by tumor growth by day 60. This patient's CAR T cells failed to expand, likely explaining the poor response. Subsequently, the patient received CD22.CAR T cells and 64 days later achieved a CR. Although promising, given small sample sizes and lack of correlative studies explaining differential responses, additional questions remain regarding this sequential approach.

Another report detailed an 8-year-old with relapsed BL who experienced disease progression 50 days after autologous 4-1BB/CD3ζ.CD19.CAR T cells and Cy/Flu. Seventy days later, he received lymphodepletion before 4-1BB/CD3ζ.CD22.CAR T cells manufactured from the initial cryopreserved apheresis product. Although a CT scan on day 35 indicated PR, biopsy revealed disease progression within 11 days with retained expression of CD19/CD20/CD22, eliminating antigen escape as the mechanism of failure. Finally, ~70 days after receiving CD22.CAR T cells, he received Cy/Flu, then 2 weekly infusions of 4-1BB/CD3ζ.CD20.CAR T cells (manufactured from a new apheresis). PET-CT performed 64 days later showed CR.[33] Although ultimately beneficial for this patient, this sequential treatment approach is labor intensive, cost prohibitive, and logistically challenging. More important, sequential CAR T-cell therapy may increase the risk of antigen escape, as demonstrated by a 12-year-old with refractory DLBCL who developed a largely CD19- and CD22-negative tumor following sequential treatment with CD19 and CD22.CAR T cells.[27] Thus, many groups prefer to target multiple antigens simultaneously.

A phase 1/2 trial simultaneously targeting multiple antigens in a single product using tandem CD19/20.4-1BB/CD3ζ.CAR T cells after Cy/Flu enrolled 28 patients with r/r B-NHL and reported an ORR of 79% and a striking CR rate of 71%. Among patients with DLBCL, ORR was 75%. Rates of CRS and neurotoxicity were similar to single-antigen–targeting CAR T cells.[34] In a preclinical report, Fousek et al[35] manufactured CD19/20/22 CAR

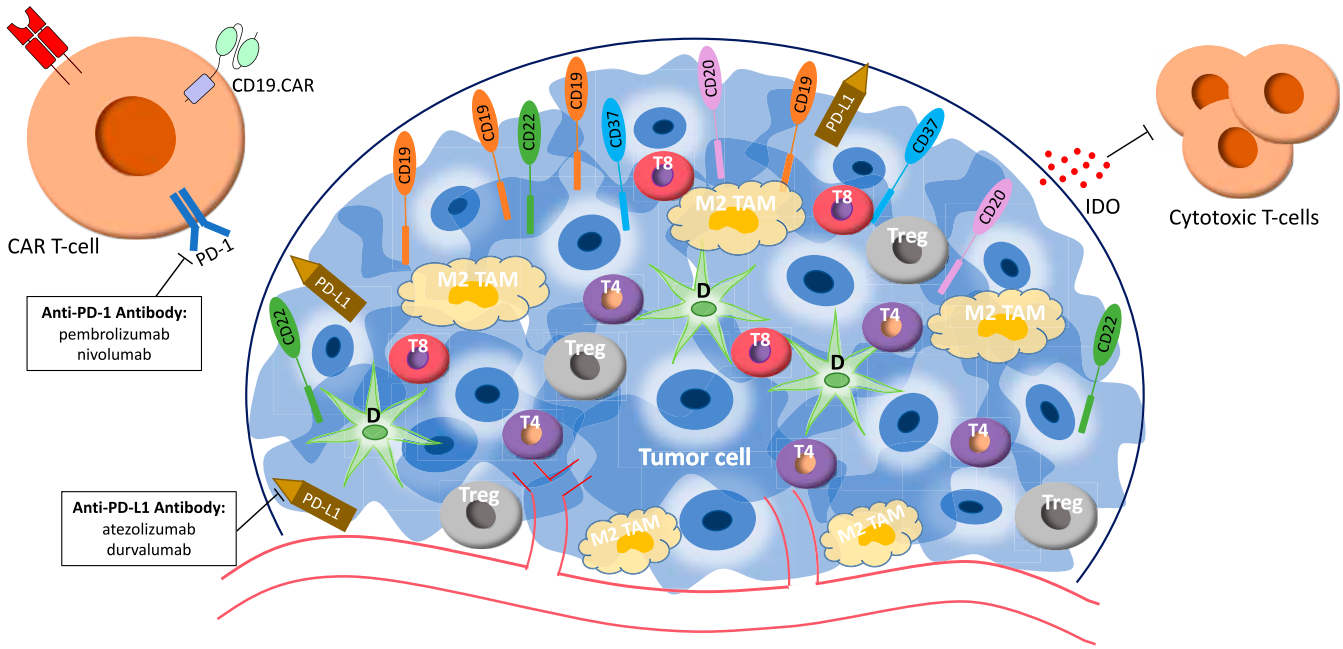

Figure 1. Schematic diagram of the lymphoma tumor microenvironment and treatment modalities currently being evaluated to target aspects of the tumor microenvironment. D, dendritic cell; IDO, indoleamine 2,3-dioxygenase; M2 TAM, M2 tumor-associated macrophage; T4, intratumoral CD4⁺ cell; T8, intratumoral CD8⁺ cell; Treg, regulatory T cell.

Table 2. Actively recruiting CAR T-cell trials for relapsed B-cell lymphomas using tumor antigen targets other than CD19

Target	ClinTrials.gov identifier	Institution/sponsor	Age criteria (y)	Phase	Results reported (Y/N)
CD20	NCT03277729	Fred Hutchinson Cancer Research Center, Seattle, WA	≥18	1/2	N
	NCT04316624	Institute of Hematology and Blood Diseases Hospital, Tianjin, China	18-75	1	N
	NCT04169932	First Affiliated Hospital with Nanjing Medical University, Jiangsu, China	18-70	1	N
	NCT04036019*	Shanghai Tongji Hospital, Tongji University School of Medicine, Shanghai, China	14-70	1	N
CD22	NCT04088890	Stanford Medical Center, Stanford, CA	≥18	1/1b	N
	NCT04088864*	Stanford Medical Center, Stanford, CA	1-30	1	N
	NCT02650414*	Children's Hospital of Philadelphia, Philadelphia, PA	1-24	1	Y (in ALL cohort) Ruella et al, 2017[25]
	NCT03262298	Affiliated Hospital to Academic of Military Medical Sciences, Beijing, China	18-65	1/2	N
	NCT04007978*	Union Hospital, Tongji Medical College, Hubei, China	14-70	1	N
Dual target: CD19/CD20	NCT04186520	Medical College of Wisconsin and Froedtert Hospital, Milwaukee, WI	≥18	1/2	N
	NCT04007029	UCLA/Jonsson Comprehensive Cancer Center, Los Angeles, CA	18-70	1	N
	NCT04215016	Fujian Medical University, Fujian, China	≥18	1	N
	NCT03881761*	Cancer Hospital Affiliate to Zhengzhou University and Henan Cancer Hospital, Henan, China	17-70	1	N
	NCT03097770*	Chinese PLA General Hospital, Beijing, China	16-70	1/2	Y (74% CR) Zhang et al, 2020[32]
Dual target: CD19/CD22	NCT03233854	Stanford University School of Medicine, Palo Alto, CA	≥18	1	N
	NCT04204161*	Xiangya Hospital Central South University, Hunan, China	1 mo to 18 y	1	N
Dual target: CD19/CD22^{+} anti-PD1 Ab	NCT03287817	Autolus Therapeutics	≥18	1/2	Y (55% CR) Osborne, 2020[46]
Dual target: CD19/CD20 or CD19/CD22	NCT03398967*	Chinese PLA General Hospital, Beijing, China	12-70	1/2	N

Ab, antibody; ALL, acute lymphoblastic leukemia; PLA, People's Liberation Army; UCLA, University of California, Los Angeles.
*Trials enrolling pediatric/adolescent patients.

T cells that eliminated CD19-negative blasts from patients who experienced relapse after CD19.CAR T cells, as well as primary B-ALL in a CD19-knockout mouse model. Early evidence of CD22-targeting CARs and the success of CD20 monoclonal antibodies indicate this trispecific strategy holds promise for B-NHL. Identification of novel tumor antigen targets continues to be an active area of research, as shown in Figure 2, along with modifications to CAR design (as discussed in the CAR T cells for mature B-cell lymphomas section).

Product-specific characteristics may impact responses

Patient age and T-cell quality both affect CAR T-cell function. Itzhaki et al[36] analyzed differences in features and phenotypes of CD28-CD3ζ.CD19.CAR T cells manufactured from patients with r/r ALL or B-NHL. Although 100% and 94% of patients with ALL and B-NHL, respectively, reached the target dose of 1×10^6 CAR T cells per kilogram, products from patients with ALL had almost twice as many CAR T cells on expansion day 10 as those from patients with NHL. Furthermore, ALL products contained significantly more naive T cells and fewer central memory T cells than NHL products did. When expansion data were analyzed according to the age of patients with ALL (1-19 vs ≥20 years), CAR T cells from patients younger than 20 years old had significantly increased fold expansion compared with older patients. Aligning with previous studies, ORR (CR) was 84% (67%) in patients with ALL and 62% (31%) in patients with NHL. Although T-cell naivety from patients with ALL may contribute to superior in vitro expansion and cytokine secretion,[37] the absence of other phenotypic differences (effector memory T-cell phenotype, expression of coinhibitory receptors) between the groups cannot fully explain differences in efficacy. Similarly, Singh et al[38] reported pediatric patients with ALL with more naive T cells had in vitro expansion superior to that of the T cells from pediatric patients with NHL (few naive and central memory T cells). Thus, the quality of CAR T cells manufactured from patients with lymphoma may be inferior due to intrinsic T-cell features.

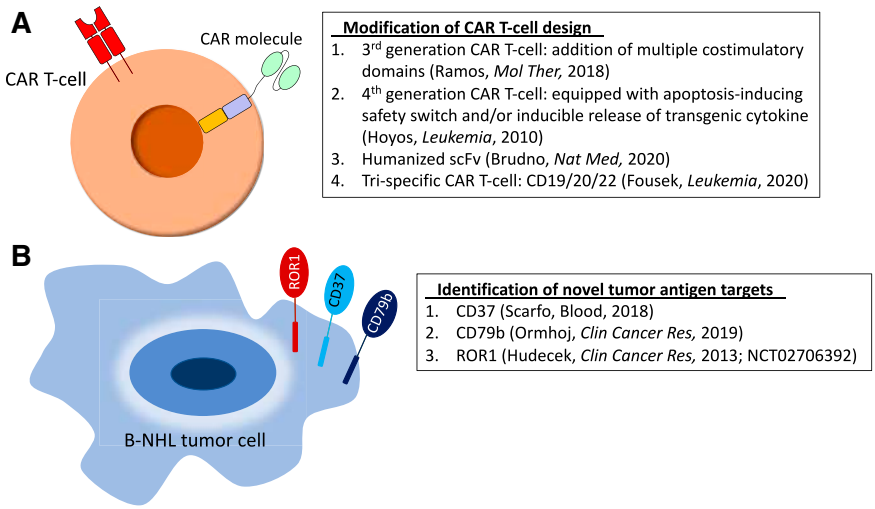

Figure 2. Preclinical and early clinical strategies to optimize CAR T-cell therapy in B-NHL. (A) Modification of CAR T-cell design. (B) Identification of novel tumor antigen targets. ROR1, receptor tyrosine kinase-like orphan receptor 1; scFv, single-chain variable fragment.

"Off-the-shelf" strategies

Though the percentage of adult patients with lymphoma in whom manufacture of autologous CAR T cells fails has decreased from its original 20%, manufacture failure remains a problem.[6] Furthermore, patients with tumors such as BL often cannot wait the obligate 3 to 4 weeks for autologous CAR T-cell manufacture. Immediately available off-the-shelf options could overcome these limitations. HLA-mismatched allogeneic NK and NK-T cells, which are unlikely to cause graft-versus-host disease, have been used as off-the-shelf effectors with safe and promising early-phase results.[39] In addition to eliminating manufacture failure/delays, off-the-shelf cord blood or healthy donor–derived, CAR-modified immune effectors minimize product heterogeneity, allowing selection of the product predicted to have the best in vivo activity. Our group is actively enrolling patients with r/r B-cell malignancies in a phase 1 clinical trial of CD19.CD28.CAR-NKT cells (NCT03774654). Other groups have used transcription activator–like effector nuclease–mediated gene editing of T-cell receptor α-chain and CD52 gene loci of non–HLA-matched donor cells transduced with a CD19.CAR to create a universal CAR T cell. These cells have been used successfully as a bridge to transplant in two pediatric patients.[40] Phase 1 trials for CD19.CAR to create a universal CAR T-cell therapy are currently active and recruiting pediatric patients with r/r ALL (NCT02808442). Last, we and others are actively exploring alternative off-the-shelf CAR approaches using virus-specific T cells, which do not mediate graft-versus-host disease. Whether third-party CAR T cells can overcome the risk of allorejection to persist for the long term in relatively immunocompetent hosts is an open question.

Summary

Data for CAR T cells in pediatric mature B-cell lymphoma remain limited, lagging behind data in adult patients. Aside from accepted obstacles plaguing CD19.CAR T cells, lymphoma-specific characteristics may impact efficacy in pediatric B-NHL. Intrinsic differences in T cells collected from heavily pretreated patients with B-NHL, such as naivety and memory potential, affect CAR T-cell expansion, functionality, and persistence. Immunosuppressive cells, checkpoint molecules, and cytokines within the TME represent lymphoma-specific challenges. Ongoing trials combining checkpoint blockade or simultaneous targeting of inhibitory molecules as well as off-the-shelf strategies that may overcome two major obstacles of CAR therapy in lymphoma—manufacture failure and product heterogeneity—all hold promise. When revisiting the case above, we propose a stepwise approach: reimage in 3 to 4 weeks, given clinical improvement and the possibility of ongoing CAR T-cell response; maintain a low threshold for biopsy of an accessible PET-avid lesion to discriminate tumor flare from active disease; and consider addition of PD-1/PD-L1 inhibition in an effort to augment response.

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Conflict-of-interest disclosure

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Off-label drug use

None disclosed.

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CAR T cells for other pediatric non–B-cell hematologic malignancies

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As CAR T-cell therapy has advanced in B-cell acute lymphoblastic leukemia, research is now underway to develop similar therapies for other lymphoid and myeloid malignancies for pediatric patients. Barriers, including antigen selection and on-target/off-tumor toxicity, have prevented the rapid development of immune-based therapies for T-lineage and myeloid malignancies. More recently, unique strategies have been developed to overcome these barriers, with several products advancing to clinical trials. For T-lineage diseases, targets have focused on CD5, CD7, and CD38, whereas myeloid disease targets have predominately focused on CD123, CD33, and, more recently, CLL-1. This review provides a comprehensive overview of these targets and approaches to overcoming safety concerns in the development of CAR T-cell therapies for pediatric patients with T-lineage and myeloid malignancies.

LEARNING OBJECTIVES

- Recognize the barriers to CAR T-cell therapy for children with T-cell or myeloid hematologic malignancies
- Understand strategies to overcome these barriers, including a review of current studies of CAR T cells for children with T-cell or myeloid malignancies

Introduction

Chimeric antigen receptor (CAR) T cells redirected against B-cell antigens (eg, CD19, CD22) have demonstrated remarkable clinical activity in children and adults with relapsed/refractory B-cell malignancies.[1] Successful development of CAR T cells for non–B-cell hematologic malignancies has been far more challenging, primarily due to antigen selection and on-target/off-tumor toxicity.

Early efforts in non–B-cell hematologic malignancies focused on finding near-universal targets with permissible on-target/off-tumor toxicity, analogous to CD19. As the field has evolved, it has become apparent that such targets are unlikely to exist; instead, we must come up with more sophisticated strategies to support the implementation of CAR T cells in these diseases. Using a patient case scenario, we review the major obstacles to successfully implementing CAR T-cell strategies for two hematologic malignances in pediatrics with the highest unmet needs: acute myeloid leukemia (AML) and T-cell acute lymphoblastic leukemia and lymphoblastic lymphoma (T-ALL/T-LL). We then present strategies investigators are implementing to overcome these obstacles and review recent and current clinical trials.

Clinical case

A 3-year-old boy presented to a clinic with a large mediastinal mass and was diagnosed with T-LL. Flow cytometric immunophenotyping confirmed a diagnosis of T-LL on the basis of the presence of CD7, CD2, CD38, and cytoplasmic CD3. At the end of induction, his mediastinal mass had decreased in size, but following consolidation chemotherapy, his disease had progressed. After two salvage attempts, he continued to have disease progression as well as the development of new sites of disease. On the basis of his unresponsiveness to chemotherapies, his treating team began investigating immunotherapeutic options.

CAR T-cell therapy for children with T-ALL and T-LL

T-ALL is a more aggressive and more chemoresistant disease than B-cell acute lymphoblastic leukemia (B-ALL).[2] Although outcomes for upfront disease have neared those for B-ALL, relapsed and/or refractory T-ALL is particularly difficult to treat and has dismal outcomes.[3] Survival after relapsed/refractory T-LL is equally poor, with an overall survival of <10%.[4] The poor prognoses associated with these diseases are compounded by a lack of immunotherapeutic salvage options. Barriers to the use of CAR T cells for patients with T-cell malignancies include product contamination with malignant T lymphoblasts, fratricide, and on-target/off tumor toxicity and the associated risk for T-cell aplasia (Figures 1 and 2).

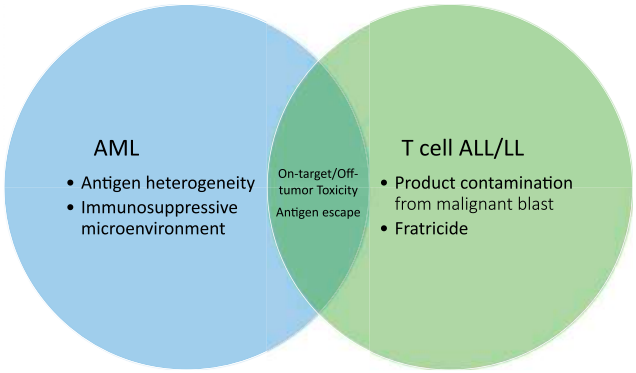

Figure 1. An overview of the barriers facing chimeric antigen receptor T-cell development for acute myeloid leukemia (AML) and T-cell acute lymphoblastic leukemia (ALL) and lymphoblastic lymphoma (LL).

There are two issues unique to the manufacturing of a CAR T-cell product for T-cell disease: contamination with malignant cells and fratricide. Contamination of a CAR T-cell product with malignant cells is a theoretical risk for any patient with a hematologic malignancy. The implications of product contamination were highlighted by a recent case report of a patient with B-ALL who developed a CD19-negative relapse 8 months after a CD19 CAR T-cell infusion that was determined to have been from a leukemic cell that was transduced during manufacturing of the CAR product.[5] This risk can be mitigated when the product undergoes upfront T-cell selection.[6] However, the ability to select for T lymphocytes in patients with T-cell malignancies is more technically difficult because of the higher likelihood for circulating tumor cells and the shared antigen expression between malignant and normal T cells. Surface CD3 is commonly absent on T-ALL; thus, with meticulous separation techniques, contamination can be greatly reduced but not eliminated.

One proposed solution for preventing product contamination is the use of allogeneic, or "off-the-shelf," T cells from healthy donors. Along with an inherent lack of risk for product contamination, this strategy has the additional benefit of being readily available, which is critically important for patients with rapidly progressing disease or those unfit to undergo apheresis. The major limitation of this approach is the risk for significant graft-versus-host disease (GVHD) without a full allogeneic match. Alternatively, a host immune system may reject a mismatched product, preventing engraftment. To eliminate the risk of GVHD, several groups are using gene-editing technology to knock out the T-cell receptor (TCR) α-chain. By knocking out this component, TCR-mediated signaling is blocked and GVHD is prevented while preserving the function of the CAR T cell.[7] In the current state, the efficacy of allogeneic CAR T-cell products has lagged behind autologous products for treatment of B-cell malignancies.[8,9]

Natural killer (NK) cells are being explored as an alternative off-the-shelf product, with early clinical trial results in B-cell malignancies rivaling the outcomes of autologous CAR T cells, albeit in a small number of patients.[10] There are numerous potential advantages to using NK cells over CAR T cells. Owing to their lack of a TCR, NK cells do not pose a risk for GVHD and therefore require no additional gene editing to be used as a universal product. Theoretically, NK cells may retain lytic activity against the tumor cells in a non–antigen-dependent manner, which may prove useful in settings where antigen modulation is frequently encountered. In addition, there is less antigen overlap

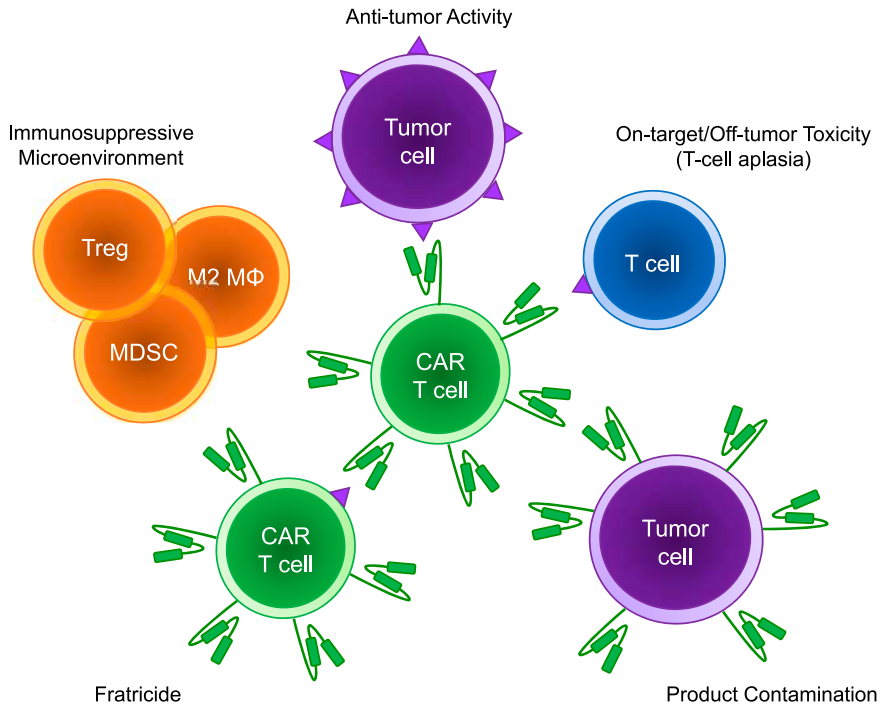

Figure 2. Schema of the barriers to implementation of chimeric antigen receptor T cells for non–B-cell hematologic malignancies. Treg, T regulatory cells; M2 MΦ, M2 macrophage; MDSC, myeloid-derived suppressor cell.

between NK cells and non–B-cell hematologic malignancies, which may allow a greater number of possible targets.

Fratricide is a unique form of ex vivo on-target/off-tumor toxicity commonly referred to as "self-killing." Fratricide is when CAR T cells attack each other during the manufacturing process and occurs if the target antigen is concurrently expressed on the CAR T cell (Figures 1 and 2). This is a major theoretical barrier preventing the targeting of T-cell malignancies because there is an inherent overlap of surface antigens between malignant and CAR T cells. Without alteration of the target antigen on the CAR T cells, CAR-mediated self-killing may occur, undermining T-cell expansion during the manufacturing phase. Using NK cells may be advantageous in avoiding fratricide when the T-cell antigen is not shared with the NK cells.

Aside from a move toward NK cells, a straightforward solution to avoid fratricide is choosing a tumor antigen that is highly specific for the malignant cells and not expressed on normal T cells. Unfortunately, the number of these potential targets is limited, and they are usually restricted to a subset of patients with T-ALL (Table 1). One example is CD1a, which, in the normal state, is an antigen largely restricted to developing cortical thymocytes. CD1a is also expressed in cortical T-ALL, a major subset of T-ALL in adult and pediatric patients, making it relatively tumor specific. Preclinical modeling has shown an absence of fratricide, and, notably, the CAR T cells appear to respond to viral antigens, suggesting they retain some anti-infectious function, reducing the concern for opportunistic infections.[11]

Another example of an antigen with relative tumor specificity is CD38.[12] Targeting CD38 has proved to be safe and well tolerated in multiple myeloma, with some supporting preclinical and emerging clinical evidence in T-ALL.[13,14] Along with restricted expression on normal T cells, additional attractive properties of CD38 are its plasticity and its ability to be upregulated in a variety of hematologic malignancies, enhancing the cytolytic activity of CAR T cells in preclinical studies.[15] Related to the tolerability of other CD38-targeting strategies in multiple myeloma, clinical investigations involving CD38-targeting CAR T cells are quickly advancing.[16]

Strategies to avoid fratricide are required when a tumor-specific antigen cannot be identified and may involve indirect or direct modulation of universally expressed T-cell antigens on the CAR T cell. An example of indirect modulation is modeled through the targeting of CD5. After transduction of CAR T cells with a CD28 costimulatory domain, CD5 is downregulated either by internalization or by masking of the CD5 antibody epitope by the CAR. This downregulation limits the degree of fratricide in this product while preserving the ability of the CAR to target malignant cells. This product has been advanced to clinical trials, with some encouraging preliminary results available (Table 2).[17,18] In contrast, when a 4-1BB costimulatory domain was incorporated, significant fratricide occurred, suggesting different costimulatory molecules affect antigen stabilization.[19]

Alternatively, forced inhibition of the target antigen can be engineered in order to prevent fratricide. Examples of direct antigen modulation include gene editing or protein expression blockers (PEBLs); both are currently being implemented in CD7-targeting strategies. Early studies involving CD7 as a CAR target showed extensive fratricide because CD7 is not downregulated in a fashion similar to CD5. By using a CRISPR/Cas9 system, CD7 can be knocked out, eliminating surface expression and the risk of fratricide, with some of these products advancing to clinical trials (Table 2).[7,20,21] Similarly, a PEBL couples an anti-CD7 single-chain variable fragment with an endoplasmic reticulum/Golgi-retention motif, preventing any newly synthesized CD7 from being expressed. This technique was remarkably effective at abrogating CD7 expression and eliminating fratricide without compromising CAR T-cell function in a preclinical model.[22] An additional advantage to the PEBL approach is that it does not require additional gene editing to the T cells.

A final primary concern, discussed in more detail below, is the concept of on-target/off-tumor toxicity and the risk of targeting healthy T cells in vivo, leading to T-cell aplasia (Figures 1 and 2). Strategic antigens that may be suitable for circumventing prolonged T-cell aplasia include CD4 and/or TRBC1/2[23,24] (Table 1). These antigens are expressed in a subset of normal T cells, but, given the clonal nature of malignancies, they are universally expressed on the tumor in a subset of patients. By targeting these antigens, there would be a significant reduction in a patient's normal T-cell compartment but potentially sufficient T-cell populations to limit life-threatening infections if only transiently eliminated.

CAR T-cell therapy for children with AML

Relapsed and/or refractory AML remains a major source of childhood cancer–associated mortality.[25] Similarly, treatment-related leukemia, including lineage switch after immunotherapeutic pressures, is particularly difficult to salvage.[26] These challenging diseases have prompted an interest in novel immunotherapeutic strategies for myeloid diseases.

Table 1. Currently identified T-cell antigens for immunotherapeutic targeting under preclinical or clinical study

Target	T-ALL/T-LL frequency	Normal tissue expression	Selected references
CD1a	80%/67%	Cortical thymocytes, immature dendritic cells, Langerhans cells	11
CD3	33%/46%	Thymocytes, mature T cells, NK cells	40
CD4	82%/72%	T-helper cells, thymocytes, granulocytes, monocytes, dendritic cells, Langerhans cells	23
CD5	88%/95%	Mature T cells, thymocytes, B cells	17
CD7	98%/97%	Mature T cells, thymocytes, NK cells	20
CD38	100%/100%	Plasma cells, hematopoietic progenitor cells, T cells, NK cells, B cells	12
CD123	43%/—	Plasmacytic dendritic cells, eosinophils	41
TRBC1	30%/—	T cells	24

NK, natural killer; T-ALL, T-cell acute lymphoblastic leukemia; T-LL, T-cell lymphoblastic lymphoma.

Table 2. Active chimeric antigen receptor T-cell clinical trials for T-cell acute lymphoblastic leukemia/T-cell lymphoblastic lymphoma

Target	ClinicalTrials.gov identifier	Institution/sponsor	Age restriction	Status	Reference
CD4	NCT03829540	Stony Brook University	18+ y	Recruiting	23
CD5	NCT03081910	Baylor College of Medicine	<75 y	Recruiting	18
CD7	NCT04033302	Shenzhen Geno-Immune Medical Institute	6 mo-75 y	Recruiting	—
	NCT04004637	First Affiliated Hospital of Zhengzhou University	7-70 y	Recruiting	21
	NCT03690011	Baylor College of Medicine	<75 y	Not yet recruiting	7
	NCT04264078	Xinqiao Hospital of Chongqing	2-70 y	Not yet recruiting	—

Summary of active and completed clinical trials according to www.clinicaltrials.gov as of 1 June 2020.

The major challenge in the treatment of AML is antigen selection. This is due in part to the phenotypic heterogeneity not only between patients but also within an individual patient, limiting the identification of a universal target (Table 3). When choosing an antigen with restricted expression, there is a risk of incompletely treating the tumor or precipitating an antigen escape. On the opposite end of the spectrum is choosing a target too widely expressed, leading to substantial on-target/off-tumor toxicity (Figures 1 and 2).

Leading immunotherapeutic targets in AML include CD33 and CD123. CD33 is expressed in a majority of AML cases and, given the pediatric experience with gemtuzumab ozogamicin, is a natural candidate for CAR T-cell therapy. Similarly, CD123 is an appealing target for CAR T-cell therapy, given the expanding landscape of CD123-targeting strategies. For both targets, there are several CAR T-cell trials either currently recruiting or in development that include pediatric participation (Table 4). Preliminary results from adult trials have been largely limited to case reports, but both targets appear to have shown benefit, at least in a subset of patients.[27,28] There are numerous emerging targets, including Lewis Y antigen, NKG2DL, CD44v6, CLL-1, CD7, CD38, and FLT3, that may show similar benefit in a subset of patients (Table 3).[29-31]

Because of the phenotypic heterogeneity of myeloblasts, none of the above antigens are likely to serve as universal targets, and targeting one alone may be insufficient to clear disease. It is reasonable to assume that antigen escape will emerge as a limitation of single-antigen–targeting CAR T cells in AML. Early results from CD123-specific CAR T cells in patients with AML demonstrate an efficacy signal without myeloablation but a lack of durable remission.[27] Different genotypes of CD33 are widespread, and variants can cause a truncated CD33 protein to be expressed.[32] There is a higher prevalence of these CD33 splice variants than of CD19, raising concerns for the frequency of antigen escape after CD33-directed CAR T-cell therapy. Given these concerns over the potential for antigen escape, most clinical trials in AML recommend consolidating a remission after CAR T cells with hematopoietic stem cell transplant (HSCT).

An alternative strategy to overcome this restricted antigen expression is through the use of dual targeting strategies. This is done by combining different targeting populations of CAR T cells, expressing more than one distinct CAR molecule on a single T cell, or engineering two different binding domains into a single CAR. Dual targeting allows multiple antigens to activate the cells synchronously. In theory, this could mitigate the risk of antigen escape and is currently being investigated in other hematologic malignancies.[33] An added risk of a combinatorial targeting strategy is the possibility of increased on-target/off-tumor toxicity, and thorough investigations of individual targets is required before multiplexed targets.

A unique challenge to treating AML with CAR T cells is the growing evidence surrounding the immunosuppressive microenvironment resident to the bone marrow niche (Figures 1 and 2). Multiple groups have shown infiltration of regulatory T cells, myeloid-derived suppressor cells, and macrophages in the tumor microenvironment as well as coinhibitory checkpoint expression on effector T cells.[34] This microenvironment appears to be as heterogeneous as the disease itself, with patients clustering into subgroups based on immune gene expression profiling.[34] This immunosuppressive environment may further hinder our ability to provide effective cellular therapies by dampening CAR T-cell function. Alternatively, phenotyping this environment might serve as a biomarker to identify those patients who would benefit most from CAR T-cell therapy. Similarly, the immunosuppressive environment might represent a novel opportunity for enhancing the potency of CAR T cells via immune checkpoint blockade or other mechanisms.[35]

On-target/off-tumor toxicity

Unless a truly specific tumor antigen is targeted, there will always be a risk of on-target/off-tumor toxicity. A shared concern between T-cell and myeloid targeting strategies is the risk of prolonged immune suppression and life-threatening infections, with many groups exploring strategies to circumvent this challenge (Figures 1 and 2).

For T-cell and myeloid targeting, the ongoing persistence of CAR T cells may be detrimental. The B-cell aplasia seen with CD19 and CD22 targeting strategies is considered a permissive toxicity because it is readily managed with periodic immunoglobulin infusions. In contrast, prolonged T-cell or myeloid aplasia is a life-threatening immunodeficiency. The degree of myeloablation with different targets is yet to be defined and will require ongoing clinical investigations to understand the implications, including the time to recovery and/or ability to recover from myeloid cell aplasia with removal of the antigenic targeting. Early results of CD33/CLL-1 dual targeting therapy has demonstrated that, at least in some patients, complete myeloablation is noted and is used as a component of conditioning for HSCT.[28] Conversely, early experience with CD123 CAR T cells has not resulted in treatment-related cytopenias.[27] Therefore, strategic antigen selection, limitation of persistence, or rapid transition to HSCT may need to be employed to mitigate this risk.

Table 3. Currently identified acute myeloid leukemia antigens for immunotherapeutic targeting under preclinical or clinical study

Target	Frequency in AML	Normal tissue expression	Selected references
CD7	10%-35%	Mature T cells, thymocytes, NK cells	42
CD13	75%-95%	Granulocytes, monocytes, mast cells, osteoclasts	43
CD33	75%-95%	Hematopoietic progenitor cells, basophils, granulocytes, mast cells, monocytes, Kupffer cells	42
CD38	43%	Plasma cells, hematopoietic progenitor cells, T cells, NK cells, B cells	12
CD44v6	64%	Keratinocytes	44
CD70	95%	B cells, T cells, dendritic cells	45
FLT3 (CD135)	50%	Hematopoietic stem cells, neurons, testis	46
CD123	97%	Myeloid progenitors, plasmacytic dendritic cells, eosinophils, endothelial cells	42
Lewis Y (CD174)	46%	Hematopoietic progenitor cells, intestinal epithelial cells	47
CD244	95%	NK cells, T cells, monocytes, basophils, eosinophils, spleen	42
TIM3 (CD366)	85%	T cells, lung tissue	42
CLL-1 (CLEC12A, CD371)	80%	Granulocytes, monocytes	30,31
Folate receptor B	70%	Myeloid cells	48
NKG2DL	70%	T-regulatory cells, endothelial cells	49

NK, natural killer; T-ALL, T-cell acute lymphoblastic leukemia; T-LL, T-cell lymphoblastic lymphoma.

One strategy is designing CAR T cells for transient in vivo persistence. This can be accomplished up front by using the more potent but less persistent CD28 costimulatory domain over the more persistent 41-BB costimulatory domain. Modulation of persistence can also be accomplished through the incorporation of suicide switches (eg, herpes simplex virus thymidine kinase, inducible caspase 9, coexpression of synthetic surface proteins targetable by monoclonal antibodies).[36] Recovery from myeloid cell aplasia or T-cell depletion after CAR T-cell therapy can also be accomplished through a consolidative HSCT in which the CAR T cells are ablated.

An alternative to suicide switches or a consolidative HSCT is the ability to include "on-and-off" switches, which in theory can

Table 4. Active chimeric antigen receptor T-cell clinical trials for acute myeloid leukemia

Target	ClinicalTrials.gov identifier	Institution/sponsor	Age restriction	Status
CD7	NCT04033302	Shenzhen Geno-Immune Medical Institute	6 mo-75 y	Recruiting
CD33	NCT03971799	Center for International Blood and Marrow Transplant Research	1-30 y	Recruiting
CD44v6	NCT04097301	Horizon 2020/MolMed	1-75 y	Recruiting
CD123	NCT02159495	City of Hope Medical Center	>12 y	Recruiting
	NCT03766126	University of Pennsylvania	>18 y	Recruiting
	NCT04109482	Mustang Bio	>18 y	Recruiting
	NCT03190278	Cellectis	18-64 y	Recruiting
	NCT04318678	St. Jude Children's Research Hospital	<21 y	Recruiting
NKG2D	NCT04167696	Celyad Oncology	>18 y	Recruiting
	NCT03018405	Celyad Oncology	>18 y	Recruiting
FLT3	NCT03904069	Amgen	>12 y	Not yet recruiting
CD123/CLL1	NCT03631576	Fujian Medical University	<70 y	Recruiting
CD123/CD33	NCT04156256	iCell Gene Therapeutics	None	Recruiting
CCL1/CD22/CD123	NCT04010877	Shenzhen Geno-Immune Medical Institute	6 mo-75 y	Recruiting
Muc1/CLL1/CD22/CD38/CD56/CD123	NCT03222674	Shenzhen Geno-Immune Medical Institute	2-75 y	Recruiting
CD33/CD28/CD56/CD123/CD117/CD133/CD34/Muc1	NCT03473457	Zhujiang Hospital	>6 mo	Recruiting

Summary of active and completed clinical trials according to www.clinicaltrials.gov as of 1 June 2020.

allow precise control of the CAR T-cell activity. For B-ALL, persistence has been demonstrated as a key attribute in the prevention of relapse.[37] It is currently unclear how essential ongoing persistence of CAR T cells will be for non–B-cell hematologic malignancies, but one would hypothesize that it will be required for ongoing remission, thus limiting the applicability of CAR T-cell specifically engineered for limited in vivo persistence. By controlling the activity of the CAR, one could allow ongoing persistence while also managing the off-tumor toxicity. An example of this is the dimerizing agent–regulated immunoreceptor complex CAR T cells composed of separate antigen-targeting and signal transduction polypeptides that will only dimerize, and therefore function, in the presence of rapamycin.[38] Finally, similar to the strategy of dual targeting antigens to enhance efficacy, there are strategies to combine an activating CAR that targets a tumor antigen with an inhibitory CAR that engages with an antigen on normal tissue, thereby reducing on-target/off-tumor toxicity.[39] Until one of these strategies becomes consistently successful, CAR T-cell therapy for T-cell and myeloid disorders will likely serve as a bridge to an allogeneic HSCT to both reverse any ongoing on-target/off-tumor toxicity and increase the chance of cure.

Summary

Justifiable excitement exists surrounding successful integration of CAR T cells into the treatment of children and adolescents with high-risk non–B-cell hematologic malignancies. There are several unique barriers that exist which have limited implementation of this therapy, but novel strategies are currently in development and being investigated in clinical trials that seek to overcome these barriers. It is anticipated that several iterations will be required before we are able to parallel the success that has been seen in B-ALL.

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Conflict-of-interest disclosure

The authors declare no competing financial interests.

Off-label drug use

None disclosed.

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CAR T cells vs allogeneic HSCT for poor-risk ALL

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For subgroups of children with B-cell acute lymphoblastic leukemia (B-ALL) at very high risk of relapse, intensive multiagent chemotherapy has failed. Traditionally, the field has turned to allogeneic hematopoietic stem cell transplantation (HSCT) for patients with poor outcomes. While HSCT confers a survival benefit for several B-ALL populations, often HSCT becomes standard-of-care in subsets of de novo ALL with poor risk features despite limited or no data showing a survival benefit in these populations, yet the additive morbidity and mortality can be substantial. With the advent of targeted immunotherapies and the transformative impact of CD19-directed chimeric antigen receptor (CAR)–modified T cells on relapsed or refractory B-ALL, this approach is currently under investigation in frontline therapy for a subset of patients with poor-risk B-ALL: high-risk B-ALL with persistent minimal residual disease at the end of consolidation, which has been designated very high risk. Comparisons of these 2 approaches are fraught with issues, including single-arm trials, differing eligibility criteria, comparisons to historical control populations, and vastly different toxicity profiles. Nevertheless, much can be learned from available data and ongoing trials. We will review data for HSCT for pediatric B-ALL in first remission and the efficacy of CD19 CAR T-cell therapy in relapsed or refractory B-ALL, and we will discuss an ongoing international phase 2 clinical trial of CD19 CAR T cells for very-high-risk B-ALL in first remission.

LEARNING OBJECTIVES

- Recognize the need for novel mechanistic approaches for children with poor-risk B-ALL
- Identify the indications and evidence for allogeneic HSCT in first complete remission for poor-risk ALL
- Understand the efficacy data for CD19 CAR T-cell therapies in refractory B-ALL and review the ongoing trial of CD19 CAR T cells in frontline therapy

Clinical case

A 15-year-old girl diagnosed with B-cell acute lymphoblastic leukemia (B-ALL) receives 4-drug induction chemotherapy according to a high-risk (HR) protocol. Minimal residual disease (MRD) at the end of induction (EOI) is detectable at 0.26%. After HR consolidation chemotherapy, MRD remains positive at 0.23%. The poor prognosis with continuation of standard HR chemotherapy regimens and alternative therapy options are discussed.

Introduction

Survival rates for children with de novo B-ALL approach 90% in the modern era, improving dramatically over the past 60 years with intensification of multiagent chemotherapy, risk stratification, and further intensification for subgroups of patients at higher risk of relapse.[1] Despite these improvements in outcome for the majority of patients, subgroups of patients remain at very high risk of relapse with current intensive chemotherapy regimens. Some of these patients are identified by the underlying genomics of the leukemic blasts, but many poor-risk ALLs can be identified on the basis of a poor early response to therapy. Multiple studies have shown MRD response to be the single most important independent prognostic indicator.[2-5] Data from a Children's Oncology Group (COG) phase 3 study for HR B-ALL, AALL0232 (ClinicalTrials.gov identifier: NCT00075725), showed extremely poor 5-year disease-free survival (DFS) in patients with persistent MRD by multiparameter flow cytometry after 2 cycles of chemotherapy. Patients with MRD ≥0.01% at the end of consolidation (EOC) had a 5-year DFS of 39% compared with 79% for those with EOI MRD ≥0.1% but EOC MRD <0.01% (P < .0001).[2] Persistent disease after 3 months of multiagent chemotherapy and high risk of relapse despite intensive multiagent chemotherapy suggests that these leukemias are chemotherapy refractory. These patients are in need of novel therapeutic approaches.

One approach used for poor-risk ALL that carries a high risk of relapse with standard chemotherapy regimens alone is hematopoietic stem cell transplantation (HSCT). With the advent of targeted immunotherapies such as CD19-directed

chimeric antigen receptor (CAR)–modified T cells, which have demonstrated transformative outcomes in relapsed/refractory (R/R) B-ALL, this approach is being studied in 1 poor-risk ALL population, HR B-ALL with persistent MRD, which is defined as very high risk (VHR) on current COG ALL protocols. We will discuss the evidence and ongoing questions for both of these approaches.

Allogeneic transplantation for poor-risk ALL in first complete remission

Traditionally, HSCT in first complete remission (CR1) has been considered in ALL for poor-risk subgroups that have demonstrated poor outcomes with standard chemotherapy.[6] Indications for HSCT in CR1 vary across cooperative trial groups and are evolving,[7] and the benefit of HSCT varies by indication or is not well studied.

Indications

Common indications for transplantation for ALL in CR1 across most trial groups have included induction failure and severe hypodiploidy (Table 1).[8] Several other criteria that were common in previous studies, such as a high white blood cell (WBC) count at presentation, Philadelphia chromosome positivity (Ph$^+$), or translocation (4;11), are no longer considered strict indications for transplantation.[8,9] Cooperative groups differ in their response-based indications for HSCT, with some European

Table 1. Summary of HSCT indications and outcomes for ALL in CR1 on pediatric trials

Trial	Years of enrollment	Region	Criteria for HSCT in CR1	N*	Outcomes	Comments
AALL0031[6]	2002-2006	USA	Hypodiploidy, MLL rearrangement plus SER, induction failure	30	Hypodiploidy: 4-year DFS for chemotherapy, 50% ± 11% vs HSCT, 62% ± 14% (P = .65). Induction failure: 4-year DFS for chemotherapy, 44% ± 23%; chemotherapy vs HSCT, 75% ± 19% (P = .14)	Ph$^+$ ALL excluded from analysis; nonrandomized study
Total Therapy 13[12,49]	1991-1998	USA	Ph$^+$ ALL, induction failure	57	Combined Total Therapy 13/14 trials 5-year OS: 28% (95% CI, 17%-40%)	Nonrandomized study
Total Therapy 14[4,12]	1998-1999	USA	Ph$^+$ ALL, induction failure			Nonrandomized study; trial terminated early because of excess toxicity
Total Therapy 15[12,50]	2000-2007	USA	Ph$^+$ ALL, induction failure; MRD ≥1% after 6 weeks of induction	37	5-year OS: 65% (95% CI, 46%-78%)	Nonrandomized study
ALL BFM-90[51]	1990-1995	Europe	Induction failure, Ph$^+$ ALL or PPR and T-ALL; myeloid marker BFM-RF ≥1.7 or t(4;11)	35	NR	Nonrandomized study
ALL BFM-95[11]	1995-2000	Europe	Induction failure, Ph$^+$ ALL, t(4;11), PPR and T-ALL or WBC ≥100 × 10^9/L; only patients with matched related donor underwent HSCT	77	5-year DFS for chemotherapy: 40.6% (SE, 3.1%); chemotherapy vs HSCT, 56.7% (SE, 5.7%) (hazard ratio, 0.67; 95% CI, 0.46-0.99; P = .02)	Patients were randomly assigned between chemotherapy and related-donor HSCT
AEIOP-BFM ALL 2000[24]	2000-2006	Europe	(1) PPR and T-cell ALL or WBC ≥100 ×10$^{9/L}$ or pro-B ALL or MRD ≥10^{-2} at day 33; (2) MRD ≥10^{-3} at day 78 or t(4;11) and PGR; (3) induction failure or MRD>10^{-2} at day 78 or t(4;11) and PPR	81	5-year DFS for chemotherapy vs HSCT: (1) 67.7% (SE=6.3) vs 83.3% (SE=10.8; p=0.31); (2) 47.2% (SE=6.6) vs 51.1% (SE=9.6; p=0.74); (3) 54.7% (SE=13.6) vs 50.5% (SE=8; p=0.79)	Ph$^+$ ALL excluded; nonrandomized study; required matched donor (related for subgroups 1/2) for HSCT
NOPHO ALL-92[5]	1992-2001	Scandinavia	No uniform criteria	57	NR	Nonrandomized study
NOPHO ALL-2000[5]	2002-2007	Scandinavia	Induction failure, Ph$^+$ ALL, WBC ≥200 × 10^9/L, MLL rearrangement and age older than 10 years, hypodiploidy (<34); optional: MRD ≥10^{-3} at 3 months	62	NR	Nonrandomized study
NOPHO ALL-2008[25]	2008-2016	Scandinavia	Induction failure, MRD ≥0.1% at day 79; optional: hypodiploidy (<44) with good response	71	DFS, 79.1% (95% CI, 69.8%-89.6%) at median follow-up of 5.5 years since HSCT	Ph$^+$ ALL patients excluded; nonrandomized study

Berlin-Frankfurt-Muenster risk factor (BFM-RF) calculated by 0.2 × log (peripheral blood blasts per μL + 1) + 0.06 × liver size in centimeters below the costal margin + 0.04 × spleen size in centimeters below the costal margin.
NR, not reported; NOPHO, optional indication for HSCT; OS, overall survival; PGR, prednisone good; PPR, poor prednisone response; SE, standard error; SER, slow early response; WBC, white blood cell count.
*Number of patients who underwent HSCT.

groups taking children to HSCT in CR1 on the basis of a poor response to a prednisone prophase in combination with other criteria: Ph+, t(4;11), WBC >×10^{9}/L, or slow response.[10] Many groups also consider HSCT if MRD is positive at ≥10^{-3} at week 12.[10] The COG used a different response-based criteria on a previous generation of trials, with HSCT considered for patients with MRD ≥1% at EOI that persists despite receiving 2 additional weeks of induction therapy.[6] Because most studies combine indications, and HSCT may be of greater benefit for some children than for others, it is challenging to assess outcomes in specific subsets.

Outcomes

The prospective BFM-95 trial of chemotherapy vs HSCT for children with VHR ALL (defined in Table 1) demonstrated a survival benefit to HSCT.[11] A retrospective analysis of patients with ALL treated on 3 consecutive St Jude trials (Total Therapy 13, -14, -15) examined HSCT for HR patients (Table 1) and showed improved survival for HSCT patients over time, regardless of donor type.[12] A retrospective analysis of the consecutive Italian trials AEIOP-88, -91, -95, and -2000 examined the role of HSCT for patients with HR ALL in CR1. Indications for HSCT varied between the trials, but the overall 10-year DFS was 61%.[13] In summary, several trials have demonstrated a survival benefit with HSCT for poor-risk ALL in CR1 (Table 1); however, it should be noted that these trials were not randomized and they carried the inherent bias of necessitating remission and a matched donor for HSCT; several of the studied indications would no longer be considered, with improvements in risk stratification, chemotherapy regimens, and targeted therapies.[8]

Ph+ ALL

Previously, Ph+ ALL was considered an absolute indication for HSCT in CR1. However, the risk-benefit ratio of transplantation has been drastically altered by the availability of the tyrosine kinase inhibitor imatinib. A retrospective analysis published in 2000, before the availability of imatinib, demonstrated a 65% event-free survival (EFS) in children with Ph+ ALL with transplant vs 25% EFS with chemotherapy alone, establishing Ph+ ALL as a clear indication for HSCT.[14] The COG AALL0031 trial later demonstrated that patients with Ph+ ALL treated with imatinib had significantly improved survival relative to historical controls and that there was no survival benefit for patients treated with imatinib who underwent HSCT.[15,16] On the basis of these data, an ongoing international intergroup trial reserves HSCT for patients with induction failure or persistent MRD.

Hypodiploidy

Children with hypodiploid ALL, here defined as modal chromosome number <44 chromosomes or DNA index <0.81, have an inferior prognosis with standard or intensified chemotherapy and thus have been historically considered for HSCT in CR1, but improved outcomes have not been demonstrated.[17-19] A recent retrospective analysis of 131 children with hypodiploid ALL enrolled on the COG biology study AALL03B1 failed to demonstrate a survival benefit of HSCT in CR1 (5-year EFS: 57.4% with HSCT vs 47.8% without HSCT; P = .49).[17] Another recent retrospective study of patients with hypodiploid ALL enrolled in several international cooperative trials also failed to demonstrate a benefit of HSCT.[18] Lack of randomized direct comparisons limit these data; nevertheless, they call into question the consideration of HSCT based solely on poor prognosis.

Induction failure

Most cooperative groups consider induction failure, variably defined as M3 marrow (>25% leukemic blasts) or failure to achieve morphologic remission (<5% leukemic blasts) after 1 month of induction chemotherapy, as an indication for allogeneic transplantation in CR1.[7] In a retrospective analysis of 1041 children with ALL and induction failure treated across 14 cooperative groups between 1985 and 2000, HSCT from any donor seemed to benefit children with T-cell ALL and some patients with B-ALL.[20] Matched sibling transplants were of benefit to patients older than age 6 years with B-ALL, but not other types of transplants. In children younger than age 6 years with B-ALL, chemotherapy provided significantly better survival rates than HSCT.[20] These results must be interpreted with caution because treatment-related mortality of HSCT has improved significantly in the intervening period since 1985.[21]

Persistent MRD

With the increased availability of MRD and the recognition of its prognostic utility, persistent MRD is increasingly used as an indication for transplantation.[22,23] The AIEOP-BFM ALL 2000 trial stratified children with persistent MRD ≥10^{-3} at day 78 of therapy to HSCT, but found no statistically significant difference in DFS between HSCT and chemotherapy alone (Table 1).[24] The Nordic Society of Paediatric Haematology and Oncology (NOPHO) cooperative group used MRD >5% at EOI or persistent MRD ≥10^{-3} after 3 months of therapy as a potential indication for transplant on the NOPHO ALL-2000 trial and allocated these patients to intensified chemotherapy and HSCT on the subsequent ALL2008 trial.[5] NOPHO recently retrospectively examined HSCT outcomes

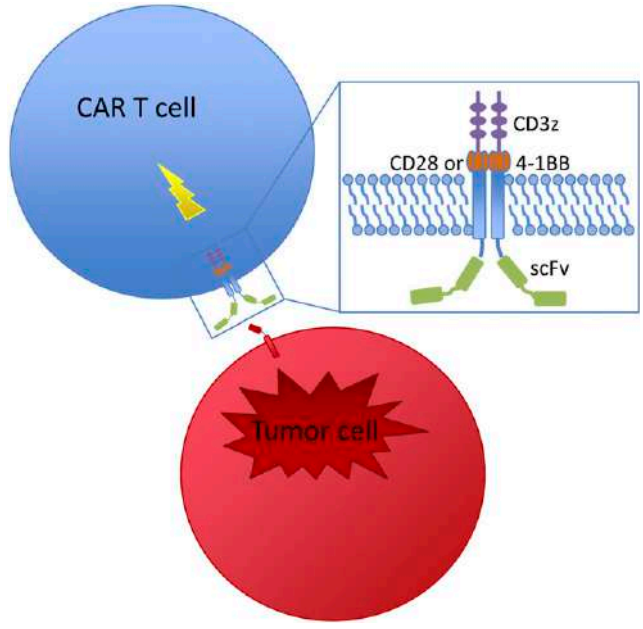

Figure 1. Structure and mechanism of CAR-modified T cells. T cells are engineered to express a CAR, which links an extracellular antibody domain (scFv) to intracellular T-cell signaling domains, the CD3 zeta cytoplasmic domain and a costimulatory domain (CD28 or 4-1BB). Once engaged by their target, CARs activate a cytotoxic T-cell response that kills the bound antigen-expressing cell.

Table 2. Summary of CD19-directed CAR T-cell trials in pediatric R/R ALL

Trial	Costimulatory domain	Population	N*	CR rate (%)	Subsequent HSCT n (%)†	Outcome	CAR T-cell persistence
Penn/ CHOP phase 1/ 2a[37,39]	4-1BB	R/R ALL: refractory, relapse after HSCT, ineligible for HSCT	60[39]	93[39]	7 (13)[39]	60% RFS at 12 months (95% CI, 48-75%)[39]	68% at 6 months (95% CI, 50-92%)‡[37]
NCI phase 1[36,52]	CD28	R/R B-ALL: second relapse or greater, refractory, ineligible for HSCT	51[52]	60.8[52]	21 (75)[52]	49.5% LFS at 18 months[52]	Longest, 68 days§[36]
Seattle phase 1/ 2[32]	4-1BB	R/R ALL: second relapse or greater, refractory, MRD after HSCT, ineligible for HSCT	43	93	11 (28)	50.8% EFS at 12 months (95% CI, 36.9-69.9%)	Median, 3 months (range, 2.07-6.44 months)‖
MSKCC phase 1[53]	CD28	R/R B-ALL: second relapse or greater, very early (CR1 <18 months) BM relapse, refractory, ineligible for HSCT	25	75	15 (83)	8 of 18 in remission, median follow-up, 28.6 months	Median, 7 days (range, 0-234 days)
ELIANA phase 2[33]	4-1BB	R/R B-ALL: second relapse or greater, refractory, relapse after HSCT	75	81	8 (13)	80% RFS at 6 months, 59% RFS at 12 months	Median, 168 days (range, 20-617 days)

CHOP, Children's Hospital of Philadelphia; EFS, event-free survival; LFS, leukemia-free survival; MSKCC, Memorial Sloan Kettering Cancer Center; NCI, National Cancer Institute; Penn, University of Pennsylvania; RFS, relapse-free survival; R/R, relapsed/refractory.
*Numbers of patients infused.
†Percentage of patients in remission.
‡Probability of persistence.
§Longest duration in any patient.
‖Measured by B-cell aplasia.

in CR1 and reported a 5-year DFS of 79.1%.[25] Persistent MRD was the indication for HSCT in only 35% of this cohort, and 17% received a transplant without a protocol indication. The population studied differed from the COG AALL0232 population referenced above in several potentially relevant features; however, these and other promising results in CR1 led many to consider HSCT for persistent MRD, for example, the current prospective examination on the European trial AEIOP-BFM-ALL 2009.[26] Nevertheless, HSCT outcomes are also affected by persistent MRD, but these studies measure MRD later, primarily in the peritransplant period. MRD that remains detectable at a level of 10^{-3} or 0.1% pre-HSCT increases the risk of relapse,[23,27] prompting several groups to incorporate intensive HR chemotherapy blocks before HSCT. Although the clearance or reduction of MRD to a low level before HSCT may improve DFS, data on MRD reduction are limited, and these blocks are associated with a high rate of grade 3 to 4 toxicities, notably infections in two-thirds of patients.[28] Finally, post-HSCT MRD was more predictive of relapse risk on a multicenter observational study.[23] It is not clear whether the level of MRD itself is prognostic, or if MRD is a marker of the underlying leukemia biology. Although chemotherapy refractory leukemias may be responsive to the immune surveillance provided by allogeneic HSCT, aggressive leukemias may not tolerate the delay in immune surveillance before donor T-cell engraftment.

Toxicity

The risk-benefit analysis for HSCT necessarily considers the morbidity and mortality associated with HSCT, both of which have improved dramatically over the past 30 years.[21,29] These improvements are due in part to improved matching of donors and recipients, leading to decreased graft-versus-host disease (GVHD), improved treatments for GVHD, and improved supportive care.[21,27,29] Nevertheless, rates of grade 3 to 4 acute GVHD and nonrelapse mortality remain substantial, between 10% and 15% and between 5% and 15%, respectively.[27,29,30] In addition, long-term toxicities related to chronic GVHD or conditioning regimen include endocrinopathies, growth delay, organ dysfunction, and infertility. The role of HSCT in pediatric ALL continues to be a moving target, as survival benefit and significant but improving toxicities are balanced against novel and targeted therapies.

CAR T-cell therapy for VHR B-ALL in CR1

Chemotherapy refractory disease remained an insurmountable challenge for most novel therapies and HSCT until the advent of CAR T-cell therapy. T cells engineered to express a CAR, which links an antigen recognition domain with T-cell signaling domains, are activated by CAR engagement by their target, producing a cytotoxic T-cell response that kills the bound antigen-expressing cell (Figure 1). In clinical trials for multiply relapsed/refractory (R/R) B-ALL, CAR-modified T cells targeting CD19 produced CR rates exceeding 80% to 90% (Table 2).[31-37] Tisagenlecleucel (CTL019) became the first CAR T-cell therapy approved by the US Food and Drug Administration in August 2017. This CAR T-cell product, developed by the University of Pennsylvania (Penn), Children's Hospital of Philadelphia (CHOP), and Novartis, uses an anti-CD19 scFv domain for B-cell targeting and the 4-1BB domain for costimulation.[38]

Efficacy in R/R ALL

In a phase 1/2a single-institution trial of tisagenlecleucel conducted at CHOP (Clinical Trials.gov identifier: NCT01626495), a CR rate of 93% was observed in 60 patients with R/R ALL; relapse-free survival was 60% (95% confidence interval [CI], 48%-75%) at 12 months and 53% (95% CI, 39%-70%) at 24 months, with a median follow-up of 15 months.[37,39] A phase 2

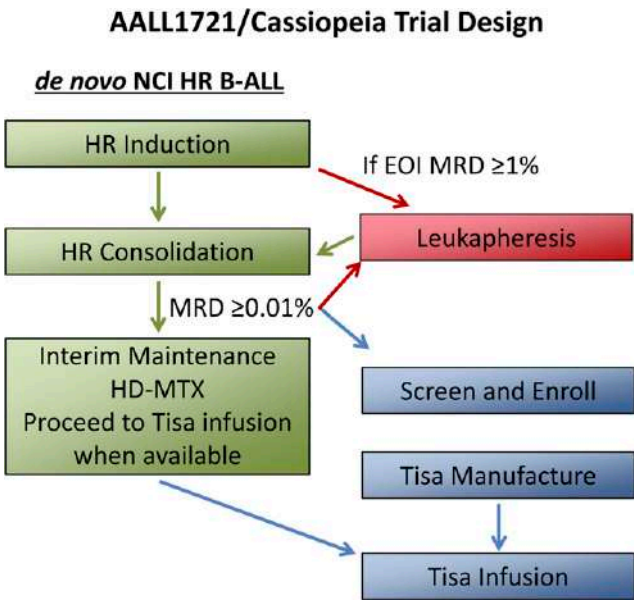

Figure 2. AALL1721/Cassiopeia trial design. AALL1721/Cassiopeia is a phase 2, single-arm, international multicenter trial of tisagenlecleucel in children and young adults with persistent MRD. Patients age 1 to 25 years diagnosed with CD19-expressing National Cancer Institute (NCI) HR (age 10 years or older or presenting with a white blood cell count ≥50 × $10^{9/L}$) B-ALL are eligible in first remission after induction/protocol IA and consolidation/protocol IB chemotherapy if MRD is detected by central multiparameter flow cytometry at ≥0.01%. Leukapheresis can occur after induction, if EOI MRD ≥1%, or after consolidation, once a patient has a qualifying MRD result. Enrolled patients proceed to the next phase of standard-of-care therapy, interim maintenance (IM), during the period of tisagenlecleucel manufacture. After stopping IM chemotherapy, patients will receive a lymphodepleting chemotherapy regimen of fludarabine and cyclophosphamide followed by a single infusion of tisagenlecleucel. After infusion, no further cancer-directed chemotherapy (including intrathecal chemotherapy) will be administered per protocol. HD-MTX, high-dose methotrexate; tisa, tisagenlecleucel.

single-arm, multicenter, global registration trial (ELIANA; Clinical Trials.gov identifier: NCT02435849) conducted across 25 centers demonstrated a CR rate of 81% in 75 patients with R/R B-ALL treated with tisagenlecleucel, with undetectable MRD in 100% of responses.[33] At 12 months, relapse-free survival was 59% (95% CI, 41%-73%) and overall survival was 76% (95% CI, 63%-86%), with a median follow-up of 13 months. Remissions were achieved in patients with chemotherapy refractory disease who had high leukemic burden in the bone marrow and across a wide range of disease burden. In the phase 1/2a trial, 73% of patients had detectable disease: 20% with MRD, 53% with >5% leukemic blasts, and 38% with >50% leukemic blasts in the bone marrow at the time of infusion. Both of these trials demonstrated durable tisagenlecleucel persistence (as long as 39 months at data cutoff) and durable remissions without consolidative HSCT (11% underwent HSCT in remission after tisagenlecleucel).[33,39] On the basis of data from the ELIANA trial, with supporting data from the Penn/CHOP phase 1/2a trial and a Novartis US multicenter phase 2 trial (Clinical Trials.gov identifier: NCT02228096), tisagenlecleucel was granted approval by the US Food and Drug Administration for children and young adults up to age 25 years with B-ALL that is refractory or in second or greater relapse.

AALL1721/Cassiopeia

For patients with poor early response to chemotherapy, a therapy with a distinct mechanism of action and demonstrated efficacy in chemotherapy refractory disease is desirable. To improve outcomes in the VHR population with persistent MRD, the COG chose to study CAR T-cell therapy using tisagenlecleucel on the basis of the excellent MRD-negative remission rates and durable remission rates reported in B-ALL refractory to standard chemotherapy. Although HSCT is often considered for patients with anticipated poor survival with chemotherapy alone, relevant to this population, detectable MRD at the time of HSCT has been consistently associated with an increased risk of relapse, as is MRD post-HSCT.[23,25,27,40,41] Further intensification of chemotherapy to decrease or eliminate MRD and HSCT itself are not without significant risk of morbidity and mortality.[3,22,28,42] Therefore, the possibility of durable remission without consolidative HSCT is one aim of an ongoing trial developed by the COG and Novartis.

AALL1721/Cassiopeia (Clinical Trials.gov identifier: NCT03876769), a phase 2 single-arm trial of tisagenlecleucel in children and young adults with National Cancer Institute HR B-ALL and persistent MRD at EOC, is the first trial of CAR T-cell therapy in CR1. Participating sites include COG centers in the United States and Canada as well as pediatric centers in the United Kingdom and Europe. Patients age 1 to 25 years diagnosed with CD19-expressing National Cancer Institute HR B-ALL are eligible in CR1 after induction/protocol IA and consolidation/protocol IB chemotherapy if MRD is detected by central multiparameter flow cytometry at ≥0.01% (Figure 2). To closely match the population studied on AALL0232, patients with Ph^+ ALL and hypodiploid ALL are excluded, as are those who have received tyrosine kinase inhibitor therapy. AALL1721/Cassiopeia, which opened in March 2019, aims to determine the efficacy and safety of tisagenlecleucel in CR1 in this patient population through a primary end point of 5-year DFS and secondary end points including overall survival, safety, the percentage of patients in remission without HSCT at 1 year, and time to B-cell recovery, a surrogate marker of CD19 CAR T-cell functional persistence. A potential advantage of CAR T-cell therapy is rapid, targeted immune surveillance; however, durability of this surveillance is determined by persistence, which can vary between products and individuals (Table 2). In addition, the potential for antigen escape leading to relapse is a concern with targeted immunotherapy that has been observed in ~20% to 25% of patients.[33,39] Poor T-cell expansion resulting in manufacture failure or toxicities precluding infusion are also potential limitations to CAR T-cell therapy; therefore, these rates will be monitored.

Toxicity

The principle toxicity of CAR T-cell therapy, cytokine release syndrome (CRS), is anticipated to be less severe in this population in CR, based on experience in early-phase clinical trials.[37] CRS is a hyperinflammatory syndrome associated with rapid exponential proliferation of CAR T cells.[43-45] On previous clinical trials of tisagenlecleucel, CRS was observed in close to 90% of ALL patients, with grade 4 CRS reported in 25% of patients on the ELIANA trial.[33,39] CRS symptoms range from mild flu-like symptoms, including persistent high fevers, myalgias, headache, fatigue, nausea/vomiting, and anorexia, that are self-limited and typically fully resolve in the first month, to life-threatening complications and multiorgan system failure. In patients with ALL, high

bone marrow disease burden is associated with an increased risk of severe CRS, an association reproduced with several CD19 CAR T-cell products.[31,35-37,45] Conversely, the risk of severe CRS is low for patients in morphologic remission.

Neurotoxicity is a second common toxicity associated with T-cell–engaging immunotherapies, reported in 40% to 45% of patients on clinical trials of tisagenlecleucel.[33,46] The spectrum of neurotoxicity symptoms is broad and includes confusion, delirium, hallucinations, global encephalopathy, aphasia, tremor and, less commonly, seizure.[33,37,46-48] Although neurotoxicity has been observed at low disease burden and can occur in the absence of CRS symptoms,[47,48] increased incidence and severity of neurotoxicity has been associated with higher-grade CRS.[33,46,48]

The association of CRS severity with disease burden and with incidence and severity of neurotoxicity suggests improved tolerance in a low disease burden state of MRD during frontline therapy. However, the long-term effects of CAR T-cell therapy, its acute toxicities, and chronic B-cell aplasia remain unknown; therefore, it will be important to monitor patients for late toxicities.

Clinical case update

After discussing the risk of relapse with EOC MRD, HSCT or enrollment on the AALL1721/Cassiopeia trial is offered, and the patient and family elect to enroll on the study. The patient continues standard-of-care chemotherapy while tisagenlecleucel is manufactured, receiving 2 courses of high-dose methotrexate. Before infusion, MRD remains stably positive. The patient receives tisagenlecleucel and achieves an MRD-negative remission 1 month after infusion.

Conclusions

The data for poor outcomes with standard chemotherapy in HR B-ALL with persistent MRD are strong; however, the data for alternative therapy approaches are limited or lacking. HSCT improves outcomes in specific ALL populations, and CD19 CAR T-cell therapy has demonstrated efficacy in patients who are not candidates for HSCT. It is reasonable to hypothesize that either approach will improve outcomes relative to chemotherapy alone, but it is difficult to directly compare these approaches with differing eligibility criteria and toxicity profiles. The AALL1721/Cassiopeia trial aims to address part of the question of whether outcomes for VHR B-ALL can be improved without the toxicity of HSCT. Results of this trial may inform broader study of CAR T-cell therapy in frontline therapy for poor-risk B-ALL.

Conflict-of-interest disclosure

S.L.M. received clinical trial support from Novartis and participated in consulting, advisory boards, or study steering committees for Novartis, Kite, and Wugen. C.D. declares no competing financial interests.

Off-label drug use

Tisagenlecleucel is investigational in the frontline setting.

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EVIDENCE-BASED MINIREVIEW

What is the role for HSCT or immunotherapy in pediatric hypodiploid B-cell acute lymphoblastic leukemia?

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LEARNING OBJECTIVES

- Understand the role of hematopoietic stem cell transplantation in pediatric patients with hypodiploid acute lymphoblastic leukemia (ALL)
- Understand the rationale for immunotherapy and the need for clinical trials of novel therapies in hypodiploid B-cell ALL

Clinical case

A 6-year-old boy was diagnosed with B-cell acute lymphoblastic leukemia and received induction chemotherapy on an institutional protocol. Cytogenetic analysis of the leukemic blasts revealed hypodiploidy (near haploid; 25 chromosomes); no other high-risk disease or patient characteristics were present. End-of-induction disease response showed complete remission, with minimal residual disease (MRD) by flow cytometry of 0.018%. The patient was risk stratified to high-risk therapy and received consolidation chemotherapy, after which MRD remained detectable at 0.039%. What is the role for hematopoietic stem cell transplantation or another immunotherapeutic approach for this patient?

Introduction

Pediatric hypodiploid acute lymphoblastic leukemia (ALL) is a rare subtype (<5% of B-cell ALLs [B-ALLs]) associated with adverse prognosis, with reported 5-year event-free-survival (EFS) of 50% to 55% overall.[1-4] Outcomes are increasingly dismal with decreasing modal chromosome number, with 5-year EFS as low as 30% in some subsets.[1-4] Subsets of hypodiploid ALL categorized by chromosome number carry distinct genetic lesions that may contribute to greater risk of treatment failure and/or toxicity, including mutations in *TP53*, *RB1*, and *IKZF2*, recurrent in low hypodiploid ALL with 32 to 39 chromosomes, and *IKZF3* and alterations in RAS and receptor tyrosine kinase signaling, recurrent in near-haploid ALL with 24 to 31 chromosomes.[5,6] Furthermore, increased incorporation of upfront response monitoring by minimal residual disease (MRD) testing has demonstrated that end-of-induction (EOI) MRD is more common and significantly decreases survival in hypodiploid ALL.[3-5] This suggests that chemotherapy resistance may play a role in poor outcomes, raising the question of whether immune-based approaches could improve responses and potentially avoid toxicity associated with *TP53* mutations, which can be germline in a significant fraction of pediatric patients. Because of its extremely poor prognosis, attempts to improve survival in hypodiploid ALL through empiric intensification of therapy have been employed, including allogeneic hematopoietic stem cell transplantation (HSCT) in first complete remission (CR1). We will review the evidence for HSCT and other immunotherapies in hypodiploid ALL.

Allogeneic HSCT for hypodiploid ALL

Randomized trials evaluating the efficacy of allogeneic HSCT compared with standard therapy for pediatric patients with hypodiploid ALL in CR1 are lacking, given the rarity of this disease subset and the poor outcomes with chemotherapy alone, making randomization undesirable. Using data submitted to the Center for International Blood and Marrow Transplant Research, Mehta et al[7] reported on 78 pediatric patients with hypodiploid B-ALL who underwent HSCT (CR1, 55%; CR2, 38%) between the years 1990

Table 1. Studies of empiric allogeneic HSCT in the treatment of pediatric hypodiploid ALL

Study	Design	Study years	Population, N (n with HSCT)	CR (% of patients)	Median age (range), y	N of chromosomes (% of patients)	EOI MRD (% of patients)	Outcomes (95% CI)
Mehta et al[7] (CIBMTR)	Retrospective, nonrandomized, multicenter	1990-2010	78 (78)	CR1 (55)	10 (3-18)	≤43 (50)	NR	5-y LFS: 51% ≤43 ch, 37% (23-51) vs 44-45, 64% (48-76); *P* = .01
				CR2 (38)		44 (15)		5-y OS: 56% ≤43 ch, 38% (24-52) vs 44-45, 71% (56-82); *P* = .001
				CR3 (7)		45 (35)		
Pui et al[4] (Ponte di Legno)	Retrospective, nonrandomized, multicenter	1997-2013	272 (42 of 228)*	CR1 (100)	9.8 (0.6-19.5)	25-29 (37) 30-39 (43) 40-43 (5) 44 (15)	$<10^{-4}$ (54)	5-y EFS: 55.1% (49.3-61.5)
							10^{-4}-10^{-3} (16)	Favorable: 44 ch, 74% (61-89); *P* = .021
							$\geq 10^{-3}$ (30)	MRD $<10^{-4}$, 75% (66-85); *P* = .003
								5-y DFS: HSCT vs no HSCT*: 59.8% (45.7-78.2) vs 53% (45.9-61.2); *P* = .47 MRD $<10^{-4}$: 70% (46.7-100) vs 73.6% (63.3-85.7); *P* = .81 MRD $\geq 10^{-3}$: 55.9% (37.2-84) vs 40.3% (27.2-59.7); *P* = .29 30-39 ch, 63.5 (43.2-93.3) vs 61.6 (51.8-73.1); *P* = .89 25-29 ch, 50.8 (32.5-79.4) vs 44 (34.2-56.6); *P* = .60
								5-y OS: HSCT vs no HSCT*: 68.9% (55.8-85.2) vs 57.7% (50.7-65.7); *P* = .21
McNeer et al[2] (COG)	Retrospective, nonrandomized, multicenter	2003-2011	131 (61 of 113)	CR1 (100)	10 (1-30)†	25-29 (42) 30-39 (36) 40-43 (2) Masked (20)	<0.01% (68)	5-y EFS: 52.2% ± 4.9%
							≥0.01%, 32%	HSCT vs no HSCT: 56.4% ± 7.3% vs 48.8% ± 7.8%; *P* = .62 NCI SR: 68.8% ± 10.3% vs 57.1% ± 13.2%; *P* = .64 NCI HR: 48.3% ± 9.0% vs 44.4% ± 9.2%; *P* = .75 MRD <0.01%: 66.3% ± 7.9% vs 60.3 ± 9.2%; *P* = .77 MRD ≥0.01%: 29.4% ± 14.3% vs 16.7% ± 10.8%; *P* = .67
								5-y OS: 58.9% ± 4.8% HSCT vs no HSCT: 65.6% ± 6.9% vs 53.8% ± 7.8%; *P* = .32

ch, chromosomes; CI, confidence interval; CIBMTR, Center for International Blood and Marrow Transplant Research; COG, Children's Oncology Group; DFS, disease-free survival; HR, high risk (age ≥10 y or white blood cell count ≥50,000/μL); LFS, leukemia-free survival; NCI, National Cancer Institute; NR, not reported; SR, standard risk (age 1 to <10 y and white blood cell count <50 x 10^3/μL).

*HSCT analyses limited to patients with <44 chromosomes.

†Age range eligible for study (actual age range not reported).

and 2010. Overall survival (OS) for the entire cohort was similar to that in prior reports (Table 1), and modal number of chromosomes significantly affected both 5-year OS (≤43: 38%; 95% CI, 24-52 vs 44-45: 71%; 95% CI, 56-82; *P* = .001) and leukemia-free survival (≤43: 37%; 95% CI, 23-51 vs 44-45: 64%; 95% CI, 48-76; *P* = .01), when adjusting for both CR number and transplantation era.[7] Although these data suggest those with higher modal number of chromosomes fare well with HSCT, this subset also fares better with chemotherapy alone. Therefore, improved survival post-HSCT may not be attributable to HSCT.

Relapsed hypodiploid ALL portended a significantly worse prognosis (mortality hazard ratio for CR2/3 vs CR1, 2.28; 95% CI, 1.02-5.10; *P* = .04); while expected, this suggests hypodiploid ALL is exceedingly difficult to salvage. The authors acknowledged the limitations of modest sample size, only including patients who achieved CR and were candidates for HSCT, and lack of EOI MRD response risk stratification. Conclusions regarding superiority of undergoing consolidative HSCT compared with receiving chemotherapy alone cannot be drawn from this study.

Does HSCT improve outcomes over chemotherapy alone?

More recently, reports on 2 larger cohorts of pediatric patients with hypodiploid ALL included comparative analyses of consolidative HSCT in CR1 or chemotherapy alone. The first included 272 evaluable patients treated among 16 cooperative groups/institutions between the years 1997 and 2013 (Ponte di Legno Childhood ALL Working Group).[4] Among 228 patients with <44 chromosomes, 18% underwent HSCT in CR1, and the remainder received chemotherapy alone. Between these 2 cohorts, no significant difference was seen in adjusted 5-year DFS or OS (Table 1). Importantly, HSCT did not significantly affect DFS, regardless of high-risk features, including EOI MRD and ploidy. However, MRD-stratified protocols were associated with improved EFS and OS.[4] The COG reported on 131 patients with hypodiploid ALL treated from 2003 to 2011, 113 of whom were evaluable for impact of consolidative HSCT in CR1 (n = 61) vs chemotherapy alone (n = 52).[2] HSCT did not confer a survival advantage (Table 1), including in subgroup analyses using National Cancer Institute risk group and EOI MRD. Notably, patients in the HSCT cohort had a higher incidence of secondary malignant neoplasms.[2] Although germline *TP53* mutations were not evaluated in this study, >90% of patients with low hypodiploid ALL harbor *TP53* mutations, nearly half of which may be germline, underscoring the importance of critically evaluating the role of HSCT in populations that may have higher toxicity risk.[6] Both studies adjusted for time to HSCT to partially mitigate the selection bias inherent to nonrandomized studies of HCST, which necessitate patients achieving and maintaining CR.

Although HSCT has been shown to improve outcomes for subsets of patients with ALL with poor-risk features, the large international, multicenter studies demonstrate that HSCT in CR1 does not significantly improve outcomes for pediatric patients with hypodiploid ALL.[2,4] Despite these 2 larger patient cohorts, analyses regarding the role of empiric HSCT are likely still underpowered as a result of the rarity of this disease subtype. Collectively, these data highlight that EOI MRD may be a useful indicator of leukemic chemosensitivity. Therefore, empiric intensification of existing therapies is likely not needed for patients who achieve MRD^- status nor effective for MRD^+ patients; alternative approaches with distinct mechanisms of action are needed.

Immunotherapy: beyond HSCT

Although allogeneic HSCT is an effective immunotherapy for many patients, it is not curative for all, particularly subgroups of patients with persistent MRD at the time of HSCT. Chemorefractory leukemias may be responsive to the immune surveillance provided by allogeneic HSCT, but aggressive leukemias with detectable disease at the time of HSCT may not tolerate the delay in immune surveillance before donor T-cell engraftment. Increased resistance to HSCT may also be in part due to increased inherent capability of mutagenesis and acquisition of immune escape mechanisms. As we strive to improve outcomes for hypodiploid ALL, targeted immunotherapeutic approaches could provide an alternative treatment strategy with increased benefit in these high-risk patients. More contemporary immunotherapeutic strategies specifically target leukemic cells, often through recognition of tumor-associated antigens in conjunction with an effector cell–mediated response. Many of these therapies have seen great success, particularly CD19-specific chimeric antigen receptor (CAR) T-cell therapy for the treatment of pediatric $CD19^+$ B-ALL.[8-10] Although data are limited in rare subsets, an analysis of outcomes with the CD19 CAR T-cell therapies CTL019 and CTL119 demonstrated similar outcomes for ALL with high-risk cytogenetics, including hypodiploidy, which accounted for 3.5% of the cohort of 231 patients, relative to other cytogenetic subgroups.[11] Similarly, a retrospective study of the CD22 antibody-drug conjugate inotuzumab in relapsed/refractory pediatric ALL included hypodiploidy (6% of the cohort of 51) and found no predictive effect of cytogenetic subtype on response.[12] However, as with chemotherapy and HSCT, remission is not attainable or durable for all patients, likely in part due to inherent leukemic resistance and/or mutagenicity. Mutagenic leukemic clones may have a higher risk of developing immune escape after antigen-directed therapy. With current data, it is impossible to draw conclusions yet regarding the role of these newer immunotherapeutic modalities for hypodiploid ALL, because the number of reported patients treated thus far is quite limited. Both strategies are being studied in the frontline setting in clinical trials that include hypodiploid ALL. A single-center single-arm trial is investigating CTL019 for indications not previously studied, including hypodiploid ALL (registered at www.clinicaltrials.gov as #NCT04276870). The current frontline COG trial for de novo high-risk B-ALL, AALL1732 (registered at www.clinicaltrials.gov as #NCT03959085), is randomly assigning patients to standard chemotherapy or the intercalation of inotuzumab into the chemotherapy backbone. Although sample size is likely to remain limiting, by offering therapies with mechanisms of action distinct from those of cytotoxic chemotherapy, these studies have the potential to provide improved outcomes and valuable insights into the resistance of hypodiploid ALL to current standard therapies.

Conclusion

Pediatric hypodiploid ALL is a high-risk disease subtype, with continued poor outcomes despite intensification of both upfront and relapse therapies. The use of empiric allogeneic HSCT in CR1 has not proven to add a survival benefit for this patient population. However, improvements have been reported with risk-stratified protocols based on EOI MRD response, which remains highly predictive of outcome in this subtype. Using modal chromosome number, genomics, and EOI MRD, patients with hypodiploid ALL can be categorized into distinct biologic subsets with different responses to traditional therapies. This may allow for the opportunity to evaluate the role of newer treatment strategies, including immunotherapy, for patients with the worst predicted outcomes. The rarity of such subgroups necessitates continued collaboration and prospective study to sufficiently power analyses and gain further biologic insight into this difficult-to-treat patient population.

Recommendations

1. MRD response is prognostic and can guide treatment considerations in pediatric hypodiploid ALL (grade 1A).
2. There is insufficient evidence to recommend HSCT for all children with hypodiploid ALL in CR1 (grade 2C).
3. Novel immunotherapies, such as CD19 CAR T cells and inotuzumab, with strong evidence of efficacy in the relapsed/refractory setting can be considered for hypodiploid ALL with poor MRD response in the context of a clinical trial (grade 2D).

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Off-label drug use

CTL019 and inotuzumab are investigational in the frontline setting.

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Does ABO and RhD matching matter for platelet transfusion?

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Platelets express ABO antigens and are collected in plasma, which contains ABO antibodies as would be consistent with the donor ABO group. Platelet ABO antigens that are incompatible with recipient ABO antibodies may have accelerated clearance from circulation and result in lower count increments. ABO antibodies that are passively transferred from donor plasma may result in hemolysis of recipient red blood cells. Although platelets do not express Rh antigens, they contain small numbers of intact red blood cells or fragments, which can lead to alloimmunization in the recipient. Alloimmunization to the RhD antigen may occur when platelets obtained from RhD-positive donors are transfused to RhD-negative recipients. All of these compatibility considerations must be balanced against the available supply, which may be limited due to the 5- to 7-day shelf life of platelets. This articles describes considerations for platelet ABO and RhD selection for platelet transfusions, including the impact of major ABO incompatibility on count increments, the risks of hemolysis associated with minor ABO incompatibility, and the risk of RhD alloimmunization when RhD-negative patients receive platelets obtained from RhD-positive donors.

LEARNING OBJECTIVES

- Describe the role of ABO matching in platelet transfusions, including the impact of major ABO incompatibility on count increments and the risks for hemolysis associated with minor ABO incompatibility
- Recognize the risk of RhD alloimmunization when RhD-negative patients receive platelets obtained from RhD-positive donors and describe how Rh immune globulin is used to mitigate this risk

Clinical case

A 60-year-old woman with newly diagnosed acute myeloid leukemia is admitted for induction therapy. She eventually develops chemotherapy-associated thrombocytopenia. The clinical team requests platelet transfusion when her platelet count drops below 10×10^3 per microliter. The patient is A negative. The blood bank has a limited supply of apheresis platelets, including a "low-titer" O-positive unit expiring today at midnight, a B-negative unit expiring in 2 days, and an A-positive unit expiring in 3 days.

What are the risks and benefits associated with each of the platelet options for this patient, and which unit should the blood bank provide?

Platelet compatibility

When considering a platelet transfusion, one must consider the ABO compatibility of the platelets themselves, as well as the accompanying plasma. Platelets, like red blood cells (RBCs), express ABO antigens, although expression is variable and strongly expressed in only 4% to 7% of individuals.[1] Platelets are also suspended in roughly 1 unit of donor plasma, which contains the ABO antibodies predicted by the donor blood type (Table 1). Platelets do not express Rh antigens but contain small numbers of RBCs or fragments, which can cause alloimmunization to red cell antigens, including the RhD antigen when platelets obtained from RhD-positive donors are transfused to RhD-negative recipients.

Unlike RBCs, platelets are not at risk for intravascular hemolysis when transfused out of group. However, the accompanying plasma may cause hemolysis when ABO incompatible with recipient RBCs. Because of this risk, AABB standards require transfusion services to have policies concerning transfusion of components that contain significant amounts of incompatible ABO antibodies.[2] Additionally, because of the risk for alloimmunization to the RhD antigen, AABB standards also require transfusion services to have policies for the use of RhD-positive RBC-containing components in RhD-negative recipients.[2]

Table 1. Platelet antigens and plasma antibodies based on donor ABO group

Platelet ABO group	AB	A	B	O
Antigens expressed on platelet surface	A and B	A	B	None
Antibodies present in plasma	None	Anti-B	Anti-A	Anti-A and anti-B

In terms of ABO compatibility, platelets may be identical, major incompatible, minor incompatible, or bidirectional incompatible (Table 2). In major incompatibility, donor ABO antigens are incompatible with recipient ABO antibodies. In minor incompatibility, donor ABO antibodies are incompatible with recipient ABO antigens. Both types of incompatibility are present in bidirectional incompatible transfusions.

RhD compatibility is more nuanced (Table 2). The formation of antibodies against the RhD antigen (ie, anti-D) requires exposure to red cell antigens, such as through pregnancy, transfusion, or transplantation. Thus, transfusion recipients with anti-D antibodies are much less common than those with the anti-A and/or anti-B antibodies that are predictably present based on ABO group. This is because ABO antibodies are formed without red cell exposure (ie, "naturally occurring").

Platelet transfusions from RhD-positive donors to recipients with anti-D antibodies do not result in hemolysis, because they contain very few RBCs. Only products containing >2 mL of incompatible RBCs require a serologic crossmatch per AABB standards.[2] In addition, all blood component donors undergo antibody screening to ensure that plasma-containing components, such as platelets, do not contain non-ABO antibodies (eg, anti-D). Therefore, RhD-positive patients may receive platelets obtained from RhD-positive or RhD-negative donors without risk. Only RhD-negative recipients of platelets obtained from RhD-positive donors are at risk for RhD alloimmunization.

ABO identical platelets

In an ideal world with unlimited resources, platelet transfusions would be ABO identical to the recipient and obtained from RhD-negative donors when the recipient is RhD negative. However, the short shelf life (5-7 days) and supply limitations make this challenging for most transfusion services.[3] In addition, the ABO composition of the platelet donor pool may not reflect the ABO distribution of the patient population.[4] In an effort to conserve resources, most transfusion services use a first in/first out platelet-transfusion strategy.

Not surprisingly, use of ABO identical platelets has been associated with increased outdate rates.[5] Further, provision of ABO identical platelet transfusions has not been shown to provide any clear benefit in terms of clinical outcomes.[6] One study in autologous hematopoietic stem cell transplant recipients did not find any impact on morbidity or survival among recipients of ABO nonidentical platelets.[7]

Major ABO incompatible platelets

Although the name implies increased patient risk, major incompatible platelet transfusions are common and typically uneventful. One study observed higher transfusion reaction rates among recipients of major incompatible platelet transfusions,[8] but other studies have not shown an association between ABO compatibility and transfusion reaction rates.[9]

Unlike major incompatible RBC transfusions, there is no risk for hemolysis. Recent data indicate that ~31% of platelet transfusions in the United States are major incompatible.[10] In this scenario, the recipient has antibody directed against platelet ABO antigens, as would be predicted based on ABO group, which may result in accelerated platelet clearance. Major compatible platelet transfusions are associated with higher count increments. However, this difference is small and not clinically significant in terms of

Table 2. Platelet ABO compatibility

ABO		Platelet recipient ABO group			
		AB	A	B	O
Platelet donor ABO group	AB	Identical	Major	Major	Major
	A	Minor	Identical	Bidirectional	Major
	B	Minor	Bidirectional	Identical	Major
	O	Minor	Minor	Minor	Identical
RhD		**Platelet recipient RhD type**			
		Positive		**Negative**	
Platelet donor RhD type	Positive	Identical		Mismatch At risk for RhD alloimmunization	
	Negative	Mismatch No risk for RhD alloimmunization		Identical	

ABO identical: donor and recipient have the same ABO antigens and antibodies. Major ABO mismatch: donor ABO antigens are incompatible with recipient ABO antibodies. Minor ABO mismatch: donor ABO antibodies are incompatible with recipient's ABO antigens. ABO bidirectional mismatch: donor ABO antibodies are incompatible with recipient ABO antigens and donor ABO antigens are incompatible with recipient ABO antibodies. Rh mismatch: Rh type of the recipient is different from the Rh type of the donor. RhD-negative recipients of platelets obtained from RhD-positive donors are the only ones at risk for RhD alloimmunization.

bleeding risk.[11] Because major incompatible platelet transfusions result in slightly lower increments, a decreased platelet transfusion interval has also been observed.[11] When I investigate patients who appear to be refractory to platelet transfusions, major ABO incompatibility is always in the differential. Patients who are found to have high-titer anti-A or anti-B antibodies may respond better to major compatible platelet transfusions. I typically support these patients with ABO compatible platelet transfusions, when possible.

