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Hematological ONCOLOGY

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Articles from the 16th International Conference on Malignant Lymphoma, Lugano, Switzerland
Virtual edition, June 18–22, 2021

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Hematological Oncology

16th International Conference on Malignant Lymphoma
Virtual Edition
18–22 June, 2021

Bayer-Sponsored Satellite Symposium at ICML 2021

The Evolving Treatment Landscape and Emerging Novel Therapies in *Indolent Non-Hodgkin's Lymphoma (iNHL)*

Friday, 18 June 2021 16:00 – 17:30 CET
16th ICML, Virtual Edition

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Current Treatment Landscape and Emerging Therapies in iNHL
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The Evolving Therapeutic Options for Patients With Follicular Lymphoma After First Relapse
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All panelists
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Aims and Scope

Hematological Oncology considers for publication articles dealing with experimental and clinical aspects of neoplastic diseases of the hemopoietic and lymphoid systems and relevant related matters. Translational studies applying basic science to clinical issues are particularly welcomed. Manuscripts dealing with the following areas are encouraged:

- Clinical practice and management of hematological neoplasia, including
  - Acute and chronic leukemias
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  - Myeloproliferative disorders
- Diagnostic investigations, including imaging and laboratory assays
- Epidemiology, pathology and pathobiology of hematological neoplasia
- Therapeutic issues including Phase 1, 2 or 3 trials as well as allogeneic and autologous stem cell transplantation studies
- Aspects of the cell biology, molecular biology, molecular genetics and cytogenetics of normal or diseased hematopoeisis and lymphopoiesis, including stem cells and cytokines and other regulatory systems.

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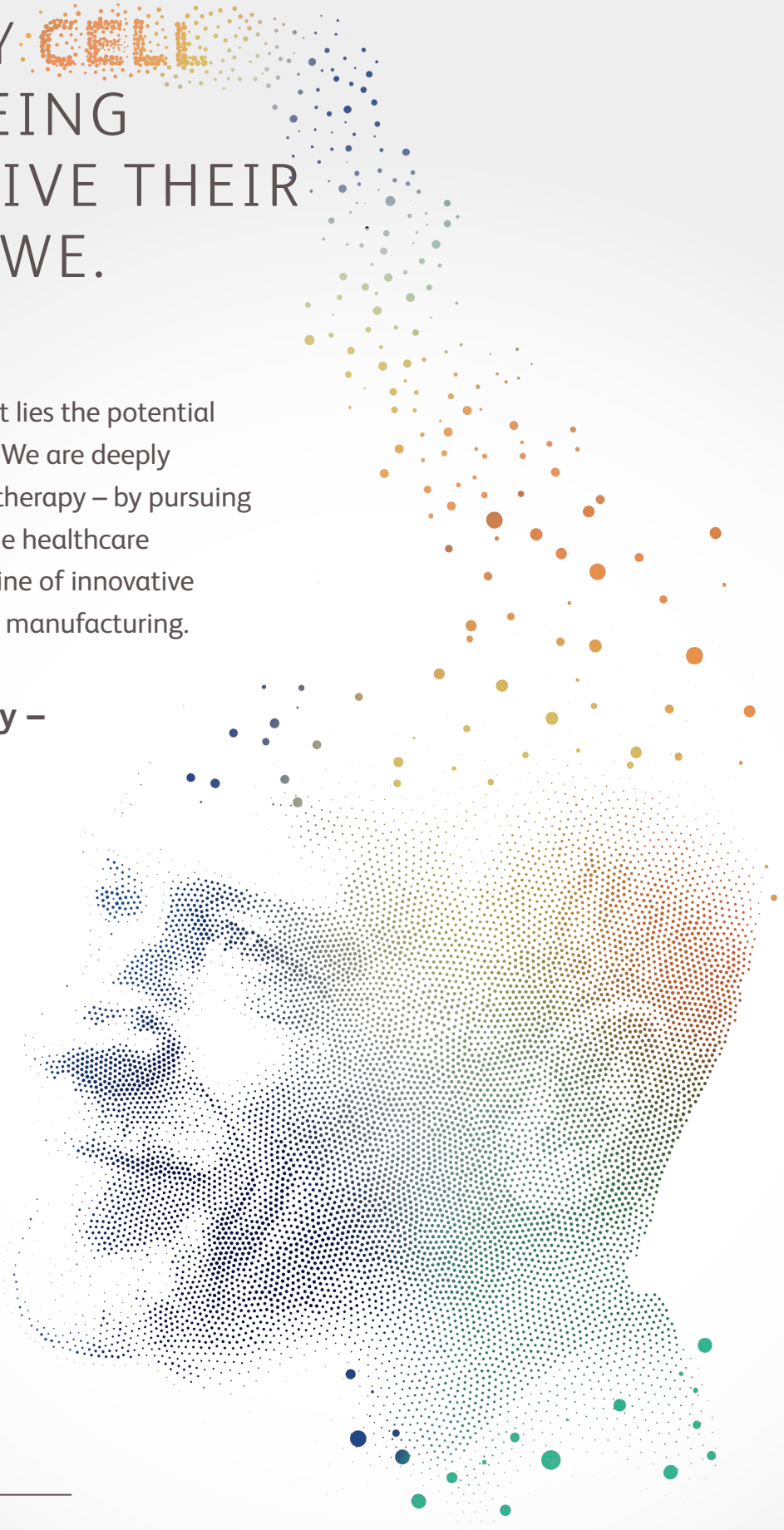