Minor ABO incompatible platelets

Currently, ~19% of platelet transfusions in the United States are plasma incompatible.[10] Although the name implies negligible risk, minor incompatible transfusions can result in hemolysis of recipient RBCs, which may even be fatal. Although this complication is rare, there have been 6 fatalities reported to the US Food and Drug Administration from 2007 to 2017 that were due to hemolysis from minor ABO incompatible platelet transfusions (Table 3).[12] Clinically detectable reactions that are not fatal are also reported in the literature.[13] Most case reports of hemolysis following minor ABO incompatible platelets involve transfusion of group O apheresis platelets to group A or AB recipients.[14] This is due to that fact that a subset of group O donors produce high-titer anti-A antibodies.[15] However, there are case reports associated with non-O donors.[16]

Hemolytic reactions occur most commonly if the antibody titer exceeds 100 in saline or 400 with antiglobulin reagent.[14] Some centers attempt to mitigate this risk by performing screening titers.[17] In the United States, there is no standardized titer method and no agreement on the definition of a high-titer unit.[18-20] One center, which investigated using a saline titer cutoff of 250, found that 25% of group O platelets would be identified as high titer and, therefore, limited to group O recipients.[21]

Interestingly, units retrospectively identified as high titer are not always predictive of hemolysis.[22,23] This suggests that risk may be impacted by other recipient factors. Protective mechanisms include the presence of ABO antigen on endothelial cells and the presence of soluble A and/or B substance in the plasma of secretors, both of which may decrease the interaction of incompatible antibodies with recipient RBCs.[24]

Other risk-mitigation approaches have been used to address the volume of ABO incompatible plasma (Table 4). Some centers limit the allowable volume of incompatible plasma over defined periods to decrease the risk of a cumulative effect of transfused incompatible antibody.[17,25] Pooled whole blood–derived platelets may dilute the plasma from any high-titer donors, but case reports exist of hemolysis following pooled platelet transfusions.[26]

Another dilution strategy is the use of platelets suspended in platelet additive solution (PAS), a manufacturing modification that replaces 65% of the donor plasma.[27] Studies have shown that PAS can reduce ABO antibody titers by 50%, which would reduce the number of high-titer group O units and expand the potential for out-of-group transfusions.[28] However, only 8.5% of surveyed facilities in the United States were using PAS platelets in 2017.[29] Case reports also exist of hemolysis following PAS platelet transfusions.[30]

Volume reduction and washing are component-modification strategies that also reduce risk from ABO incompatible plasma. Both of these component modifications increase the time required for component preparation and reduce the product shelf life to 4 hours. One center that implemented volume reduction for all minor ABO incompatible platelet transfusions estimated that the blood bank workload increased by 0.34 full-time equivalent.[25]

Neither volume reduction nor washing is practical in the setting of urgent transfusion. These modifications are also associated with platelet loss. They are estimated to result in a 15% to 33% decrease in platelet content,[15] leading to decreased transfusion intervals among transfusion recipients.[31]

RhD compatibility

Platelets do not express Rh antigens, but platelet components contain residual intact RBCs or fragments that can result in alloimmunization to RBC antigens, including RhD.[3] The volume of contaminating RBCs varies by component-manufacturing method. Whole blood platelet production in the United States utilizes the platelet-rich plasma method, whereas Europe and Canada use the buffy coat method.[32] However, use of whole blood–derived platelets in the United States is limited, and 91% of platelet doses in 2017 were apheresis platelets.[33] Whole blood–derived pooled platelets may

Table 3. Fatalities associated with incompatible platelet transfusions reported to the US Food and Drug Administration from 2007 to 2017

Fiscal year	Patient ABO	Platelet ABO	Antibody titer
2015	B	A	Anti-B (1:2048)
2014	A	O	Anti-A (1:2048)
2014	AB	O*	Anti-A and anti-B (1:128)
		O*	Anti-A and anti-B (1:128)
2012	A	O†	"High titer"
		O†	Data not provided
2011	A‡	O	Anti-A 1:512 for IgM and 1:2048 for IgG[25]
2008	B‡	O	"High titer" Data not provided.

IgG, immunoglobulin G; IgM, immunoglobulin M.
*Recipient received 2 units of group O platelets from the same donor.
†Recipient received 2 units of group O platelets from 2 different donors.
‡Recipient's blood group recently changed following ABO-mismatched hematopoietic stem cell transplant.

Table 4. Summary of platelet minor ABO incompatibility risk-mitigation strategies

Mitigation strategy	Drawbacks
Issue only ABO identical or compatible units	Identical or compatible units may not always be available in inventory Potential for increased outdate rates
Limit volume of incompatible plasma	Compatible units may not always be available in inventory Potential for increased outdate rates
Provide pooled whole blood–derived platelets	Pooled platelets are not widely available in the United States Hemolytic transfusion reactions have been reported in pooled platelet units
Provide units suspended in PAS	PAS platelets are not widely available in the United States Hemolytic transfusion reactions have been reported in PAS units
Provide low-titer units	Titer method is not standardized No agreed upon high-titer cutoff Substantial percentage of group O units may be high titer, depending on cutoff Saline titer methods may miss units with high-titer IgG antibodies Hemolytic transfusion reactions have been reported in low-titer units
Volume reduction	Shortens shelf life Decreases platelet content Increases the time required for unit preparation and blood bank workload Not feasible for emergency transfusions
Washing	Shortens shelf life Decreases platelet content Increases the time required for unit preparation and blood bank workload Not feasible for emergency transfusions

IgG, immunoglobulin G; PAS, platelet additive solution.

contain up to 0.3 mL of residual RBCs, whereas apheresis platelets contain <0.001 mL.[34] This is likely why the risk of alloimmunization appears to be higher for whole blood–derived platelets compared with apheresis platelets.[35]

Although there have been reports of RhD alloimmunization following platelet transfusions, this appears to be an uncommon complication.[36] One center that attempts to provide platelets obtained from RhD-negative donors to RhD-negative patients has observed that this practice results in fewer of these patients receiving ABO identical transfusions.[4]

In a recent study, only 39% of platelet transfusions to RhD-negative patients were obtained from RhD-negative donors.[10] Current clinical practice guidelines recommend prophylaxis with Rh immune globulin (RhIg) when RhD-negative girls or women of childbearing potential receive platelets obtained from RhD-positive donors.[37] A standard 300-µg dose is sufficient for prevention of alloimmunization.[38] RhIg has a half-life of 3 weeks, and the volume of RBCs in each platelet dose is quite small. Therefore, a 300-µg dose should provide prophylaxis for multiple transfusions of platelets obtained from RhD-positive donors over a 2- to 4-week period. Given the low risk, some centers advocate the use of platelets without consideration of RhD status and without RhIg prophylaxis.[39,40]

Clinical case

Returning to the 60-year-old woman with newly diagnosed acute myeloid leukemia who requires platelet transfusion for chemotherapy-induced thrombocytopenia, in the example provided, the patient is A negative and the blood bank has a "low-titer" O-positive unit expiring today at midnight, a B-negative unit expiring in 2 days, and an A-positive unit expiring in 3 days.

The oldest platelet, and the one that should be used if inventory management is the only consideration, is the "low-titer" O-positive unit expiring today at midnight. The fact that it is "low titer" indicates that the blood bank routinely performs titer assessment of group O platelets and that this unit has anti-A and anti-B titers below the critical cutoff defined by this laboratory. Therefore, the risk for hemolysis is low if the unit is selected for this group A patient. This platelet is from an RhD-positive donor and poses a theoretical risk for RhD alloimmunization if given to this RhD-negative patient. However, given her age, there is no concern for a future pregnancy complicated by HDFN.

The second option is the B-negative platelet expiring in 2 days. Use of this unit may result in outdate of the O-positive unit that is expiring sooner. This unit is also minor ABO and major ABO incompatible (ie, bidirectional). Because it is not designated a "low-titer" unit, it suggests that titer of ABO antibodies has not been assessed. However, this unit is from an RhD-negative donor, which negates any risk for RhD alloimmunization in this patient.

The final option is the A-positive unit expiring in 3 days. As above, use of this unit may result in outdate of the O-positive and the B-negative units, which both expire sooner. This unit is ABO identical but is obtained from an RhD-positive donor.

Given these options, my hospital blood bank would most likely issue the O-positive platelet. If the group O unit were high titer, however, we would select the A-positive unit over the B-negative unit without anti-A titer. In either case, I would not offer prophylaxis with RhIg to this patient.

Summary

Management of the platelet inventory requires careful consideration, given the short shelf life of this product. The supply does

not always allow for provision of ABO identical and/or platelets obtained from RhD-negative donors for RhD-negative patients. Risks of giving non-ABO identical transfusions include decreased count increments and shorter transfusion intervals for major ABO incompatible transfusions and hemolysis of recipient RBCs for minor ABO incompatible transfusions. Provision of platelets obtained from RhD-positive donors for RhD-negative patients may result in alloimmunization to the RhD antigen. This risk appears to be quite low but can be mitigated by the use of RhIg in at-risk populations.

Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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How well do platelets prevent bleeding?

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Prophylactic platelet transfusions are used to reduce the risk of spontaneous bleeding in patients with treatment- or disease-related severe thrombocytopenia. A prophylactic platelet-transfusion threshold of <10 × 10^3/μL has been shown to be safe in stable hematology/oncology patients. A higher threshold and/or larger or more frequent platelet doses may be appropriate for patients with clinical features associated with an increased risk of bleeding such as high fevers, sepsis, disseminated intravascular coagulation, anticoagulation therapy, or splenomegaly. Unique factors in the outpatient setting may support the use of a higher platelet-transfusion threshold and/or dose of platelets. A prophylactic platelet-transfusion strategy has been shown to be associated with a lower risk of bleeding compared with no prophylaxis in adult patients receiving chemotherapy but not for autologous transplant recipients. Despite the use of prophylactic platelet transfusions, a high incidence (50% to 70%) of spontaneous bleeding remains. Using a higher threshold or larger doses of platelets does not change this risk. New approaches to reduce the risk of spontaneous bleeding, including antifibrinolytic therapy, are currently under study.

LEARNING OBJECTIVES

- Define the appropriate threshold for prophylactic platelet transfusion in a stable hematology/oncology patient
- List the clinical conditions for which a higher threshold may be indicated
- Describe the limitations of prophylactic platelet transfusions and the potential role of additional strategies to reduce the risk of bleeding

Clinical case

A 60-year-old woman with newly diagnosed acute myelogenous leukemia (AML) is admitted for induction therapy. Her platelet count on admission is 14 × 10^3/μL. She reports a minor nosebleed the day before that lasted for <5 minutes. She denies headaches or visible blood in her sputum, urine, or stools. Her physical examination is only remarkable for a few scattered petechiae on both arms. Urinalysis and stool guaiac for blood is negative.

Treatment-related questions

What is the role of prophylactic platelet transfusion in this patient? What is her risk of significant bleeding at a platelet count of 14 × 10^3/μL? Would platelet transfusion change her risk of bleeding?

The next day, her platelet count is 5 × 10^3/μL. No new bleeding is identified.

More treatment-related questions

Is there a role for prophylactic platelet transfusion at this point in her course? Has her risk of bleeding changed, and would platelet transfusions reduce her risk of bleeding?

Rationale for prophylactic platelet transfusions

It has been known for decades that patients with treatment- or hematologic disease–related severe thrombocytopenia have an increased risk of bleeding. Although most bleeding episodes are mild, there is a risk of severe or even fatal bleeding involving critical sites such as the lungs, brain, or eyes.[1] The biologic basis for prophylactic platelet transfusion was provided in a radiolabeled platelet-transfusion study, which suggested that 7 × 10^3/μL per day are required to maintain endothelial integrity.[2] Since then, prophylactic platelet transfusions have been routinely used to reduce the risk of bleeding, and they account for nearly 50% of all platelets transfused to patients with thrombocytopenia due to hematologic/oncologic disease or treatment-related thrombocytopenia.[3]

Prophylactic platelet-transfusion threshold

The prophylactic platelet-transfusion threshold has been evolving over the years since platelet transfusions first became available in the 1960s. For decades, a threshold of <20 × 10^3/μL was used for most patients. As treatment regimens changed and improvements were made in the

clinical management of thrombocytopenic patients, particularly in the management of infections, it became apparent that lower platelet counts could be safely tolerated than had been previously believed. This spawned a series of observational and randomized clinical trials comparing the risk of major bleeding using a prophylactic platelet count of ≤10 × 10^3/μL vs <20 × 10^3/μL.[4] Four randomized trials[5-8] demonstrated the safety of a lower threshold in that there was no difference in the rate of major bleeding events using a prophylactic threshold of <10 × 10^3/μL vs <20 × 10^3/μL or higher (Table 1). The lower threshold was also safe even in patients undergoing autologous or allogeneic hematopoietic stem cell transplantation (HSCT).[7,8] Admittedly, these are relatively small studies, however, additional supportive data came from a secondary analysis of a large randomized trial of the prophylactic platelet-transfusion dose.[1] The Platelet Dosing (PLADO) study used a platelet-transfusion threshold of ≤10 × 10^3/μL in 1272 patients with over 24 000 days of bedside observation, demonstrating that bleeding occurred on 25% of the study days when the platelet count was 5 × 10^3/μL or lower, as compared with 17% of study days when platelet count was >5 × 10^3/μL (P < .001) (Figure 1). These data suggest that the risk of bleeding exhibits a threshold effect and does not appear to change once the platelet count is above 5 × 10^3/μL, consistent with the estimate of ~7.1 × 10^3/μL platelets per day required to maintain vascular integrity in a nonfebrile, stable patient.[2] The results of the randomized trials and the results of the PLADO study support the use of ≤10 × 10^3/μL as a prophylactic platelet-transfusion threshold in stable hematology/oncology patients.

The data supporting a prophylactic platelet-transfusion trigger in pediatric hematology/oncology patients are less robust. Three of the randomized trials[5,7,8] included pediatric patients, but 1 study, by Rebulla et al,[5] only included children who were age 16 years or older; another trial, by Zumberg et al,[7] did not state how many of the 159 subjects were pediatric patients nor their mean age. Only Diedrich et al[8] indicated that 51 of the 166 patients in the trial were pediatric patients. The PLADO study included 198 pediatric patients transfused using a trigger of ≤10 × 10^3/μL.[9] Currently, there is no prospective randomized controlled trial (RCT) comparing platelet-transfusion thresholds exclusively in pediatric hematology/oncology patients. Thus, there are a limited number of pediatric patients and data upon which the recent guidelines promulgated by the American Society of Clinical Oncology (ASCO)[10] and the International Collaboration for Transfusion Medicine Guidelines[11] have been based. These guidelines recommend 10 × 10^3/μL as the prophylactic platelet-transfusion trigger for stable pediatric hematology/oncology patients.

Clinical factors that may impact the prophylactic platelet-transfusion threshold

Currently, most physician and hospital transfusion guidelines recommend a threshold platelet count of ≤10 × 10^3/μL as an indication for platelet transfusion in stable hematology/oncology patients. The platelet count is not the only consideration in determining bleeding risk in individual patients. Patients may have clinical factors associated with an increased risk of bleeding requiring an individualized approach. Increased platelet consumption and/or an increased risk of bleeding has been reported in patients with high fever, sepsis/infections, antifungal therapy, splenomegaly, coagulopathy, anticoagulant therapy, graft-versus-host disease, and veno-occlusive disease/sinusoidal obstruction syndrome.[12-14] A higher prophylactic platelet-transfusion threshold (eg, <20 × 10^3/μL) and/or larger or more frequent doses is a reasonable approach to ensure that the nadir platelet count prior to the next transfusion is maintained above the critical 5 × 10^3/μL. For prophylactic platelet transfusions in the outpatient setting, logistics, a lack of daily patient observation, and a potential delay in receiving therapy should bleeding occur need to be taken into consideration when formulating transfusion decisions. Outpatients may benefit from using a higher threshold or larger doses to lengthen the intervals between transfusion[1]; however, this strategy has been extrapolated from inpatient studies and has not been studied in outpatients.

The prophylactic platelet-transfusion threshold data from the RCTs[5-8] and other supportive observational trials[4] demonstrate that the threshold can be safely lowered to ≤10 × 10^3/μL for stable patients including HSCT recipients who do not have clinical factors for increased risk of bleedings such as high fever, sepsis, anatomical lesions, or other abnormality of hemostasis.[10,11,15] There are limitations to the available data when applied to subsets of hematology/oncology patients such as pediatric patients and outpatients.

Optimal dose of prophylactic platelets

The dose of platelets used for prophylaxis has gradually declined over the years from 10 whole-blood platelets in a pool to as few as 4 U in a pool, roughly equivalent to a single unit of apheresis platelets.[16] The impact of prophylactic platelet dose on bleeding outcomes has been assessed in 6 RCTs.[17] The largest and most recent of these studies is PLADO.[16] This trial randomized 1272 evaluable patients to 1 of 3 platelet-dosing strategies: low dose (1.1 × 10^{11} platelets per m^2) vs medium dose (2.2 × 10^{11} platelets per m^2) vs high dose (4.4 × 10^{11} platelets per m^2), which are equivalent to roughly one-half unit of apheresis platelet vs 1 U of

Table 1. RCTs comparing prophylactic platelet-transfusion thresholds

Study/Year	10 × 10^3/μL threshold		20 × 10^3/μL threshold		P
	No. of patients	Major bleeding	No. of patients	Major bleeding	
Rebulla et al[5]/1997	135	22%	120	20%	.41
Heckman et al[6]/1997	37	4 episodes/patient	41	2 episodes/patient	.12
Zumberg et al[7]/2002	78	14%	81	17%	.66
Diedrich et al[8]/2005*	79	18%	87	14%	NS

NS, not significant.
*Compared 10 × 10^3/μL vs 30 × 10^3/μL.

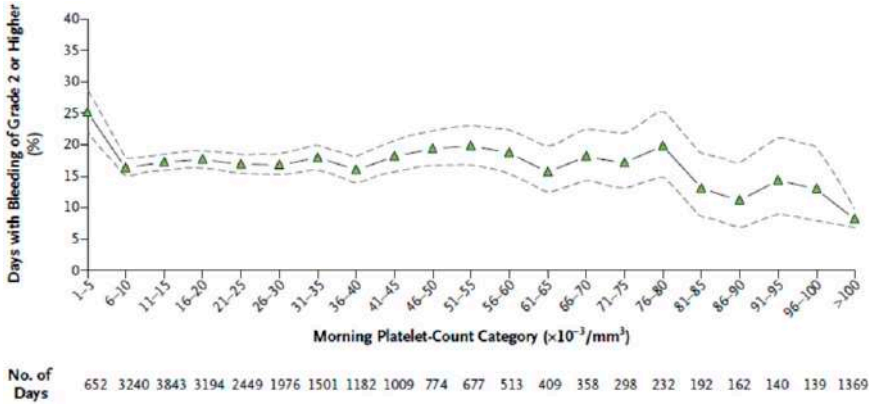

Figure 1. Days with bleeding of grade 2 or higher in all 3 treatment groups, according to morning platelet-count categories. Days with bleeding of grade 2 or higher in all 3 treatment groups, according to morning platelet-count categories. The percentage of days on which patients had bleeding of grade 2 or higher is shown, along with the associated 95% confidence intervals (dashed lines), according to the morning platelet-count category. Data are based on the 24 309 days during the study period on which patients had both a morning platelet count and information on bleeding of grade 2 or higher. Reprinted from Slichter et al[1] with permission.

apheresis platelet vs 2 U of apheresis using a prophylactic platelet-transfusion threshold of ≤10 × 10^3/μL. The study's primary end point was World Health Organization (WHO) grade ≥2 bleeding assessed daily through physical examination and medical record review. The study found that the bleeding rate was the same in each of the 3 arms, occurring in ~70% of subjects regardless of platelet-dose strategy. The higher-dose arm not only had the longest intertransfusion interval but also used the most platelets. A subset analysis of the PLADO study limited to 198 pediatric patients also found no difference in bleeding rates between the groups but did find a higher overall rate of bleeding of 84% compared with 67% in adults.[9] A meta-analysis of the 6 RCTs concluded that a low-dose strategy for prophylactic platelet transfusion was not associated with an increased risk of bleeding compared with medium- or higher-dose strategies.[17]

These data support the safety of a low-dose platelet strategy for prophylactic platelet transfusion for stable adult and pediatric hematology/oncology inpatients including HSCT recipients. In clinical practice, low-dose platelets are typically reserved for periods of platelet shortages. The low-dose strategy may not be appropriate for outpatients in whom a longer transfusion interval would be desirable as was seen with the higher-dose strategy in PLADO.[1] These data derived in the prophylactic setting should not be extrapolated to patients who need platelet transfusions for active bleeding as lower-dose platelets in this setting have not been adequately studied.

Prophylactic vs therapeutic platelet transfusion

The strategy of using platelets prophylactically balances the benefits of reducing the risk of bleeding vs the risks and expense of multiple platelet transfusions. As treatment regimens evolve, supportive care improves, and the duration of severe thrombocytopenia declines, particularly for autologous stem cell transplants, investigators have questioned whether a prophylactic platelet-transfusion strategy is still beneficial. The alternative would be to only transfuse platelets when patients exhibit evidence of bleeding; this is known as a therapeutic platelet-transfusion strategy. This concept is not new as 3 small RCTs totaling 99 patients were performed >30 years ago and did not find a difference in bleeding outcomes between the prophylactic and therapeutic transfusion strategies.[17] More recently, 2 larger RCTs compared bleeding outcomes between a prophylactic vs therapeutic platelet-transfusion strategy (Table 2).[18,19] Wandt et al[18] studied 391 adults with AML undergoing chemotherapy or autologous stem cell transplantation primarily for multiple myeloma or lymphoma. The primary end point of the study was the number of platelet transfusions. The therapeutic strategy resulted in a 33.5% reduction in number of transfusions, however, the incidence of WHO grade 2 or higher bleeding was significantly higher in the therapeutic group (42%) vs the prophylactic group (19%). Importantly, the rates of more serious grade 3 and grade 4 bleeding were also higher in the therapeutic group. Grade 4 bleeding, including central nervous system bleeding events, occurred in 5% in the therapeutic group vs 1% in the prophylactic group (P = .16). Subgroup analyses in the AML group were similar to the overall results. However, in the 201 patients who underwent autologous stem cell transplantation, grade 2 hemorrhages were more common in the therapeutic group, but there were no differences in the more serious grade 3 or 4 hemorrhages between groups. The TOPPS trial by Stanworth et al[19] studied 600 patients age 16 years or older who received chemotherapy or HSCT for hematologic malignancy (Table 2). This trial was designed as a noninferiority trial. The primary end point of WHO grade 2 or higher bleeding was higher in the therapeutic group (50% vs 43%), thus noninferiority was not achieved (the therapeutic strategy was inferior to the prophylactic strategy). The patients who received prophylactic platelet transfusions also had fewer days of bleeding, a shorter time to the first bleed, and a trend toward fewer serious grade 3 or 4 bleeding events (6 of 301 vs 1 of 299; P = .13). A prespecified subgroup analysis of the 410 patients (70%) in the trial who underwent autologous transplantation found no difference in WHO grade 2 or higher bleeding (47% vs 45%) between the arms.

Thus, both of these trials provide evidence supporting the benefit of prophylactic platelet transfusions in reducing overall and serious bleeding events compared with a therapeutic platelet-transfusion strategy in adults. The evidence for benefit is predominantly in adult hematology/oncology patients treated with chemotherapy; interestingly, both studies found little benefit

Table 2. Large RCTs of prophylactic vs therapeutic platelet transfusion

Study/Year	Prophylactic strategy 10 × 10³/μL				Therapeutic strategy				P†
	No. of patients	Bleeding grade,* %			No. of patients	Bleeding grade,* %			
		≥2	3	4		≥2	3	4	
Wandt et al[18]/2012	194	19	3	4	197	42	7	14	<.0001
Stanworth et al[19]/2013	299	43	0.3	0	301	50	1.3	0.7	.06**

*WHO bleeding grading system.[1]
†*P* value for primary end point WHO grade 2 or higher bleeding.
***P* value for inferiority.

in autologous stem cell transplant recipients, prompting the ASCO to recommend a therapeutic transfusion strategy in autologous stem cell transplants recipients.[10] There were too few patients who underwent allogeneic stem cell transplant to draw a conclusion about the safety of a therapeutic platelet-transfusion strategy, and pediatric patients <16 years old were not studied in either trial.

New approaches to reducing the risk of spontaneous bleeding

Acute leukemia patients and HSCT transplant recipients are at higher risk of bleeding compared with patients with other causes of severe thrombocytopenia, such as immune thrombocytopenic purpura, due to chemotherapy-induced damage to the endothelium, graft-versus-host disease, infection, and their primary disease. The PLADO[1] and TOPPS[19] studies used a ≤10 × 10³/μL prophylactic platelet-transfusion threshold, and found the incidence of WHO grade 2 or higher bleeding was quite high, ranging from 43% to 79% in adults and up to 84% in children.[9] Using a higher threshold[5-8] or using a higher-dose platelet-transfusion strategy[1] does not change bleeding rate. This led investigators to explore alternative strategies to enhance hemostasis. For many years, antifibrinolytic drugs such as ε amino caproic acid or tranexamic acid (TXA) have been used to reduced bleeding in thrombocytopenic patients.[20,21] Anecdotal experience and observational studies suggest efficacy, but rigorous studies are lacking. There are 2 phase 3 double-blinded placebo-controlled RCTs under way studying the efficacy of TXA on bleeding in hematology/oncology patients with severe thrombocytopenia. The National Heart, Lung, and Blood Institute (NHLBI)-funded American Trial Using Tranexamic Acid in Thrombocytopenia (A-TREAT; NCT02578901) recently completed its planned enrollment of 330 evaluable adult patients with hematologic malignancy or aplasia. Patients were randomized to receive TXA or placebo when their platelet count fell below 30 × 10³/μL until they experienced platelet count recovery or 30 days on study, whichever came first. The primary end point was WHO grade 2 or higher bleeding assessed daily at the bedside. The results of this trial are expected soon. The Trial to Evaluate Tranexamic Acid Therapy in Thrombocytopenia (B-TREATT; NCT03136445) is the UK version of this study. This double-blinded and placebo-controlled trial plans to enroll ~600 patients with hematologic malignancy and severe thrombocytopenia. The study intervention is similar to A-TREAT and the primary end point is the proportion of patients with WHO grade 2 or higher bleeding or death within the first 30 days. Enrollment is ongoing but is expected to be completed in 2020. These studies should provide high-quality data defining the role of TXA in addition to prophylactic platelet transfusion in mitigating the risk of spontaneous bleeding in adult hematology oncology patients with severe thrombocytopenia. The use of TXA in children has been limited to surgical patients and has not been studied in pediatric hematology/oncology patients. Studies of TXA or other strategies to reduce bleeding in this population are needed.

Summary

In stable hematology/oncology patients, a prophylactic platelet-transfusion threshold of ≤10 × 10³/μL would appear to provide a reasonable safety margin to ensure a nadir circulating platelet count above 5 × 10³/μL, a level below which the risk of bleeding appears to increase. A higher threshold and/or larger or more frequent doses may be appropriate for patients with clinical features associated with an increased risk of bleeding or in the outpatient setting. There is a high incidence of spontaneous bleeding despite the use of a prophylactic platelet-transfusion strategy. New approaches to reducing the risk of bleeding including antifibrinolytic therapy are currently under study.

Clinical case revisited

Returning to the 60-year-old woman with newly diagnosed AML admitted for induction therapy, her platelet count on admission was 14 × 10³/μL. She reported a minor nosebleed the day before lasting <5 minutes. She denied headaches or visible blood in her sputum, urine, or stools. Her physical examination was only remarkable for a few scattered petechiae on both arms. Urinalysis and stool guaiac for blood were negative.

What is the role of prophylactic platelet transfusion in this patient?

Since she is stable with only minor WHO grade 1 bleeding and a platelet count >10 × 10³/μL, prophylactic platelet transfusion is not indicated.

What is her risk of significant bleeding at a platelet count of 14 × 10³/μL?

She would be expected to have a grade 2 or higher hemorrhage on 17% of days or roughly 1 in 6 days when her platelet concentration is >10 × 10³/μL (Figure 1).

Would platelet transfusion change her risk of bleeding?

No, the risk of bleeding remains unchanged even if she were to be transfused to a higher platelet count (Figure 1). The next day, her platelet count is 5 × 10³/μL. No new bleeding is identified.

Is there a role for prophylactic platelet transfusion at this point in her course?

Yes, based on her platelet count, prophylactic platelet transfusion is indicated.

Has her risk of bleeding changed, and would platelet transfusions reduce her risk of bleeding?

Yes. She is at increased risk of bleeding with a platelet count of 5×10^3/μL and her risk will be decreased with a platelet transfusion (Figure 1).

Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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Platelet transfusion refractoriness: how do I diagnose and manage?

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Platelet refractoriness continues to be a problem for thrombocytopenic patients because the risk of a major spontaneous or life-threatening bleed significantly increases when platelet counts drop below 10 × 10⁹/L. The majority of patients have nonimmune causes driving the refractoriness, such as bleeding, medications, or diffuse intravascular coagulation; however, this article is dedicated to the diagnosis and support of patients with immune-based platelet refractoriness. Antibodies to class I HLA molecules (A and B alleles) are responsible for most immune-based refractory cases, with antibodies to platelet antigens seen much less frequently. Patients may be supported with either crossmatch-compatible or HLA-matched/compatible platelet units. When trying to select HLA units it can be difficult to find a perfect "4 of 4" match for the patient's class IA and IB alleles. In these cases, it is better to use the antibody specificity prediction method, which identifies compatible units that lack antigens recognized by the patient's anti-HLA antibodies. For an algorithmic approach to the patient with platelet refractoriness, see Visual Abstract.

LEARNING OBJECTIVES

- Describe the role of immune-based factors in causing platelet refractoriness
- Describe how to diagnose platelet refractoriness
- Understand the availability and relative advantages of different compatible platelet products for a patient with platelet refractoriness

Clinical case

A 60-year-old woman with newly diagnosed acute myeloid leukemia is admitted for induction therapy. She has a history significant for multiple pregnancies (G5P5). Her platelet count on admission is 14,000/μL (150,000/μL to 450,000/μL). She reports a minor nosebleed the day before, lasting for <5 minutes. She reports no visible blood in her sputum, urine, or stools. Her physical examination is remarkable only for a few scattered petechiae on both arms. A peripheral smear reveals normal red cell morphology, with no spherocytes or schistocytes, and marked thrombocytopenia. Urinalysis and stool guaiac for blood are negative. The next day her platelet count is 5,000/μL. No new bleeding is identified. The clinical team orders a platelet transfusion and notes that her posttransfusion platelet count (taken the next morning) is 4,000/μL. Over the next 2 days this trend of lower-than-expected platelet increments continues. Her team requests a clinical consult with the transfusion service to better understand her refractory state and develop a plan to correct her thrombocytopenia.

Background

Platelet refractoriness is defined as a repeated suboptimal response to platelet transfusions with lower-than-expected posttransfusion count increments. Refractoriness can be caused by immune and nonimmune factors, with nonimmune factors (Table 1) responsible for 60% to 80% of cases.[1] Immune factors, which play a role in 10% to 25% of patients with platelet refractoriness, include antibodies against four antigen classes: HLA class I, human platelet antigens (HPAs), ABO, and drug-dependent antibodies. In most cases HLA antibodies have been implicated.[1] Antibodies against HLA arise because of pregnancy, solid organ transplantation, or blood transfusions. It is the residual white blood cells found in cellular blood components that cause HLA alloimmunization.[2] Before the widespread use of leukoreduction, platelet refractoriness was seen in 30% to 70% of patients with bone marrow failure[3]; however, a Canadian study found that leukoreduction lowered HLA alloimmunization from 19% to 7% and alloimmune platelet refractoriness from 14% to 4% for patients undergoing chemotherapy for acute leukemia or stem cell transplantation (SCT).[4] Despite this reduction, platelet refractoriness is still an important clinical problem in SCT and for patients with hematologic disorders.

Table 1. Immune and nonimmune causes of platelet refractoriness

Nonimmune causes	Immune-mediated causes
Fever, infection, or sepsis	Antibodies against HLA class I
Bleeding	ABO-mismatched platelets
Accelerated platelet consumption (DIC, microangiopathic hemolytic anemia)	Antibodies against human platelet antigens
Drugs (amphotericin B, vancomycin, ATG, interferons)	Antibodies against drug–platelet glycoprotein complex
Splenic sequestration	
Graft-versus-host disease	
Poor platelet quality or greater storage age	

ATG, antithymoglobulin; DIC, diffuse intravascular coagulation.

Defining immune-based refractoriness

To define platelet refractoriness, one must follow posttransfusion platelet increments in a systematic fashion. The corrected count increment (CCI) and the percent platelet response (PPR) are the most frequently used formulas for tracking the posttransfusion increment adjusted for the size of the patient and the dosage administered.[5] In both cases the pretransfusion platelet count is subtracted from the posttransfusion count and divided by the number of platelets transfused (Figure 1). The important difference is that the CCI uses the patient's body surface area to normalize the calculation, whereas the PPR uses the patient's blood volume. Most studies define refractoriness as a CCI of <5,000 after 2 sequential transfusions.[2] However, a CCI of <7,500 or a PPR of <30% are also accepted values.[6]

Confirming immune-based refractoriness

When we suspect that a patient is refractory to platelet transfusions, we try to answer two questions: Is the patient truly refractory? And is the refractoriness caused by immune or nonimmune factors? To begin a workup, 2 posttransfusion platelet counts are taken within 10 to 60 minutes after the transfusion is completed. Studies have shown that platelets need at least 60 minutes to equilibrate within the intravascular space.[7] Logistically however, a 1-hour posttransfusion count can be difficult to obtain. As a result, many use a 10-minute postcount for the CCI calculation.[8] Some believe that a reduced 1-hour CCI points to an immune cause for refractoriness. However, the evidence to support this concept is confounded and highly variable.[5,9-12]

Once refractoriness has been confirmed, immune and nonimmune factors should be considered (Table 1) A good history and physical examination are usually sufficient to rule out nonimmune factors such as active bleeding, diffuse intravascular coagulation, and drug-induced thrombocytopenia. Sepsis and fever can also contribute to the nonimmune refractory state; however, these comorbidities are often present in patients with acute leukemia or SCT. Because most chronically thrombocytopenic patients with hematologic disorders are complicated, some immune and nonimmune factors may be present simultaneously.[13,14]

Antibody specificity

Platelets display class I HLA molecules, platelet-specific glycoproteins, and a low level of ABO on their surface membrane. These antibodies can bind to cognate antigens on the surface of transfused platelets and remove them from the patient's circulation.

Class I HLA

The HLA system is composed of highly polymorphic cell surface proteins that are responsible for distinguishing self from nonself in the immune response. Class I HLA molecules are present on platelets and most nucleated cells in the body, whereas class II molecules are mostly restricted to cells involved in antigen presentation. Class I consists of three loci: HLA-A, HLA-B, and HLA-C; however, platelets predominantly express HLA-A and HLA-B alleles, and antibodies against HLA-C are not a significant cause of immune-based refractoriness. HLA antigens are highly immunogenic: The risk of alloimmunization is 11% with 1 pregnancy, 32% with ≥4 pregnancies,[15] and 23% for multiply transfused patients.[16]

Platelet-specific antigens

There are 35 known human platelet antigens (HPAs). The HPA system has less antigenic variability when compared with the HLA system, which may be why far fewer antibodies against HPA are implicated in immune-based platelet refractory cases. Alloimmunization to HPA antigens has been reported in 2% to 8% of multiply transfused thrombocytopenic patients,[2,16] and refractoriness due to HPA antibodies is rarely seen.[17-19] Indeed, antibodies to HPA are usually found in combination with HLA antibodies.[20]

ABO

If a patient has high titers of anti-A or anti-B antibodies, then substantial clearance of donor platelets bearing cognate ABO antigens could occur. This problem may be avoided by transfusing ABO-identical platelets, because these units typically cause a better platelet increment than ABO-nonidentical units.[21,22] For a full exploration of this topic, see the accompanying article by Dunbar.[41]

Drug-induced antibodies

Although several mechanisms for drug-induced antibody formation have been described, most clinically relevant drug-dependent platelet antibodies are thought to result when a drug interacts with platelet membrane glycoproteins.[23,24] Drugs commonly implicated are listed in Table 2.[25,26] Drug-induced antibodies can cause a rapid onset of thrombocytopenia that usually resolves within 3 to 4 days after drug discontinuation. There are also non–drug-dependent antibodies that do not require the continued presence of the drug for reactivity.

Table 2. Drugs reported to cause drug-dependent platelet antibodies[26]

Drugs
Abciximab
Carbamazepine
Ceftriaxone
Eptifibatide
Heparin
Oxaliplatin
Phenytoin
Piperacillin
Piperacillin/tazobactam
Quinidine
Quinine
Rifampin
Sulfamethoxazole/trimethoprim
Tirofiban
Vancomycin

These drugs were associated with drug-dependent antibodies in ≥10 patients.

Testing for antibodies

When faced with a new patient, we often begin with a screening test that confirms the presence of HLA or HPA antibodies. At our institution we use a standard enzyme-linked immunosorbent assay that is rapid and, if negative, will save the time and expense needed for a full refractory workup. This assay determines only whether HLA or HPA antibodies are present; other tests are needed to define antibody specificity.

HLA antibody testing was initially performed with a lymphocytotoxic assay, which consists of incubating serum from a potential recipient with lymphocytes from prospective donors. Antibody binding caused complement-mediated lysis, indicating incompatibility. By using a panel of HLA phenotyped donors that were representative of the regional ethnic pool, the number of compatible donors could be assessed and the percent panel reactivity could be calculated. Variations of this test were used for >50 years, but the advent of solid phase testing has largely supplanted it.

Solid phase testing (also known as single-antigen bead or Luminex assay; Luminex Corp., Austin, TX) uses beads coated with individual HLA antigens. Antibody binding is detected by staining with fluorescently labeled antihuman globulin, and the level of antibody is characterized via flow cytometry, flow microarrays, or enzyme-linked immunosorbent assay. The strength (avidity) or amount of antibody binding is expressed as the mean fluorescent intensity (MFI). Single-antigen assays allow identification of multiple HLA antibody specificities that could not be readily distinguished via cytotoxic assays. The assay results in a list of antibody specificities and their MFIs. A calculated panel reactivity can also be used for an overall estimate of alloimmunization.

The increased sensitivity of the solid phase assay can create problems, because the clinical significance of low-level antibodies (MFI <500 to 1,000) is unclear. One study showed that weak to moderate HLA antibodies detectable by solid phase assay (but negative by lymphocytotoxic assay) were not associated with platelet refractoriness.[27] This finding creates a problem for HLA labs, which must determine an MFI threshold that corresponds to a "positive" or clinically significant antibody. Unfortunately, there is wide interlaboratory variability, with a range of 500 to 6,000 MFI used as a cutoff value.[28]

In an effort to identify clinically significant antibodies, an adaptation of the solid phase assay was developed that targets complement-fixing antibodies. Although some platelets are removed from the circulation by macrophages stimulated by antigen–antibody interactions, a subset is bound by the C1q protein, which activates the classic complement cascade, ending with direct platelet lysis.[29] The evidence is mixed as to the clinical importance of complement-fixing antibodies. For solid organ transplants, C1q-binding anti-HLA antibodies appear to be correlated with antibody-mediated rejection.[30] However, no similar association was found for platelet-refractory patients with weak to moderate HLA antibody levels.[31] More studies are needed to confirm or dismiss the utility of this assay for platelet-refractory patients.

Key question 1

Why are the patient's posttransfusion increments lower than expected?

Answer

The patient's epistaxis and petechiae are not likely to be significant contributing factors to her refractory state, and a review of her medications was noncontributory. Because there are no other obvious nonimmune causes, a workup for immune-based refractoriness should begin. A screening test should be ordered to confirm alloimmunization against HLA or HPA. If antibodies are present, the next step involves identification of compatible platelet units.

Identifying compatible platelet units

Finding a compatible platelet unit for an alloimmunized patient depends on the tests available, the frequency of the patient's HLA type relative to the pool of HLA-matched donors, and the level of alloimmunization. Different methods can be used to identify a compatible unit (Table 3). Platelet crossmatching and HLA matching are frequently used, and the success rate of these strategies is comparable.[32-34]

Platelet crossmatch

This method is usually the fastest and easiest way to obtain a compatible unit. The solid phase red cell adherence assay mixes a panel of donor platelets with the patient's serum. Antibodies against HLA or HPA that bind to the platelets are visualized with indicator red cells coated with anti–immunoglobulin G. The crossmatch approach is commonly used because compatible units usually can be obtained within 24 hours, and both HLA and HPA compatibility issues are covered with no additional testing. Despite these advantages, HLA-matched units are sometimes preferred because the provision of HLA-matched platelets may reduce future alloimmunization. Most importantly, platelet crossmatching tends to be problematic with highly alloimmunized patients, which can make it difficult to find enough compatible units.

Table 3. Comparison of methods used to identify compatible platelet units for alloimmunized patients

	Crossmatched	HLA matched	HLA compatible
Method	Test patient's serum against a panel of platelets to determine compatibility	Identify platelet donors with perfect (4/4) match for patient's HLA class IA and IB alleles	ASP: Use antibody specificities to select donor units that lack corresponding antigens
Pros	• Rapid turnaround-time	• 4/4 match ensures HLA compatibility	• Larger donor pool
	• Obtain compatible units without HLA genotype or HLA antibody testing	• Reduced risk of future alloimmunization	• Reduced risk of future alloimmunization
	• Compatible with HLA and HPA antibodies		
Cons	• Difficult to find compatible units for highly alloimmunized patients	• HLA genotyping required	• Not useful for HPA antibodies
	• Risk of alloimmunization for mismatched HLA antigens	• Limited donor pool for some patients	• HLA antibody testing required

Table adapted from Forest and Hod.[1]
ASP, antibody specificity prediction.

HLA match

Patients with platelet refractoriness can be supported with apheresis platelets from donors whose HLA-A and HLA-B antigens match those of the patient. However, obtaining a supply of 4/4 matches is possible only for blood centers that have a large number of HLA-typed donors and a well-organized inventory. Blood centers that cannot track the HLA type of units in inventory must resort to recruiting known donors, which causes delays in obtaining units and usually allows a very limited supply of platelet units.

When exact matches are unavailable, one can use the antibody profile determined by the single-antigen bead test to select donor units that lack the corresponding cognate antigens (ie, HLA compatible).[13,14,35] This antibody specificity prediction (ASP) method is equivalent to HLA matching in terms of efficacy. In addition, the ASP method increases the pool of compatible donors when compared with the number available under traditional HLA-matching criteria, making it easier to support patients with platelet refractoriness.[35] It is important to note that an HLA-compatible unit carries a potential risk for further alloimmunization, similar to a crossmatch-compatible unit.

HLAMatchmaker is another method for identifying HLA-compatible platelet units.[36] Using the derived amino acid sequence of HLA class I alleles, Duquesnoy et al[36] developed software that predicts epitopes within alloantibody-accessible regions of HLA molecules. These epitopes, or "eplets," consist of clusters of amino acids that are brought together by the tertiary structure of the HLA molecule. The HLAMatchmaker algorithm (http://www.hlamatchmaker.net) predicts compatibility based on these defined epitopes. Studies have found that the HLA-Matchmaker approach successfully identified donors associated with good transfusion outcomes in refractory recipients.[37]

The older antigen match grade system (A, BU, B2U, BX, C, and D matches) has been rendered obsolete by molecular typing methods and the specificity of the single-bead assay, and it should no longer be used.[38] Compatibility across cross-reactive groups is also less of a concern in selecting compatible platelet units, because the single-antigen assay allows the specific delineation of relevant antibodies.

Corrected Count Increment (CCI)

$$CCI = \frac{\text{Post-transfusion plt count} - \text{Pre-transfusion plt count (/L)} \times \text{BSA } (m^2)}{\text{platelets transfused } (10^{11})^*}$$

Percentage Platelet Recovery (PPR)

$$PPR = \frac{\text{Post-transfusion plt count} - \text{Pre-transfusion plt count (/L)} \times \text{TBV} \times 100\%}{\text{platelets transfused } (10^{11})^*}$$

*If exact platelet count is unavailable use 3×10^{11}
plt – platelet; BSA – body surface area; TBV – total blood volume

Figure 1. Formulae used in the diagnosis of platelet refractoriness.[5]

HPA match

Although the incidence of HPA antibodies causing transfusion refractoriness is small, this possibility should be investigated when most of the crossmatches are incompatible or when HLA-matched transfusions fail. If antibodies against HPA are present, then donors of known platelet antigen phenotype may be recruited. The patient's relatives, who may share the patient's phenotype, should also be tested.

Other options

Family members can sometimes provide directed platelet units that are a good match. However, if the patient is scheduled for SCT from a related donor, antibodies against minor-HLA antigens can develop and possibly create problems with engraftment.[39] The complement inhibitor eculizumab has been used to increase the CCI in a limited number of transfusion refractory patients with severe thrombocytopenia.[40] Finally, in extreme cases when a patient's count must be increased for a procedure, we have resorted to an "in vivo adsorption" strategy: If a patient has a strong antibody against HLA-A2, we repeatedly transfuse an HLA-A2 positive unit to deplete the antibody, then drip-in an A2 positive unit during the procedure.

Key question 2

What steps can the clinical team take to manage the patient's thrombocytopenia?

Answer

The clinical team must first order 10- to 60-minute posttransfusion platelet counts on 2 sequential transfusions to confirm refractoriness. The next step is a trial of crossmatch-compatible platelets, closely monitored with 10- to 60-minute CCI. If this trial fails, then HLA-matched or HLA-compatible platelets should be tested. See Visual Abstract for greater detail.

Clinical recap

For our 60-year-old thrombocytopenic patient, the first step in management would be calculating CCIs for the next 2 successive platelet transfusions and ruling out nonimmune factors for her refractory state (Visual Abstract). Given her multiparous history, it is reasonable to assume that she has HLA antibodies; however, an initial screen for HLA/HPA antibodies can be used to confirm this.

With an immune-driven refractory state high on the differential, the next step is a limited trial of crossmatch-compatible units. Two or three crossmatch-compatible platelet transfusions with 10- to 60-minute CCI can indicate the success or failure of this strategy. If the CCI improves, then continuing with crossmatch-compatible units is the best way to manage her thrombocytopenia. However, if the patient continues to have poor posttransfusion increments, then switching to an HLA matching strategy is indicated. For this patient, an HLA genotype and single-bead assay for class I HLA antibodies must be ordered. The chance of finding several units that are a perfect match for the patient is unlikely; therefore, a mixed strategy of HLA matching and antibody avoidance is probably necessary.

To keep things moving quickly, we order the required HLA tests if the first crossmatch unit fails to increase the CCI. We may find a few perfect 4/4 matches, but usually we turn to the ASP method to identify additional compatible units. If the patient is highly alloimmunized and we cannot find fully compatible units, we work closely with the HLA laboratory to decide which antibodies to honor. A 10- to 60-minute CCI should be obtained with the transfusion of each HLA-matched/compatible unit. For some patients, an extended trial of HLA-selected units does little to improve the CCI. In these cases, we return to transfusing random units, because obtaining HLA-matched units is not worth the time and expense. In most cases, however, the platelet count begins to rebound when either engraftment occurs or chemotherapy abates.

Conflict-of-interest disclosure

The author has received honoraria from Octapharma and Terumo Corporation. No other conflicts of interest to disclose.

Off-label drug use

None disclosed.

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EVIDENCE-BASED MINIREVIEW

The use of premedications for platelet transfusions in pediatric patients

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LEARNING OBJECTIVES

- Understand the epidemiology of transfusion-related adverse events and their impact on care
- Review the evidence for premedication prior to platelet transfusion

Clinical case

An 11-year-old boy with acute lymphoblastic leukemia undergoing induction therapy received a platelet transfusion without premedication. Midway through the transfusion, he experienced a fever of 38.5°C, increasing from a baseline of 37.1°C, accompanied by chills and rigors. His other vital signs remained stable. The transfusion was stopped, and his symptoms resolved within 30 minutes with a decline in temperature to baseline. Workup confirmed unit compatibility with the patient and did not reveal any evidence of bacteremia or hemolytic transfusion reaction. Should this patient be premedicated prior to subsequent platelet transfusion?

Discussion

Over half of pediatric oncology patients require transfusion support with platelet products during therapy for their malignancy. The median number of platelet transfusions required per patient ranges from 2 to 25 and varies on the basis of diagnosis and therapy.[1] Transfusion-related adverse events (TRAEs) complicate ≤14% of all platelet transfusions.[2,3] The most commonly occurring reactions are febrile nonhemolytic transfusion reactions (FNHTRs) and allergic reactions.[4] FNHTRs are identified by fever (>38°C and a change in temperature of >1°C) during or shortly following transfusion and can be associated with additional symptoms such as chills, headache, nausea, or vomiting.[5] Allergic reactions occur in 1% to 2% of platelet transfusions and are typically mild or moderate, with cutaneous symptoms of erythema, urticaria, and pruritus. However, severe allergic reactions, including anaphylaxis, can occur as well.[5] The risk of TRAEs may be greater in pediatric patients than in adult patients. In a retrospective analysis of data from a 6-year period, children experienced an increased rate of allergic reactions (833 vs 358 per 100 000 platelet transfusions) and FNHTR (155 vs 126 per 100 000 platelet transfusions) compared with adults.[6]

The are several factors that contribute to the pathophysiology underlying platelet transfusion reactions (Figure 1). In FNHTRs, cytokines (including interleukin-1, interleukin-6, and tumor necrosis factor-α) accumulate within plasma supernatant prior to transfusion. When administered to a recipient, these inflammatory mediators interact with the hypothalamic thermoregulatory center, leading to an increase in body temperature.[5,7] Allergic reactions occur secondary to interactions between donor proteins and immunoglobulin E, leading to mast cell and basophil activation.[8] Premedication with antipyretics and antihistamines has been used in an attempt to decrease the incidence of FNHTRs and allergic reactions. The earliest published research on antihistamines to prevent TRAEs was produced in the 1950s after investigators inoculated whole blood with diphenhydramine, chlorpheniramine, or other antihistamines and noted a subsequent decrease in the rate of febrile and allergic reactions.[9,10] A Cochrane review on the topic of premedication for the prevention of TRAEs concluded there was insufficient evidence to support the use of premedication on the basis of existing data.[11] Despite this, the use of premedication remains widespread. In a survey of pediatric physicians from 15 tertiary hospitals, the majority of respondents reported administering premedication prior to platelet transfusion in ≤25% of transfusions. Twelve percent of respondents reported administering premedication in 26% to 50% of transfusions.[12] Observational studies have reported 60% to 80% of adult patients receive premedication prior to transfusion.[13,14] However, data supporting the efficacy of this practice are limited.

Two studies have evaluated the use of premedication prior to platelet transfusion in pediatric patients.[15,16] The first retrospectively reviewed all blood and platelet transfusions administered to pediatric hematology-oncology patients

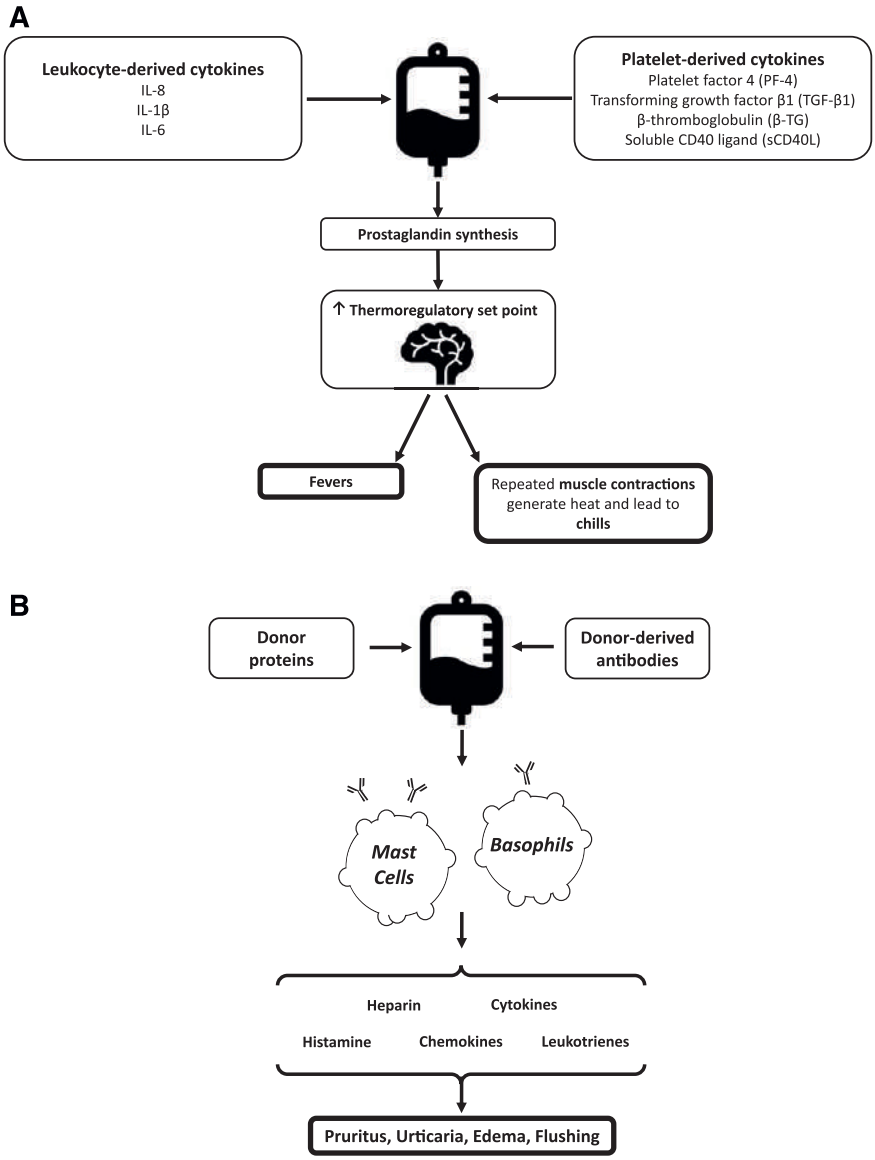

Figure 1. Pathophysiology of FNHTRs (A) and allergic reactions (B) in platelet transfusions. IL, interleukin.

over the course of 1 year.[15] In 385 patients receiving 8277 blood product transfusions, a total of 4280 platelet concentrates were administered. Premedication was provided in 68% of transfusions, including in 63% of patients who had no prior history of TRAEs. Allergic reactions and FNHTRs complicated 0.86% and 0.21% of platelet transfusions, respectively, and 0.61% and 0.36% of red blood cell transfusions. However, premedication with diphenhydramine did not significantly affect the development of allergic reaction (odds ratio, 1.74; 95% confidence interval [CI], 0.99-3.06). Likewise, acetaminophen premedication was not associated with a decrease in FNHTRs in multivariate analysis (odds ratio, 1.74; 95% CI, 0.71-4.23).[15]

Patterson et al[16] collected data from both adult and pediatric patients across 5 hospitals. They reported the rate of TRAEs following platelet transfusion at 3 points in time: prior to implementation of a protocol standardizing premedication use, following protocol institution, and after applying universal prestorage leukoreduction of all platelet products. The protocol dictated that premedication was indicated only if a febrile or allergic reaction had occurred in both of the 2 prior transfusions. Following protocol implementation, the use of premedication prior to platelet transfusion decreased from 73.1% to 50%. Despite this decrease in premedication use, among pediatric patients, the number of platelet transfusions complicated by TRAEs did not significantly change (27.9% vs 25.8% of transfusions). However, a decrease in TRAEs was noted after implementing universal leukoreduction, with only 13.9% of subsequent transfusions in pediatric patients complicated by TRAEs.[16]

If data from the adult literature are considered, evidence for the efficacy of premedication remains limited. In a large retrospective study of 34 867 platelet transfusions over a 5-year period, Ezidiegwu et al[14] found the incidence of FNHTRs within their population to be 0.09%. In their institution, acetaminophen is routinely prescribed to all patients prior to transfusion. The authors compared the incidence of FNHTR within their hospital with published rates in the literature of 0.11% to 38%, and they

considered this indirect evidence of premedication efficacy. However, the results of a randomized trial conducted by Wang et al[17] did not conclude premedication was effective. In this study, 55 hematology-oncology patients received 122 platelet transfusions. Randomization to premedication (consisting of 650 mg of acetaminophen and 25 mg of diphenhydramine) or placebo occurred prior to each platelet transfusion. There was no significant difference in the number of transfusions affected by nonhemolytic transfusion reactions between the 2 groups (15.4% vs 15.2%). There were only 3 cases of urticarial reaction, all of which were experienced in the placebo group.[17]

A second randomized trial, conducted by Kennedy et al, also did not provide convincing evidence for the routine use of premedication.[18] In their study, 315 patients admitted to either the leukemia or bone marrow transplantation services were assigned to receive either placebo or a combination of 500 mg of acetaminophen and 25 mg of diphenhydramine 30 minutes prior to transfusion. A total of 2333 platelet transfusions were administered, which represented 55% of all blood products transfused to study patients. The authors found no significant difference in the incidence of allergic reactions (1.05 vs 0.68 reactions per 100 transfusions) between the intervention and placebo groups. There was a slight reduction in the incidence of FNHTRs in the intervention group (0.35 vs 0.64 reactions per 100 transfusions), which was not significant. In multivariate regression analysis, premedication was associated with decreased hazard of FNHTRs (hazard ratio, 0.48; 90% CI, 0-0.89) after adjusting for age, race, sex, and diagnosis. On the basis of these results, the authors estimated 344 transfusions would require premedication to prevent a single FNHTR.[18]

It is important to consider that there is significant heterogeneity between the studies discussed, which makes direct comparison of results difficult. Studies varied in terms of patient population, outcome measures, and inclusion and exclusion criteria (such as exclusion of patients with a history of prior FNHTRs). Importantly, both acetaminophen and diphenhydramine have an onset of action within 30 to 60 minutes. Of the studies described, only the one by Kennedy et al[18] included a recommendation regarding the timing of premedication administration in their protocol (30 minutes prior to transfusion), though no data were provided regarding compliance with this recommendation. Other cited works did not include information on the temporal relationship between premedication and transfusions. Therefore, it can be difficult to conclude if a lack of demonstrated premedication efficacy was secondary to suboptimal use of medications. An additional historical element to take into account is a history of TRAEs with a prior transfusion, which seems to be associated with an increased risk of recurrent transfusion reactions.[17] A history of reaction often influences practitioner decision making when determining if premedication use is appropriate.[16,19] This group of patients was excluded from the study by Kennedy et al[18]; however, Sanders et al[15] found that regardless of the number of prior reactions, premedication was not associated with a decreased incidence of TRAEs.

Although the majority of TRAEs are considered to be mild in severity, they can have serious implications for patient quality of life and cost of care. In one study describing 437 FNHTRs, 93% of patients required interruption of transfusions for evaluation, and transfusions were resumed in only 15% of cases. Blood cultures and imaging studies were frequently obtained to determine the source of symptoms, and in 15% of cases, patients required admission from the outpatient setting due to FNHTRs.[20] These interventions can lead to significant cost burdens.[14,20] Pediatric oncology patients are at a heightened risk of developing fevers, with febrile neutropenia as a result of myelosuppressive chemotherapy being one of the most common adverse effects of therapy. In some cases, the benefit of therapy with antipyretics prior to transfusion may lie in their efficacy in reducing temperature elevation associated with coincidental neutropenia or infection and therefore avoiding costly evaluation of fevers falsely attributed to blood products.[21]

Other options exist to decrease the risk of TRAEs through modifications to the platelet product itself. Prestorage leukoreduction is associated with a >90% reduction in the risk of TRAEs due to a decrease in cytokine levels stored in platelet products.[22,23] Through a similar mechanism, the use of concentrated or washed products reduces transfused plasma volume and decreases TRAEs.[24] The use of platelet additive solution may also be associated with fewer TRAEs.[25] In conclusion, although the utility of premedication prior to transfusion is yet to be proved, careful consideration of premedications based on individual circumstances and the use of blood product modifications may be beneficial in conserving limited resources.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

Off-label drug use

None disclosed.

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Management of heavy menstrual bleeding on anticoagulation

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Heavy menstrual bleeding (HMB) is a common complication of anticoagulation, affecting ~70% of menstruating women receiving oral anticoagulants. The risk of HMB is lower with apixaban and/or dabigatran than with rivaroxaban. HMB can result in iron deficiency with or without anemia, increased need for medical interventions, decreased quality of life, and missed school/work. Mainstays of treatment include hormone therapies such as the levonorgestrel intrauterine system, subdermal implant, and other progesterone-based therapies, which can result in decreased blood loss and, in some cases, amenorrhea. Combined hormone therapies can be used while patients continue receiving anticoagulation and are also highly effective for decreasing menstrual blood loss. Rarely, procedure-based interventions such as endometrial ablation may be required. Patients should be evaluated for iron deficiency and anemia and offered supportive therapies as needed. Abbreviating the course of anticoagulation or skipping doses can increase the risk of recurrent venous thromboembolism by as much as fivefold, but switching oral anticoagulants may be considered. Awareness of HMB and careful history taking at each visit are crucial to avoid a missed diagnosis.

LEARNING OBJECTIVES

- Understand the risks, signs/symptoms, and consequences of heavy menstrual bleeding in women treated with various oral anticoagulants
- Review options for the treatment of heavy menstrual bleeding in the anticoagulated woman

Clinical case

A 27-year-old woman presented to a clinic with fatigue and decreased exercise tolerance. Her past medical history was remarkable for deep vein thrombosis (DVT) of the left lower extremity, which had been diagnosed ~2 months ago in the setting of oral contraceptive pill (OCP) use. She was undergoing treatment with rivaroxaban 20 mg daily and had discontinued the OCPs upon diagnosis of her DVT. She denied chest pain, dyspnea at rest, and lightheadedness. The symptoms of her DVT, lower extremity pain, and swelling resolved within 2 weeks of initiating therapy. She denied epistaxis, melena, hematochezia, or other bleeding but described her menses as "very heavy." Since discontinuing her OCPs and initiating rivaroxaban therapy, her periods had been lasting 10 to 14 days, and, because of a need to change a pad or tampon every 30 minutes, she was now wearing both at once and changing protection every 45 minutes on her heaviest day. On further questioning, she also reported passing multiple clots up to 1.5 inches in diameter each cycle. A complete blood count (CBC) revealed that her hemoglobin concentration was 8.0 g/dL, her mean corpuscular volume was 72.0 fL, and her other parameters were normal. At the time of her DVT diagnosis, her hemoglobin was 10.8 g/dL, and her mean corpuscular volume was 81.0 fL.

Diagnosing heavy menstrual bleeding

Heavy menstrual bleeding (HMB) is defined as menstrual blood loss (MBL) of >80 mL per cycle.[1] For research purposes, the Pictorial Blood Loss Assessment Chart, a chart with which patients can report the number of hygiene products used and the degree of saturation throughout a menstrual cycle, is a commonly used and accurate diagnostic tool with a sensitivity and specificity of >80% for scores >100.[2] Although this tool is also available in the clinical setting, administration may not be practical, and therefore many clinicians must rely on a snapshot history and physical examination in combination with laboratory findings.

The authors of the landmark study Menorrhagia 1 identified 3 key predictors of MBL >80 mL/cycle that can

be captured in a single visit. These include patient-reported passing of clots >1 inch in diameter, low ferritin, and need to change protection more often than hourly during the heaviest days of menses.[3] Subject description of her menses as "very heavy" as opposed to "moderate" or "heavy" was significantly associated with increased MBL (64 mL vs 40 mL; $P < .001$), suggesting that patient reporting is more accurate than previously considered. Additional factors associated with increased mean MBL are listed in Table 1. Ultimately, there is no gold standard outside of measuring blood loss and/or using the Pictorial Blood Loss Assessment Chart, and thus a combination of all these factors and clinical judgment are required. It is also important to note that, in addition to medical outcomes such as iron deficiency, HMB is associated with decreased quality of life and missed time from work and school,[4] which impact overall well-being.

HMB while receiving anticoagulation

Up to one-third of women will meet criteria for HMB at some point in their lifetime. For women receiving anticoagulation, the risk of HMB or otherwise abnormal uterine bleeding (AUB) increases to ~70% and varies on the basis of choice of anticoagulant,[5] although comparisons between agents can be challenging because of inconsistency in outcome definitions throughout the literature (Table 2). Standard major bleeding (MB) and clinically relevant nonmajor bleeding (CRNMB) definitions, used almost exclusively in large trials of direct oral anticoagulants, are problematic because they fail to account for the chronic and recurrent nature of HMB. Although patients with HMB rarely require transfusions or experience a rapid decline in hemoglobin, they often develop iron deficiency, sometimes severe, over the course of multiple cycles and may hesitate to seek needed medical attention. Subsequently, true HMB is underreported and underappreciated in virtually all trials of anticoagulants.

Table 1. Clinical and laboratory features associated with heavy menstrual bleeding and/or increased menstrual blood loss (MBL)

Clinical and laboratory features	Odds ratio (95% CI) or *P* value
Features associated with MBL >80 mL[3]	
Finding	
Changing protection more often than hourly	3.08 (1.4-68)
Clots >1.1 inch in diameter	4.80 (1.9-12.2)
Low ferritin	5.71 (1.9-17.4)
Features associated with increased mean MBL[3]	
Finding	
Subjective report of "very heavy" periods	<.001
Hemoglobin <12.0 g/dL	.002
Need to change protection during the night	<.001
Leaking through protection	<.001
Need to wear double protection	<.001

CI, confidence interval.

Vitamin K antagonists

When evaluating specifically for AUB and/or HMB, rates as high as 67% are reported among subjects using vitamin K antagonists such as warfarin.[5] Other focused studies report somewhat lower but still impressive rates ranging from 18% to 39%.[6,7] Large trials using MB and CRNMB criteria, however, report much lower rates, consistently <10%,[8,9] suggesting that most HMB goes unreported and undiagnosed in the anticoagulated population.

Anti-Xa agents

Analysis of data from the large registry trials of each of the 3 oral anti-Xa agents has demonstrated a greater than a twofold increased risk of uterine MB or CRNMB in female subjects of all ages using rivaroxaban as compared with warfarin and low-molecular-weight heparin. Users of apixaban were no more likely to meet such criteria than users of warfarin/low-molecular-weight heparin. Data from registry trials of edoxaban, as reported in product monographs, demonstrated uterine MB and CRNMB rates similar to those seen with rivaroxaban (9%). Subjects receiving warfarin in these studies, however, reported higher rates of uterine bleeding events than those seen in trials of rivaroxaban or apixaban, ultimately resulting in a relative risk of 1.26 for all-age female users of edoxaban as compared with warfarin (Table 3).[8]

Although additional data on uterine bleeding events in these registry trials have been published in the form of post hoc analyses, comparison is challenging because of inclusion of different populations (based on age group or menopausal status) in each analysis. Post hoc analysis of the Hokusai-VTE study,[10] when excluding subjects >50 years of age, reported an incidence of 15 cases of uterine MB or CRNMB per 100 person-years (95% confidence interval [CI], 11-19), translating to a 1.7-fold (95% CI, 1.1-2.5) increased risk with edoxaban as compared with warfarin.[11] Post hoc analysis of a slightly older (≤60 years of age) group of women included in the EINSTEIN trials demonstrated a hazard ratio of 2.13 (95% CI, 1.57-2.89) for uterine bleeding events in users of rivaroxaban vs warfarin. Incidence densities of uterine bleeding events were 28.9% or 30.7%/year for users of rivaroxaban and 15.5% or 13.4%/year for users of warfarin who were or were not receiving hormone therapy, respectively.[12] Age-specific rates of uterine MB or CRNMB events from the AMPLIFY studies were not published, although one post hoc analysis reported the odds ratio of a combined outcome of intermenstrual bleeding, HMB, and anemia to be 1.3 (95% CI, 0.2-7.3) for premenopausal women using apixaban vs warfarin.[13]

Studies using actual AUB and HMB definitions are much rarer, but rates from 20% to 73%[5,14] have been reported with rivaroxaban. Women using rivaroxaban have also been demonstrated to experience more prolonged menses and to be more likely to require medical or surgical intervention, unscheduled contact with a medical provider, and/or modification of anticoagulation for menstrual bleeding.[5] Furthermore, HMB in users of rivaroxaban resulted in an increased risk of recurrent venous thromboembolism (VTE) as high as fivefold, potentially because of increased rates of modification of anticoagulation, including abbreviated courses and missed doses.[6] One observational study of women receiving apixaban reported the incidence of true HMB to be 9.3%,[15] but studies using such definitions in users of edoxaban are lacking.

Table 2. Definitions of terms used to capture heavy or otherwise abnormal menstrual bleeding in the literature

Term	Definition
Heavy menstrual bleeding (HMB)/ menorrhagia	Menstrual blood loss (MBL) of >80 mL/cycle or Pictorial Blood Loss Assessment Chart score >100
Abnormal uterine bleeding (AUB)	Menstrual bleeding of abnormal quantity, duration, or schedule. Includes heavy bleeding; excessively frequent, infrequent, or irregular bleeding; and intermenstrual bleeding.
Clinically relevant non-major bleeding (CRMNB)	Uterine bleeding that requires medical intervention by a health care professional, leads to hospitalization or increased level of care, or prompts a face-to-face evaluation
Major bleeding (MB)	Uterine bleeding that is fatal or causes a fall in hemoglobin level of ≥20 g/L (≥2 g/dL) or leading to transfusion of ≥2 units of whole blood or red cells

Dabigatran

Post hoc analysis of the RE-COVER and RE-MEDY studies suggested that dabigatran carries a lower risk of uterine MB and CRNMB (4.7%) than warfarin, although AUB occurred more frequently in the warfarin arm than in any other study (9.6%).

Evaluation for HMB while receiving anticoagulation

Observational data suggest that HMB is underrecognized in anticoagulated women, in part because of an absence of discussion of symptoms. The first assessment for HMB should be conducted at the time of initial oral anticoagulant prescription and should include both current and past symptoms, particularly for women whose VTE occurred in the setting of use of OCPs or other hormone agents that may have temporarily improved symptoms. Women who report symptoms consistent with HMB or increased MBL (Table 1) should undergo a CBC and a ferritin check. For women who report additional bleeding symptoms beyond HMB, completion of the International Society on Thrombosis and Haemostasis/Scientific and Standardization Committee Bleeding Assessment Tool followed by workup for any potential underlying bleeding disorders, such as von Willebrand disease, should be considered.

Patients who do not immediately report symptoms of HMB should be educated on the signs of it and instructed to notify the prescriber if these symptoms develop. Patients should be asked about symptoms again at subsequent evaluations, at least annually for those women receiving long-term anticoagulation, keeping in mind that HMB can develop at any point, particularly during perimenopausal years, when bleeding becomes more irregular and can be heavier. Periodic laboratory monitoring, including CBC and ferritin checks, is also appropriate. Ongoing and open dialogue is critical because patients may be hesitant to report symptoms promptly due to embarrassment or the belief that nothing can be done to manage them. Once HMB is identified, there are many options for management.

Table 3. Relative risk of heavy menstrual bleeding by choice of oral anticoagulant in women aged ≥18 years

OAC	Incidence of uterine CRNMB/MB	Relative risk
Warfarin	4.5%-9.6%	Reference
Apixaban	5.4%	1.18
Edoxaban	9.0%	1.26
Rivaroxaban	9.5%	**2.10***
Dabigatran	4.7%	**0.53***

OAC, oral anticoagulant.
*Statistically significant, $P < 0.01$.

Management of HMB while receiving anticoagulation

Hormone therapies

Although somewhat counterintuitive, hormone therapies are a first-line option for managing HMB in this population. In addition to being effective for the reduction of bleeding, hormone therapies provide contraception, which is particularly important in women receiving teratogenic agents, such as warfarin, and in women with a recent history of VTE due to the dramatically increased risk of recurrence with pregnancy.

The levonorgestrel intrauterine system (LNG-IUS) can be incredibly effective for management of HMB, boasting a 44% amenorrhea rate at 6 months of use, increasing to 50% by 1 year,[16] as well as >99% effectiveness for prevention of pregnancy. Individuals who do not achieve amenorrhea can still be expected to experience a reduction in median MBL of 80% by 4 months, accompanied by an increase in hemoglobin of 7.8%.[17] Of note, the LNG-IUS has been associated with an increased risk of ovarian cysts, particularly in the first 12 months of use.[18] The subdermal implant can result in amenorrhea in ~20% of cases, although irregular spotting is common and can be troublesome.[19] The subdermal implant also has a >99% efficacy for prevention of pregnancy.

Depo-medroxyprogesterone acetate results in amenorrhea in 55% of women at 1 year and in 68% at 2 years,[20] and it is >99% effective for prevention of pregnancy when used perfectly. However, it is associated with a 2.2- to threefold increased risk of VTE and therefore is not recommended in the absence of anticoagulation.[21] Progestin-only pills also result in amenorrhea in 5% to 10% of women but frequently cause other menstrual irregularities[22] and require precise adherence to avoid reduction in contraceptive efficacy.

Combined contraceptives, including both an estrogen and a progesterone component, are highly effective for the management of HMB. OCPs are considered by many to be the first-line treatment of HMB and provide the option of prescribing with or without scheduled interruptions in hormone exposure, allowing the potential to induce amenorrhea. Because of the increased risk of VTE associated with estrogen, many consider estrogen-containing therapies to be contraindicated in women with a history of thrombosis. However, no difference in rates of recurrent VTE were found retrospectively in a comparison of

women who were and were not prescribed combined contraceptives while receiving anticoagulation,[12] suggesting that, for many women, the benefits of OCPs outweigh the risks while receiving anticoagulation. High-quality prospective studies of the safety and efficacy of combined contraceptives in anticoagulated women are urgently needed. In particular, those women who develop VTE while receiving OCPs for treatment of HMB, such as the patient in our case study, will be especially vulnerable to heavy bleeding with the additive effects of anticoagulation and withdrawal of hormone therapy. Strong consideration should be given to either continuing the current hormone therapy regimen, with appropriate risk–benefit counseling, in the setting of anticoagulation or rapidly transitioning to an alternative therapy, such as a progestin-based option. OCPs should be discontinued before anticoagulation withdrawal, preferably in the setting of a transition to an alternative agent, such as the LNG-IUS. If an estrogen-based therapy is newly initiated in the setting of a recent clot, ensuring therapeutic anticoagulation is already in place before the first administration is imperative.

Procedural therapies

In extreme cases, surgical therapies such as endometrial ablation or uterine artery embolization may be considered. Such therapies should, in general, be limited to refractory bleeding in women who either require long-term anticoagulation or who have HMB even in the absence of anticoagulation. These options are also limited to women who have completed childbearing, because they either guarantee infertility or dramatically increase the odds of morbidity with future pregnancies. Therapeutic options include endometrial ablation, uterine artery embolization, and hysterectomy. Approximately 87% of women undergoing endometrial ablation for HMB will perceive improvement in symptoms at 1 year, although 12% will require further surgery for HMB.[23] Although uterine artery embolization is typically limited to women with fibroids, women who meet this qualification may enjoy up to a 90% reduction in MBL.[24] Hysterectomy, a true permanent solution, requires discontinuation of anticoagulation in the perioperative setting, carries an increased risk of both bleeding and VTE, and thus is a last resort.

Antifibrinolytics

Antifibrinolytics, such as tranexamic acid, have established efficacy in the management of HMB in non-anticoagulated populations, including women with bleeding disorders. Although antifibrinolytics have historically been considered to be contraindicated in women with a history of thrombosis, large studies of patients at high risk of VTE, including postpartum women,[25] trauma patients,[26] and patients undergoing orthopedic surgery,[27] have failed to demonstrate increased incidence of VTE. One study of patients with gastrointestinal bleeding noted a very small increased risk (0.4%) of VTE with high-dose (4 g over 24 hours) intravenous tranexamic acid compared with placebo.[28] The vast majority (90%) of these patients were not anticoagulated, and the difference in rates of thrombosis between treatment groups was most notable in patients with underlying cirrhosis.

No studies have been done of the combination of anticoagulants and antifibrinolytics, and, although use in the immediate post-VTE setting would be inadvisable because of the desire for ongoing fibrinolysis of the thrombosis, the benefit may outweigh the risks in specific situations, particularly if it enables continuation of anticoagulation without interruptions. Antifibrinolytic use was reported in a few subjects in the EINSTEIN DVT and pulmonary embolism cohorts, although not in association with VTE outcomes.[12] As with use for HMB in other circumstances, antifibrinolytics should be prescribed during only the heaviest days of the cycle.

Modification of anticoagulation

In the setting of HMB, temporary or early discontinuation of anticoagulation may be tempting. However, because this has been shown to result in increased risk of recurrent VTE,[6] alternative approaches are strongly recommended. Although high-quality data, such as randomized controlled trials, comparing HMB between agents are lacking, a preponderance of observational data seem to suggest increased rates of HMB with rivaroxaban. Therefore, consideration of alternative agents, such as dabigatran or apixaban, in women at high risk for, or perhaps even those who experience, HMB while receiving anticoagulation is reasonable in addition to the above recommended approaches. Data on temporary dose reduction (eg, to prophylactic doses) during menses are lacking, and this approach is not currently recommended outside of the research setting.

Adjunctive therapies

An important but often forgotten aspect of the care of patients with HMB is treatment of iron deficiency anemia. All women reporting HMB should be tested with a CBC and a ferritin level measurement. Iron therapy, either oral or intravenous, should be administered as necessary.

Gynecological evaluation

Patients receiving anticoagulation are vulnerable to all the same gynecological causes of bleeding as non-anticoagulated patients, and therefore the possibility of underlying causes beyond anticoagulation must be considered. Postmenopausal bleeding is worrisome for a potential diagnosis of endometrial cancer, and periods that are both heavy and painful can be indicative of other problems, such as fibroids or endometriosis, which may improve with targeted management strategies offered by gynecologists.

Clinical case continued

On the basis of her reported history of changing protection more frequently than hourly, passing clots >1 inch, and reported "very heavy" bleeding, our patient can be diagnosed with HMB. Management options, including hormone therapies and switching from rivaroxaban to an alternative agent, should be offered. Because this patient is likely to discontinue anticoagulation in 1 month, progesterone-based therapies, in particular the LNG-IUS, are preferred to OCPs, although continuing OCPs at the time of diagnosis and later transitioning to a progesterone-based therapy may have reduced the severity of the clinical picture. This patient almost certainly has iron deficiency and, after a ferritin check, should be offered iron therapy. She should also be counseled on the importance of using effective contraception, regardless of menstrual management strategy, until and unless pregnancy is desired, in which case preconception counseling by a hematologist and/or obstetrician experienced with the use of anticoagulation in pregnancy is strongly advised.

In summary, an ideal approach to HMB while receiving anticoagulation includes addressing it at the time of anticoagulation

initiation, counseling patients about the fact that they may experience new or worsened HMB, and asking specifically about menstrual bleeding at subsequent visits. A key aspect of prevention and management is having detailed risk–benefit discussions about choice of anticoagulant (rivaroxaban vs other) and the potential to continue estrogen-based therapies or start progestin-only therapy for the management of HMB. In general, hormone therapy is the most effective strategy to manage HMB while allowing continued anticoagulation and is the one I most often recommend.

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Prevention and treatment of postpartum hemorrhage: focus on hematological aspects of management

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Postpartum hemorrhage (PPH) is the leading cause of global maternal mortality and accounts for approximately one-quarter of all maternal deaths worldwide. Prevention of excess maternal deaths requires a coordinated approach to prevention, early recognition, and intervention by a multidisciplinary team. Although some women have risk factors for PPH that can be identified during pregnancy or during labor or birth, most women with severe PPH do not have any risk factors. Therefore, all pregnant women must be considered to be at risk of PPH. Common causes include uterine atony, retained placenta, trauma to the genital tract or uterus, and coagulopathy. The pivotal role of fibrinogen and hyperfibrinolysis in the evolution and as a treatment target for PPH is increasingly recognized. Coagulopathy can be an early feature in PPH that may be unrecognized, as it can be present before massive transfusion has occurred. Identification of coagulopathy by viscoelastic point-of-care testing or conventional laboratory assays can be helpful in guiding management of PPH and preventing severe maternal outcomes.

LEARNING OBJECTIVES

- Recognize the importance of risk assessment of pregnant women to identify PPH risk factors in the antenatal period and during labor and birth
- Recognize the importance of coagulation tests in women with PPH, to enable early identification and treatment of coagulopathy and hyperfibrinolysis

Clinical case

The patient was a 29-year-old Black woman in her first pregnancy. Her body mass index was 33 kg/m^2. She had no other medical history of note, and antenatal care had been uncomplicated, apart from iron deficiency treated with oral iron supplements from 28 weeks' gestation. At the 38-week scan, the fetus was well developed (estimated weight, 4100 g).

The patient had spontaneous onset of labor at 39^{+5} weeks' gestation. Her admission observations were normal: afebrile, pulse 88 per minute, blood pressure (BP) 110/68 mm Hg, and respiratory rate 14 per minute. A complete blood count on admission showed hemoglobin (Hb) 10.4 × 10^9/L, platelets 152, white blood cell count 7.8 × 10^9/L. She made slow progress in the first stage of labor, requiring augmentation with IV oxytocin. At 11 hours, an epidural was placed after the IV oxytocin was started. The first stage of labor was complete at 17 hours, and initial effective pushing occurred in the second stage, with the head on the perineum at 65 minutes with no further advancement. A successful vacuum extraction (ventouse) was performed after episiotomy by the senior resident, with birth of the infant after 80 minutes in the second stage. Active management of the third stage of labor appeared complete, with controlled cord traction, intramuscular oxytocin, and delivery of the placenta. Immediate postpartum blood loss was estimated at 1200 mL. The pediatric team was called to review the infant, who had a 3990-g birth weight and some initial floppiness but responded rapidly to basic resuscitation.

Ongoing vaginal blood loss continued in the postpartum period. The patient's uterus remained atonic but responded well to "rubbing up," and IV oxytocin infusion was started. The team agreed to move her to an operating room (OR) for examination under anesthesia to determine whether there were any retained products of conception or any genital tract trauma that would explain the ongoing

Table 1. Risk factors for PPH

Uterine atony	**Placental problems**
Previous PPH	Retained placenta
Labor >12 h	Placental abruption
Induction of labor	Placenta previa
Prolonged third stage of labor	Placenta accreta
Baby >4 kg	**Coagulopathy**
Multiple pregnancies	Amniotic fluid embolism
Increased body mass index	Acute fatty liver of pregnancy
Infection	Maternal sepsis
Genital tract trauma	Massive transfusion
Instrumental delivery	**Bleeding tendency**
CS	Inherited
Uterine rupture	Acquired (receiving anticoagulant therapy)

blood loss. Maternal observations were pulse, 106 per minute; BP, 98/60 mm Hg; and respiratory rate, 18 per minute.

On arrival in the OR, the observations were pulse, 114 per minute; BP, 94/60 mm Hg; and respiratory rate, 20 per minute. Vaginal blood loss continued, with estimated blood loss of 400 mL on new drapes and swabs in the OR.

Blood taken in the OR and tested on the blood gas analyzer showed Hb of 8.2 g/dL. Two units of packed red cells were ordered from the blood bank. The uterus remained atonic, and there were some abrasions of the vaginal wall and bleeding from the episiotomy; no other cause was identified. Ongoing vaginal blood loss was noted, with loss estimated at 1600 mL. Uterine atony persisted and further uterotonics were given, after which the maternal observations were pulse, 118 per minute; BP, 90/58 mm Hg; and respiratory rate, 22 per minute.

A senior obstetrician and anesthesiologist were called for support.

Discussion

Obstetric hemorrhage is the leading cause of maternal mortality and bleeding after childbirth. Postpartum hemorrhage (PPH) accounts for two-thirds of cases of obstetric hemorrhage and for approximately one-quarter of all maternal deaths worldwide. There is no universally accepted definition of PPH, with some suggesting that blood loss volume >500 or 1000 mL represents standard or severe PPH.[1] Most otherwise fit and healthy pregnant women will have minimal physiological response to this degree of blood loss, leading some clinicians to suggest more relevant clinical definitions, such as persistent PPH: ongoing active bleeding >1000 mL occurring within 24 hours after birth that continues despite the use of measures such as first-line uterotonic therapy and uterine massage.[2]

Maternal deaths represent only the tip of the iceberg in terms of the overall impact of major bleeding on maternal health. Women who have life-threatening hemorrhage but do not die of PPH can face long-term health complications including loss of fertility and psychological trauma. Although nearly all women with severe PPH live in countries with limited economic resources, PPH and its complications can affect women living in any resource setting. Data from the United States show that rates of severe PPH are increasing.[3] Well-resourced health care settings with access to skilled practitioners, drugs, and blood banks offer the best opportunity to provide optimal care for women. In any care setting, early recognition of abnormal postpartum bleeding and mobilization of appropriate staff and resources is essential to stop the bleeding promptly and minimize morbidity and mortality.

Postpartum hemorrhage should not be viewed as a diagnosis but rather a clinical manifestation of an underlying condition or conditions that require identification and treatment. The differential diagnosis is not wide and includes one or more of the following: uterine atony, retained placenta, and placental malimplantation (previa, accreta, increta, or percreta), and genital tract trauma or coagulopathy, often referred to as the "4 T's" (tone, tissue, trauma, and thrombin). Some women enter pregnancy with risk factors for PPH or develop these risk factors during the course of pregnancy or labor and birth (Table 1). Women with risk factors identified antenatally should be managed in the appropriate setting with access to skilled staff and a blood bank and with precautionary steps taken during labor and childbirth to minimize the risk of PPH and respond early if it occurs (Figure 1). However, it is critical that all staff caring for women in labor and childbirth be aware that most women who have severe PPH have no identifiable antenatal risk factors and that a high level of awareness be maintained. Risk factors should be reassessed frequently during labor and birth.

Setting the scene for the "PPH perfect storm"

Most women with PPH respond to initial measures of uterine massage and therapeutic uterotonics. However, if bleeding continues despite these interventions, the situation can rapidly escalate with more severe blood loss, maternal morbidity, and even mortality. Failure to recognize and respond to an evolving situation of severe PPH is frequently described in reviews of adverse outcomes caused by hemorrhage. This delay in response can be explained by several factors that create the "perfect storm" where the clinical team fails to recognize the severity of the blood loss and to take the appropriate steps.

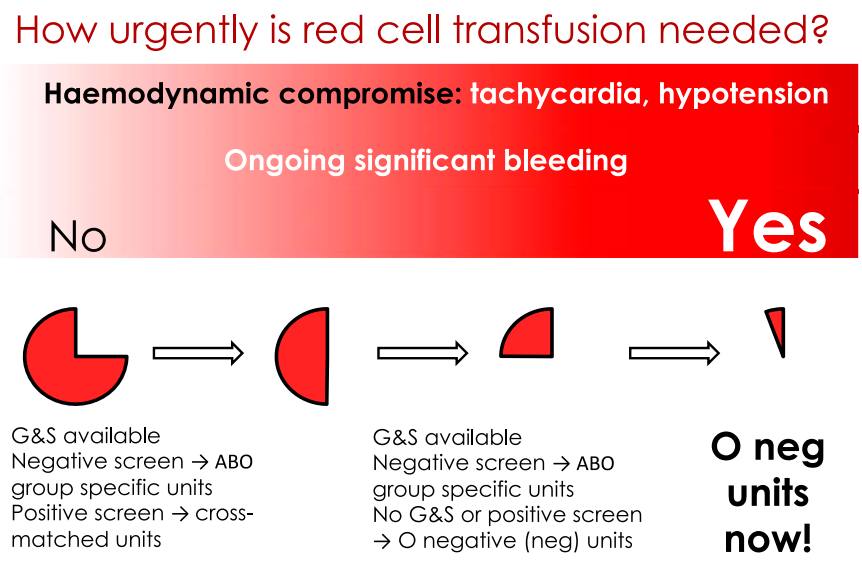

Figure 1. How urgently is red cell transfusion needed?

Potential for rapid loss of a large volume of blood

In pregnancy, the total blood volume is ~5 to 7 L (70-80 mL/kg lean body mass). By term, the blood supply to the uterine arteries is ~500 to 600 mL per minute, increased from its normal level of 10 to 15 mL per minute outside of pregnancy.[4] After delivery of the placenta, the uterine muscles contract, effectively staunching blood flow from the uteroplacental bed. Uterine atony, retained placental tissue, and abnormal placental implantation impede the normal action of the uterus in completing this critical mechanical hemostatic process. Given the high blood flow to the uterine arteries, it is easy to appreciate how rapidly a large volume of blood can be lost in a short time.

Underestimation of the degree of blood volume loss

In many clinical settings the volume of blood loss is estimated by using visual assessment rather than objective measurement, which significantly underestimates the actual blood volume, especially at higher volumes.[5] Accurate measurements of blood volume using graduated containers and gravimetric measurement of blood-soaked pads and swabs reported to the clinicians in real time can help alert them to the development of severe continued bleeding. Providing a cumulative total of blood volume loss is especially important when women are moved during the process of PPH management (for example, from the delivery room to the OR) if required for an examination while the patient is under anesthesia, to assess for retained products of conception or genital tract trauma.

Most pregnant women are healthy and physiologically robust

Healthy pregnant women show minimal physiological response to blood loss of 1000 to 1500 mL, perhaps only becoming slightly tachycardic with a minor decline in systolic BP. By the time women have significant hypotension or tachycardia or an increased respiratory rate or become distracted or agitated, they usually have lost in excess of 2000 to 2500 mL. Careful and repeated clinical assessments and documentation of vital signs, such as pulse rate, BP, temperature, and respiratory rate is essential for identifying a trend indicating physiological decompensation in response to hypovolemia. The use of maternity early warning scoring to improve early detection of clinically deteriorating patients and escalation of the clinical response is increasing.[6,7] These systems assign a score to a range of clinical vital signs to form a total maternity early warning score. An increasing score suggests a deviation from a normal physiological state and indicates clinical deterioration, which should prompt an escalation in response by clinicians who have the appropriate level of skill to care for the patient.

Lack of anticipation of the presence of early coagulopathy with PPH, before a massive transfusion is needed

Dilutional coagulopathy is common in patients with hemorrhage after multiple transfusions. Urgent red cell transfusion is preferred to infusion of significant volumes of crystalloid, but in an unstable patient, volume replacement with up to 2 to 3 L of crystalloid may be necessary prevent severe hypotension.[2] Coagulopathy resulting from conditions, such as amniotic fluid embolism, placental abruption, and sepsis, can be present early, before administration of IV fluids or blood products. Early identification of coagulopathy by testing basic hemostatic function, with either laboratory-based assays or point of care (POC) testing, can help identify coagulopathy early and enable directed transfusion of the appropriate blood products.

Standard thrombin-based functional clotting assays for fibrinogen, such as the Clauss fibrinogen assay, measure the time it takes for a fibrin clot to form. In most clinical settings the final result will not be available for at least 45 to 60 minutes. Viscoelastic point of care (VE-POC) tests, such as rotational thromboelastometry (ROTEM) and thromboelastography, are increasingly used to assess global hemostasis, determining the activity of the coagulation factors and the amount of fibrinogen available to form a fibrin clot, as well the resistance of the clot to fibrinolysis.[8] In the setting of PPH, hyperfibrinolysis is not uncommon, especially in more severe PPH. The results of POC tests are usually available within 15 to 20 minutes, especially if the instrument is available in the OR.

Fibrinogen and fibrinolysis in PPH

The importance of fibrinogen and fibrinolysis in major postpartum bleeding has been brought into focus in recent years. Fibrinogen

levels in pregnant women at term are increased at ~4 to 6 g/L, compared with levels of 2 to 4 g/L in nonpregnant patients. In 2007, Charbit et al showed that fibrinogen levels were lower in women who developed severe PPH than in women with nonsevere PPH (median levels, 3.3 and 4.4 g/L, respectively).[9] Importantly, this difference was evident early in the evolution of PPH and before any blood or blood products had been administered. Other studies have confirmed this finding[10,11]

Although these studies suggested that a low level of fibrinogen is predictive of development of severe PPH, they did not ascertain whether early fibrinogen replacement could modify the degree of blood loss. A controlled study of women with moderate PPH (blood loss >1000 mL after Cesarean section [CS], >500 mL in women who required manual removal of placenta, and >1000 mL in women who required exploration of the uterus) randomized women to 2 g of fibrinogen or placebo.[12] Transfusion rates were the same in women (n = 25 of 123; 20%) given fibrinogen as in those who received placebo (n = 26 of 121; 22%). Of note, the mean fibrinogen level in each group was normal (4.5 g/L) and the median blood loss was <1500 mL, indicating that perhaps fibrinogen would not be a key factor in this population.

A second multicenter, double-blind, randomized, placebo-controlled trial of early fibrinogen replacement in women with severe PPH (>1000-1500 mL measured blood loss, with ongoing bleeding and reduced fibrinogen on ROTEM: FIBTEM A5 <15 mm, equivalent to ~3 g/L fibrinogen).[13] Of 606 women eligible for inclusion, only 57 (9.4%) were randomized, with 55 women analyzed for the primary outcome (the number of allogeneic units transfused: red blood cells and plasma products). There was no difference in blood products transfused or in any of the secondary outcomes, such as invasive procedures for control of blood loss or transfer to intensive care. Analysis of prespecified subgroups showed that, in women with fibrinogen >2 g/L (n = 22), there was no difference in blood loss or blood transfusion after administration of the study medication, whereas the median blood loss was lower in the fibrinogen arm than in the placebo arm in women with fibrinogen <2 g/L. The researchers concluded that a fibrinogen level of >2 g/L appeared sufficient for hemostasis in the setting of PPH. Too few women in the cohort had very low fibrinogen (<2 g/L) for them to determine whether early administration of fibrinogen would modify outcomes in that patient group.

Although Collins et al[13] did not demonstrate an improvement in outcomes in women who were given fibrinogen concentrate, they observed that, over the course of the clinical trial, their approach of routine risk assessment for PPH, objective measurement of cumulative blood loss, and early involvement of senior staff enabled early recognition and intervention in the course of the PPH to take steps to respond to and control blood loss. The prompt recognition allowed for timely and appropriate escalation of care and involvement of senior staff at an early stage. Also, the study led to the practice of early assessment of hemostasis, Hb, and lactate by using POC tests. The clinicians used the study protocol to inform the development of a nationwide interventional program for standardized management of PPH, "OBS Cymru," which has been effectively implemented across all obstetric units in Wales.[14]

Approach to transfusion of blood and plasma products in management of PPH

Using empiric fixed ratios of red blood cells, fresh frozen plasma (FFP), and platelets in women with PPH >1500 mL has been shown to reduce progression to severe PPH. Most women with PPH <2000 mL do not have low levels of fibrinogen or other clotting factors, and fibrinogen does not appear to decrease to <2 g/L until blood volumes of >4000 mL are lost, although early coagulopathy is a feature of placental abruption and amniotic fluid embolism. Empiric transfusion in the absence of hemostatic testing may lead to overtransfusion of blood and plasma products increased risk of complications, such as transfusion-associated circulatory overload and transfusion-associated lung injury. Collins et al advocate using targeted transfusion protocols with viscoelastic-POC (VE-POC) testing.[15] They reported that fibrinogen levels decrease sooner than other coagulation factors, so that correction of low fibrinogen levels may be a more important therapeutic target.

The International Society on Thrombosis and Haemostasis (ISTH) recommends using either cryoprecipitate (~15 g/1000 mL) or fibrinogen concentrate (20 g/1000 mL) to maintain fibrinogen >2 g/L when managing PPH.[16] The fibrinogen concentration of FFP is much lower (2 g/1000 mL), and its use for fibrinogen replacement could lead to hemodilution.

Deficiencies of other clotting factors tend to occur at a later stage in PPH, suggesting that there is a more limited requirement for FFP. When VE-POC testing is not available, conventional laboratory testing may be helpful and the ISTH Scientific and Standardization Committee recommends targeting using 15 mL/kg FFP to maintain activated partial thromboplastin time/prothrombin time >1.5 × normal.[16]

Severe thrombocytopenia is uncommon in most women with PPH, leading to a recommendation (ISTH) to limit platelet transfusions, unless platelet count is <75 × 10^9/L.[16]

Inhibition of fibrinolysis

Over the past 20 years or so, the impact of hyperfibrinolysis in major bleeding has been recognized. The CRASH-2 study demonstrated tranexamic acid, a potent inhibitor of fibrinolysis, reduction in death related to bleeding by 21% (risk ratio [RR], 0.79; 95% confidence interval [CI], 0.64-0.97) in trauma patients who received it within 3 hours and by 32% (RR, 0.68; 95% CI, 0.57-0.82) in patients who received it within 1 hour.[17]

The WOMAN study is a placebo-controlled trial conducted in 21 countries that assessed the impact of tranexamic acid in 20 021 women with PPH >500 mL after vaginal birth or >1000 mL after CS.[18] The maternal mortality rate was 2.4% (n = 483) with 72% (n = 346) of deaths caused by hemorrhage. Administration of 1 g of tranexamic acid (with a second dose given for ongoing bleeding) resulted in an overall reduction in death related to bleeding of 19% (RR, 0.81; 95% CI, 0.65-1.00) when given within 3 hours.

As a result of this study, the World Health Organization now strongly recommends early use of IV tranexamic acid (within 3 hours of birth) in addition to standard care for women with clinically diagnosed PPH after vaginal birth or CS.[19]

Recognition and response to major PPH also requires rapid response with transfusion of red blood cells to maximize oxygen delivery and prevent tissue hypoxia, development of acidosis, organ failure, and worsening of shock. The urgency of red cell transfusion depends on the degree of maternal clinical instability and rapidity of blood loss. Transfusion of O^- red blood cells may be required in women who are clinically unstable or who are losing blood rapidly when cross-matched blood is not available.

Comments on the case

Antenatal risk factors

In the clinical case, the only identifiable antenatal risk factors for PPH were increased body mass index and fetal macrosomia. Although the patient was iron deficient, she was not anemic. However, during labor she needed augmentation with oxytocin and had prolonged first and second stages of labor, with an assisted delivery. The immediate estimated postpartum blood loss was high, confirming a PPH, and importantly, the bleeding did not stop after initial interventions of uterine massage and therapeutic uterotonics.

An opportunity for escalation of intervention and a call for backup was missed at the time the patient was transferred to the OR. The degree of tachycardia and the increase in the respiratory rate were signs that this otherwise healthy, physiologically robust woman had lost a significant amount of blood. As a rule of thumb, a pulse rate higher than the systolic BP indicates a problem.

Estimated rather than measured blood loss

It is likely given the drop in the Hb that the woman had lost >1600 mL blood. Accurate cumulative measurement of blood loss would have alerted the clinicians to the severity of blood loss and the potential for progression.

Failure to perform an early coagulation test

A test of coagulation is helpful in identifying unanticipated coagulopathy. If available, a VE-POC test (ROTEM or thromboelastography) can provide a result within 15 minutes. Even though a conventional laboratory-based fibrinogen assay may be delayed by 60 minutes, it could still provide additional help if the PPH is not being controlled by initial maneuvers.

Ongoing significant postpartum bleeding in a woman with a well-contracted uterus with no evidence of genital tract trauma or retained placenta should alert the clinicians to the possible presence of coagulopathy.

Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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Women and bleeding disorders: diagnostic challenges

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Women with bleeding disorders suffer from multiple bleeding symptoms, including easy bruising, epistaxis, bleeding from minor wounds and the oral cavity, and bleeding after dental work or surgery. However, women with bleeding disorders especially suffer from gynecologic and obstetrical bleeding. These symptoms often are not recognized as abnormal, and many women are left undiagnosed and without access to appropriate medical care. Additional challenges to diagnosing women with bleeding disorders include lack of access to appropriate laboratory testing and issues around disease classification and nomenclature. Efforts have been undertaken to address these challenges, including the development and validation of bleeding assessment tools and strategies to clarify diagnostic thresholds and algorithms for von Willebrand disease (VWD) and platelet function disorders. Efforts to improve communication with the nomenclature used for hemophilia carriers are also underway.

LEARNING OBJECTIVES

- Understand the burden of disease for women with bleeding disorders
- Explore the barriers facing women in achieving an accurate bleeding disorder diagnosis

Clinical case

An 18-year-old woman seeks medical attention from her rural family physician for heavy periods. They typically last 8 to 10 days, with the heaviest bleeding on days 2 and 3. She has struggled with iron deficiency since menarche and had excessive bleeding following extraction of wisdom teeth at age 14 that required a return to the dentist. She bruises easily, and her gums bleed when she brushes her teeth. She has no family history of bleeding disorders.

Background

Up to 30% of all women report heavy menstrual bleeding (HMB) at some point during their reproductive years and up to half seek medical attention for this symptom.[1,2] Multiple studies have shown that fully 15% to 30% of those with HMB have an underlying inherited bleeding disorder.[2-4] Among women known to have a bleeding disorder, HMB is the most common symptom.[3] These women are also at risk for postpartum hemorrhage and postoperative bleeding as well as hemorrhagic ovarian cysts.[5] HMB is defined as loss of more than 80 mL menstrual blood per cycle; however, this is difficult to determine clinically. Studies have shown that having to change sanitary protection more often than every hour, soaking through pajamas and sheets at night, passing clots >1 inch in diameter, and low ferritin all correlate with menstrual blood loss of more than 80 mL.[6] HMB is an important cause of work and school absenteeism and historically has led to two thirds of the hysterectomies in women of reproductive age.[3,5] It has also been shown to have a profoundly negative impact on quality of life.[7]

The population prevalence of a symptomatic inherited bleeding disorder is approximately 1 in 1000[8,9]; however, far fewer have ever been diagnosed. In Canada, a predicted 37 500 are affected but only ~8000 (~20%) have been diagnosed (http://fhs.mcmaster.ca/chr/; data up to the end of 2018). This problem particularly affects women, and in addition to patients not being diagnosed, those who are diagnosed report delays of up to 15 years after the onset of symptoms until they receive appropriate medical attention.[10] There are multiple barriers to diagnosis, including the lack of recognition of the difference between normal and abnormal bleeding (especially gynecologic and obstetric), challenges in terms of laboratory testing, and issues around disease classification and nomenclature.

Bleeding assessment tools

In the past decade, significant work has been done to develop and validate bleeding assessment tools (BATs).[11-14] BATs are

questionnaires about bleeding that result in a quantitative bleeding score. In addition to standardizing a bleeding history, BATs have been shown to accurately distinguish normal from abnormal bleeding.[15] Several studies have validated an abnormal bleeding score as a screening test for inherited bleeding disorders, particularly von Willebrand disease (VWD).[11-14] In addition, BATs have been shown to be an effective measure of bleeding severity in patients known to have a bleeding disorder.[12,14-17] BATs contain a series of questions about the presence or absence of bleeding symptoms and the level of medical attention and treatment required for each (ie, epistaxis that required medical consultation with a medical professional, treatment with desmopressin, or blood transfusion). Each symptom is then scored, with higher levels of medical intervention receiving a higher score. An overall bleeding score is then calculated by adding together the scores for each symptom (see Table 1 for an example of a BAT scoring key).

The majority of BATs are administered by experts; however, a self-administered version (the Self-BAT) was published in 2015.[14] The Self-BAT has been validated for use as a screening tool for VWD and has been studied in hemophilia carriers.[14,18] It was designed to be made widely and freely available to the general public and can be found at https://letstalkperiod.ca. The Let's Talk Period website (Figure 1) was launched in May 2016, and in September 2016, 2 complementary social media accounts were also launched on Facebook (https://www.facebook.com/letstalkperiod) and Instagram (https://www.instagram.com/lets_talk_period/). Four years after launch, the website has had 168 855 page views from 200 countries (see Figure 2 for global reach). A total of 19 365 individuals have completed the Self-BAT, and 8512 (44%) had a positive or abnormal bleeding score. Anyone with an abnormal score is advised to speak with a physician about the result. The Facebook page has 4428 followers and a reach of 866 520 individuals; the Instagram account has 385 followers. Preliminary studies show that patients referred to a hematologist because of a positive Self-BAT bleeding score had more significant bleeding symptoms and were more likely to require intervention (ie, iron replacement; referral to an ear, nose, and throat specialists; or a gynecologist) than those referred by their primary care provider for bleeding or bruising symptoms, abnormal laboratory results, or a positive family history of a bleeding disorder.[19] The project has been expanded to include a grade 9 outreach program to raise awareness about normal vs abnormal menstruation, iron deficiency, and bleeding disorders.[20] Future plans include launching toolkits for teachers and nurses as well as providing resources for primary care practitioners.

Importantly, Let's Talk Period is not the only initiative of its kind. There are worldwide efforts to raise awareness about HMB and bleeding disorders in women. Other examples include the Irish Know Your Flow website (https://www.knowyourflow.ie), Better You Know (https://betteryouknow.org) from the National Hemophilia Foundation in the United States, and the work of the Foundation for Women & Girls with Blood Disorders (https://www.fwgbd.org).

Laboratory testing

An important barrier to the diagnosis of an underlying bleeding disorder is the lack of access to accurate laboratory testing, even if bleeding symptoms are recognized as abnormal. Unfortunately, most bleeding disorders cannot be diagnosed by the commonly available screening tests of coagulation, the complete blood count, prothrombin time/international normalized ratio (PT/INR), and activated partial thromboplastin time (aPTT), which can be normal even in affected individuals. Therefore, special coagulation assays that include coagulation factor levels and/or platelet aggregation and release must be performed. These are available only in special coagulation laboratories and require significant expertise and experience to perform. In addition, pre-analytical variables are known to have a major impact on the results.[21] Thus, patients often need to be referred to a center with a special coagulation laboratory which, in many cases, necessitates travel and missing work or school. This issue was highlighted very clearly in a recent publication by Jaffray et al,[22] which showed that <40% of post-menarchal females referred with abnormal von Willebrand factor (VWF) offsite test results were confirmed to have VWD with onsite testing. Even when assays are performed properly, there remain challenges in terms of interpretation and lack of international agreement on diagnostic thresholds, classification, and nomenclature.

Issues with diagnosis, classification, and nomenclature for bleeding disorders that affect women

von Willebrand disease

VWD is the result of deficiency or dysfunction of VWF, a hemostatic protein essential for normal hemostasis. It is characterized by excessive mucocutaneous bleeding such as HMB, epistaxis, easy bruising, prolonged bleeding from minor wounds, oral cavity and gastrointestinal bleeding, and bleeding after dental work, childbirth, or surgery, with musculoskeletal bleeding seen in more severe cases.[23] It is the most common inherited bleeding disorder in humans, with prevalence estimates ranging from ~1 in 100 to 1 in 10 000.[23-26] At the level of primary care, ~1 in 1000 individuals is affected and requires medical attention for bleeding.[9,27] Although VWD is autosomally inherited, only women suffer from the gynecologic and obstetrical manifestations. The current International Society on Thrombosis and Haemostasis (ISTH) classification recognizes 3 types: type 1 is a partial quantitative deficiency of VWF, type 2 is caused by qualitative abnormalities of VWF, and type 3 is a virtual absence of the VWF protein with associated very low levels of factor VIII (FVIII). Type 2 VWD is further divided into 4 subtypes: type 2A is characterized by a loss of high molecular weight VWF, type 2B results from a gain of function in VWF that increases its affinity to platelets, type 2M is caused by reduced VWF interactions with platelets or collagen, and type 2N results from reduced binding of VWF to FVIII.[28] Although it is not included in the ISTH classification, type 1C VWD which is caused by increased VWF clearance, is also recognized.[29,30]

Even within the bleeding disorder community, there remains debate about diagnostic cutoffs for diagnosis and classification of VWD, especially for type 1 VWD. In addition to the classification reviewed above, the term "low VWF" has been used for patients with milder reductions in VWF levels (ie, VWF antigen [VWF:Ag] and/or VWF ristocetin cofactor [VWF:RCo] between 0.30 and 0.50 IU/mL). Importantly, the work of Lavin et al[18,31] has shown that the bleeding phenotype is similar in patients with VWF levels <0.30 IU/mL and those with milder reductions, including gynecologic bleeding. The American Society of Hematology/International Society on Thrombosis and Haemostasis/National Hemophilia Foundation/World Federation of Hemophilia (ASH/ISTH/NHF/WFH) Guideline on VWD diagnosis has taken an evidence-based approach to this problem with publication of the final guideline expected in December 2020. Priorities for the guideline were informed by >600 responses from international

Table 1. ISTH-BAT scoring key for bleeding episodes

Type of bleeding	BAT score				
	0	1	2	3	4
Epistaxis	None/ trivial	>5 per year or more than 10 min	Consultation only	Packing, cauterization, or antifibrinolytics	Blood transfusion or replacement therapy (use of hemostatic blood components or rFVIIa) or desmopressin
Cutaneous	None/ trivial	≥5 bruises (>1 cm) in exposed areas	Consultation only	Extensive	Spontaneous hematoma requiring blood transfusion
Bleeding from minor wounds	None/ trivial	>5 per year or more than 10 min	Consultation only	Surgical hemostasis	Blood transfusion, replacement therapy, or desmopressin
Oral cavity	None/ trivial	Present	Consultation only	Surgical hemostasis or antifibrinolytics	Blood transfusion, replacement therapy, or desmopressin
Gastrointestinal bleeding	None/ trivial	Present (not associated with ulcer, portal hypertension, hemorrhoids, angiodysplasia)	Consultation only	Surgical hemostasis, antifibrinolytics	Blood transfusion, replacement therapy, or desmopressin
Hematuria	None/ trivial	Present (macroscopic)	Consultation only	Surgical hemostasis, iron therapy	Blood transfusion, replacement therapy, or desmopressin
Tooth extraction	None/ trivial or none performed	Reported in ≤25% of all procedures, no intervention	Reported in >25% of all procedures, no intervention	Resuturing or packing	Blood transfusion, replacement therapy, or desmopressin
Surgery	None/ trivial or none performed	Reported in ≤25% of all procedures, no intervention	Reported in >25% of all procedures, no intervention	Surgical hemostasis or antifibrinolytics	Blood transfusion, replacement therapy or desmopressin
Menorrhagia	None/ trivial	Consultation only or changing pads more frequently than once every 2 hours or clot and flooding or pictorial bleeding assessment chart score >100	Time off work or school more than twice per year or requiring antifibrinolytics or hormonal or iron therapy	Requiring combined treatment with antifibrinolytics and hormonal therapy or present since menarche and for more than 12 months	Acute menorrhagia requiring admission and emergency treatment or requiring blood transfusion, replacement therapy, desmopressin, or requiring dilatation and curettage or endometrial ablation or hysterectomy
Postpartum hemorrhage	None/ trivial or no deliveries	Consultation only or use of syntocin or lochia for >6 weeks	Iron therapy or antifibrinolytics	Requiring blood transfusion, replacement therapy, desmopressin or requiring examination under anesthesia and/or the use of a uterine balloon or package to tamponade the uterus	Any procedure requiring critical care or surgical intervention (eg, hysterectomy, internal iliac artery ligation, uterine artery embolization, uterine brace sutures
Muscle hematomas	Never	Posttrauma, no therapy	Spontaneous, no therapy	Spontaneous or traumatic, requiring desmopressin or replacement therapy	Spontaneous or traumatic, requiring surgical intervention or blood transfusion
Hemarthrosis	Never	Posttrauma, no therapy	Spontaneous, no therapy	Spontaneous or traumatic, requiring desmopressin or replacement therapy	Spontaneous or traumatic, requiring surgical intervention or blood transfusion
Central nervous system bleeding	Never	—	—	Subdural, any intervention	Intracerebral, any intervention
Other bleeding	None/ trivial	Present	Consultation only	Surgical hemostasis, antifibrinolytics	Blood transfusion or replacement therapy or desmopressin

Table adapted from Rodeghiero et al.[13]

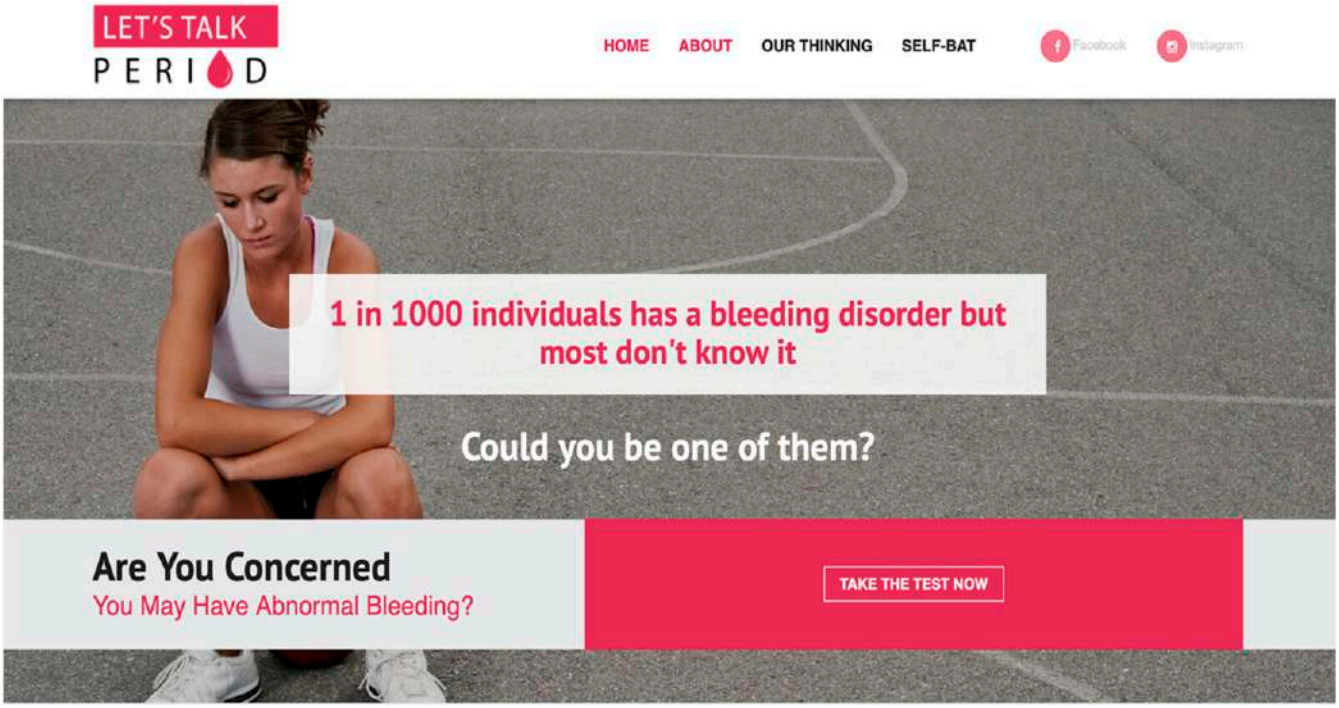

Figure 1. Homepage of Let's Talk Period.

stakeholders, including health care providers, patients, and caregivers.[32]

Hemophilia carriers

The hemophilias are X-linked bleeding disorders, with a frequency of 1 in 4000 live male births for hemophilia A and 1 in 20 000 live male births for hemophilia B.[33] In contrast, the true prevalence of hemophilia carriers is not known because many come to the attention of physicians only as the result of a male relative being diagnosed, although it has been estimated that for every male with hemophilia, there are 3 to 5 hemophilia carriers.[34] Approximately 30% of hemophilia carriers manifest low FVIII/FIX levels.[35,36] A number of variables have been proposed to explain these low levels, including skewed X-chromosome inactivation (lionization), ABO blood type, VWF level, and *F8/F9* mutation severity, although published studies show conflicting results about the relative contribution of each.[35-38]

In a multinational study of 168 hemophilia carriers, 65 (38%) had abnormal or positive bleeding scores (BS) with the mean BS in carriers of 5.7 compared with a BS of 1.43 in normal controls ($P < .0001$). The correlation between coagulation factor levels and abnormal bleeding was weak ($r^2 = -0.36$; $P < .001$), and even carriers with normal levels of factors were shown to have excessive bleeding.[37] Many other studies have also evaluated the bleeding symptoms experienced by hemophilia carriers by using a variety of other bleeding assessment tools, and it is clear that these patients experience multiple bleeding symptoms, including menorrhagia, postpartum hemorrhage, excessive postsurgical bleeding, epistaxis, easy bruising, oral cavity bleeding, and musculoskeletal bleeding.[35,36,39-41] Despite this, the underlying pathophysiology of bleeding is not completely understood, although recent work by Candy et al[42] suggests that a decreased and less sustained response to hemostatic stress is a contributor. Unfortunately, many of these women continue to receive suboptimal care and have an impaired quality of life.[43,44]

To improve communication around the issue of bleeding in hemophilia carriers, a joint initiative of the Factor VIII and Factor IX as well as Women's Issues in Thrombosis and Hemostasis ISTH Scientific and Standardization Committees was undertaken. This group recommends that the term "hemophilia carrier" be reserved for discussions regarding genetic counseling and terms such as "symptomatic/asymptomatic hemophilia carrier" and "women and girls with hemophilia" be used in clinical management. Thus, a hemophilia carrier with factor levels >5% to 40% should be referred to as a woman or girl with mild hemophilia, 1% to 5% as moderate hemophilia, and <1% as severe hemophilia. Carriers with factor levels >40% should be referred to as

Figure 2. Global reach of Let's Talk Period.

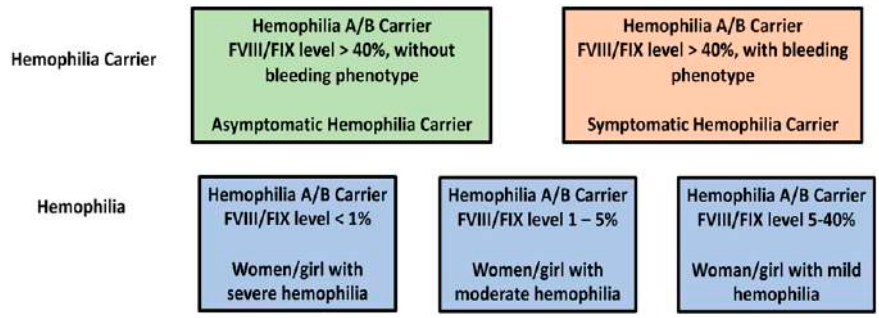

Figure 3. Proposed nomenclature for hemophilia carriers and women and girls with hemophilia.

symptomatic or asymptomatic, depending on their bleeding phenotype (see Figure 3 for a schematic of this nomenclature).

Platelet function disorders

Disorders of platelet function present with a similar pattern of clinical bleeding in women, including HMB. In a study of adolescents presenting to a tertiary care center with HMB, platelet function disorders were second only to VWD in terms of the those who were found to have an underlying bleeding disorder.[44] Although the severe disorders, such as should be Glanzmann Thrombasthenia and Bernard-Soulier Syndrome are straightforward to identify from a laboratory perspective, the diagnosis of the more common, milder forms presents many challenges similar to those of other inherited bleeding disorders. Assays of platelet aggregation and release are not widely standardized, are technically challenging to perform, and have poor reproducibility. International efforts by ISTH and the International Society for Laboratory Hematology (ISLH) provide guidance for addressing these issues.[45,46]

Return to the clinical case

This young woman had heard about the Let's Talk Period website at her school, so she went online and took the Self-BAT. Her quantitative BS was calculated to be 7 (≥6 is positive or abnormal). Her family physician performed initial blood work that showed a hemoglobin of 112 g/L (normal, 120-160 g/L) with a mean corpuscular volume of 75 fL (normal, 81-98 fL). Her ferritin was low at 8 µg/L (normal, 15-205 µg/L), so she was started on oral iron supplementation. PT and aPTT were normal, so she was referred to an urban center for hematologic consultation. There, the hematologist determined that her bleeding symptoms were indeed abnormal (ISTH-BAT BS of 7 [≥6 is abnormal or positive]) and that she was not able to tolerate the oral iron because of abdominal pain and constipation. Blood work showed a hemoglobin of 105 g/L with a ferritin of 5 µg/L, so intravenous iron was arranged. PT and aPTT were again normal, and special coagulation blood work was sent to the local laboratory. VWF:Ag was 0.25 IU/mL (normal, 0.50-1.50 IU/mL), VWF:GPIbM was 0.21 IU/mL (normal, 0.50-1.50 IU/mL), and FVIII:C was 0.56 IU/mL (normal, 0.50-1.50 IU/mL), with normal VWF multimers. Platelet aggregation and release testing were normal. Therefore, she was diagnosed with type 1 VWD and a desmopressin trial was arranged. She was started on tranexamic acid 1 g orally twice per day during menses pending an appointment with a gynecologist to discuss other strategies for managing her menstrual cycles.

Conclusion

The accurate diagnosis of women with bleeding disorders is critical to ensuring appropriate medical care. An accurate assessment of bleeding symptoms is critical, as is access to high-quality laboratory assays. There are several effective treatments for women with bleeding disorders that can vastly improve quality of life.

Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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EVIDENCE-BASED MINIREVIEW

Abnormal uterine bleeding in users of rivaroxaban and apixaban

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Up to two-thirds of menstruating women experience abnormal uterine bleeding (AUB) when treated with oral anticoagulants. However, the true prevalence of AUB for specific agents remains uncertain, as many of these episodes, while interfering significantly with quality of life and overall health, are not captured by definitions of major bleeding (MB) or clinically relevant nonmajor bleeding (CRNMB) used in clinical trials. A 2017 systematic review determined that women taking rivaroxaban, but not edoxaban or apixaban, had a twofold higher risk of AUB than women taking warfarin. Since then, new data have become available from extension trials, cancer-associated venous thromboembolism trials, pediatric trials, and a few observational studies specifically examining AUB as an outcome. Reported rates of uterine CRNMB were low (around 1%) and similar for rivaroxaban and apixaban in all these studies, and no episodes of uterine bleeding meeting MB criteria were reported. Rates of AUB not meeting MB or CRNMB criteria were much higher, affecting up to 50% of women on rivaroxaban. Only 1 such study included women on apixaban, and no AUB was reported. In pediatric trials, 19% of girls experienced menorrhagia when treated with rivaroxaban. In conclusion, rates of uterine MB and CRNMB were low in all studies, but rates of other types of AUB not meeting these criteria ranged from 15.8% to 50%. We conclude that AUB is underreported due to the limitations of MB/CRNMB criteria despite its substantial impact on quality of life. We urge future investigators to include broader definitions of AUB to better capture the impact of this outcome in menstruating women treated with oral anticoagulants.

LEARNING OBJECTIVES

- Quantify the risk of abnormal uterine bleeding with rivaroxaban and apixaban
- Manage a patient with a history of heavy menstrual bleeding and venous thromboembolism

Clinical case

A 22-year-old woman is found to have a pulmonary embolism 3 months after starting a combined oral contraceptive for the management of heavy menstrual bleeding severe enough to require a blood transfusion. She is concerned about a worsening of her menstrual bleeding with starting an anticoagulant. Which is the most appropriate oral anticoagulant to offer?

Discussion

"Abnormal uterine bleeding" (AUB) is defined as uterine bleeding that is excessive and/or occurs outside of the normal menstrual cycle and encompasses heavy menstrual bleeding/menorrhagia, intermenstrual bleeding, prolonged menstrual bleeding, or postmenopausal bleeding. Up to two-thirds of menstruating women experience AUB when treated with oral anticoagulants, which can, in turn, lead to premature discontinuation and increased risk of venous thromboembolism (VTE) recurrence.[1] The majority of studies of anticoagulants define uterine bleeding as that meeting International Society on Thrombosis and Haemostasis definitions of major bleeding (MB) and clinically relevant nonmajor bleeding (CRNMB),[2] which do not capture many forms of AUB. A 2017 review by Godin et al[3] summarized existing data on AUB in women receiving direct oral anticoagulants (DOACs) for VTE and included uterine bleeding events from registry trials of apixaban, rivaroxaban, and edoxaban, including those reported only in product monographs. The authors concluded that women receiving rivaroxaban have a twofold increased risk of AUB (relative risk [RR], 2.10; 95% confidence interval, 1.64-2.69; $P < .0001$) as compared with those receiving vitamin K antagonists (VKAs), an increase not seen with apixaban or edoxaban (RR, 1.18; $P = .37$; RR, 1.26; $P = .044$, respectively). One observational study included in this

review found that, although rates of AUB were similar in users of rivaroxaban and VKAs, women receiving rivaroxaban had significantly increased incidence of prolonged menstrual bleeding and intermenstrual bleeding. Women receiving rivaroxaban also had more medical or surgical interventions as a result of AUB and had more modifications of anticoagulant therapy than women receiving VKAs.[3] Since the publication of this review, new data have become available from extension trials, cancer-associated VTE trials, and pediatric trials. The present review provides an update on the incidence of AUB in women treated with full and reduced doses of rivaroxaban and apixaban, the most commonly prescribed DOACs for women of childbearing age.

We conducted a literature review of the MEDLINE, PubMed, and Cochrane databases from the earliest available date until 18 May 2020 to retrieve any study in English reporting AUB as an outcome or adverse effect in women receiving rivaroxaban or apixaban for VTE. Newly published randomized controlled trials (RCTs) of rivaroxaban or apixaban for VTE were also included when MB and CRNMB events were reported by site, allowing the identification of uterine bleeds. Each abstract was screened by 2 reviewers.

Our literature search identified a total of 11 new publications specifically addressing AUB in anticoagulated menstruating women. Seven did not include new data, and 4 did not include AUB outcomes specific to drug; thus, all were excluded. We identified 2 additional studies of apixaban and rivaroxaban for extended prophylaxis after VTE; 3 on cancer-associated thrombosis; and 5 prospective studies in unique VTE populations, including pediatric patients. Three publications did not report bleeding events by site, but the authors of one were able to provide us a detailed breakdown via personal communication. The remaining studies were excluded. Outcomes were defined differently between studies but included AUB, menorrhagia, uterine MB, and uterine CRNMB (Table 1).

Pivotal extension and cancer-associated VTE trials

The randomized, double-blind extension trials compared standard and reduced doses of apixaban (AMPLIFY-EXT)[4] and rivaroxaban (EINSTEIN CHOICE)[5] with placebo (apixaban) or aspirin (rivaroxaban) for the treatment of VTE beyond the initial 6 to 12 months. Uterine bleeds were reported as uterine MB (0%) or CRNMB (apixaban, 0.9% to 1.1%; rivaroxaban, 0.8% to 1.2%) (Table 2).[4,5] Other types of AUB were not included as outcomes. In the 2 randomized, open-label cancer-associated thrombosis trials, standard dose rivaroxaban (SELECT-D)[6] or apixaban (Caravaggio)[7] was compared with low-molecular-weight heparin (LMWH). Outcomes included uterine MB (0%) and uterine CRNMB (apixaban, 1.4%; rivaroxaban, 1.1%).[6,7]

Observational studies

Three observational studies reported AUB in adult women receiving rivaroxaban or apixaban (Table 2). In a study of rivaroxaban in patients with upper extremity deep vein thrombosis, 1 (5.9%) of 17 women reported uterine bleeding classified as CRNMB, and none reported uterine MB events.[8] In a study of patients treated with DOACs for cerebral venous sinus thrombosis, menorrhagia was reported in 2 (20%) of 10 women treated with rivaroxaban, whereas no AUB was reported in women treated with apixaban or dabigatran.[9] In a study that included patients with sickle cell disease treated with rivaroxaban for VTE, 4 (50%) of 8 women reported menorrhagia.[10] Neither of the latter 2 studies classified uterine bleeds as MB or CRNMB.

Pediatric trials

In the EINSTEIN-Jr phase 2 single-arm study of rivaroxaban in pediatric patients previously treated with LMWH, VKAs, or fondaparinux, 15.8% of girls in the 6- to 17-year-old age group reported menorrhagia.[11] In the randomized, open-label, phase III study comparing rivaroxaban with heparin, LMWH, or VKAs, 19% of girls (ages 6-17 years) in the rivaroxaban group reported menorrhagia compared with 7.1% of girls in the control group (Table 3).[12] No uterine bleeding events meeting criteria for MB or CRNMB were reported.

Conclusions

No uterine MB events were reported in any of the 10 studies included in this updated review. Reported rates of uterine CRNMB

Table 1. Uterine MB and CRNMB definitions

Uterine MB bleeding was defined as uterine bleeding with one or more of the following:
A decrease in hemoglobin ≥2 g/dL
Transfusion of ≥2 U of packed red blood cells
Fatal bleeding
Bleeding requiring surgical intervention
Uterine CRNMB was defined as uterine bleeding with one or more of the following:
Acute clinically overt uterine bleeding that does not meet criteria for MB and consists of:
Any bleeding compromising hemodynamics
Any bleeding considered to have clinical consequences for the patient, such as medical intervention, need for an unscheduled visit or phone call with a physician, temporary cessation of study drug, or associated with pain or impairment in activities of daily living
AUB was defined as uterine bleeding with the following:
Uterine bleeding that is excessive and/or occurs outside of the normal menstrual cycle, including heavy menstrual bleeding, intermenstrual bleeding, prolonged menstrual bleeding, or postmenopausal bleeding
Heavy menstrual bleeding or "menorrhagia" was defined as follows:
A menstrual period with excessively heavy flow (>80 mL of blood loss per cycle, soaking a pad/tampon at least every 2 h, or bleeding lasting >7 d)

Table 2. AUB rates in adult studies of rivaroxaban and apixaban for the treatment of VTE

Reference (duration)	Study design/population	Study drug/comparator (maintenance dose)	Number of women	Uterine CRNMB,* n (%)
AMPLIFY-EXT[4] (12 mo)	Randomized, double-blind comparison of 2 doses of apixaban vs placebo for extended (beyond 6-12 mo) therapy in patients with VTE	Apixaban 5 mg twice daily	334	3 (0.9%)
		Apixaban 2.5 mg twice daily	353	4 (1.1%)
		Placebo	361	2 (0.6%)
EINSTEIN CHOICE[5] (12 mo)	Randomized, double-blind comparison of 2 doses of rivaroxaban vs aspirin for extended (beyond 6-12 mo) therapy in patients with VTE	Rivaroxaban 20 mg daily	505	6 (1.2%)
		Rivaroxaban 10 mg daily	507	4 (0.8%)
		Aspirin 100 mg daily	488	1 (0.2%)
SELECT-D[6] (6 mo)	Randomized, open-label pilot in patients with cancer and VTE	Rivaroxaban 20 mg daily	87	1 (1.1%)
		Dalteparin 150 IU/kg/d	105	0 (0.0%)
Caravaggio[7] (6 mo)	Randomized, open-label, noninferiority trial in patients with cancer and VTE	Apixaban 5 mg twice daily	284	4 (1.4%)
		Dalteparin 150 IU/kg/d	303	3 (1.0%)
Schastlivtsev et al[8] (3-6 mo)	Single-center prospective observational study on patients with upper extremity DVT	Rivaroxaban 20 mg daily	17	1 (5.9%)
Rusin et al[9] (3-6 mo)	Single-center prospective case series on patients with CSVT treated with DOACs	Dabigatran 150 mg twice daily	18	0 (0.0%)
		Rivaroxaban 20 mg daily	10	2 (20.0%)†
		Apixaban 5 mg twice daily	8	0 (0.0%)
Christen et al[10] (variable)	Prospective cohort study of patients with sickle cell disease undergoing VTE treatment with DOACs	Rivaroxaban 20 mg daily	8	4 (50%)†

CSVT, cerebral sinus venous thrombosis; DVT, deep vein thrombosis.
*No major uterine bleeding was reported in any of the studies.
†Bleeding was menorrhagia, which was classified as MB and not CRNMB.

were overall very low (0% to 5.9%). Rates of menorrhagia or other types of AUB not meeting MB or CRNMB criteria, however, ranged between 15.8% and 50% in females treated with rivaroxaban and were higher than rates of bleeding in females treated with apixaban (0%) or LMWH/VKA (7.1%) when these groups were directly compared. This finding is congruent with the conclusion of Godin et al's review.[3] Though AUB in women receiving anticoagulants has significant effects on quality of life and can lead to early discontinuation of anticoagulation, we suspect rates are underreported because many such bleeds do not meet criteria for MB or CRNMB.

Rates of uterine MB or CRNMB in women in the extension RCTs who were receiving both full and reduced doses of rivaroxaban and apixaban were virtually identical (0.9% vs 1.1% for apixaban; 1.2% vs 0.8% for rivaroxaban). Once again, the overall low rates of uterine MB or CRNMB events, compared with the broader scope of AUB outcomes reported in observational studies, suggest that cases of AUB went unreported, and therefore a true difference may have been missed. In addition, because subjects in the extension RCTs had already completed 6 months of full-dose anticoagulation and were deemed eligible for longer-term anticoagulation, it is likely that any who experienced intolerable AUB in the early months of treatment were either successfully treated for it or were excluded from these studies.

This dramatic increase in rates of AUB when including broader definitions, such as menorrhagia, in addition to uterine MB or CRNMB events, is also a likely explanation for the substantially

Table 3. AUB rates in pediatric studies of rivaroxaban for treatment of VTE

Reference (duration)	Study design/population	Study drug/comparator	Number of girls, ages 6-17 y*	Menorrhagia, n (%)
EINSTEIN-Jr phase II[11] (30 d)	Single-arm study of children with VTE initially treated with LMWH, VKA, or fondaparinux	Rivaroxaban (age and weight based, therapeutic dosing)	19	3 of 19 (15.8%)
EINSTEIN-Jr phase III[12] (3 mo)	Randomized (2:1), open-label, parallel group trial in children with acute VTE grouped by age	Rivaroxaban (age and weight based, therapeutic dosing)	121	23 of 121 (19.0%)
		Standard anticoagulants (heparin, LMWH, and/or VKA)	70	5 of 70 (7.1%)

*Only girls from 6- to 17-year-old age groups are reported here; younger girls are excluded because they would be premenarchal.
†No uterine MB or CRNMB in pediatric studies occurred; all bleeding was menorrhagia classified as grade 1-2 (minor bleeding).

higher rates of AUB reported in the pediatric studies than in adult studies. An overall higher prevalence of AUB in adolescents due to anovulatory cycles likely also played a role.

Limitations of our review include, as discussed, inconsistent definitions used between studies (uterine MB and CRNMB only vs inclusion of menorrhagia). We were also unable to clearly define denominators of the population at risk (menstruating women), and therefore rates of AUB are, in general, underestimates. Our findings are consistent with those of Godin et al,[3] suggesting increased rates of AUB with rivaroxaban vs apixaban. An RCT specifically assessing menstrual blood loss in patients receiving apixaban vs rivaroxaban (RAMBLE; NCT02761044) is currently enrolling patients, and 2 additional cohort studies of AUB in women receiving DOACs (NCT4477837, NCT03772366) will be enrolling patients soon. We hope that the results of these trials will provide more conclusive data. We strongly urge investigators of future RCTs of anticoagulants to evaluate broader definitions of AUB in addition to standard MB and CRNMB criteria to avoid an ongoing underestimation of the significance of this problem.

Graded recommendations

1. In menstruating women with an indication for oral anticoagulant therapy, we recommend taking a careful menstrual history both before and after initiation of anticoagulation (strong recommendation, very low certainty in the evidence of effects).
2. In menstruating women who are experiencing ongoing consequences of AUB, we recommend apixaban over rivaroxaban as first-line oral anticoagulant therapy for VTE (strong recommendation, moderate certainty of the evidence about effects).
3. In menstruating women without a history of AUB or with a successful, ongoing management strategy for AUB, we suggest apixaban over rivaroxaban as first-line oral anticoagulant therapy for VTE (conditional recommendation, moderate certainty of the evidence about effects).

Conflict-of-interest disclosure

The authors declare no competing financial interests.

Off-label drug use

None disclosed.

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Neuropathic pain in sickle cell disease: measurement and management

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The identification of chronic pain and neuropathic pain as common contributors to the overall pain experience of patients with sickle cell disease (SCD) has altered the way we should evaluate difficult-to-treat pain. The recognition of these 2 entities is not generally routine among various medical specialties and provider levels that treat SCD. Due to the relative recency with which neuropathic pain was first described in SCD, validated assessment tools and evidence-based treatments remain lacking. Although clinical assessment and judgment must continue to inform all decision making in this understudied area of SCD pain management, a number of validated neuropathic pain assessment tools exist that can make possible a standardized evaluation process. Similarly, investigation of available neuropathic pain treatments for the uniquely complex pain phenotypes of SCD has only just begun and is better established in pain conditions other than SCD. The aim of this review is to briefly summarize the proposed basic pathophysiology, assessment, and treatment of neuropathic pain in patients with SCD. Furthermore, the aim of this review is to encourage an expanded framework for the assessment and treatment of SCD pain that appreciates the hidden complexities of this common complication of SCD.

LEARNING OBJECTIVES

- Recognize the complex nature of sickle cell pain
- Understand the proposed basic pathophysiology of neuropathic pain in sickle cell disease
- Recognize that although robust, disease-specific evidence does not exist for all neuropathic pain assessment tools and treatments, a nuanced approach to pain management can be considered

Clinical case

A 25-year-old woman with hemoglobin SS disease presents to the emergency department with low back and leg pain refractory to repeated dosing of her home oxycodone. She rates her pain as 10/10 and is very fatigued and anxious due to its persistence. She states she has missed work for 2 separate weeklong hospital admissions recently and may lose her job. She is given IV doses of morphine and ketorolac, and after minimal relief, she is started on a morphine patient-controlled anesthesia pumps and admitted to the hospital. Over the next 8 days, there is little improvement, and she continues to have frequent demands on the patient-controlled anesthesia despite 3 escalations in the continuous morphine rate. The findings of magnetic resonance imaging of her spine, hips, and upper legs are unremarkable.

The evolution of sickle cell disease pain

An outdated model

Until relatively recently, the pain of sickle cell disease (SCD) was classified as only acute in nature and was subdivided into vaso-occlusive crisis (VOC) or noncrisis pain on the basis of severity and health care use.[1] This oversimplification was in large part due to the information bias inevitable when providers only see patients whose symptoms are intolerable enough to necessitate urgent or emergent care in a health care setting. In addition, in the not so distant past, the lifespan of these patients was so greatly reduced relative to the general population that there was not sufficient time to develop chronic complications, a reality that has improved with modern preventive care. We now have evidence that almost one-third of adults with SCD experience pain on 95% of the days of their lives, despite seeking treatment in the hospital on <5% of the days.[2] Thus, it is very evident that SCD pain encompasses both acute and chronic components.

Old complexities, newly defined

The accepted sickle cell pain model has grown significantly in complexity in the last decade and now includes

hematological, neurological, immunological, and psychological contributions.[3,4] In an effort to improve comparisons between studies targeting these various facets, the ACTTION-APS Pain Taxonomy and ACTTION-APS-AAPM Pain Taxonomy initiatives published definitions of acute and chronic SCD pain that take into consideration both the lack of previous evidence-based consensus and the factors that have guided definitions for other pain syndromes.[5,6] The consensus classification is illustrated in Figure 1 on the basis of those publications, with emphasis on an understudied and clinically undertreated subcategory: neuropathic pain (NP). The remainder of this review focuses on the measurement and management of the neuropathic component of SCD pain.

Clinical case continued

On day 9 of hospitalization, the patient requests another increase in the continuous morphine. She reports 10/10 pain in her legs. When the resident asks her to describe it, she says, "It just hurts" and starts to cry, but she later describes it as burning and shooting down her leg. The resident notes that she recoils at the slightest touch and decides to try a dose of ketorolac because it is due soon, then consults psychiatry and social work to investigate for secondary gain.

NP in SCD

The clinical case presented illustrates the complex nature of pain assessment and treatment. The resident's assessment is likely influenced by the negative connotations surrounding pain and opioids, as well as by a lack of consideration of NP as the etiology of the examination findings. Although the contribution of NP to SCD pain has generally become established among most hematologists specializing in SCD, it is undoubtedly less recognized among other care providers who may not be familiar with the nuances of SCD pain management. In fact, general reviews of NP published in the past 3 years do not mention SCD as an etiology of NP.[7,8] The slow recognition of this entity may be due in part to the lack of a clearly demonstrated "lesion or disease of the somatosensory nervous system," as required to meet the strict definition put forth by the International Association for the Study of Pain.[9] The lack of discrete nerve injury or lesion (eg, compression, degeneration, infarction) in the case of SCD does not necessarily preclude a diagnosis of NP, however, as much as it suggests a broader interpretation of which neurologic changes represent a "lesion or disease." As discussed below, the available evidence in SCD includes demonstrated pathologic changes to the nervous system (eg, altered cortical processing networks, altered biochemical structure and function of pain-sensing neurons), as well as patient-reported pain descriptors widely associated with NP, the combination of which seems to meet the aforementioned criteria.[10-15] Whatever the reason, due to its lack of broad recognition and the significant heterogeneity among patients, NP is often overlooked and is potentially an important factor in the labeling of difficult-to-treat patients as drug seeking. In this case, the exaggerated response to light touch suggests nervous system sensitization and should prompt specific questions regarding NP descriptors. NP can contribute to both acute and chronic pain. As Figure 1 illustrates, the complex interplay between acute and chronic pain is such that various acute pain types can contribute to chronic pain development. However, acute pain can also result from exacerbations of chronic pain, making it difficult to parse the causative role of NP in a given patient's pain phenotype. Using quantitative sensory testing (QST), patients have been shown

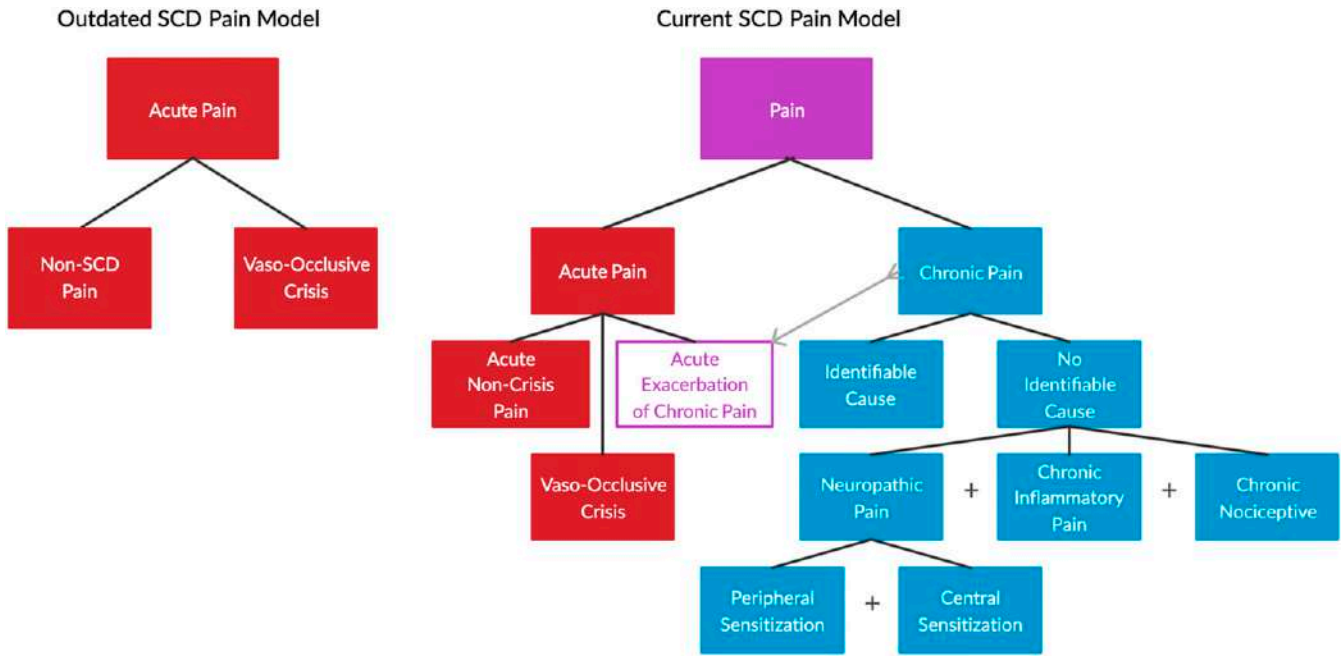

Figure 1. Taxonomy of pain in SCD. The previously simple classification of pain in SCD assumed all pain was episodic and acute in nature. The current model includes the chronic pain that most patients with SCD are now known to experience, with potential contributions from multiple etiologies to varying degrees. Superimposed acute pain episodes can result from the classic vaso-occlusion or from exacerbations of chronic pain. Any one category of pain shown can likely indirectly contribute to the development of any other category, introducing even further complications.

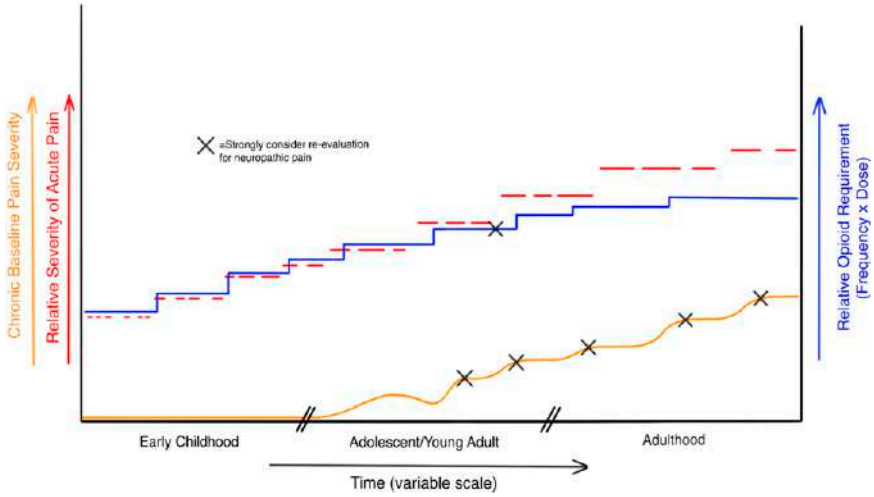

Figure 2. Hypothetical model of SCD pain. Red dashed lines represent acute pain episodes worsening in severity and duration over the lifespan. Blue line represents the required opioid dose to achieve analgesia, which becomes harder and harder to achieve as chronic pain and NP develop over time. Chronic pain can be a result of acute pain episodes and their cumulative damage as well as a cause, because exacerbations of chronic pain/NP may be a trigger for acute pain. Because current assessment tools cannot definitively isolate the contributions of inflammatory pain, nociceptive pain, and NP to overall chronic pain, we suggest formally evaluating for NP when chronic pain shows clear worsening, as well as when pain seems to become refractory to escalating doses of opioid.

to have global hypersensitivity to mechanical stimuli (assessed at nonpainful reference sites) both at baseline and during hospitalization for acute pain.[15,16] Thus, NP should be considered in all settings (eg, acute or chronic pain).

General mechanisms

NP represents a maladaptive response of the somatosensory nervous system to injury, disease, or medications, among other etiologies, whereby a "ramp-up" in sensitivity occurs that results in either pain out of proportion to painful stimuli (hyperalgesia) or pain in response to innocuous stimuli (allodynia).[7] The broadest classification divides NP according to etiology as secondary to either central nervous system (CNS) or peripheral nervous system (PNS) insult. PNS and/or CNS injuries can result in sensitization of either the CNS or PNS, which can in turn cause positive (gain of uncomfortable sensation) or negative (loss of normal function) symptoms.[7,17] There is evidence of both central and peripheral sensitization in patients with SCD.[13,18-20]

In SCD, a likely inciting insult to peripheral nerves is chronic inflammatory and oxidative stress that intensifies during recurrent VOCs.[17] Recurrent stimulation/damage of nociceptive (pain-sensing) neurons results in increased sensitivity and density of various membrane channels (eg, TRPV1) responsible for the initial transduction of pain signals into action potentials. Altered nociceptors fire at lower stimulation thresholds and from ectopic depolarizations along their axons.[7,17,21] Aberrant signaling is then transmitted to dorsal root ganglia (DRG) of the spinal cord, responsible for the synthesis of signals from many localized nociceptors with modulatory inputs from descending pathways. The second-order interneurons originating there, which can be similarly hypersensitized, transmit the resulting signal to the thalamus for further processing and modulation. The importance of DRG hypersensitization is evidenced by studies showing that peripheral amyloid-β fibers normally responsible for light touch signaling will actually trigger the transmission of pain signals to the brain via sensitized DRG interneurons.[21] *N*-methyl-D-aspartate receptors responsible for long-term potentiation of pain signals in the DRG are also believed to be very important to durable central sensitization.[22] Cytokines released by overactive glial and mast cells of the CNS cause neuroinflammation that further contributes to abnormal activity of descending inhibitory pathways and subcortical pain-processing networks, resulting in activation rather than inhibition of interneurons of the DRG.[17,23,24] The contributions of these alterations to SCD pain are just beginning to be understood.

Evidence of NP in SCD

Some of these pathologic changes have been identified in murine studies of SCD, with their clinical effects confirmed in patients with SCD.[17] Clinical evidence for NP in SCD has been found using patient-reported outcome (PRO) measures developed for other NP states, QST, and functional magnetic resonance imaging (fMRI). The reported prevalence of NP in adult patients with SCD ranges from 25% to 40% based on studies using PRO questionnaires, and its presence is significantly associated with older age.[15,25] The association with older age supports the mechanistic explanation centered on the cumulative effects of chronic SCD pathobiology.[15,25,26] Psychophysical testing results have provided further support for the presence of both central and peripheral sensitization, because on the basis of QST, approximately two-thirds of patients with SCD studied were found to have reduced thresholds for pain in response to thermal stimuli, and approximately one-third in response to mechanical stimuli, compared with healthy control subjects.[20,25] One study of patients with SCD compared those with high central sensitization with those with low central sensitization based on QST and found worse pain in the higher-sensitization group, as evidenced by increased VOC frequency, opioid intake,

Table 1. Comparison of NP assessment tools

Modality	Tool	Description	Utility	Limitations
Patient-reported outcome (PRO) (gold standard)	Douleur neuropathique 4 questions	• 10 items • Incorporates physical examination findings* • Sensitivity 83% • Specificity 90%	• Validated for NP as a self-report tool	• Not validated in SCD • Validated for NP secondary to injury or discrete insult
	Identification Pain questionnaire	• 6 items • Sensory descriptors only	• Validated for NP • More useful as a screening tool	• Not validated in SCD • Limited descriptors interrogated
	Leeds Assessment of Neuropathic Symptoms and Signs	• **7 items** • **Incorporates physical examination findings*** • **Sensitivity 82%-91%** • **Specificity 80%-94%**	• **Validated for NP as a self-report tool** • **Studied in SCD** • **Demonstrated sensitivity to treatment effect**	• **Not formally validated in SCD**
	Neuropathic Pain Questionnaire	• 12 items • Incorporates affect-type symptoms • Sensitivity 66% • Specificity 74%	• Validated for NP • Short form with only 3 items performed similarly	• Not validated in SCD • Questionable accuracy
	PainDETECT	• **9 items** • **Incorporates temporal and spatial pain characteristics** • **Sensitivity 85%** • **Specificity 80%**	• **Validated for NP as a self-report tool** • **Scaled answers add nuance vs yes/no answers** • **Studied in SCD**	• **Developed for back pain** • **Not formally validated in SCD**
	PAINReportIt	• **>90 items** • **Assesses pain quality, intensity, location, pattern, treatment response**	• **Validated for NP as a computerized self-report tool** • **Formally validated in SCD[35]** • **Less bias (patient selects descriptors)** • **Some answers scaled rather than yes/no**	• **More time intensive (15-20 min)** • **Language may be difficult for less well-educated patients** • **Cost (commercially available)**
	PROMIS	• More comprehensive than others • Customizable for assessment goal • Assesses many domains, including sensory, affect, quality of life	• Includes NP domain • Comprehensive assessment of pain experience	• Minimal data thus far for SCD and NP • More time intensive
QST	**Manual von Frey filaments for mechanical QST, various digital instruments available for mechanical and thermal QST**	• **Assesses patient response to different stimuli to determine detection and pain thresholds**	• **Potential for future treatment monitoring** • **Differentiation of central from peripheral sensitization** • **Operator training relatively simple**	• **Expensive instrument cost (subsequently low cost to operate)** • **Time intensive** • **No defined diagnostic cutoffs** • **Used only on a research basis**
Imaging	• **MRI** • **fMRI** • **PET** • **EEG**	• **Established chronic pain patterns for each (on a research basis)**	• **Potential as a more objective measure than others**	• **Expensive with every test** • **Subspecialist training required for interpretation/ analyses** • **Patterns identified for chronic pain but not NP** • **Needs further study** • **Not widely available**

Tools used in published studies of SCD are in bold. Sensitivity/specificity numbers are reported on the basis of performance relative to expert clinical diagnosis. None have been formally validated against expert clinical examination in the SCD population.
*Tools incorporating physical examination findings have higher reported sensitivity and specificity than interview-only tools.[36]

catastrophizing, negative mood, and poorer sleep continuity.[27] Additional evidence for central sensitization has been found via fMRI and electroencephalogram studies demonstrating increased activity in pain-processing networks with associated increased pain frequency.[11,19,24] Although they have not been validated as true diagnostic biomarkers, elevations in markers of neuroinflammation known to contribute to nervous system sensitization are present in SCD, including substance P at baseline and with further increases during acute pain.[24,28,29]

Clinical case continued

The patient is eventually discharged with a pain score of 4/10 after noticing slight improvement and after a slow wean of her oxycodone to a slightly higher home dose than she had received previously. After discharge, the shooting pain again worsens, but she continues to take oxycodone most days in order to avoid another hospitalization. She is seen 3 weeks later in the clinic, asking for a better pain management strategy after losing her job.

NP evaluation

Despite extensive research into its pathobiology, there persists for NP a lack of objective measures or specific treatments. This is especially true with regard to SCD, for although the core nervous system changes may be the same as in other conditions, NP is not an entirely new symptom for patients long experiencing recurrent vaso-occlusive pain. Because of this gradual transition in phenotype from nociceptive to neuropathic/mixed-type pain, these patients may have a harder time recognizing and describing unique NP symptoms. The identification of NP therefore becomes even more heavily dependent on active investigation by the provider in response to subtle clues from the history and physical examination. Evidence that <5% of patients take an NP drug despite its much higher demonstrated prevalence suggests this active investigation is not routine.[15,30] Currently, no standardized protocols exist regarding evaluation timing, but we suggest reassessment when there is a clear worsening of pain frequency or an increased opioid dose needed for effective analgesia (Figure 2).

Acute pain encounters such as the one described represent another important opportunity to inquire about NP symptoms. Descriptors such as burning, electric shocks, pins and needles, pricking, shooting, radiating, allodynia (as evident in the presented clinical case), and pain exacerbated by temperature changes should trigger investigation for NP.[26,31,32] The best way to conduct this evaluation is unclear, and currently, no one tool can reliably guide management or measure treatment response. Nonetheless, the measures described in the next subsections can be used to support the diagnosis in concert with clinical indicators and sound judgment based on currently available evidence.

PROs

PRO measures (assessing subjective elements such as pain intensity, character/quality, and interference with daily life) represent the gold standard for pain assessment and cannot ethically be replaced by any single objective measure at this time. As such, new and more specific PROs should be used in conjunction with known biomarkers, imaging, and psychophysical testing to assess and phenotype pain.[33] Several PROs for NP evaluation exist, but none have been developed specifically for NP in SCD. The National Institutes of Health has funded the development of 3 PROs applicable to SCD pain assessment: the Patient-Reported Outcome Measurement Information System, the Adult Sickle Cell Quality of Life Measurement Information System, and the Pediatric Quality of Life Inventory Sickle Cell Disease Module. These PROs evaluate and track changes in pain, function, and quality of life.[33,34] Only the Patient-Reported Outcome Measurement Information System has tools specifically geared for NP, but the performance of these tools in SCD and their ability to differentiate pain states and track NP changes over time requires further evidence.[34] Other PROs that have been used to investigate NP in SCD are briefly summarized in Table 1, but because these measures have thus far been used primarily on a research basis, no algorithms exist to guide timing of their implementation as far as age, frequency, relationship to pain status, or as outcomes evaluating treatment response. Although NP has been demonstrated in pediatric patients, the typical age of onset and risk factors for its development remain unknown.[15,35] There is a clear need to further validate the ability of PROs, both alone and in combination with other assessment tools, to confirm the presence of NP and guide treatment. Furthermore, cutoff scores on NP-specific PROs need to be established for SCD that would trigger more time-/resource-intensive comprehensive testing. Given all of these unknowns, a specific recommendation regarding a universally "best" tool for assessing SCD-related NP cannot be made. On a research basis, all questionnaires listed in Table 1 offer unique benefits. For clinicians choosing from among those PROs previously studied in SCD, however, consideration should be given to the intent of the assessment, cost, test length, comprehensive vs "quick" screen, sensitivity/specificity, and the age of study participants.

QST

QST represents a more direct method of assessing altered pain sensation to multiple types of stimuli, including mechanical (eg, pressure, pinprick, tactile) and thermal (ie, heat, cold), and has been used in attempts to differentiate peripheral from central sensitization. As more treatments for NP become available, it is possible that they will be divided between those targeting central sensitization and those targeting peripheral sensitization. With continued research correlating QST findings with both imaging and PROs, QST could represent a means of tailoring treatment of individual phenotypes and assessing treatment response. This research has begun in patients with SCD, but continued investigation is needed to better differentiate these states with confidence.[25,27,36,37] The psychophysical nature of QST as dependent on patient responses lends to a possible role in supporting PROs as a link between underlying pathophysiology and patient experience. Currently only used on a research basis, QST is time intensive and costly to conduct and is not widely available.

Imaging

Magnetic resonance imaging, fMRI, electroencephalogram, and positron emission tomography have been investigated in the field of NP, but due to the underlying complexity of discovered and yet to be discovered neural networks, a pathognomonic imaging pattern for chronic pain and NP has yet to be described.[26] The abnormalities typical of chronic pain have been identified in patients with SCD,[11,14,19] but although these studies support the

Table 2. Pharmacologic options for NP treatment

Therapy	2015 *Lancet Neurology* meta-analysis of treatments for NP[39,*]	2020 ASH guidelines for SCD chronic pain without identifiable cause	Interventional studies of NP in SCD?	Considerations
Gabapentinoids (gabapentin, pregabalin)	• First line: strong recommendation • Best studied • NNT = 7.2 (gabapentin) • NNT = 7.7 (pregabalin)	• Conditional recommendation based on very low certainty in evidence • Recommendation for adults only	• One completed study (pregabalin): safe, trend toward pain reduction • One ongoing (gabapentin) for acute pain	• Fatigue and dizziness most common side effects • May be intolerable/ trigger workup in SCD
Serotonin and norepinephrine reuptake inhibitors	• First line: strong recommendation • NNT = 6.4	• Conditional recommendation based on very low certainty in evidence • Recommendation for adults only	• None	• Risk of suicidal ideation in children
Tricyclic antidepressants	• First line: strong recommendation • NNT = 3.6	• Conditional recommendation based on very low certainty in evidence • Recommendation for adults only	• None	• Risk of suicidal ideation in children • Imipramine also used for enuresis
Topical patches (lidocaine 5%, capsaicin 8%)	• Second line: weak recommendation • NNT = 10.6 (capsaicin) • Only for peripheral NP	• Not addressed	• Phase 2 single-arm pediatric study (lidocaine for acute pain): well tolerated, evidence for clinical efficacy	
Tramadol	• Second line: weak recommendation • NNT = 4.7 • Least studied of recommended drugs	• Not addressed	• None	
Strong opioids	• Third line: weak recommendation • NNT = 4.3 • Third line due to abuse and adverse effect potential	• Current standard, no robust comparison studies found between chronic opioid and nonopioid treatment	• None for neuropathic pain	• Difficult to replace due to unique interplay between acute and chronic pain, nociceptive pain and NP
Ketamine	• Inconclusive evidence	• Oral ketamine: not addressed • IV ketamine (for refractory acute pain): conditional recommendation based on very low certainty in evidence	• Studied for VOC/ acute pain treatment with demonstrated benefit	• Not mentioned by either resource but used for acute SCD pain and for NP in separate studies
Trifluoperazine	• Not addressed	• Not addressed	• Phase 1 open label study: safe, evidence of efficacy	

NNT, number needed to treat.
*The *Lancet Neurology* meta-analysis was selected for simplicity as being representative of broader NP recommendations from the pain literature (which do not include SCD). American Society of Hematology guidelines for chronic pain are based on indirect evidence from patients with fibromyalgia.

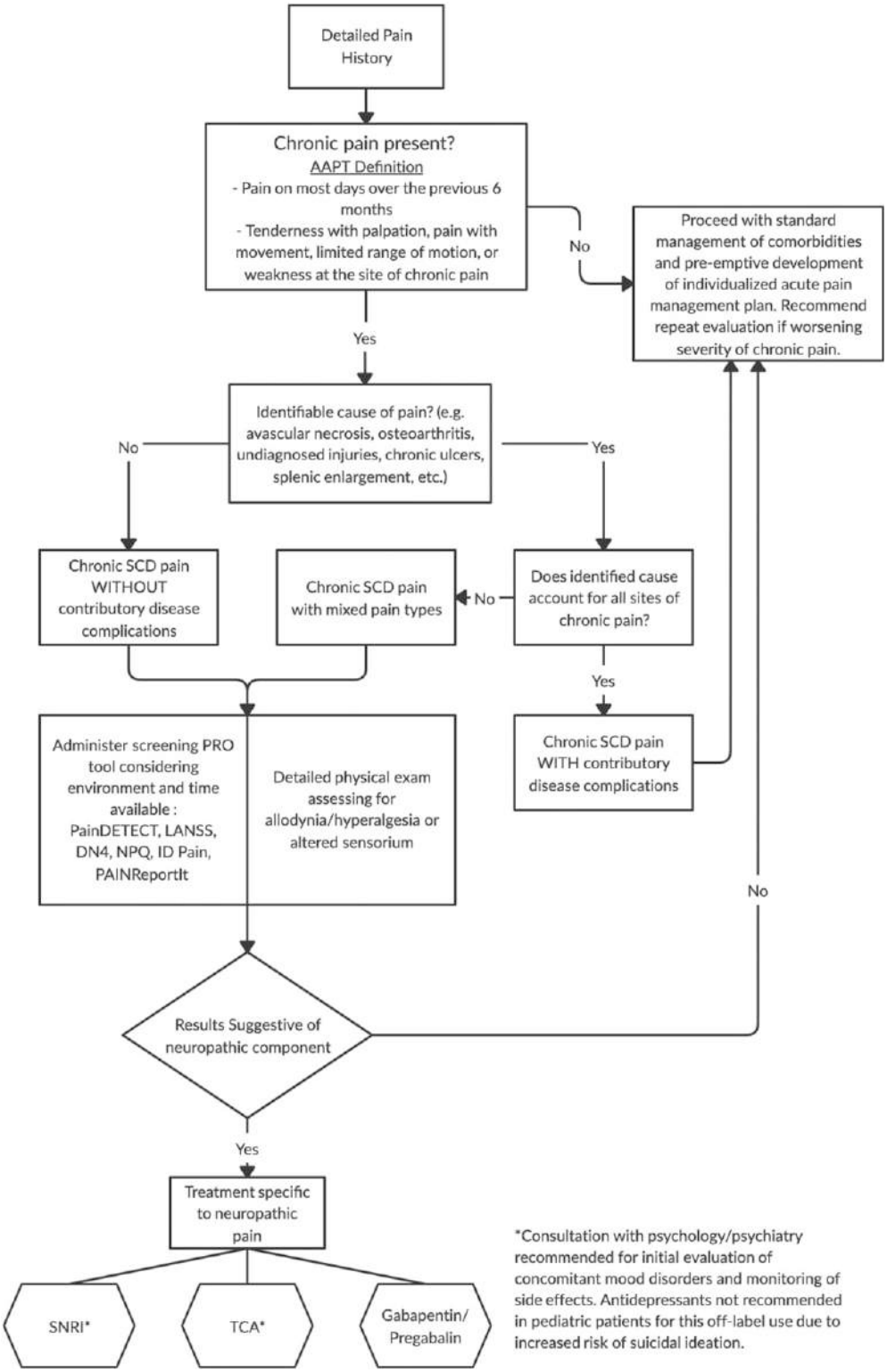

Figure 3. Suggested assessment/management algorithm for NP in SCD based on the best available evidence.

existence of altered central pain-processing mechanisms, similarly to QST, they cannot currently replace patient reports of NP symptoms in the diagnostic process. The majority of centers treating SCD do not have regional access to teams with experience in the identification of pain signatures based on fMRI, but that may change in the years to come as NP becomes more synonymous with SCD and further evidence is uncovered surrounding this modality.

Treatment of NP in SCD

Even in the broader NP literature, there is a lack of strong evidence for any one treatment resulting in significant and consistent improvement. What is widely recommended (and is inconsistent with general practice for SCD), however, is the downgrading of opioids to second- or third-line treatment of NP. The reasons for this recommendation include the likelihood that opioids ineffectively treat NP and the risks of tolerance and significant side effects in the face of escalating doses for chronic pain.[24,38-40] The unique parallel (but not independent) development of NP and nociceptive chronic pain in SCD, wherein NP contributes to chronic pain and chronic nociceptive/inflammatory pain contributes to nervous system sensitization, make completely eliminating opioids from a pain regimen not feasible (Figure 2). Similar to other aspects of NP in SCD, there is a paucity of evidence regarding the utility of other therapies. Thus, the recent American Society of Hematology guidelines for chronic SCD pain without an identifiable cause were informed using indirect evidence from patients with fibromyalgia, deemed the disease state most comparable to SCD chronic pain without an identifiable cause.[41] These recommendations are in line with those for general NP management with some caveats (in SCD, most medications have not been in widespread use, so no longitudinal data exist).[38,42] These medications are listed in Table 2.[26,43] Medication choices must be individualized and influenced by side effect profiles in relation to SCD-related comorbidities. Consultation with psychology/psychiatry should also be considered because chronic pain and NP have been shown to negatively affect neurocognitive/psychosocial functioning and quality of life, respectively.[10,15]

Conclusion

Given the lack of validated assessment tools or well-studied treatments for SCD-related NP, detailed, evidence-based recommendations cannot be made at this time. Currently available evidence of its prevalence and treatment of related conditions, however, are at least sufficient to prevent the passive allowance of suffering in affected patients. To that end, we offer a potential algorithm for evaluation and management (Figure 3) that, with ongoing research, may continue to expand in the near future.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

Off-label drug use

None disclosed.

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Optimizing the management of chronic pain in sickle cell disease

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Chronic pain in sickle cell disease (SCD) refers to pain present on most days lasting over six months. It can start during childhood and the prevalence increases with age. By adulthood, over 55% of patients experience pain on over 50% of days; 29% reporting pain on 95% of days. The true prevalence of chronic pain in SCD is likely underappreciated as it is mostly managed at home. Patients with chronic pain and SCD frequently seek acute care for exacerbation of underlying chronic pain difficult to distinguish from their usual acute vaso-occlusive crises. When treating chronic pain in SCD, the challenge is distinguishing between non-SCD related etiologies versus chronic pain resulting from SCD pathophysiological processes. This distinction is important to delineate as it will drive appropriate management strategies. Chronic pain in SCD has profound consequences for the patient; is often associated with comorbid psychiatric illnesses (depression and anxiety), not dissimilar from other chronic pain syndromes. They may also experience challenges with sleep hygiene, various somatic symptoms, and chronic fatigue that impair quality of life. How best to treat chronic pain in SCD is not definitively established. Both acute and chronic pain in SCD is typically treated with opioids. Emerging data suggests that chronic opioid therapy (COT) is a suboptimal treatment strategy for chronic pain. This review will discuss the complexity of managing chronic pain in SCD; pain that may be dependent or independent of the underlying SCD diagnosis. We will also describe alternative treatment approaches to high-dose COT.

LEARNING OBJECTIVES

- Review common presentations and characterize complex pathophysiology of chronic pain in SCD, recognizing the contribution of non-SCD comorbidities
- Identify limitations of and alternatives to chronic opioid therapy (COT) for chronic pain in SCD and the overlap between opioid toxicity and SCD symptoms

Introduction

Chronic pain in sickle cell disease (SCD) refers to pain that is present on most days and has lasted at least 6 months.[1] It can start as early as childhood, and its prevalence increases with age. By adulthood, more than 55% of patients experience pain on more than half of days, with nearly one third (29%) reporting pain on 95% of days.[2] The true prevalence of chronic pain in SCD is likely underappreciated, because most patients manage their pain at home.[2] Patients with SCD and chronic pain frequently seek acute care for an exacerbation of their underlying chronic pain, which is difficult to distinguish from their usual acute vaso-occlusive crises (VOCs). No laboratory markers exist to help define this distinction. When an individual with SCD experiences chronic pain, the challenge is distinguishing the chronic pain that is from non–SCD-related etiologies from chronic pain that results from the SCD pathophysiologic process itself. Understanding and delineating this distinction is critical to determine appropriate disease management strategies.

Chronic pain in SCD has profound consequences for the patient; it is often associated with comorbid psychiatric illnesses such as depression and anxiety, not dissimilar from other chronic pain syndromes.[1] Patients with SCD and chronic pain may also experience challenges with sleep hygiene, various somatic symptoms, and chronic fatigue that impair quality of life.[1]

How best to treat chronic pain in SCD is not definitively established. Both acute and chronic pain in SCD is typically treated with opioids, but emerging data suggest that chronic opioid therapy (COT) is a suboptimal treatment strategy for chronic pain.[3] This review will discuss the complexity of managing chronic pain in the patient with SCD; pain that may

be dependent or independent of the underlying SCD diagnosis. We will also describe alternative treatment approaches to high-dose COT.

Clinical case 1

A 25-year-old African American woman with hemoglobin (Hb) SS presented to the emergency department (ED) with acute pain. She reports sudden onset of pain in her lower back and thighs, rated 9 of 10 on the numeric pain rating scale. Her pain was not alleviated by her usual home pain regimen of oxycodone 20 mg every 4 to 6 hours as needed. Her outpatient morphine equivalent daily dose (MEDD) is 120 mg, based on prescription fills. Pain was described as throbbing and aching of all joints and thigh muscles that progressed to "pain all over." She reports chills, sweating, and nausea. She is not currently taking any SCD-modifying therapy and does not have a primary adult hematologist. She receives most of her care via the ED. In the past year, she has had significantly high acute care utilization, with 25 ED visits for pain exacerbations, 20% of which resulted in a hospital admission that has lasted 3 days on average.

Her medical history revealed 1 episode of acute chest syndrome at age 13, a laparoscopic cholecystectomy at age 14, and a normal transcranial Doppler ultrasound screen at age 16. She was lost to hematology follow-up after leaving pediatric care at age 19 when she became pregnant. She struggled with depression as a teenager. She scored a 14 on the Patient Health Questionnaire-9 after the birth of her daughter; she was diagnosed with postpartum depression but declined antidepressant medication and psychotherapy. There are no reports of suicidal ideation or suicide attempts. While in pediatric care, she was prescribed hydroxyurea (maximum tolerated dose of 1500 mg daily) for frequent VOCs with moderate-to-low adherence. Her Hb F peaked at 8%.

In the ED, her vital signs revealed a temperature of 37.3°C, pulse of 110 beats/min, respiratory rate of 12 breaths/min, blood pressure of 129/76 mm Hg, and oxygen saturation of 99% on room air. Physical examination revealed no focal findings, with normal range of motion in all joints. Her hemoglobin level was 8.9 g/dL (at baseline), mean corpuscular volume (MCV) was 83 fL, reticulocyte fraction was 15%, platelet count was 690 000/mm^3, and white cell count was 16 800/mm^3. Serum electrolytes and kidney function were normal for her age. Chest radiograph showed no infiltrates.

She received intravenous (IV) hydration with NaCl 0.5% at 125 mL/h, 1 dose of IV ketorolac 30 mg, and 3 doses of IV hydromorphone 2 mg every 30 to 60 minutes. She was subsequently admitted for ongoing management of presumed acute VOC, and the hematology team was consulted.

Clinical case 2

A 40-year-old African American man with Hb SC presented for a follow-up hematology visit. He has a 10-year history of daily chronic pain in his low back, knees, and hips. He describes his pain as tingling, aching, and throbbing, with occasional bouts of sharp radiating pain down both legs. His pain intensity fluctuates but at baseline is a 6 of 10 on the numeric pain rating scale and is worst in the mornings. The pain is exacerbated by physical activity, partially relieved by rest, and marginally improved by taking opioid analgesics. Lately, he has had frequent exacerbations of his chronic pain to an intensity of 8 of 10. He presented to the ED 3 times in the last 2 months with acute pain because he ran out of his pain medication early. He reports intermittent swelling of his hands and feet, morning stiffness in all joints, chronic fatigue, feelings of sadness, and difficulty sleeping. He worked in construction but went on disability 6 years ago because of chronic pain. His prescribed home pain regimen includes extended-release morphine sulfate 60 mg 3 times daily and immediate-release morphine sulfate 30 mg up to 6 doses daily. However, he reports taking additional breakthrough doses of his immediate release morphine for pain exacerbations at home; 8 or more doses a day. His daily prescribed outpatient MEDD is 360 mg with no current SCD-modifying therapy.

His medical history includes bilateral hip avascular necrosis (AVN), exaggerated lumbar lordosis, and chronic constipation, with a bowel movement every 3 days.

His vital signs revealed a temperature of 37.6°C, pulse of 69 beats/min, respiratory rate of 10 breaths/min, blood pressure of 139/71 mm Hg, and oxygen saturation of 94% on room air. His weight is 265 lbs, height is 5 ft, 11 inches, and body mass index is 37 kg/m^2, which is considered morbidly obese. He has bilateral swelling of knees, feet, and hands. Range of motion evoked pain in all joints. His abdomen is protuberant with soft mobile fecalith masses palpated in his left lower abdomen. Hemoglobin level was 9.0 g/dL (at baseline), MCV was 63 fL, reticulocyte fraction was 4.5%, platelet count was 656 000/mm^3, and white cell count was 6100/mm^3. Serum electrolyte levels and kidney function were normal for his age. Serum 25-hydroxyvitamin D level was 7 ng/mL. The patient would like to discuss increasing the number of immediate-release morphine sulfate doses prescribed per month so that he can avoid going to the ED.

Causes of chronic pain in SCD

Chronic pain in SCD is a debilitating complication that is associated with increased morbidity and mortality. Pain may be present in 1 or more locations, with or without contributing disease complications such as leg ulcers, AVN, bone infarcts, vertebral body collapse, or any combination thereof.[1] SCD complications accumulate over a lifespan; therefore, individuals have an increasing burden of chronic pain with age. Multiple underlying causes of pain can contribute to the evolution of chronic pain in SCD, including repeated acute nociceptive pain from VOCs, inflammatory pain, neuropathic pain, and opioid-induced hyperalgesia (OIH) as depicted in Figure 1, all of which lead to central sensitization (CS).[4-7] CS is a condition of hypersensitivity to pain that is associated with both allodynia and hyperalgesia. Allodynia occurs when an individual experiences pain from stimuli that do not normally cause pain. Hyperalgesia occurs when an individual experiences an enhanced sensitivity to a familiar painful trigger.

Chronic exposure to high doses of opioids may contribute to chronic pain in SCD from either OIH and/or cyclic opioid withdrawal. Although poorly described in the literature, cyclic opioid withdrawal refers to a pattern of repeated cycles of pain exacerbations along with symptoms of opioid withdrawal. This typically occurs when high doses and/or chronic opioid therapy are abruptly discontinued or when the dose is precipitously decreased after treatment of presumed VOC pain. Effective reductions in plasma opioid levels that can precipitate withdrawal symptoms may occur when there is a change in the administration route, which can lead to a change in the relative potency of the drug; for example, the oral dosing of morphine

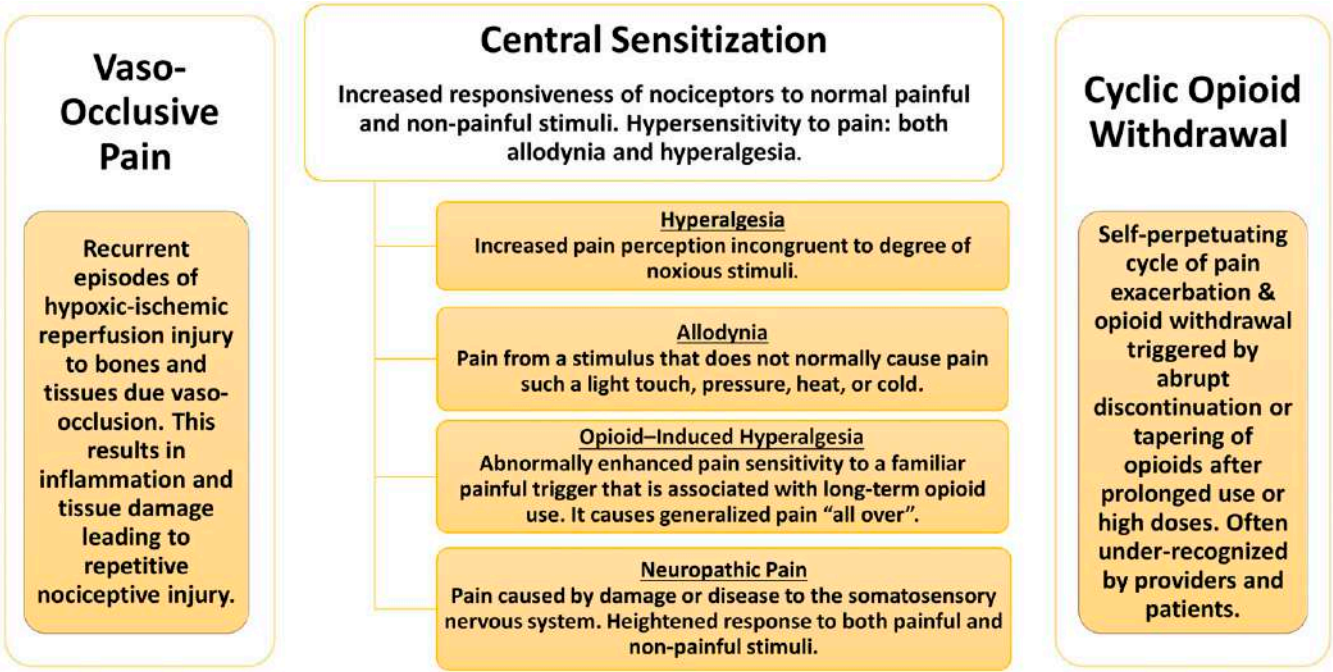


Figure 1. Distinction between 3 different types of pain experienced by patients with SCD.

sulfate needs to be 3 times higher than the parenteral dosing to maintain the same potency. It can also occur when there is a change in opioid potency, which can occur when switching between 2 opioid drugs.