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SUPPLEMENT ARTICLE

16th International Conference on Malignant Lymphoma Virtual Edition 18 - 22 June, 2021

Organized by the Foundation for the Institute of Oncology Research (IOR) in cooperation with the American Association for Cancer Research (AACR)

Note: The articles published in the 16-ICML Educational Book represent the presentations made at the "Meet the Professor" sessions and "Educational Symposia" during the 16th International Conference on Malignant Lymphoma (16-ICML Virtual Edition - Lugano, Switzerland) on June 18-22, 2021.

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SCIENTIFIC PROGRAM - MEET THE PROFESSOR SESSION AND EDUCATIONAL SYMPOSIUM SCHEDULE

All session times for the 16-ICML are Central European Summer Time (CEST)

Saturday, June 19, 2021			
Time	**Channel**	**Session**	**Article/ abstract nr.**
13:00 – 13:30	Channel 1	"Meet the Professor" session	
		What's new in peripheral T-cell lymphomas	EB 06
		Stefano Luminari, Reggio Emilia (Italy)	
13:30 – 13:45		Live Q&A	
13:00 – 13:30	Channel 2	"Meet the Professor" session	
		Cutaneous T-cell lymphoma – an update 2021	EB 05
		Werner Kempf, Zürich (CH)	
13:30 – 13:45		Live Q&A	
13:00 – 13:30	Channel 3	"Meet the Professor" session	
		Personalized medicine for Hodgkin lymphoma: mitigating toxicity while preserving cure	EB 04
		Peter W.M. Johnson, Southampton (UK)	
13:30 – 13:45		Live Q&A	
13:00 – 13:30	Channel 4	"Meet the Professor" session	
		Use of available prognostic scores in treatment decision: beyond standard prognostic scores to include molecular/genetics/imaging	
		John F. Seymour, Melbourne (Australia)	
13:30 – 13:45		Live Q&A	
Sunday, June 20, 2021			
Time	**Channel**	**Session**	**Article/ abstract nr.**
15:00 – 16:30	Channel 1	Educational symposium on High risk follicular lymphoma	
		Co-chairs: Elias Campo, Barcelona (Spain) and Carla Casulo, Rochester, NY (USA)	
15:00 – 15:25		Upfront identification of high risk follicular lymphoma	EB 12
		Carla Casulo, Rochester, NY (USA)	
15:25 – 15:50		Vulnerabilities in the tumor and microenvironment in follicular lymphoma	EB 11
		Elias Campo, Barcelona (Spain)	
15:50 – 16:15		High risk follicular lymphoma: treatment options	EB 10
		Brad Kahl, Saint Louis, MO (USA)	
16:15 – 16:30		Live Q&A	
16:45 – 17:15	Channel 1	"Meet the Professor" session	
		Molecular diagnostics and reporting in lymphoid malignancies: current status and beyond	EB 09
		Richard Rosenquist Brandell, Stockholm (Sweden)	
17:15 – 17:30		Live Q&A	
16:45 – 17:15	Channel 2	"Meet the Professor" session	
		New drugs and pharmacological interactions in real life	EB 10
		Anastasios Stathis, Bellinzona (CH)	
17:15 – 17:30		Live Q&A	
16:45 – 17:15	Channel 3	"Meet the Professor" session	
		Mantle cell lymphoma - advances in molecular biology, prognostication and treatment approaches	EB 03
		Martin Dreyling, Munich (Germany)	