The patient's clinical course often reveals a gradual increase in multifocal bone, joint, and muscle pain or aching associated with the classic symptoms of opioid withdrawal such as low-grade fevers, chills, nausea, vomiting, diarrhea, abdominal cramps, and depressed mood.[8] The pain is often relieved with oral or parenteral opioids, and symptoms of severe acute pain recur once the patient is no longer receiving opioids (Figure 2). Patients with SCD receiving opioids may not be asked about symptoms of withdrawal when they present with worsening pain, and constitutional symptoms that may indicate withdrawal may often be attributed to their usual VOC. As a result, opioid withdrawal in SCD may be grossly underdiagnosed. Failure to treat opioid withdrawal promptly with opioids, via either the oral or parenteral route, and supportive care may lead to dehydration and autonomic distress, resulting in hospitalization for severe pain.

Patients with SCD may also experience a myriad of painful conditions that are unrelated to SCD. Therefore, clinicians should keep an open mind when developing a differential diagnosis for chronic pain in SCD and not assume that all pain in a person with SCD is related to their SCD. Indeed, it may be difficult to discern the true cause of chronic pain because the symptoms of many painful conditions overlap with those of SCD.

Autoimmune diseases and chronic pain

Diagnoses of autoimmune disorders in patients with SCD are often delayed or missed because the symptoms of SCD are similar to those of many autoimmune disorders. Chronic musculoskeletal complications, like AVN, compression spine deformities, and leg ulcers, are not uncommon in SCD. Thus, clinicians may overlook the possibility of a coexisting autoimmune disorder such as rheumatoid arthritis (RA) in patients with chronic pain in SCD.[9,10] Although not well documented in the literature, comorbid autoimmune disorders observed in patients with SCD include RA,[11] autoimmune hepatitis,[12] Crohn's disease,[13] myasthenia gravis,[14] and juvenile idiopathic arthritis.[15]

Clinicians should inquire about a family history of autoimmune disorder and document symptoms that may be suggestive of one, such as alopecia, generalized fatigue, rashes, and pain in multiple joints or involving both large and small joints. If symptoms are suggestive, screening for laboratory markers of

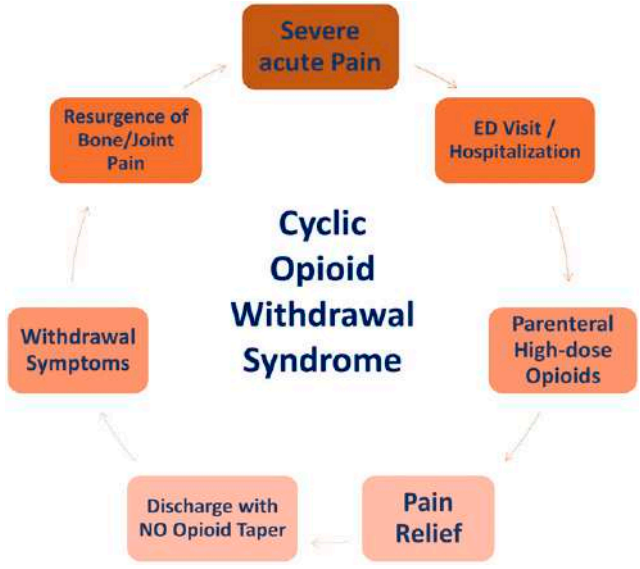


Figure 2. Cycle of opioid withdrawal syndrome in SCD.

Figure 3. Multidisciplinary team approach to optimizing the management of chronic pain in SCD.

autoimmunity, such as antinuclear antibody titers, C-reactive protein, and erythrocyte sedimentation rates, will help guide appropriate rheumatology referral. It is important to note that both erythrocyte sedimentation rate and C-reactive protein may both be elevated in SCD, because they are nonspecific markers of inflammation, and SCD is a chronic inflammatory state. However, if abnormal at baseline, an upward trend may be more indicative of a surrogate marker of rheumatologic disease. Treatment of autoimmune disorders in SCD requires careful orchestration between rheumatology and hematology, because many immunomodulatory agents like steroids and other disease-modifying antirheumatic drugs can induce severe SCD complications such as VOCs or cause cytopenias.[16] Therefore, it is important to have a coordinated multidisciplinary team approach to managing patients with both chronic pain in SCD and a comorbid autoimmune disorder.

Neuropathic pain

Neuropathic pain is an increasingly recognized subtype of chronic pain in SCD. This is pain caused by a lesion, medication, or disease of the somatosensory system.[17] The somatosensory nervous system allows for the perception of sensations such as touch, temperature, pain, pressure, position, movement, and vibration.[18] Individuals with neuropathic pain experience a heightened response to painful and typically nonpainful stimuli - touch, heat, and cold, for example. Patients commonly report sensations of burning, tingling, pins and needles, and numbness.

The lack of a standardized approach for diagnosing neuropathic pain has resulted in unreliable estimates of its prevalence in the general population and among patients with SCD. The prevalence of neuropathic pain in SCD is estimated to be 25% to 40%.[19-22] A number of validated screening tools in the form of patient–reported questionnaires can now be used to support a neuropathic pain diagnosis.[21,23-25]

Because neuropathic pain in SCD is under-recognized, it is also undertreated. Very few patients with SCD are reported as taking neuropathic medications for their chronic pain.[20] Examples of medications that have strong supporting evidence as first-line treatment of neuropathic and other pain include tricyclic antidepressants, serotonin noradrenaline reuptake inhibitors, selective serotonin reuptake inhibitors, and gabapentinoids such as gabapentin and pregabalin.[26]

A recent systematic review by Asnani et al[27] found that just 1 randomized neuropathic drug clinical trial has been conducted in patients with SCD with only 22 subjects.[28] More trials are needed to support the efficacy of neuropathic medication in treating chronic pain in SCD.

Psychiatric disorders and chronic pain

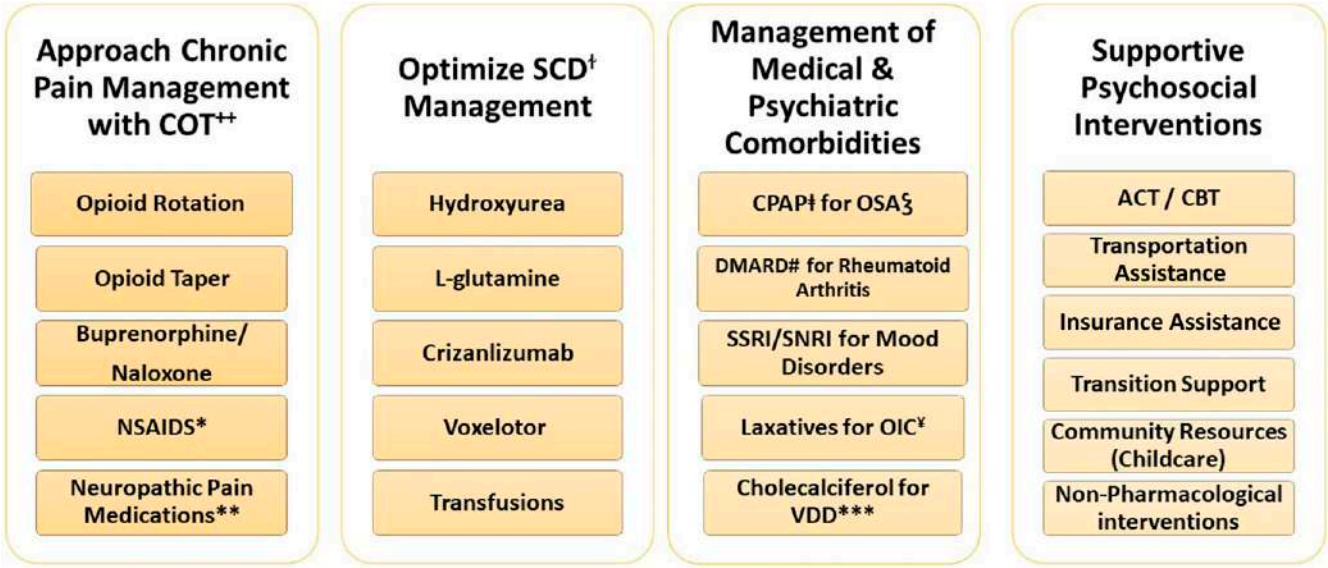
Comorbid psychiatric disorders (mood disorders, anxiety, pain catastrophizing, posttraumatic stress disorder, and substance use disorder) are documented in patients with SCD and influence all aspects of the patient experience.[29,30] Mood disorders range in spectrum from dysthymia and depressed mood to major depressive episodes and major depressive disorder.

One study reported that more than 35% of adults with SCD had depression,[31] a much higher incidence rate than the general population. Among children with SCD, chronic or recurrent pain increases the risk of depression.[32] Parent catastrophizing may influence the pain experience of children with SCD[33] and significantly contribute to their depressive symptoms.[34,35] These studies highlight the bidirectional link between mental illness and pain, with both conditions mutually reinforcing one another.

Opioid therapy has long been associated with depressed mood in persons with chronic noncancer pain. An increasing body of literature suggests that longer duration of opioid therapy of greater than 90 days is associated with a higher incidence of newly reported depressed mood and depressive disorders.[36] Similarly, the chronic use of opioids may adversely impact the psychological health of patients with SCD. A recent descriptive study showed that SCD patients on COT reported depression more often than those not on COT[5] and self-reported long-acting opioid use in SCD varied as a function of daily negative affect.[37] To effectively address chronic pain in SCD, we must also address the mental health of the patient. The American Society of Hematology recently published the 2020 guidelines for sickle cell disease management of acute and chronic pain.[38] They suggest that given the high prevalence of psychologic comorbidities that often coexist in the context of pain, routinely screening for depression and anxiety, and targeted screening for other psychologic comorbidities is considered good clinical practice.

COT

Opioids are central to the management of acute SCD pain, and strong evidence supports their use in this setting.[39] However, opioids are also used in the management of chronic pain in SCD, despite the lack of evidence supporting their use in treating chronic noncancer pain.[40,41] The perceived benefits of COT are often negated by its myriad adverse effects such as CS,[42] poor oral health,[43] opioid-induced constipation, somnolence, sleep disturbance, cognitive dysfunction, depression, endocrinopathy, tolerance, physiologic opioid dependence, hyperalgesia, and increased overdose- and cardiovascular-associated mortality.[44] CS is often mistaken for increased opioid tolerance,



++ Chronic Opioid Therapy; * Nonsteroidal anti-inflammatory drugs; ** Neuropathic Pain Medication e.g. Gabapentinoids, Tricyclic antidepressants, SSRIs and SNRIs; ǂ sickle cell disease, ǂ continuous positive airway pressure; ʒ obstructive sleep apnea; # disease modifying autoimmune drug; ¥ opioid induced constipation; ** *Vitamin D deficiency

Figure 4. Management approach to chronic pain in SCD.

causing providers to further escalate opioid dosages in an attempt to ameliorate symptoms. In a study by Karafin et al,[45] SCD patients on high doses of opioids had lower health-related quality of life than those on lower doses. Furthermore, patients with chronic pain in SCD on COT had higher pain scores than those who were not on COT.[5] Therefore, it is critically important that we explore alternatives to opioids for the management of chronic pain SCD.

Management decisions for case 1: addressing cyclic opioid withdrawal and comorbid mental illness in a young adult with SCD

Initially, this patient's treatment strategy centered on engaging psychosocial and crisis intervention services. Several barriers to care were identified, including financial difficulty, housing insecurity, transportation challenges, intimate partner violence, and childcare difficulties. Addressing these social determinants became the primary focus of her initial care plan. An extensive psychologic assessment led to a diagnosis of depression, anxiety, and moderate physiologic opioid dependence. Her social worker provided solution-focused therapy and individualized support to encourage her to start an selective serotonin reuptake inhibitor for depression.

The patient was using a standardized opioid risk assessment tool, the patient was identified as being high risk for adverse opioid-related outcomes.[46] Without a coordinated multidisciplinary approach to overall disease management, many patients with SCD are inadvertently prescribed opioid doses that far exceed the safety threshold of 90 MEDD recommended by the Centers for Disease Control and Prevention in 2016.[47,48] The potential analgesic benefits of a higher MEDD must be carefully weighed against opioid-related toxicity. If pain is not relieved by increasing opioid doses, clinicians should consider clinical explanations such as tolerance, OIH, poorly managed SCD, an unrecognized psychiatric comorbidity, a non–SCD pain–related syndrome, or any combination thereof. The treatment of each of these conditions diverge; higher opioid doses with or without opioid rotation could address physiologic tolerance, whereas lower opioid doses could ameliorate OIH. Cyclic opioid withdrawal should also be considered as a plausible reason for more frequent acute care utilization. Clinicians should be vigilant for signs of withdrawal such as yawning, piloerection, rhinorrhea, increased lacrimation, and diarrhea. Performing an objective assessment of withdrawal using a practice-preferred withdrawal scale, such as the Clinical Opiate Withdrawal Scale (COWS)[49]or the Clinical Institute Narcotic Assessment may be beneficial.

At the patient's most recent ED presentation, an opiate withdrawal assessment was performed using the COWS. Her score of 18 was indicative of moderate opioid withdrawal. This score, her medical history, and her presenting symptoms led to a diagnosis of physiologic opioid dependence and cyclic opioid withdrawal. Transitioning this patient from as-needed oxycodone and frequent IV opioids to sublingual buprenorphine/naloxone may control her symptoms of cyclic opioid withdrawal and physiologic opioid dependence while providing a safer alternative for pain relief. Several buprenorphine preparations are approved for the treatment of acute and chronic pain. Buprenorphine/naloxone is approved for the treatment of opioid use disorder; however, it is increasingly prescribed off-label for chronic pain.[50] We used motivational interviewing techniques and shared decision making to transition our patient from daily short acting opioid therapy to buprenorphine/naloxone. Under the guidance of an experienced provider, our patient received her first sublingual dose of buprenorphine/naloxone during an ambulatory clinic visit after overnight abstinence from oxycodone. Her COWS score at induction showed evidence of mild-to-moderate withdrawal, and her induction course followed a modified version of the treatment improvement

protocol for buprenorphine induction that was previously reported by McNicholas et al.[51]

After transitioning to buprenorphine/naloxone, her acute care utilization dropped dramatically, adherence to hydroxyurea improved, and Hb F increased from 8% at baseline to 42% after 12 months. Remarkably, the patient had no ED visits in those 12 months. With ongoing psychosocial support from social work and community liaisons, her Patient Health Questionnaire-9 score gradually improved, and adherence to scheduled outpatient appointments increased by 75%.

Management decisions for case 2: addressing RA, depression, obstructive sleep apnea, vitamin D deficiency, and opioid-induced constipation

Early morning joint stiffness and swelling experienced by this patient were suggestive of an autoimmune disorder. Imaging showed early erosive changes and osteopenia involving radius and ulna with carpal crowding. Laboratory markers that were indicative of an autoimmune disorder include an antinuclear antibody titer of 1:1280 with a speckled pattern, rheumatoid factor positive, C-reactive protein level of 5.5 mg/L, erythrocyte sedimentation rate of 89 mm/h, and anti–cyclic citrullinated peptide antibody titer of 69 U/mL. His low hemoglobin and MCV and elevated platelet count were consistent with anemia of chronic disease superimposed with preexisting hemoglobinopathy. Anemia of chronic disease is common in autoimmune disorders. He was referred to rheumatology and diagnosed with seropositive RA.

A comprehensive psychosocial assessment revealed feelings of hopelessness from multiple comorbid diagnoses. His treatment plan consisted of supportive cognitive behavior therapy, acceptance commitment therapy, and the serotonin noradrenaline reuptake inhibitor duloxetine that also treats neuropathic pain. A physiatry assessment recommended physical therapy to improve joint mobility and function. Vitamin D deficiency (VDD) has been associated with chronic pain in SCD.[52] VDD often presents as pain in back, bones, and muscles, low bone density, fatigue, and depressed mood. These symptoms confound the myriad of musculoskeletal and constitutional symptoms that patients with chronic pain in SCD often experience.[53] His severe VDD was treated per the American Association of Clinical Endocrinologists guidelines,[54] with oral vitamin D_3 50 000 IU weekly for 6 weeks. He was subsequently maintained on 5000 IU oral vitamin D_3 daily.

An Epworth Sleepiness Scale questionnaire was administered to evaluate the patient's history of nonrestorative sleep. His score of 12 prompted referral to a sleep specialist, and he was diagnosed with obstructive sleep apnea (OSA). Chronic pain poses limitations on physical activity that can lead to weight gain and worsening OSA symptoms. COT may also contribute to sleep-disordered breathing via suppression of respiratory and sleep centers.[44] His treatment plan for OSA included the use of a continuous positive airway pressure machine at night and dietary modifications to support weight loss. Sleep disorders are common among patients with SCD.[53] They are associated with increased risk of cardiovascular comorbidities such as higher mean systolic blood pressure, impaired left ventricular diastolic dysfunction, and shorter time to first stroke.[53] The combination of nocturnal hypoxemia, increased oxidative stress, increased proinflammatory cytokines, and endothelial dysfunction seen when a patient has both SCD and OSA may potentiate the harmful clinical effects, including increased frequency of pain exacerbations and the associated chronic end-organ damage of SCD.[55]

The hematology and rheumatology care teams discussed optimal choice of disease-modifying therapy for this patient. Autoimmune disorders may cause pain from the destructive inflammation of multiple joints; therefore, definitive therapy is indicated. Patients with seropositive RA may experience more aggressive pain symptoms and more severe damage to affected joints than with seronegative RA. In addition, the proinflammatory state and ischemic changes from VOCs can exacerbate the bone erosions and cartilage damage associated with RA.[56] OSA is often associated with nighttime hypoxia. This may trigger increased sickling and further exacerbate inflammatory pain and VOC; therefore, the underlying SCD must also be meticulously managed. When choosing therapy for concomitant autoimmune disorders in SCD, clinicians must carefully consider the risks of steroid-induced SCD pain exacerbations, and steroid-induced progression of AVN. Caution is warranted with the use of methotrexate and sulfasalazine, because these medications have potential hematologic toxicities.[56] The decision was made to treat with a short course of corticosteroids and anti–tumor necrosis factor biologic therapy.

The patient was started on L-glutamine to mitigate ongoing ischemia-mediated VOC from SCD. In a phase 3 trial, oral L-glutamine twice daily was shown to reduce the incidence of pain crises requiring parenteral analgesia.[57] L-Glutamine improves nitric oxide bioavailability and mitigates oxidative stress, which could potentially reduce the oxidative damage from RA.[56]

After initiating RA therapy and SCD-directed therapy with L-glutamine, the patient reported significant improvement in RA-specific symptoms - joint pain, swelling, and stiffness, and successfully tapered his opioids to <90 MME/day within 6 months with and experienced improved bowel motility. He continues to report mild neuropathic pain symptoms that are tolerable.

Discussion

Our cases highlight important concepts for the optimal management of chronic pain in SCD.

- A multidisciplinary team approach that addresses the medical and psychosocial drivers of pain is required to adequately treat chronic pain in SCD. The team must include a hematologist and the appropriate subspecialist but, critically, must also include psychosocial and mental health providers, such as a social worker, behavioral health specialist, case manager, and community-based health worker or advocate (Figure 3). This ensures a holistic patient-centered approach is used that adequately addresses social determinants of health in addition to the medical complications that contribute to persistent pain.
- Clinicians should keep an open mind when treating chronic pain in SCD and consider a broad differential diagnosis (Figure 4). For each patient, the pathologic basis for chronic pain can evolve; therefore, periodic re-evaluation is warranted, and treatment recommendations should be revised accordingly.
- SCD-modifying therapies should be optimized for each patient. The patient in case 1 was treated with hydroxyurea; the patient in case 2 with L-glutamine. Other SCD treatment options for overall disease management should also be

considered including chronic hyper-transfusion therapy and the newer agents (crizanlizumab, voxelotor); however, there are limited data available on their efficacy for chronic pain treatment. When appropriate, clinicians should also inform patients of available clinical trials.

- We recommend a treatment strategy that tracks MEDD and focuses on harm reduction by transitioning from high-risk opioids to safer options when appropriate. The use of buprenorphine/naloxone is worth considering for patients with SCD on COT who have a high opioid risk score, are experiencing cyclic opioid withdrawal, or meet criteria for physiologic opioid dependence.[58] Large studies are needed to further support the use of buprenorphine products for chronic pain in SCD.

Conflict-of-interest disclosure

I.O. has consulted for Novartis, Pfizer, Cyclerion, Forma Therapeutics, Acceleron Pharma, MagellanRx; been on the speakers' bureau for Novartis, Terumo, and Global Blood Therapeutics; been on the advisory board for Novartis, Pfizer, Acceleron, Cyclerion, and Global Blood Therapeutics; received grants from Health Resources and Services Administration, Patient Centered Outcomes Research Institute, NC State Dept. of Health and Human Services, and Data and Safety Monitoring Boards; has membership for Micella Biopharma ASH Guidelines Panel and the National Academies of Sciences, Engineering, and Medicine SCD study committee; and is the *Hematology News* board editor-in-chief. E.S. is on the advisory board for FORMA therapeutics and has received Health Resources and Services Administration funding. H.F.O. declares no competing financial interests.

Off-label drug use

None disclosed.

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Next-generation cell therapies: the emerging role of CAR-NK cells

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T cells engineered with chimeric antigen receptors (CARs) have revolutionized the field of cell therapy and changed the paradigm of treatment for many patients with relapsed or refractory B-cell malignancies. Despite this progress, there are limitations to CAR-T cell therapy in both the autologous and allogeneic settings, including practical, logistical, and toxicity issues. Given these concerns, there is a rapidly growing interest in natural killer cells as alternative vehicles for CAR engineering, given their unique biological features and their established safety profile in the allogeneic setting. Other immune effector cells, such as invariant natural killer T cells, γδ T cells, and macrophages, are attracting interest as well and eventually may be added to the repertoire of engineered cell therapies against cancer. The pace of these developments will undoubtedly benefit from multiple innovative technologies, such as the CRISPR-Cas gene editing system, which offers great potential to enhance the natural ability of immune effector cells to eliminate refractory cancers.

LEARNING OBJECTIVES

- CAR-T cell therapy has revolutionized the field of cell therapy but has limitations
- Natural killer cells and other immune effectors with unique biological features offer advantages for CAR-based cell therapies
- CRISPR-Cas9 gene editing offers new opportunities to increase the safety and efficacy of cell therapies

Clinical case

A 46-year-old woman with no previous medical problems presented to her primary care physician with complaints of neck swelling and pressure in her throat. She denied any history of fever, night sweats, or weight loss. On physical examination she was noted to have palpable lymph nodes in the neck and inguinal areas. Computed tomography scanning of the neck, chest, abdomen, and pelvis showed diffuse lymphadenopathy above and below the diaphragm. Laboratory values revealed a hemoglobin of 11 g/dL and a lactate dehydrogenase of 431 U/L. Excisional biopsy of a left inguinal lymph node and a bone marrow biopsy confirmed the diagnosis of grade 3, stage IV follicular lymphoma with bone marrow involvement. The Follicular Lymphoma International Prognostic Index score was 4, indicating high-risk disease. After receiving 6 cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy, the patient achieved a complete remission. Four years later, she relapsed and was treated with multiple lines of therapy, including rituximab, obinutuzumab plus bendamustine, and rituximab, gemcitabine, and oxaliplatin. The treatments were ineffective, and the disease became refractory, with the patient entering a leukemic phase with leukocytosis (white blood cells >200 × 10^3/μL with 90% lymphocytes). A positron emission tomography–computed tomography scan showed increased fluorodeoxyglucose uptake (up to a standardized uptake value of 14) in multiple lymph nodes above and below the diaphragm, with bulky abdominal lymphadenopathy. Biopsy of an inguinal lymph node showed follicular lymphoma grade 2 (90%) and grade 3A (10%). Bone marrow biopsy revealed extensive involvement with follicular lymphoma, and flow cytometry showed an aberrant λ-restricted B-cell population positive for CD19, CD20, CD22, CD38 dim, and CD10 dim and negative for CD5, CD43, and CD200. The patient was treated with hyperfractionated cyclophosphamide plus dexamethasone and achieved a partial response, although persistent bulky abdominal lymph nodes were still apparent.

CAR-T cell therapy: advantages and limitations

T cells modified to express a chimeric antigen receptor (CAR) represent a major advance in the fields of cell therapy and personalized medicine.[1] In this strategy, a

patient's own T cells are isolated and engineered to express a synthetic receptor that binds a tumor antigen to induce tumor cell death. These CAR-engineered T cells are then expanded ex vivo to clinically significant numbers and infused back into the patient as cancer immunotherapy. The potency of these engineered cells lies in merging the effector functions of T lymphocytes with the specificity and binding affinity of antibodies. The extracellular domain of a CAR comprises an antigen-binding single-chain variable fragment made up of the variable heavy and variable light chains of an antibody, fused by a short peptide linker.[2] The intracellular domain consists of a signaling molecule, traditionally from the T-cell receptor (TCR) CD3ζ chain, and other (optional) features depending on the generation of the CAR construct.[2] Whereas first-generation CARs contain CD3ζ alone, second-generation CARs incorporate an additional costimulatory endodomain, such as CD28 or 4-1BB, and third-generation CARs contain >1 costimulatory domain fused to CD3ζ.[1] Finally, fourth-generation CARs harbor an extra transgenic payload such as cytokines to boost their effector function.[3-5]

CAR-T cells were first tried against B-cell malignancies with CD19 used as a target antigen, resulting in remarkable clinical responses in diseases that were multiply relapsed and refractory to chemotherapy.[6] This success led to the US Food and Drug Administration approval of 2 autologous CAR-T cell products: tisagenlecleucel (Kymriah) and axicabtagene ciloleucel (Yescarta).[7-9] Kymriah was approved for patients ≤25 years of age with relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL) based on the results from the phase 2 pivotal ELIANA trial that reported an overall response rate (ORR) of 81%, with 60% of patients having achieved complete remission (CR).[7] Kymriah is also approved for adults with relapsed or refractory large B-cell lymphoma, including patients with diffuse large B-cell lymphoma (DLBCL) not otherwise specified, high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma for whom ≥2 lines of systemic therapy have failed, based on the JULIET trial reporting an ORR of 52% with a CR rate of 40%.[9] The ZUMA-1 trial led to the approval of Yescarta for use for adult patients with large B-cell lymphoma after ≥2 lines of therapy, including DLBCL, primary mediastinal large B-cell lymphoma, high-grade B-cell lymphoma, and DLBCL arising from follicular lymphoma. The trial reported an ORR of 82% and a CR rate of 54%, with ongoing responses observed in 42% of patients.[8]

Despite the elegance of this therapy and its clinical successes, autologous CAR-T cells have some limitations.[10] First and foremost, from a clinical standpoint, not all patients can be candidates for this therapy. For example, some patients with cancer are heavily pretreated and have significant T-cell lymphopenia, which could potentially hamper collection of autologous T cells in sufficient numbers for a clinically relevant dose of CAR-T cells.[11] Moreover, the process of producing an autologous CAR-T cell product is lengthy and logistically cumbersome; therefore, patients who have rapidly progressive disease are usually not candidates for this therapy. Another limitation of CAR-T cells is their toxicity profile, because clinical experience has shown a substantial risk of cytokine release syndrome (CRS) and immune effector cell–associated neurotoxicity syndrome (ICANS) among patients receiving this therapy,[12] especially those with bulky or high-burden disease.[13,14] Fortunately, advances in consensus grading and management guidelines for these inflammatory syndromes have significantly improved outcomes for patients with CAR-T cell–related toxicities.[15] Target antigen loss after CAR-T cell therapy can pose another clinical problem, because CAR-T cell killing depends on CAR engagement; that is, target antigen loss renders these immune cells ineffective.[16] Finally, allogeneic CAR-T cells are being explored for off-the-shelf therapy,[17,18] but given their associated risk of graft-versus-host disease (GVHD), allogeneic CAR-T cells would need further genetic modification, such as deletion of the TCR, to mitigate this risk.[19]

Back to our patient

Given the advanced stage of her disease, which had resisted multiple lines of therapy, and the leukemic phase of her follicular lymphoma, which predicts poor prognosis,[20] our patient was considered for treatment with autologous CAR-T cell therapy. However, she was not a candidate for this therapy because Yescarta and Kymriah are approved only for follicular lymphoma that has transformed to high-grade lymphoma, and even if the disease did fit the transformation criteria, the patient's severe T-cell lymphopenia and rapidly progressing disease would not permit the collection of autologous T cells and the necessary wait for the manufacture of an autologous CAR-T cell product. Next, the patient was considered for a clinical trial of allogeneic CAR-T cell therapy but was not eligible in view of multiple antibodies against the mismatched HLA alleles with the donors. In addition, our patient had bulky abdominal disease, which would have led to an increased risk of CRS and ICANS with use of CAR-T cell therapy.[21]

NK cells as an alternative platform for CAR engineering

In view of the aforementioned limitations of CAR-T cell therapy and of the patient's specific clinical and disease-related features, a decision was made to take an alternative approach using CAR natural killer (NK) cells that might circumvent these problems.

NK cells are CD3-CD56+ innate lymphoid cells that play a fundamental role in host defenses against infectious agents and malignancies.[22] Unlike T cells, NK cells can kill transformed cells without the need for prior antigen priming, and their killing capacity is not restricted by the target cell's expression of major histocompatibility complex (MHC) molecules.[23] In fact, NK cell activity is governed by a broad repertoire of activating and inhibitory receptors (Figure 1) whose complex integration of positive and negative signals determines the final disposition of NK cells to kill or not to kill a target cell.[24] As a result, NK cells are capable of distinguishing between normal and "stressed" cells. Healthy cells are spared as they express self-MHC class I molecules that bind to inhibitory receptors on NK cells, thus delivering a "no kill" signal, whereas transformed or infected cells that downregulate MHC class I molecules or upregulate stress-induced molecules such as MICA/MICB and ULBP, which bind to activating receptors such as NKG2D, deliver an activating signal to the NK cells to kill.[25] Thus, tumor cells that escape T-cell killing by downregulating MHC class I are still susceptible to NK cell killing. Another major advantage of NK cells over T cells is that they do not cause GVHD in the allogeneic setting,[26] making them a safe choice as a third-party, off-the-shelf cellular therapy option. Allogeneic NK cells may also be less prone to rejection by recipient alloreactive T cells. A number of preclinical studies have shown that NK cells in the graft can target and kill host lymphohematopoietic cells that may participate in the rejection

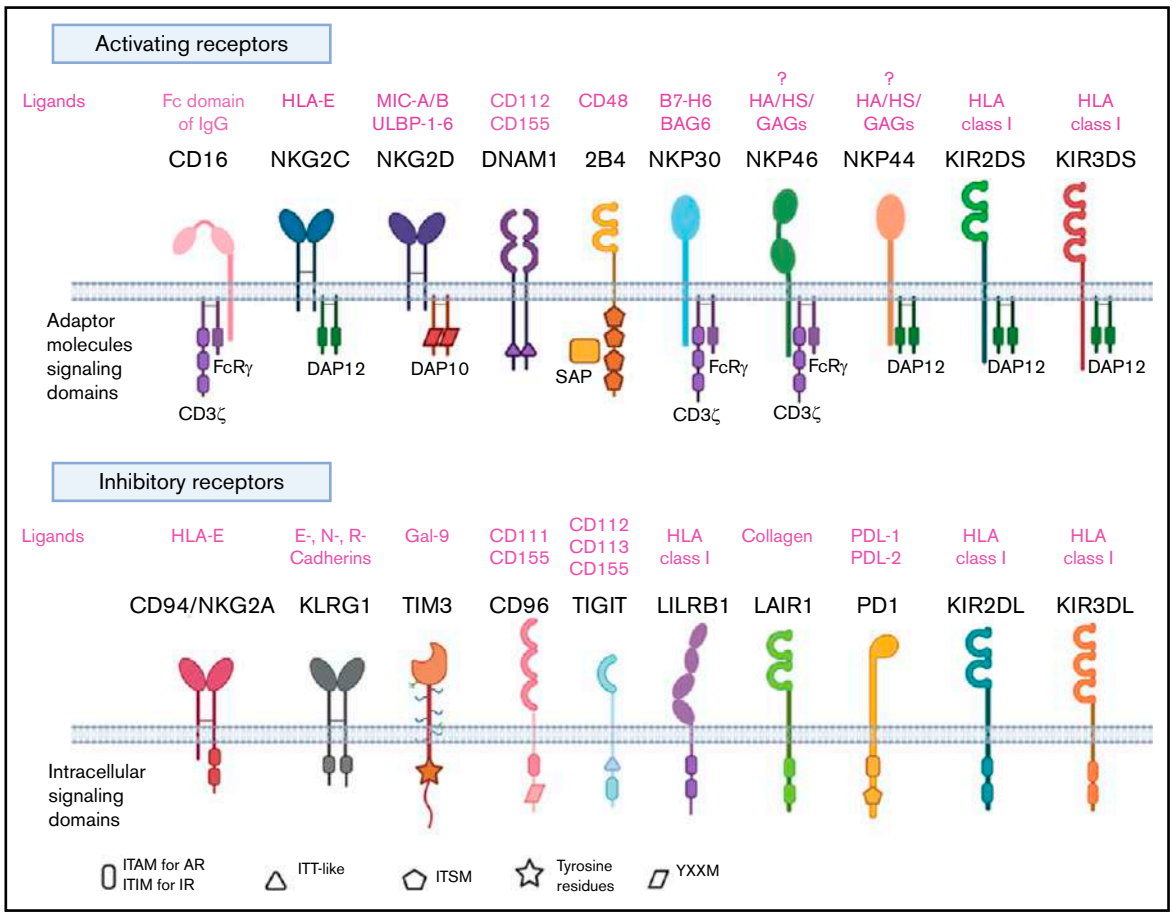

Figure 1. NK cell repertoire of activating and inhibitory receptors. AR, activating receptor; BAG6, BCL2-associated athanogene 6; DAP10, DNAX activating protein of 12 KDa; DAP12, DNAX activating protein of 12 KDa; DNAM1, DNAX accessory molecule 1; GAGs, glycosaminoglycans; Gal-9, galectin-9; HA, hemagglutinin; HS, heparan sulfate; IR, inhibitory receptor; ITAM, immunoreceptor tyrosine-based activation motif; ITIM, immunoreceptor tyrosine-based activation motif; ITSM, immunoreceptor tyrosine-based switch motif; ITT-like, immunoglobulin tail tyrosine-like; KIR, killer immunoglobulin like receptor; KLRG1, killer cell lectin-like receptor G1; LAIR1, leukocyte-associated immunoglobulin like receptor-1; LILRB1, leukocyte Ig-like receptor B1; MIC-A/B, MHC class I chain-related proteins A and B; PD1, programmed cell death protein 1; PDL-1, programmed cell death ligand 1; PDL-2, programmed cell death ligand 2; TIGIT, T-cell immunoreceptor with Ig and ITIM domains; TIM3, T-cell immunoglobulin mucin domain-3; YXXM, Y stands for tyrosine, X for any amino acid residue, and M for methionine.

of donor cells.[27] Indeed, our clinical data confirm the persistence of adoptively transferred CAR-NK cells at low levels in patients for at least a year, despite HLA mismatching,[26] supporting the notion that NK cells may be less susceptible to host-versus-graft rejection. Therefore, these favorable features of NK cells with regard to GVHD and host-versus-graft reactions may obviate HLA matching in the allogeneic setting.

Despite their advantages, NK cells have a number of limitations that could affect their efficacy. These include a short lifespan of only 1 to 2 weeks in the absence of cytokine support, limited cell numbers often requiring ex vivo expansion and activation, and, in common with other immune cells, susceptibility to the immunosuppressive tumor microenvironment that could in turn limit their trafficking and effector function. Advances in engineering have enabled investigators to overcome some of these limitations. For example, the incorporation of cytokine transgenes (eg, interleukin [IL]-2 or IL-15) in NK cells can enhance their proliferation and persistence, and the incorporation of chemokine receptors can promote their trafficking to tumor sites.[25] Other engineering strategies to improve NK cell performance are reviewed elsewhere.[25,28]

In view of their unique biological features, their potent innate antitumor activity, and their favorable safety profile in the clinic,[25,26] NK cells have garnered considerable attention over the past few years as an emerging alternative platform for CAR engineering. Pure populations of NK cells can be derived from autologous or allogeneic sources, such as peripheral blood; umbilical cord blood; stem cells, including induced pluripotent stem cells and hematopoietic stem cells; and in vitro propagated NK cell lines such as NK-92.[29] Limitations of autologous NK cells derived from patients with cancer, including reduced effector function and the need for a patient-specific product (similar to autologous T cells), have led to the rise of allogeneic NK cells as a platform for CAR engineering.[30] Allogeneic NK cells used for CAR engineering can be derived from multiple sources, each with unique advantages and limitations.[29] Cord blood is a

Table 1. Clinical trials evaluating alternative immune cells for CAR-based cancer therapy

National Clinical Trial identifier	Clinical trial phase	Cancer type	Antigen target	NK cell source	Construct/ method	Dosage	Status	Location
Active CAR NK cell trials								
NCT03056339	1/2	B-lymphoid malignancies, ALL, CLL, NHL	CD19	Cord blood	CAR.CD19-CD28-CD3ζ-iCasp9-IL15	3 dosage levels: 10^5/kg 10^6/kg 10^7/kg	Phase 1 portion completed, phase 2 recruiting	MD Anderson Cancer Center, Houston, TX USA
NCT00995137	1	B-ALL	CD19	Haploidentical donor	CAR.19-41BB-CD3ζ	Unknown	Completed	St Jude Children's Research Hospital, Memphis, TN
NCT04245722	1	B-cell lymphoma, CLL	CD19 ± CD20 antibody (rituximab or obinutuzumab)	Induced pluripotent stem cell–derived NK cells	CAR.19-NKG2D-2B4-CD3ζ-IL15RF-hnCD16	Dose escalation, exact dosages unknown	Recruiting	University of Minnesota Masonic Cancer Center, Minneapolis, MN
NCT03940833	1/2	Multiple myeloma	BCMA	NK-92 cell line	Unknown	Unknown	Recruiting	China
NCT03415100	1	Solid tumors	NKG2D ligands	Autologous or allogeneic NK	mRNA electroporation	Unknown	Recruiting	China
NCT03940820	1/2	Solid tumors	ROBO1		Unknown	Unknown	Recruiting	China
NCT03941457	1/2	Pancreatic cancer	ROBO1	Unknown	Unknown	Unknown	Recruiting	China
NCT03383978	1	Glioblastoma	HER2	NK-92	CAR.HER2-CD28- CD3ζ	1×10^7-1×10^8 intracranial infusion	Recruiting	Germany
CAR-NK T cell trials								
NCT03774654	1	Relapsed or refractory B-cell malignancies	CD19	Allogeneic iNKT cells	CAR.19-CD28-CD3ζ -IL15	4 dosage levels: $1 \times 10^7/m^2$ $3 \times 10^7/m^2$ $1 \times 10^8/m^2$ $3 \times 10^8/m^2$	Not yet recruiting	Baylor Methodist–Texas Children's, Houston, TX
NCT03294954	1	Relapsed or refractory neuroblastoma	GD2	Autologous iNKT cells	CAR.GD2-CD28-CD3ζ-IL15	4 dosage levels: $3 \times 10^6/m^2$ $1 \times 10^7/m^2$ $3 \times 10^7/m^2$ $1 \times 10^8/m^2$	Recruiting	Baylor Methodist–Texas Children's, Houston, TX
CAR γδ T cell trials								
NCT02656147	1	B-cell leukemia and lymphoma	CD19	Allogeneic γδ T cells	Unknown	Unknown	Not yet recruiting	China
NCT04107142	1	Solid tumors	NKG2D ligands	Haploidentical or allogeneic γδ T cells	Unknown	3×10^8-3×10^9 cells	Not yet recruiting	Malaysia

ALL, acute lymphoblastic leukemia; BCMA, B-cell maturation antigen; CLL, chronic lymphocytic leukemia; iCasp9, inducible caspase 9; mRNA, messenger RNA; NHL, non-Hodgkin lymphoma.

Table 1. (Continued)

National Clinical Trial identifier	Clinical trial phase	Cancer type	Antigen target	NK cell source	Construct/ method	Dosage	Status	Location
CAR CIK trial								
NCT03389035	1/2	Relapsed B-ALL	CD19	Allogeneic (donor derived peripheral blood) CIK	CAR.19-CD28-OX40- CD3ζ Sleeping beauty transposon	4 dose levels: 1 × 10^6, 3 × 10^6 7.5 × 10^6 15 × 10^6 CAR+ cells/ kg	Recruiting	Italy

ALL, acute lymphoblastic leukemia; BCMA, B-cell maturation antigen; CLL, chronic lymphocytic leukemia; iCasp9, inducible caspase 9; mRNA, messenger RNA; NHL, non-Hodgkin lymphoma.

readily available off-the-shelf source of allogeneic NK cells, which, though numerically few, can be expanded to large, highly functional doses because of their inherently high proliferative capacity.[31] In addition, access to hundreds of thousands of cord blood units in the global inventories allows selection of units without cross-reactivity to allosensitized patients, as for the case presented here. Peripheral blood NK cells, on the other hand, are phenotypically mature and highly functional; however, their use requires a willing healthy donor to undergo leukapheresis or blood donation. NK cells derived from induced pluripotent stem cells are immature, with low expression of antibody-dependent cellular cytotoxicity–inducing CD16 receptors, but are highly proliferative and can be made readily available for use in biobanks.[32] Finally, NK cell lines such as NK-92 cells can be easily expanded and manipulated, but because they originate from undifferentiated NK cell precursors from patients with NK lymphoma, they lack expression of CD16 and some killer immunoglobulin-like receptors, and because of their malignant potential they need irradiation before clinical use, which in turn could limit their in vivo persistence and efficacy.[33] A multitude of preclinical studies have confirmed the activity of CAR-engineered NK cells derived from these sources against a range of cancer models, both in vitro and in vivo (reviewed by Pfefferle et al).[29] More recently, the efficacy of CAR-NK cells is being explored in different malignancies (completed or recruiting clinical studies are summarized in Table 1). We conducted the first-in-human phase 1/2 clinical trial of CAR-NK cell therapy for patients with relapsed or refractory B-cell hematologic malignancies (ClinicalTrials.gov number NCT03056339).[26] The NK cells were derived from cord blood and were HLA mismatched with the recipient. The retroviral vector used to transduce the NK cells encoded a CAR against the CD19 antigen and IL-15 to enhance NK cell persistence and function. An inducible caspase 9 suicide gene (*iCas9*) was added as a safety switch.[26] Eleven heavily pretreated patients have received this therapy at 3 different dose levels (1 × 10^5, 1 × 10^6, or 1 × 10^7 cells per kilogram). Eight responded, for an overall response rate of 73%, with 7 achieving a complete remission (64%). These responses were rapid and seen at all dosage levels. Importantly, serious toxicities including CRS, ICANS, or GVHD did not develop in any of the patients.[26] Based on these data, our patient is being considered for participation in the CAR-NK cell study. A schematic diagram of the ultimate goal of producing off-the-shelf cord blood derived CAR-NK cells for the treatment of patients with cancer is represented in panel A of the visual abstract.

Other immune effector cells as vehicles for CAR engineering

Immune effectors other than NK cells are also being investigated as alternative platforms for CAR engineering. These cell populations possess a number of specific biological features that could significantly expand and diversify the repertoire of CAR-based therapies. For example, invariant NK T cells have attracted growing attention as possible CAR vehicles, because they possess features of both innate and adaptive immune cells. Much like innate immune cells, they can mount a rapid response to antigen exposure, but they can also display precise antigen recognition in the manner of adaptive cells.[34] Unlike conventional T cells, their TCR recognizes lipid antigens presented by CD1d, a monomorphic MHC class 1–like molecule.[34] Another group of immune effector cells being explored as potential platforms for CAR engineering are γδ T cells. These T cells are predominant at epithelial surfaces and express γδ TCRs, which are triggered in an MHC-independent fashion (eg, by aminobisphosphonates), contrary to αβ TCR activation.[35] γδ T cells can also cross-present antigens to αβ T cells and thus can serve as a link between innate and adaptive immunity.[36] Cytokine-induced killer cells (CIK) are a group of immune effector cells featuring a mixed T and NK cell–like phenotype and therefore can kill tumor targets in both MHC-dependent and MHC-independent manners.[37,38] Preclinical studies exploring CAR-transduced CIK cells have reported promising results both in hematological[39,40] and solid tumor[41-43] settings and have led to an ongoing clinical trial (NCT03389035) to test the safety of CAR CIK-CD19 cells in adult and pediatric patients with relapsed or refractory B-cell acute lymphoblastic leukemia. Other than lymphocytes, a different type of immune cell that offers the advantage of being able to penetrate tumor beds and naturally engulf malignant cells, macrophages, has garnered attention as a possible effector in CAR-based therapies.[44] The advantages and disadvantages of alternative immune effector cells for CAR-based cancer therapy are summarized in Table 2. Preclinical evaluations of these types of immune cells as CAR platforms support this prediction (reviewed by Rotolo et al[45]), and clinical trials using these cell types are either planned or ongoing (see Table 1).

CRISPR-mediated gene editing as the next frontier for cell therapies

Will a bacterial and archaeal immune system adapted for eukaryotic gene editing further elevate the field of cell therapy?

Table 2. Advantages and disadvantages of alternative immune effector cells as platforms for CAR engineering

Alternative immune effector cell	Advantages	Disadvantages	Safety profile
NK cell	Multiple innate activating receptors that can mediate killing Can harness KIR-ligand mismatch and "missing self" to reduce risk of relapse Multiple mechanisms of cytotoxicity No need for previous antigen priming Rapid tumor killing	Low persistence in the absence of cytokine Numerically few necessitating ex vivo expansion Suboptimal trafficking and penetration into solid tumors	In early clinical results of CAR-NK cells: -No GVHD -No CRS -No ICANS
iNKT	Innate and adaptive features Invariant TCR recognizes lipid antigens presented by CD1d	Can have immunosuppressive properties (Th2, Th17) Numerically few requiring ex vivo expansion	Limited clinical data with iNKT-CAR NK cells; reports in 2 patients showed no toxicity In non–CAR-engineered cells: -No GVHD -No toxicities
γδ T cells	Links innate and adaptive immune systems MHC independent γδ TCR Cross-present antigens to αβ T cells	Can have immunosuppressive properties (γδ T17, Vδ1 γδ T cells, γδ Treg) Numerically few necessitating ex vivo expansion	No clinical data with CAR γδ T cells In non–CAR-engineered cells: No GVHD No toxicities
Macrophages	Good penetration into solid tumors Mediates phagocytosis and cytotoxicity Cross present antigens to αβ T cells	Can have immunosuppressive properties (M2) Numerically few necessitating ex vivo expansion	No clinical data with CAR macrophages In non–CAR-engineered cells: No GVHD No toxicities
CIK	Multiple killing mechanisms including MHC-dependent and MHC-independent	Heterogeneous products Numerically few necessitating ex vivo expansion	No clinical data with CAR CIK In non–CAR-engineered cells: Lower GVHD risk than T cells[60]

Genome editing technologies allow researchers to modify the genome by adding, removing, or otherwise altering the DNA. Several approaches have been devised, including zinc fingers, transcription activator–like effector nucleases, and most recently the clustered regularly interspaced short palindromic repeats (CRISPR) system.[46] The discovery of CRISPR revolutionized the field of gene editing because of its simplicity, efficiency, reproducibility, and low cost.[47] The CRISPR-Cas system is an RNA-mediated bacterial defense system against viruses (bacteriophages) and plasmids that was repurposed for precise RNA-programmable genome editing in mammalian cells.[48]

Briefly, the CRISPR-Cas9 technology used in the laboratory for gene editing relies on 2 key elements: the Cas9 enzyme, which acts as a pair of molecular scissors to cut DNA at a specific locus; and a piece of RNA, called guide RNA, which consists of 2 fragments (the *trans*-activating CRISPR RNA, which binds to the Cas9 enzyme, and the CRISPR RNA, an 18- to 20-nucleotide sequence that is predesigned to recognize a complementary DNA target site in a gene of interest). The guide RNA can therefore guide the Cas9 enzyme to the desired target sequence for gene editing.[49,50] The CRISPR-Cas9 tool can also be used to target multiple genes simultaneously by using multiple single-guide RNAs.[51]

It is important to note that nonspecific and unintended genetic modifications such as insertions or deletions at off-target cleavage sites can arise through the use of engineered nuclease technologies such as CRISPR gene editing.[52] For clinical applications, identification of even low-frequency alterations will be critically important. Thus, careful evaluation of off-target effects via technologies such as GUIDE-Seq,[53] CIRCLE-Seq,[54] and rhampSeq[55] is essential before CRISPR-based therapies can be translated to the clinic. The use of ribonucleoprotein complexes and high-fidelity Cas9 was recently shown to significantly reduce the occurrence of such unwanted DNA changes.[56]

Given the versatility of this gene editing technology, one can imagine its potential applications in cell therapy. A good starting point would be to modify the function of immune effector cells by selectively suppressing negative regulators of cytolytic activity and increasing activation signals. This technology can also be used to fine-tune the safety of cellular therapy products by targeting genes associated with toxicity.[57] Positive steps in CRISPR-modified adoptive cell therapy in cancer were recently reported by Stadtmauer et al.[58] In this first-in-human pilot study, the investigators isolated autologous T cells from the blood of patients with refractory cancer and electroporated them with CRISPR-Cas9 ribonucleoprotein complexes targeting *TRAC*, *TRBC1*, and *TRBC2* to suppress the endogenous TCR and *PDCD1* to reduce programmed cell death protein 1 expression. The cells were then transduced with a lentiviral vector to express a TCR specific for the cancer–testis antigens NY-ESO-1 and LAGE-1, ex vivo expanded, and then returned to the patients via intravenous infusion.[58] This phase 1 study established the feasibility and initial safety of multiplex CRISPR-Cas9–mediated genome engineering of human T-cells. Other clinical trials evaluating

Table 3. CRISPR-Cas modified cell therapies for cancer

National Clinical Trial identifier	Clinical trial phase	Cancer type	CRISPR target	Cell source	Method of CRISPR	Other cell engineering	Status	Location
NCT04037566	1	Relapsed or refractory ALL and B-cell lymphoma	HPK1	Autologous T cells	Electroporation of RNP	CD19 CAR lentiviral vector	Recruiting	China
NCT03399448	1	Multiple myeloma Melanoma Synovial sarcoma Myxoid/round cell liposarcoma	Endogenous TCRα, TCRβ, and PD-1	Autologous T cells	Electroporation of RNP	NYESO-1 TCR lentiviral vector	Active, not recruiting	University of Pennsylvania
NCT03164135	1	Hematologic malignancies with HIV infection	CCR5	CD34+ hematopoietic stem/progenitor cells	Unknown	None	Recruiting	China
NCT03545815	1	Mesothelin positive Solid tumors	Endogenous TCR and PD-1	T cells, unknown source	Unknown	Anti-mesothelin CAR	Recruiting	China
NCT04244656	1	Refractory multiple myeloma	TCR and β2 M gene	Allogeneic T cells	Unknown	Anti-BCMA CAR insertion at the TRAC locus	Recruiting	USA (Nashville and Oregon) and Australia
NCT03747965	1	Mesothelin positive Solid tumors	PD-1	T cells, unknown source	Unknown	Anti-mesothelin CAR	Recruiting	China
NCT04035434	1/2	B-cell malignancies	TCR and β2 M gene	Allogeneic T cells	Unknown	Anti-CD19 CAR insertion at the TRAC locus	Recruiting	Multiple sites in USA and Australia
NCT03166878	1/2	B-cell leukemia and lymphoma	TCR and β2 M gene	Allogeneic T cells from healthy unrelated donors	Electroporation of RNP	Anti-CD19 CAR, lentiviral vector 41BB-CD3ζ	Recruiting	China
NCT03044743	1/2	EBV associated malignancies	PD-1	EBV CTL from autologous source	Unknown	None	Recruiting	China

BCMA, B-cell maturation antigen; EBV, Epstein–Barr virus; HPK1, hematopoietic progenitor kinase 1; NYESO-1, New York esophageal squamous cell carcinoma 1; RNP, ribonucleoprotein; TRAC, T-cell receptor-α constant.

CRISPR-modified adoptive cell therapy are under way, as summarized in Table 3. Our group has developed a method to combine CAR engineering with CRISPR-Cas9 gene editing in primary NK cells,[59] and we are working on developing a good manufacturing practice–compliant strategy for the production of off-the-shelf CRISPR-modified cord blood–derived CAR-NK cells for the treatment of patients with cancer (visual abstract, panel B).

Conclusions

CAR-T cell therapy has emerged from an exciting concept at the beginning of this century to a highly effective treatment with curative potential in B-cell malignancies. Nonetheless, despite its many advantages over other forms of cancer therapy, including in vivo expansion and long-term persistence, treatment with CAR-T cells remains a work in progress. Current limitations of this therapy are being overcome by the introduction of alternative platforms for CAR engineering, including NK cells, and the testing of innovative methods such as CRISPR-Cas9 gene editing to counteract tumor-initiated immunosuppressive tactics. If these efforts are successful, we can look forward to a time when clinical applications of cell therapies for cancer are routine rather than investigational strategies at the margins of frontline treatment.

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K.R., M.D., R.B., and The University of Texas MD Anderson Cancer Center (MDACC) have an institutional financial conflict of interest with Takeda Pharmaceutical for the licensing of the technology related to the research mentioned here. MD Anderson has implemented an Institutional Conflict of Interest Management and Monitoring Plan to manage and monitor the conflict of interest with respect to MDACC's conduct of any other ongoing or future research related to this relationship.

Off-label drug use

None disclosed.

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Data sharing statement
Data are available from the corresponding author, Katayoun Rezvani (KRezvani@mdanderson.org).

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Practical aspects of building a new immunotherapy program: the future of cell therapy

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Cellular-redirecting therapies, including bispecific T-cell engagers and chimeric antigen receptor (CAR) T cells, are rapidly changing the treatment landscape of hematologic malignancies and solid tumor malignancies. I will discuss the unique safety profile and logistical aspects that pose challenges and opportunities for the safe and successful delivery of these therapies. Close interaction, communication, and established partnerships between the primary oncologist, the disease specialist, and the immune effector cell provider will be needed to provide optimal care longitudinally for any patient. I will discuss practical ways for any program to deliver these therapies and how future advances may widen availability beyond just a few centers.

LEARNING OBJECTIVES
- Identify best practices for safe and successful delivery of cellular redirecting therapies
- Identify opportunities to anticipate and address acute phase and late phase toxicity
- Prepare and plan for a clinical model that best fits your practice

Clinical case

A 52-year-old man with germinal center B-cell diffuse large B-cell lymphoma (DLBCL), without MYC translocation, is referred for consideration of chimeric antigen receptor (CAR) T therapy. The patient was diagnosed 2 years ago and underwent 6 cycles of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone, achieving complete remission (CR). One year later he relapsed and underwent salvage with 3 cycles of rituximab, ifosfamide, carboplatin, and etoposide followed by autologous hematopoietic cell transplantation with a carmustine, etoposide, cytosine arabinoside, and melphalan conditioning regimen. He achieved CR, but 6 months later he presents with relapse. Clinically he is doing well, and aside from hypertension and hypercholesterolemia he is healthy. He continues to perform activities of daily living without difficulty and has an excellent performance status. You agree he is a good candidate for CAR T therapy. Are you and your institution ready to proceed with this type of therapy? What are the next steps in shepherding this patient safely and successfully through the CAR T process?

Introduction

In 2017 the US Food and Drug Administration (FDA) approved blinatumomab, a CD19:CD3 bispecific T-cell engager, for the treatment of relapsed or refractory B-cell acute lymphoblastic leukemia, heralding a new era of immunotherapy.[1] This approval was shortly followed by the approval of 2 CD19-directed CAR T cell products, tisagenlecleucel for the treatment of relapsed or refractory B cell acute lymphoblastic leukemia [2,3] and DLBCL and axicabtagene ciloleucel[4] for the treatment of DLBCL. Since then the number of ongoing clinical trials and therapies in development is mind boggling. In 2020, the FDA predicted the development of >200 investigational new drugs per year and expected to approve 10 to 20 cell and gene therapy products per year by 2025.[5] Furthermore, it is becoming clear that although there are common themes across the different therapies, individual products behave differently between disease types and even within diseases; some of these differences include significant variation in dosage levels, degree and duration of cytokine release syndrome (CRS) and neurotoxicity, variable duration of cytopenias, and variable time to onset of CRS. [6-15] These unique qualities may pose both a challenge and an opportunity for various delivery and access methods.

Practical aspects

Autologous CAR T process

Without a doubt, the resource-heavy processing and delivery of autologous CAR T products present unique

challenges. A pictorial representation of the basic autologous CAR T process is shown in Figure 1. Initially the process mirrored the one already established by transplant programs. But even then, the processes in place needed adjustments, and specific immune effector cell (IEC) programs and paradigms were created. Figure 2 depicts some of the practical and logistic differences between the standard autologous hematopoietic cell transplantation process and the autologous CAR T process. Although the discussion that follows is aimed at the delivery of autologous CAR Ts, the development of off-the-shelf cellular and antibody-based products may allow simplification of at least the initial processes.

Toxicity profile

The toxicity profiles associated with cellular redirecting therapies have been extensively described elsewhere and are summarized in Table 1.[6-9] In general, toxicity can be divided into an acute phase (30 days) and a late phase (beyond 30 days). In the acute phase, CRS, neurotoxicity, and cytopenias predominate. The rapidity and onset of these potential toxicities require multidisciplinary expert vigilance, availability, and management. These are treated mostly in the inpatient setting but are slowly starting to be managed on an outpatient basis for some patients.

Late phase toxicity for the most part is dominated by persistent or recurrent cytopenias and increased infection risk. Issues pertaining to infectious prophylaxis, revaccination, immunoglobulin deficiency, and need for replacement predominate. Longer-term sequelae such as fatigue, memory loss, and lapses in concentration are becoming better described.[9,12,13] Although the optimal management continues to evolve and be defined, clear processes and resources are needed for the safe delivery of these therapies.

Requirements of an IEC program

Perhaps the best way to identify the practical needs for a new program is to address the steps that a patient undergoing CAR T therapy will traverse. Figure 3 breaks this process into 8 different steps.

1. The first is intake and triage, initiated by the patient or by a referring provider. Ideally, it involves a dedicated triage nurse or navigator, a dedicated CAR T coordinator, and an insurance benefits or financial coordinator.
2. This first step leads to a consultation with an IEC provider, who will determine eligibility. Given the current indications of the FDA-approved products in the relapsed or refractory setting of aggressive diseases, moving forward quickly is important. If the patient is found eligible, the CAR T coordinator then sets in motion all necessary procedures and communications with all appropriate parties, including apheresis staff assessment to determine the type of catheter needed, usually large-bore peripheral IV versus a temporary trifusion catheter, and to secure the apheresis date and time; and social work evaluation to assess for any concerns about compliance, caregiver support, financial resources, and ability to secure local housing if needed.
3. The next step is the collection, processing, and shipping or manufacturing of cells. The vein-to-vein process will vary, but anticipate on average 4 to 6 weeks. Once the CAR T product has been manufactured, it is shipped and received by the cell processing laboratory in preparation for infusion. A thorough analysis of expected volume increase and additional infrastructure needs must be undertaken to anticipate space, equipment, and staffing needs.
4. After apheresis and during the manufacture of the CAR T product, many patients will need bridging therapy. The goal of bridging therapy is to control the patient's disease but not induce toxicity that may make the patient ineligible to receive the CAR T product. This therapy can be delivered by the IEC provider or the referring oncologist or disease specialist.
5. Once the product is ready for infusion, the patient is reevaluated by the IEC provider to ensure that no new problems have arisen that would make the patient ineligible to proceed as planned. Currently, most CAR T products are indicated in aggressive tumors in the relapsed and refractory setting. Therefore, deterioration of performance status and organ function and concurrent infections can often halt the

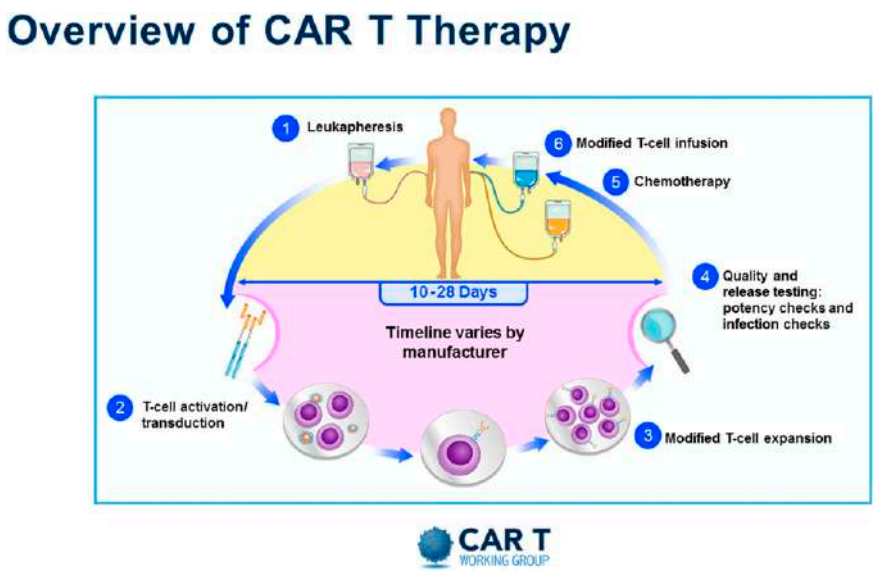

Figure 1. Overview of CAR T therapy. Reproduced with permission from the Slide Library of the CAR T Working Group (v2 9.4.2019).

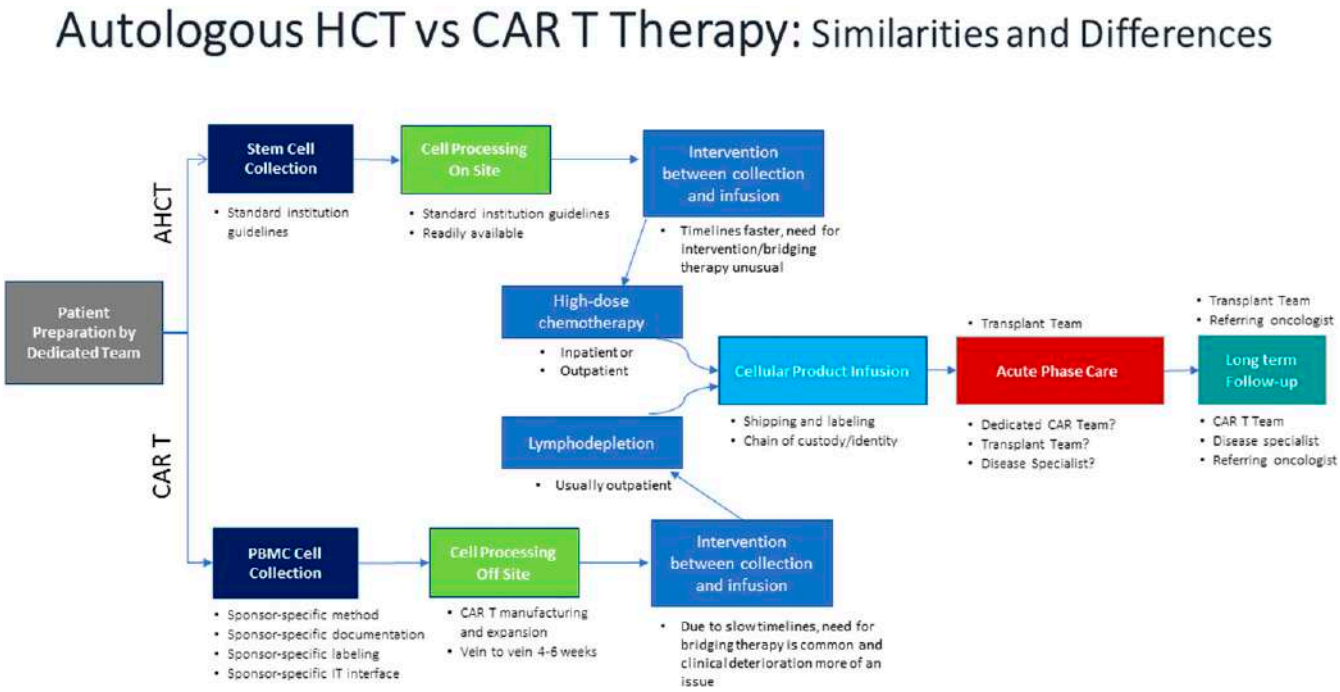

Figure 2. Autologous hematopoietic cell transplantation versus CAR T therapy: similarities and differences.

process. It is important that these are resolved before treatment begins. If the patient is found eligible, the team (IEC provider, outpatient nursing, and pharmacy) prepare and proceed with lymphodepleting chemotherapy, usually delivered over 3 days. This is often done on an outpatient basis but can be done on an inpatient basis if logistically more feasible; the inpatient setting is the most common. The patient then undergoes infusion of the product, usually 2 to 7 days after completion of lymphodepletion.

6. After infusion, the patient is considered to be in the acute phase of their potential toxicity. This phase usually lasts 30 days. With current products, the first 2 weeks are the highest risk for development of CRS, neurotoxicity, and pancytopenia. Careful evaluation, coordination, observation, and treatment are carried out by the IEC provider in conjunction with the medical intensive care unit, the emergency room team, neurology, and other consultants as needed. Of course, experienced nurses and pharmacists are paramount. Ensuring emergent availability of blood products and anticytokine drugs such as tocilizumab and anakinra is mandatory.
7. At day 30, if clinically well, the patient is released to the referring provider, and long-term surveillance begins. This is usually done by the referring oncologist in consultation with the IEC provider. During this time, patients are in their late phase of potential toxicity and often still cytopenic, and they may need transfusion and growth factor support. Patients continue to have both humoral and cellular immunosuppression. Although the exact antimicrobial prophylaxis has not been standardized, most experienced centers recommend prophylaxis for at least varicella-zoster virus and *Pneumocystis jirovecii* pneumonia and possibly intravenous immunoglobulin (IVIG) infusion. A plan must be put in place to ensure the safety of these patients. Frequent communication between the primary oncologist and the IEC provider is paramount.
8. Finally, continuous reporting of outcomes is currently required by the Centers for Medicare and Medicaid (CMS). This can be accomplished in various ways; one is to use the established Centers for International Blood and Marrow Transplant Research reporting already being done by many centers.

Regulatory hurdles

CMS has established a risk evaluation and mitigation strategy (REMS) program to define criteria for a center to qualify as a provider of FDA-approved IECs and be reimbursed (https://www.cms.gov/). REMS is a safety program for medications with serious safety concerns. It is designed to reinforce safe medication use and applies only to commercial, licensed products and not research products. It is the responsibility of the manufacturer to develop the REMS program based on FDA guidelines and to implement and monitor compliance. It is the responsibility of the IEC program or provider to enroll and become certified in the REMS program and comply with REMS regarding training, consenting, prescribing, patient management, and compliance. This includes all staff who will be involved in the care of these patients. Centers can usually meet these requirements by being accredited by the Foundation for Accreditation of Cellular Therapies or Joint Accreditation Committee, International Society for Cell & Gene Therapy/European Society for Blood and Marrow Transplantation, although this is not necessary (http://www.factwebsite.org/). Each center must establish standard operating procedures that conform and meet all the REMS requirements. CMS has also established a requirement to provide continuous long-term outcome data under the coverage with evidence development program (https://www.cms.gov/Medicare/Coverage/Coverage-with-Evidence-Development). Finally, a thorough understanding and

Table 1. CAR T–associated toxicities

Acute phase (D0-30)	Late phase (D30+)

• CRS
• Immune effector cell–associated neurotoxicity syndrome
• Cytopenias
  • Macrophage activation syndrome or hemophagocytic lymphohistiocytosis, is a very rare and severe form
  • Disseminated intravascular coagulopathy
• B-cell aplasia and hypogammaglobulinemia
• Life threatening if not managed by expert multidisciplinary team
• Tumor lysis is rare and probably varies by disease and disease burden.
	• Persistent cytopenias
	• B-cell aplasia and hypogammaglobulinemia
	  • ?IVIG replacement
	• T-cell deficiency
	  • *Pneumocystis jirovecii* pneumonia and varicella-zoster virus prophylaxis, other?
	• Infection prophylaxis
	• Residual effects of acute toxicity
	• Delayed CRS and neurotoxicity is rare but can occur.
	• Impaired quality of life: fatigue, memory issues, not yet well described

assessment of the reimbursement process are necessary before a center decides to become a provider for CAR T therapy. The financial considerations are complex, continue to evolve, and can lead to significant unreimbursed costs of care. A description of these requirements is beyond the scope of this discussion but is well documented, and centers are urged to review them for guidance before moving forward in establishing a new IEC program.

Building a team and best practices from experienced centers

It is imperative to develop a core team of providers dedicated to IEC therapy. For some centers, this will be a new, independent team or clinical unit; for others the existing transplant team will absorb this task; and for others it may be a hybrid entity where resources are shared between the IEC team and the transplant program. Each center will determine which model makes the most sense for them. Regardless of the underlying structure, the IEC team should be multidisciplinary and include the IEC provider, CAR navigators, dedicated intensive care physicians, neurologists and other consultants, pharmacy, and collection and processing facilities. Ideally, ongoing education and communication should occur among the various stakeholders. Each program should develop and disseminate written guidance for management of complications, including the use of anticytokine therapy and corticosteroids.

- Communication between all providers is imperative. All involved providers should be continuously educated on all aspects of IEC therapy. Multidisciplinary rounds, weekly grand rounds, or similar rounds are encouraged. Standard operating

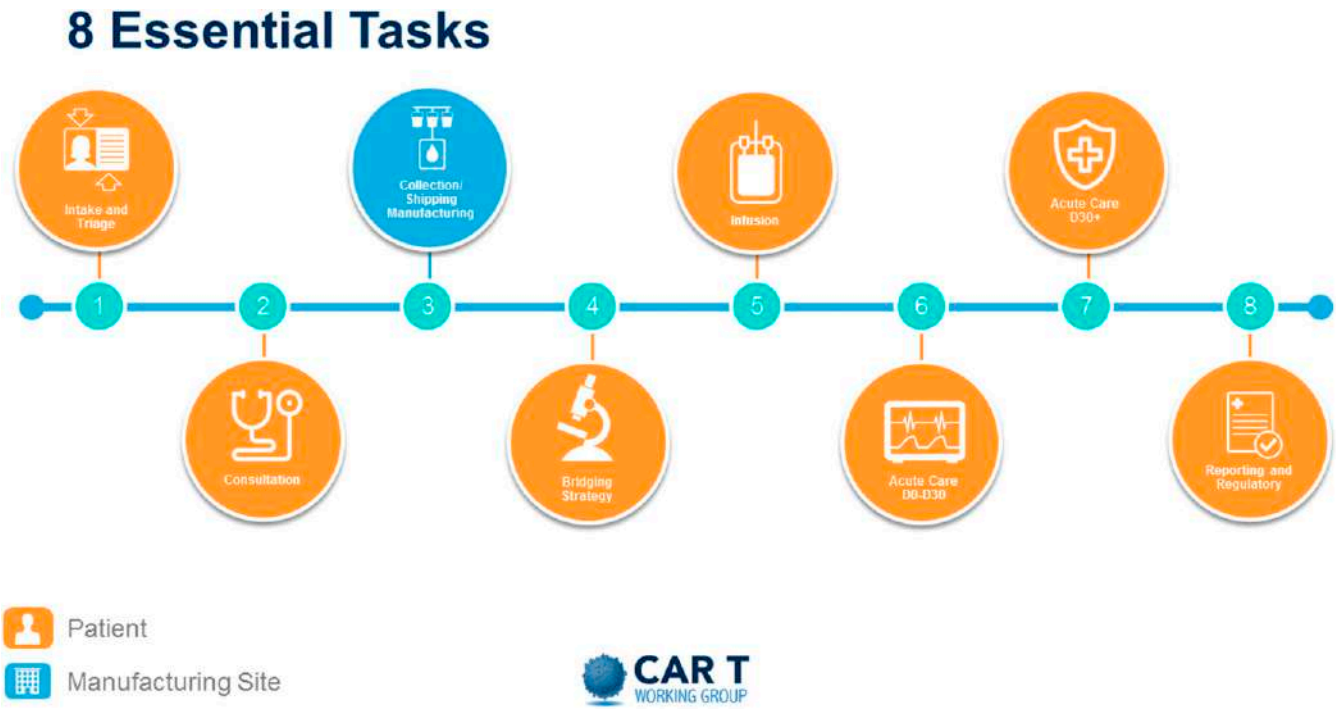

Figure 3. Illustration of a patient's journey through the CAR T process. Reproduced with permission from the Slide Library of the CAR T Working Group (v2 9.4.2019).

procedures should be developed to facilitate communication for after-hours providers. Ensure rapid availability of ICU support, neurology, infectious disease, and other consultants as needed. The ICU should be aware of all CAR T recipients admitted to the IEC service.

- Nurses should be well trained and dedicated to the care of CAR T recipients. Nurses should undergo continuous education in CAR T therapy and adverse event management. Incorporating a symptom checklist and neurologic assessment at least once per shift often reinforces the need for continued surveillance and early detection of symptoms.
- Pharmacy should be aware of the plans for lymphodepletion and CAR T infusion. The pharmacist should provide supervision to ensure proper management of CAR T therapy side effects and ensure that anticytokine therapy (ie, tocilizumab) is available for use emergently, ideally infusion within 1 hour of the order being placed.
- Emergency room staff should be educated about the specific needs of this patient population. They should have access and knowledge of CRS and neurotoxicity management algorithms, and they should promptly communicate with the IEC team and medical intensive care unit if needed. Ideally, they should attend multidisciplinary team meetings.
- Perhaps the most important measure is embracing the patient and caregiver as integral members of the care team. This is especially true in the outpatient setting, but even in the inpatient setting, educating patients and caregivers will lead to prompt detection of CRS, neurotoxicity, and other side effects. Early identification of symptoms can significantly improve intervention and hopefully decrease high-grade toxicity and poor outcomes.

Although these recommendations focus mostly on the acute phase of management, the paradigm applies to the late phase management as well. Although unusual, delayed CRS and neurotoxicity could occur. Infections become a higher risk during this time. At this point the referring oncologist becomes the primary caregiver, and education and communication are of the utmost importance. Best practices for long-term management after CAR T therapy are still evolving. However, standards of care and practice must be developed for any center, and all members of the team, including the referring providers, must be familiar with these recommendations. Written guidelines for the use of prophylactic antimicrobials, use of growth factors, use of IVIG replacement, revaccination strategies, and disease monitoring and restaging are extremely helpful to minimize errors of omission. A long-term coordinator could be the liaison between the primary oncologist and the IEC team and can ensure proper communication and implementation of the various long-term needs for the patient.

Future

For now, most FDA-approved therapies require a period of inpatient treatment. However, it appears there may be certain intrapatient variables such as burden of disease, high inflammatory markers, comorbidities that may predict higher risk of high-grade toxicity.[14-16] Similarly, the disease being treated, the specific target (CD19 vs BCMA), the cell modified (T cell vs natural killer cell), and other characteristics may continue to challenge the need for inpatient administration and supervision.[10,11,17-20] Currently, most products are autologous; they not only require particular resources but also can make the coordination and prompt delivery of therapy cumbersome. Off-the-shelf cellular and antibody-based products are being evaluated in clinical trials.[1,21,22] Theoretically these therapies could simplify the treatment algorithm. Furthermore, as these therapies continue to expand beyond the current disease indications and eventually solid tumors,[19] with the ability to predict and mitigate toxicity, these therapies will become more and more amenable to outpatient dosing for many patients if not the majority. Therefore, the facilities that will be able to deliver these therapies are likely to expand beyond the current structure. In anticipation of this potential, various groups such as the Community Oncology Alliance and the Association of Community Cancer Centers have been active in establishing educational resources, reviewing requirements, and providing guidance in preparation for possible incorporation of these therapies. Each individual program will need to determine the approach that makes best use of its resources.

Conclusion

As IEC therapy continues to expand, it is likely that current providers will need to adjust, and more facilities will need to become providers of these therapies. It is important to realize that establishing an IEC program requires very specific resources and structures to allow the safe delivery of these therapies. A careful assessment of an individual program's capabilities and potential need for partnership is imperative. Each individual program will need to determine the best approach that makes best use of resources. Paramount will be communication between all involved parties, including referring providers, disease specialists, IEC providers, and consultants.

Conflict-of-interest disclosure

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Off-label drug use

None disclosed.

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Fundamentals of immunology for understanding immunotherapy for lymphoma

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An effective antitumor immune response in patients with lymphoma would eradicate the malignant B cells and cure the patient of the disease. This, however, does not occur, and a suboptimal antitumor response results in persistence and subsequent progression of the patient's disease. The goals of immunotherapy are therefore to restore an effective antitumor immune response by promoting immune recognition, optimizing immune activation, and supporting persistence of the immune response resulting in subsequent immunological memory. Multiple mechanisms, however, are present within the tumor microenvironment that account for an inadequate immune response. These include loss of major histocompatibility complex expression on tumor cells and subsequent inadequate antigen presentation, increased expression of immunosuppressive ligands on malignant cells, populations of immune cells with suppressive function present in the tumor, and cytokines secreted by the malignant cell or other cells in the microenvironment that promote immune exhaustion or suppress the immune response. Successful immunotherapeutic strategies are specifically addressing these issues by promoting antigen presentation, improving recognition of the malignant cell, directly activating T cells and natural killer cells, and blocking immune checkpoint signaling that would suppress the immune response. Many of these approaches have proven highly successful in patients with various subtypes of lymphoma and are now being incorporated into standard clinical practice.

LEARNING OBJECTIVES

- Define the components of an effective T-cell–mediated immune response
- Identify deficiencies in the immune response in lymphoma
- Describe strategies to overcome the immune deficiencies including the use of immune checkpoint blockade, bispecific molecules, CD47/signal-regulatory protein α blockade, and chimeric antigen receptor T cells

Case presentation

To illustrate the potential clinical efficacy of modulating the immune system, the case of an 83-year-old male patient, initially diagnosed in August 2015 with diffuse large B-cell lymphoma (DLBCL), activated B-cell type, is presented. At diagnosis, the patient presented with extensive bone marrow involvement and was treated with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone chemotherapy for 6 cycles. He only achieved a partial remission, and a repeat bone marrow biopsy done at the end of therapy showed residual DLBCL. The patient was then treated with a combination of a Bruton tyrosine kinase inhibitor, acalabrutinib, plus an anti–programmed death-1 (PD-1) antibody, pembrolizumab, on a clinical trial as neither agent is approved for this indication. The hypothesis for using this combination was that acalabrutinib, similar to ibrutinib, may inhibit both Bruton tyrosine kinase and interleukin 2 (IL-2)-inducible T-cell kinase, thereby promoting a TH1-dominant T-cell response, whereas pembrolizumab would prevent suppression of activated T cells by blocking PD-1 signaling. The patient responded well to the combination and achieved a complete remission based on a negative bone marrow biopsy and a negative positron emission tomography scan. Per protocol, treatment was then discontinued and the patient was observed. In January 2019, the patient had biopsy-proven evidence of disease progression with multiple bone lesions seen on positron emission tomography scan. The patient was retreated with pembrolizumab alone, again achieved a complete remission, and remains on treatment and in complete remission to date.

This clinical case illustrates a number of important immunological points. First, intratumoral T cells are able to recognize and suppress the malignant B-cell clone when inhibitory signals that downregulate their function are

blocked. Second, despite the intratumoral T cells suppressing the malignant clone, they do not appear to eradicate all malignant cells and patients commonly progress when treatment is stopped. Third, as the disease recurs, intratumoral effector cells remain susceptible to suppression, and fourth, no immunological memory appears to be generated by exposure to the B-cell malignancy. All of these issues need to be addressed if effective and curative immunotherapy is to be developed.

What constitutes an effective antitumor T-cell response in lymphoma?

Immune homeostasis, and, particularly, T-cell activation due to engagement with presented antigens, is a very closely regulated process not only to ensure lysis of infected or malignant cells, but also to prevent autoimmunity and indiscriminate tissue destruction.[1-3] Although it is incredibly important for the immune system to be activated by threats to the host including pathogens and malignancies, it is equally important for the immune response to be regulated so that the level of activation remains appropriate to the threat. As the threat is contained, it is important for activation and cytotoxicity to proportionally decrease. To achieve this, there is a complex network of antigen-presenting cells (APCs) and immune-regulatory cells, all of which express or secrete immune-activating and -suppressing ligands, which modulate the immune response.

In patients with malignancies, such as lymphoma, optimal activation of, particularly, effector T cells is a complex process requiring 2 activating signals.[3] The first signal is typically provided by recognition by the T-cell receptor of a tumor antigen presented in the context of the major histocompatibility complex (MHC) molecules expressed either on the surface of APCs or the lymphoma cell itself. Optimal activation of the T cell then requires a second costimulatory signal that is typically provided by engagement between B7 molecules, including B7-1 (CD80) and B7-2 (CD86), on APCs or lymphoma cells, and CD28 on the T cell.[4] Receipt of these 2 signals results in activation, differentiation, and expansion of effector T cells (Table 1). Failure to deliver a specific second activating signal, despite the appropriate presentation of tumor antigens, typically results in T-cell anergy and subsequent apoptosis.

The activation process can be modulated by additional costimulatory or coinhibitory signals that may be delivered to the T cell to further fine-tune immune stimulation and the subsequent activation of the cell.[4-7] Activation of T cells typically results in upregulation of receptors that are primed to receive these immune-modulatory signals. Additional activating signals may be provided through CD137 (4-1BB), CD134 (OX40), CD27, and glucocorticoid-induced tumor necrosis factor receptor. In contrast, inhibitory signals are typically delivered through cytotoxic T-lymphocyte antigen 4 (CTLA4), PD-1, B- and T-lymphocyte attenuator (BTLA), lymphocyte-activation gene 3, T-cell immunoglobulin mucin-3, and T-cell immunoreceptor with immunoglobulin and ITIM domains.[5] Additional activating and inhibitory receptors have been described and are also likely to be important. Activation of T cells results in upregulation of inhibitory signals in particular to regulate the intensity of the immune response. Signaling through these inhibitory signals suppresses immune activation, as inhibition of an activated T-cell response is quite appropriate after effective eradication of a biological threat.

Aside from appropriate activation and subsequent suppression of T cells, additional mechanisms are also important to optimize the antitumor immune response in lymphoma.[3] Activated T cells need to proliferate to ensure adequate numbers are present to overcome the threat. Furthermore, tumor-specific T cells need to persist to ensure that malignant cells can be continually inhibited should they show any sign of re-expansion after initially being suppressed by the effector T cells. Ideally, immunological memory needs to develop so that effector T cells can rapidly re-expand should dormant malignant B cells reactivate and become a threat to the patient.

Table 1. Necessary components of an effective antitumor immune response

Components
• Recognition of tumor antigens as foreign
• Activation and expansion of immune effector cells
• Persistence of activated cells until all tumor cells are lysed
• Immunological memory to prevent recurrence

Table 2. Mechanisms responsible for the inadequate immune response in lymphoma

Mechanisms
• Loss of MHC expression on malignant B cells
• Increased expression of immunosuppressive ligands on lymphoma cells
• Suppressive immune cell populations in the TME
• Secreted cytokines that promote immune exhaustion or suppression

Mechanisms accounting for an inadequate immune response

The tumor microenvironment (TME) in patients with lymphoma appears to be an ideal niche for an adequate antitumor immune response.[8-11] There is typically a substantial presence of immune cells in close proximity with the malignant clone and both APCs and malignant B cells are commonly located close to effector T cells. Despite this, malignant cells do not appear to be suppressed by the immune response and commonly continue to proliferate, resulting in disease progression.

Prior research has shown that there are multiple mechanisms that account for the lack of an effective immune response to the malignant B-cell clone (Table 2). A substantial issue in both Hodgkin lymphoma and aggressive non-Hodgkin lymphomas is loss of MHC class I and class II molecules.[12-17] In Hodgkin lymphoma, decreased or absent expression of MHC class I may be present in ~75% of patients, whereas decrease or loss of MHC class II expression may be identified in one-third of patients. Decreased expression of MHC class I molecules particularly has been shown to be associated with decreased progression-free survival in patients treated with standard therapy.[12] Similarly, loss of MHC class II molecules in DLBCL, primary mediastinal large B-cell lymphoma, and aggressive lymphomas in immune-privileged sites, is associated with an inadequate immune response and a poor outcome.[14-17] Additional molecular and genetic changes in the tumor cell may further compromise

antigen presentation as well as the ability of the immune system to respond in an adequate fashion. These results suggest that inadequate presentation of tumor antigens due to loss of MHC molecules is a substantial barrier to an effective antitumor immune response and is an issue in multiple subtypes of lymphoma.

A second barrier to an effective antitumor immune response in lymphoma is the upregulation or overexpression of immunosuppressive ligands on the tumor cells or on other cells in the TME. Overexpression of programmed death ligand 1 (PD-L1; CD274) and PD-L2 (CD273) by malignant lymphoma cells is a mechanism by which malignant cells protect themselves from activated effector T cells and the expression is commonly driven by viral or genetic causes.[18-21] Many intratumoral T cells in lymphoma express PD-1, the receptor for these ligands, making them susceptible to PD-L1/2 signaling.[22] As PD-1 is increasingly expressed on T cells as they become activated, PD-L1 and PD-L2 signal through PD-1 to inhibit T-cell function, promote immune exhaustion, and result in subsequent T-cell apoptosis. Over time, many T cells in the microenvironment of lymphoma express multiple immune-inhibitory receptors, including PD-1, T-cell immunoglobulin mucin-3, and lymphocyte-activation gene 3, all of which are associated with immune exhaustion.[23,24] These findings suggest that many of the T cells present at sites of lymphoma are in fact suppressed or exhausted.

Despite the presence of multiple immune cell populations at sites of lymphoma involvement, many of these cell types do not target the malignant B cell but rather have a suppressive and regulatory effect on the immune response. Many intratumoral T cells express FoxP3 and CD25, are regulatory T cells with the ability to suppress both $CD8^+$ and $CD4^+$ T-cell function, and are located in close proximity to the malignant cell.[25] Malignant B cells may further induce FoxP3 expression in $CD4^+$ T cells and promote differentiation of T cells to a suppressive phenotype.[26] Monocytes and macrophages are typically abundant in the tumor and commonly promote malignant cell growth.[27] They also commonly express PD-L1 and PD-L2, which inhibit the function of effector T cells; $PD\text{-}L1^+$ macrophages may surround the malignant cell, effectively providing it with protection from effector T cells.[28] Myeloid-derived–suppressive cells (MDSCs) also increase in patients with lymphoid malignancies and the presence of these cells profoundly inhibits T-cell activation and proliferation.[29] The presence of all of these cells in the TME, therefore, counteracts the T-cell response and prevents eradication of the malignant cell.

Additionally, multiple cytokines are secreted by the malignant cell or by cells in the TME that directly suppress T-cell function or subsequently induce T-cell exhaustion. For example, IL-10 may be secreted by the malignant B cell and expands the population of MDSCs.[30] Serum IL-10 levels have been shown to be increased in patients with lymphoma, and IL-10–induced MDSCs substantially suppress T-cell proliferation. Transforming growth factor β may be expressed on the surface of lymphoma cells and results in immune suppression.[31] Transforming growth factor β may in fact activate T cells but these activated cells commonly express immune-exhaustion markers and are dysfunctional.[31] Furthermore, T-cell–activating cytokines like IL-12 may initially promote T-cell function but with sustained exposure to IL-12, T cells become exhausted and poorly functional.[23] Clearly, there are many immunological barriers to circumvent to ensure an optimal antitumor T-cell immune response.

Strategies to overcome immune dysfunction

Although there are multiple mechanisms that inhibit an effective antitumor T-cell response in lymphoma, many of these specific mechanisms are being overcome with novel immunotherapeutic strategies (Table 3). To prevent immune suppression and thereby activate intratumoral T cells, blocking immune checkpoint signaling has been a successful strategy.[8] Immune checkpoint blockade, particularly blocking PD-1 signaling, has been highly successful in patients with Hodgkin lymphoma. It is also been very effective in select non-Hodgkin lymphoma subtypes in which overexpression of PD-1 ligands is due to copy-number gain or amplification at the chromosome 9p24.1 locus or activation of the JAK/STAT pathway due to viral causes. Although blockade of other immune checkpoints such as CTLA4 and killer cell immunoglobulin-like receptor has been less effective,[32] combination approaches that include activating signals through receptors such as CD27 and CD137 may be more promising.[33,34]

A further way to activate T cells or natural killer (NK) cells and specifically direct them to lyse malignant B cells is to use molecules with bispecific binding ability. These antibodies are able to bind to both the malignant B cell and also to receptors such as CD3 or CD16 expressed on T cells and NK cells.[35,36] These agents bring the effector cells into very close proximity to the malignant cell, thereby directly activating the effector cells while also directing them specifically to the malignancy. This therapy has shown substantial clinical efficacy in single-agent trials and has also shown substantial promise when used in combination with immune checkpoint blockade.

Because loss of MHC molecules effectively "hides" the malignant cell from activated T cells, immunotherapy that promotes presentation of tumor antigens to the immune system or allows for the T cells to directly engage with malignant cells is likely to be effective. Chimeric antigen receptor (CAR) T-cell treatment introduces an engineered receptor into T cells that provides all of the machinery necessary to activate the cell.[37] The chimeric receptor construct has a binding domain that engages proteins on the lymphoma cell without the requirement for typical T-cell receptor signaling. The same receptor also includes a costimulatory domain that automatically provides the second required activation signal, thereby allowing for activation and expansion of the T cells. However, because CAR T cells are susceptible to similar suppressive signals as normal T cells, further strategies are now being tested to promote persistence of CAR T cells and to provide them with protection against suppression by blocking or removing inhibitory receptors such as PD-1.

An additional way to promote presentation of tumor antigens to the immune system, and thereby induce a more effective T-cell response, is to use a tumor-specific vaccine,[38] often using

Table 3. Clinical strategies in lymphoma to overcome immune suppression

Strategies
• Target immune checkpoints to prevent immune suppression
• Promote tumor cell phagocytosis and antigen presentation by engaging macrophages
• Improve malignant cell engagement using CARs
• Activate T cells and NK cells using bispecific antibodies

dendritic cells to promote antigen presentation. This approach allows tumor-specific antigens to be presented in the appropriate immunological context and thereby induce an adaptive immune response. A similar strategy is to induce macrophages to phagocytose lymphoma cells and thereby promote tumor antigen presentation to T cells. Blockade of the CD47 "don't-eat-me-signal" on lymphoma cells, which inhibits phagocytosis of the malignant cells by macrophages, has been found to substantially increase their ability to phagocytose malignant cells and triggers T-cell–mediated destruction of the tumor.[39] Clinically, CD47 blockade in combination with rituximab in patients with lymphomas has shown very promising results.[40]

Conclusions

Optimizing immune function in lymphoma patients is clearly a new therapeutic frontier. Although all of the components for an antitumor immune response are present at sites of lymphoma involvement, there are many immunological barriers to the response being effective. These barriers include downregulation of MHC expression on malignant cells, increased expression of immunosuppressive ligands on lymphoma cells, suppressive immune cell populations in the TME, and secreted cytokines that promote immune exhaustion. Strategies to prevent suppression and promote immune activation, as well as approaches that increase malignant cell engagement by the immune system, are all resulting in promising activity. In the future, however, we will need to consider combination approaches to comprehensively overcome all of the immune dysfunction so that we will be able to achieve durable responses that will ultimately result in cures for lymphoma patients.

Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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Immunotherapy with cells

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Both older and newer cell therapies have demonstrated impressive responses in otherwise poor-prognosis lymphomas. Consequently, cellular therapy now plays a major role in the management of many non-Hodgkin lymphomas. In this article, we examine the role of chimeric antigen receptor (CAR) T cells, allogeneic stem cell transplantation, and virus-directed T cells for treatment of lymphomas. We review the current indications for CAR T cells and discuss our clinical approach to selecting and treating patients with aggressive B-cell lymphomas to receive CD19-directed CAR T cells. In addition, we highlight newer cell therapies and provide an overview of promising future approaches that have the potential to transform immunotherapy with cells to treat lymphomas.

LEARNING OBJECTIVES

- Describe the role of CAR T cells in the treatment of lymphoma
- Identify limitations to currently available CAR T-cell therapies
- Review the role of allogeneic stem cell transplantation in B-cell lymphomas
- Discuss novel approaches to cell therapies

Clinical case 1

A 50-year-old woman with diffuse large B-cell lymphoma (DLBCL) initially received 6 cycles of R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone) chemotherapy but relapsed within 3 months. She then received 2 cycles of R-ICE (rituximab, ifosfamide, carboplatin, and etoposide), and positron emission tomography/computed tomography (PET/CT) showed stable disease. After discussing therapeutic options, including alternative chemotherapy vs chimeric antigen receptor (CAR) T cells (CAR-T), she proceeded with CAR-T. After leukapheresis for T-cell collection, she presented with rapidly enlarging lymphadenopathy. Bridging therapy with polatuzumab-bendamustine/rituximab was initiated, and, after 2 cycles, PET/CT showed an excellent partial response and a normalized level of lactate dehydrogenase (LDH). She underwent lymphodepletion with cyclophosphamide/fludarabine followed by infusion of tisagenlecleucel.

This case raises the following questions: which patients should be referred for consideration of cellular therapy, when should therapy be initiated, and what should the management considerations be for patients undergoing CAR-T?

CD19-directed CAR T cells

Aggressive B-cell lymphomas: US Food and Drug Administration–approved products

CD19-directed CAR-T is an option for patients with DLBCL, high-grade B-cell lymphoma (HGBCL), transformed follicular lymphoma (tFL), and primary mediastinal large B-cell lymphoma (PMBL) that is relapsed/refractory after 2 or more lines of therapy.

The SCHOLAR-1 study provided a benchmark for the very poor outcomes in patients with refractory DLBCL before the availability of CAR-T. Median overall survival (OS) was 6.3 months, and only 20% of patients remained alive at 2 years.[1]

The currently approved CAR-T products improve on these historical outcomes. CARs are synthetic molecules containing an extracellular single-chain variable fragment directed against a tumor antigen such as CD19, as well as a hinge region, a transmembrane domain, and an intracellular signaling domain. CAR-Ts are manufactured from T-cells and are genetically modified to express the CAR on the cell surface.[2] The "first-generation" CAR-Ts contained the CD3ζ signaling domain and had limited expansion, persistence, and antitumor activity. A major breakthrough came with the

addition of a costimulatory domain, such as 4-1BB or CD28, to the CAR molecule, resulting in dramatic improvement in expansion, persistence, and T-cell killing. CAR-Ts recognize their target in a major histocompatibility class–unrestricted manner and activate the T-cell signaling and costimulatory pathways. The CARs best studied in lymphoma are diagrammed in the accompanying visual abstract; Table 1 summarizes the properties of these CAR-Ts.

Axicabtagene ciloleucel (axi-cel) is a CD28-containing CAR-T and has been studied for relapsed/refractory DLBCL, tFL, PMBL, and HGBCL. The complete remission (CR) rate was 59%, median duration of response (DOR) was 11.1 months, and 2-year OS was 50.5%.[3,4] Tisagenlecleucel (tisa-cel) is a 4-1BB–containing CAR-T and has been studied for relapsed/refractory DLBCL, tFL, and HGBCL. After tisa-cel, 40% of patients achieved CR, median DOR was not reached, and OS was 10.3 months (Table 1).[5,6]

Responses to CAR-Ts are generally rapid and occur within 1 to 3 months. For patients in remission beyond 6 to 12 months, most of the responses remain durable, as noted at 3- and 4-year follow-ups.[7-9]

Table 1. CD19-directed CAR T-cell products for DLBCL: 1-year outcomes

	Axicabtagene ciloleucel ZUMA-1 trial[3,4]			Tisagenlecleucel JULIET trial[5,6]		Lisocabtagene maraleucel TRANSCEND NHL 001 trial[10]				
US FDA approved	Yes			Yes		No				
CAR construct	Anti-CD19, CD28, CD3z			Anti-CD19, 4-1BB, CD3z		Anti-CD19, 4-1BB, CD3z (tEGFR)				
Costimulatory domain	CD28			4-1BB		4-1BB				
Vector	Retrovirus			Lentivirus		Lentivirus				
CAR T-cell manufacturing	Bulk, fresh			Bulk, cryopreserved		$CD8^+$ and $CD4^+$ T cells: separate, fresh				
CAR T-cell dose	2.0×10^6 cells/kg, max 2.0×10^8 cells			0.6-6 × 10^8 cells		1.0 × 10^8 $CD8^+$ and $CD4^+$ cells				
Bridging therapy	No			Yes: 92%		Yes: 59%				
Lymphodepletion	Flu/Cy (30 mg/m², 500 mg/m²) × 3 d			Flu/Cy (25 mg/m², 250 mg/m²) × 3 d or bendamustine (90 mg/m²) × 2 d		Flu/Cy (30 mg/m², 300 mg/m²) × 3 d				
Secondary CNS lymphoma	No			No		Yes: small number				
ALC cutoff for manufacturing, per µL	ALC ≥100			ALC ≥300		None				
Lymphoma subtypes enrolled	DLBCL/ HGBCL	PMBL	tFL	DLBCL/ HGBCL	tFL	DLBCL	HGBCL	t-iNHL	PMBL	FL3B
Evaluable patients, n	77	8	16	89	22	137	36	78	15	3
Follow-up time, mo	15.4			14		12.3				
Efficacy, n	101			93		256				
Best ORR, % (CR%)	82 (54)			52 (40)		73 (53)				
DOR at 12 mo	11.1 mo/NR*			NR		NR (all patients)				
						5.6 mo	10.8 mo	NR (tFL)	NR	—
DOR for CR at 12 mo	NR			NR		NR				
OS at 12 mo, %	59			49		58				
Median follow-up for trial, mo	27			24		12				
Safety, n	101			111		269				
CRS ≥grade 3, %	13†			22‡		2‡				
CRS time to onset median duration (range)	2 d (range, 1-12)			3 d (range, 1-9)		5 d (range, 1-14)				
	8 d (not reported)			7 d (range, 2-30)		5 d (1-17)				
Neurotoxicity ≥grade 3, %	28			12		10				
Neurotoxicity time to onset median duration (range)	5 d (range, 1-17)			6 d (range, 1-17)		9 d (range 1-66)				
	not reported			14 d (not reported)		11 d (range, 1-86)				

Flu/Cy, fludarabine/cyclophosphamide; t-iNHL, transformed indolent non-Hodgkin lymphoma; FL3B: FL grade 3B; NR, not reached.
*Per the independent review committee.
†Graded according to the Lee scale.[5]
‡Graded according to the Penn scale.[4]

Taken together, the results suggest that at least some patients may be cured of lymphoma after CAR-T.

In our practice, patients with aggressive B-cell lymphoma are considered for anti-CD19 CAR-T when they have stable or progressive lymphoma after second-line chemotherapy, have a relapse after autologous stem cell transplantation (SCT), or require a third-line or greater therapy. Currently, patients who achieve CR and select patients with a partial response after second-line chemotherapy are offered autologous SCT. There are ongoing randomized trials comparing the role of CD19-directed CAR-T vs autologous SCT for second-line therapy (Figure 1), as well as first-line CAR-T trials for high-risk patients (www.clinicaltrials.gov #NCT03761056).

Patient selection

An important aspect of CAR-T is appropriate patient selection and management before the cells are infused. Aside from active infection at the time of CAR-T infusion, there is no absolute contraindication to CAR-T. Performance status and organ function are major considerations. Although clinical trials have enrolled only relatively fit patients (Eastern Cooperative Oncology Group performance status 0-1), real world data substantiates that patients with higher performance status can be treated safely,[10] as is also our practice. If the patient is fit, there is no age at which we deem the patient ineligible for CAR-T. Both clinical trial and real-world data have failed to demonstrate an age cutoff for those who benefit from CAR-T.[3,4,10,11] Nevertheless, cytokine release syndrome (CRS) and neurologic toxicity appear to increase with CD28-containing CAR-Ts, and we often select a CAR-T product with less toxicity in older or frail patients (Table 1). Because CRS can result in physiologic stress, including high fevers, capillary leak, and hemodynamic instability, patients must have adequate organ function reserve to tolerate potentially severe CRS. Clinical trials and real-world practice typically exclude patients with significant cardiomyopathy (New York Heart Association grades 3 and 4 or left ventricle ejection fraction <40%-45%), renal dysfunction (creatinine <1.6-2 mg/dL or creatinine clearance <40), liver disease, or poor lung function. Furthermore, given potential neurologic toxicity with all CAR-T products, we are hesitant to administer cells to patients with underlying cognitive impairment related to difficulty in monitoring for neurotoxicity. Responses do not seem to be affected by cell of origin or double-hit lymphoma status.[3,5] However, patients with uncontrolled lymphoma, reflected by tumor volume,[6,12] may have inferior outcomes. Data suggest that patients with poor performance status and elevated LDH levels

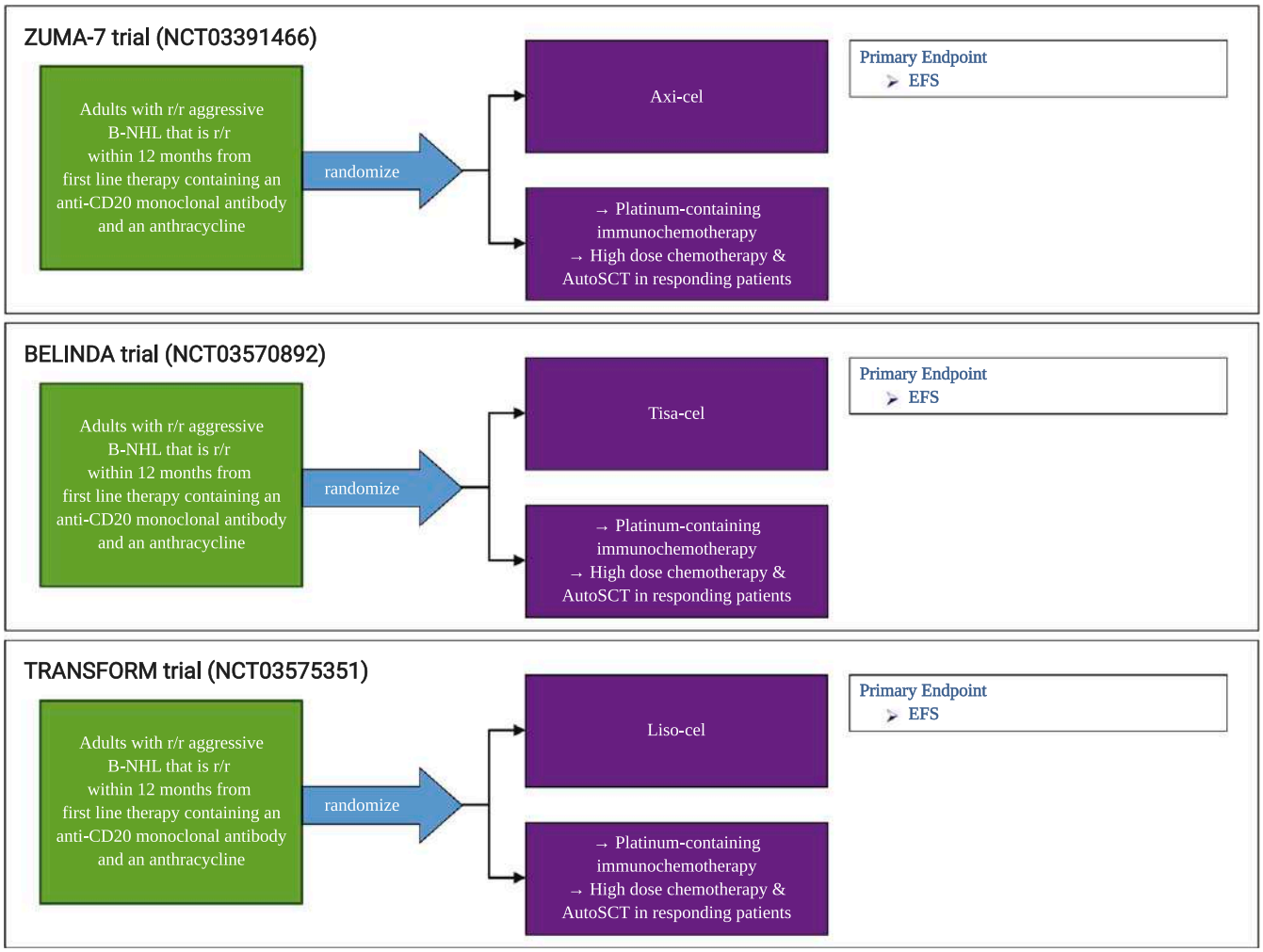

Figure 1. Randomized trials comparing autologous SCT to CAR T cells.

also have shorter PFS and OS.[10] These patients represent a high-risk group that should be addressed in future trials.

Although commercial CAR-T is not approved for primary central nervous system (CNS) lymphoma, small subsets of patients with secondary CNS lymphoma have been treated.[10,11,13] Currently, there is no evidence that these patients have a higher incidence of CNS toxicity.

Another consideration is selection of therapy for patients who require disease control. Before T-cell collection, the intent is to preserve an intact immune system, especially the number of lymphocytes. We try to avoid therapies that deplete lymphocytes, including radiation, bendamustine, corticosteroids, or cytotoxic chemotherapy, immediately before collecting a patient's T-cells for CAR-T manufacturing.

After leukapheresis, bridging therapy may be necessary to maintain control of aggressive lymphoma during commercial CAR-T manufacturing. The foremost goal of bridging therapy is to maintain functional status and organ function. Compared with therapy before T-cell collection, the choices for bridging therapy are less limited because the T-cells needed for manufacturing have already been removed from the patient. Bridging therapy can be individualized. In the current case we chose polatuzumab-bendamustine/rituximab; other options include corticosteroids, chemotherapy, radiation, monoclonal antibodies, and immunomodulatory agents. Another interesting approach that we have used for bridging therapy is ibrutinib, which is active in non-germinal center DLBCL and may also improve the number and function of T-cells.[14] Some data suggest that patients who receive bridging therapy while awaiting axi-cel have an outcome inferior to that of patients who do not receive bridging therapy.[10,15,16] Although bridging therapy may have a negative impact, it is more likely that this effect identifies a higher risk group of patients based on the need for disease control after T-cell collection. Theoretically, it is possible that immunosuppressive bridging therapy can enhance CAR-T expansion and persistence.

Post-CAR T-cell toxicity

The 2 well-described therapy-specific toxicities after CAR-T are CRS and neurologic toxicity (immune effector cell-associated neurotoxicity [ICANS]). The diagnosis of CRS requires the presence of fever. More severe cases progress to capillary leak and hypotension. Grading is dependent on presence and severity of hypotension and hypoxia.[17] Nevertheless, manifestations can affect almost any organ.[18] ICANS often manifests with headaches and various degrees of encephalopathy that adversely affect speech, level of consciousness, and motor function and can progress to seizures and cerebral edema.[17]

Known risk factors for CRS and ICANS include elevated LDH and high tumor burden before CAR-T infusion.[6,10] Management of CRS and ICANS is now more consistently defined by consensus American Society for Transplantation and Cellular Therapy guidelines.[17,19] Tocilizumab, an anti-IL6 receptor antibody, remains the mainstay of therapy for CRS. Corticosteroids are the recommended treatment for ICANS, although treatment approaches for neurologic complications are less well defined. Mitigation of CRS and ICANS is an ongoing field of study. Approaches include prophylactic or early steroids[20] and tocilizumab,[21,22] anakinra, and itacitinib.

Other toxicities after CAR-T infusion are cytopenias, infection, B-cell aplasia, and hypogammaglobulinemia. Patients in long-term remission may require intravenous immune globulin, although many patients have B-cell and immunoglobulin recovery.[4,23]

Clinical case 1 (continued)

The patient in our case was at lower risk of complications because she had a normal LDH level and decreased tumor volume after bridging therapy. She developed grade 1 CRS that spontaneously resolved. Her 3-month PET/CT showed complete remission and she continued in remission 55 months after CAR-T. Her only long-term complication was hypogammaglobulinemia. Per our institutional practice, she did not require intravenous immune globulin for the condition, because she did not have recurrent sinopulmonary infections.

Mantle cell lymphoma: FDA-approved product

Brexucabtagene autoleucel (KTE-X19) has been approved by the US Food and Drug administration (FDA) for relapsed/refractory MCL. Sixty patients previously treated with Bruton tyrosine kinase (BTK) inhibitors, anti-CD20 antibodies, and chemotherapy had an overall response rate (ORR) of 93%, a CR rate of 67%, and 1-year progression free-survival (PFS) of 61%, although longer-term follow-up is needed.[24] Toxicity was similar to that reported for axi-cel (CRS, 91%; neurologic toxicity, 63%). CAR-T appeared to be efficacious regardless of historically poor-risk groups, including those with blastoid MCL.

Newer agents and non-FDA–approved indications

Lisocabtagene maraleucel for aggressive B-cell lymphomas

In addition to axi-cel and tisa-cel, another important anti-CD19 CAR-T product is lisocabtagene maraleucel (liso-cel; JCAR017). Similar to tisa-cel, this product uses a 4-1-BB costimulatory domain and lentivirus transfection. The major difference is that equal doses of $CD8^+$ and $CD4^+$ CAR-Ts are infused sequentially. In a trial for relapsed/refractory aggressive B-cell lymphomas, the CR rate was 53%, median PFS was 12.3 months, and OS was 58%.[11] This product is currently not approved for this indication; however, the data are promising, and liso-cel may join the products that are currently commercially available for aggressive B-cell lymphomas.

In Table 1, we present a summary of the 3 CAR-T products that are furthest along in development for relapsed/refractory lymphoma. However, we stress that it is not possible to directly compare outcomes across these clinical trials. Trial design, patient characteristics and enrollment, and even toxicity grading differ across studies. Generally, responses are impressive and relatively similar for a group of patients with highly refractory lymphoma and an otherwise dismal prognosis. Toxicity varies across studies but there is low treatment–related mortality.

CD19-directed CAR T cells for chronic lymphocytic leukemia

Although CAR-T is not currently FDA approved for chronic lymphocytic leukemia (CLL), relapsed/refractory CLL was one of the first diseases treated with it. We tested anti-CD19 CAR-Ts (ultimately developed as tisa-cel) in 14 heavily pretreated patients who had relapsed/refractory CLL. Responses were durable in most patients; the first 2 patients treated remained in remission beyond 10 years.[25] Liso-cel also showed promising early results for CLL.[26] To further improve CAR-T efficacy, ibrutinib has been combined with CAR-T for CLL[27,28] based on data that ibrutinib enhances CAR-T function in CLL.[14] Optimization of CAR-T dosing may be another strategy to improve outcomes.[29]

CD19-directed CAR T cells for FL and marginal zone lymphoma

Studies have demonstrated high response rates[8,9,23,30] and long-term remissions[8,9,23] after CAR-T administration for FL (Table 2). In 80 patients with FL and 14 patients with marginal zone lymphoma that relapsed after 2 prior therapies, axi-cel treatment produced a 94% ORR with CR of 80%.[30] For the tisa-cel construct, similar FL responses were observed (80% ORR) with 60% of patients responding at 4 years.[8,23] Taken together, the limited long-term data suggest that remissions after anti-CD19 CAR-T in indolent lymphoma are durable.

One challenge in the application of CAR-T to FL and other indolent lymphomas will be determining the appropriate sequencing for therapy, given the large array of therapeutic options. CAR-T has a higher early toxicity profile than most other available noncurative therapies, but preliminarily appear to induce long-term remission.

EBV-directed cytotoxic T cells

Autologous LMP1/LMP2-directed cytotoxic T lymphocytes (CTLs) have been shown to induce durable remissions in Epstein-Barr virus (EBV)–associated lymphomas.[31] Tabelecleucel is an allogeneic T-cell product that utilizes donor-derived EBV-specific CTLs to treat EBV$^+$ post-transplant lymphoproliferative disorder. HLA restriction and matching are used to select a donor product for a given patient from a preexisting library. This therapy depends on recognition of EBV by the donor T-cell receptor, which differs from the chimeric receptor used in CAR-Ts. Posttreatment follow-ups are currently short, but early results are encouraging.[32,33] A multicenter phase 3 trial (NCT03394365) is under way.

Clinical case 2

A 56-year-old woman with HGBCL was treated with R-EPOCH (rituximab, etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin) but relapsed 6 months after concluding chemoimmunotherapy. After R-DHAP (rituximab, dexamethasone, cytarabine, cisplatin) followed by autologous SCT, she achieved CR; however, she relapsed within 6 months. She then received CAR-Ts and relapsed again, 4 months later, with diffuse adenopathy. A biopsy showed HGBCL. She received aggressive combination chemotherapy and achieved CR after 2 cycles of therapy. She was referred for consideration of allogeneic SCT (allo-SCT).

Allogeneic SCT

Allo-SCT is one of the oldest and most successful forms of cellular immunotherapies. The efficacy of allo-SCT is based, in part, on the immunologic graft-versus-lymphoma (GVL) protection provided by the donor graft.[34]

Allo-SCT may be curative in a subset of patients who have relapsed/refractory non-Hodgkin lymphoma (NHL) that is responsive to therapy; however, this potential cure is at the expense of high treatment-related mortality. In DLBCL, 503 patients from the Center for International Blood and Marrow Transplant Research database who relapsed after autologous SCT underwent allo-SCT and had a 5-year PFS of 29%, nonrelapse mortality (NRM) of 31%, and OS of 34%.[35] Another retrospective study of 396 allo-SCT recipients with DLBCL reported that myeloablative conditioning (MAC) yielded the lowest rate of relapse at 5 years (26% vs 40% for the less intense, non-MAC treatment). In contrast, MAC had the highest rate of NRM (56% vs 36% for non-MAC).[36] These results illustrate the efficacy of allo-SCT and highlight the

Table 2. Selected CD19-directed CAR T-cell trials

Trial registration no. and name	Lymphoma status	N, Lymphoma subtypes	Ongoing?	ORR, %	PFS	DOR,%	Median follow-up, mo
NCT00924326 NCI trial (axi-cel construct)[9]	R/r	N = 43 DLBCL, 28; low-grade B-NHL, 8; CLL, 7	No	—	EFS: DLBCL, 15 mo; Low grade-B NHL, 55 mo; CLL, 40.5 mo	3 y DOR: DLBCL+PMBL, 48; Low-grade B-NHL, 63; CLL, 50	42
NCT02030834 Penn trial (tisa-cel construct)[8,23]	No curative treatment options, <2-y life expectancy	N = 38 DLBCL, 24; 14 FL, 14	No	DLBCL, 50; FL, 79	DLBCL, 5.8 mo; FL, 32.4 mo	49 mo DOR: DLBCL, 60; FL, 60	49
NCT02601313 ZUMA-2[24]	R/r after BTKi, chemotherapy, and anti-CD20 antibody	N = 60 MCL (primary analysis)	No	85	61%	Not reached	12.3
NCT03105336 ZUMA-5[30]	R/r after 2 therapies, including anti-CD20 and alkylating agent	N = 94 FL, 80; MZL, 14	Yes	94	—	—	—
NCT03331198 TRANSCEND CLL 004[26]	R/r after 3 therapies if standard risk; r/r after 2 therapies if high risk; all received prior ibrutinib	N = 23 CLL	Yes	82	—	—	—

BTKi, BTK inhibitor; r/r, relapsed/refractory.

importance of selecting an appropriate conditioning regimen based on the clinical features of individual patients.

Clinical case 2 (continued)

In our practice, allo-SCT is considered for aggressive B-cell lymphoma in patients who have relapsed/refractory disease, largely after autologous SCT and recently after CAR-T, but who have responsive disease. This patient underwent reduced-intensity, matched-sibling allo-SCT. A reduced-intensity regimen was chosen because of the extent of prior therapy, including autologous SCT. After allo-SCT, she developed grade 2, skin-only graft-versus-host disease (GVHD). The disease was successfully treated with systemic corticosteroids. All immunosuppression was tapered off within 9 months of transplantation. She had no chronic GVHD and remained disease-free 3 years after allo-SCT.

Efficacy of allogeneic SCT in other lymphomas

Allo-SCT may also be an effective cell therapy for select patients with relapsed indolent lymphoma in whom autologous SCT has failed, in whom bone marrow involvement is extensive, or in whom adequate autologous stem cells are not available.[37] Compared with patients who underwent autologous SCT, patients who received allo-SCT had longer PFS (48% vs 57%, respectively), but 5-year OS was similar (72% autologous SCT vs 67% allo-SCT). These and other FL data suggest that allo-SCT is a potentially curative treatment in select patients.[37] Allo-SCT has also been an effective cell therapy for select patients with other subtypes of lymphoma, including MCL[37,38] and TCL.[39,40]

The results of donor lymphocyte infusions (DLIs) in patients who relapse after allo-SCT also provide direct evidence of GVL induction by cellular immunotherapy. In patients with relapsed lymphoma after allo-SCT, DLI is effective in a minority, although it is also associated with significant GVHD.[41] More targeted cell therapies may provide antitumor activity with less nonspecific toxicity.

Future directions

Limitations and new targets

Despite the unprecedented results of autologous CAR-T, the therapy requires that each product be manufactured specifically for an individual, making manufacturing complex and expensive. The manufacturing of cells can take between 2 and 5 weeks, and may be unsuccessful. Patients with highly refractory lymphoma may develop progressive disease while awaiting CAR-T. Furthermore, heavily pretreated patients may have dysfunctional T cells, which may lead to manufacture of autologous CAR-Ts with poor efficacy.[42]

Moreover, at least half of patients ultimately do not have long-term responses to anti-CD19 CAR-T. Dual antigen–directed therapies are being studied to address the loss of the CD19 target antigen and to improve tumor cytotoxicity. There are multiple ways to achieve this goal, including tandem CARs (a single CAR with 2 linked single-chain variable fragments with different affinities in which T-cell activation occurs only when target cells coexpress both targets, such as CD19 and CD20), bicistronic CARs (one vector genetically modifies the T-cell to express 2 CARs, such as CD19 and CD20), and CAR pools (infusion of separately manufactured CAR-Ts with different specificities). There are also multicenter trials investigating CAR-T in combination with other active agents to increase CAR-T efficacy, such as CAR-T with checkpoint blockade, BTK inhibitors, or immunomodulatory agents (Table 3).

Allogeneic CAR T cells

For patients with relapse after allogeneic SCT, donor-derived CAR-T has been effective, with minimal GVHD.[43]

Another allogeneic approach to CAR-T is to use a premanufactured, "off-the-shelf," or universal, immediately available product. Donor T-cells presumably are free of prior exposure to cytotoxic therapies and may be more "fit" than a patient's autologous T-cells. Because the cells can be premanufactured in bulk, there are also potential economic advantages.[44]

Theoretical complications from the use of allogeneic CAR-Ts are rapid rejection related to limited HLA matching and induction of GVHD. Gene engineering techniques are being developed and studied to produce allogeneic T cells that express a CAR while knocking out HLA expression, to limit rejection, and the native T-cell receptor, to limit the risk of GVHD.[45]

CAR NK cells

Allogeneic natural killer (NK) cells have been safely administered as adoptive immunotherapy for various malignancies.[37] Cell engineering techniques are now available to generate and expand CAR-modified NK cells for clinical use. NK cells derived from umbilical cord blood have been engineered to express an anti-CD19 CAR with IL-15 to enhance in vivo expansion and persistence of the CAR NK cells.[46] Importantly, in that study, no patient developed CRS, neurotoxicity, or GVHD. Most patients received additional therapy; thus, it is not yet possible to assess the DORs after CAR NK cells.

Conclusion

Scientific breakthroughs and rigorous clinical trials have led to rapid progress in cell therapy for B-cell lymphomas. The powerful GVL effect of allo-SCT and DLI provided evidence years ago that cellular immunotherapy could be effective for lymphoma. CAR-modified immune cells represent a more targeted, specific, and safer approach to use the immune system to treat, and likely cure, otherwise incurable lymphomas. We anticipate that CAR-T will be approved for other types of lymphoma in the future.

Table 3. Selected CAR T-cell combination trials

Clinicaltrials.gov registration no.	Trial	Lymphoma subtypes	Regimen
NCT02926833	ZUMA-6	DLBCL	axi-cel+atezolizumab
NCT03630159	PORTIA	DLBCL	tisa-cel+pembrolizumab
NCT03310619	PLATFORM	DLBCL, FL 3B, EBV+DLBCL, PMBL, HGBCL, T-cell histocyte-rich large B-cell lymphoma	liso-cel+durvalumab; liso-cel+CC-122; liso-cel+CC-220; liso-cel+ibrutinib
NCT03876028	—	DLBCL	tisa-cel+ibrutinib

Challenges are to decrease toxicity while improving efficacy, cost, and availability of CAR-T products. Novel transplantation approaches, allogeneic CTLs, new CAR constructs, novel target antigens, universal CAR-T or CAR NK cells, and CAR-T in combination with other agents all will be developed and tested to further harness the potential of immune cells for use in treatment of patients with lymphoma.

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Conflict-of-interest disclosure

E.A.C. has received honoraria from Juno Therapeutics and Kite Pharma. D.L.P. has received honoraria from Novartis, Kite, Incyte, and Janssen; royalties from Novartis as a patent inventor for CTL019 (CAR T cells for CD19 disease, managed according to University of Pennsylvania patent policy); and research funding from Novartis. His spouse was formerly employed by Genentech.

Off-label drug use

None disclosed.

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Immunotherapy with drugs

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The treatment of lymphomas has undergone a shift in the last few decades, from traditional cytotoxic chemotherapy toward immune-targeting agents that supplement or, in some cases, even supplant direct tumor killing with activation of antitumor systemic immunity. Since the introduction of the first known immunomodulatory modality, allogeneic hematopoietic cell transplantation, multiple immunotherapeutic approaches have been developed including monoclonal antibodies (mABs), antibody-drug conjugates, bispecific T-cell engagers, checkpoint inhibitors, small molecule inhibitors, chimeric antigen receptor (CAR) T-cell therapies, and vaccines. Many of these agents, either as monotherapies or as a component of a combination strategy, have shown impressive results, combining efficacy with tolerability. Immunotherapy ranging from mABs to checkpoint inhibitors and CAR T-cell therapy are now integrated into lymphoma treatment from the earliest lines of therapy to the relapsed and refractory setting for both Hodgkin (HL) and non-Hodgkin lymphoma (NHL). Although further studies are needed to improve our understanding of the unique side effects of immunomodulation, to determine the optimal sequence and combinations of these agent with targeted therapies and standard chemotherapy, and to identify predictive biomarkers, they clearly represent a growing list of treatment options for both HL and NHL and an important step on our road toward cure of these diseases.

LEARNING OBJECTIVES

- Understand different immunotherapeutic approaches being explored in Hodgkin and non-Hodgkin lymphoma, including monoclonal antibodies, antibody-drug conjugates, bispecific T-cell engagers, immune checkpoint inhibitors, and small molecule inhibitors
- Review clinical efficacy and safety data for these approaches in frontline and relapsed or refractory settings

Case presentation: part 1

A 53-year-old woman, diagnosed with stage III classical Hodgkin lymphoma (cHL) and treated with 6 cycles of doxorubicin, bleomycin, vinblastine, and dacarbazine, presented with a relapsed disease 7 months after completing therapy. She was treated with 2 cycles of salvage chemotherapy, ifosfamide, carboplatin, and etoposide, followed by an autologous stem cell transplantation (ASCT). One year after ASCT, she had a second relapse that was salvaged with brentuximab vedotin (BV); she subsequently underwent allogeneic hematopoietic cell transplantation (allo-HCT) from a matched related donor. Now relapsed 1 year after allo-HCT, she presents to discuss treatment options for her third relapse.

Introduction

The treatment of lymphomas has undergone a shift in the last few decades, from traditional cytotoxic chemotherapy to chemoimmunotherapy with the addition of rituximab, and more recently toward immune targeting of the tumor cells or the tumor microenvironment (TME). Since the introduction of the first known immunomodulatory modality, allo-HCT, as a treatment option for relapsed or refractory (R/R) lymphoma, multiple immunotherapeutic approaches have been developed including antibodies, checkpoint inhibitors, chimeric antigen receptor (CAR) T-cell therapies, and vaccines (Figure 1). This article focuses on the role of immunologic antitumor therapies in Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) and reviews the clinical results of recently developed agents. Discussion of cell-based approaches including vaccines, CAR T-cell therapies and HCT is covered in separate articles.

Monoclonal antibodies

Since the type I anti-CD20 monoclonal antibody (mAB) rituximab changed the therapeutic landscape of B-cell NHL in the 1990s, there have been multiple attempts to improve

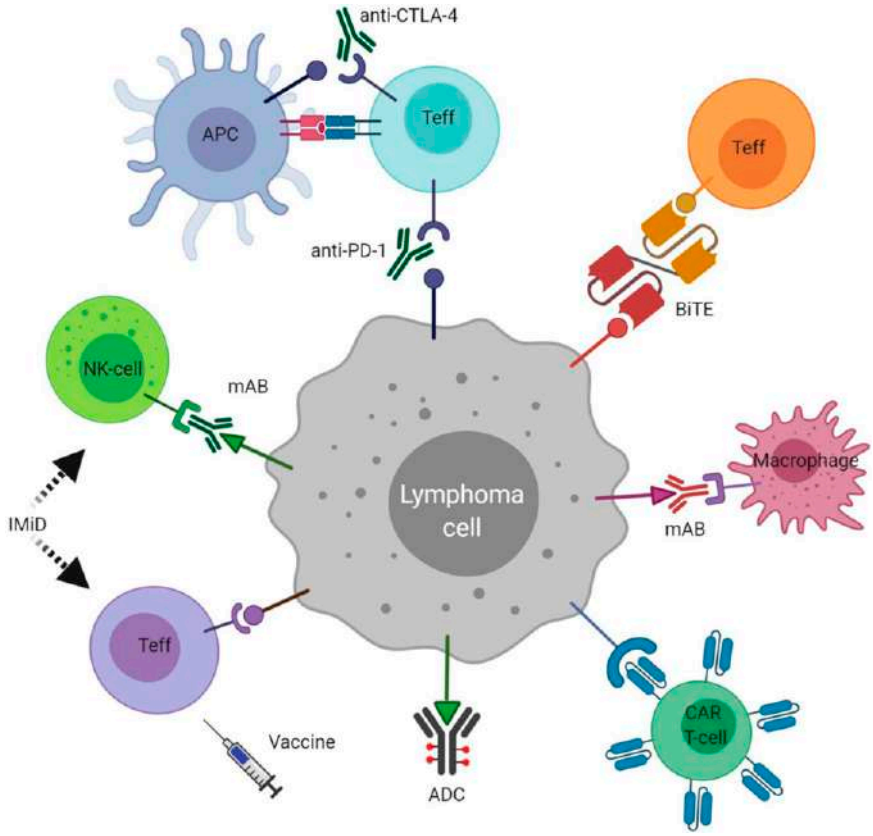

Figure 1. Immune optimization in lymphoma. ADC, antibody-drug conjugate; APC, antigen-presenting cell; BiTE, bispecific T-cell engager; CAR T-cell, chimeric antigen receptor T-cell; CTLA-4, cytotoxic T-lymphocyte antigen 4; IMiD, immunomodulatory drug; mAB, monoclonal antibody; NK-cell, natural killer cell; PD-1, programmed cell death-1; Teff, effector T-lymphocyte.

the efficacy and the duration of tumor killing with CD20-targeting mABs. Ofatumumab, a type I humanized mAB, binds to a different epitope with higher affinity and improved complement-dependent cytotoxicity. Despite these intended enhancements, efficacy in lymphomas has been limited, and ofatumumab's only approved indication in hematologic malignancies is in chronic lymphocytic leukemia. Selected studies of novel monoclonal antibodies in lymphoma are shown in Table 1. Obinutuzumab is a glycoengineered type II mAB with increased binding affinity to the FcγRIII receptor on immune effector cells[1] and decreased FcγRIIb-mediated internalization of CD20 in lipid rafts,[2] leading to enhanced antibody-dependent cell-mediated cytotoxicity (ADCC). In the GALLIUM trial, obinutuzumab, combined with chemotherapy followed by 2 subsequent years of maintenance, had a superior 3-year progression-free survival (PFS) compared with rituximab for the frontline treatment of follicular lymphoma (FL), although no overall survival (OS) advantage was observed.[3] Improved outcome was also observed in patients with indolent NHL refractory to rituximab in the GADOLIN trial, with a significantly longer PFS for the bendamustine-obinutuzumab combination compared with bendamustine monotherapy.[4] Despite promising results in indolent lymphomas, obinutuzumab with cyclophosphamide, doxorubicin, vincristine, prednisone has not demonstrated superior outcome over the rituximab combination (R-CHOP) in the frontline treatment of diffuse large B-cell lymphoma (DLBCL).[5]

In R/R setting, CD19, which is highly expressed on malignant B cells, is an attractive alternative target in B-cell lymphomas as it continues to be expressed in setting of CD20 downregulation, a primary resistance mechanism in rituximab-refractory disease.[6] Tafasitamab is a humanized mAB directed against CD19 with an engineered Fc domain to decrease the binding affinity to inhibitory receptor FcγRIIa and increase its binding to stimulatory FcγRIIIa on the effector cells, resulting in more potent ADCC. Single-agent tafasitamab has shown promising antitumor activity and favorable safety profile in a phase IIa study of R/R NHL patients, including those with rituximab-refractory disease, with an overall response rate (ORR) of 26% in DLBCL.[7] Tafasitamab has also shown encouraging antitumor activity in combination with lenalidomide in R/R DLBCL in the phase II L-MIND study, with an ORR of 54% and a complete response (CR) rate of 32%.[8]

CD47 is an antiphagocytic protein with increased expression in NHL cells compared with normal B cells. Overexpression of CD47 by lymphoma cells enables immune evasion of antitumor macrophages and has been shown to be an independent predictor of unfavorable clinical outcomes in multiple NHL subtypes including DLBCL and FL. Antibodies to CD47 block the interaction between CD47 and its ligand SIRPα on macrophages, enhancing recognition and phagocytosis of lymphoma cells. CD47-targeting mABs are under investigation both as single agents and in combination in T- and B-cell NHL. Magrolimab, a humanized mAB against CD47, has demonstrated an ORR of 50% and a CR rate of 36% in combination with rituximab in a heavily pretreated population of DLBCL and FL patients in a phase Ib/II study while causing no clinically significant safety events.[9]

Table 1. Selected studies of monoclonal antibodies in lymphoma

mAB	Target	Study phase	Patient population	Outcome(s)
Obinutuzumab	CD20	III[3]	Previously untreated FL	3-y PFS: 80.0% (obinutuzumab-based chemotherapy) vs 73.3% (rituximab-based chemotherapy)
				ORR: 88.5% vs 86.9%
				Grade 3-5 AEs: 74.6% vs 67.8%
				SAEs: 46.1% vs 39.9%
		III[4]	Indolent NHL refractory to rituximab	Median PFS: not reached (obinutuzumab plus bendamustine) vs 14.9 mo (bendamustine monotherapy)
				Grade 3-5 AEs: 68% vs 62%
				SAEs: 38% vs 33%
Tafasitamab	CD19	IIa[7]	R/R NHL	Tafasitamab monotherapy
				ORR: 26% (DLBCL), 29% (FL), 27% (other)
				AEs: IRR (12%), neutropenia (12%)
		II[8]	R/R DLBCL	Tafasitamab with lenalidomide
				ORR of 54%, CR rate of 32%, PR rate of 22%
				Treatment-related SAEs: infections (10%), neutropenic fever (5%)
Magrolimab	CD47	Ib/II[9]	R/R NHL	Magrolimab with rituximab
				TRAEs: chills (41%), headache (41%), anemia (41%), IRR (36%)
				ORR of 50%, CR rate of 36%
Mogamulizumab	CCR4	III[10]	R/R CTCL	Median PFS: 7.7 (mogamulizumab) vs 3.1 mo (vorinostat)
				Grade 3-4 AEs: 41% vs 41%

AEs, adverse events; CR, complete response; CTCL, cutaneous T-cell lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; IRR, infusion-related reaction; mAB, monoclonal antibody; NHL, non-Hodgkin lymphoma; ORR, overall response rate; PFS, progression-free survival; PR, partial response; SAEs, serious adverse events; TRAEs, treatment-related adverse events.

In T-cell lymphomas, C-C chemokine receptor 4 (CCR4), which is highly expressed on the surface of tumor cells in mature T-cell malignancies including cutaneous T-cell lymphoma (CTCL), is an attractive therapeutic target. Mogamulizumab, a mAB against CCR4, is now approved for R/R mycosis fungoides (MF) and Sézary syndrome (SS) based on the data from a phase 3 randomized trial that demonstrated a superior PFS compared with vorinostat (7.7 vs 3.1 months) and comparable toxicity profile.[10]

Antibody-drug conjugates

In an effort to deliver cytotoxic agents selectively to tumor cells, antibody-drug conjugates (ADCs) have been developed. An ADC consists of a tumor-specific mAB conjugated to a tumor-killing agent that is released after internalization of the ADC. Several ADCs have shown activity in lymphoid malignancies, selected studies are shown in Table 2. Brentuximab vedotin targets the CD30-expressing HRS cells in HL with the antimicrotubule agent monomethyl auristatin E as its payload. Since its approval by the US Food and Drug Administration in 2011 in R/R cHL, BV has gained additional indications in CD30-expressing lymphomas including in HL (post-HCT consolidation therapy, first-line advanced-stage in combination with chemotherapy) and in peripheral T-cell lymphoma (untreated CD30-expressing peripheral T-cell lymphoma, relapsed systemic anaplastic large cell lymphoma, and relapsed primary cutaneous anaplastic large cell lymphoma and CD30-expressing MF after failure of prior systemic therapy).

In hairy cell leukemia (HCL), moxetumomab pasudotox, a recombinant immunotoxin with anti-CD22 mAB and truncated *Pseudomonas* exotoxin, is approved for the treatment of HCL relapsed after at least 2 systemic therapies, based on a phase 3 trial that showed a durable CR rate of 30% and 85% of complete responders achieving minimal residual disease negativity by immunohistochemistry.[11]

Polatuzumab vedotin, recently approved in combination with the alkylating agent bendamustine and rituximab (BR) for R/R DLBCL, uses the same toxic payload as BV and targets CD79b-expressing B cells. The approval was based on phase 2 data demonstrating improved ORR and CR rate with the addition of polatuzumab to BR.[12] Polatuzumab is also being studied in a novel combination with the mAB obinutuzumab and the immunomodulatory drug (IMiD) lenalidomide in R/R FL and in R/R DLBCL. For the frontline treatment of DLBCL, polatuzumab is being evaluated in a combination with rituximab, cyclophosphamide, doxorubicin and prednisone compared with R-CHOP in a phase 3 trial[13] and may challenge R-CHOP as the long-standing standard of care in newly diagnosed DLBCL patients (Table 5).

Bispecific T-cell engagers

With the improved understanding of the immunomodulatory effects of antibodies on the TME, bispecific antibodies that bind two different antigens have been developed to promote the engagement of peritumoral effector cells with tumor cells. A subtype of bispecific antibodies, bispecific T-cell engager

Table 2. Selected studies of antibody-drug conjugates in lymphoma

ADC	Target	Study phase	Patient population	Outcome(s)
Brentuximab vedotin	CD30	II[38]	R/R cHL after ASCT failure	BV monotherapy
				ORR of 75%, CR rate of 34%
				Median PFS of 5.6 mo
				Median DoR of 20.5 mo
				TRAEs: peripheral sensory neuropathy (42%), nausea (35%), fatigue (34%), neutropenia (19%), diarrhea (18%)
		III[39]	R/R cHL after ASCT	Median PFS: 42.9 (BV) vs 24.1 mo (placebo)
				AEs: peripheral sensory neuropathy (56% vs 16%), neutropenia (35% vs 12%)
		III[40]	Previously untreated stage III or IV cHL	2-y modified PFS: 82.1% (A+AVD) vs 77.2% (ABVD)
				AEs: neutropenia (58% vs 45%), peripheral neuropathy (67% vs 43%), grade 3 or higher pulmonary toxicity (< 1% vs 3%)
Moxetumomab pasudotox	CD22	III[11]	R/R HCL after at least 2 therapies	Moxetumomab pasudotox monotherapy
				Durable CR rate of 30%, CR rate of 41%, ORR of 75%
				AEs: peripheral edema (39%), nausea (35%), fatigue (34%), headache (33%)
				Treatment related SAEs: HUS (7.5%), capillary leak syndrome (5%)
Polatuzumab vedotin	CD79b	II[12]	R/R DLBCL	Nonhematologic AEs: diarrhea (41% with pola+BR vs 21% with BR), infections (39% vs 41%), fatigue (36% vs 28%), pyrexia (33% vs 23%), IRR (31% vs 21%), peripheral neuropathy (39% vs 3%)
				Hematologic AEs: neutropenia (54% vs 39%), thrombocytopenia (49% vs 23%), anemia (44% vs 15%)
				Grade 5 AEs: 18% in each arm
				ORR: 70% vs 33%
				CR: 58% vs 20%
				Median DoR: 8.8 vs 3.7 mo

A+AVD, BV-doxorubicin-vinblastine-dacarbazine; ABVD, doxorubicin-bleomycin-vinblastine-dacarbazine; ADC, antibody-drug conjugate; AEs, adverse events; ASCT, autologous stem cell transplantation; BR, bendamustine-rituximab; BV, brentuximab vedotin; cHL, classical Hodgkin lymphoma; CR, complete response; DLBCL, diffuse large B-cell lymphoma; DoR, duration of response; HCL, hairy cell lymphoma; HUS, hemolytic uremic syndrome; IRR, infusion-related reaction; ORR, overall response rate; PFS, progression-free survival; pola, polatuzumab vedotin; SAEs, serious adverse events; TRAEs, treatment-related adverse events.

(BiTE), links 2 antibody fragments, one specific for an antigen on tumor cells and the other that binds a surface antigen on T cells, and brings the 2 cells in close proximity, triggering T-cell cytotoxicity without a need for costimulation. Table 3 shows selected studies of bispecific T-cell engagers in lymphoma. Despite its success in R/R B-cell acute lymphoblastic leukemia, the efficacy of blinatumomab, a CD3/CD19 BiTE composed of tandem single-chain variable fragments,[14] in NHL, it has been overshadowed by significant neurotoxicity. Mosunetuzumab, a BiTE targeting CD3 and CD20, has shown promising activity and tolerable side effects in a phase 1/1b dose escalation and expansion study in heavily pretreated FL and DLBCL patients, including those who had failed CAR T-cell therapy. Response rates (ORR and CR) were 64.1% and 42.2%, respectively, for indolent NHLs and 34.7% and 18.6%, respectively, for aggressive NHLs.[15] Neurotoxicity and cytokine release syndrome (CRS), common sconcern with blinatumomab and CAR T-cell therapy, were reported in 44% and 28.4% of patients, respectively, with most toxicity grades 1 to 2, transient and reversible. The study was modified to include step-up dosing with ascending doses of mosunetuzumab during cycle 1; this appeared to mitigate CRS and neurotoxicity further. The structure of mosunetuzumab, a full-length humanized IgG1 molecule with a near-native antibody structure and an Fc region engineered to enhance its pharmacokinetic properties, increases its serum half-life,[16] obviating the need for a continuous infusion that is required with other BiTEs such as blinatumomab. Another CD3/CD20 BiTE, CD20-TBC, is designed to increase avidity for tumor antigen with binding of Fab regions of CD20 and CD3 in a 2:1 configuration and to increase half-life with a modified heterodimeric Fc region. CD20-TCB has shown clinical activity and safety as a single agent in heavily pretreated NHL patients; in a dose-escalation study, the ORR was 47% including CRs in 34% of patients, and CRS occurred in 55% of patients, primarily grades 1 to 2.[17]. In addition, CD20-TCB has been safely combined with another CD20-targeted agent, obinutuzumab, in a phase 1b study of R/R B-cell NHL, with only 8% of patients experiencing grade 3 or higher CRS.[18] REGN1979, another CD3/C20 BiTE, is also being studied in R/R NHL and has shown activity with a tolerable safety profile as a single agent.[19]

Case presentation: part 2

You discuss treatment options with your multiple-relapsed HL patient including BV retreatment, checkpoint inhibitor, standard

Table 3. Selected studies of bispecific T-cell engagers in lymphoma

BiTE	Target	Study phase	Patient population	Outcome(s)
Mosunetuzumab	CD20/CD3	I/Ib[15]	R/R B-cell NHL	CRS in 28.4% including 27.1% grade 1-2
				NAEs in 44% including 40.8% grade 1-2, headache (14.7%), insomnia (10.1%), and dizziness (9.2%)
				ORR of 64.1% and CR rate of 42.2% for indolent NHL, ORR of 34.7% and CR rate of 18.6% for aggressive NHLs
CD20-TCB	CD20/CD3	I[17]	R/R NHL	CD20-TCB monotherapy
				CRS in 55.1% including 22% grade 1 and 31% grade 2, pyrexia in 34.7% and neutropenia in 34.7%
				ORR of 47%, CR rate of 34% in aggressive NHL patient
	CD20/CD3	I/Ib[18]	R/R B-cell NHL	CD20-TCB with obinutuzumab
				AEs: anemia (21%), thrombocytopenia (21%), neutropenia (14%), pyrexia (14%), hypokalemia (14%)
				CRS in 57% of patients including 50% grade 1-2, rare NAEs
				ORR of 48% and CR rate of 43%

CR, complete response; CRS, cytokine release syndrome; NAEs, neurologic adverse events; NHL, non-Hodgkin lymphoma; ORR, overall response rate; R/R, relapsed or refractory.

chemotherapy, or some combination of these agents. She chooses to enroll in a phase 1 trial of the combination of BV, ipilimumab, and nivolumab. She is treated for 24 months per protocol. Now more than 2 years from her last treatment, she remains in CR with no treatment-related toxicity.

Checkpoint inhibitors

Classical HL is an atypical hematologic malignancy, characterized by Hodgkin Reed-Sternberg (HRS) cells that account for less than 1% of cells in the affected lymph node and are dispersed in a background of extensive infiltrate of immune cells. To thrive in an immune-rich TME, HRS cells have developed multiple mechanisms to evade immune surveillance, including a high expression of PD-L1/PD-L2 as a result of copy-number amplification of the PD-L1 and JAK2 gene loci on chromosome 9p24,[20] a lack of β_2-microglobulin and MHC class I on the HRS cells[21] to dampen effector T-cell function, and a lack of MHC class II expression on the surface of HRS cells to suppress helper T-cell function.[22] In addition, Epstein-Barr virus, present in 30% to 40% of HRS cells, can promote PD-L1 expression by activating the AP1 transcription factor pathway. Table 4 highlights selected studies of checkpoint inhibitors in lymphoma. The PD-1 inhibitors, pembrolizumab and nivolumab, have shown striking antitumor activity in patients with R/R HL with a manageable safety profile[23,24] and are approved for the treatment of patients of cHL after at least 3 prior therapies (pembrolizumab) and who have relapsed or progressed after ASCT and post-ASCT BV (nivolumab). The approvals were based on phase 2 studies that showed an ORR of 69% including 22% CRs, median duration of response (DoR) that was not reached, and 6-month PFS and OS of 72.4% and 99.5%, respectively, for pembrolizumab,[23] and an ORR of 69% including 40% CRs and median DoR of 16.6 months for nivolumab.[24] Pembrolizumab was superior to BV for patients with R/R cHL who have relapsed after or are ineligible for ASCT in a phase 3 trial, which showed a median PFS of 13.2 months in the pembrolizumab arm compared with 8.3 months in the BV arm; whether this will replace BV as a standard in this space remains to be seen.[25] Combination immunotherapies, including BV and nivolumab or the cytotoxic T-lymphocyte antigen 4 (CTLA-4) inhibitor ipilimumab and a triplet regimen of BV plus ipilimumab and nivolumab, are being studied and have shown promising results in early-phase trials of R/R HL.[26,27]

In NHLs, results of checkpoint blockade studies have been less impressive. Pembrolizumab has the sole US Food and Drug Administration approval in R/R primary mediastinal large B-cell lymphoma, a subtype of DLBCL that shares features with nodular sclerosis HL and also overexpresses PD-L1,[28] for the treatment of patients who have failed 2 or more prior therapies. The KEYNOTE-170 trial supported the approval with an ORR of 45% including 13% CRs, median DoR not reached after a median follow-up time of 12.5 months, and grade 3 or 4 treatment-related adverse effects occurring in 23% of patients.[29] Pembrolizumab has shown durable antitumor activity in R/R MF and SS, subtypes of CTCL also with frequent genomic alterations in PD-L1 and PD-L2,[30] with an ORR of 38% and median DoR not reached with a median follow-up time of 58 weeks.[31] Although the response rates in NHL have been modest, there are data to suggest that checkpoint blockade therapy may sensitize some R/R patients to subsequent therapy regardless of their response to the checkpoint inhibitor.[32]

The use of checkpoint inhibitors in lymphoma poses a unique challenge in monitoring treatment response because of pseudoprogression in which imaging findings suggest progression of disease despite clinical improvements likely from increased tumor infiltration by activated immune cells in setting of immunotherapy. In a phase II study of R/R cHL, patients were allowed to continue treatment with nivolumab beyond disease progression, and 61% of such patients had stable or reduced tumor burdens.[24] This approach of treating beyond conventional disease progression appears to be safe, and a revised response criteria is needed for immune checkpoint inhibitor therapies.

Table 4. Selected studies of checkpoint inhibitors in lymphoma

Checkpoint inhibitor	Target	Study phase	Patient population	Outcome(s)
Nivolumab	PD-1	II[24]	R/R HL after ASCT failure	Nivolumab monotherapy
				ORR of 69%, CR rate of 40% CRs
				Median DoR of 16.6 mo
				Median PFS of 14.7 mo
				Grade 3-4 drug-related AEs: lipase increases (5%), neutropenia (3%), ALT increases (3%)
		I/II[27]	R/R HL	Nivolumab with BV
				ORR of 82%, CR rate of 61%
				AEs: 98%, mostly grades 1-2, IRRs in 44%
		I/II[26]	R/R HL	Nivolumab with BV
				AEs: grade 3 or higher TRAEs in 16%, grade 5 in 1.5%
				ORR of 89%, CR rate of 61%
				Median PFS not reached, with median follow-up of 2.4 y
Pembrolizumab	PD-1	II[23]	R/R HL	Pembrolizumab monotherapy
				ORR of 69%, CR rate of 22.4%
				Response lasting 6 mo or longer in 75.6%
				TRAEs: hypothyroidism (12.4%), pyrexia (10.5%)
				AEs: immune-mediated AEs and IRRs in 28.6%
		III[25]	R/R HL	Median PFS: 13.2 (pembrolizumab) vs 8.3 mo (BV)
				ORR: 65.6% vs 54.2%
				CR rate: 24.5% vs 24.2%
				Median time to response: 2.8 vs 2.8 mo
				Median DoR: 20.7 vs 13.8 mo
				Grade 3-5 TRAEs: 19.6% vs 25%
		II[29]	R/R PMBCL	Pembrolizumab monotherapy
				ORR of 45%, CR rate of 13%
				Median DoR not reached with median follow-up of 12.5 mo
				Grade 3-4 TRAEs: 23%
		II[31]	R/R MF and SS	Pembrolizumab monotherapy
				ORR of 38%
				Median DoR not reached, with median follow-up of 58 wk
				Immune-related AEs leading to treatment discontinuation in 4 patients
Nivolumab + BV +/− ipilimumab	PD-1, CD30, CTLA-4	I/II[26]	R/R HL	Ipilimumab with BV
				AEs: grade 3 or higher TRAEs in 43%, no grade 5
				ORR of 76%, CR rate of 57%
				Median PFS 1.2 y, with median follow-up of 2.6 y
		I/II[26]	R/R HL	Ipilimumab with nivolumab and BV
				AEs: grade 3 or higher TRAEs in 50%, grade 5 in 1.5%
				ORR of 82%, CR rate of 73%
				Median PFS not reached, with median follow-up of 1.7 y

AEs, adverse events; ALT, alanine aminotransferase; ASCT, autologous stem cell transplantation; BV, brentuximab vedotin; CR, complete response; CRS, cytokine release syndrome; CTLA-4, cytotoxic T-lymphocyte-associated protein-4; DoR, duration of response; IRR, infusion-related reaction; MF, mycosis fungoides; NHL, non-Hodgkin lymphoma; ORR, overall response rate; PFS, progression-free survival; PMBCL, primary mediastinal large B-cell lymphoma; R/R, relapsed or refractory; SS, Sézary syndrome; TRAEs, treatment-related adverse events.

Table 5. Selected ongoing upfront trials of immunotherapy in lymphoma

Trial ID	Study regimen	Phase	Study objective(s)
NCT03274492	Polatuzumab with R-CHP vs R-CHOP in previously untreated DLBCL	III[13]	Primary end point: investigator-assessed PFS
			Secondary end points: IRC-assessed PET/CT CR rate at EOT, EFS, 2-y PFS, and OS
NCT03907488	BV vs nivolumab plus AVD in newly diagnosed stage III-IV cHL	III	Primary end point: PFS
			Secondary end points: OS, EFS, CR rate, and TRAE rate

AVD, doxorubicin- vinblastine-dacarbazine; BV, brentuximab vedotin; cHL, classical Hodgkin lymphoma; CR, complete response; DLBCL, diffuse large B-cell lymphoma; EFS, event-free survival; EOT, end of treatment; IRC, independent review committee; OS, overall survival; PFS, progression-free survival; R-CHOP, rituximab-cyclophosphamide-doxorubicin-vincristine-prednisone; R-CHP, rituximab-cyclophosphamide-doxorubicin-prednisone; TRAE, treatment-related adverse event.

Small molecule inhibitors

Lenalidomide, an IMiD best known for its activity in multiple myeloma, has pleotropic effects on immune cells of the TME in addition to its direct antitumor mechanism via an activation of cereblon's E3 ligase. These effects may include repair of defective immune synapses and enhanced T-cell expansion resulting in improved cytotoxicity, enhancement of ADCC by natural killer cells, and increased efficiency of antigen presentation to $CD8^+$ T cells by dendritic cells.[33] Lenalidomide plus rituximab is a chemotherapy-free regimen with a PFS benefit over rituximab monotherapy in indolent lymphoma, 39.4 vs 14.1 months,[34] and is approved for the treatment of previously treated FL and marginal zone lymphoma patients. Although the pathophysiology of tumor flare reaction, seen in 10% of patients in the study, remains unclear, it likely occurs via an immunomodulatory mechanism. Lenalidomide is also used to treat patients with mantle cell lymphoma after a failure of 2 prior therapies and has shown an ORR of 28% and median DoR of 16.6 months as a monotherapy.[35]

Although not specifically designed to target immune cells in the TME, a number of small molecule inhibitors appear to have off-target immunomodulatory effects. Ibrutinib, an irreversible inhibitor of Bruton tyrosine kinase, also inhibits interleukin-2–inducible T-cell kinase, thereby tipping the balance of T helper-1 versus T helper-2 (Th1/Th2) immunity in favor of Th1-based immune activation against tumors.[36] In the class of phosphoinositide 3-kinase inhibitors, PI3Kα/δ isoform inhibition has been shown to promote $CD8^+$ T-cell activation and enhance effector T-cell function, in addition to directly targeting tumor cells.[37]

Conclusion

Since the introduction of allo-HCT, optimizing antitumor immunity has taken an expanding role in the treatment of lymphomas with many new classes of drugs such as mABs, ADCs, BiTEs, and checkpoint inhibitors, which have shown impressive efficacy and manageable tolerability. Further studies are needed to improve the understanding of the unique side effects of immunomodulation, to determine the optimal sequence and combinations of these agents, and to identify predictive biomarkers that may drive personalization, yet it is clear that they represent a growing list of treatment options for both HL and NHL patients.

Conflict-of-interest disclosure

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Off-label drug use

This article covers off-label and investigational uses of many novel drugs, and drug combinations including: investigational combinations of nivolumab, brentuximab, and nivolumab in first line in for HL, and iplimumab, magrolizumab, mogamulizumab, CD20 TCB, and polatuzumab vedotin in first line for NHL.

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Role of venous stenting for venous thromboembolism

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Endovenous stenting has emerged as the method of choice to treat iliofemoral venous outflow obstruction. It is used in patients with established postthrombotic syndrome (PTS) after previous deep vein thrombosis (DVT) to reduce symptoms of chronic pain and swelling and to aid ulcer healing in severe cases. Venous stenting is used to alleviate symptoms of obstruction in patients presenting with acute DVT, with the aim of preventing development of PTS. There is a low risk of morbidity and mortality associated with the use of endovenous stenting, and although significant advances have been made, particularly improvements in stent design for use in the venous circulation, data are lacking on beneficial long-term outcomes. Unmet research needs include optimal patient selection, anticoagulant choice and duration, best practice for postoperative surveillance, and use of validated assessment tools to measure outcomes. In this article, I address the potential benefits, as well as the challenges, of endovenous stenting.

LEARNING OBJECTIVES

- Understand the role of venous stenting in patients who present with acute deep vein thrombosis or in patients with postthrombotic syndrome
- Address the challenges associated with venous stenting

Introduction

The majority of patients presenting with venous thromboembolism (VTE) receive treatment with anticoagulation to prevent thrombus extension and embolization in order to reduce morbidity and mortality. However, up to 20% to 50% of patients develop PTS as a consequence of DVT despite anticoagulation.[1]

Symptoms of PTS include chronic limb swelling and pain, often leading to major disability and impaired QoL.[2] In severe cases, development of leg ulceration leads to significant costs for health care services. Clinical scoring systems often used as outcome measures in studies of VTE have been developed to assess the severity of PTS, including the Villalta score[3]; Clinical, Etiology, Anatomic, Pathophysiology classification[4]; and Venous Clinical Severity Score.[5]

Mechanical methods such as thrombectomy and/or local thrombolysis to remove thrombus, potentially reducing the incidence or severity of PTS in patients with symptomatic iliofemoral DVT, have been in place since the 1990s. Endovenous stenting is increasingly used as a further treatment modality for management of underlying symptoms relating to venous outflow obstruction in patients with acute or chronic symptoms of VTE (Table 1). Although it appears to be a safe and promising treatment for prevention and management of PTS, data are lacking. This article aims to update the reader on the current use of venous stenting in VTE.

Case 1

Patient 1 is a 27-year-old woman who presented with a 3-day history of left lower limb pain and extensive leg swelling. She had experienced a postpartum left-sided deep vein thrombosis (DVT) 13 years previously and received short-term anticoagulation. She had been asymptomatic since.

A venous duplex scan demonstrated acute thrombus in the left external iliac, common femoral, superficial femoral, and popliteal veins, confirmed on the basis of a computed tomographic venogram (obtained while the patient was being considered for thrombolysis). She was anticoagulated with low-molecular-weight heparin (LMWH) and had catheter-directed thrombolysis (CDT) (without venous stenting) due to persistent symptoms. Her symptoms improved within 24 hours of CDT, and she was discharged to home with rivaroxaban.

Table 1. Patient selection criteria for endovenous stenting

When to consider venous stenting
Acute DVT
Iliofemoral DVT
Symptoms present for <14 d and candidate for thrombolysis
Evidence of residual thrombus or obstruction following thrombolysis
Chronic DVT
Established PTS with significant moderate to severe symptoms using validated clinical assessment score (eg, Villalta or CEAP)
Iliofemoral stenosis or obstruction confirmed on imaging amenable to stent placement

CEAP, Clinical, Etiology, Anatomic, Pathophysiology.

She developed worsening symptoms within 2 to 3 weeks. Repeat imaging using magnetic resonance (MR) venography demonstrated an occluded left iliac vein. She can walk only short distances due to leg swelling and pain, uses crutches to mobilize, and is unable to work. She continues to take rivaroxaban (20 mg daily).

Case 2

Patient 2 is a 16-year-old boy who presented with gradual onset of right lower limb swelling and was diagnosed with iliofemoral DVT. Several small areas of ulceration were present on his shin, indicating chronic venous hypertension.

He was anticoagulated with LMWH followed by warfarin. In view of his age and presentation of acute-on-chronic DVT (leg ulceration caused by chronic venous insufficiency), it was recommended that he receive lifelong anticoagulation. His Villalta score was consistent with severe postthrombotic syndrome (PTS). His quality of life (QoL) had significantly deteriorated, and he had nonhealing leg ulcers despite treatment with compression bandaging.

Imaging demonstrated high-grade stenosis of the lower inferior vena cava and right common iliac vein. Venous stents were inserted in the inferior vena cava (IVC) and iliofemoral vein (one 14-mm × 90-mm and two 14-mm × 60-mm stents). Anticoagulation postoperatively was with warfarin and was later switched to rivaroxaban. His symptoms improved over 3 to 4 months, and his leg ulcers healed. He has intentionally lost 25 kg with the ability to exercise.

What is the history of endovascular stenting?

Endovascular stents have been in use since the 1960s. Traditionally, stents were designed for placement in the arterial system.

Endovenous recanalization of iliofemoral stenosis or occlusion with venoplasty and stent placement was first reported by Berger et al in 1995[6] and is now the treatment of choice to treat deep venous disease secondary to venous outflow tract obstruction.

Early attempts at endovenous stenting used arterial stents but were associated with high rates of reocclusion, since arterial stents are unsuitable for use in the venous system due to their small diameters and high radial force. Arterial and venous anatomies have significant differences. Venous stents need to have stent flexibility, radial strength and crush resistance (force required to compress the stent), and the ability to allow precise deployment (see Table 1).

Most endovenous stents are "bare" (ie, uncovered), but expanded polytetrafluoroethylene-covered nitinol stents and drug-eluting stents or heparin-coated stents have been developed. The Vici (Boston Scientific [Figure 1] and Venovo (BD interventional) nitinol venous stents are currently the only US Food and Drug Administration–approved stents for use in iliofemoral venous occlusive disease, but studies of other devices are underway.

When are venous stents used?

Venous stenting is used as an adjunctive treatment in patients presenting with acute iliofemoral DVT if there is a residual venous obstruction (RVOO) following thrombolysis and balloon angioplasty,[7] with the aim of restoring vein patency and preventing PTS (Table 1). There appears to be a higher incidence of PTS and VTE recurrence if balloon angioplasty is used alone in patients with RVOO.[8] Appropriate diagnostic methods to define the lesion using a combination of computed tomography or MR venogram and intravascular ultrasound (IVUS) should be used prior to treatment.[9]

Severe PTS is often due to a chronic outflow obstruction, mainly the iliac vein, since there is usually poor collateralization of this vessel. Studies suggest when patients have severe symptoms; venous stenting is indicated when the obstruction is >50%, superficial collaterals form (Figures 2 and 3), and there is reflux in the deep and/or superficial veins.[10] Femoropopliteal DVTs are best treated with anticoagulation only.

Challenges of placing venous stents

Case selection is a key factor when patients are considered for endovascular treatment. Patients presenting with acute DVT require considerations different from patients with chronic symptoms. For acute iliofemoral DVT, strict selection criteria apply to use of thrombolysis with or without venous stenting, including bleeding risk, life expectancy, anatomy of the DVT, and severity of presenting symptoms; thrombolysis is usually reserved for those presenting with clinically severe thrombosis. There is a lack of convincing data, particularly medium- to long-term outcomes, to support use of venous stents in addition to CDT. Chronic venous obstruction can be postthrombotic or nonthrombotic, secondary to a number of causes, such as intrinsic, mural, and extrinsic pathology. External compression can be from surrounding tissues or localized compression from a pulsatile artery, as seen in May-Thurner configurations (compression of the left common iliac vein by the right common iliac artery). Chronically occluded veins are usually composed of collagen and often more difficult to treat. Outcomes vary according to whether a lesion is a postthrombotic or nonthrombotic iliac vein lesion (NIVL); therefore, the timing of stenting and the choice of stent design require careful consideration. The evidence for intervention is much less clear in NIVL, and it should be avoided until there are more convincing data.

Figure 1. The Vici venous stent system.

Table 2. Ideal venous stent structure

Stent structure	Material composition: steel/nickel/titanium/nitinol stent design, cell design (laser cut vs braided)
Mechanical properties	Radical strength, radical stiffness, acute recoil, foreshortening, crush resistance
Deployment method	Self-expandable vs balloon expandable
Stent covering	Bare metal vs coated vs drug eluting

The main complication of venous stents is in-stent restenosis or occlusion. Surveillance of stented limbs with duplex ultrasound is recommended on the day after stent placement, at 6 weeks, and yearly thereafter.[11] It is our practice to perform duplex ultrasound the day after stent placement and at 6 weeks, 6 months, and yearly thereafter. If stents are >50% stenosed, reintervention, usually in the form of angioplasty to the in-stent stenosis, is required to maintain stent patency to prevent worsening of symptoms or occlusion.[11]

The risk of stent reocclusion is associated with a number of factors, including poor inflow to the obstructed vessel, external compression, or inappropriate stent design. Patient-related risk factors may include underlying thrombophilia.[12] Other risks include stent misplacement or migration, stent fracture, and bleeding, although risk of major bleeding appears to be low (<1%).[13]

Patients presenting for surgical intervention usually have significantly limited function and a severe Villalta score before intervention. When venous stenting is offered, decision-making should be based on the patient's premorbid condition, anatomical extent of disease, and likelihood of symptomatic improvement. Patients need to be advised of the potential to reintervene and risks of bleeding and in- stent thrombosis.

What is the evidence for use of venous stenting in acute DVT?

Current American College of Chest Physicians, National Institute for Health and Care Excellence, and European Society of Cardiology guidelines do not provide recommendations for endovenous stenting after CDT or pharmacomechanical thrombectomy, likely due to lack of evidence. Most studies are retrospective, cohort series, or smaller trials with variable study design. Earlier studies used arterial stents rather than dedicated venous stents. Stent patency is the most frequently measured outcome, and few studies examined improvements in severity of PTS or QoL scores.

A systematic review of deep venous stenting in acute DVT identified 27 studies (542 patients). It included 3 randomized controlled trials (RCTs) and 21 cohort studies (8 prospective, 12 retrospective), and all patients included had undergone lysis, venoplasty, and stenting. The overall patency rates were 87.8% over a follow-up period of 12 to 19.7 months.[14] PTS was assessed in 26 of 27 studies, with an observed rate of 14.6%, but PTS clinical scores and QoL were assessed in only 3 of 27 studies over a short period of 3 months. Chronic venous insufficiency questionnaires (CIVIQs) were used; only one compared stenting versus no stenting, but it did show a significant improvement in QoL using CIVIQ scores (22.67 ± 3.01 vs 39.34 ± 6.6; $P < 0.01$).[15] Only 1 of the 3 randomized trials included[15-17] was randomized between stenting and no stenting.[15] In that study, the patency rate was 86% vs 54.8% in the stented vs nonstented group, and there was a significant reduction in CEAP score (1.61 ± 0.21 vs 0.69 ± 0.23).

The ATTRACT (Acute Venous Thrombosis: Thrombus Removal with Adjunctive Catheter-Directed Thrombolysis)[18] and CAVENT (Catheter-Directed Venous Thrombolysis in Acute Iliofemoral Vein Thrombosis)[19] trials were large RCTs designed to address whether thrombolysis led to a reduction of PTS. The CAVENT trial[19] showed a significant reduction in the incidence of PTS with CDT (49% vs 63%) but did not show any difference in QoL outcomes. The ATTRACT trial[18] showed less reduction in the incidence of PTS, but it showed a reduction in severity of PTS at 6-, 12-, 18-, and 24-month follow-ups and, similar to CAVENT, showed no improvement in QoL. However, only a small number of patients included in the intervention arms of these trials had venous stenting after thrombolysis (28% in the ATTRACT trial and 18% in CAVENT), which may partly explain the poor outcomes of these trials.

Overall, although outcomes appear promising, there are few data to support use of stenting in patients with acute DVT, and it should be used only for highly selected patients.

Table 3. Venous stent devices

Device	CE mark approval	FDA approved
Abre (Medtronic)	2017	—
blueflow (plus medica GmbH & Co. KG)	2018	—
sinus-Obliquus (optimed Medizinische Instrumente GmbH)	2013	—
sinus-Venous (optimed Medizinische Instrumente GmbH)	2015	—
WALLSTENT (Boston Scientific)	2015	—
Venovo (BD)	2015	2019
Vici (Boston Scientific)	2013	2019
Zilver Vena (Cook Medical)	2010	—

CE mark, administrative marking that indicates conformity with health, safety, and environmental protection standards for products sold within the European Economic Area; FDA, US Food and Drug Administration.

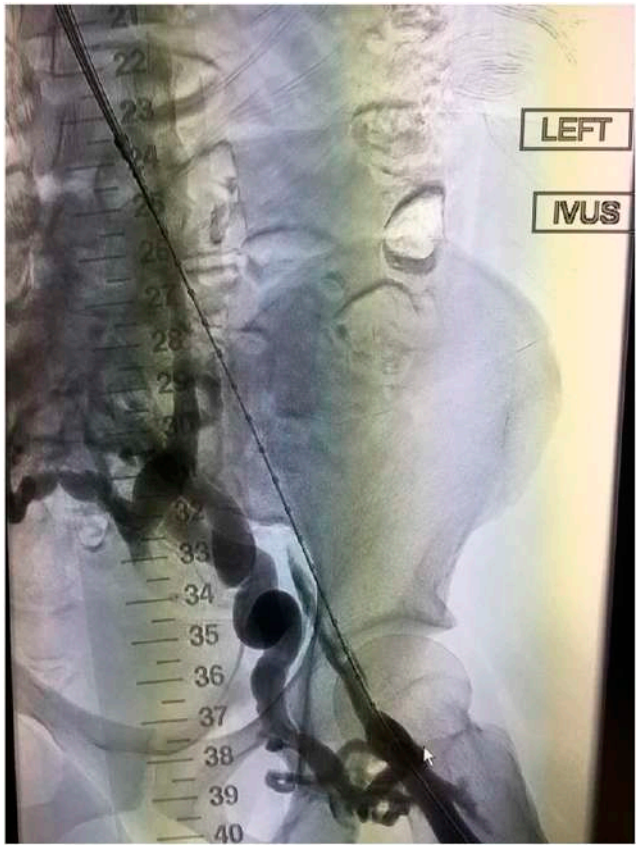

Figure 2. Example of thrombosed iliofemoral lesion with collateralization before venous stenting.

Placement of a venous stent in patient 1 in addition to thrombolysis at the time of presentation of DVT might have prevented reocclusion and reduced her risk of developing significant PTS.

What is the evidence for the use of venous stenting in chronic DVT?

European Society for Vascular Surgery guidelines recommend angioplasty and stenting as first-line treatment for patients with clinically relevant chronic iliocaval or iliofemoral obstruction or those with symptomatic NIVL (class IIa, level B),[20] while the American Heart Association guidelines recommend that it be considered in these situations (class IIb, level of evidence B).[21]

One of the first large single-center retrospective studies of endovenous stenting included 139 patients (78 patients with PTS secondary to previous DVT and 61 with NIVL). Patients with PTS showed a significant improvement in symptoms of pain and swelling (reduction of visual analog pain score from 4.2 to 2.2); 50% of 24 patients with leg ulcers showed complete healing.[22] Stent reocclusion rates were 17%. While these initial results appeared promising, most later studies of venous stents were also retrospective, using stent patency as the main outcome measure. Few studies looked at reduction in symptom severity over the medium to long term.

A recent systematic review of venous stenting in chronic disease included 16 (mainly retrospective) studies of 2373 postthrombotic and 2586 nonthrombotic patients. Primary patency rates (open stent without need for reintervention) were 32% to 98%.[23] Primary assisted patency rates (open stent but additional procedures required to prevent occlusion) were between 66% and 96%, and secondary patency rates (open stent required an additional procedure following occlusion) were between 68% and 96%. Twelve of the 16 studies reported on ulcer healing, which occurred in 56% to 100% of patients. Only 5 studies reported on severity of symptoms, but 4 of them showed a significant reduction in symptoms using validated scoring systems (CEAP and Villalta).

Similarly, only 3 studies reported on QoL outcomes (using CIVIQ and VEINESQOC scores) and showed general improvement in venous disease–related QoL scores.

Major complications were less than 2% across all studies (including bleeding, prolonged hospitalization, or need for further intervention), but reporting was variable between studies. No postoperative mortality was reported in 6 of the 16 studies, and no pulmonary emboli were reported.

It is accepted that chronic venous disease is more difficult to treat than NIVL and often leads to higher rates of stent reocclusion, particularly in patients with postthrombotic lesions.[10,24,25] Significant drawbacks of most studies are that NIVLs are included with postthrombotic lesions, while control groups were rarely included, making it difficult to interpret whether stenting really improves outcomes.

Preliminary data from multicenter, multinational single-arm prospective studies (the VERNACULAR and VIRTUS trials) using newer dedicated venous stents have shown primary patency rates at 12 months to be 88.3%.and 84%, respectively.[26,27] The

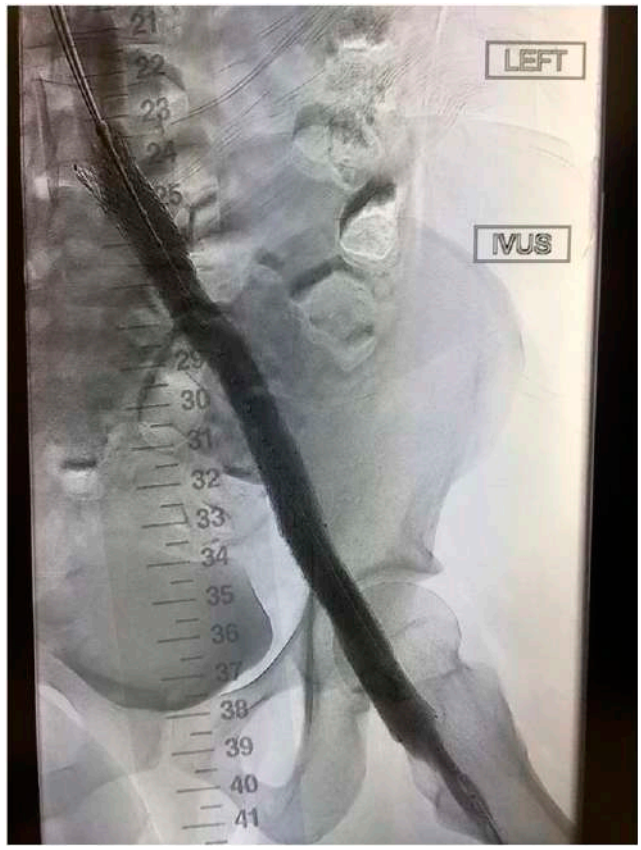

Figure 3. Improved vascular flow after venous stenting using the Abre stent (Medtronic).

VERNACULAR study showed a significant improvement in QoL scores (venous clinical severity score [VCSS] reduced at 12 months [4.0 ± 3.9]); further data from the VIRTUS trial are pending. The Arnsberg venous registry of 79 patients has shown a 6-month primary patency rate of 98% and significant decreases in revised VCSS scores, as well as ulcer healing in all 8 patients with ulcers.[28] No major complications were seen.

Overall, evidence for use of venous stenting for treatment of chronic venous disease is weak, but potential particular benefits in improvement of QoL scores and ulcer healing have been shown. Randomized controlled trials using dedicated venous stents are needed to provide robust data on improvements in severity of PTS using clinical scores and QoL indicators, as well as complication rates over the long term.

Patient 2 underwent venous stenting and had significant reduction in the severity of his PTS, and his leg ulcers healed. Importantly, his QoL significantly improved, demonstrating that, in carefully selected patients, stenting can lead to improved outcomes.

How is anticoagulation managed in patients with venous stents, and how should they be followed up?

Few studies specifically address management of anticoagulation in patients with venous stents. The duration of anticoagulation is usually determined by underlying risks for VTE recurrence and not by the presence of a venous stent.

Most studies to date have involved the use of unfractionated heparin, LMWH, and vitamin K antagonists because many predate the use of direct oral anticoagulants. Eijgenraam et al concluded that anticoagulation regimens made no difference to outcomes in their systematic review of antithrombotic management.[34] Taha et al could not draw any firm conclusions regarding anticoagulation, particularly extended use, in the setting of stenting in acute VTE.[14] Use of antiplatelet agents did not seem to show any significant benefit.[14]

Pregnancy in women who have had previous VTE and stenting procedures may need careful consideration. Retrospective data suggest they are not at increased risk of stent occlusion or recurrent VTE during pregnancy or in the postpartum period,[30] but further data are required.

Future research will need to focus on defining optimal peri- and postoperative management.

Conclusions

Endovenous stenting is a minimally invasive, relatively safe procedure with promising outcomes in reducing the severity of PTS and improving QoL. However, further research is needed to establish its usefulness in management of deep venous disease, particularly the long-term outcomes and cost-benefits.

Conflict-of-interest disclosure

K.B. has received speaker fees from Boston Scientific and Bayer.

Off-label drug use

None disclosed.

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Thrombolytic therapy in acute venous thromboembolism

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Although anticoagulation remains the mainstay of treatment of acute venous thromboembolism (VTE), the use of thrombolytic agents or thrombectomy is required to immediately restore blood flow to thrombosed vessels. Nevertheless, systemic thrombolysis has not clearly been shown to improve outcomes in patients with large clot burdens in the lung or legs as compared with anticoagulation alone; this is in part due to the occurrence of intracranial hemorrhage in a small percentage of patients to whom therapeutic doses of a thrombolytic drug are administered. Algorithms have been developed to identify patients at high risk for poor outcomes resulting from large clot burdens and at low risk for major bleeding in an effort to improve outcomes in those receiving thrombolytic therapy. In acute pulmonary embolism (PE), hemodynamic instability is the key determinant of short-term survival and should prompt consideration of immediate thrombolysis. In hemodynamically stable PE, systemic thrombolysis is not recommended and should be used as rescue therapy if clinical deterioration occurs. Evidence is accumulating regarding the efficacy of administering reduced doses of thrombolytic agents systemically or via catheters directly into thrombi in an effort to lower bleed rates. In acute deep venous thrombosis, catheter-directed thrombolysis with thrombectomy can be used in severe or limb-threatening thrombosis but has not been shown to prevent postthrombotic syndrome. Because the management of acute VTE can be complex, having a rapid-response team (ie, PE response team) composed of physicians from different specialties may aid in the management of severely affected patients.

LEARNING OBJECTIVES

- Describe risk stratification strategies in patients with acute pulmonary embolism
- Review current evidence on the efficacy and safety of systemic and catheter-directed thrombolytic therapy in pulmonary embolism and deep vein thrombosis
- Examine the role of pulmonary embolism response teams

Clinical case

A 36-year-old woman was brought to the emergency department with a 1-day history of progressive shortness of breath and pleuritic chest pain. Vital signs showed pulse of 142 beats per minute, respiratory rate of 38 breaths per minute, blood pressure of 128/94 mmHg, and weight of 200 lbs; before being given oxygen, her oxygen saturation on room air was 75%. D-dimer level was very elevated at 8238 ng/mL, lactate was 3.7 mmol/L (normal range, 0.5-2 mmol/L), and pro–B-type natriuretic peptide (proBNP) was 2636 pg/mL (reference range, 0-178 pg/mL). Computed tomography pulmonary arteriography showed pulmonary emboli with a saddle embolus and extension into all lobar pulmonary arteries; there was evidence of right heart strain, with interventricular septal flattening and right ventricular (RV)/atrial dilatation. Her risk factors were use of an oral contraceptive for 10 years and obesity. She was started on a heparin infusion, and the pulmonary embolism (PE) response team (PERT) was consulted. Shortly after the heparin infusion was initiated, the patient became hypotensive, with BP of 90/60 mmHg. Because she had no contraindications to systemic thrombolysis, she was administered half-dose tissue plasminogen activator (tPA) IV (10 mg bolus followed by 40 mg over 2 hours along with unfractionated heparin). She clinically improved over several hours, with marked improvement of hypoxia. Fibrinogen level was monitored, reaching a nadir at 83 mg/dL (reference range, 180-400 mg/dL); she had a brief episode of epistaxis. She was discharged on therapeutic anticoagulation on the fourth hospital day.

Introduction

Venous thromboembolism (VTE), which includes deep vein thrombosis (DVT) and PE, is a common vascular disease with significant morbidity and mortality. The mortality rate of VTE has substantially decreased over the last few decades as a result of advances in diagnosis and management.[1,2] Nonetheless, early mortality remains a major complication, occurring in 3.1% to 4% of PEs and 0.7% of DVTs.[1,2]

The cornerstone of treatment of VTE is anticoagulation. In a majority of patients, therapeutic anticoagulation is effective in preventing thrombus propagation and distal embolization while allowing the endogenous fibrinolytic system to dissolve the existing clots. In severe cases, such as those with acute RV failure, hemodynamic instability, and sudden cardiac arrest in PE or phlegmasia cerulea dolens in DVT, reperfusion therapy aimed at thrombus dissolution with immediate restoration of vascular patency is warranted to save life or limb function. Methods of reperfusion are categorized as pharmacological (systemic or catheter-directed thrombolysis [CDT]), mechanical (surgical or catheter-based embolectomy), or a combination of both.

In VTE, the potential benefits of thrombolysis include immediate symptom relief, prevention of clinical deterioration and short-term mortality, and prevention of long-term complications, such as chronic thromboembolic pulmonary hypertension and postthrombotic syndrome (PTS). However, evidence supporting the benefits of thrombolysis are inconclusive, and debate continues over whether, when, and which modality of thrombolysis should be used for a given patient with VTE. In this article, we present an update on evidence regarding the efficacy and safety of thrombolytic therapy in PE and DVT.

PE

Are we able to effectively stratify high-risk patients who will benefit from thrombolytic therapy?

The clinical presentation of PE represents a continuous spectrum, ranging in severity from no symptoms to hemodynamic instability and sudden death. The prognosis, as well as the risk/benefit ratio of thrombolytic therapy, varies widely based on severity at presentation. During the initial assessment of PE, it is therefore mandatory to identify those patients at risk of early mortality to guide management decisions. An ideal strategy would allow us to identify (1) patients who require immediate reperfusion therapy; (2) patients who require hospitalization and, within this group, those who may benefit from early advanced therapy, and (3) patients who can be safely discharged and treated as outpatients.

The presence of hemodynamic instability is the most important determinant of short-term mortality and should prompt immediate reperfusion therapy. Acute PE with hemodynamic instability, manifested as cardiac arrest, profound bradycardia, or persistent hypotension, represents a high-risk cohort with massive PE.[3,4] In this group, the 90-day mortality rate can be as high as 52.4%.[5] In a metaanalysis of 40 363 patients with acute PE, 3.9% had unstable PE. These patients had increased risk of short-term all-cause mortality (odds ratio [OR], 5.9; 95% confidence interval [CI], 2.7-13.0) and PE-related mortality (OR, 8.2; 95% CI, 3.4-19.7) compared with their stable counterparts.[6] Given this dire prognosis, systemic thrombolytic therapy is justified to rapidly resolve pulmonary vascular obstruction. To date, there has been only 1 randomized control trial (RCT) comparing systemic thrombolysis with anticoagulation alone in patients with massive PE.[7] In this trial, 8 patients with massive PE and cardiogenic shock were enrolled and randomly assigned to receive either 1.5 million IU of streptokinase and heparin or heparin alone. The trial was terminated after all 4 patients (100%) in the heparin group died compared with none (0%) in the streptokinase group. The streptokinase group had clinical and echocardiographic improvement within the first hour of treatment. Notwithstanding its methodological limitations, this early evidence suggests a mortality benefit of systemic thrombolysis in massive PE. In recent VTE registries, unstable PE patients who received thrombolytic therapy had a lower risk of short-term mortality than those who did not (OR, 0.69; 95% CI, 0.49-0.95).[6] Barring contraindications (Table 1), systemic thrombolytic therapy is indicated for acute PE with hemodynamic instability.[3,4] Surgical embolectomy or percutaneous catheter-directed treatment is an alternative for those with contraindications to systemic thrombolysis.

A large majority of hemodynamically stable PE patients (~50% to 60%) fall between the 2 extremes of hemodynamically unstable and low-risk PE. Among these intermediate-risk PE patients, the short-term mortality rate ranges from 3.2% to 11.4%.[8] Many clinical (concomitant DVT and respiratory index), imaging (RV dysfunction on echocardiogram or computed tomography), laboratory (troponin, BNP, N-terminal proBNP, lactate, and heart-type fatty acid-binding protein levels), or combined parameters have been shown to be associated with higher risk of clinical deterioration and early mortality in hemodynamically stable patients with PE.[9] However, none have been shown to effectively identify patients who will benefit from routine early advanced therapies, including systemic thrombolysis.

In hemodynamically stable PE, RV dysfunction, detected by echocardiography or computed tomography, and elevation of myocardial injury markers such as troponins are associated with increased risk of short-term mortality.[10,11] Patients presenting with both are classified as intermediate-high–risk or submassive

Table 1. Contraindications to thrombolysis[4]

Contraindication
Absolute
History of hemorrhagic stroke or stroke of unknown origin
Ischemic stroke in previous 6 mo
Central nervous system neoplasm
Major trauma, surgery, or head injury in previous 3 wk
Bleeding diathesis
Active bleeding
Relative
Transient ischemic attack in previous 6 mo
Oral anticoagulation
Pregnancy or first postpartum week
Traumatic resuscitation
Refractory hypertension (systolic blood pressure >180 mmHg)
Advanced liver disease
Infective endocarditis
Active peptic ulcer

PE. The role of early systemic thrombolysis to prevent short-term adverse outcomes in this group of patients has been investigated in the PEITHO trial.[12] In this large RCT, tenecteplase (single weight-based IV bolus; dose range, 30-50 mg) plus heparin was compared with placebo plus heparin in 1005 patients with intermediate-high–risk PE. The primary outcome, which was death or hemodynamic decompensation within 7 days after randomization, occurred more commonly in the placebo group (5.6%) than the tenecteplase group (2.6%; OR, 0.44; 95% CI, 0.23-0.87; P = .02). The effect was largely driven by the difference in hemodynamic decompensation (5.0% vs 1.6%) and not by mortality (1.8% vs 1.2%). The potential benefit was offset by the higher bleeding events from thrombolysis. The tenecteplase group had a fivefold higher risk of major bleeding (11.5% vs 2.4%) and 10-fold higher risk of hemorrhagic stroke (2.0% vs 0.2%). Long-term follow-up was continued in 709 randomly assigned patients from the PEITHO study. Over the median follow-up time of 37.8 months, thrombolysis did not have a positive impact on overall mortality rate, functional limitation, persistent symptoms, or chronic thromboembolic pulmonary hypertension.[13] These results suggest that the combination of RV dysfunction and myocardial injury is not sufficient to identify intermediate-risk PE patients who will benefit from systemic thrombolysis. Nevertheless, given the substantial risk of early hemodynamic deterioration, close monitoring is warranted, and rescue therapy should be considered for patients who develop hemodynamic instability. In a recent metaanalysis, after excluding studies with high risk of bias, systemic thrombolysis did not show a mortality benefit over heparin alone (OR, 0.66; 95% CI, 0.42-1.06; N = 2054; P = .08). Moreover, the incidence of major bleeding was significantly higher in the thrombolysis group (OR, 2.90; 95% CI, 1.95-4.31; N = 1897; P < .001).[14]

In light of this evidence, full-dose systemic thrombolysis is not routinely recommended for intermediate-risk PE and should be reserved for patients presenting with hemodynamic instability or with clinical deterioration after anticoagulation. Additional studies to improve the risk/benefit ratio of thrombolysis should focus on developing more effective risk stratification tools to identify high-risk patients and minimize the bleeding risk from thrombolysis using alternatives such as low-dose or CDT.

What is the evidence for low-dose thrombolysis?

Because the bleeding risk associated with thrombolysis is dose dependent, lower doses of thrombolytic drugs may provide a more favorable safety profile with comparable efficacy. Several studies have been conducted to explore the feasibility of low-dose thrombolysis. In the MOPETT study, 121 moderate PE patients were randomly assigned to receive low-dose tPA (50 mg for patients ≥50 kg and 0.5 mg/kg for patients <50 kg) or anticoagulation alone. At 28 months, the low-dose tPA group had a lower rate of pulmonary hypertension, with no difference in mortality rate or recurrent PE. Interestingly, bleeding events were not observed in either group.[15] Low-dose tPA was also compared with full-dose tPA in an RCT enrolling 127 acute PE patients with hemodynamic instability or massive obstruction. In this study, 50 mg of tPA (10 mg bolus followed by 40 mg by IV clinical integration over 2 hours) was comparable to 100 mg of tPA (10 mg bolus followed by 90 mg by IV continuous infusion) with respect to improvement of RV dysfunction, lung perfusion defects, and pulmonary obstruction. Although statistical significance was not reached, bleeding was numerically lower in the low-dose group.[16] In a systematic review and metaanalysis, low-dose tPA was associated with lower risk of major bleeding than full-dose tPA (OR, 0.33; 95% CI, 0.12-0.91), with no difference in recurrent PE or all-cause mortality.[17] In contrast, a propensity score–matched analysis of an administrative database concluded that half-dose alteplase was associated with more frequent treatment escalation, with similar rates of mortality and major bleeding.[18] At present, more evidence is needed to support the use of low-dose thrombolysis. PEITHO-III (NCT04430569) is an ongoing placebo-controlled RCT evaluating the efficacy of low-dose alteplase administered as bolus (0.6 mg/kg) in intermediate-high–risk PE; the premise is that bleeding will be reduced if tPA is administered over a short period.[19]

What is the role of CDT?

In clinical practice, only a fraction (30%) of eligible high-risk PE patients receive systemic thrombolysis, possibly because of contraindications and risk of bleeding.[8] Catheter-directed therapy provides an alternative reperfusion approach that allows localized drug delivery and can be combined with mechanical thrombus removal. Catheter-based modalities include mechanical thrombectomy (thrombus fragmentation, aspiration, and rheolytic thrombectomy), pharmacologic CDT (via thrombolytic infusion catheter or ultrasound-facilitated CDT), or a combination of both.

The major advantage of CDT is the lower bleeding risk. In a metaanalysis of outcomes of CDT in 1168 patients, the rates of major bleeding were 6.7% and 1.4% in high- and intermediate-risk PE, respectively, which seem more favorable than those associated with systemic thrombolysis (up to 20% in high- and 12% in intermediate-risk PE).[20] In a propensity score-matched administrative database analysis, CDT was associated with lower in-hospital mortality and intracranial hemorrhage rates compared with systemic thrombolysis in acute PE.[21] Nevertheless, the bleeding risk associated with CDT is still greater than anticoagulation alone (1.1% to 1.7%).[4] The procedure also requires specialized resources and expertise that might not be readily available in many centers. Most importantly, current evidence supporting the use of CDT in acute PE is limited to a small RCT or single-arm studies focusing on short-term surrogate outcomes rather than clinical outcomes (Table 2).[22-26] Therefore, the decision to use CDT should be based on individualized risk/benefit considerations.

In patients with high-risk PE, CDT is recommended when systemic thrombolysis is contraindicated or has failed.[3,4] In a recent prospective registry, catheter-directed aspiration thrombectomy with low-dose thrombolysis was administered to 54 patients with acute unstable PE. In-hospital PE-related death occurred in 6 patients (11%), whereas hemodynamic stability was achieved in the remaining 48 patients. One patient (2.1%) developed hemorrhagic stroke.[27]

The role of routine CDT in intermediate-risk PE remains controversial. In the ULTIMA trial, ultrasound-assisted CDT was superior to anticoagulation alone in terms of RV/left ventricular ratio reduction from baseline at 24 hours.[22] However, there was no difference in mortality, recurrent VTE, or major bleeding at 90 days. Given the lack of evidence regarding short- and long-term clinical benefits, CDT should be reserved for intermediate-risk PE patients who develop signs of hemodynamic instability despite adequate anticoagulation.[4] Additional studies with larger sample sizes are required to elucidate the optimal use of CDT.

Table 2. Summary of key studies of DCT in intermediate-risk PE

Study	N	Study design	Study population	Treatment	Comparison	Efficacy	Safety
ULTIMA[22]	59	RCT	Intermediate-risk PE	USAT: tPA at 10 mg via EKOS catheter + therapeutic anticoagulation (n = 30)	UFH alone (n = 29)	Mean difference in RV/LV ratio from baseline to 24 h USAT tPA: 0.30 ± 0.20 UFH alone: 0.03 ± 0.16 (P < .001) No difference in hemodynamic decompensation, recurrent VTE, mortality at 90 d	No major bleeding Minor bleeding USAT rtPA: 10% UFH alone: 3% (P = .61)
SEATTLE II[23]	150	Prospective single arm	Massive PE (n = 31; 21%) Submassive PE (n = 119; 79%)	USAT: tPA at 24 mg via EKOS catheter + therapeutic anticoagulation	None	Mean RV/LV ratio decreased from baseline (1.55) to 48 h (1.13; P < .001) PASP decreased at 48 h	30-d major bleeding, 10% 30-d mortality, 2.7%; no ICH
PERFECT[24]	101	Prospective single arm	Massive PE (n = 28; 28%) Submassive PE (n = 73; 72%)	Standard CDT (64%) or USAT via EKOS catheter (36%) with tPA at 0.5-1.0 mg/h or urokinase 100 000 IU/hr + therapeutic anticoagulation	None	Clinical success* achieved in 85.7% massive PE and 97.3% submassive PE PASP decreased post-CDT No difference in PASP change, tPA dose, or infusion between USAT and standard CDT	In-hospital mortality, 5.9% No major bleeding or ICH at 30 d
OPTALYSE-PE[25]	101	Randomized comparison of 4 USAT regimens	Intermediate-risk PE	USAT: tPA at 8-24 mg via EKOS catheter + therapeutic anticoagulation	4 USAT regimens	Mean RV/LV ratio decreased at 48 h in all 4 regimens	Major bleeding at 72 h, 4% 2 ICHs (1 attributable to USAT tPA) Recurrent PE, 1% 30-d mortality, 1%
FLARE[26]	104	Prospective single arm	Intermediate-risk PE	Catheter-directed mechanical thrombectomy without thrombolysis + therapeutic anticoagulation	None	Mean RV/LV ratio decreased from baseline (1.56) to 48 h (1.15; P < .0001)	1 major bleeding No ICH 4 clinical deterioration 1 death at 23 d

EKOS, EndoWave Infusion Catheter System; ICH, intracranial hemorrhage; LV, left ventricular; PASP, pulmonary artery systolic pressure; rtPA, recombinant tPA; UFH, unfractionated heparin; USAT, ultrasound-assisted CDT.
*Clinical success was defined as stabilization of hemodynamics, improvement in pulmonary hypertension and/or right-sided heart strain, and survival to hospital discharge.

What is the role of a PERT?

Given the limitations of risk stratification and availability of advanced therapies, the optimal management of acute PE can be challenging. Treatment decisions, especially for intermediate- and high-risk PE, require individualized and timely use of these therapies. To aid this process, institutions caring for patients with severe PE have established multidisciplinary PERTs. Although the composition of the team varies by institution, a PERT often includes specialists in cardiology, pulmonology, vascular medicine, critical care, emergency medicine, hematology, interventional radiology, and vascular or cardiothoracic surgery. Upon activation, a PERT evaluates, triages, and provides treatment and follow-up plans for patients with acute PE. The impact of PERTs on management and outcomes has varied among institutions. Compared with historical controls, the initiation of a PERT led to increased use of advanced therapies, particularly CDT, shorter time to therapeutic anticoagulation, and decreased use of inferior vena cava filters.[28-31] The early involvement of interventional radiologists may help facilitate the identification of patients who are suitable for catheter-directed therapies and avoid the bleeding risk from systemic

thrombolysis. Major bleeding and 30-day mortality rates were lower after PERT involvement in 1 study,[31] but this was not demonstrated in others.[32]

In our clinical case, the patient was normotensive on presentation, with an elevated proBNP level and evidence of right heart strain on computed tomography pulmonary arteriography; this placed her in the intermediate-high–risk group. Although immediate systemic thrombolysis was not clearly required, she hemodynamically decompensated after anticoagulation was initiated. Because she was at low risk for bleeding, the recommendation of the PERT was to administer low-dose tPA, which was associated with clinical improvement.

DVT

How can we predict the risk of PTS in DVT patients?

In patients with acute DVT that is limb threatening or who have progressive symptoms despite adequate anticoagulation, thrombolysis and/or thrombectomy is indicated to improve blood flow. Another proposed benefit of thrombolysis with or without thrombectomy is the prevention of PTS by rapidly relieving venous obstruction. PTS is a common long-term complication occurring in up to 50% of patients with lower-extremity DVT. Risk factors for PTS include preexisting venous insufficiency, iliofemoral DVT, high body mass index, older age, inadequate anticoagulation during the first 3 months, and ipsilateral DVT recurrence.[33] Several models have been developed to predict the risk of PTS in patients with DVT (Table 3).[34-36] On the basis of these models, the highest risk groups have a risk of 25% to 80.7% for developing PTS. Although external validation is needed, elements of these models may be useful in selecting DVT patients at high risk for PTS who may benefit from strategies employing thrombolysis with or without thrombectomy.

Should thrombolysis be used to prevent PTS?

In the early clinical trials comparing systemic thrombolysis with anticoagulation alone in DVT, thrombolysis was associated with a nonsignificant reduction of PTS and a twofold higher bleeding risk, particularly intracranial hemorrhage.[37] Therefore, systemic thrombolysis was not recommended as an adjunct to anticoagulation for the initial treatment of DVT. Pharmacological and pharmacomechanical CDT have been investigated to prevent PTS in selected patients with DVT. To date, 3 multicenter RCTs have been conducted to assess the efficacy and safety of these interventions (Table 4).[38-40] In CaVenT, CDT prevented PTS at 2 and 5 years. In contrast, the occurrence of PTS at 2 years was not significantly different in ATTRACT, although CDT decreased PTS severity and rate of moderate to severe PTS in the subgroup with iliofemoral DVT. In CAVA, which enrolled only patients with iliofemoral DVT, the rates of PTS at 1 year were not different between the 2 groups. The risk of bleeding increased with CDT in all studies. Although CDT led to quality of life (QoL) improvement at 1 and 6 months in the ATTRACT trial, none of the studies found long-term QoL to be improved with CDT.

Table 3. Risk prediction models for PTS

	SOX-PTS score[34]	Points	Amin et al[35]	Points	Méan et al[36]	Points
Age, y	—		>56	2	≥75	1
BMI, kg/m²	≥35	2	>30	2	—	
DVT anatomy	Iliac DVT	1	Iliofemoral DVT	1	Multilevel thrombosis	1
Signs of preexisting venous insufficiency	Baseline Vilalta score		Varicose veins	4	Prior varicose vein surgery	1
	>14 (severe)	2				
	10-14 (moderate)	1				
					N of leg signs and symptoms*	1 (for each)
Other	—		Smoking	1	Concomitant antiplatelet/NSAID therapy	1
			Female sex	1		
			Provoked DVT	1		
			History of DVT	1		
Risk category	**Total score**	**PTS risk, %**	**Total score**	**PTS risk, %**	**Total score**	**PTS risk, %**
Low	0	6.4	0-2	10	0-3	24.4
	1	13.4				
Intermediate	2	16.4	3-4	20	4-5	38.4
	3	25				
High	≥4	30	≥5	40	≥6	80.7

BMI, body mass index; NSAID, nonsteroidal antiinflammatory drug.
*Pain, cramps, heaviness, pruritus, paraesthesias, edema, skin induration, hyperpigmentation, venous ectasia, redness, and pain during calf compression.

Table 4. Summary of RCTs evaluating CDT in DVT

Study	N	Study population	Treatment	Comparison	Efficacy	Safety
CaVENT[38]	209	Iliofemoral DVT within 21 d	CDT: tPA at 20 mg + therapeutic anticoagulation (n = 90)	Anticoagulation alone (n = 99)	PTS at 24 mo: CDT, 37 (41.1%) vs control, 55 (55.6%; *P* = .047) PTS at 5 y: CDT, 37 (42.5%) vs control, 63 (70.8%; *P* < .001)	Major bleeding: CDT, 3 (3.3%) vs control, 0 (0%) No intracranial hemorrhage
ATTRACT[39]	692	Iliac, femoral, common femoral DVT within 14 d	PMCDT: tPA at <35 mg + therapeutic anticoagulation (n = 336)	Anticoagulation alone (n = 355)	PTS at 24 mo: PMCDT, 157 (47%) vs control, 171 (48%) RR, 0.96 (95% CI, 0.82-1.11; *P* = .56) Moderate to severe PTS at 24 mo: PMCDT, 60 (18%) vs control, 84 (24%) RR, 0.73 (95% CI, 0.54-0.98; *P* = .04)	Major bleeding in 10 d: PMCDT, 1.7% vs control, 0.3% RR, 6.18 (95% CI, 0.78-49.2; *P* = .049) No intracranial hemorrhage
CAVA[40]	184	Iliofemoral DVT within 14 d	USAT: urokinase via EKOS catheter + therapeutic anticoagulation (n = 77)	Anticoagulation alone (n = 75)	PTS at 12 mo: USAT, 22 (29%) vs control, 26 (35%) OR, 0.75 (95% CI, 0.38-1.50)	Major bleeding in 10 d: USAT, 4 (5%) vs control, 0 (0%) OR, 9.25 (95% CI, 0.49-174.7) No intracranial hemorrhage

EKOS, EndoWave Infusion Catheter System; PMCDT, pharmacomechanical CDT; RR, relative risk; USAT, ultrasound-assisted CDT.

In summary, CDT has not consistently been shown to reduce the occurrence of PTS or improve long-term QoL and is associated with an increased bleeding risk. Therefore, CDT should be restricted to selected patients with severe symptoms and a higher risk of PTS (iliofemoral DVT) who have a low risk of bleeding. The use of validated prediction models for PTS in the future may allow us to successfully reduce its occurrence in future studies of CDT with or without thrombectomy.

Systemic or CDT can lead to a rapid improvement in vascular patency in patients with severe PE and DVT. Because improved clinical outcomes have not clearly been demonstrated in RCTs, the selection of suitable candidates for these therapies remains critical. Management of these patients can be facilitated by taking a multidisciplinary team approach to their care, with consideration of each patient's clinical presentation, disease severity, comorbidities, and bleeding tendency.

Conflict-of-interest disclosure

K.A.B. has served as a consultant to Bristol-Myers Squibb and Takeda. T.C. declares no competing financial interests.

Off-label drug use

None disclosed.

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Inferior vena cava filters: a framework for evidence-based use

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Venous thromboembolism (VTE) is a common cause of morbidity and mortality. Although most patients can be managed safely with anticoagulation, inferior vena cava filters (IVCFs) represent an important alternative to anticoagulation in a small subset of patients. IVCF use has expanded exponentially with the advent of retrievable filters. Indications for IVCFs have liberalized despite limited evidence supporting this practice. Because indiscriminate use of IVCFs can be associated with net patient harm, knowledge of the risks and benefits of these devices is essential to optimal evidence-based practice. Patients with acute VTE and absolute contraindications to anticoagulation or major complications from anticoagulation are universally agreed indications for IVCFs. However, the reliance on IVCFs for primary VTE prophylaxis in high-risk patients is not substantiated by the available literature. This review examines trends in IVCF use, practice-based recommendations on IVCF use in various clinical scenarios, complications associated with indwelling IVCFs, and indications for IVCF retrieval.

LEARNING OBJECTIVES

- Review current evidence on therapeutic and prophylactic indications for inferior vena cava filters (IVCFs)
- Summarize immediate, early, and late complications associated with IVCFs
- Discuss considerations for timely IVCF retrieval
- Outline a "best practices" approach to incorporating IVCFs into clinical practice

Introduction

Despite advances in prevention strategies, venous thromboembolism (VTE) remains a leading cause of preventable hospital mortality.[1] In most patients, pharmacologic and/or mechanical thromboprophylaxis are sufficient to prevent VTE. For acute VTE, anticoagulation remains the treatment of choice without need for additional interventions. However, in patients with active bleeding or high risk of bleeding, inferior vena cava filters (IVCFs) are used to mechanically interrupt the inferior vena cava (IVC), thereby preventing pulmonary embolism (PE). Unlike anticoagulation, IVCFs neither treat VTE nor prevent deep vein thrombosis (DVT) or in situ PE.

Designing an IVCF that can be deployed safely, traps thrombi, preserves laminar flow, and minimizes the inherent thrombogenicity of an intravascular device is challenging. IVC interruption advanced over the course of 100 years from surgical IVC ligation to percutaneous placement of permanent filters (eg, Greenfield filter). In 2003, the first retrievable inferior vena cava filter (rIVCF) was approved for patients at risk for VTE with short-term contraindication to anticoagulation. rIVCFs have largely supplanted permanent inferior vena cava filters (pIVCFs), although there is no evidence that they are either safer or more effective.[1] IVCF use has increased due to liberalized indications, bedside placement techniques, increased numbers of specialists with skills of insertion, improved detection of PE with modern imaging, and the unconfirmed belief that rIVCFs are safer than older pIVCFs. Therapeutic indications for IVCFs have increased linearly, whereas placement for prophylactic indications has increased supralinearly.[2] Globally, the United States surpassed the 5 largest European nations in IVCF insertion by 25-fold despite similar annual VTE mortality.[2] Herein, we present safety and efficacy data surrounding IVCFs in common clinical scenarios, complications of IVCFs, considerations for timely IVCF retrieval, and a holistic approach for how to incorporate IVCFs into practice (Figure 1; Table 1).

Indications for IVCF placement

IVCF placement can be grouped into 2 categories: (1) therapeutic indications for known VTE and (2) prophylactic

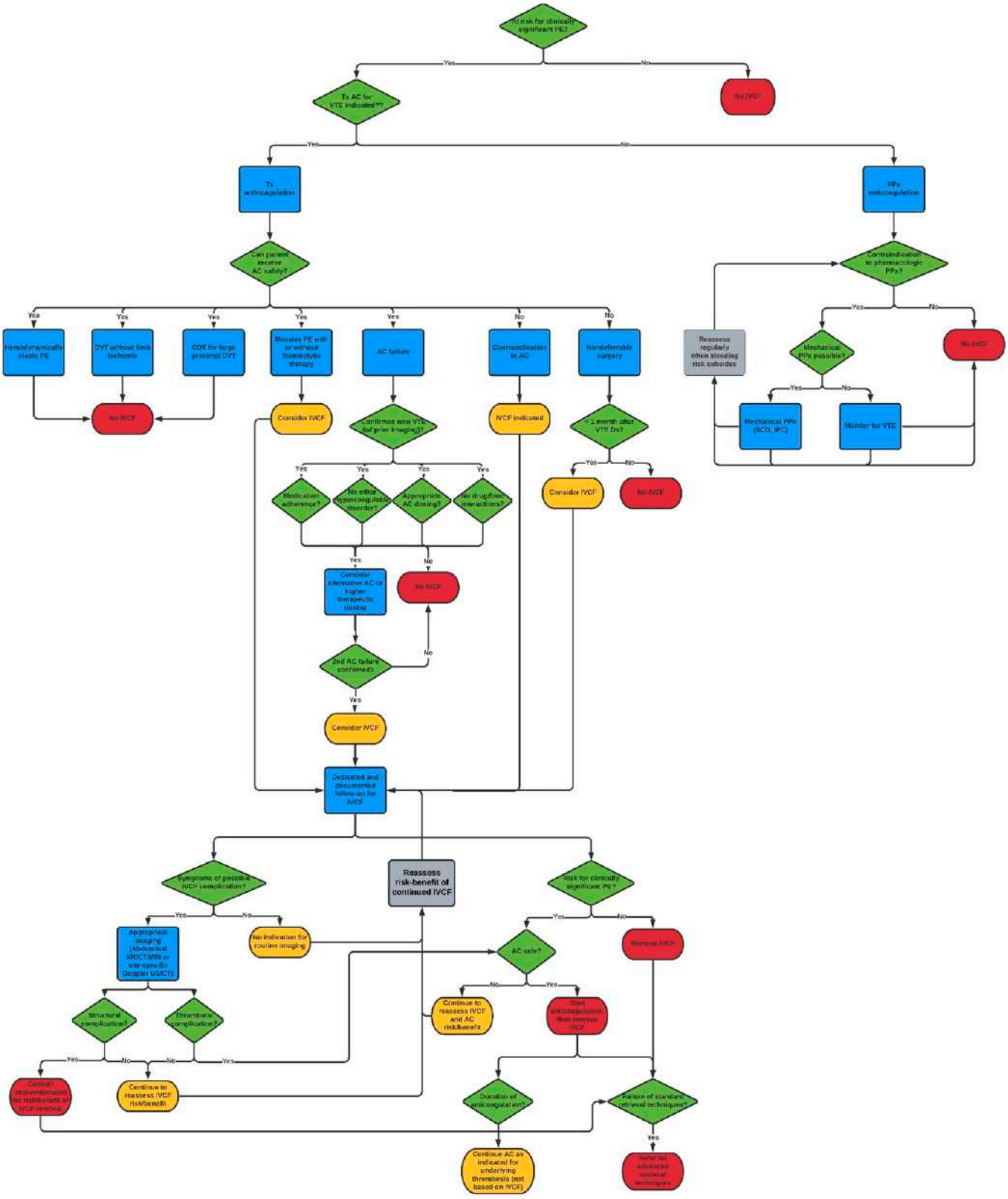

Figure 1. IVCF decision tree. AC, anticoagulation; CT, computed tomography; IPC, intermittent pneumatic device; MRI, magnetic resonance imaging; PPx, prophylaxis; SCD, sequential compression device; Tx, treatment; US, ultrasound; XR, x-ray.

Table 1. Dos, doubts, and don'ts of IVCFs

Do
• Evaluate risks/benefits of AC before considering IVCF placement
• Assess regularly the safety of resuming prophylactic or therapeutic AC in those with initial CI
• Place rIVCF in patients with acute VTE and absolute CI to AC (eg, active major bleeding or major complication while receiving AC)
• Screen for modifiable reasons of AC "failure" in suspected recurrent VTE before considering rIVCF placement
• Document systematic follow-up plan for retrieval at the time of rIVCF insertion
• Schedule periodic assessments for filter integrity and complications
• Report IVCF complications to FDA MAUDE database
• Remove rIVCF as soon as PE risk subsides and/or AC can be resumed safely
• Refer to center with expertise in advanced retrieval techniques if standard techniques fail
Doubt
• Benefit of routine IVCF placement for extended indications (CDT for DVT, massive PE, surgery requiring interruption of AC, free-floating DVT)
• Benefit of long-term AC for the sole purpose of an indwelling IVCF
Don't
• Place IVCFs for primary VTE prophylaxis in high-risk patients (trauma, bariatric surgery, spinal cord injury, high-risk orthopedic surgery)
• Place IVCFs in exquisitely hypercoagulable patients (eg, cancer, APLS) with acute VTE outside of classic indications
• Use permanent IVCFs because most patients have only temporary CI to AC

AC, anticoagulation; APLS, antiphospholipid syndrome; CDT, catheter-directed lysis; CI, contraindication; FDA MAUDE, US Federal Drug Administration Manufacturer and User Facility Device Experience.

indications for patients labeled "high risk" for VTE. Because of a dearth of high-quality studies, recommendations from clinical practice guidelines are incongruent, leading to wide practice variation (Table 2).[3-15]

IVCFs for therapeutic indications

Case 1

A 63-year-old-man presents 2 days after a fall with headache, vision changes, and nausea. Computed tomography of his head reveals an acute, moderate-sized right subdural hematoma (SDH) without midline shift. Two days after admission, he complains of right leg tenderness and is diagnosed with an acute right iliofemoral vein DVT. The result of computed tomography with pulmonary angiography is negative for pulmonary embolism. An IVCF is considered.

The standard of care in patients with acute VTE is therapeutic anticoagulation.[8] However, when anticoagulation is contraindicated, such as in major bleeding or emergent surgery, anticoagulation may be delayed or interrupted. Estimated rates of recurrent VTE without anticoagulation are 40% in the first month and 10% in the second and third month after the diagnosis of acute VTE.[16] Large observational studies evaluating the benefit of IVCFs in patients with acute VTE and contraindications to anticoagulation have reported conflicting results, and no prospective studies have been performed.[17,18] Nevertheless, as in case 1, therapeutic IVCF placement for acute VTE with contraindication to anticoagulation is the only consensus indication for routine IVCF placement.[4-9,11,13-15] In some cases in which therapeutic anticoagulation is temporarily contraindicated, prophylactic dose anticoagulation with titration to a therapeutic dose may be preferable to IVCF placement.

Data on the use of IVCFs in patients with known VTE come predominantly from 2 randomized controlled trials (RCTs) that explored IVCFs as an adjunct to therapeutic anticoagulation (Table 3).[19-21] In PREPIC, patients randomized to a pIVCF compared with no filter had a sustained reduction in PE at the cost of long-term increase in DVT and no change in mortality. This benefit would likely be diminished with currently recommended durations of anticoagulation for similar high-risk patients.[19,20] In PREPIC 2, the low rate of recurrent PE observed in the nonfilter group is consistent with successful contemporary therapeutic anticoagulation.[21] Taken together, the results of PREPIC and PREPIC 2 do not provide justification for routine IVCF placement for VTE that can be treated with anticoagulation.

Extended IVCF indications in patients with VTE

Cancer-associated thrombosis

Malignancy is an independent risk factor for VTE. The increasing incidence of VTE is possibly due to longer survival of patients with cancer, administration of prothrombotic systemic therapies, and improved VTE diagnostic measures. The high frequency of recurrent VTE and bleeding cannot be explained by over- or under-anticoagulation.[13] Low-molecular-weight heparins or direct oral anticoagulants are the preferred anticoagulants for cancer-associated thrombosis.[22] A meta-analysis found no difference in recurrent VTE in patients with cancer receiving an IVCF as an adjunct to anticoagulation.[23] A small RCT explored the benefits of an IVCF in addition to fondaparinux compared with fondaparinux alone in patients with cancer with acute DVT and reported higher DVT resolution rates in the nonfilter arm without any difference in PE, DVT, or 90-day mortality.[24] Furthermore, because the hypercoagulable state of cancer affects all vascular beds, regional therapies, such as IVCFs, are likely insufficient for prevention of recurrent thrombosis and may instead be thrombogenic. Nevertheless, patients with cancer are twice as likely as patients without cancer to receive an IVCF, and retrieval rates are lower.[25] Evidence-based guidelines recommend against the routine

Table 2. Comparison of clinical practice guidelines for IVCF indications

Proposed indications	EAST, 2002[3]	BSH, 2006[4]	AHA, 2011[5]	ESC, 2014[6]	ACCP, 2012[7]/ 2016[8]	ISTH, 2013[9]	ESA, 2018[10]	ACR, 2019[11]	ASH, 2019[12]	ASCO, 2019[13]	NICE, 2020[14]	SIR, 2020[15]
Acute VTE without CI to AC	NR	Not supported (grade A, level 1b)	NR	Not supported (class III, level A)	Not supported (grade 1B)	Not supported	NR	NR	NR	NR	Not supported	Not supported
VTE and CI to AC	NR	Supported (grade B, level III)	Supported (class I, level B)	Supported (class IIa, level C)	Supported (grade 1B)	Supported if high-risk PE	NR	Supported	NR	Supported if life-threatening acute VTE (within 4 wk). Moderate recommendation, low-quality evidence	May be appropriate	Supported
Acute VTE and major complication of AC	NR	NR	Supported (class I, level B)	NR	NR	Supported	NR	Supported	NR			Supported
Recurrent VTE despite appropriate AC ("failure of AC")	NR	May be appropriate after alternative AC discussed (grade C, level IV)	May be appropriate (class IIa, level C)	Supported (class IIa, level C)	NR	NR	NR	Supported	NR	May be appropriate. Weak recommendation, low-quality evidence	May be appropriate after other options explored	Not supported
CTEPH	NR	NR	NR	Not supported	NR	NR	NR	May be appropriate if CI to AC	NR		NR	NR
Nondeferrable surgery requiring AC interruption with recent history of VTE (<1 mo)	NR	May be appropriate (grade C, level IV)	NR	NR	NR	NR	May be appropriate (grade 2C)	NR	NR	Not supported. Moderate recommendation, low-quality evidence	NR	NR
Thrombolysis for DVT	NR	Not supported (grade C, level IV)	Not supported (class III, level C)	NR	NR	NR	NR	May be appropriate	NR		NR	May be appropriate
Poor cardiopulmonary reserve (eg, massive PE)	NR	NR	May be appropriate (class IIb, level C)	Not supported	May be appropriate	NR	NR	May be appropriate	NR		NR	May be appropriate
Free-floating iliofemoral or IVC thrombus	NR	Not supported (grade B, level III)	NR	Not supported	NR	NR	NR	May be appropriate	NR		NR	NR
Patient with cancer with acute VTE as an adjunct to AC	NR	Not supported	NR	May be appropriate if AC CI	NR	Not supported	NR	Not supported	NR		NR	NR
Primary prophylaxis trauma	Supported if PP CI (level III)	NR	NR	NR	Not supported (grade 2C)	NR	May be appropriate if PP/MP CI (grade 2C)	May be appropriate	Not supported		NR	Not supported
Primary prophylaxis bariatric surgery	NR	NR	NR	NR	Not supported (grade 2C)	NR	May be appropriate if PP/MP CI (grade 2C)	May be appropriate	Not supported		NR	Not supported
Primary prophylaxis high-risk orthopedic surgery	NR	NR	NR	NR	Not supported (grade 2C)	NR	May be appropriate if PP/MP CI (grade 2C)	May be appropriate	Not supported		NR	Not supported

AC, anticoagulation; ACCP, American College of Chest Physicians; ACR, American College of Radiology; AHA, American Heart Association; ASCO, American Society of Clinical Oncology; ASH, American Society of Hematology; BSH, British Committee for Standards in Haematology; CI, contraindicated; CTEPH, chronic thromboembolic pulmonary hypertension; EAST, Eastern Association for the Surgery of Trauma; ESA, European Society of Anaesthesiology; ESC, European Society of Cardiology; ISTH, International Society on Thrombosis and Haemostasis; MP, mechanical prophylaxis; NICE, National Institute for Health and Care Excellence; NR, not reported; PP, pharmacologic prophylaxis; SIR, Society of Interventional Radiology.

Table 3. RCTs on efficacy and safety of IVCFs

	Study/year	Population	Intervention	Comparator	Outcome in IVCF group vs non-IVCF group (ratios presented with 95% CI)
Therapeutic trials	Decousus, PREPIC 1998,[19] 2005[20]	Acute proximal DVT ± PE	Permanent IVCF + therapeutic AC	Therapeutic AC	12 d PE, 1.1% vs 4.8% OR, 0.22 (0.05-0.90); *P* = .03 2 y Symptomatic PE, 3.4% vs 6.3% OR, 0.5 (0.19-1.33); *P* = .16 DVT, 20.8% vs 11.6% OR, 1.87 (1.10-3.20); *P* = .02 8 y Symptomatic PE, 6.0% vs 15.0% HR, 0.37 (0.17-0.79); *P* = .008 DVT, 35.7% vs 27.5% HR, 1.52 (1.02-2.27); *P* = .042 No difference in mortality at 12 d, 2 y, 8 y
	Barginear, 2012[24]	Patients with cancer with acute DVT ± PE	Permanent IVCF + therapeutic AC	Therapeutic AC	2 mo DVT, 64% vs 58%; *P* = .63 PE, 24% vs 24.8%; *P* = NS DVT resolution, 37.5% vs 61%; *P* = .02 No difference 90-d mortality or major bleeding
	Sharifi, 2012[30] PEVI-CDT	Proximal DVT undergoing PEVI	PEVI + IVCF + AC	PEVI + AC	24 h after PEVI Iatrogenic symptomatic PE, 1.4% vs 11.3%; *P* = .048 No difference in mortality
	Mismetti, PREPIC2 2015[21]	Symptomatic PE and lower-limb vein thrombosis + additional risk factor for severity	Retrievable IVCF + therapeutic AC	Therapeutic AC	3 mo Recurrent PE, 3% vs 1.5%; RR, 2.00 (0.51-7.89); *P* = .50 6 mo Recurrent PE, RR 1.75 (0.52-5.88); *P* = .54 No difference in recurrent DVT, major bleeding, or mortality at 3 or 6 mo
Prophylaxis trials	Rajasekhar, 2011[39]	High-risk trauma without VTE	Retrievable IVCF + pharmacologic prophylaxis	Pharmacologic prophylaxis	6 mo PE, 0 vs 1 DVT, 1 vs 0 No difference in mortality
	Ho, 2019[40]	High-risk trauma without VTE and CI to AC	Retrievable IVCF	No IVCF	90 d Composite symptomatic PE or death 13.9% vs 14.4%; HR, 0.99 (0.51 to 1.94); *P* = .98 Mortality, 13.1% vs 9.3%; RR, 1.41 (0.69-2.87)

AC, anticoagulation; CDT, catheter-directed lysis; CI, confidence interval; HR, hazard ratio; OR, odds ratio; RR, relative risk.

use of IVCFs in cancer-associated thrombosis outside of classic indications.[9,13]

Anticoagulation failure

Recurrent VTE while receiving therapeutic anticoagulation is uncommon and often represents inadequate treatment or noncompliance. Patients with suspected recurrent VTE should continue therapeutic anticoagulation without IVCF placement, ensuring attention to proper dosing, malabsorption issues, medication adherence, and potential drug or food interactions that may reduce anticoagulant efficacy. Current and past imaging should be compared to distinguish acute from chronic thrombosis. In confirmed anticoagulation failure, it is preferable to use alternative anticoagulants or dose escalation.[8] In such

cases, hypercoagulable conditions, such as active malignancy, antiphospholipid syndrome, heparin-induced thrombocytopenia, paroxysmal nocturnal hemoglobinuria, or myeloproliferative syndromes, should be explored.[26] In prothrombic conditions, IVCFs will not prevent recurrent VTE and may serve as a thrombotic stimulus. Data from the PREPIC and PREPIC2 provide ample evidence that supplementing anticoagulation with IVCFs did not improve outcomes.[19-21] Recurrent lower-extremity DVT or non–catheter-related upper-extremity DVT in the same location, especially in young patients, should prompt evaluation for vascular anomalies such as May-Thurner syndrome (abnormal compression of the left common iliac vein by the right iliac artery) or Paget-Schroetter syndrome (thoracic outlet syndrome). In these anatomic variants, thrombolysis, pharmacomechanical thrombectomy, endovascular stenting, and decompressive surgery are preferred interventions over IVCFs, which can exacerbate the underlying chronic obstruction to venous flow.[26]

Interruption of anticoagulation

IVCFs have been proposed for major surgery requiring interruption of anticoagulation. Clinicians must determine if the surgery is urgent or elective and the necessary duration off anticoagulation. If surgery can be delayed to allow completion of 3 months of therapeutic anticoagulation or if anticoagulation will be held for only a short period of time (eg, <48 hours for a low–bleeding risk procedure), aggressive pharmacologic prophylaxis with dose escalation to therapeutic doses may be preferred over an IVCF. If the surgery cannot be deferred in the setting of recently diagnosed DVT (≤30 days), an rIVCF can be considered.[4,10] The rationale for this approach is based on the estimated 40% risk of VTE recurrence in the first month after diagnosis.[16] If an rIVCF is placed, a systematic plan should be delineated before insertion to ensure timely removal and avoid filters being left indwelling permanently. Beyond 30 days, VTE recurrence risk is reduced, and thus the risks of IVCF at that point likely outweigh the benefits.

Other extended indications

Patients with massive PE (large-volume PE accompanied by hemodynamic instability) are considered at high risk for fatal recurrent PE and thus are potential candidates for rIVCFs. The concern is that additional PE could lead to poor outcomes due to limited cardiopulmonary reserve. However, most patients with massive PEs will receive emergent systemic or catheter-directed thrombolysis (CDT) and promptly experience reduced thrombus burden, questioning the utility of an IVCF at that point. Large registry studies have provided mixed results but overall have demonstrated short-term survival benefit in patients with massive PE, regardless of thrombolytic use.[27] However, given the inherent selection and survival biases of registry studies, we cannot currently recommend routine use of rIVCFs as short-term adjuncts to anticoagulation and thrombolysis.[5,8,15,27]

IVCF deployment before CDT has been suggested as an appropriate indication. Retrospective studies of CDT combined with IVCFs showed no difference in PE or mortality compared with CDT alone, but increased complications were noted in patients who had filters placed.[28,29] Conversely, the FILTER-PEVI RCT demonstrated that IVCFs lowered the incidence of immediate post-procedural symptomatic PE without mortality benefit compared with patients receiving anticoagulation alone.[30] The authors advised a selective approach to filter placement in those with specific predictors of PE. IVCFs have also been considered for proximal free-floating DVT; however, anticoagulation alone is sufficient for treatment.[4,6]

IVCFs for primary prophylaxis

IVCF placement for primary VTE prophylaxis is controversial but accounts for more than half of IVCFs placed in the United States.[31] The rationale for inserting a prophylactic IVCF is to offer mechanical protection against PE during the limited high-risk period when pharmacologic prophylaxis may be contraindicated.

Case 2

A 42-year-old morbidly obese woman (body mass index, 55 kg/m^2) presents for elective gastric bypass surgery. She has no personal or family history of VTE. The surgeon asks whether a rIVCF preoperatively for VTE prophylaxis is appropriate.

VTE is an important cause of preventable postoperative mortality after bariatric surgery owing to obesity, immobility, surgery, and possible underdosing with standard pharmacologic prophylaxis. The reported incidence of DVT is 1% to 3% and that of PE is 0.3% to 2%, but mortality with PE may be as high as 40%.[32] Incidence of PE is highest within 1 month after bariatric surgery and may occur despite pharmacologic prophylaxis. A practice pattern survey revealed that 28% of respondents used IVCFs routinely before bariatric surgery.[33] No RCTs of prophylactic IVCFs in this population exist, leading to incongruent guideline recommendations.[7,12,15] A National Inpatient Sample study and a meta-analysis reported no difference in PE, increased rates of DVT, and increased risk of mortality, calling into question empiric placement of IVCFs before bariatric surgery.[32,34] Therefore, in case 2, aggressive pharmacologic prophylaxis rather than an IVCF should be used for prevention of postoperative VTE.

A similar lack of benefit of prophylactic placement of IVCFs is apparent in other high-risk surgical patients. In major trauma, VTE occurs in up to 58% of patients without thromboprophylaxis.[35] Although pharmacologic prophylaxis is effective and unanimously recommended by evidence-based guidelines, many patients with trauma have ongoing or perceived risk of bleeding and are not considered to be candidates for initial anticoagulation, owing to their underlying injuries.[3,7,12] Conflicting guidelines on IVCF use in patients with trauma has led to inconsistent practice patterns.[3,7,10-12] Three meta-analyses and 2 RCTs did not demonstrate a reduction in fatal PE or death with prophylactic IVCF placement in patients with trauma (Table 3).[36-40] In recent years, IVCF use in patients with trauma has declined without an increase in PE rates, further supporting a restrictive strategy.[41] For additional information on IVCF use in trauma, refer to the evidence-based minireview in Hematology 2020.[42]

Prophylactic IVCFs also have unproven benefit perioperatively in patients undergoing spinal surgery, total hip arthroplasty, or total knee arthroplasty and are therefore not recommended.[7,11,12,15] Importantly, IVCF insertion may lead to a delay in initiation of pharmacologic prophylaxis.

IVCF-related complications

Case 3

A 39-year-old man presents with abdominal pain and melena. He has a history of rIVCF placement 7 years ago for primary VTE prevention after a motor vehicle accident. The IVCF was never removed. Computed tomography of his abdomen and pelvis

Table 4. Complications associated with IVCFs

	Complications	Definition*	Reported Rates*	Comments
Immediate	Insertion problems	Incomplete filter opening, filter tilt more than 15 degrees from the IVC axis, misplacement of filter outside the intended area, or prolapse of filter components	5%-23%	Filter tilt may contribute to impaired filtration efficiency and increased difficulty with removal.
	Pneumothorax	Pneumothorax developing after filter insertion due to filter or guidewire complications	0.02%	
	Air embolism	Air embolism of the pulmonary arteries developing after filter insertion	0.2%	
	Carotid artery puncture	Carotid artery puncture developing after filter insertion due to filter or guidewire complications	0.04%	
	Arteriovenous fistula	Arteriovenous fistula developing after filter insertion due to filter or guidewire complications	0.02%	
	Insertion site hematoma	Hematoma developing at the venotomy site after filter insertion	0.6%	
Early	Insertion site thrombosis	Thrombus developing at the venotomy site after filter insertion	0-25%	
	Infection	Infection developing at or from the venotomy site after filter insertion		
Late	Filter migration	Movement of the filter >2 cm from its initial placement position	0-18%	In extreme migration, embolization of the entire filter or strut components to a distant anatomic location have been reported (0.1%).
	IVC penetration or perforation	Filter component extending >3 mm beyond the caval wall or into an adjacent structure	0-41%	Limited IVC wall penetration is required to secure the struts of an IVCF at the desired location during deployment. Risk can be reduced by using fluoroscopy during interventional radiology procedures and straight-tipped guidewires. Conical devices are associated with higher IVC perforation.
	Filter/IVC thrombosis	Acute or chronic thrombus in the IVC or filter after filter insertion	2%-30%	Thrombus can be related to new local thrombus, trapped embolus within IVCF, or extension of a distal DVT proximally. Histopathologic evidence of thrombus is evident on removed IVCFs within 2-11 d after placement. Risk increases with time. Cylindrical or umbrella-shaped filters have more IVC occlusion. For diagnosis, contrast-enhanced CT is most useful, whereas ultrasound has limited value. Venography should be limited to when catheter-directed intervention is pursued.
	Recurrent DVT	Thrombosis of proximal lower extremities after filter insertion	5%-35%	
	PE	Thrombosis of pulmonary arteries after filter insertion	0.5%-6%	
	Post-thrombotic syndrome	Post-thrombotic syndrome of the proximal lower-extremity vessels developing after filter insertion	15%-40%	
	Filter tilting or fracture	Filter tilting or fracture occurring after filter insertion		
	Entrapment of guidewire	Entrapment of guidewire after filter insertion		

CT, computed tomography.
*Definitions and reported rates modified from BSH 2006,[4] Angel 2011,[31] and Caplin 2011.[43]

Table 5. IVCF research portfolio and priorities

Ongoing studies and outcomes
• PRESERVE (NCT02381509) - Prospective observational study of safety and effectiveness of 6 commercially available permanent and retrievable IVCFs
• RIPT (NCT03070834) - RCT comparing rIVCF vs no rIVCF for primary VTE prophylaxis in trauma
• Safety and Efficacy Study of Fitaya Vena Cava Filter (NCT03691753) - RCT comparing implantation success and prevention of VTE between 2 rIVCFs
• EPICT (NCT04066764) - RCT in patients with IVCF comparing VKA vs DOAC for prevention of VTE and filter-related thrombosis
• FILTER (NCT01158482) - Prospective observational study of outcomes of IVCF placement and removal procedure
• Inferior Vena Cava Filters: Analysis of a Database (NCT04330170) - Retrospective observational cohort study of IVCF occlusion and filter removal rates
• REFiVeC (NCT02757001) - Prospective observational registry evaluating successful planned retrieval and adverse events during dwell time
• Bioconvertible Sentry IVC Filter (NCT04208139) - Prospective observational study of patency and thrombus formation of a bioabsorbable filter
Future research priorities (When RCTs are not feasible/ethical, then prospective observational studies should be undertaken.)
• Does rIVCF vs no rIVCF prevent post-procedural PE in patients undergoing advanced therapies (eg, thrombolysis for massive PE or phlegmasia cerulea dolens)?
• Does rIVCF vs no rIVCF prior to urgent/emergent major surgery in patients with acute VTE (<1 mo) improve postoperative PE rates?
• Does rIVCF vs no rIVCF in high-risk patients without VTE and contraindication to pharmacologic/mechanical VTE prophylaxis affect mortality or symptomatic PE rates?
• Does change in AC (dose or drug) vs rIVCF in patients with confirmed recurrent VTE despite therapeutic AC reduce recurrent VTE or mortality rates?
• Standardized criteria for optimal retrieval strategies (including time frame for retrieval and preprocedure imaging)
• Cost-effectiveness studies in patients with therapeutic or prophylactic IVCFs
• Multi-institutional clinical IVCF registry for systematic and standardized reporting of efficacy, safety, and complications
• What is the most effective system-, provider-, and patient-focused structured follow-up program that maximizes IVCF retrieval rates?
• In patients with IVCFs left indwelling long term, does extended duration AC vs no AC reduce thrombotic IVCF complications?

AC, anticoagulation; DOAC, direct oral anticoagulant; VKA, vitamin K antagonist.

reveals IVCF tines extending beyond the wall of the IVC into the lumen of the duodenum. Esophagogastroduodenoscopy confirms that the IVCF perforated the distal duodenum with evidence of recent bleeding. General surgery is consulted for laparotomy with IVCF removal.

Recognizing the paucity of evidence showing benefit of IVCFs in most circumstances, providers must consider the mounting evidence for adverse events with these devices. Complications may occur in the immediate post-procedural period, early after IVCF placement, or years later (Table 4). Immediate and early complications are uncommon, and fatal complications are rare, occurring in only 0.12% of insertions.[43] Late complications are more common, particularly when filters are left indwelling beyond when risk–benefit analysis favors removal. Recurrent DVT, even in patients who are receiving anticoagulation, may reflect filter-mediated changes in venous flow, the underlying hypercoagulable condition of the patient, or a synergistic effect of both. Furthermore, PE can still occur despite the presence of an IVCF due to thrombus extension proximally off the device.

From 2009 to 2012, 1606 IVCF-related adverse events were reported to the US Food and Drug Administration Manufacturer and User Facility Device Experience database on IVCF complications.[44] These rates likely underestimate the true incidence of complication rates because reporting is voluntary. Published rates of specific IVCF complications are disparate due to variance in filter types, follow-up duration, complication definitions, use of concurrent anticoagulation, and use of screening imaging. Thrombotic and device-related adverse events are 6 times more likely to be reported with indwelling rIVCFs than with pIVCFs (86.8% vs 13.2%; $P < .0001$).[44] Optimal management of nonthrombotic device-related complications is unknown. Management decisions should be made in collaboration with the interventionalist on a case-by-case basis, weighing risks of intervention vs continued monitoring in asymptomatic patients.

Retrieval

Case 1b

The patient in case 1 with acute SDH and acute lower-extremity DVT has an rIVCF placed. He recovers gradually from SDH without operative intervention. Two weeks later, neurosurgery is comfortable with initiating anticoagulation. The patient remains stable after initiation of therapeutic anticoagulation without any signs of recurrent bleeding.

Over 50% of IVCFs are placed for temporary prevention of VTE, but only 12% to 45% are retrieved.[45] This reflects a combination of overconfidence in the long-term safety of indwelling rIVCFs, lack of provider and patient education on the importance of retrieving filters, and loss to follow-up.[31,45] When attempted, >90% of IVCF retrievals are successful in the first month. At 12 months, the success rate drops to 37%.[31] Procedural factors associated with retrieval failure include prolonged dwelling time, advanced patient age, filter tilting, adherence to the IVC

wall, or large clot volume within the filter.[31] Clinical factors that influence the rate of IVCF retrieval include comorbidities, concurrent anticoagulation, insurance coverage, primary indication for placement, and documented plans for removal.[45] The urgency of early retrieval was highlighted in a 2014 US Food and Drug Administration safety alert.[46] Though no guidelines recommend a specific time frame for removal, a decision analysis study found that retrieval between 29 and 54 days after insertion was optimal.[47] Notably, an IVCF left indwelling permanently is not of itself an indication for indefinite anticoagulation.[4,7] The underlying thrombotic event and perceived bleeding risk should guide duration of anticoagulation.

To improve provider- and system-related factors associated with low retrieval rates, providers have focused on increased clinician education and oversight, novel technical aspects of retrieval, and streamlining systems-based approaches for patient follow-up.[44,48] Poor patient education is a barrier to IVCF removal. In one qualitative study 12% of patients interviewed were not aware of having an IVCF, 77% did not know an IVCF can be removed, and 79% were not aware of long-term risks of IVCFs, highlighting patient education and engagement as an important strategy to improve retrieval rates.[49] Ultimately, the decision to retrieve an IVCF should be based on the patient's current risk of thrombosis and bleeding risk with anticoagulation. In case 1b, the patient has initiated anticoagulation for DVT without progression of SDH; therefore, IVCF retrieval should be planned. Optimizing appropriate IVCF removal requires a collaborative approach with multidisciplinary providers and shared decision making with the patient.

Conclusion

Despite the availability of safe and effective anticoagulants, a small group of patients with acute VTE and absolute contraindication to anticoagulation will continue to require IVCFs. However, for extended indications, there is insufficient evidence to support routine IVCF use. If IVCFs are employed, close follow-up is vital with attention to resumption of anticoagulation when safe, monitoring for filter complications, and IVCF removal when no longer needed. Further research is essential to address these popular, but overall unsubstantiated, devices (Table 5). Until well-designed trials are available, IVCFs will remain a contentious topic. Clearly, a "more is better" approach is not appropriate when incorporating IVCFs into clinical practice, and therefore clinicians must assimilate the available evidence to make case-by-case decisions.

Conflict-of-interest disclosures

A.R. has served on advisory boards for Alexion, Baxter, Bayer, Kedrion Biopharma, and Octapharma Plasma. A.H.K. declares no competing financial interests.

Off-label drug use

None disclosed.

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EVIDENCE-BASED MINIREVIEW

Do prophylactic inferior vena cava filters in trauma patients reduce the risk of mortality or pulmonary embolism?

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LEARNING OBJECTIVES

- Review evidence for efficacy of prophylactic inferior vena cava filters (IVCFs) in trauma patients in preventing pulmonary embolism (PE)
- Review evidence for efficacy of prophylactic IVCFs in trauma patients in preventing mortality

Clinical case

A 25-year-old man with no significant medical history presents to the emergency department after a motor vehicle collision. He sustained multiple fractures (injury severity score of 27). He is deemed to be high risk for bleeding with anticoagulant prophylaxis. A pneumatic compression device was placed on the lower extremities, and the surgeon on duty would like to discuss placing a prophylactic inferior vena cava filter for primary thromboprophylaxis.

pIVCFs in trauma patients

Venous thromboembolism (VTE), which includes deep vein thrombosis (DVT) and pulmonary embolism (PE), is a common but potentially life-threatening complication of major trauma. Without appropriate thromboprophylaxis for trauma patients, the incidence of DVT and PE is as high as 18% and 11%, respectively.[1] Importantly, 37% of symptomatic PE occur early after trauma, often within the first 4 days. Although fatal PE is uncommon (0.4-4.2%), 12% of deaths in trauma patients can be attributed to PE.[1] Thus, PE is consistently identified as a preventable cause of death and target for improved prevention strategies. The 2019 American Society of Hematology guidelines for prevention of VTE in surgical hospitalized patients recommend routine anticoagulant prophylaxis with either low-dose unfractionated heparin or low-molecular-weight heparin over no prophylaxis in major trauma patients at low to moderate risk of bleeding.[2] However, because of high bleeding risk, anticoagulant thromboprophylaxis is often contraindicated, and mechanical prophylaxis with graduated compression stockings, intermittent pneumatic compression devices, or prophylactic inferior vena cava filters (pIVCFs) are considered. With the advent of retrievable IVCFs, utilization of these devices for primary VTE prophylaxis has expanded disproportionately to the data supporting their use and guideline recommendations.[3]

To examine current best evidence of pIVCFs on PE and mortality risk in trauma patients, we conducted a PubMed search of articles published from inception to 31 May 2020 using the following search terms: (trauma[MeSH Terms]) AND ("vena cava filters"[MeSH Terms]). To evaluate the highest quality data available, PubMed "Article Type" filters were applied for clinical trial, meta-analysis, randomized controlled trial, review, and systematic reviews. No language filters were used. All articles identified were reviewed for the following inclusion criteria: (1) meta-analysis, randomized controlled trial (RCT), or observational study, (2) trauma patients comprised at least a portion of the study population, (3) at least a portion of IVCFs used were for prophylaxis (before any known VTE), (4) a statistical comparison between patients with and without pIVCFs in trauma patients was reported, and (5) clinical outcomes for PE or mortality were provided. To broaden our search, references of review articles and known guidelines on the topic were screened for studies not previously identified. Both authors screened selected articles first by titles, then by abstracts, and subsequently by full-text articles. Disagreement between authors was resolved by consensus. Our systematic search identified a total of 10 articles: 3 clinical trials, 4 study-level meta-analyses, and 3 additional observational studies not included in these meta-analyses (Table 1; Figure 1).[1,3-12] Data from all identified studies were extracted by both authors.

Table 1. Clinical trials, systematic review and meta-analyses, and observational studies

Study	Study design	Studies	Population	Intervention	Comparator	Efficacy outcomes	Safety outcomes
Clinical trials							
Fullen et al 1973[4]	Quasi-RCT	N/A	Traumatic fracture of the proximal femur without VTE	Permanent IVCF (n = 41)	No IVCF (n = 59)	PE: 4 (10%) in IVCF group vs 19 (32%) in non-IVCF group Mortality: 4 (10%) in IVCF group vs 14 (24%) in non-IVCF group	IVCF complications: none reported in either group
Rajasekhar et al 2011[5]	Randomized pilot feasibility study	N/A	High-risk trauma patient without VTE	Retrievable IVCF and anticoagulant prophylaxis (n = 18)	Standard anticoagulant VTE prophylaxis (n = 16)	PE: 0 in IVCF group vs 1 in non-IVCF group	DVT: 1 in IVCF group vs 0 in non-IVCF group
						Mortality: 1 in IVCF group vs 0 in non-IVCF group	IVCF retrieval: 0% at 3 mo; 15% at 6 mo
Ho et al 2019[1]	RCT	N/A	High-risk trauma patient (ISS > 15) without VTE and contraindication to AC	Retrievable IVCF (n = 122)	No IVCF (n = 118)	90-d composite symptomatic PE or mortality: 17 (13.9%) in IVCF group vs 17 (14.4%) in non-IVCF group (HR 0.99; 95% CI 0.51-1.94; *P* = .98)	Symptomatic PE (after 7 d with continued contraindication to AC): 0 in IVCF group vs 5 in non-IVCF group (RR 0, 95% CI 0.00-0.55)
						Mortality: 16 (13.1%) of IVCF group vs 11 (9.3%) of non-IVCF group (RR 1.41; 95% CI 0.69-2.87)	IVCF complications (at time of filter retrieval): 15%
							90-d IVCF retrieval: 71 of 108 (66%)
Systematic reviews and meta-analyses							
Rajasekhar et al 2011[3]	Systematic review and meta-analysis	7 observational cohort studies	High-risk trauma patient without VTE	IVCF (n = 428) (6 studies with permanent IVCFs, 1 not reported) and anticoagulant prophylaxis (in 5 studies)	No IVCF (n = 1472) and anticoagulant prophylaxis (in 5 studies)	PE: 4 of 428 (0.9%) in IVCF group vs 76 of 1472 (5%) in non-IVCF group (OR 0.21; 95% CI 0.09-0.49)	DVT*: 17 of 94 (18%) in IVCF group vs 18 of 138 (13%) in non-IVCF group (OR 1.6; 95% CI 0.76-3.37)
Singh et al 2013[6]	Systematic review and meta-analysis	8 controlled studies: 1 pilot feasibility, 7 observational; 2 excluded from meta-analysis	Mixed trauma patient	IVCF (2 permanent IVCF; 6 not reported) (n = 432)	No IVCF (i.e. mechanical ± anticoagulant prophylaxis) (n = 4160)	PE*: 2 of 334 (0.5%) in IVCF group vs 48 of 730 (6.5%) in non-IVCF group (RR 0.20; 95% CI 0.06-0.70)	Fatal PE*: 0 of 163 (0%) in IVCF group vs 20 of 407 (4.9%) in non-IVCF group (RR 0.09; 95% CI 0.01-0.81)
						Mortality*: 21 of 166 (12.6%) in IVCF group vs 60 of 312 (19.2%) in non-IVCF group (RR 0.7; 95% CI 0.4-1.23)	DVT*: 18 of 112 (16%) in IVCF group vs 18 of 154 (11.7%) in non-IVCF group (RR 1.76; 95% CI 0.49-6.18; *P* = .38)
							Filter complication* 1.0%-5.7%
Haut et al 2014[7]	Systematic review and meta-analysis	8 controlled studies: 1 pilot feasibility, 7 observational – 2 excluded from meta-analysis	Mixed trauma patient	IVCF and standard VTE prophylaxis (n = 334)	Standard VTE prophylaxis (n = 730)	PE: 2 (0.5%) in IVCF group vs 48 (6.5%) in non-IVCF group (RR 0.20; 95% CI 0.06-0.70)	Fatal PE*: 0 in IVCF group vs 20 of 407 (4.9%) in non-IVCF group (RR 0.09; 95% CI 0.01-0.81)
						Mortality*: RR 0.70 [95% CI 0.4-1.23]	DVT*: RR 1.76; 95% CI 0.50-6.19, *P* = .38)
Shariff et al 2020[8]	Systematic review and meta-analysis	10 controlled studies: 2 RCTs, 8 observational studies	High-risk trauma patients (ISS > 15 or trauma delaying initiation of VTE prophylaxis)	IVCF (n = 573)	Standard VTE prophylaxis (n = 1717)	Symptomatic PE: 5 (0.87%) in IVCF group vs 90 (5.2%) in non-IVCF group (RR 0.27; 95% CI 0.12-0.58; *P* < 0.05)	Fatal PE*: 0 in IVCF group vs 23 of 619 (3.7%) in non-IVCF group (RR 0.29; 95% CI 0.08-1.10; *P* = .07)

AC, anticoagulation; CI, confidence interval; HR, hazard ratio; ISS, Injury Severity Score; N/A, not applicable; NR, not reported; OR, odds ratio; RR, relative risk.
*Outcome not reported in all studies.

Table 1. (Continued)

Study	Study design	Studies	Population	Intervention	Comparator	Efficacy outcomes	Safety outcomes
Observational studies							
Batty et al 2012[9]	Prospective cohort study with concurrent controls	N/A	High-risk trauma patient without VTE	IVCF (n = 511)	No IVCF (n = 5833)	PE: OR 0.28; 95% CI 0.088-0.890; *P* = .031	NR
Hemmila et al 2015[10]	Retrospective cohort study	N/A	High-risk trauma patient without VTE	IVCF (n = 803)	No IVCF (n = 39456)	PE: 9 (1.1%) in IVCF group vs 187 (0.5%) in non-IVCF group (*P* = .01) Mortality: 42 (5.2%) in IVCF group vs 1369 (3.5%) in non-IVCF group (*P* = .01)	DVT: 54 (6.7%) in IVCF group vs 483 (1.2%) in non-IVCF group (*P* < .001)
Sarosiek et al 2017[11]	Retrospective cohort study	N/A	High-risk trauma patient without VTE	IVCF (n = 451)	No IVCF (n = 1343)	Mortality: IVCF group with higher mortality than non-IVCF group (*P* = .14)	NR

AC, anticoagulation; CI, confidence interval; HR, hazard ratio; ISS, Injury Severity Score; N/A, not applicable; NR, not reported; OR, odds ratio; RR, relative risk.
*Outcome not reported in all studies.

Does the literature support IVCFs in trauma patients for primary VTE prophylaxis? Numerous nonrandomized cohort studies have evaluated the efficacy of pIVCFs in trauma patients. These studies have inherent limitations including lack of a control group not receiving an IVCF, variance in reporting details of patient baseline characteristics, different definitions for high-risk trauma patients, lack of documented or standardized contemporary anticoagulant prophylaxis, different screening patterns for VTE, and limited follow-up for clinical outcomes after hospital discharge. Only 3 clinical trials have investigated the role of pIVCFs in trauma patients. In 1973, Fullen et al[4] randomized 129 patients with traumatic femoral fractures to pIVCF vs. no IVCF. Significant reductions in PE and mortality were reported, driven primarily by a reduction in fatal PE. This study was limited by absence of anticoagulant prophylaxis in any group, high crossover from the filter to nonfilter group, and the inclusion of possible PE without radiologic confirmation of diagnosis. In 2011, Rajasekhar et al[5] performed a prospective pilot feasibility study randomizing high-risk trauma patients to pIVCF vs no IVCF. All patients also received anticoagulant prophylaxis. At 6 months, 1 PE occurred in the non-IVCF arm and 1 death in the IVCF arm. This study was not powered to detect differences in clinical outcomes but showed feasibility of conducting a larger scale study. In 2019, Ho et al[1] randomized 240 major trauma patients with contraindications to anticoagulation to an IVCF, placed within 72 hours of admission, vs no IVCF. After 90 days, early IVCF placement was not associated with a lower risk of the composite end point (symptomatic PE) and death. Within 7 days of injury, 67% had started anticoagulant prophylaxis. Of those with ongoing contraindications to prophylactic anticoagulation, symptomatic PE developed in none in the IVCF group and 5 in the non-IVCF group, including 1 fatal PE. Notably survivor bias could have affected this subgroup analysis.

Numerous reviews discuss primary VTE prophylaxis with IVCFs in trauma patients, but only 4 met our inclusion criteria.[3,6-8] One study-level meta-analysis by Rajasekhar et al[3] critically appraised results from comparative observational studies and found a lower risk for PE but no difference in DVT or mortality in trauma patients treated with pIVCFs compared with a non-IVCF group. However, significant heterogeneity among studies limited firm conclusions. A 2013 meta-analysis by Singh et al[6] included the 2011 pilot RCT and 5 observational studies. All included studies compared an IVCF group in addition to standard VTE prophylaxis against standard prophylaxis alone, although there was variance in types of outcomes reported. Notably, the definition of standard prophylaxis varied between trials, ranging from either anticoagulant or mechanical prophylaxis alone or combined prophylaxis. All studies were judged to have a moderate to high risk of bias. A precise and consistent reduction in PE with IVCFs was reported in all studies without evidence of statistical heterogeneity. IVCFs were found to be protective against fatal PE, although sensitivity analysis did not reveal robustness in this outcome. Mortality and DVT were not different between groups. Retrieval rates were not consistently reported. A subsequent 2014 meta-analysis arrived at the same conclusions based on the same body of evidence.[7] In this meta-analysis, Haut et al estimated 109 to 962 trauma patients (assuming a baseline PE risk of 0.13-1.15%) would need to receive an IVCF to prevent 1 PE. With a case fatality rate of 10%, 1099 patients would need to receive a pIVCF to prevent 1 fatal PE. The most recent meta-analysis on this topic by Shariff et al[8] included the 2011 and 2019 clinical trials and 8 observational studies, all deemed to have high risk of bias, and was the only review to include the highest quality data from the large clinical trial by Ho et al.[1] A lower risk of symptomatic PE without difference in fatal PE was observed in patients that received a pIVCF compared with no pIVCF. No significant heterogeneity in the pooled estimates or publication bias was present.

We found 3 additional observational studies in our systematic review that were not included in the above meta-analyses.[9-11] A

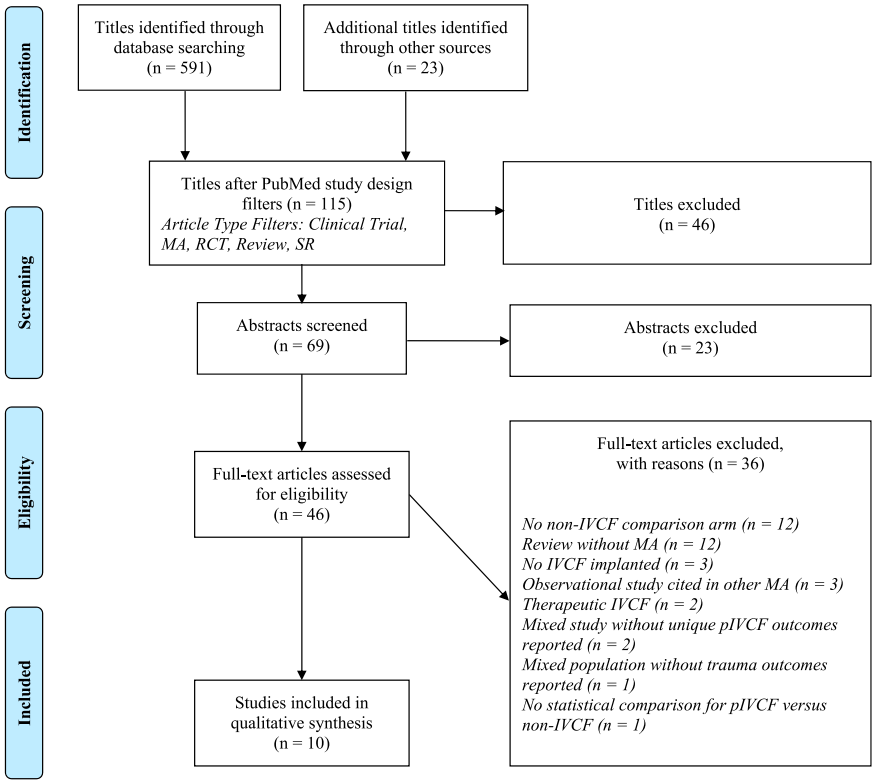

Figure 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram. PRISMA methodology: Moher et al.[12] MA, meta-analysis; SR, systematic review.

trauma registry study by Batty et al[9] found that the presence of a pIVCF was independently associated with lower risk for PE. However, this study was limited by its retrospective nature and low incidence of PE overall compared with those without an IVCF. Sarosiek et al[11] evaluated mortality outcomes in trauma patients who received an IVCF compared with propensity-matched controls without an IVCF. Placement of an IVCF did not affect short-term or long-term mortality in those that survived their initial injury after 24 hours, regardless of whether the IVCF was placed for prophylactic or therapeutic indications. Finally, analysis of a collaborative registry from 26 trauma centers by Hemmila et al[10] found that pIVCFs were associated with higher rates of mortality, DVT, PE, and overall VTE, although IVCF patients had higher baseline injury severity scores compared with non-IVCF patients.

In conclusion, there is low quality evidence based on the Grading of Recommendations Assessment, Development, and Evaluation approach that prophylactic IVCF use in patients with major trauma reduces the incidence of PE in the absence of a reduction in mortality.[13] Given the potential increased risk of DVT, known complications associated with these devices, low retrieval rate in the relatively young healthy trauma population, and lack of cost-effectiveness with this approach, IVCFs should not be used routinely in trauma patients for primary VTE prophylaxis.[14]

Conflict-of-interest disclosure

The authors declare no competing financial interests.

Off-label drug use

None disclosed.

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Does aspirin prevent venous thromboembolism?

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Venous thromboembolism (VTE; deep vein thrombosis and/or pulmonary embolism) is a well-established cause of morbidity and mortality in the medical and surgical patient populations. Clinical research in the prevention and treatment of VTE has been a dynamic field of study, with investigations into various treatment modalities ranging from mechanical prophylaxis to the direct oral anticoagulants. Aspirin has long been an inexpensive cornerstone of arterial vascular disease therapy, but its role in the primary or secondary prophylaxis of VTE has been debated. Risk-benefit tradeoffs between aspirin and anticoagulants have changed, in part due to advances in surgical technique and postoperative care, and in part due to the development of safe, easy-to-use oral anticoagulants. We review the proposed mechanisms in which aspirin may act on venous thrombosis, the evidence for aspirin use in the primary and secondary prophylaxis of VTE, and the risk of bleeding with aspirin as compared with anticoagulation.

LEARNING OBJECTIVES

- Understand the evidence supporting the use of low-dose aspirin in the primary prophylaxis of VTE in specific medical and surgical contexts
- Understand the evidence related to the use of low-dose aspirin in the secondary prophylaxis of VTE
- Review the safety profile and bleeding risk of aspirin use in comparison with anticoagulation

Clinical case 1

A 65-year-old man with no prior medical history undergoes an elective total knee replacement for chronic degenerative disease. The surgery is uneventful, and he is discharged the next day. He has no personal or family history of venous thromboembolism (VTE) and he is not obese. He is educated on the importance of early mobility and rehabilitation. He is motivated and asks how best to minimize his postoperative risk of VTE.

The physiology behind venous thrombosis

Hemostasis is a balance between clot formation and clot degradation, a tightly regulated system of procoagulant and anticoagulant forces. Thrombosis occurs when this equilibrium is disrupted. Clinicians have historically approached the prevention and treatment of arterial and venous thrombosis somewhat differently, in part because of perceived pathophysiologic differences.

Arterial thrombosis is a platelet-predominant phenomenon, often associated with atherosclerotic damage and inflammation. Ruptured plaque and high shear forces promote the binding and unfolding of von Willebrand factor, inciting platelet aggregation and activation. Histopathology of the arterial clot is characterized by fibrin, leukocytes, and an abundance of platelets, providing a classic "white" appearance.[1] Arterial thrombi most often present clinically as acute stroke, myocardial infarction, or peripheral arterial disease.

Venous thrombosis, on the other hand, is generally thought of as a disorder in plasma coagulation. Venous thrombi are fibrin-rich, originating in areas of slower blood flow such as the deep veins of the legs. It has been proposed that the endothelium becomes activated and sets off a cascade of inflammation and activation of the coagulation pathway. On histopathology, venous clots are composed of fibrin, leukocytes, and red blood cells, providing a classic "red" appearance; platelets are less prominent than they are in arterial thrombi (Figure 1).[1-3]

These distinctions notwithstanding, there is significant mechanistic overlap between arterial and venous thrombosis. The presence of platelets in venous thrombi provides a biologic rationale for the hypothesis that antiplatelet therapy may reduce the risk of VTE in some settings.

Aspirin

Mechanism of action

Acetylsalicylic acid, also known as aspirin, was the first synthetic drug produced, in 1897.[4] Cyclooxygenase (COX)

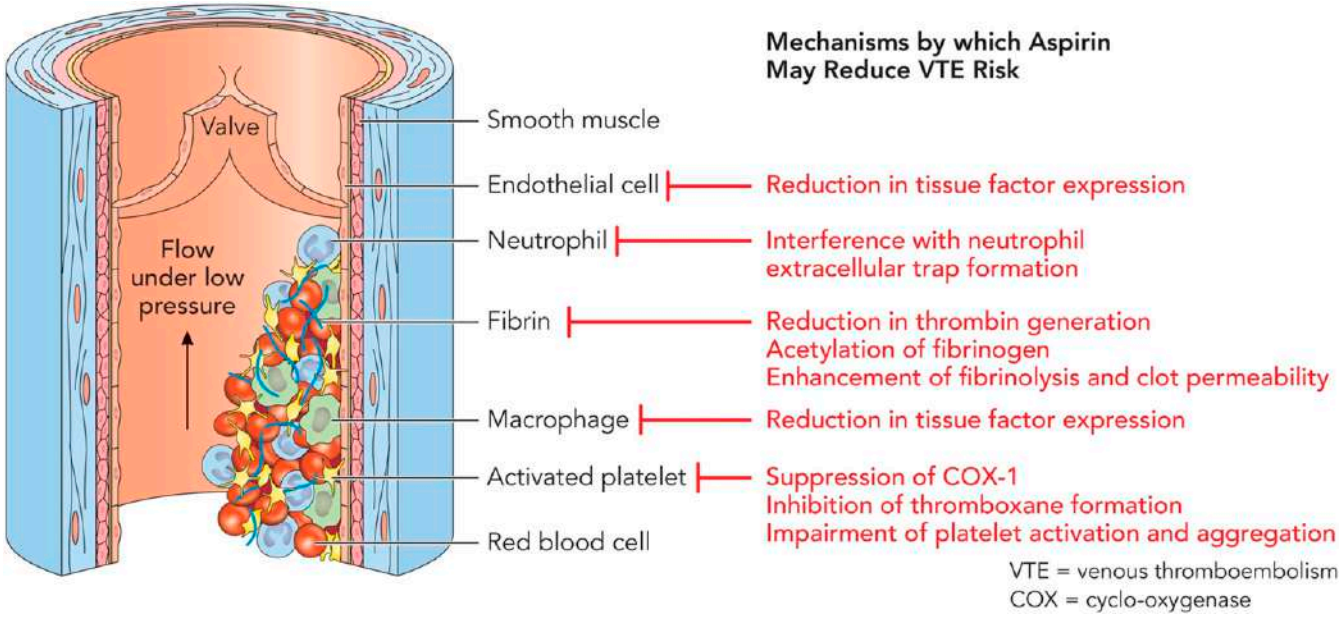

Figure 1. Composition of venous thrombosis and the antithrombotic effects of aspirin. Venous thrombosis typically originates in areas of slower blood flow, such as the venous anatomy near valves. Venous clots consist primarily of fibrin, red blood cells, and leukocytes. Platelets are involved, but are less prominent in comparison with the platelet-rich arterial thrombus. Aspirin exerts various antithrombotic effects on the participating cells and proteins of thrombus formation, and fibrinolysis via cyclooxygenase (COX) and COX-independent pathways. Professional illustration by Patrick Lane, ScEYEnce Studios.

isoenzymes, COX-1 and COX-2, catalyze the formation of prostaglandins, thromboxane, and levuloglandins.[5] Aspirin inhibits COX activity (mainly COX-1) irreversibly. The suppression of COX-1 decreases the generation of thromboxane A2 (TXA2), an important cofactor for platelet activation and aggregation.[6] Aspirin is also suspected to downregulate tissue factor expression, thrombin formation, and downstream thrombin-mediated coagulant reactions. In addition, aspirin may participate in the acetylation of various proteins to catalyze more efficient fibrinolysis (Figure 1).[7-9] Aspirin may also exert influence COX-independent pathways to inhibit platelet aggregation and dense granule secretion.[8,9]

Dosing

Aspirin is absorbed primarily in the stomach and upper small intestine. Doses of 30 to 100 mg of aspirin daily are sufficient to inhibit platelet TXA2 synthesis.[10] Paradoxically, higher doses of aspirin appear to have weaker effects on fibrin properties than the lower 75-mg daily dose.[11] Low-dose aspirin is typically considered optimal for the primary and secondary prophylaxis of arterial thrombosis.[12,13] In the setting of VTE prophylaxis following total joint arthroplasty, a pooled analysis of numerous studies found no significant differences in symptomatic pulmonary embolism (PE), symptomatic deep vein thrombosis (DVT), 90-day mortality, or major bleeding across patient groups receiving low-dose or high-dose aspirin (defined as >162 mg).[14]

Primary prophylaxis of venous thrombosis

VTE (DVT and PE) is a well-established cause of morbidity and mortality in the medical and surgical patient populations.[15,16] The orthopedic surgery community has long embraced aspirin for postsurgical VTE prophylaxis, mainly after total hip arthroplasty [THA] and total knee arthroplasty [TKA].[17] Aspirin is widely available and inexpensive, does not require monitoring, and is conventionally thought to confer a lower bleeding risk than anticoagulants in the perioperative period. Many studies support the use of aspirin for primary VTE prophylaxis, but much of the available evidence is considered low quality because it is retrospective and/or subject to selection bias.[18] On the other hand, there is a significant amount of high-quality evidence relevant to aspirin use in this postarthroplasty setting; we review this evidence here.

In a meta-analysis of randomized studies by the Antiplatelet Trialists' Collaboration in 1994, antiplatelet therapy (not exclusive to aspirin) was found to effect a significant reduction in VTE risk and a favorable trend toward mortality benefit (compared with no prophylaxis).[19] This finding was reinforced by the multinational and prospective Pulmonary Embolism Prevention (PEP) study. In the PEP study, 17 000 patients undergoing surgery for hip fracture or elective arthroplasty were randomized to either 160 mg of aspirin daily or placebo, starting preoperatively and continued for 35 days. Aspirin reduced the risk of symptomatic VTE by ~36% when compared with placebo.[20] Other forms of thromboprophylaxis were concurrently allowed. The statistical significance of benefit was seen primarily in the hip-fracture group, but not observed in the subgroup receiving low-molecular-weight heparin (LMWH) or in patients who were undergoing elective arthroplasty. There was also a trend toward more major nonfatal bleeding and nonfatal myocardial infarctions with aspirin, practically balancing out the benefit of VTE reduction. However, the evidence of efficacy in the PEP study supported aspirin's inclusion as an option to consider for postorthopedic surgical VTE prophylaxis, even in the early 2000s (Table 1). Although aspirin may be better than placebo in

Table 1. Summary of clinical practice guidelines involving the use of aspirin for the pharmacologic prophylaxis of VTE

Society/Year VTE indication	Recommendation
ACCP[24]/2016	
Secondary prophylaxis	In patients with an unprovoked proximal DVT or PE who are stopping anticoagulant therapy and do not have a contraindication to aspirin, we suggest aspirin over no aspirin to prevent recurrent VTE
AAOS[43]/2012	
Elective hip or knee arthroplasty	We suggest the use of pharmacologic agents and/or mechanical compressive devices for the prevention of VTE in patients undergoing elective hip or knee arthroplasty, and who are not at elevated risk beyond that of the surgery itself for VTE or bleeding
ASH[44]/2019	
THA or TKA	The ASH guideline panel suggests using aspirin (ASA) or anticoagulants
	When anticoagulants are used, the panel suggests using DOACs over LMWH
	The panel suggests using any of the DOACs approved for use
	If a DOAC is not used, the panel suggests using LMWH rather than warfarin and recommends LMWH rather than UFH
ESA[45]/2018	
Hip fracture, hip arthroplasty, or knee arthroplasty	We recommend using aspirin, considering that it may be less effective than or as effective as LMWH for prevention of DVT and PE after THA, TKA, and hip-fracture surgery
	Aspirin may be associated with less bleeding after THA, TKA, and hip-fracture surgery than other pharmacological agents
General orthopedic procedures	Aspirin may be less effective than or as effective as LMWHs for prevention of DVT and PE after other orthopedic procedures
General surgery	We do not recommend aspirin as thromboprophylaxis in general surgery; however, this type of prophylaxis could be interesting especially in low-income countries and adequate large-scale trials with proper study designs should be carried out
NICE[46]/2018	
Hip arthroplasty	Offer VTE prophylaxis to people undergoing elective hip-replacement surgery whose risk of VTE outweighs their risk of bleeding
	Choose any 1 of: LMWH (for 10 d) followed by aspirin (75 or 150 mg) for a further 28 d; LMWH (for 28 d) combined with antiembolism stockings (until discharge); rivaroxaban
Knee arthroplasty	Offer VTE prophylaxis to people undergoing elective knee-replacement surgery whose VTE risk outweighs their risk of bleeding
	Choose any 1 of: aspirin (75 or 150 mg) for 14 d; LMWH (for 14 d) combined with antiembolism stockings (until discharge); rivaroxaban
Multiple myeloma patients on immunomodulator therapy	Consider pharmacological VTE prophylaxis for people with myeloma who are receiving chemotherapy with thalidomide, pomalidomide, or lenalidomide with steroids
	Choose either: aspirin (75 or 150 mg) or LMWH
SIGN[47]/2010	
General surgical patient	Aspirin is not recommended as the sole pharmacological agent for VTE prophylaxis in surgical patients, as other available agents are more effective
Orthopedic surgical patient	As other agents are more effective for prevention of DVT, aspirin is not recommended as the sole pharmacological agent for VTE prophylaxis in orthopedic patients
Medical patient	When the assessment of risk favors use of thromboprophylaxis, UFH, LMWH, or fondaparinux should be administered
	Aspirin is not recommended as the sole pharmacological agent for VTE prophylaxis in medical patients

AACP, American College of Chest Physicians; AAOS, American Academy of Orthopaedic Surgeons; ASA, acetylsalicylic acid; ASH, American Society of Hematology; DOAC, direct oral anticoagulant; ESA, European Society of Anaesthesiology; NICE, National Institute for Health and Clinical Excellence; SIGN, Scottish Intercollegiate Guidelines Network; UFH, unfractionated heparin.

regard to reducing VTE risk, there was still debate around the overall efficacy and safety of low-dose aspirin when compared with low-dose anticoagulants.

In the Extended Prophylaxis Comparing Low-Molecular-Weight Heparin to Aspirin in Total Hip Arthroplasty (EPCAT) trial, patients undergoing hip arthroplasty received 10 days of prophylaxis-dose dalteparin, and then were randomized to 28 days of low-dose aspirin or continued dalteparin. Aspirin was noninferior to dalteparin in the prevention of symptomatic VTE over a 90-day follow-up period; bleeding event rates were

similar.[21] The study was limited by low adherence in the LMWH group and an imbalance in risk factors for thrombosis between the 2 groups. A second randomized trial, Extended Venous Thromboembolism Prophylaxis Comparing Rivaroxaban to Aspirin Following Total Hip and Knee Arthroplasty II (EPCAT II), established low-dose aspirin as noninferior to rivaroxaban for VTE prevention in patients undergoing hip or knee arthroplasty (all patients received low-dose rivaroxaban for the first 5 postoperative days).[22] Patients at high risk for VTE, such as those with known thrombophilia, prior VTE, cancer, or morbid obesity were underrepresented or excluded. Bleeding was uncommon in both groups and usually occurred within 10 days of surgery, possibly because rivaroxaban was used by all patients during the first 5 postoperative days. Addition of mechanical compression to either regimen was optional and relatively uncommon in both groups (approximate rate, 16%).

In a more recent systematic review that pooled data from 13 randomized trials, aspirin was found to be comparable to other antithrombotic agents in preventing postoperative VTE after total joint arthroplasty.[23] The safety data from this pooled comparison did not identify a significant difference in major bleeding rates between aspirin and the comparator anticoagulants (~0.5% of patients in both groups experienced major bleeding). There was a trend toward lower rates of wound hematoma and wound infection in patients receiving aspirin, but the differences were not statistically significant. The findings of the meta-analysis should be interpreted with caution as the event rates were low and there was significant heterogeneity across trials.

With the continued uncertainty about how aspirin compares to anticoagulants, the upcoming Pulmonary Embolism Prevention after Hip and Knee Replacement (PEPPER) and VTE Prevention Following Total Hip and Knee Arthroplasty (EPCAT III) trials (NCT02810704 and NCT04075240, respectively) will be of particular interest. EPCAT III will randomize patients undergoing hip and knee arthroplasty to receive either aspirin alone or aspirin and rivaroxaban for the prevention of VTE, using similar inclusion and exclusion criteria as EPCAT II; patients with metastatic cancer, existing need for long-term anticoagulation, and previously documented VTE will be excluded. The PEPPER trial will randomize a similar patient population to 4 weeks of VTE prophylaxis with either rivaroxaban, aspirin, or warfarin. PEPPER appears to have less stringent exclusion criteria than EPCAT II and will not explicitly exclude patients with prior VTE or cancer. However, patients who are already on chronic anticoagulation will not be eligible to enroll. All patients will receive in-hospital pneumatic compression (along with modern surgical techniques and postoperative care); this design should provide insight into the possibility that combination mechanical prophylaxis and aspirin may lead to even lower VTE rates.

In summary, all low-risk patients in the postarthroplasty surgical setting are candidates for thromboprophylaxis with aspirin. Whether a few days of an anticoagulant prior to low-dose aspirin has benefit, and whether prolonged administration of a low-dose anticoagulant may be the best choice for high-risk patients, remain unanswered questions. For many patients, the addition of mechanical VTE prophylaxis (eg, with sequential compression devices) effectively closing any efficacy gap between low-dose aspirin and a low-dose anticoagulant (if such a gap exists) is a possibility.

The patient in our case is at low risk for VTE following TKA. We would recommend the use of a hybrid strategy with 10 mg of rivaroxaban daily for 5 days followed by low-dose aspirin for an additional 9 days, as was done in EPCAT II. Pending the results of the upcoming PEPPER and EPCAT-3 trials, available evidence would also support low-dose direct oral anticoagulants (DOACs), low-dose LMWH, or, because he is low risk, low-dose aspirin for an entire 14-day course.

Clinical case 2

A 58-year-old woman with a history of coronary artery disease, morbid obesity, and a prior unprovoked proximal DVT 3 years ago presents to her primary care office prior to embarking on a long international flight to a low-resource setting. For her past unprovoked DVT, she completed 6 months of warfarin and decided to forgo further anticoagulation. She had no bleeding complications while on therapy. She takes 81 mg of aspirin daily as recommended by her cardiologist. She is concerned about her risk to develop DVT or PE, either while traveling or at some point later in life. She wonders whether she should consider adding 1 of the new anticoagulants to her medication regimen.

Aspirin

Secondary prophylaxis of venous thrombosis

If anticoagulant therapy is stopped 6 to 12 months after a first unprovoked VTE, the 5-year risk of recurrence is ~30%.[24] Whereas aspirin is widely accepted as a secondary prevention strategy for stroke and myocardial infarction, the role of aspirin to prevent recurrent VTE is less defined.

The International Collaboration of Aspirin Trials for Recurrent Venous Thromboembolism (INSPIRE) investigators pooled analyses from 2 underpowered trials: the Warfarin and Aspirin (WARFASA) and the Aspirin to Prevent Recurrent Venous Thromboembolism (ASPIRE) studies.[25-27] Across the WARFASA and ASPIRE trials, the combined study population completed anywhere from 6 weeks to 24 months of initial anticoagulation therapy before randomization to low-dose aspirin or placebo. The median follow-up time was 30.4 months, in which there was a statistically significant 32% reduction in VTE recurrence with aspirin when compared with placebo. The authors also suspect, through refined estimate modeling, that with full medical adherence, aspirin would prevent closer to 40% of recurrent events. The major bleeding rate was low (0.5%) and essentially identical between the aspirin and placebo groups.

In the Reduced-dosed Rivaroxaban in the Long-term Prevention of Recurrent Symptomatic Venous Thromboembolism (EINSTEIN CHOICE) trial, patients who completed 6 to 12 months of anticoagulation for VTE were randomized to rivaroxaban (either 10 mg or 20 mg daily) or aspirin (up to 100 mg daily) to prevent recurrence.[28] With a mean follow-up of 1 year, VTE occurred in 1.5% of patients receiving 20 mg of rivaroxaban, 1.2% of patients receiving 10 mg of rivaroxaban, and 4.4% of patients receiving aspirin. Whether DOACs and/or warfarin reduce the risk of myocardial infarction or noncardioembolic stroke as effectively as aspirin is not yet known.

The results of these trials suggest that aspirin has some efficacy in preventing VTE recurrence; patients who use aspirin as a long-term secondary prevention strategy can expect a VTE recurrence risk lower than if they took no medication but higher than if an anticoagulant was used instead. However, the safety benefit of aspirin (vs anticoagulant therapy), if there is one, may not offset the lower efficacy of aspirin. Aspirin, though easily

Table 2. Summary of major bleeding outcomes across major SRs and RCTs comparing aspirin and placebo

Study reference	Indication	Aspirin dose compared with placebo	No. of major bleeding events: aspirin vs placebo	RR/HR
ECLAP[34]	Primary thromboprophylaxis for polycythemia vera	Aspirin 100 mg daily	3 vs 2	RR, 1.62 (0.27-9.71)
RCT, 2004; n = 518				
INSPIRE[25]	Recurrent VTE prevention	Aspirin 100 mg daily	9 vs 7	HR, 1.31 (0.48-3.53)
RCT, 2014; n = 1224				
POISE-2[48]	Perioperative administration of aspirin in patients undergoing noncardiac surgery	Aspirin 200 mg daily	230 vs 188	HR, 1.23 (1.01-1.49)
RCT, 2014; n = 10 010				
Mahmoud et al, 2019[49]	Primary cardiovascular prevention	Aspirin mostly, 75-100 mg	1301 vs 901	RR, 1.47 (1.31-1.65)
SR, 11 RCTs; n = 157 248				
Zheng et al, 2019[50]	Primary cardiovascular prevention	Aspirin ≤100 mg daily	1195 vs 834	HR, 1.54 (1.35-1.76)
SR, 11 RCTs; n = 134 470				

Major bleeding as defined by criteria set in each individual randomized control trial or systematic review.
HR, hazard ratio; INSPIRE, International Collaboration of Aspirin Trials for Recurrent Venous Thromboembolism; n, number of patients; POISE-2, Perioperative Ischemic Evaluation 2; RR, relative risk; SR, systematic review and meta-analysis.

acquired over the counter, can cause serious adverse effects including renal dysfunction, gastrointestinal pathology, and, most importantly, serious bleeding.[6]

The risks

Long-term stroke-prevention trials in patients with atrial fibrillation provide an excellent assessment of the relative bleeding risk with aspirin compared with the anticoagulants. In review of recent randomized controlled trials (RCTs) and systematic reviews of RCTs in which aspirin was compared with placebo, the major bleeding rate seen with aspirin is unsurprisingly higher than that seen with no antithrombotic therapy (Table 2). When aspirin is compared with warfarin or the DOACs across various indications, there is a trend suggesting that aspirin may cause less bleeding than anticoagulants, but in most studies, the difference fails to achieve statistical significance (Table 3). In a systematic review of patients older than 65 years on antiplatelet therapy, the risk of major hemorrhage associated with chronic antiplatelet drug use is very close to the risk associated with the oral anticoagulants.[29] Overall, major bleeding was as frequent among patients taking antiplatelet therapy as among patients taking warfarin in RCTs. Of course, the difficulty in establishing a safety benefit from aspirin (vs anticoagulants) may be due to a lack of power to detect a difference; however, excellent safety profiles of modern anticoagulant strategies (at least within randomized trials) casts some doubt on the assumption that aspirin is a much safer long-term alternative to anticoagulant therapy.

Primary VTE prevention in selected medical populations

In specific medical contexts, such as in some patients with myeloproliferative neoplasms (MPNs) and in some patients with multiple myeloma, aspirin is widely used to reduce the risk of both VTE and arterial thrombosis. There is evidence that supports platelet activation and dysfunction in both MPNs and in multiple myeloma.[30,31] In newly diagnosed multiple myeloma, the VTE rate is estimated to be at least 10% with the majority of events occurring in the first 6 months of induction.[32] A systematic review of 6 studies, encompassing 1125 patients receiving lenalidomide-based therapy, demonstrated a 10.7% total risk of VTE with aspirin compared with 1.4% with LMWH in multiple myeloma patients.[33] More evidence is needed to determine the best strategy to reduce the risk of arterial and venous thrombosis in myeloma patients starting immunomodulatory drugs.

In the European Collaboration on Low-Dose Aspirin in Polycythemia Vera (ECLAP) trial, low-dose aspirin (100 mg per day), when compared with placebo, reduced a composite end point of thrombotic complications without a significantly increased incidence of major bleeding.[34] On the other hand, in essential thrombocythemia, a systematic review of 24 observational studies concluded that patients who received antiplatelet therapy (mainly low-dose aspirin) derived a modest relative risk reduction of 26% with a median increase in major bleeding of 30%.[35] Unfortunately, with a lack of randomized trial data, these observational studies were deemed to have a high risk of bias and the evidence was rated very uncertain.

The use of aspirin for secondary VTE prevention is perhaps best reserved for situations in which antiplatelet therapy is already strongly recommended for another indication (eg, coronary stent placement), or anticoagulation is contraindicated or simply cannot be acquired due to cost or logistics. For example, in clinical case 2, aspirin could be an option for secondary thromboprophylaxis because she already has an existing cardiac indication for aspirin. Although the combination of antiplatelet

Table 3. Summary of major bleeding outcomes across major SRs and RCTs comparing anticoagulants and aspirin

Study reference	Indication	Comparators	No. of major bleeding events	RR/HR
AVERROES[51]	Stroke prophylaxis in atrial fibrillation	Apixaban 5 mg BID vs aspirin 81 to 324 mg daily	44 vs 39	HR, 1.13 (0.74-1.75)
RCT, 2011; n = 5599				
Warkentin et al, 2012[52]	Any indication for long-term antithrombotic therapy	Warfarin vs aspirin 75 to 300 mg daily	69 vs 54	OR, 1.27 (0.83-1.94)
SR, 8 RCTs; n = 2904				
COMPASS[53]	Secondary cardiovascular prevention	Rivaroxaban 5 mg BID vs aspirin 100 mg daily	288 vs 170	HR, 1.51 (1.25-1.84)
RCT, 2017; n = 27 395				
EINSTEIN-CHOICE[28]	Extended treatment of VTE	Rivaroxaban 10 mg daily vs aspirin 81 mg daily Rivaroxaban 20 mg daily vs aspirin 81 mg daily	5 vs 3 6 vs 3	HR, 1.64 (0.39-6.84) HR, 2.01 (0.50-8.04)
RCT, 2017; n = 3365				
EPCAT II[22]	Post-joint arthroplasty extended VTE prophylaxis	Rivaroxaban 10 mg daily vs aspirin 81 mg	5 vs 8	RR, 0.62 (0.20-1.90)
RCT, 2018; n = 3424				
NAVIGATE ESUS[54]	Secondary stroke prophylaxis	Rivaroxaban 15 mg daily vs aspirin 100 mg daily	62 vs 13	HR, 2.72 (1.68-4.39)
RCT, 2018; n = 7213				
Xie et al, 2019[55]	Prevention of VTE	Rivaroxaban mostly 10 mg daily vs aspirin mostly 100 mg daily or less	16 vs 11	RR, 0.81 (0.42-1.55)
SR, 9 RCTs; n = 7656				
Ng et al, 2020[56]	Stroke prophylaxis in atrial fibrillation	Aspirin (subgroup, dose not specified) vs warfarin	Not provided	RR, 0.63 (0.41-0.96)*
SR, 37 RCTs; n = 100 142				
Matharu et al, 2020[23]	Post-joint arthroplasty VTE prophylaxis	Low- and high-dose aspirin (subgroup) vs comparator anticoagulants	11 vs 10	RR, 1.11 (0.47-2.59)*
SR, 13 RCTs; n = 6060				

Major bleeding as defined by criteria set in each individual randomized control trial or systematic review.
AVERROES, Apixaban Versus Acetylsalicylic Acid to Prevent Stroke in Atrial Fibrillation Patients Who Have Failed or Are Unsuitable for Vitamin K Antagonist Treatment; BID, twice daily; COMPASS, Cardiovascular Outcomes for People Using Anticoagulation Strategies; EINSTEIN-CHOICE, Reduced-dosed Rivaroxaban in the Long-term Prevention of Recurrent Symptomatic Venous Thromboembolism; EPCAT II, Extended Venous Thromboembolism Prophylaxis Comparing Rivaroxaban to Aspirin Following Total Hip and Knee Arthroplasty II; NAVIGATE ESUS, New Approach Rivaroxaban Inhibition of Factor Xa in a Global Trial versus ASA to Prevent Embolism in Embolic Stroke of Undetermined Source; OR, odds ratio. See Table 2 for expansion of other abbreviations.
*Note relative risk ratio is reported with respect to aspirin in contrast to the format in the rest of the table.

and anticoagulant therapy would likely reduce the risk for VTE compared with antiplatelet therapy alone, the marginal benefit in this patient (who had 1 unprovoked DVT 3 years ago) would likely not offset the bleeding risk.[36-42]

There are other intriguing hypotheses that would be interesting to test. For example, might primary VTE prophylaxis with aspirin be of benefit to some patients during and/or after hospitalization for acute medical illness? Would selected persons undertaking long-distance travel benefit from taking low-dose aspirin? Which patients would benefit and for how long would such treatment be recommended? For now, these questions remain unanswered.

Conclusion

For many patients, aspirin is an inexpensive, safe and effective VTE-prevention strategy following total joint arthroplasty. Although ongoing clinical trials (EPCAT III and PEPPER) will further clarify the roles of low-dose aspirin and low-dose anticoagulants after joint replacement surgery, there is already robust evidence to support low-dose aspirin as part of a hybrid strategy after an initial period of low-dose anticoagulant administration.

For secondary VTE prophylaxis, aspirin is less effective than anticoagulants but more effective than placebo. Establishing that long-term aspirin use is clearly safer than long-term anticoagulant exposure (especially compared with low-dose, oral

factor Xa inhibitors) has been surprisingly difficult. Unless a safety benefit from aspirin can be established in well-designed prospective studies, patients who need long-term antithrombotic therapy for VTE will often choose a low-dose factor Xa inhibitor, once presented with the risk-benefit tradeoffs.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

Off-label drug use

None disclosed.

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Combining antiplatelet and anticoagulant therapy in cardiovascular disease

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Up to 10% of the >3 million Americans with atrial fibrillation will experience an acute coronary syndrome or undergo percutaneous coronary intervention. Therefore, concurrent indications for multiple antithrombotic agents is a common clinical scenario. Although each helps reduce thrombotic risk, their combined use significantly increases the risk of major bleeding events, which can be life threatening. In the past 5 years, a number of randomized clinical trials have explored different combinations of anticoagulation plus antiplatelet agents aimed at minimizing bleeding risk while preserving low thrombotic event rates. In general, shorter courses with fewer antithrombotic agents have been found to be effective, particularly when direct oral anticoagulants are combined with clopidogrel. Combined use of very low-dose rivaroxaban plus aspirin has also demonstrated benefit in atherosclerotic diseases, including coronary and peripheral artery disease. Use of proton pump inhibitor therapy while patients are taking multiple antithrombotic agents has the potential to further reduce upper gastrointestinal bleeding risk in select populations. Applying this evidence to patients with multiple thrombotic conditions will help to avoid costly and life-threatening adverse medication events.

LEARNING OBJECTIVES

- Select appropriate patients with both atrial fibrillation and coronary artery disease for dual therapy (oral anticoagulation and P2Y12 inhibitor therapy) to reduce bleeding risk
- Select appropriate medication combinations for prevention of major adverse cardiovascular events for patients with atherosclerotic disorders
- Apply multiple strategies to reduce bleeding risk for patients with multiple indications for anticoagulant and antiplatelet therapy

Clinical case

The patient is a 65-year-old man who presented to the hospital with new chest discomfort at rest. He was diagnosed with a non–ST segment elevation acute coronary syndrome (ACS) and underwent percutaneous coronary intervention (PCI). He has a history of atrial fibrillation (AF), for which he takes warfarin to prevent stroke, but no prior history of atherosclerotic cardiovascular disease or bleeding events. He is overweight but not obese (90 kg, body mass index of 27.0). He was initially treated with aspirin 325 mg once, then 81 mg daily. He was placed on an unfractionated heparin infusion before his PCI, when the infusion was discontinued. He was initiated on atorvastatin 80 mg daily and metoprolol tartrate 25 mg twice a day. His baseline laboratory studies included normal coagulation tests (prothrombin time, activated partial thromboplastin time, and international normalized ratio), normal complete blood count, and normal renal function (serum creatinine 1.1 mg/dL). Before he is discharged from the hospital, his physician wonders what the safest antithrombotic regimen to balance bleeding and thrombotic risk would be, given his known AF and recent ACS with PCI.

Introduction

Antithrombotic agents, consisting of antiplatelet and anticoagulant medications, are some of the most commonly prescribed medications. They are currently used by millions of Americans to prevent thrombotic complications in a wide variety of cardiovascular conditions.[1] When combined, these medications increase the risk of significant bleeding complications. Recent studies have compared different combinations of antiplatelet and anticoagulant medications for a variety of cardiovascular conditions. Applying the findings from these trials will help individual patients and their health care providers balance potential

benefits and risks when selecting appropriate antithrombotic regimens.

Indications for antithrombotic therapies

Aspirin therapy has been used for decades to prevent and treat cardiovascular disease, including myocardial infarction (MI) and ischemic stroke. This usage is based, in part, on a series of studies published before 2005 demonstrating reductions in MI risk.[2] Daily aspirin therapy was widely recommended in both clinical guidelines and the lay media, leading to broad application both with and without health care provider involvement. Aspirin is often combined with a P2Y12 receptor antagonist (clopidogrel, prasugrel, or ticagrelor) for dual antiplatelet therapy (DAPT) after PCI or ACS.[3,4] Aspirin monotherapy or DAPT may also be used to prevent major adverse cardiovascular events for patients with peripheral artery disease.[5] Oral anticoagulants, including warfarin and the direct oral anticoagulants (DOACs), are used for a wide range of thrombotic disorders, most commonly to prevent stroke and systemic embolism associated with AF and to prevent or treat venous thromboembolism (VTE).

Many patients have comorbid conditions that each have indications for different antithrombotic medications. In fact, up to half of patients with AF needing anticoagulation have comorbid coronary artery disease (CAD), nearly 10% of whom will undergo PCI and need antiplatelet therapy.[6] However, with each additional antithrombotic agent that a patient is taking, their risk of major and life-threatening bleeding increases.[7] Therefore, efforts to reduce bleeding risk for patients with comorbid prothrombotic conditions (eg, AF and PCI) are needed.

Combined anticoagulant–antiplatelet use by patients with multiple indications

Numerous trials have explored reducing the number of antithrombotic medications used by patients taking chronic oral anticoagulants, usually for AF, who then undergo PCI or experience an ACS that necessitates antiplatelet therapy. The first of these was the WOEST trial, an open-label trial comparing oral anticoagulation plus clopidogrel alone (double therapy) to oral anticoagulation plus DAPT (triple therapy).[8] As might be expected, any bleeding was less common among patients in the double therapy group (19.4% vs 44.4%; hazard ratio [HR] 0.36; 95% confidence interval [CI], 0.26-0.50). However, patients in the double therapy group also had fewer thrombotic events or deaths (11.1% vs 17.6%; HR 0.60; 95% CI, 0.38-0.94), including fewer MI, stroke, and stent thrombosis events.

As shown in Table 1, a number of subsequent trials compared variable numbers of antithrombotic medications, different anticoagulants (warfarin vs DOACs), and different dosages of anticoagulants (full dose vs reduced dose).[9-12]

The largest trial comparing different oral anticoagulant dosages and number of antithrombotic medications is the AUGUSTUS trial.[11] This trial used a 2 × 2 factorial design to compare treatment dosages of both warfarin and apixaban and to compare DAPT to clopidogrel alone in patients with AF who had undergone PCI or experienced an ACS. The findings suggest a marked reduction in major bleeding associated with the use of apixaban as compared with warfarin and with omission of aspirin therapy. Collectively, when the warfarin–clopidogrel–aspirin triple therapy combination was compared with the apixaban–clopidogrel double therapy combination, only 9 patients needed to be treated with the apixaban–clopidogrel regimen to avoid 1 major or clinically relevant nonmajor bleeding event. Of note, not all trials used full treatment dosages of anticoagulants, which limits the ability to compare the impact of different anticoagulant drugs and dosage combinations with the inclusion or omission of aspirin therapy on bleeding outcomes. Although some suggest that clinicians select the lowest possible anticoagulant dosage when combining with antiplatelet therapy, others favor use of an anticoagulant dosage that has been proven effective for stroke prevention in AF.

Equally important, the risk reduction in cardiovascular death, MI, stroke, and stent thrombosis associated with aspirin use is concentrated in the first 30 days but does not extend beyond that time point.[13] In fact, there is a nearly equal increased risk of ischemic events and decreased risk of bleeding events in the first 30 days after PCI or ACS. But after those initial 30 days, the increased risk of bleeding associated with aspirin use persists, whereas the ischemic risk is equal with and without aspirin therapy.

Independent of the need for ongoing anticoagulant therapy, recent studies have suggested that shorter courses of DAPT (sometimes ≤3 months) may be appropriate for many patients undergoing PCI.[14,15] Therefore, many cardiovascular specialists, including interventional cardiologists, are recommending shorter courses of DAPT for patients after PCI or an ACS if they are taking concurrent anticoagulant medications (Figure 1). In fact, recent guidelines and expert consensus documents recommend shorter courses of triple therapy for most of these patients.[16-18] This recommendation is supported by 2 recent meta-analyses showing lower rates of bleeding when dual therapy (an anticoagulant plus P2Y12 inhibitor) rather than triple therapy is used.[19,20] This is particularly true for the combination of a DOAC plus P2Y12 inhibitor and is similar in both stable CAD and ACS. Fortunately, the meta-analyses have also demonstrated no significant increased risk in all-cause mortality, cardiovascular mortality, MI, stent thrombosis, major adverse cardiac events, or stroke.

In general, oral anticoagulant monotherapy is recommended for patients with AF who need anticoagulation for stroke prevention and have concomitant stable CAD (last ACS or PCI >12 months earlier). Although evidence in favor of this recommendation is less robust than evidence for therapy in the first 6 to 12 months after PCI, 2 recent trials demonstrated relative safety with regard to both bleeding outcomes and thromboembolic events (eg, MI, death).[21,22] Some degree of caution is advised because 1 study was terminated prematurely for failure to enroll,[22] and the other was conducted in a purely Japanese population.[21] Nevertheless, concurrent use of oral anticoagulation with aspirin for patients with AF and stable CAD remains common and will probably require further efforts to promote deprescribing, including rigorous evaluation of these deprescribing efforts.[23]

Although the data on anticoagulation alone versus anticoagulation plus single antiplatelet therapy are limited for patients with stable CAD, there is more robust evidence that aspirin may have net clinical harm for primary prevention of atherosclerotic disease.[2] This is particularly true for patients taking chronic anticoagulant therapy but without a clear indication for concurrent antiplatelet treatment.[24] Efforts to reduce aspirin use in this population may lead to reductions in medication-related adverse events, including hospitalizations.[25]

Table 1. Trials of combined antithrombotic therapy for AF and CAD

Name	WOEST[8]	PIONEER AF-PCI[11]	RE-DUAL PCI[9]	AUGUSTUS[12]	ENTRUST-AF PCI[13]
Total patients	573	2124	2725	4614	1506
Population	Patients taking OAC undergoing PCI	Patients with AF undergoing PCI	Patients with AF undergoing PCI	Patients with AF and recent ACS or PCI	Patients with AF and recent ACS or PCI
ACS	155 (27.1%)	1096 (51.6%)	1744 (64.0%)	2811 (60.9%)	777 (51.6%)
Treatments	• OAC + clopidogrel (double therapy) • OAC + DAPT with clopidogrel (triple therapy)	• Group 1: Rivaroxaban 15 mg daily + clopidogrel • Group 2: Rivaroxaban 2.5 mg twice daily + DAPT (1, 6, or 12 mo) • Group 3: VKA + DAPT (1, 6, or 12 mo)	• Dabigatran 110 mg twice daily + clopidogrel or ticagrelor • Dabigatran 150 mg twice daily + clopidogrel or ticagrelor • VKA + DAPT with clopidogrel or ticagrelor	• Apixaban 5 mg twice daily + DAPT • Apixaban 5 mg twice daily + P2Y12 only • VKA + DAPT • VKA + P2Y12 only	• Edoxaban 30-60 mg daily + P2Y12 • VKA + DAPT
Notable exclusion criteria	Prior intracranial bleed, cardiogenic shock, recent peptic ulcer or major bleeding, or thrombocytopenia	Prior stroke, recent GI bleeding event, CrCl <30 mL/min, or anemia (Hg <10 g/dL)	Cardiac valve replacement (mechanical or bioprosthetic) or CrCl <30 mL/min	Anticoagulant use for indications other than AF, severe renal insufficiency, prior intracranial bleed, recent or planned coronary artery bypass graft surgery, or ongoing bleeding	Mechanical heart valve, moderate to severe mitral stenosis, and end-stage renal disease
Bleeding outcome: rate and definition	Double therapy, 19.4% Triple therapy, 44.4% Double vs triple, HR 0.36 (95% CI, 0.26-0.50)	Group 1, 16.8% Group 2, 18.0% Group 3, 26.7% 1 vs 3, HR 0.59 (95% CI, 0.47-0.76) 2 vs 3, HR 0.63 (95% CI, 0.50-0.80)	D110 + P2Y12, 15.4% D150 + P2Y12, 20.2% VKA + DAPT, 26.9% D110 vs TT, HR 0.52 (95% CI, 0.42-0.63) D150 vs TT, HR 0.72 (95% CI, 0.58-0.88)	Apixaban, 10.5% VKA, 14.7% DAPT, 16.1% P2Y12 only, 9.0% Apixaban vs VKA, HR 0.69 (95% CI, 0.58-0.81) DAPT vs P2Y12, HR 1.89 (95% CI, 1.59-2.24)	Edoxaban, 17% VKA, 20% Edoxaban vs VKA, HR 0.83 (95% CI, 0.65-1.05)
Bleeding outcome definition	Any bleeding	TIMI major + minor bleeding	ISTH major + CRNM bleeding	ISTH major + CRNM bleeding	ISTH major + CRNM bleeding

CrCl, creatinine clearance; CRNM, clinically relevant nonmajor; Hg, hemoglobin; ISTH, International Society on Thrombosis and Haemostasis; OAC, oral anticoagulant; TIMI, Thrombolysis in Myocardial Infarction; TT, triple therapy; VKA, vitamin K antagonist.

Although data have rapidly emerged on the risks and benefits of double versus triple antithrombotic therapy for patients taking oral anticoagulants for stroke prevention in AF, much less data is available for patients with VTE who need PCI. For most patients with VTE on oral anticoagulation, an approach similar to that of patients with AF can be taken. Namely, if a patient on chronic oral anticoagulation for VTE experiences an ACS or PCI, dual therapy with an oral anticoagulant (preferably DOAC) and P2Y12 inhibitor is generally recommended. However, for patients with acute VTE in the first 1 to 3 weeks of therapy, caution is advised if DAPT is combined with higher daily doses of either apixaban or rivaroxaban. For any patient with acute VTE early in their course of therapy, it may be advisable to delay PCI until after induction dosing for VTE is complete when possible (eg, PCI for stable angina). This also allows time to discuss the role of dual therapy (anticoagulation plus P2Y12 inhibitor) versus triple therapy with the interventional cardiologist. For patients whose recurrent risk for VTE is low, discontinuing anticoagulant therapy may be reasonable if a strong indication for antiplatelet therapy exists. Though less effective at reducing VTE recurrence risk, aspirin monotherapy is associated with a 32% relative risk reduction.[26]

Combined anticoagulant–antiplatelet use by patients with atherosclerotic disease

Although the most common combined anticoagulant–antiplatelet use involves patients with multiple indications (eg, AF and CAD), trial data support the use of select combined regimens for patients with various atherosclerotic disorders (Table 2). In the ATLAS ACS 2-TIMI 51 study, patients with ACS were randomly assigned to receive either rivaroxaban 2.5 mg twice daily, rivaroxaban 5 mg twice daily, or placebo in addition to DAPT for a mean of 13 months.[27] Although the primary composite efficacy end point of cardiovascular death, MI, and stroke was reduced for both rivaroxaban dosages as compared with placebo, there was also a significantly increased risk of major bleeding, including intracranial hemorrhage. A similar study, APPRAISE-2, randomly assigned patients with ACS to receive apixaban 5 mg twice a day or placebo in addition to DAPT.[28] This study was stopped prematurely because of an excess of major

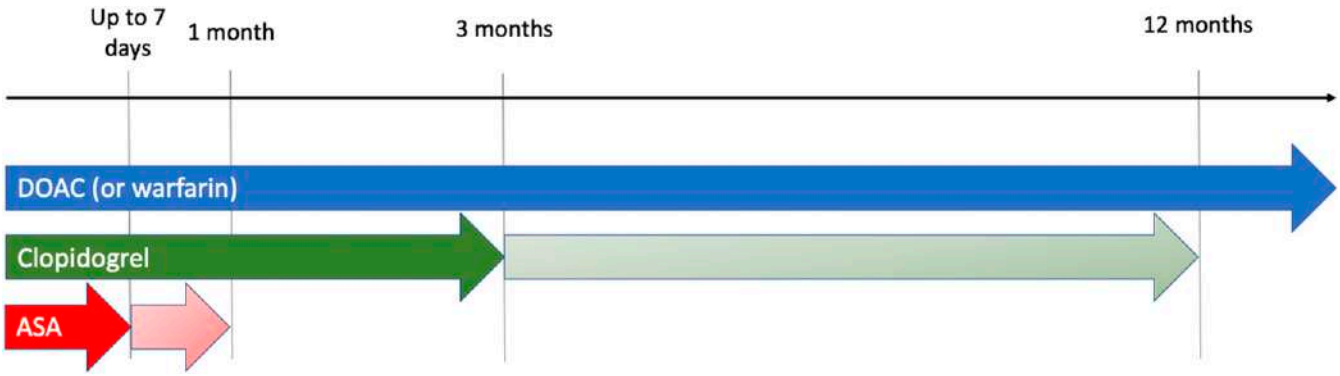

Figure 1. Timeline of antithrombotic therapy in atrial fibrillation and coronary artery disease. For patients with high thrombotic risk (including ACS), ≥3 months (and ≤12 months) of clopidogrel and ≤1 month of aspirin (ASA) is recommended. Longer courses of clopidogrel use may be appropriate for patients with high ischemic risk or who experience an ACS. For PCI for stable angina, a shorter course of clopidogrel and ASA (≤7 days) may be more appropriate (indicated by dark shaded arrows).

bleeding events among patients taking apixaban plus DAPT (2.4 vs 0.9 per 100 patient-years, HR 2.59; 95% CI, 1.50-4.46).

In the subsequent COMPASS trial, patients with stable CAD or peripheral artery disease (PAD; including carotid artery disease) were randomly assigned to receive rivaroxaban 2.5 mg twice daily plus aspirin 100 mg daily, rivaroxaban 5 mg twice daily without aspirin, or aspirin 100 mg daily.[29] The composite primary outcome of cardiovascular death, stroke, or MI occurred less often among patients randomly assigned to rivaroxaban 2.5 mg twice daily plus aspirin than among patients taking aspirin alone. Although major bleeding was higher in the rivaroxaban–aspirin combination group, there was no increased in intracranial or fatal bleeding as compared with aspirin monotherapy, a key distinction from the ATLAS ACS 2-TIMI 51 study results.[27] Most recently, the VOYAGER study randomly assigned patients with PAD who had undergone revascularization to receive rivaroxaban 2.5 mg twice daily or placebo in addition to aspirin.[30] Patients receiving both rivaroxaban and aspirin experienced fewer thrombotic events (composite of acute limb ischemia, major amputation for vascular causes, MI, ischemic stroke, or cardiovascular death) than patients receiving aspirin monotherapy. Major bleeding was more common in the rivaroxaban plus aspirin group than the aspirin monotherapy group according to the International Society on Thrombosis and Haemostasis (ISTH) definition but not the Thrombolysis in Myocardial Infarction (TIMI) definition. There was no difference in intracranial or fatal bleeding between the two groups (0.52% vs 0.58%; HR 0.91; 95% CI, 0.47-1.76).

Taken together, these 4 trials outline a few key findings for combined anticoagulant–antiplatelet use in atherosclerotic disease. First, bleeding is a significant concern when anticoagulants are combined with DAPT, as has been shown in the AF plus CAD studies outlined earlier. Second, although major bleeding often increases with combined anticoagulant–antiplatelet combinations, fatal and intracranial hemorrhage risk appear to be increased when a third antiplatelet medication (eg, P2Y12 inhibitor) is included. Third, the anticoagulant drug and dosage selection is critical. Full-dose anticoagulation in the APPRAISE-2 study (apixaban 5 mg twice daily) was associated with higher rates of major bleeding.[28] However, very low dosages of rivaroxaban (2.5 mg twice daily) were overall safe and efficacious in the COMPASS and VOYAGER studies.[29,30]

Other strategies to reduce bleeding risk

Although reducing the total number of antithrombotic medications is highly effective at reducing bleeding risk, this is not always feasible and does not completely eliminate bleeding risk for patients. Other strategies may be recommended (Figure 2).

First, the use of clopidogrel is recommended over other P2Y12 inhibitors (eg, prasugrel, ticagrelor) for patients taking concurrent oral anticoagulants. In fact, most patients in the randomized trials detailed earlier used clopidogrel rather than prasugrel or ticagrelor. This recommendation is also supported by a class IIa recommendation from the 2019 American Heart Association/American College of Cardiology guideline on AF management and the 2018 European Consensus guidelines.[17,31]

Second, use of a DOAC is preferred to warfarin when combined with either single antiplatelet or DAPT therapy. Although only the AUGUSTUS trial was designed for a head-to-head comparison of warfarin and a DOAC independent of antiplatelet therapy, data from randomized trials in AF, VTE, and other indications have generally demonstrated safety with the entire class of DOAC medications, especially with regard to intracranial hemorrhage.[32] This finding has been reinforced in a number of guidelines and expert consensus documents favoring DOAC use over warfarin, both in general and when combined with antiplatelet therapy.[16,31,33]

Third, patients who need combined use of anticoagulants and antiplatelet medications are at increased risk for upper gastrointestinal (GI) bleeding. Proton pump inhibitors (PPIs) can be highly effective at reducing this risk, but they are often underused.[34-37] Data supporting reductions in hospitalizations for upper GI bleed exist for patients taking anticoagulants and concurrent aspirin, P2Y12 inhibitors, and nonsteroidal anti-inflammatory medications.[34,35] Although the primary bleeding outcome was not significantly reduced in the PPI arm of the COMPASS trial (HR 0.88; 95% CI, 0.67-1.15), there was a reduction in overt GI bleeding events (HR 0.52; 95% CI, 0.28-0.94).[36] The low dosage of anticoagulant used in this study may have affected the overall bleeding rates. However, concerns remain regarding long-term PPI use and risk of cardiovascular disease, renal insufficiency, *Clostridium difficile* infection, and fracture risk.[38] Guidelines from both North America and Europe recommend PPI use for patients taking combined anticoagulant–anticoagulant therapy given that the reduction in elevated GI

Table 2. Trials of combined antithrombotic therapy for atherosclerotic disease

Name	APPRAISE-2	ATLAS ACS 2-TIMI 51	COMPASS	VOYAGER
Total patients	7392	15 526	27 395	6564
Population	Patients with recent ACS and additional risk factors for recurrent ischemic events	Patients with recent ACS	Patients with stable CAD or PAD	Patients with PAD undergoing revascularization
Treatments	• Apixaban 5 mg twice daily • Placebo All patients received standard antiplatelet therapy (usually DAPT)	• Rivaroxaban 2.5 mg twice daily • Rivaroxaban 5 mg twice daily • Placebo All patients received standard antiplatelet therapy (usually DAPT)	• Rivaroxaban 2.5 mg twice daily + aspirin 100 mg daily • Rivaroxaban 5 mg twice daily • Aspirin 100 mg daily	• Rivaroxaban 2.5 mg twice daily • Placebo All patients received aspirin therapy
Notable exclusion criteria	Severe hypertension, CrCl < 20 mL/min, active bleeding, recent ischemic stroke, NYHA class IV heart failure, prior intracranial bleeding, anemia (Hg <9 g/dL), thrombocytopenia, ongoing use of anticoagulation or aspirin >325 mg daily	Thrombocytopenia, anemia (Hg <10 g/dL), CrCl <30 mL/min	High risk of bleeding, recent stroke, severe heart failure, estimated glomerular filtration rate <15 mL/min, use of dual antiplatelet therapy, or anticoagulation use	Unstable clinical condition, high risk for bleeding, or long-term use of clopidogrel (beyond 6 mo)
Efficacy outcome	• Apixaban, 13.2 per 100-patient-years • Placebo, 14.0 per 100 patient-years HR 0.95 (95% CI, 0.80-1.11) Composite of cardiovascular death, MI, ischemic stroke	• Rivaroxaban 2.5 mg, 9.1% • Rivaroxaban 5 mg, 8.8% • Placebo, 10.7% Rivaroxaban 2.5 mg vs placebo, HR 0.84 (95% CI, 0.72-0.97) Rivaroxaban 5 mg vs placebo, HR 0.85 (95% CI, 0.73-0.98) Composite of cardiovascular death, MI, ischemic stroke	• Rivaroxaban 2.5 mg + aspirin, 4.1% • Rivaroxaban 5 mg, 4.9% • Aspirin, 5.4% Rivaroxaban + aspirin vs aspirin, HR 0.76 (95% CI, 0.66-0.86) Rivaroxaban vs aspirin, HR 0.90 (95% CI, 0.79-1.03) Composite of cardiovascular death, MI, ischemic stroke	• Rivaroxaban 2.5 mg, 17.3% • Placebo, 19.9% HR 0.85 (95% CI, 0.76-0.96) Composite of acute limb ischemia, major amputation for vascular causes, MI, cardiovascular death
Primary safety outcome	• Apixaban, 2.4 per 100 patient-years • Placebo, 0.9 per 100 patient-years HR 2.59 (95% CI, 1.50-4.46) TIMI major bleeding	• Rivaroxaban 2.5 mg, 1.8% • Rivaroxaban 5 mg, 2.4% • Placebo, 0.6% Rivaroxaban 2.5 mg vs placebo, HR 3.46 (95% CI, 2.08-5.77) Rivaroxaban 5 mg vs placebo, HR 4.47 (95% CI, 2.71-7.36) TIMI major bleeding not related to coronary artery bypass graft	• Rivaroxaban 2.5 mg + aspirin, 3.1% • Rivaroxaban 5 mg, 2.8% • Aspirin, 1.9% Rivaroxaban + aspirin vs aspirin, HR 1.70 (95% CI, 1.40-2.05) Rivaroxaban vs aspirin, HR 1.51 (95% CI, 1.25-1.84) Modified ISTH major bleeding (including all bleeding leading to an acute care facility presentation or hospitalization)	• Rivaroxaban 2.5 mg, 2.65% • Placebo, 1.87% HR 1.43 (95% CI, 0.97-2.10) TIMI major bleeding • Rivaroxaban 2.5 mg, 5.94% • Placebo, 4.06% HR 1.42 (95% CI, 1.10-1.84) ISTH major bleeding
Intracranial bleeding	• Apixaban, 0.6 per 100 patient-years • Placebo, 0.2 per 100 patient-years HR 4.06 (95% CI, 1.15-14.38)	• Rivaroxaban 2.5 mg, 0.4% • Rivaroxaban 5 mg, 0.7% • Placebo, 0.2% Rivaroxaban 2.5 mg vs placebo, HR 2.83 (95% CI, 1.02-7.86) Rivaroxaban 5 mg vs placebo – HR 3.74 (95% CI, 1.39-10.07)	• Rivaroxaban 2.5 mg + aspirin, 0.3% • Rivaroxaban 5 mg, 0.5% • Aspirin, 0.3% Rivaroxaban + aspirin vs aspirin, HR 1.16 (95% CI, 0.67-2.00) Rivaroxaban vs aspirin, HR 1.80 (95% CI, 1.09-2.96)	• Rivaroxaban 2.5 mg, 0.40% • Placebo, 0.52% HR 0.78 (95% CI, 0.38-1.61)

CrCl, creatinine clearance; Hg, hemoglobin; ISTH, International Society on Thrombosis and Haemostasis; PAD, peripheral artery disease; TIMI, Thrombolysis in Myocardial Infarction; TT, triple therapy.

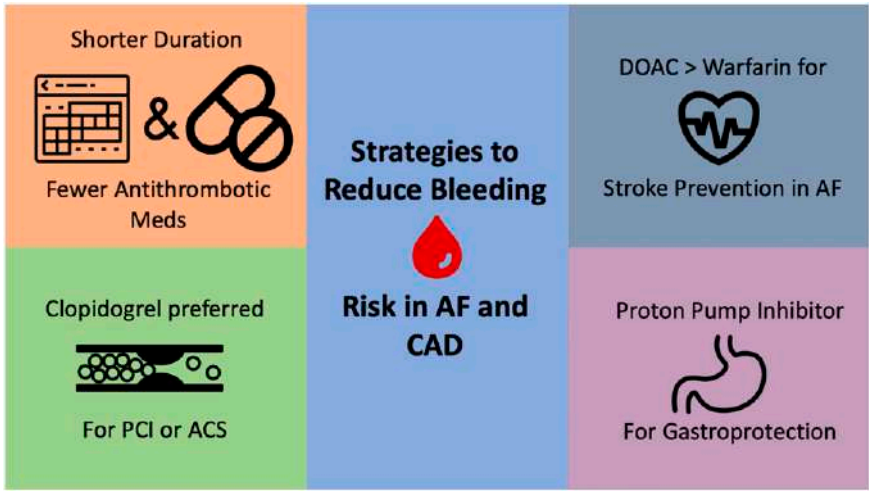

Figure 2. Strategies to reduce bleeding risk for patients with AF and CAD.

bleeding risk probably outweighs any potential drug-related adverse event risk.[17,39] It is also important to address PPI deprescribing once the bleeding risk has been mitigated (eg, transition to anticoagulation monotherapy).

Return to the case

The patient's hospitalist and interventional cardiologist discuss the risks and benefits of various combinations of antithrombotic agents. Overall, the patient is thought to be at low bleeding risk given that he has not had a prior history of bleeding, has normal renal function, and has normal blood counts. Nonetheless, to minimize bleeding risk, they elect to change his warfarin to apixaban 5 mg twice daily, following data from the AUGUSTUS trial. Because he experiences an ACS, the interventional cardiologist feels more comfortable continuing aspirin 81 mg daily for 30 days, but then agrees to stop aspirin and continue dual therapy (apixaban and clopidogrel) for the remainder of the 12 months. This duration is selected because the patient experienced an ACS event. The hospitalists recommended use of a PPI to help minimize bleeding risk while the patient was taking multiple antithrombotic medications. The interventional cardiologist agrees to follow the patient for ≥12 months so that he can reassess the need for ongoing antiplatelet therapy in the future and address PPI deprescribing when a transition to apixaban monotherapy is initiated.

Conclusion

Combined use of anticoagulant and antiplatelet medications is common for patients with comorbid cardiovascular conditions, including CAD, AF, and VTE. Recent trial evidence has outlined the safety and efficacy of reducing the number of antithrombotic agents, favoring dual therapy (oral anticoagulant plus a single antiplatelet agent) in many clinical contexts. Additional strategies, including the use of clopidogrel over other P2Y12 inhibitors, preferential use of DOACs over warfarin, and use of PPI therapy, to reduce bleeding risk, should be explored for many patients who need multiple antithrombotic agents.

Conflict-of-interest disclosure

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Off-label drug use

None disclosed.

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Allogeneic hematopoietic stem cell transplantation in adults with primary immunodeficiency

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With recent advances in genetic sequencing and its widespread adoption for clinical diagnostics, the identification of a primary immunodeficiency (PID) as the underlying cause of diseases presenting to hematologists including refractory autoimmunity, cytopenias, immune dysregulation, and hematologic malignancy, is increasing, particularly in the adult population. Where the pathogenic genetic variants are restricted to the hematopoietic system, selected patients may benefit from allogeneic hematopoietic stem cell transplantation (allo-HSCT). Although it is generally accepted that *early* allo-HSCT (ie, in infancy or childhood) for PID is preferable, this is not always possible. The clinical phenotype of non–severe combined immune deficiency forms of PID can be very heterogeneous, in part because of the high number of genetic and functional defects affecting T, B, and natural killer cells, neutrophils, and/or antigen presentation. As a result, some patients have less severe disease manifestations in childhood and/or a later de novo presentation. For others, a delayed diagnosis, lack of a genetic diagnosis, or a previous lack of a suitable donor has precluded prior allo-HSCT. Specific issues which make transplantation for adult PID patients particularly challenging are discussed, including understanding the natural history of rare diseases and predicting outcome with conservative management alone; indications for and optimal timing of transplant; donor selection; conditioning regimens; and PID-specific transplant management. The role of gene therapy approaches as an alternative to allo-HSCT in high-risk monogenic PID is also discussed.

LEARNING OBJECTIVES

- Understand and evaluate the role of allo-HSCT in adults with PID; although allo-HSCT can be curative, patient selection and optimal timing of transplant is complex
- Understand the importance of always taking a careful family history in younger adults presenting with refractory autoimmunity, HLH, or lymphoproliferation/lymphoma to help identify an underlying PID
- Understand the need to integrate genetic, laboratory, clinical, and family history data to predict prognosis
- Understand that alternative therapies instead of, or as a bridge to, transplant are increasingly available

Clinical cases

Figure 1 illustrates the clinical course of 2 adult primary immunodeficiency (PID) patients with (i) autoimmune lymphoproliferative syndrome and (ii) hypomorphic Rag2 deficiency (combined immune deficiency).[1] Both patients presented in childhood or adolescence with anemia (autoimmune hemolytic anemia in patient 1 and red cell aplasia in patient 2). Both subsequently underwent allogeneic hematopoietic stem cell transplantation (allo-HSCT) in adulthood.

Patient 1 presented with autoimmune hemolytic anemia at the age of 12 weeks, which was successfully treated with steroids and intravenous immunoglobulin (ivIg). However, refractory immune thrombocytopenic purpura (ITP) and splenomegaly developed soon after requiring splenectomy at the age of 2 years. There was a known family history of autoimmune lymphoproliferative syndrome affecting her mother and 2 brothers. Genetic diagnosis was made in early childhood. Childhood and adolescence were complicated by inflammatory arthropathy, colitis secondary to lymphocytic infiltration of the gut mucosa, extensive genital herpes simplex virus infection, and recurrent, widespread reactive lymphadenopathy. Classical Hodgkin lymphoma (HL) was diagnosed on inguinal lymph node biopsy when the patient was 18 years old. The decision to proceed to allo-HSCT was made after relapse of HL 2 years later at the age of 20 years.

Patient 2 initially presented at 14 years of age to dermatology with a nontraumatic leg ulcer and localized granulomatous skin lesions. There was no response to

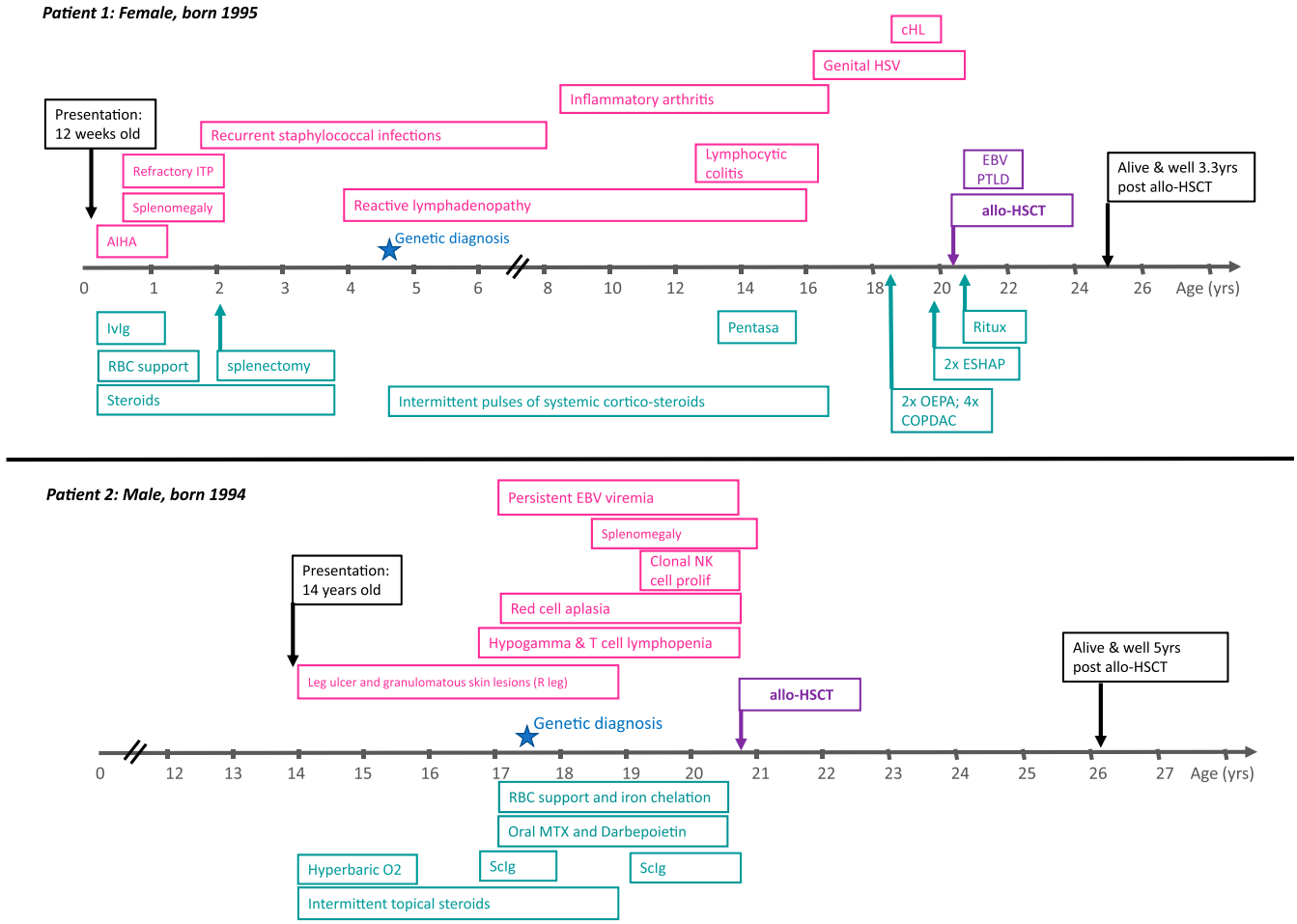

Figure 1. Clinical cases.

hyperbaric oxygen or topical steroids. Other therapies were declined. There was no family history of immunodeficiency. At 16 years, he was noted to have hypogammaglobinemia and T-cell lymphopenia. He was started on immunoglobulin replacement therapy but soon after developed severe, refractory red cell aplasia. Two years later, he developed a clonal natural killer (NK)-cell proliferation (no T-cell clone detected), and genetic diagnosis was confirmed. He was then referred for allo-HSCT, which was performed at the age of 20 for NK-cell clonal proliferation and associated bone marrow failure.

Introduction

For the last 30 years, allo-HSCT has been considered the standard of care and the major therapeutic option for children with serious inborn errors of immunity or PID.[2] Early transplant is particularly important for infants or children presenting with serious or life-threatening infections, because without definitive treatment, patients with severe PID, such as severe combined immune deficiency (SCID), rarely survive beyond 1 year of age. Until recently, the vast majority of transplant procedures for PID were performed in childhood, with clinical expertise in these rare diseases residing almost exclusively in pediatric specialist centers.

However, as an increasing number of nontransplanted PID (almost all non-SCID) patients are now surviving, or only being diagnosed, beyond childhood, most patients with PID are now found within the adult population.[3] The role of allo-HSCT for adults is therefore being carefully evaluated.

Uncorrected PID can lead to recurrent, progressive, or life-threatening infections, autoimmunity, autoinflammatory manifestations, and malignant disease (typically lymphoma or epithelial neoplasia). This places a significant burden on health care resources with frequent hospital admissions, the need for ongoing antimicrobial therapy, and expensive therapy including biological-modifying drugs (eg, monoclonal antibodies) and immunoglobulin replacement therapy. The result can be poor quality of life and early death.

Within the last 3 years, evidence has been published that demonstrates selected adults with PID can be transplanted safely using reduced intensity conditioning regimens, achieving outcomes equivalent to that achieved in pediatric transplant practice.[4,5] However, questions remain regarding the indications for and optimal timing of transplant in adults.

Basic principles of allo-HSCT for nonmalignant disease

Allo-HSCT allows the replacement of defective or dysregulated recipient immune and hematopoietic cells with long-term repopulating cells from a healthy donor. Apart from gene therapy, in the case of selected monogenic forms of PID, it is the only potentially curative therapy. PID patients undergoing allo-HSCT (as for other

nonmalignant diseases) do not benefit from the graft-versus-malignancy effect unless they have a prior history of blood cancer (most commonly lymphoma). The prevention of graft-versus-host disease (GVHD) and successful long-term immune-hematopoietic engraftment are therefore of paramount importance. Long-term stable donor chimerism in the previously affected cell lineage/s and correction of the clinical phenotype is the ultimate goal of transplant.

Major factors influencing outcome after allo-HSCT are broadly independent of the underlying disease and include preceding comorbidity, active infection at the time of transplant, end organ function, type of donor, and age of the patient.[6,7] For PID patients, the shorter the time from onset of clinical symptoms to transplant, the lower the risk of developing resistant or refractory infections (bacterial, viral, or fungal) and end organ damage caused by uncontrolled inflammation or autoimmunity.

Who and when?

One of the biggest challenges for hematologists and immunologists looking after adults with PID is knowing which patient and when to refer for consideration of allo-HSCT. The clinical decision is straightforward if the underlying condition is known to be life-threatening or life-limiting, and the patient has a predicted poor prognosis with conservative management alone. For rarer forms of PID, the natural history with conservative management alone is often not known, which necessitates careful, shared decision making with a wider team of specialist health care professionals, the patient, and their family. This is essential for the patient to be fully informed of the risks and uncertainties. However, as with all potential allo-HSCT recipients, for maximal benefit, transplant must occur before serious end organ damage has occurred, which could make the risk of transplant unacceptably high. With respect to comorbidities, the HCT-CI score (hematopoietic stem cell transplantation comorbidity index, a validated comorbidity index predicting high risk patients for HSCT in the setting of hematologic malignancies) has recently been validated as having predictive value for patients with nonmalignant diseases including PID,[8] with the caveat that most patients included in the analysis were children.

Background on natural history of PID in adults and data in allo-HSCT

Every year an international expert committee provides a genotypic and phenotypic classification of all human inborn errors of immunity. There are now 406 distinct clinical disorders with 430 different gene defects,[1] and although individually rare, these disorders are enriched in patients with hematologic malignancies and autoimmune cytopenias. In the more recent classifications, the concept of pure immunodeficiencies with predisposition to infections has been replaced with newly described autoimmune, autoinflammatory conditions or syndromal disorders associated with immunodeficiency. Many of these disorders can be cured by allo-HSCT, whereas in syndromal disorders, only the hematopoietic portion of the disease can be corrected, which may nevertheless be indicated with improved survival and quality of life in selected patients.

PID patients who survive to adulthood without having undergone an allo-HSCT in infancy or early childhood will almost all have some residual immune function, even if grossly abnormal. Therefore, most nontransplanted adult PID patients have non-SCID PID, such as combined immune deficiency (CID, affecting both cellular and humoral immunity), CID with syndromic features, predominantly antibody deficiencies, diseases of immune dysregulation, phagocytic/innate immunity disorders, and autoinflammatory disorders.[1] In many of these, the predominant clinical features include immune dysregulation, such as refractory autoimmunity, lymphoproliferation, or autoinflammation; the presentation may be much later, and the role and timing of allo-HSCT are currently less clear.

Studies that address the long-term prognosis of PID patients are very difficult to perform, but natural disease outcome data are desperately needed to be able to determine the optimal role of allo-HSCT,[9] particularly with newly described or PID with a wide phenotypic heterogeneity in disease severity. For example, most patients with common variable immunodeficiency (CVID), the most common form of PID in adults, have an excellent prognosis and few complications if receiving adequate immunoglobulin replacement therapy, whereas others with additional features of immune dysregulation develop severe complications of the lung and liver and have a very poor prognosis.[10] Published retrospective data for HSCT in adult patients with complex CVID have indicated worse outcomes than for other PID patients after transplant,[11] underlining the need for better information to select patients and timing for HSCT in CVID. For other PID subtypes, there is accumulating evidence of continued disease progression throughout adulthood, including chronic granulomatous disease (CGD), X-linked lymphoproliferative syndrome, and CD40 ligand (CD40L) deficiency,[12-15] resulting in serious morbidity and mortality in these studies. Importantly, for other PID, such as dedicator of cytokinesis 8 (DOCK8) deficiency, recent studies have indicated that a plateau in overall survival may be reached in adulthood, but nevertheless, there is a progressive fall in event-free survival with conservative management alone.[16]

There is a growing body of publications detailing outcome data on approximately 200 cases of allo-HSCT for PID in adults.[4,5,11,17-25] Additional data on a similar number of adults have been published in abstract form. In most published series, the overall survival was equivalent to that achieved in children and infants being in excess of 80% at a median follow-up of between 14 months and 5 years. Most data relate to patients with CGD, hemophagocytic lymphohistocytosis (HLH), and GATA2 deficiency, for whom the data are therefore most robust. The published data are summarized in Table 1.

Because published adult transplant outcome data are limited for most PID subtypes, a large retrospective study is underway coordinated by the Inborn Errors Working Party of the European Society for Blood and Marrow Transplantation to address this issue. Ideally, decisions about allo-HSCT would be based on prospective randomized studies. However, these are not feasible in rare and ultra-rare diseases. In an attempt to use retrospective data, a large Franco-British retrospective case-controlled study is comparing outcomes of transplanted and matched nontransplanted adults with various subtypes of severe PID with the aim of identifying whether allo-HSCT in adulthood confers genuine benefit in terms of overall survival, event-free survival, and cumulative incidence of PID-associated events. Provisional, unpublished data suggest that allo-HSCT in adult PID patients may prevent the progressive morbidity associated with PID in adults and outweigh the negative impact of transplant-related mortality (TRM) (Cheminant M, Fox T, et al, unpublished data).

Table 1. Summary of published allo-HSCT outcome data for adult (and adolescent) PID patients

Reference	No. of patients	PID subtype	Age at HSCT, y (range)	Donor (no.)	Conditioning	OS	EFS	TRM	Engraftment	Median follow-up (range)
Albert et al 2018[4]	18	6 CGD 12 other PID	18 y (15-22)	MRD (2) MUD (5) 1Ag MMUD (11)	Full Bu/Cy (2); Full Bu/Flu (1); Sub Bu/Flu (7); Flu/Mel ±TT (1); Treo/Flu ±TT (7). All had serotherapy.	94%	94%	6%	100%	5 y (2-9 y)
Fox et al 2018[5]	29	11 CGD 18 other PID	24 y (17-50)	MRD (11) MUD (13) 1Ag MMUD (5)	Flu/Mel/Alem (20) Flu/Bu/ ATG (8) Flu/ Bu/Alem (1)	89% at 1yr 85% at 3yrs	90%	14%	100%	3.5 y (4 mo to 12 y)
Jin et al 2018[18]	8	Primary HLH	25 y (18-54)*	HaploID (6) MUD (2)	TBI/VP16/ Cyclo (6); VP16/Flu/Bu/ ATG (2)	88%	NS	12%	100%	27 mo
Leiding et al 2018[19]	5 (AYA) 10 (ped <12 y)	STAT1 GOF	29 y (18-35) 8 y (1-17)	MRD (4); MUD (8); MMUD (1); HaploID (2)‡; UCB (2, 2‡).	Flu/Mel/Alem (4); Bu/Cy (3); Flu/Bu or Treo/ATG or Alem (6); Various other (4‡)	20% at 1yr (AYA) 60% at 1yr (ped) 40% at 1yr (all)	NS NS NS	NS	50%†	NS
Parta et al 2017[20]	17 (AYA) 20 (ped)	CGD	24 y (18-32) 8 y (4-17)	MRD (6); MUD (30); MMUD (1)	Bu/Alem (6); Bu/TBI/Alem (31)	82.5% (all)	80% (all)	17.5% (all)	85% (all)	3.4 y (range, NS)
Shah et al 2017[21]	7	DOCK8 Defic	20 y (7-25)	HaploID (7)	Flu/Bu/Cy + low dose TBI (7)	86% (all)	NS	14%	100%	2 y (3 mo to 5.7 y)
Fu et al 2016[22]	30	1° HLH; EBV-HLH; Tu-HLH; Undef- HLH	23 y (14–52) 19 y (14–55) 24 y (14–44) 29 y (16–32)	HaploID (23); MRD (6); MUD (1).	Bu/Cyclo/ VP16 (6) for MRD TBI/ Cyclo/VP16 + ATG (24) for HaploID/MUD	63.3% at 2 y (100% in 1° HLH; 64% in EBV-HLH; 17.7% in Tu-HLH; 25% in Undef)	NS		100%	26 mo
Wehr et al 2015[11]	14 (adult) 11 (ped)	CVID	34 y (18-50) 14 y (8-17)	MRD (14); MUD (10); MMUD (1)	BCNU/Flu/ Mel (5); Flu/ Mel (7); Flu/ Mel/Treo (2); Flu/Bu ±TT (4); Bu/Cy ±AraC (6); Flu/ Cy (1).	57% (adults) 52% (all patients)		44% at 1 y (all)	79% (adult)	NS
Grossman et al 2014[23]	14	GATA2 Defic	33 y (15-46)	MRD (4); MUD (4); UCB (4); HaploID (2).	Flu/low dose TBI (8); Flu/ Cy/low dose TBI (6).	57% (all)	NS	28%	100%	3.5 y (1-5 y)
Gungor et al 2014[24]	13 (adult) 43 (ped)	CGD CGD	21 y (18-39) 9 y (0.8 – 17)	MRD (21); MUD (25); MMUD (10).	Flu/Bu ATG or Alem (all).	92% (adult) 96% (all)	91% (all)	7%	93% (adult)	21 mo
Spinner et al 2014[25]	21	GATA2 Defic	NS (15-49 y)	NS	NS	72% at 1 y; 65% at 2 y; 54% at 4 y	NS	NS	NS	14 mo (0-180 mo)

Adult ≥ 18 years at transplant. Ag, antigen; Alem, alemtuzumab; AraC, cytarabine; ATG, anti thymocyte globulin; BCNU, carmustine; Bu, busulfan; CGD, chronic granulomatous disease; CVID, common variable immunodeficiency; Cy, cyclophosphamide; def, deficiency; EBV, Epstein-Barr virus; EFS, event free survival; Flu, fludarabine; GOF, gain of function; HaploID, haploidentical; HLH, hemophagocytic lymphohistiocytosis; Mel, melphalan; MMUD, mismatched unrelated donor; MRD, matched related donor; MUD, matched unrelated donor; ns, not stated; OS, overall survival; PID, primary immunodeficiency; TBI, total body irradiation; Treo, treosulfan; TRM, transplant related mortality; TT, thiotepa; UCB, umbilical cord blood; VP16, etoposide.
Adapted from Morris and Albert[41] with permission.
*Age at diagnosis.
†Secondary graft failure
‡As second or third procedure.

Optimal timing of allo-HSCT in adults with PID

In pediatric practice, well infants/children with a diagnosis of severe PID are typically offered preemptive allo-HSCT before the development of significant infections, autoimmunity, and/or autoinflammation to reduce the risk of peritransplant complications. Where the TRM risk is small and the severity/prognosis of the underlying disease is well understood and predictable, the preemptive use of allo-HSCT is justifiable; examples of specific PID in this category include SCID, CGD, Wiskott-Aldrich syndrome (WAS), and primary HLH. For specific PID with well-described high risks of developing lymphoma or other malignancies, there may be a role for preemptive allo-HSCT after careful discussion with the patient. However, for adults with known PID who remain clinically well beyond early adulthood, the risk-benefit balance of transplant is less clear, particularly for PID subtypes with less well-studied allo-HSCT outcomes. In these circumstances, proceeding to transplant without a clear trigger or indication is not currently recommended.

Most adult PID patients referred for transplant are not at immediate risk of death but are at risk of ongoing recurrent, progressive, or life-threatening infection, autoimmunity, autoinflammation, and malignant disease, typically lymphoma or epithelial neoplasias. For other younger adults, there may have been gradual disease progression through adolescence, and therapeutic options at the time of referral include a trial of biologics and/or immunomodulatory therapies (where appropriate) or allo-HSCT. In rarer or more recently described PID, the long-term efficacy and toxicity of nontransplant treatment modalities (examples include phosphoinositide 3-kinase delta inhibitors, jak/stat inhibitors, or abatacept) is also unknown. Whether to use such agents (singly or sequentially) as a bridge to transplant by reducing PID-associated complications before transplant with the aim of reducing TRM or using them longer term, which may eventually lead to refractoriness, is open to discussion. However, some universal truths of transplantation can guide us: outcomes are generally better when disease is in remission, and the recipient is younger and has less comorbidities at the time of transplant. Such uncertainty requires hematologists and immunologists to work together effectively to build consensus and where possible recruit patients to international studies.

It is well understood that patients proceeding to transplant incur an immediate risk associated with transplant conditioning and immunosuppression. If the TRM is high because of comorbidities at the time of transplant, the potential benefit from transplant can be lost for a given individual. Because many patients with PID have several comorbidities at the time of transplant, early differences in survival between transplanted and nontransplanted patients can be small or nonexistent because of the negative impact of a high TRM.

There are clear data demonstrating that within pediatric cohorts, transplanting earlier in the disease course improves outcomes.[26-29] However, in adolescent and young adult cohorts, the impact of age appears less significant.[4,5] There are very few older adults (>50 years) included in published series detailing outcome after allo-HSCT for PID patients,4,5,11,18-25 and until further data are available, patients should only be transplanted with a very clear indication.

Similarly, for patients with very rare PID diagnoses or with PID for which the number of transplants performed to date are very small (eg, <10 reported cases), the data to support allo-HSCT over trials of biologics/targeted therapies is likely to be scarce.

Unfortunately, in real-world clinical practice, many adult PID patients are referred for consideration of allo-HSCT too late. Typically, referrals for transplant are triggered by a severe PID-related event (eg, a life-threatening infection, malignancy, or major organ dysfunction), which acts as a declaration of the severity of the clinical phenotype and predictor of future complications. If these complications do not respond promptly to treatment, the window of opportunity for transplant may be missed.

Indications for allo-HSCT in adults with PID

Figures 2 and 3 suggest algorithms for identifying adult PID patients who may benefit from allo-HSCT and for whom referral to a specialist transplant unit is appropriate. The process requires close multidisciplinary patient-specific discussion between the immunology and transplant teams. Additional input from pediatric immunologists who have previously cared for the patient and/or other family members may be beneficial. Decisions are made with consensus based on the experience of the group also taking account pediatric transplant data, any reported adult transplant outcomes, known natural history of the specific condition, and international advice for very rare cases.

In the United Kingdom, we established a national virtual multidisciplinary team expert panel to discuss all adults with PID being considered for allo-HSCT. The multidisciplinary team serves to share expertise and discuss diagnosis, further investigations where indicated, appropriateness of transplant, conditioning regimens, and donor selection. Discussion of nontransplant treatment options is also included.

In summary, for patients who have either known pathogenic variants in PID genes (Figure 2) and/or a family history of serious PID, or clinical and laboratory findings consistent with PID (Figure 3), the currently accepted indications for transplant include the following:

1. Recurrent, persistent, or life-threatening infections (bacterial, fungal, and/or viral, including chronic active Epstein-Barr virus [EBV], CAEBV);
2. Refractory autoimmune cytopenias;
3. Bone marrow failure;
4. HLH;
5. PID-associated hematologic malignancy; and/or
6. Refractory autoinflammation (eg, severe colitis).

Evolution of clinical cases

Patient 1 was referred for allo-HSCT at the age of 20 years after relapse of HL, fulfilling the indication criteria of PID-associated hematologic malignancy/lymphoma. Patient 2 was referred for allo-HSCT after the development of a clonal NK-cell proliferation and associated bone marrow failure, fulfilling 2 of the accepted transplant indications.

Role of genetic diagnosis and family history

It is our practice to perform genetic sequencing for all PID patients being considered for HSCT if this has not already been done. Although it is sometimes necessary to proceed to HSCT for adults with PID, based on clinical disease progression alone, it is our preference to know the precise genetic diagnosis, which permits maximal prior consideration of natural history, published transplant experience, and specific extra-hematopoietic features of the disease.

For many patients, their underlying PID is well characterized, genetically, functionally, and clinically, but treatment decisions can

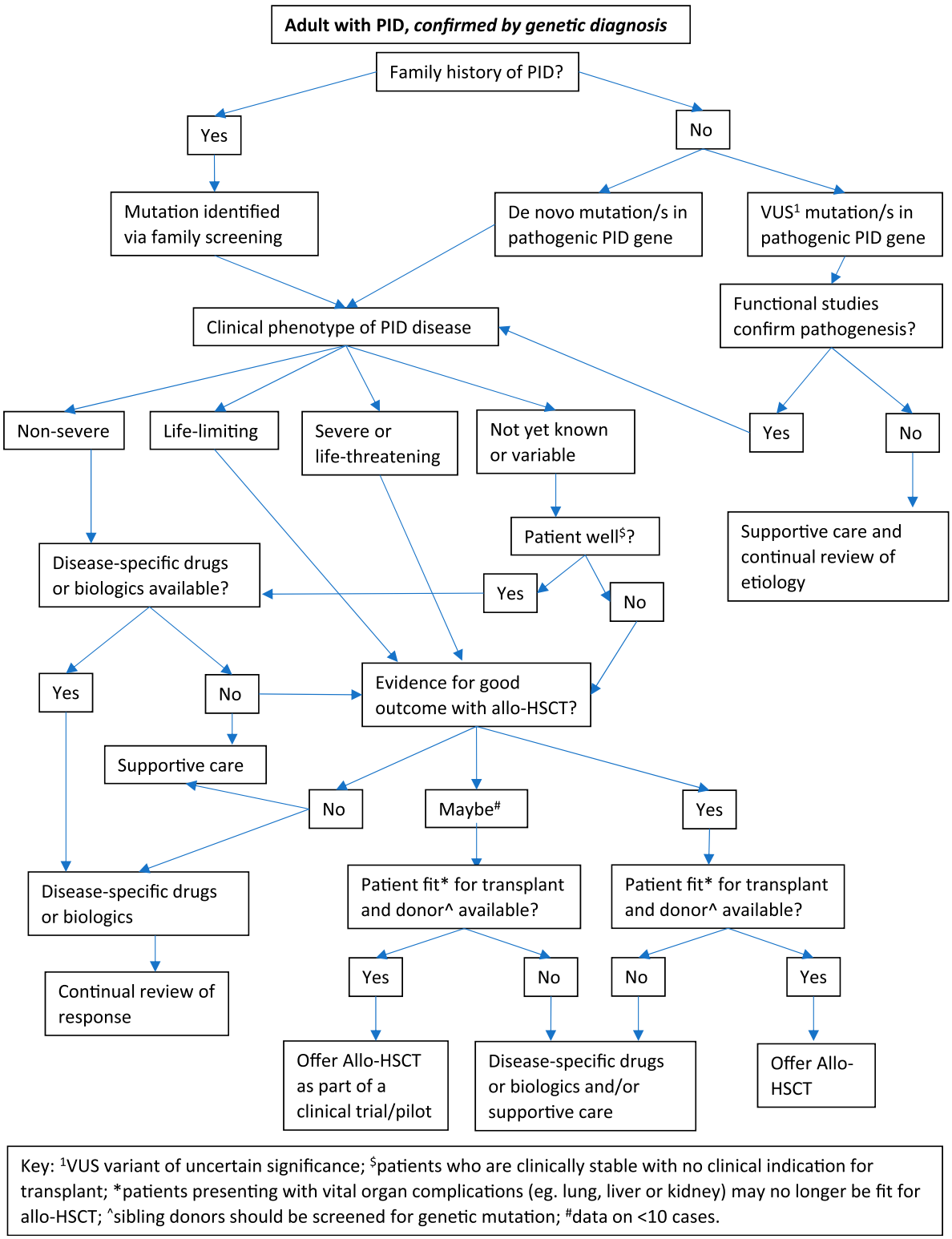

Figure 2. Decision flowchart (algorithm) for adult PID patients with *known genetic diagnosis* referred for allogeneic HSCT.

still be challenging. As with other rare or ultra-rare diseases, predicting outcome with conservative management alone is difficult because of very small numbers of affected individuals and limited published data. Similarly, if the genetic variant has only recently been described as potentially pathogenic or is a variant of uncertain significance, therapeutic decisions need to be based on the clinical picture alone, including the cumulative rate of serious PID-related complications, family history, and quality of life.

For other adult patients, the genetic cause remains undefined despite sequencing or the urgency of transplant

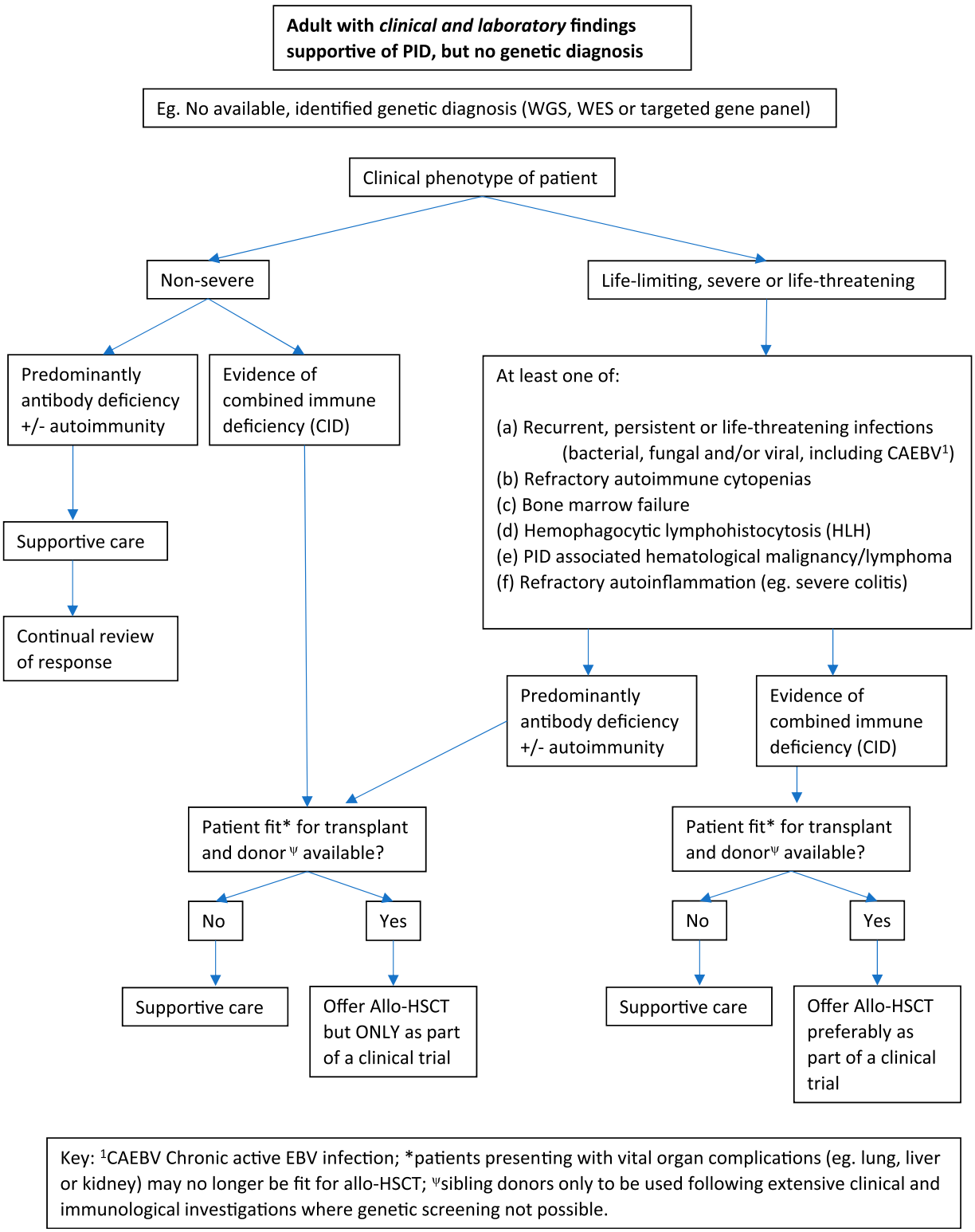

Figure 3. Decision flowchart (algorithm) for adult PID patients *without a genetic diagnosis* referred for allogeneic HSCT.

precludes obtaining genetic sequence results in advance. It should be remembered that serious monogenic PID are, in general, more likely to present at an early age, resulting in a lower rate of genetic diagnosis for PID presenting in adulthood.

For some PID disorders, members of the same family/kindred sharing the same genetic variants can have highly variable clinical presentations, such as nuclear factor κB1 haploinsufficiency,[30] lipopolysaccharide (LPS)-responsive and beige-like anchor protein deficiency,[31] and cytotoxic T lymphocyte associated protein 4

deficiency,[32] so that some have severe disease and others are asymptomatic. The reasons for this are poorly understood but may relate to environmental triggers, such as infection, other genetic background, and epigenetic factors. Highly variable clinical phenotypes may also arise as a result of incomplete penetrance, variable expressivity and gain-of-function (GOF) or loss-of-function variants in the same gene, and finally, hypomorphic (leaky) defects where residual gene expression and function may be preserved. Clinical case 2 illustrates exemplifies this. *Rag* gene mutations can cause SCID with life-threatening presentations in infancy if recombinase activity is lacking, whereas hypomorphic mutations in the same PID gene, which permit 5% to 30% recombinase activity, result in a CID clinical phenotype, often much later in onset.[33]

Where possible, functional analyses of genetic diagnoses should be performed before transplant. Exceptions include rapidly deteriorating patients requiring urgent intervention and/or novel genetic variants for which no validated functional assay exists. Functional assay development for novel variants predicted to be pathogenic is also critical for the informed selection of HLA-matched family donors, who may be heterozygous carriers for the same genetic variant. This is complicated further in phenotypically variable and late-onset PID. In such cases, although TRM risks are generally higher with unrelated donors, these may be preferable.

In all cases of adult PID being considered for allo-HSCT, the final decision is a clinical one, and the absence of genetic and functional data should not necessarily preclude transplant if the clinical evidence is compelling.

Evolution of clinical cases

Both patients described in Figure 1 have heterozygous pathogenic variants in PID-associated genes. Patient 1 had a heterozygous pathogenic variant in the death domain region of TNFRSF6 gene (also known as Fas and CD95), resulting in defective apoptosis in lymphoid cells (p.I246T). This patient also had other affected family members (mother and 2 brothers) with a milder clinical phenotype, illustrating the additional complexity resulting from varied clinical phenotypes within the same kindred and/or incomplete penetrance. Patient 2 was found to have compound heterozygous-predicted pathogenic variants in the RAG2 gene [c.104G>T (Gly35Val)/het and c.814G>A (Val72Ile)/het and c.965T>C (Met 322Thr)/Het]. This patient had no family history of PID.

Donor selection

Preferred stem cell donors are 12/12 HLA-matched, cytomegalovirus (CMV) sero-matched, unaffected related donors to minimize TRM and the risk of GVHD. However, most series published to date include large numbers of matched or 1 antigen mismatched unrelated donor transplants (matched unrelated donor (MUD), typically 10/10 or mismatched unrelated donor (MMUD), typically 8/10 or 9/10), with good results. HLA-matched family members should be genetically screened if a known pathogenic variant has been identified in the recipient.

In pediatric practice, unmanipulated haploidentical transplants using PTCy (posttransplant cyclophosphamide) and αβTCR/CD19-depleted haploidentical (Haplo) transplants have achieved excellent results for infants and children with PID and other inborn errors, where no matched donors are available.[34] However, there are currently insufficient published data in older PID patients to definitively support the use of haploidentical donors in preference to MMUDs. In non-PID settings, a number of prospective randomized controlled trials are being planned to determine the safety and efficacy of MMUD vs Haplo in patients >18 years at HSCT.

Conditioning regimens

PID patients surviving to adulthood typically have residual functional cellular immunity necessitating conditioning to permit engraftment of allogeneic stem cells and prevent graft rejection; as a consequence, unconditioned transplants are not indicated.

To limit TRM in older PID patients, either reduced toxicity or reduced intensity conditioning is recommended in all cases. However, it is increasingly evident that achieving stable donor chimerism is important for PID transplants, and this is not always reliably achieved with reduced intensity conditioning regimens. Typically, 10% to 20% of children undergoing allo-HSCT for PID require a second procedure or donor lymphocyte infusion (DLI), with HLA mismatch and the use of reduced intensity conditioning being significant risks.[35]

Most experience to date has been with fludarabine/busulfan (Flu/Bu), fludarabine/melphalan (Flu/Mel), or fludarabine/treosulfan (Flu/Treo)-based reduced intensity conditioning incorporating serotherapy (alemtuzumab or ATG) for in vivo T-cell depletion. Recent improvements in conditioning regimens for nonmalignant diseases such as PID have primarily come from pediatric centers and include the introduction of treosulfan,[36,37] the use of targeted busulfan,[24,38] and personalized serotherapy dosing.[34,35,39]

The use of PTCy or αβTCR depletion to remove alloreactive T cells has facilitated the use of haploidentical donors in older recipients with hematologic malignancies, without prohibitive risks of GVHD and/or graft rejection.[40] Similarly, in pediatric series, these approaches have been used with excellent results in nonmalignant disease and PID.[41-43] However, as the cumulative experience transplanting older adults with PID using haploidentical donors remains very small, further studies are warranted.

Evolution of clinical cases

Patient 1 had an unaffected sibling who was HLA matched and CMV matched (+/+). Patient 2 had a fully matched unrelated donor, also CMV matched (−/−). Both patients received reduced intensity conditioning with fludarabine, melphalan, and alemtuzumab, together with single-agent cyclosporine as GVHD prophylaxis.

Adult PID-specific transplant management

Additional pretransplant investigations are indicated in many PID patients to document the presence or absence of specific pathogens, antimicrobial sensitivities, and/or degree of organ specific inflammation. Examples include the following: colonoscopy and gastrointestinal tract imaging for patients with inflammatory colitis or prior chronic norovirus or cryptosporidial diarrhea; high-resolution computed tomography chest imaging, bronchoscopy, lavage, and/or biopsy in patients with granulomatous lymphocytic inflammatory lung disease, previous aspergillosis, or atypical mycobacterial infections; and liver biopsy, fibroscan, and/or measurement of portal pressures in patients with abnormal liver function tests and a history of nodular regenerative hyperplasia or autoimmune hepatitis.

Other pretransplant investigations occasionally indicated are lumbar puncture with cerebrospinal fluid culture and biopsies of atypical granulomatous lesions, where relevant, to exclude or identify persistent pathogens requiring tailored antimicrobial prophylaxis throughout the transplant period.

Where possible, control of autoimmunity or inflammation should be achieved before transplant. This reduces the inflammatory cytokine milieu at the time of transfer of allogeneic cells, thus reducing the risk of acute GVHD and ensures end organ function is optimized before transplant. Preexisting PID-associated malignancies should be treated and in remission as per routine practice in HSCT for lymphoid malignancies. For patients with EBV handling disorders, the inclusion of rituximab in the conditioning regimen can bridge the gap until functional immune reconstitution is achieved after transplant.

Specialist infectious disease advice should be sought for patients with a history of refractory or atypical infections preceding transplant. Regular posttransplant monitoring for recurrence of previous, persistent, or latent opportunistic pathogens is indicated on a per-patient basis. We recommend the continued use of prophylactic antimicrobials until cessation of immune suppression as GVHD prophylaxis or treatment.

Splenomegaly in PID is not uncommon and has multiple causes. A number of studies have clearly demonstrated that poor graft function and hematologic recovery after allo-HSCT is associated with splenomegaly.[44,45] Although prior splenectomy may improve hematologic recovery after transplant, it is associated with perioperative complications and lifelong impaired immunity to encapsulated bacteria; therefore, it is only rarely performed.

Recurrence or de novo autoimmunity after transplant has also been reported in PID transplant cohorts, particularly in the context of mixed chimerism,[6,46] which is discussed below. The exact pathophysiology underlying persistent or late onset autoimmunity is poorly understood.

Evolution of clinical cases

Patient 1 developed early EBV-associated post transplant lymphoproliferative disease at 2 months after transplant. She responded to 4 cycles of rituximab and prompt withdrawal of immune suppression, without the development of GVHD. EBV viremia (without lymphadenopathy) resolved by 5 months after transplant.

Importance of donor chimerism after transplant

Conditioning regimens using reduced doses of cytoreductive agents are better tolerated in older recipients with higher comorbidities, but they carry the risk of failing to eradicate host hemopoiesis. This can result in mixed chimerism in differentiated lineages including neutrophils, B cells, red cells, and platelets, although the T-cell compartment is most commonly affected. Mixed T-cell chimerism after transplant can follow re-expansion of residual recipient T cells not effectively depleted by lymphodepleting conditioning +/− serotherapy (alemtuzumab or ATG).[47,48]

For very long-term functional immune reconstitution, multilineage stable donor chimerism is considered optimal, although full donor chimerism in any given lineage is not per se required for correction of a functional deficit. However, mixed donor/recipient stem cell chimerism is expected to only partially correct the underlying immunologic disorder with a risk of late complications or disease recurrence.

During the first year after transplant, chimerism may fluctuate. The use of DLI to promote/force conversion from mixed chimerism to full donor chimerism should be evaluated carefully because it carries with it a risk of GVHD. Previously the use of DLI was only considered where worsening mixed chimerism raised concerns of incipient graft rejection. However, recent data suggest that persistent mixed chimerism after transplant is associated with poorer event-free survival as long as 20 years after transplant (E.C.M., unpublished data, October 2020) and late complications such as autoimmunity.[6,46] Future studies should investigate the role of prophylactic or preemptive DLI early after transplant in the setting of allo-HSCT for PID.

For patients with neutrophil defects, achieving high level stable myeloid chimerism is essential. Studies in male patients and female carriers of the X-linked form of CGD have demonstrated that symptoms arising from inflammation or autoimmunity may be present in carriers with extreme degrees of lyonization (resulting in 20%-30% normally functioning phagocytes), although the risk of serious infection is rarely present if >10% of circulating neutrophils are functional.[49,50]

Where at all possible, known carriers should not be used as stem cell donors in PID, because even with full donor chimerism, full immunologic correction may not be achieved.

For rarer non-SCID PID and immune dysregulatory syndromes such as activated PI3k Delta syndrome, CTLA4 deficiency, LRBA deficiency, and STAT1 GOF, there are insufficient data regarding the optimal chimerism required for long-term immunologic correction and cure, although insight into the disease-specific pathophysiology may predict which lineages are critical to correct.[19,51-53]

How do we measure success after allo-HSCT in adult PID?

In malignant disease, a successful transplant results in long-term survival, no recurrence of the original malignancy, normal hematopoietic reconstitution, no chronic GVHD, and no late complications. Recently the use of composite outcome measures such as GVHD-free, relapse-free survival or chronic GVHD-free, relapse-free survival has been included in studies of allo-HSCT.[54] In parallel, the Center for International Blood and Marrow Transplant Research is developing the use of patient-reported outcomes.[55] In adult PID, the indication for transplant is often the prevention of progressive decline in quality of life secondary to the accumulation of PID-related medical complications, and patient-reported outcomes are vital in evaluating success. Other important end points for PID patients include the reconstitution of normal pathogen-specific immunity, resolution of autoimmunity and/or inflammation, and reduction in future malignancy risk.

For some PID cases, functional correction of the underlying immune deficit is easy to measure, for example, the correction of neutrophil function in CGD, as measured by nitroblue-tetrazolium or dihydrorhodamine assays. In X-linked PID where carriers are asymptomatic, it is possible to achieve functional/clinical correction without full donor chimerism in the relevant lineage. In the context of gene therapy, it is relatively easy to track the gene-corrected immune cells and quantify functional correction. In allo-HSCT recipients, lineage-specific donor chimerism is a surrogate marker for functional correction, but proof of transplant efficacy relies on functional immunologic assays (eg, response to vaccination, T-cell proliferation, normalization of lymphocyte subsets) and clinical responses. For patients going into transplant with preexisting end-organ

damage, it is critical that the consent process involves a clear discussion regarding which disease-associated symptoms/complications can be improved by transplant and which cannot (eg, bronchiectasis/pulmonary fibrosis, gut strictures, and extra-hematologic complications).

Evolution of clinical cases

Patient 1 is now alive and well, off immune suppression and immunoglobulin replacement therapy, with no GVHD, no recurrence of lymphoma, and employed at 40 months after transplant. Patient 2 is alive and well, off immune suppression and immunoglobulin replacement therapy, with no GVHD, normal peripheral blood counts, and at university 5 years after transplant.

Role of solid organ transplant in combination with allo-HSCT in adult PID patients with end organ failure

The incidence of end organ failure increases over time for untransplanted PID patients. As a result, a proportion of adult patients referred for transplant may already have or be at risk of imminent end organ failure. There is a small amount of published literature on the role of solid organ transplant either before or after allo-HSCT, and in selected cases, this should be considered.[56] Most available data in PID is for combined liver transplant in combination with allo-HSCT. Physicians and patients should be aware that allocation of cadaveric organs for patients with uncorrected PID also requiring an allo-HSCT for which outcome data are scarce is not universally guaranteed. Because of limited availability of such organs, they are typically reserved for clinical scenarios for which the evidence of long-term benefit is strongest. Furthermore, in a subset of patients, the severity of their liver disease may not yet meet criteria for liver transplant but precludes safe allo-HSCT. This very specialist area of clinical practice would benefit from ongoing international collaboration and prospective pilot studies.

Role of gene therapy as an alternative to allo-HSCT

For patients without a matched related donor or a fully matched unrelated donor, the decision between using multiple mismatched cord units, a mismatched unrelated donor, a haploidentical donor, or to consider gene therapy (where available, eg, for X-linked SCID, adenosine deaminase deficiency, WAS, X-linked CGD, and in development for other diseases including autosomal recessive-CGD and CD40L deficiency) remains difficult, in part because of the relatively limited experience of all these modalities in older patients with PID.

The use of alternative donors has a proven safety and efficacy record in younger children with PID and in adults with hematologic malignancies. It is predicted that the use of PTCy and or αβTCR depletion in these older patients will also facilitate the safe use of allo-HSCT in a wider group of potential recipients lacking a perfect donor.

Gene therapy has been successfully used in adults for WAS[57,58] and X-linked CGD[59] where appropriately matched allogeneic stem cell donors were not available. Gene therapy approaches currently rely on the ex vivo modification of autologous hematopoietic stem cells with viral vectors encoding the wild-type version of the absent or mutated gene. The early clinical trials of hematopoietic stem cell gene therapy using gammaretroviral vectors resulted in clonal expansion of gene-corrected cells, mediated by potent enhancer elements in the gammaretroviral long-terminal repeats, and led to the development of leukemia in some patients.[60] In addition, CpG dinucleotide promoter methylation led to silencing of transgene expression limiting durability of effective gene correction.[61] The most recent trials have demonstrated much improved outcomes with the use of self-inactivating lentiviral vectors (developed to minimize the mutagenic risk) and enhanced promoter sequences.

The potential advantage of gene therapy is the requirement for less immunosuppressive conditioning and no risk of GVHD. Both of these potential benefits are important in adult PID patients, who typically have high comorbidity scores when referred for a definitive procedure. Theoretically, gene therapy approaches in adults could be preferred to allo-HSCT if long-term correction and immune reconstitution is proven to be effective and durable.

Role of allo-HSCT in adults and adolescents who have failed gene therapy

As a reflection of the profound impact of gene therapy in monogenic PIDs, including X-SCID, ADA-SCID, CGD, and WAS, some of the first ever patients who were treated with hematopoietic stem cell gene therapy have recently transitioned into adult care. The first gene therapy protocols for ADA-SCID and X-SCID used minimal or no chemotherapy conditioning because the objective was to secure T-cell reconstitution, with the expectation that T cells arising from gene-corrected stem cells would have a clear competitive advantage to successfully secure engraftment of gene-corrected cells. However, a small number of recipients of unconditioned gene therapy have failed to reconstitute humoral immunity, requiring long-term immunoglobulin replacement therapy,[62] and others remain lymphopenic despite persistence of gene-corrected cells. Some of these patients, now young adults, have undergone or are being considered for either repeat gene therapy or allo-HSCT as a rescue procedure.

Understanding of the durability of gene therapy efficacy is limited by its relatively recent introduction compared with allo-HSCT for PID. As more recent gene therapy protocols have adopted targeted conditioning with improved engraftment of gene-corrected stem cells and incorporated safer and more efficient vectors, it is predicted that future results will continue to improve.

Conclusion

Recent data have established that allo-HSCT in adults PID is safe, that engraftment can be reliably achieved, and that overall survival is excellent for well-selected patients. Despite the availability of next-generation gene sequencing for a large proportion of patients, the decision to proceed to transplant remains a complex clinical decision.

There is an urgent need to inform practice further with large international multicenter studies designed to assess outcome after allo-HSCT for PID in adults, together with equivalent studies describing the natural history of these rare diseases for untransplanted patients. It is important that future prospective studies include detailed analysis of functional immune reconstitution, lineage-specific chimerism, quality of life, psychosocial impact, and late effects.

Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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How to evaluate for immunodeficiency in patients with autoimmune cytopenias: laboratory evaluation for the diagnosis of inborn errors of immunity associated with immune dysregulation

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The identification of genetic disorders associated with dysregulated immunity has upended the notion that germline pathogenic variants in immune genes universally result in susceptibility to infection. Immune dysregulation (autoimmunity, autoinflammation, lymphoproliferation, and malignancy) and immunodeficiency (susceptibility to infection) represent 2 sides of the same coin and are not mutually exclusive. Also, although autoimmunity implies dysregulation within the adaptive immune system and autoinflammation indicates disordered innate immunity, these lines may be blurred, depending on the genetic defect and diversity in clinical and immunological phenotypes. Patients with immune dysregulatory disorders may present to a variety of clinical specialties, depending on the dominant clinical features. Therefore, awareness of these disorders, which may manifest at any age, is essential to avoid a protracted diagnostic evaluation and associated complications. Availability of and access to expanded immunological testing has altered the diagnostic landscape for immunological diseases. Nonetheless, there are constraints in using these resources due to a lack of awareness, challenges in systematic and logical evaluation, interpretation of results, and using results to justify additional advanced testing, when needed. The ability to molecularly characterize immune defects and develop "bespoke" therapy and management mandates a new paradigm for diagnostic evaluation of these patients. The immunological tests run the gamut from triage to confirmation and can be used for both diagnosis and refinement of treatment or management strategies. However, the complexity of testing and interpretation of results often necessitates dialogue between laboratory immunologists and specialty physicians to ensure timely and appropriate use of testing and delivery of care.

LEARNING OBJECTIVES

- Describe the phenotypic manifestations and molecular mechanism of CTLA-4 haploinsufficiency
- Discuss the approach to diagnostic evaluation of patients with autoimmune cytopenias in the context of inborn errors of immunity

Introduction

The most recent classification of inborn errors of immunity (IEIs)[1] included a total of 416 genes, 45 of which are associated with immune dysregulation[2] and 43 of which are associated with bone marrow failure syndromes, based on clinical phenotypes and inheritance patterns. In the last 6 months, since publication of the most recent classification, ~30 new monogenic IEIs have been reported. Abnormal immune regulation and persistent inflammation are hallmarks of autoimmune disease, and hematological anomalies affecting one or more cell lines are common manifestations of autoimmunity and often reflect underlying disease activity. There is also a relationship between chronic immune dysregulation and lymphoproliferative disease, likely related to persistent stimulation, and subsequent proliferative response. It is essential for hematologists and immunologists to recognize and understand the intersection of immunology and hematology, especially with regard to genetic disorders of the immune system that predispose to hematological autoimmunity, benign or malignant lymphoproliferation, and bone marrow failure.

This article uses a case-based approach to review and highlight a specific genetic inborn error as an exemplar of

immune dysregulation that results in multiple autoimmunity with hematological aberrations, lymphoproliferation, susceptibility to malignancies, and end-organ damage. This article focuses primarily on the laboratory diagnosis and work-up for suspected autoimmune cytopenias (AICs) in the context of monogenic immune dysregulation.

Clinical case

A 42-year-old woman with a 15-year history presented with progressive headache and neck pain, stiffness, and reduced hearing, with no fever or respiratory symptoms. Her symptoms of headache progressed with vomiting and horizontal diplopia, and imaging (computed tomography, magnetic resonance imaging) revealed an enhancing anomaly in the brain parenchyma. The finding of pathogen testing was negative, and treatment with IV steroids resulted in symptom resolution. About 8 months after the initial episode of the above-mentioned neurological symptoms, she developed a similar presentation and was diagnosed with multiple sclerosis, and she was initiated on immunomodulatory therapy. Approximately 4 years from the initial presentation, she was noted to be mildly anemic, and thoracic imaging revealed mediastinal and axillary adenopathy as well as splenomegaly. Multiple lymph node biopsies only revealed lymphoid hyperplasia. She later developed jaundice with extreme fatigue and shortness of breath. A complete blood count revealed a hemoglobin of 4 g/dL, and a diagnosis of hemolytic anemia was made. A few months later, she developed thrombocytopenia and shingles involving a thoracic dermatome. Her platelet count dropped to 1000 per microliter, requiring platelet transfusions. The cytopenias were treated with steroids, immunoglobulin, and rituximab. There appeared to be progression of disease over time, and the brain lesions were considered as part of an undefined lymphoproliferative process. The hematologist suggested an allogeneic hematopoietic cell transplant (HCT), despite the lack of a clear and unifying clinical diagnosis. The key questions at this point are the likely differential diagnoses and which laboratory evaluation to use to arrive at a diagnosis.

Approach to diagnostic evaluation of the clinical case

The clinical phenotype of the patient suggests an immune dysregulatory disorder. Laboratory evaluation for autoimmune lymphoproliferative syndrome (ALPS) was performed because of her autoimmune hemolytic anemia (AIHA), thrombocytopenia (presumed autoimmune), and lymphoproliferation. Flow cytometric analysis to assess for increased T-cell receptor α-β+ double-negative ($CD3^+CD4^-CD8^-$) T cells revealed they were within normal limits and not expanded, as would be seen with typical ALPS.[3-5] Her immunoglobulin levels for immunoglobulin G (IgG), IgA, and IgM revealed modest hypogammaglobulinemia with partial IgA deficiency. She had intact vaccine antibody responses to protein-based vaccines but abnormal antibody responses to pneumococcal polysaccharide vaccination.

On the basis of these findings, she was given a presumptive diagnosis of common variable immunodeficiency (CVID), which was supported by her clinical findings of interstitial lung disease and AICs. Detailed flow cytometric analysis of peripheral blood B-cell subsets revealed significant defects in B-cell differentiation with decreased total memory and class-switched memory B cells (0.1%). She appeared to have recovered total peripheral B-cell counts after multiple treatments with rituximab, but she did not have normal B-cell differentiation. She also had decreased plasmablasts with substantially increased CD21-negative B cells (marker of immature B cells). At the time of this evaluation, targeted gene sequencing panels and whole-exome sequencing (WES) were not common. CVID does not represent a single diagnostic entity, but rather encompasses several distinct monogenic defects, and the majority of patients may have an oligogenic or polygenic etiology. Several years later, as genetic testing became increasingly available and affordable, she underwent a targeted next-generation sequencing (NGS) panel for IEIs, which reported a likely pathogenic heterozygous variant in the *CTLA4* gene, c.410C>T, p.Pro137Leu, consistent with CTLA-4 haploinsufficiency. CTLA-4 haploinsufficiency with autoimmunity (CHAI) is an IEI resulting from loss of a key immunoregulatory molecule that functions as a "checkpoint" inhibitor of T-cell activation.

Understanding the predisposition to autoimmunity in the context of CTLA-4 deficiency

The cellular adaptive immune system (ie, T cells) uses several regulators to ensure there is an appropriate counterbalance to T-cell activation to prevent uncontrolled T-cell activation. Among these is CTLA-4, a key negative regulator of the peripheral immune response (Figure 1). CTLA-4 is expressed by $CD4^+$ and $CD8^+$ T cells and is only expressed on activated T cells, but it is constitutively present on regulatory T cells (Tregs). CTLA-4, like the constitutive T-cell costimulation molecule CD28, binds the ligands B7-1 (CD80) and B7-2 (CD86). The difference is CTLA-4 binds these molecules with much higher affinity and avidity than CD28, enabling it to serve as a "brake" to the T-cell activation response. In the case of Tregs, the small amounts of CTLA-4, which are constitutively expressed, are rapidly upregulated on activation of T cells. Although the immunosuppressive effects of Tregs are mediated through a variety of mechanisms, including immunomodulation via CTLA-4, the absence of this molecule affects both Treg numbers and function. This may be due to CTLA-4 being a target gene of FOXP3, which is a critical regulator of Tregs and is required for the effective suppressor function of these cells. Because CTLA-4 is typically sequestered in the intracellular compartment and substantially upregulated upon T-cell activation, it has to capture its ligands, B7-1 and B7-2, which it does via a process called *transendocytosis*, allowing these molecules to be degraded inside the cells, which express CTLA-4. This process requires sufficient CTLA-4 expression to be present on the surface of Tregs or activated T cells because the ability to remove the ligands is dependent both on cell contact and the duration of cell contact. Once CTLA-4 is internalized, it is either degraded in the lysosome or recycled to the plasma membrane. Another molecule, LPS-responsive beige-like anchor (LRBA), is colocalized in the endosome and recycles CTLA-4, allowing it to be reexpressed on the cell surface. This is why LRBA deficiency represents the "other side of the coin" with a clinical phenotype similar to CTLA-4 haploinsufficiency, especially in the predisposition to autoimmunity.[6]

The clinical significance of ineffective CTLA-4 function has been amply demonstrated through patients who have heterozygous (autosomal dominant) pathogenic variants in *CTLA4* resulting in haploinsufficiency.[6-11] The presence of multiple autoimmunity in these patients, including AICs, enteropathy, type 1 diabetes, and neurological phenotypes, all indicate that insufficient CTLA-4 protein expression (caused by haploinsufficiency)

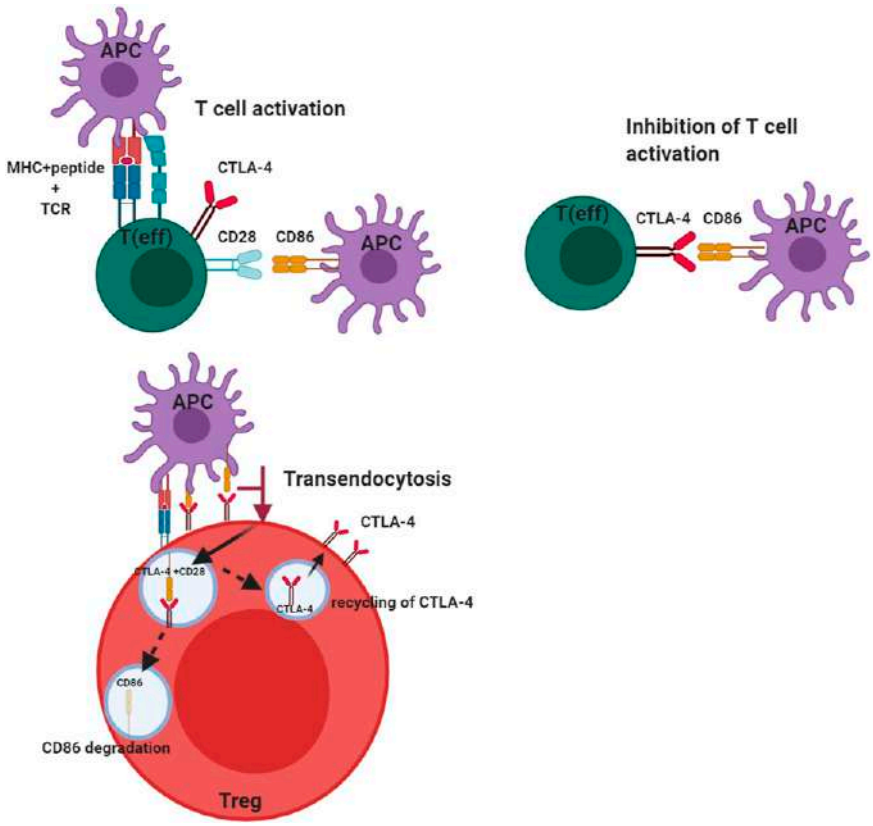

Figure 1. Role of CTLA-4 in the regulation of effector T-cell and regulatory T-cell function. CTLA-4 serves to inhibit T-cell activation in effector T cells after CD28 has interacted with its ligands B7-1 (CD80) and B7-2 (CD86). This inhibition by CTLA-4 prevents unbridled T-cell activation. CTLA-4 expressed on regulatory T cells can transendocytose the ligand CD86 by removing it from the antigen-presenting cell and internalizing it, after which the ligand is degraded and CTLA-4 is recycled to the cell membrane. This transendocytosis process is exploited in the assessment of CTLA-4 variants. This figure was created with BioRender.com.

impedes effective suppressive function of Tregs and leads to inadequate control of T-cell activation. These patients also often demonstrate decreased immunoglobulins (immunodeficiency with predisposition to respiratory infections) and nonmalignant lymphoproliferation. The inflammatory phenotype in CTLA-4 haploinsufficiency does not necessarily correlate with lymphocyte expansion and infiltration and is likely dependent on the genetic variant.[12]

Laboratory diagnosis and work-up for AICs in the context of immune dysregulatory disorders

Serological and immunophenotyping assessment

There is a growing list of single-gene defects associated with immune dysregulation and manifesting with multiple hematological and immunological phenotypes, including AICs. Therefore, it is important to know when and how to evaluate a patient with AIC for an underlying immune dysregulatory disorder, to differentiate between monogenic and polygenic causes of disease, and to recognize the implications for personalized therapy in patients with specific molecular defects. Timely laboratory evaluation in the context of clinical phenotype can reduce the diagnostic odyssey of many patients and may prevent institution of nonspecific immunomodulatory therapies, which may increase the risk of infection, while providing minimal therapeutic benefit.

It is helpful to do a preliminary serological and immunophenotyping assessment, either in parallel with obtaining genetic analysis or before it, because it is often useful to have an immunophenotype to correlate with genetic data. Because patients with monogenic immune dysregulation, also called *primary immune regulatory disorders*, may present with hypo- or hypergammaglobulinemia, depending on the underlying context, quantitative measurement of serum immunoglobulins, especially IgG, IgA, IgE, and IgM, is useful to contextualize the underlying defect. Other serological assessments for autoimmunity and/or inflammation can include evaluation of soluble (s) biomarkers, such as B-cell activating factor, sCD163 (soluble haptoglobin, a marker for macrophage activation), and sCD25, as well as autoantibodies directed against neutrophils, platelets, and erythrocytes, for the assessment of autoimmune neutropenia (AIN), thrombocytopenia (immune thrombocytopenic purpura [ITP]), and AIHA. Autoantibody testing for platelets and neutrophils is controversial and often of limited utility due to analytical constraints and the low sensitivity of many of the available assays. Therefore, although autoantibody testing is often ordered in clinical practice, a negative result is not necessarily clinically informative. A complete blood count is useful for serial monitoring of blood counts, especially in response to treatment. sCD25 is a biomarker that is significantly elevated in CTLA-4 and LRBA deficiencies.

Table 1. Laboratory diagnostic tests for example IEIs with AICs

Disease example	Genetic defect	Basic laboratory tests*	Immunophenotyping*	Functional tests*	Other tests/genetic testing
CHAI	*CTLA4*	CBC, serum immunoglobulins, MCV, reticulocyte count, haptoglobin, direct Coombs test, bilirubin, LDH, blood smear examination, neutrophil and platelet autoantibodies†	TBNK (C), T-cell subset quantitation (for naïve, memory effector, central, effector memory expressing CD45RA, activated T cells expressing HLA-DR, CD25, CD38, CD69, senescent/exhausted T cells negative for CD28, expressing CD57, activated/memory T cells expressing PD-1); follicular T helper cells, which are expanded; Tregs (expressing FOXP3, CD127), which are decreased; B-cell subset quantitation (naïve, transitional (CD24, CD38); memory B cells, including total $CD27^+$, marginal zone and switched memory B cells, $CD10^+$ immature B cells, $CD21^-$, $CD21^{dim}$, $CD21^{++}$ B cells, plasmablasts, BAFF-R/TACI-expressing total and memory B cells, NK cells (cytokine-producing, $CD56^{++}$, cytotoxic NK cells ($CD16^{++}56^{+/-}$) (C)	If recurrent infections are present, T-cell function can be assessed by proliferation to mitogens (PHA, anti-CD3/anti-CD28/IL-2), antigens (CA, TT, viral peptides, as needed) (C). T-cell proliferation to other mitogenic stimulants, such as PMA/ionomycin, is typically not available clinically but is useful for assessing the distal T-cell activation pathway (R). Production of cytokines (IL-2, TNF-α, and IFN-γ) may also be assessed in T cells after stimulation with mitogens and $CD4^+$ and $CD8^+$ T cells (R)	Expression of CTLA-4 on activated T cells and Tregs (after stimulation with PHA or PMA + ionomycin) (R), transendocytosis assay (R) if it can be assessed sBAFF, sCD25 (significantly elevated), and other biomarkers if relevant to clinical phenotype Genomic analysis (either targeted, WES, WGS, or chromosomal array may also be helpful for an initial screen of large deletions, CNVs) (C)
LATAIE	*LRBA*	Same as for CTLA-4 defects	Same as for CTLA-4 defects	Same as for CTLA-4 defects	LRBA protein expression in lymphocyte subsets, monocytes by flow cytometry (R) Transendocytosis assay (R) Genomic analysis (either targeted, WES, WGS, or chromosomal array may also be helpful for an initial screen of large deletions, CNVs) (C)
APDS1/2, PASLI	*PIK3CD* (GOF), *PIK3CD* (LOF), *PIK3R1*	Same as for CTLA-4 defects, increased serum IgM levels	Same as for CTLA-4 defects; these patients have decreased naïve T cells, increased senescent T cells, and transitional B cells.	Same as for CTLA-4 defects	Akt phosphorylation by flow cytometry or western blot (R) Genomic analysis (either targeted, WES, WGS, or chromosomal array may also be helpful for an initial screen of large deletions, CNVs) (C)

ANC, absolute neutrophil count; APDS, activated phosphatidylinositol 3-kinase δ syndromes 1 and 2; BAFF, B-cell activating factor; BAFF-R, B-cell activating factor receptor; BM, bone marrow; C, clinically available; CA, *Candida*; CBC, complete blood count; CNV, copy number variation; DC, dyskeratosis congenita; DNT, double-negative ($CD3^+CD4^-CD8^-$) T cells; GATA2, GATA2 haploinsufficiency; GOF, gain of function; HLH, hemophagocytic lymphohistiocytosis; IFN, interferon; IPF, idiopathic pulmonary fibrosis; LATAIE, LRBA deficiency with autoantibodies, Treg defects, autoimmune infiltration, and enteropathy; LDH, lactate dehydrogenase; LOF, loss of function; MCV, mean corpuscular volume; PASLI, p110-δ–activating mutation causing senescent T cell, lymphadenopathy, immunodeficiency; PHA, phytohemagglutinin; PMA, phorbol myristate acetate; R, research testing only; SDF-1, stromal cell–derived factor 1 (CXCL12); sFASL, soluble FAS ligand; STK4/MST1, serine threonine kinase 4, macrophage-stimulating factor 1; TBNK, lymphocyte subset quantitation (T, B, and NK cells); TCR, T-cell receptor; TNF, tumor necrosis factor; TT, tetanus toxoid; WAS, Wiskott-Aldrich syndrome; WHIM, warts, hypogammaglobulinemia, immunodeficiency, myelokathexis; XIAP, X-linked inhibitor of apoptosis; XLP-2, X-linked lymphoproliferative disease type 2.

*Testing to be ordered as appropriate on the basis of clinical phenotype

†Antiplatelet and antineutrophil antibody tests have limited sensitivity and therefore are not always diagnostically valuable.

‡Cousin et al.[36]

§Gifford et al.[63]

||Ammann et al.[64]

Table 1. (Continued)

Disease example	Genetic defect	Basic laboratory tests*	Immunophenotyping*	Functional tests*	Other tests/genetic testing
ALPS	*FAS*, *FASLG*, *CASP10*, others	Same as for CTLA-4 defects, vitamin B_{12}, sFASL, which are increased; IL-10	Quantitation of TCRαβ$^+$ DNT (CD3$^+$CD4$^-$CD8$^-$) T cells, which are increased; CD25, HLA-DR (increased ratio of HLA-DR to CD25), CD57 on T cells (C); switched memory B cells, which are decreased in some patients; γδ TCR$^+$ T cells (C)	As needed	In vitro assessment of apoptosis for patients not yet started on treatment (C) Genomic analysis (either targeted, WES, WGS, or chromosomal array may also be helpful for an initial screen of large deletions, CNVs) (C)
HLH	*PRF1*, *UNC13D*, *STX11*, *STXBP2*, others	CBC, sCD25, ferritin, bone marrow biopsy, liver enzymes, IL-18, CXCL9 (surrogate for IFN-γ levels) (C)	TBNK, NK cell subset quantitation (C) Lymphopenia can be observed; decreased cytotoxic NK cells	CD107 degranulation in CD8$^+$ T cells and NK cells after PMA/ionomycin and K562 stimulation, respectively, by flow cytometry (C), which is normal in perforin deficiency and abnormal in other genetic types of HLH	Perforin protein expression in NK cells and CD8$^+$ T cells by flow cytometry (C), which is abnormal in FHL2 but normal in other genetic types of HLH Genomic analysis (either targeted, WES, WGS, or chromosomal array may also be helpful for an initial screen of large deletions, CNVs) (C)
GATA2 haploinsufficiency	*GATA2*	CBC, bone marrow biopsy to look for atypical megakaryocytes	TBNK, monocyte subsets (for evaluation of monocytopenia); dendritic cell subsets (CD123, CD141, CD1c, CD11c), which are all significantly decreased (R); NK cell subsets for loss of CD56^{++} cytokine-producing NK cells (C)	T-cell function as needed (as described for CTLA-4 defects)	If concern for somatic GATA2 variant, obtain sample from sources other than blood (buccal and fibroblasts), check for presence of somatic *ASXL1* variant Genomic analysis (either targeted, WES, WGS, or chromosomal array may also be helpful for an initial screen of large deletions, CNVs) (C)
DC	*DKC1*, *RTEL1*, *TERC*, *TERT*, others	CBC, serum immunoglobulins	TBNK, additional phenotyping as needed, assessment of DNA repair defects by flow cytometry (phosphorylation of ATM and H2AX-γH2AX)# (C) Lymphopenia can be observed in one or more subsets DNA repair defects are observed in some patients and can be assessed by flow cytometry‡ (C)	T-cell function as needed (as described for CTLA-4 defects)	Telomere length analysis by flow cytometry–fluorescence in situ hybridization to estimate how short telomeres are in each subset: lymphocytes and granulocytes, assessment for IPF, depending on age of patient Genomic analysis (either targeted, WES, WGS, or chromosomal array may also be helpful for an initial screen of large deletions, CNVs) (C)

ANC, absolute neutrophil count; APDS, activated phosphatidylinositol 3-kinase δ syndromes 1 and 2; BAFF, B-cell activating factor; BAFF-R, B-cell activating factor receptor; BM, bone marrow; C, clinically available; CA, *Candida*; CBC, complete blood count; CNV, copy number variation; DC, dyskeratosis congenita; DNT, double-negative (CD3$^+$CD4$^-$CD8$^-$) T cells; GATA2, GATA2 haploinsufficiency; GOF, gain of function; HLH, hemophagocytic lymphohistiocytosis; IFN, interferon; IPF, idiopathic pulmonary fibrosis; LATAIE, LRBA deficiency with autoantibodies, Treg defects, autoimmune infiltration, and enteropathy; LDH, lactate dehydrogenase; LOF, loss of function; MCV, mean corpuscular volume; PASLI, p110-δ–activating mutation causing senescent T cell, lymphadenopathy, immunodeficiency; PHA, phytohemagglutinin; PMA, phorbol myristate acetate; R, research testing only; SDF-1, stromal cell–derived factor 1 (CXCL12); sFASL, soluble FAS ligand; STK4/MST1, serine threonine kinase 4, macrophage-stimulating factor 1; TBNK, lymphocyte subset quantitation (T, B, and NK cells); TCR, T-cell receptor; TNF, tumor necrosis factor; TT, tetanus toxoid; WAS, Wiskott-Aldrich syndrome; WHIM, warts, hypogammaglobulinemia, immunodeficiency, myelokathexis; XIAP, X-linked inhibitor of apoptosis; XLP-2, X-linked lymphoproliferative disease type 2.

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Table 1. (Continued)

Disease example	Genetic defect	Basic laboratory tests*	Immunophenotyping*	Functional tests*	Other tests/genetic testing
WHIM	*CXCR4* (GOF)	CBC (ANC often severe <500/mm3, though could be higher at times); serum immunoglobulins because some patients have hypogammaglobulinemia; BM evaluation for myelokathexis	TBNK, other phenotyping as needed	T-cell function as needed (as described for CTLA-4 defects)	Expression of CXCR4 on activated T cells, chemotaxis in response to SDF-1 (R) Genomic analysis (either targeted, WES, WGS, or chromosomal array may also be helpful for an initial screen of large deletions, CNVs) (C)
XLP-2	*XIAP/BIRC4*	Same as HLH	TBNK, other phenotyping as needed	T-cell function as needed (as described for CTLA-4 defects) CD107 degranulation; NK cell cytotoxicity can appear decreased due to NK cell lymphopenia Normal Fas-mediated T-cell apoptosis (not increased)	XIAP protein expression in lymphocyte subsets by flow cytometry§ (C) XIAP functional analysis|| (C) Genomic analysis (either targeted, WES, WGS, or chromosomal array may also be helpful for an initial screen of large deletions, CNVs) (C)
WAS	*WAS*	Same as for CTLA-4 defects, platelet size measurement	As described for CLTA-4 defects, for patients with WAS who have malignancies; early data suggest DNA repair defects, which can be assessed by flow cytometry‡ (C)	T-cell proliferation to mitogens and antigens, NK cell cytotoxicity (abnormal with K562 stimulation, but normal with IL-2 (C) or IL-15 stimulation (R)	WAS protein expression by flow cytometry (C) Assessment of immunological synapse formation (R) Genomic analysis (either targeted, WES, WGS, or chromosomal array may also be helpful for an initial screen of large deletions, CNVs) (C)
STAT3-GOF	*STAT3*	Same as for CTLA-4 defects, as needed	Same as for CTLA-4 defects, as needed T, B, and NK cell lymphopenia in some patients Decreased switched memory B cells Variable numbers of Th17 cells	Same as for CTLA-4 defects, as needed	STAT1/5 phosphorylation by flow cytometry, which is decreased (C, R) Increased SOCS3 expression (R), IL-6 levels (C) Genomic analysis (either targeted, WES, WGS, or chromosomal array may also be helpful for an initial screen of large deletions, CNVs) (C)
STK4 deficiency	*MST1*	Same as for CLTA-4 defects, serum IgE quantitation	Same as for CTLA-4 defects (phenotypic overlap with DOCK8 deficiency in some patients) $CD4^+$ T-cell lymphopenia Marginal zone B cells (nonswitched) are decreased Switched memory B cells are normal to high	Same as for CTLA-4 defects	Western blot analysis for STK4/MST1 protein expression (R) Genomic analysis (either targeted, WES, WGS, or chromosomal array may also be helpful for an initial screen of large deletions, CNVs) (C)

ANC, absolute neutrophil count; APDS, activated phosphatidylinositol 3-kinase δ syndromes 1 and 2; BAFF, B-cell activating factor; BAFF-R, B-cell activating factor receptor; BM, bone marrow; C, clinically available; CA, *Candida*; CBC, complete blood count; CNV, copy number variation; DC, dyskeratosis congenita; DNT, double-negative ($CD3^+CD4^-CD8^-$) T cells; GATA2, GATA2 haploinsufficiency; GOF, gain of function; HLH, hemophagocytic lymphohistiocytosis; IFN, interferon; IPF, idiopathic pulmonary fibrosis; LATAIE, LRBA deficiency with autoantibodies, Treg defects, autoimmune infiltration, and enteropathy; LDH, lactate dehydrogenase; LOF, loss of function; MCV, mean corpuscular volume; PASLI, p110-δ–activating mutation causing senescent T cell, lymphadenopathy, immunodeficiency; PHA, phytohemagglutinin; PMA, phorbol myristate acetate; R, research testing only; SDF-1, stromal cell–derived factor 1 (CXCL12); sFASL, soluble FAS ligand; STK4/MST1, serine threonine kinase 4, macrophage-stimulating factor 1; TBNK, lymphocyte subset quantitation (T, B, and NK cells); TCR, T-cell receptor; TNF, tumor necrosis factor; TT, tetanus toxoid; WAS, Wiskott-Aldrich syndrome; WHIM, warts, hypogammaglobulinemia, immunodeficiency, myelokathexis; XIAP, X-linked inhibitor of apoptosis; XLP-2, X-linked lymphoproliferative disease type 2.

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Flow cytometry is a tremendously valuable tool in the diagnosis of IEIs and can be used for immunophenotyping, protein expression, and functional assessment.[13] Lymphocyte subset quantitation of T, B, and natural killer (NK) cells in blood is useful in "global binning" of patients, based on whether they have numerical deficits in one or more lymphocyte subsets. However, additional immunophenotyping, particularly of the T-cell ($CD4^+$ and $CD8^+$) and B-cell compartments is also often necessary because there can be quantitative and functional dysregulation in differentiation of the adaptive immune system, which may not be apparent by a global lymphocyte subset analysis. This detailed immunophenotyping includes quantitation of naïve and memory T cells, activated T cells, senescent and exhausted T cells, follicular T helper cells, and expression of key cytotoxic proteins in NK cells and $CD8^+$ T cells (perforin, granzymes) in the T-cell compartment (Table 1). Follicular T helper cells are substantially expanded in patients with CTLA-4 and LRBA defects. Among B-cell subsets, early (transitional and naïve B cells) and late B-cell (memory B cells, plasmablasts, $CD21^{bright}$, and $CD21^{dim}$) subsets should be quantitated in blood (Table 1). $CD21^{dim}$ ($CD21^{low}$) B cells have been described in CVID as well as other secondary immune dysregulatory diseases, such as systemic lupus erythematosus, as an unusual naïve-like B cell, defective in B-cell receptor stimulation, and expanded in patients with AICs, splenomegaly, and granulomatous disease, which likely reflects the overall B-cell maturation defects in this group of immunological disorders.[14-16] In addition, because autoimmunity is often associated with either quantitative or functional defects in Tregs, immunophenotyping of this compartment ($CD4^+CD25^+/FOXP3^+$ T cells) is often considered useful for the laboratory evaluation of these conditions (Table 1). For example, in LRBA deficiency and CTLA haploinsufficiency, impairment in Treg function and quantitative reduction in Tregs, as previously noted, results in a strong predisposition to autoimmunity.[6,17,18] Therefore, the ability to assess not only Treg numbers by flow cytometry but also the function of the suppressor cells is critical; however, functional Treg assays have not been validated in most clinical diagnostic laboratories and are thus mainly available only in a research setting. Furthermore, although there are several analytical approaches to assessing Treg function, not all of them are equally robust and reliable, and these assays continue to be explored and developed for validation in the clinical diagnostic laboratory.

Global functional evaluation of the immune system, not specific to a particular molecular defect, but broad interrogation of pathways (Table 1), includes T-cell proliferation to non-specific stimuli, specific antigens, and measurement of cytokine production among others. The non-specific mitogenic stimuli include phytohemagglutinin, phorbol myristate acetate/ionomycin, anti-CD3+/anti-CD28, anti-CD3+/interleukin-2 [IL-2], anti-CD3+/IL-7, while the specific antigens include Candida, tetanus toxoid or viral peptides to Epstein Barr virus, cytomegalovirus, Varicella zoster virus among others. Assessment of the T cell response could include production of cytokines, such as IL-2, interferon gamma and TNF-alpha, by CD4+ and CD8+ T cells, after stimulation with mitogens. It could also include cellular degranulation (CD107 expression) of cytotoxic lymphocytes (CD8+ T cells, NK cells), after appropriate specific or non-specific stimulation. Dysregulation of specific pathways, such as NF-κB or STAT, can be assessed by phosphorylation of key proteins after appropriate stimuli. Protein-specific expression in relevant cellular subsets, usually lymphocytes, can be determined for proteins when there is a high likelihood of loss of protein expression contributing to the clinical phenotype. It is helpful to include a functional assay, when possible, as an adjunct to a protein expression assay, especially for novel variants or atypical phenotypes. Assessment of various immunophenotypes, expansion or contraction, of specific T- or B-cell subsets, as described above, can also be useful in creating an immunophenotype to correlate with the clinical context and/or genetic data.

When considering diagnostic immunological testing, it is important to distinguish those assessed on a research basis vs validated testing performed in a clinical diagnostic laboratory. On the one hand, research-based testing is not widely available and therefore not easily accessible by most specialty clinicians in diverse practice settings. Clinical testing, on the other hand, is usually available to all clinicians, though there can be varying degrees of complexity in ordering such testing, depending on the nature of the practice.

Functional assessment of CTLA-4 and LRBA function

To directly assess the effect of CTLA-4 haploinsufficiency or LRBA deficiency, it is essential to have assays capable of assessing the specific molecules or pathways in question. Because CTLA-4 is upregulated on activated T cells within 24 to 48 hours after activation, and because its expression of Tregs is also increased after activation,[19] flow cytometric measurement of CTLA-4 on the surface of activated T cells and Tregs provides one method of assessing if there is sufficient protein on the cell surface to mediate cellular suppressor function. However, the most definitive functional assay for these defects is the transendocytosis assay,[20-22] which measures the removal of costimulatory ligands from antigen-presenting cells using flow cytometry and confocal microscopy. Inhibition of lysosomal degradation in this assay reveals an increase in the presence of B7-2 after transendocytosis (Figure 1). The ability of CTLA-4 to bind ligand and transendocytose is very dependent on the recycling process, which is mediated by LRBA, and thus, even if CTLA-4 expression is normal, if LRBA expression is impaired, it can affect ligand uptake. This allows a distinction between CTLA-4 and LRBA defects. Also, B7-1 has much higher affinity for CTLA-4 than does B7-2, and the ability to take up B7-2 is dependent on the presence of B7-1, but not vice versa.[23] A combination of these assays is most useful, especially when characterizing novel *CTLA4* and *LRBA* variants.

The role of genetic testing in identifying monogenic defects in patients with AICs

At the start of the third decade of the 21st century, it is undisputed that genetic evaluation plays a key role in the diagnosis of patients with IEIs, including those with autoimmune phenotypes, and this represents an ever-evolving and expanding area of study.[24,25] Currently, the clinician is confronted with an embarrassment of riches when it comes to genetic testing because there are targeted NGS panels with defined sets of genes, WES, and whole-genome sequencing (WGS).[26,27] Some key rules of thumb can be employed to determine which approach to use and to deploy these in a systematic and tiered manner. An American Academy of Allergy, Asthma, and Immunology committee has developed guidelines for selection of appropriate of genetic tests and interpretation of variants for IEIs.[28] Currently, genetic testing is often done early in the diagnostic process when an IEI is suspected. However, although immunophenotyping and generic functional immune evaluation assays that are clinically available are often ordered almost immediately, genetic testing,

depending on insurance and institutional regulations, may take a little longer. However, most patients who require genetic evaluation are usually able to obtain the testing with appropriate support from the physician, genetic counselor, and geneticist. More complex functional testing, either research based or clinical, is often required once the genetic result has been obtained, especially to further validate variants of uncertain significance (VUSs) or ambiguous results.

Targeted panels are most effective when there is a clear phenotype and/or family history, which supports the likelihood of a monogenic disease, and the panel under consideration is well represented for the genes of interest.[29] The most recent International Union of Immunological Societies classification[1] includes >400 genes, and most commercially available panels include most of these, which makes these panels practical and relatively rapid screens with a turnaround time averaging 2 or rarely 3 weeks. However, if the phenotype is more amorphous and ambiguous and there is either no family history or no samples for trio analysis (proband + parents) are available or there has been a previously negative targeted panel, WES is the most logical option.[30] WES looks at a much larger sample of genes in an unbiased sequencing approach; however, it has its own limitations, including the type of bioinformatics pipeline that is used for variant calling and filtering. Clinical exomes that are available commercially are not all the same and can have variable coverage.[31] Therefore, clinicians must be aware that not all exomes are created equal, and they need to understand the coverage and the type of bioinformatics approaches used, at least at a high level. A very recent analysis of the efficacy and cost of WES vs targeted panels revealed that, overall, going directly to a WES approach might be more cost effective in the long run, though diagnostic yield, depending on the cases, could be lower,[32] indicating that an informed approach to selecting the method of genetic testing is required for each patient based on clinical and laboratory phenotype, family history, and other practical factors, including insurance coverage.

WGS has also been used for the evaluation of IEIs, but it is less common in the clinical setting as a first-tier test because of accessibility issues (payer restrictions unless other forms of genetic testing have been attempted) and complexities in data analysis and interpretation. Most often, WGS is employed for patients with complex phenotypes who have had a negative test result for either WES and/or targeted panels. WGS is particularly useful when disease may be caused by deep intronic variants (noncoding areas of the genome) or novel genes, which may not be well represented in clinical exomes.[33,34] However, in patients who may have negative genetic testing but a clinical and/or laboratory phenotype strongly suggestive of monogenic disease, an iterative approach using reanalysis of WES data, WGS, or transcriptomic studies may prove useful in identifying a diagnosis.[35] Transcriptomic studies, such as RNA sequencing, may be particularly useful in establishing the pathogenicity of variants in noncoding regions for rare disorders, such as IEIs.[36,37] In addition, the relevance of closing the loop on genetic testing with functional studies and correlation with the clinical and immunological and other laboratory phenotype cannot be overemphasized and is useful not only for VUSs but also for known pathogenic variants, which may present with an atypical or expanded clinical phenotype (Figure 2). Reconfiguring the classification of VUSs by functional testing into more distinct "benign" and "pathogenic" categories provides clinicians with the necessary direction for next steps. Besides the above-mentioned genetic analyses, copy number variations (CNVs), which can contribute to the disease phenotype, can be assessed by chromosome microarrays. These, too, come in different flavors, and the clinician has to know which microarray platform is most suitable to the clinical context.[38] There are a number of other factors that can modulate the phenotype–genotype correlation, assuming there is one,

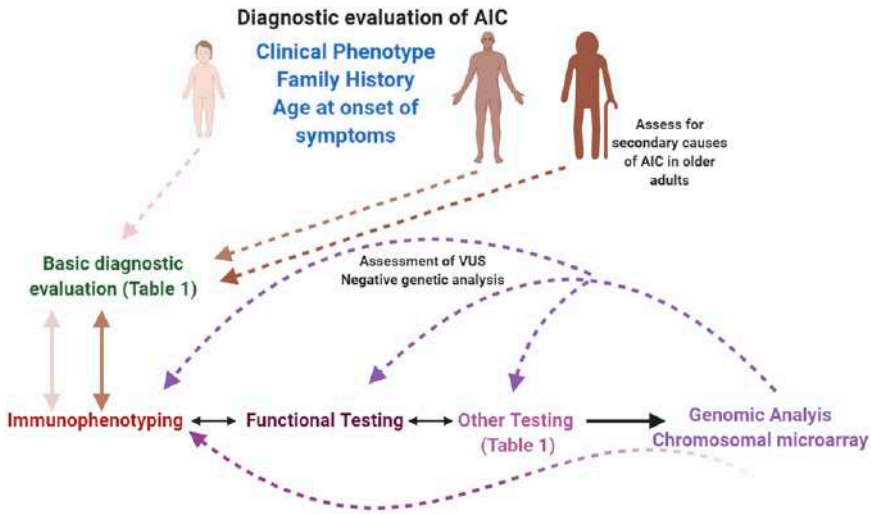

Figure 2. Diagnostic evaluation of AICs in pediatric and adult patients. A systematic, interdisciplinary approach can facilitate a timely and accurate diagnosis in these patients. All patients should receive a basic evaluation (expanded in Table 1), followed by immunological assessment (immunophenotyping, functional, and other specific assays), based on clinical phenotype, family history, and age of onset of symptoms. Molecular/genetic testing can be pursued either in parallel to the immunological assessment, depending on the clinical phenotype, or sequential to it. If a molecular diagnosis is not established at the first attempt, and if the likelihood of a genetic defect is high, recommend an iterative approach, which can also be used when characterizing VUSs. In the older adult, secondary causes of AIC should be eliminated before considering an intrinsic immune anomaly as the cause of the phenotype. This figure was created with BioRender.com.

Table 2. Standard immunomodulatory and targeted immunotherapies for management of AICs in IEIs

Genetic defect	Key clinical features	Standard immunomodulatory agents	Targeted therapy	Target
CTLA4	AICs, lymphadenopathy, splenomegaly, hypogammaglobulinemia, other organ-specific autoimmunity, lymphoid infiltration of organs, enteropathy, interstitial lung disease	Steroids, sirolimus, CsA, ATG, antibiotics, immunoglobulin replacement, allogeneic HCT	Abatacept rituximab, vedolizumab, anti-TNFα agents	CTLA-4–immunoglobulin anti-CD20 (B cell depleting), α4-β7 integrin TNF-alpha blocker
LRBA	AICs, lymphadenopathy, splenomegaly, hypogammaglobulinemia, other organ-specific autoimmunity, lymphoid infiltration of organs, enteropathy with failure to thrive, interstitial lung disease	Steroids, sirolimus, antibiotics, immunoglobulin replacement, allogeneic HCT	Abatacept anti-TNFα agents	CTLA4-Ig, TNFα blocker
PI3KCD GOF	Recurrent respiratory infections, bronchiectasis, herpes virus infections, lymphadenopathy, splenomegaly, autoimmune or autoinflammatory manifestations, lymphoid hyperplasia, increased incidence of lymphomas	Steroids, MMF, sirolimus, antibiotics, immunoglobulin replacement, allogeneic HCT	Leniolisib, idelalisib, nemiralisib, ustekinumab	PI3Kδ inhibitor IL-12/23 inhibitor
ALPS	Chronic lymphadenopathy, splenomegaly, AICs, increased risk of lymphoma	Steroids, MMF, sirolimus, splenectomy in rare cases and should be avoided, if possible	Rituximab	Anti-CD20 (B cell depleting)
PRF1, *UNC13D*, *STX11*, *STXBP2*, *RAB27A*, *LYST**	Hemophagocytosis, fever, splenomegaly, liver dysfunction, inflammatory phenotype with elevated biomarkers, CNS complications in some patients	Dexamethasone, etoposide, CsA, or hydrocortisone with intrathecal methotrexate, allogeneic HCT	Emapalumab-lszg Tadekinig alfa	IFNγ blocker IL-18BP to neutralize IL-18
GATA2	Viral and bacterial infections, cytopenias, myelodysplasia, myeloid leukemias, pulmonary alveolar proteinosis, lymphedema (Emberger syndrome)	Antibiotics and management of pulmonary disease, allogeneic HCT	None currently	—
DKC1, *RTEL1*, *TERC*, *TERT*, other DC gene defects	Triad of abnormal skin pigmentation, nail dystrophy, and oral leukoplakia in many but not all patients, bone marrow failure, short telomeres, increased risk of malignancies; adult patients have increased risk of idiopathic pulmonary fibrosis and cryptogenic cirrhosis	Allogeneic HCT	Although androgens such as danazol have been used, it is controversial and often not recommended	—
CXCR4 GOF	Warts, hypogammaglobulinemia, recurrent respiratory infections, myelokathexis	Immunoglobulin replacement, G-CSF	Plerixafor (AMD3100)	Inhibitor of CXCR4 binding to CXCL12
XIAP	HLH-like features, inflammatory bowel disease, cytopenias, splenomegaly	Allogeneic HCT	Tadekinig alfa	IL-18BP to neutralize IL-18
*WAS**	Microthrombocytopenia, eczema, diarrhea, recurrent infections, autoimmunity, increased risk of malignancies, IgA nephropathy, neutropenia with myelodysplasia (GOF variant)	Antibiotics, CsA, cyclophosphamide, high-dose immunoglobulin therapy, steroids, azathioprine, splenectomy, platelet transfusion, allogeneic HCT	Rituximab	Anti-CD20 (B cell depleting)
STAT3 GOF	Broad range of autoimmunity, including AICs, lymphoproliferation, hypogammaglobulinemia, enteropathy, interstitial lung disease	Steroids, MMF, tacrolimus, azathioprine, sirolimus, CsA, cyclophosphamide, methotrexate, allogeneic HCT	Anti-TNFα agents, anti-IL1β agents, tocilizumab, ruxolitinib	TNFα blocker, IL-1R antagonist, IL-6R blocker, JAK1/JAK2/STAT inhibitor
STK4/MST1	Overlap with DOCK8 deficiency in some patients, molluscum, warts, bacterial infections, AICs	Antibiotics, antifungals, antivirals, allogeneic HCT	None currently	—

ATG, antithymocyte globulin; CNS, central nervous system; CsA, cyclosporin A; DC, dyskeratosis congenita; G-CSF, granulocyte colony-stimulating factor; GOF, gain of function; HLH, hemophagocytic lymphohistiocytosis; MMF, mycophenolate mofetil; PI3Kδ, phosphatidylinositol 3-kinase δ; TNFα, tumor necrosis factor α; WAS, Wiskott-Aldrich syndrome.
*Experimental gene therapy is being considered or in early stages for HLH or WAS.

including mosaicism,[39,40] epigenetic factors, digenic defects, and epistatic modulation (Abraham and Butte, JACI In Practice, 2020, In Press).

An observational clinical study to identify monogenic defects in pediatric Evans syndrome is currently open (NCT03912129), and such studies will continue to add to the body of knowledge on the genetics of AICs. A recent study of 18 children with Evans syndrome who did not have FAS-associated ALPS (*FAS*) revealed that close to 38% had single gene defects, including *CTLA4* and *LRBA* defects. In the same study, of 48 children, 30 (62%) had FAS-associated ALPS, another IEI.[41]

AICs in IEIs

Although the infection phenotype was the first association made with IEI, over the last several years, numerous single-gene defects with immune dysregulation have been described and well documented.[42-44] The spectrum of immunological dysregulation can range from organ-specific autoimmunity to AICs, lymphoproliferation, and susceptibility to neoplastic disease. In many of the primary immune regulatory disorders, a multiplicity of these phenotypes are present or develop over time. The range of cytopenias can vary, and knowing the molecular defect can facilitate targeted and timely treatment. The pathophysiology behind the cytopenias, affecting one or more arms of the hematopoietic compartment, is multifaceted, and here again, the molecular defect and immunological phenotype may be helpful in determining management and therapy. AIC can also develop secondary to potentially curative therapy, such as HCT for IEI, as a result of conditioning regimens, incomplete immune reconstitution, and/or viral infections, and it requires similar approaches for management.[45] Single-lineage AIC includes only AIN, AIHA, or ITP when presenting independently, or multilineage disease, such as Evans syndrome, may be present. AIHA could be associated with either warm or cold autoantibodies. Similarly, for AIN and/or ITP, autoantibodies may be detected, which may help facilitate treatment decisions.

Returning to the clinical case

CTLA-4 haploinsufficiency was initially not included in the differential diagnosis for this patient, because the genetic defect had not been identified or described when the patient first presented. However, with hindsight, the phenotype fits the genotype. The laboratory evaluation as described above would have been useful to further narrow the diagnostic possibilities, had they been easily available at the time. The lack of effective CTLA-4–based regulation of the immune response results in aberrant activation of effector T cells, ineffective and dysregulated Treg function, and lymphocytic infiltration of organs. Patients with CTLA-4 haploinsufficiency have been shown to have a progressive loss of B cells with accumulation of these in various nonlymphoid organs. There is also an expansion of $CD21^{dim}$ B cells, which has previously been reported to be associated with autoreactivity and chronic graft-versus-host disease.[46,47] Penetrance of *CTLA4* variants is incomplete and is thought to be seen in two-thirds of cases.[11] Also, the phenotype can vary depending on whether only the *CTLA4* gene is involved or there is a larger deletion involving *CTLA4* and other genes in proximity.[12]

Although this patient received several "nonspecific" immunomodulatory therapies, including natalizumab for an incorrect diagnosis of multiple sclerosis, rituximab, steroids, intravenous immunoglobulin, and the identification of a molecular diagnosis allowed institution of a specific and personalized treatment. This case is very similar to one reported in the literature of a patient with the same pathogenic variant in *CTLA4* who had many similarities in clinical phenotype and was treated with daclizumab, targeting CD25, which resulted in further suppression of Treg function and worsening of symptoms.[48] The published case highlights the pitfalls of randomly instituting immunotherapy without fully understanding the mechanism of pathogenesis of disease. Abatacept is a fusion drug of the extracellular domain of CTLA-4 with the Fc portion of an immunoglobulin; it is available and approved for the treatment of rheumatoid arthritis, and it acts as a pharmacologic replacement for the ineffective CTLA-4 in these patients and is also efficacious in LRBA deficiency.[49,50] Sirolimus, a mammalian target of rapamycin inhibitor, can also potentiate Treg function by expanding Tregs and maintaining their immunosuppressive function, and it has been used with some success to mitigate the autoimmune manifestations in some patients. Other drugs that may be effective in CHAI include chloroquine or hydroxychloroquine, a lysosomal inhibitor (because CTLA-4 is cycled through the endolysosomal compartment before being expressed on the cell surface), though its effect in this disease has not been studied.[6] HCT has also been employed in a few select patients with CHAI with overall success, though there have been adverse outcomes in a few patients and reports of graft-versus-host disease in others.[6,51]

Approach to diagnostic evaluation of AICs in pediatric and adult patients

IEIs, though representing germline genetic defects of the immune system, can manifest at any age, ranging from infancy to adulthood. However, the likelihood of an underlying genetic defect is higher in infants and young children with AICs than in adults in the fourth decade of life and beyond, which is not to categorically state that IEIs cannot manifest in adults. In adults, statistically speaking, monogenic defects are more likely in younger adults than in older individuals. The case presented here provides an example of a patient who was diagnosed with CTLA-4 haploinsufficiency in early adulthood.

Therefore, the diagnostic evaluation of AIC in children and young adults should involve a detailed family history, immunological evaluation (as described above and in Table 1 and Figure 2), and early genetic testing. On the basis of this information, treatment can be tailored to treat the molecular defect, if identified. If a genetic diagnosis is not readily obtained, an iterative diagnostic approach, as described above (Figure 2), should be employed, especially for refractory or severe AIC.

In older adults, the cause of AIC is more often than not likely to be secondary; however, a thorough evaluation should include basic immunological testing (Table 1 and Figure 2) before initiating significant immunosuppressive therapy (baseline). If there is a family history, genetic testing is recommended along with further evaluation of other affected family members, especially younger individuals. It is also helpful to obtain information on other coexisting conditions and treatments that could potentially account for the AIC, especially in patients with no family history. The level of advanced immunological testing in such older individuals is dependent on the age of onset, clinical and family history, severity of disease, and whether the AIC can be correlated with other conditions or treatments.

Use of targeted immunotherapies in IEIs and lessons learned from CTLA-4 haploinsufficiency for checkpoint inhibitor immunotherapy in cancer

A significant advantage of improved genetic testing for IEIs has been the repurposing of targeted immunological therapies for management and treatment of these diseases.[52,53] They have added substantially to the therapeutic armamentarium, which had previously for decades consisted of antibiotics, steroids, generic immunosuppressants, immunoglobulin therapy, and HCT. Because curative gene therapy is available for only a small subset of IEIs,[54] it has been more urgent to expand the therapeutic options for patients with various clinical phenotypes, including immune dysregulation. These have been added to the existing standard immunomodulatory and immunosuppressive agents (Table 2).

Abatacept, as previously described, has been effective in many CTLA-4– and LRBA-deficient patients[50] and represents a new frontier in personalized medicine in these disorders. It has been used off-label for the last few years for various autoimmune and inflammatory manifestations of these diseases, though there is currently a phase 1/2 randomized, double-blind, placebo-controlled clinical trial open for abatacept in treatment of the chronic cytopenias in CTLA-4 haploinsufficiency (NCT03733067).

The patients with IEIs have provided many valuable lessons on the relevance of molecular pathways in the immune system and the consequences of targeting these in other diseases, such as cancer. In this article, the significance of blocking CTLA-4 is of particular relevance.[55-57] Ipilimumab is a therapeutic monoclonal antibody directed against CTLA-4[58] that has been approved for the treatment of malignant melanoma, particularly metastatic disease. Although this treatment has had dramatic effects on the survival of patients with this disease, a sizable proportion have developed immune-related adverse events,[59,60] which, though not dissimilar from those experienced by patients with CHAI, including colitis and lymphocytic infiltration, is sufficiently different in its manifestations. These adverse events require management with other immunomodulators. Therefore, pharmacological/biological manipulation of the immune system would benefit from the study of the IEIs, which offers a window into the consequences of immune manipulation and could facilitate the rational design of newer therapeutic interventions.[61,62]

Summary

Parallel to the discovery of new monogenic IEIs, including those associated with immune dysregulation, there has been a growth in the number of immunological laboratory assays available to phenotypically and functionally characterize various immune cell subsets and their alterations, which can be used to facilitate correlations of both genotype and phenotype. These immune assessment assays, coupled with genetic testing, have supported the diagnosis of the molecular basis of AICs in children and adults. The case described in this article highlights the importance of the intersection of hematology with immunology and the need for dialogue between these specialties in the care of patients with complex immunological diseases.

Conflict-of-interest disclosure

The author declares no competing financial interests.

Off-label drug use

None disclosed.

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Treatment of immune-mediated cytopenias in patients with primary immunodeficiencies and immune regulatory disorders (PIRDs)

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Severe immune cytopenias (SICs) are rare acquired conditions characterized by immune-mediated blood cell destruction. They may necessitate emergency medical management and long-term immunosuppressive therapy, strongly compromising the quality of life. The initial diagnostic workup involves excluding malignancies, congenital cytopenias, bone marrow failure syndromes, infections, and rheumatologic diseases such as systemic lupus erythematosus. Causal factors for SIC such as primary immunodeficiencies or immune regulatory disorders, which are referred to as inborn errors of immunity (IEIs), should be diagnosed as early as possible to allow the initiation of a targeted therapy and avoid multiple lines of ineffective treatment. Ideally, this therapy is directed against an overexpressed or overactive gene product or substitutes a defective protein, restoring the impaired pathway; it can also act indirectly, enhancing a countermechanism against the disease-causing defect. Ultimately, the diagnosis of an underling IEI in patients with refractory SIC may lead to evaluation for hematopoietic stem cell transplantation or gene therapy as a definitive treatment. Interdisciplinary care is highly recommended in this complex patient cohort. This case-based educational review supports decision making for patients with immune-mediated cytopenias and suspected inborn errors of immunity.

LEARNING OBJECTIVES

- Severe immune cytopenia, eg, Evans syndrome, autoimmune hemolytic anemia, or immune thrombocytopenia, is one possible, early, potentially dangerous, and difficult-to-treat manifestation of an underlying inborn error of immunity (IEI)
- Physicians must diagnose an underlying IEI to identify an effective treatment strategy for immune-mediated cytopenia
- In some cases, physicians may choose a precise, existing therapy that directly targets the pathomechanism of the IEI; in other cases, they may choose a semitargeted approach based on the category of the IEI, which may allow them to effectively adapt cytopenia-directed treatment algorithms
- Refractory or recurring cytopenia may serve as an additional indication, encouraging physicians to evaluate a patient with IEI for gene therapy or hematopoietic stem cell transplantation

Clinical case: part 1 of 3

A 17-year-old boy presented with epistaxis that necessitated tamponade and severe thrombocytopenia (5 g/L; Figure 1). Furthermore, the results of laboratory investigations showed severe neutropenia (0.4 g/L) and a positive Coombs test (1:16), normal hemoglobin, reticulocytes, and red blood cell counts. These results led physicians to suspect Evans syndrome (ES) with predominant immune thrombocytopenia and neutropenia. Because the administration of high-dose intravenous immunoglobulin (IVIG, 5 days of 0.5 g/kg/d) improved the platelet counts only transiently and moderately but did not affect absolute neutrophil counts, high-dose prednisolone was started on day 8. This regimen was successful, as platelet counts rose to around 50 g/L and the absolute neutrophil count normalized; mycophenolate mofetil (MMF) was then added on day 17 as a glucocorticosteroid-sparing measure. No clinical signs of immunodeficiency or a history suggestive of an inborn error of immunity (IEI) were present. The

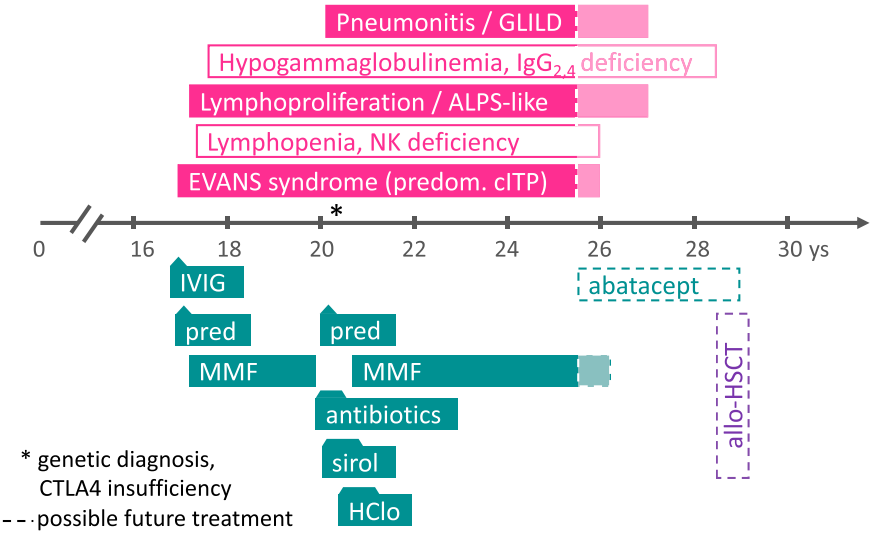

Figure 1. Clinical case presentation. The symptoms and disease phenotypes (in filled boxes) and predominant laboratory abnormalities (in empty boxes) are depicted in the upper panel, the time axis shows the patient's age in years (ys). Treatment phases with various agents are shown in the lower panel (boxes with triangles or trapezoids to indicate shorter durations of treatment courses than the boxes suggest). The dashed lines and shaded area on the right side (from 25 years of age and onward) indicate future treatment options; the asterisk indicates the time point of the genetic diagnosis. allo-HSCT, allogeneic hematopoietic stem cell transplantation; ALPS, autoimmune lymphoproliferative syndrome; GLILD, granulomatous lymphocytic interstitial pneumonitis; HClo, hydroxychloroquine; IgG, immunoglobulin G; IVIG, high-dose intravenous immunoglobulin G; MMF, mycophenolate mofetil; NK, natural killer cells; pred, prednisolone; predom. cITP, predominantly chronic immune thrombocytopenia (as part of multilineage autoimmune cytopenia); sirol, sirolimus.

prednisolone dosage was slowly reduced after the full dose (2 to 3 mg/kg/d) had been given for 4 weeks; this could be terminated around week 9 after the first presentation, rendering MMF as monotherapy. At that time, no attempts were made to perform a genetic diagnosis, although the complete absence of CD56+ natural killer cells and moderate lymphopenia (0.7 to 1 g/L, normal relative distribution of CD19+ B and CD4+ and CD8+ T cells) was noted. The total immunoglobulin (IgG, IgA, IgM) concentrations and specific antibody production amounts were normal.

Treatment options for immune cytopenia in patients with IEIs

In general, first-line therapy for severe immune cytopenia (SIC) in patients with suspected IEIs follows established guidelines for the respective "primary" condition, that is, the immune cytopenia without a known underlying disease (Figure 2, left panel, and Table 1).[1-5] However, an improvement in efficacy may be expected if an underlying condition (such as the IEI) is identified and can be treated simultaneously (Figure 2, right panel).[6-9] For some IEIs, a targeted therapy can compensate for the underlying defect, restoring the impaired signaling pathway and potentially correcting the accompanying autoimmune cytopenia. In other, more unspecific IEIs, defining at least the category of IEI (eg, whether it is a combined immunodeficiency, a predominantly antibody deficiency, or an autoinflammatory syndrome) can help the physician choose a second-line therapy that is at least directed towards the suspected pathomechanism.

Furthermore, the treatment goal must be defined: In ES and warm autoimmune hemolytic anemia (AIHA), the international standard of care is to aim at the induction of remission as soon as possible, whereas the primary aim in chronic immune thrombocytopenia is to reduce the risk of bleeding while not compromising the quality of life. In conditions with hypersplenism, structural cytopenia, or bone marrow failure and simultaneous autoimmunity (eg, thrombocytopenia in Wiskott–Aldrich syndrome or immune thrombocytopenia in Fanconi anemia[10]) it might be difficult to assess the relative contribution of each cause of cytopenia. The avoidance of irreversible, iatrogenic damage is of utmost importance in these complex situations.

Finally, physicians must consider factors other than the efficacy and target or mechanism specificity of the treatment, including assessing the off-target toxicity (eg, teratogenicity, organ toxicity, additional pharmacologic immunosuppression, reversibility) against the treatment efficacy and costs. Figure 3 presents the various categories of drugs and other therapeutic measures used in cytopenias in IEI against this background.

Clinical case: part 2 of 3

Results of more in-depth immunologic analyses at later time points showed a borderline increased proportion of T-cell receptor α-β-positive CD4- and CD8-negative (double negative) T cells (DNT, 4% to 8% of CD3+; normal <2.5). The disease course was complicated by an episode of severe respiratory problems (chest pain and respiratory distress), which initially led to polypragmatic treatment with antibacterial and antifungal antibiotics and a pause in MMF provision. Ultimately, a biopsy with subsequent histopathological assessment led to the additional diagnosis of granulomatous lymphocytic interstitial lung disease (GLILD; Figure 1). Symptoms and radiographic pathologies improved, but ES recurred; because MMF had been previously well tolerated, it could be successfully reintroduced.

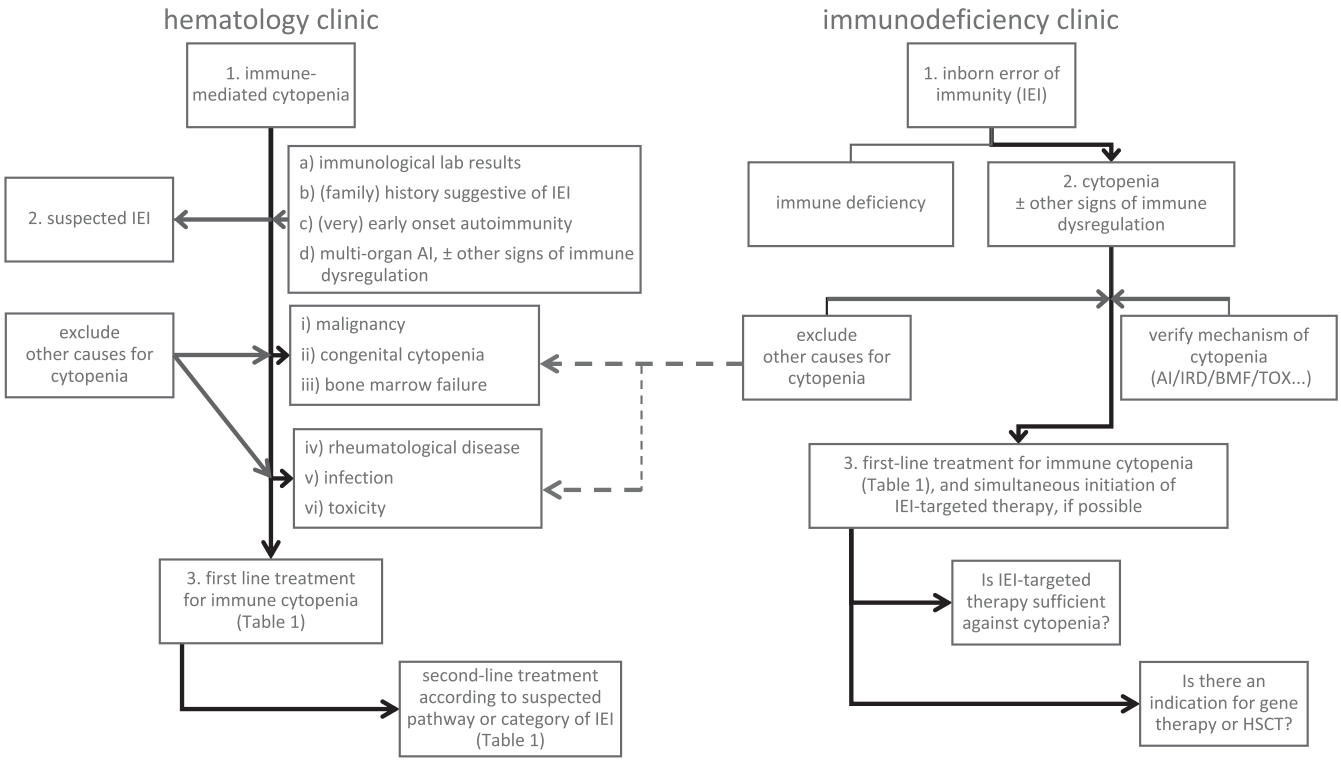

Figure 2. Management algorithm: scenarios of a patient with immune-mediated cytopenia at the hematology clinic without known IEI (left panel) or with a previously known IEI and manifestation of cytopenia at the immunodeficiency clinic (right panel). AI, autoimmune; BMF, bone marrow failure; HSCT, allogeneic hematopoietic stem cell transplantation; IEI, inborn error of immunity; IRD, immune regulatory disorder/immune dysregulation; TOX, infection- or drug-mediated myelotoxicity.

Approach to the treatment decision for SIC in patients with IEIs

Multiple scenarios arise in real life that influence early treatment decisions in this patient cohort (Figure 2).

Scenario 1: a patient with immune cytopenia at the hematology clinic

An underlying IEI is unknown but possible because of either:

1. Abnormal immunological laboratory results
2. Family history of immunodeficiency (infections) or immune dysregulation (autoimmunity, autoinflammation, etc.)
3. Very early onset, relapsing or refractory course
4. Multiorgan autoimmunity or immune dysregulation.

A physician treating any patient with a so-called primary SIC seen at a hematology clinic should subsequently exclude underlying causes in order to more precisely manage the condition. For patients with suspected IEI, various possible pathomechanisms can cause cytopenia, all of which have to be considered when selecting the treatment.[6,8] Autoimmunity in the strict sense, whether it is caused by a primary B-cell pathology, as is seen in common variable immunodeficiency (CVID), or a T-cell maturation/differentiation or functional impairment, as is seen in combined immunodeficiencies (CIDs) or primary immune regulatory disorders (PIRDs), all can result in the failure of tolerance mechanisms and autoreactivity and are usually associated with detectable autoantibodies against blood cells. However, many other features of immune dysregulation may be present in patients with IEI. These other features include hemophagocytosis, lymphoproliferation with subsequent splenic sequestration of blood cells, chronic autoinflammation or complement hyperactivation (eg, in defects of phagocytes or of intrinsic and innate immunity, autoinflammatory syndromes, or complement deficiencies), bone marrow failure, or myelotoxicity in the context of infections or drug therapies.[6,11,12]

If hematologists note the presence of any of the aforementioned risk factors (1-4; Figure 2, left panel, a-d) in a patient with immune cytopenia, they should suspect the existence of an underlying disorder, thus a "secondary" immune cytopenia, and initiate an earlier immunologic and genetic evaluation than in the general population with no detectable underlying cause for immune cytopenia (Figure 2, left panel). GLILD, as detected in the presented patient during the progress of his disease and treatment course, is one of the major, seriously compromising autoimmune and autoinflammatory manifestations in patients with certain IEIs such as CID or CVID.[13] The hematologist is well advised to consult or involve an immunologist at this point. Likewise, if the regular first-line treatment that would be provided to meet international and local standards is unsuccessful for such a patient, a rapid escalation to second-line treatment, potentially targeting IEI-linked pathomechanisms, is recommended (Table 1; Figure 2, left panel).[1,3-5,8]

Although making a definitive diagnosis of the suspected underlying IEI should be assigned first priority, it could take months until the specific IEI is identified. Meanwhile, a semi-targeted treatment that is guided by immune phenotypical clinical and laboratory parameters can be initiated before this diagnosis is achieved. For instance, if the hematologist notes signs

Table 1. Treatment options for severe immune cytopenias[a]

Treatment[a]	AIHA,[b] ES Goal: remission	Immune thrombocytopenia Goals: no risk of hemorrhage, quality of life
First-line options	• Prednisolone 2–5 mg/kg/day days 1–3, then 1–2 mg/kg/day, wean off after 4 wk > 8 wk	• IVIG 0.5–0.8 g/kg according to local standards
		• If Rh+: anti-D (25) 50–75 µg/kg s.c. or i.v.
		• Dexamethasone 5–10 (20) mg/m²/day >3–5 d
Second-line options[c]	• Prednisolone + MMF 1,200 mg/m²/day	• MMF 1,200 mg/m²/day ± prednisolone
	• If DNT ↑: prednisolone + sirolimus 1–2.8 mg/m²/day Trough level 5 (-15) ng/mL (if successful, keep as low as possible)	• If DNT ↑: sirolimus instead of MMF
		• If signs of CID, consider targeted therapy,[d] HSCT
	• If signs of CID, consider targeted therapy,[d] HSCT	◦ Wean off MMF after 6–12 mo > 3–6 mo
	◦ Wean off prednisolone after 4 wk	• TPOR agonists: eltrombopag 25–50 (–75) mg/day (0.8–1.2 mg/kg <6 y) orally or romiplostim 100–250 µg/m²/week s.c.
	◦ Wean off MMF after 6–12 mo > 3–6 mo	
	• Rituximab 375 mg/m² once per week, 4 times (consider prior vaccination against pneumococci, haemophilus influenzae type b, meningococci)	
	• Methylprednisolone 10–30 mg/kg/d > 4 d	
	• Dexamethasone 5–10 mg/m² > 4 d	
Third-line options[c]	• AZT, VCR, bortezomib,[e] danazol,[f] carfilzomib,[e] eculizumab[d] (CAD, PNH), CY, CSA, ibrutinib,[e] daratumumab[e]	• Rituximab, danazol,[f] AZT, VCR, dapson, (retinoids[e])
	• Splenectomy	• Adults: splenectomy (vaccinate before; consider OPSI prophylaxis)
	• HSCT	
Targeted treatment options[c,d]	• If underlying disease is identified or suspected on the basis of specific clinical and laboratory immune phenotypic abnormalities and typical history:	
	• In CID or PIRD: consider indication for allogeneic HSCT (eg, additional severe immunodeficiency, refractoriness of cytopenia to medical treatment, risk of malignancy)	
	• For patients with predominantly antibody deficiencies and IgG replacement therapy: consider B cell-depleting or other B- or plasma cell-directed therapy	
	• In ALPS or conditions with increased DNT cells: sirolimus or MMF	
	• In LRBA or DEF6 deficiency and CTLA4 haploinsufficiency: abatacept, sirolimus	
	• In APDS: PI3Kə/p110 inhibition	
	• In ADA2 deficiency: anti-TNFa therapy for vasculitis, HSCT for refractory cytopenias	
	• In overactivated JAK/STAT signaling: ruxolitinib or other JAK1/2 inhibitors	
	• In PNH, TMA, or TTP: eculizumab and other complement-blocking drugs	

ADA2, adenosine deaminase 2; ALPS, autoimmune lymphoproliferative syndrome; APDS, activated phosphatidylinositol-4, 5-bisphosphate 3-kinase (PI3K) δ syndrome; AZT, azathioprine; CAD, cold agglutinin disease; CSA, cyclosporin A; CTLA4, cytotoxic T lymphocyte antigen 4; CY, cyclophosphamide; DNT ↑, increased proportion of T cell receptor α-β positive CD4- and CD8-negative (double negative) T cells (ie, >2.5% of CD3+ T cells or >1.5% of total lymphocytes in the background of normal or increased lymphocyte counts); ES, Evans syndrome (defined as at least 2-lineage autoimmune cytopenia); i.v., intravenously; IVIG, intravenous immunoglobulin; JAK/STAT pathway, Janus kinase/signal transducer and activator of transcription signaling pathway; LRBA, lipopolysaccharide-responsive beige-like anchor protein; OPSI, overwhelming post-splenectomy infection; PNH, paroxysmal nocturnal hemoglobinuria; Rh+, Rhesus factor positive; s.c., subcutaneously; TMA, thrombotic microangiopathy; TPOR agonists, thrombopoietin receptor agonists; TTP, thrombotic thrombocytopenic purpura; VCR, vincristine.

[a]These treatment options and lines were collected from various international guidelines and reviews as referenced[1-4,7,8,25,33-37]; they include generic names of off-label drugs and are to be considered as selections that are somewhat biased from the perspective of treating IEIs. This table does not include treatment recommendations for malignancy-associated or post-transplant autoimmune cytopenias.

[b]In this review, AIHA refers to warm autoimmune hemolytic anemia only and does not include cold agglutinin disease or paroxysmal cold hemolytic anemia.

[c]The order depends on the immune or phenotypical abnormality; dosing rules cannot be generally provided for third-line and targeted treatment options, ideally given within clinical studies only; see also Figure 3.

[d]Selection.

[e]This is based on largely anecdotal evidence, ideally given in clinical studies only.

[f]This is used especially in bone marrow failure syndromes, eg, Fanconi anemia or telomere biology disorders but historically also in other conditions (eg, "primary" ES).

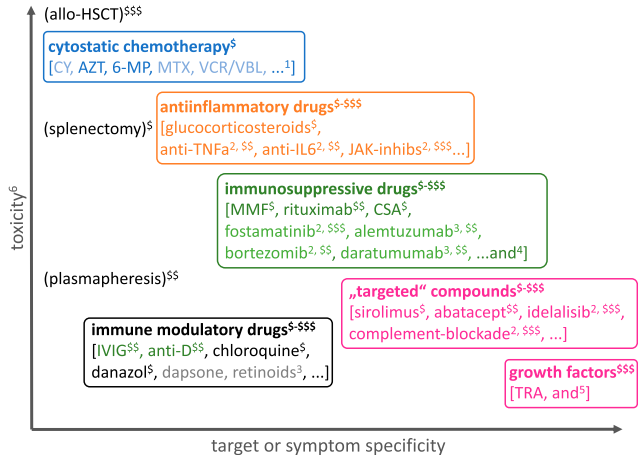

Figure 3. Classification of drugs used for the treatment of immune-mediated cytopenia according to their mechanism of action, toxicity, target, or symptom specificity and treatment costs. The large overlap of some of these subgroups (especially of anti-inflammatory drugs, immunosuppressive drugs, "targeted" drugs) is not shown for clarity. 6-MP, 6-mercaptopurine; anti-D, anti-Rh(D) antibody; AZT, azathioprine; CSA, cyclosporin A; CY, cyclophosphamide; IVIG, high-dose intravenous immunoglobulin G; JAK-inhibs, inhibitors of Janus kinases; MMF, mycophenolate mofetil; MTX, methotrexate; TNFa, tumor necrosis factor α; TRA, thrombopoietin receptor agonists; VCR/VBL, vinca alkaloids. [1]Rarely used; etoposide in hemophagocytic lymphohistiocytosis; [2]use in strictly defined indications; [3]use based on largely anecdotal evidence, ideally under clinical trial conditions or compassionate use with informed consent; [4]ibrutinib (targeting BTK, ITK), belimumab (anti-BAFF), epratuzumab (anti-CD22), carfilzomib (proteasome inhibitor); [5]G-CSF in severe aplastic anemia, CXCR4 inhibition in myelokathexis, rarely erythropoietin analogs; [6]the overall toxicity is difficult to measure, taking teratogenicity, myelo- and organ toxicity, immunosuppression, reversibility, and procedural risks into account, and is schematically illustrated here without considering individual risk factors (intolerance and acquired or inherited risk factors, eg, for thromboembolism or allergic reactions) or potential specific adverse effects; $-$$$a very rough estimate of procurement and treatment costs over repeated or continuous application is presented from "budget" ($, ≤US$1500/year) to high ($$$, ≥US$15 000-20 000/year).

of predominant antibody deficiency and impaired B-cell maturation and subset distribution, a decision to choose a B-cell–directed strategy might be more rapidly made. Alternatively, if the hematologist detects an increased proportion of DNT cells, sirolimus and MMF might be considered as preferred therapies (Table 1). Many IEIs that may manifest with autoimmune cytopenias share some immune phenotypical abnormalities, such as a phenotype of exhaustion and senescence in certain lymphocyte subsets,[14-17] but they may be distinguished from one another by specific parameters, even before a genetic diagnosis can be made. A prospective study is ongoing in which researchers strive to identify biomarkers that predict treatment responses among patients with these immediately available immune phenotypical abnormalities and analyze their transcriptome and epigenetic modifiers (www.sic-reg.org).[18]

Clinical case: part 3 of 3

Repeated attempts to reduce the MMF dosage over the years of the patient's disease course were unsuccessful. A genetic analysis that became available later in the patient's course revealed a heterozygous pathogenic variant in CTLA4 (c.2223C>T; p.R75W; ENST00000302823). On the basis of this (in the meantime known) CTLA4 haploinsufficiency, a "semitargeted" treatment attempt with hydroxychloroquine and sirolimus was made, omitting MMF temporarily (Figure 1). However, because pancytopenia recurred, MMF was reinitiated, again being successful as monotherapy. Finally, the patient was transitioned to adult hematology with mild to moderate thrombocytopenia and good partial remission from GLILD (only small residual opacities on chest computed tomography without lung function impairment) while still receiving low-dose MMF treatment (15 mg/kg/d). In future situations of deterioration, a targeted treatment using abatacept would be indicated, and in severe cases of CTLA4 insufficiency, even hematopoietic stem cell transplantation (HSCT) remains an option (Figure 1, dashed/shaded boxes).[19]

Scenario 2: a patient with cytopenia at the immunodeficiency clinic

A defined IEI is known:

1. With a targetable pathway (many monogenic IEIs, eg, CTLA4 insufficiency)
2. Without a targetable pathway (eg, CVID not further specified)

If a monogenic IEI is known (a), the therapeutic priority should be to treat the underlying disease (Figure 2, right panel). However, autoimmune cytopenia may present as an emergency situation, and not every targeted drug acts immediately; a preparative treatment of eventual HSCT also does not cure autoimmune cytopenia effectively and rapidly. Therefore, the sequence of treatment steps and bridging strategies play important roles for cytopenias in IEI. The treating immunologist should consider involving hematologists as soon as possible so they can work in close cooperation. For instance, in LRBA deficiency, abatacept may be a highly effective treatment of many autoimmune phenomena, restoring the regulatory T-cell defect in part, but the onset of its effect can be delayed for days or weeks. Thus, a short course of glucocorticoids or other first-line treatment measures may be needed to treat AIHA or ES in this context, even if a targeted drug is available (Table 1). Similarly, MMF or sirolimus may be considered as steroid-sparing therapies, for which time is needed to reach effective plasma concentrations and exploit their full therapeutic potential.

In certain situations, targeted drugs only ameliorate some of the features of an IEI, and cytopenia may arise as new manifestation in a patient despite the fact that he or she is already receiving targeted treatment. Physicians should choose drug combinations that target both the underlying pathway and the suspected mechanism of the immune-mediated cytopenia on an individual basis (eg, a combination of abatacept with sirolimus for a patient with LRBA deficiency). Likewise, a study of patients with a transplant indication with IEI and immune dysregulation showed that those who displayed reduced disease activity before HSCT had significantly better outcomes.[20] This reduction in disease activity can be achieved by inducing at least a partial remission with targeted anti-inflammatory and immunosuppressive

pretreatment.[20] If the molecular target or impaired signaling pathway is unknown (b), categorizing the primary immunodeficiency according to clinical and laboratory immune phenotype parameters and classifications[11,21,22] may help guide treatment decisions, as delineated in Table 1. Close cooperation between the immunologist and the hematologist is recommended.

Scenario 3: reevaluation of a patient with recurring cytopenia in the hematology or immunology clinic

The patient had received lines of treatment previously and had mixed responses.

1. Which earlier therapies were partially successful? Were the algorithms for the treatment of immune cytopenias in the general population followed?
2. Is there any newly detectable parameter that points toward a possible treatment target (eg, DNT cells, hypogammaglobulinemia, naive T and B cells, exhaustion and senescence markers; see Table 1)?
3. Is there any historical, clinical, or earlier laboratory parameter that points toward a different differential diagnosis other than an autoimmune condition (eg, congenital cytopenia, bone marrow failure or malignancy, splenic sequestration, hemophagocytosis, chronic infection, toxic drug exposure)? Was the evolution of bone marrow failure or of a clonal disease excluded?
4. The combination of a refractory nature of autoimmune cytopenia and severe additional immunological phenotypes or risk of malignancy (if inherent in the respective IEI) should prompt consideration of evaluating for HSCT.

In this scenario, the expert immunologist is already cooperating effectively with the expert hematologist to provide patient care. It is always warranted to repeat the evaluation and perform a thorough differential diagnostic workup to exclude other causes for patients with autoimmune cytopenias, especially in refractory or frequently recurring situations and where a monogenic cause cannot be identified or has been insufficiently described. For example, many IEIs do not exclude but rather increase the risk of malignancy or bone marrow failure as an underlying cause of cytopenia, such as in a deficiency of CTLA4, ADA2, GATA2, or dyskeratosis congenita.[23-28] Furthermore, and independent of his or her IEI, a patient with CVID may suffer from an additional trait linked to congenital, mild thrombocytopenia that is aggravated by CVID-linked splenomegaly. Therefore, composite mechanisms that contribute to the manifestation and extent of cytopenia must be considered for patients with IEI; this can ideally be achieved by a close cooperation between hematologists and immunologists.

Scenario 4: autoimmune cytopenia newly arising in a patient at the transplant clinic or ward

Autoimmune cytopenias for patients with secondary immunodeficiency, such as after allogeneic HSCT or after organ transplantation, are among the most difficult to diagnose and treat. Recent studies and reviews that address this topic specifically[29-31] provide more in-depth guidelines than this present work focusing on SIC in primary immunodeficiencies. In brief, autoimmune cytopenias in secondary immunodeficiencies such as post-transplantation settings may be caused by a complex mix of underlying pathomechanisms such as imbalanced immune reconstitution with preponderance or insufficient elimination of autoreactive T- or B-cell clones, calcineurin inhibitor–facilitated skewing of the immune system, drug-induced thrombotic microangiopathy or sinusoidal obstruction syndrome, and virus-triggered conventional autoimmune reactions, to list a few. Their manifestation probably will warrant a change of the post-transplant pharmacologic immunosuppressive treatment regimen and soon need escalation toward second- and third-line therapies, including even unconventional measures (eg, combinations of anti-T and anti-B cell–directed interventions, complement inhibition). Of note, mTOR inhibition might be a favorable alternative immunosuppression to switch to in this condition, as the enhancement of regulatory T-cell function was demonstrated under rapamycin.[32]

Conclusions

Immune cytopenias may follow a more complicated course than seen in the general population if they occur in patients with an underlying IEI. Like other features of immune dysregulation, the manifestation of immune cytopenia may precede an increased frequency or severity of infections, actually impeding the early diagnosis of an IEI. These scenarios highlight the explicit reasons hematologists and immunologists should jointly care for this selected patient cohort from the beginning of treatment and onward. Targeted, precise treatment options may exist for selected IEIs, and these options have the potential to cure or control autoimmune cytopenia and reduce off-target adverse effects. To support decision making in the future, prospective studies to define treatment response–predicting or –stratifying biomarkers for patients with autoimmune cytopenias and IEI are needed. Ultimately, a relapsing or refractory course of autoimmune cytopenia observed in patients with an IEI may represent a major indication to proceed to gene therapy or HSCT.

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Off-label drug use

None disclosed.

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Erratum

Sanz GF. In MDS, is higher risk higher reward? *Hematology Am Soc Hematol Educ Program*. 2019;2019:381-390.
Page 381: In the printed edition, the disclosure footnotes incorrectly stated that there were no competing financial interests or discussion of off-label drug use to disclose. The correct footnotes are shown below and were added to the online edition before publication.

Conflict-of-interest disclosure: G.F.S. has received honoraria from and/or played an advisory role for AbbVie, Amgen, Boehringer-Ingelheim, Celgene, Helsinn Healthcare, Hoffmann-La Roche, Janssen-Cilag, Novartis, and Onconova. He works at Hospital Universitario y Politécnico La Fe, which receives research funding from and/or participates in multiple clinical trials funded by different pharmaceutical companies, including AbbVie, Amgen, Boehringer-Ingelheim, Bristol-Myers Squibb, Celgene, Helsinn Healthcare, Hoffmann-La Roche, Janssen-Cilag, Novartis, and Onconova. He is also a member of the Spanish Group on Myelodysplastic Syndromes (Grupo Español de Síndromes Mielodisplásicos), which is sponsored by Celgene and Novartis.
Off-label drug use: Azacitidine is approved in Europe only for higher-risk MDS (IPSS intermediate-2 and high risk categories). Preliminary results for several nonapproved drugs under investigation in clinical trials for patients with MDS are presented and discussed.

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