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(Continued)

Sunday, June 20, 2021 Time	Channel	Session	Article/ abstract nr.
17:15 – 17:30		Live Q&A	
16:45 – 17:15	Channel 4	"Meet the Professor" session	
		Recognizing but not harming. Borderline B-cell lymphoid proliferations	EB 07
		Leticia Quintanilla-Martinez de Fend, Tübingen (Germany)	
17:15 – 17:30		Live Q&A	
Monday, June 21, 2021 **Time**	**Channel**	**Session**	**Article/ abstract nr.**
15:00 – 16:30	Channel 1	Educational symposium on Immunotherapy	
		Chair:	
15:00 – 15:25		Optimizing CAR T-cell therapy in lymphoma	EB 15
		Gilles Salles, New York, NY (USA)	
15:25 – 15:50		Allogeneic stem cell transplant in non-Hodgkin lymphomas: still an indication?	EB 14
		Peter Dreger, Heidelberg (Germany)	
15:50 – 16:15		Bi-specific antibodies for the treatment of lymphoma: promises and challenges	EB 16
		Stephen J. Schuster, Philadelphia, PA (USA)	
16:15 – 16:30		Live Q&A	
18:00 – 18:30	Channel 1	"Meet the Professor" session	
		Sequencing of myeloma therapy: finding the right path among many standards	EB 08
		S. Vincent Rajkumar, Rochester, MN (USA)	
18:30 – 18:45		Live Q&A	
18:00 – 18:30	Channel 2	"Meet the Professor" session	
		Langerhans Cell Histiocytosis: Version 2021	EB 01
		Carl E. Allen, Houston, TX (USA)	
18:30 – 18:45		Live Q&A	
18:00 – 18:30	Channel 3	"Meet the Professor" session	
		Molecular classification of aggressive lymphomas – past, present, future	EB 02
		Björn Chapuy, Göttingen (Germany)	
18:30 – 18:45		Live Q&A	

INDUSTRY PROGRAM – SATELLITE SYMPOSIUM SCHEDULE

Friday, June 18, 2021		
12:00 – 13:30	Channel 1	Janssen Oncology Pharmaceutical Companies of Johnson & Johnson
		PRECISION MEDICINE IN LYMPHOMA: WHAT DOES IT MEAN?
		Chair: John Gribben, London (UK)
12:00 – 13:30	Channel 2	AstraZeneca
		EVOLVING STRATEGIES USING BTK INHIBITORS IN CLL: A SELECTIVE APPROACH TO IMPROVE PATIENT OUTCOMES
		Chair: Paolo Ghia, Milan (Italy)
12:00 – 13:30	Channel 3	MSD
		EXPLORING THE UTILITY OF NOVEL TARGETS IN LYMPHOMAS
		Chair: Ulrich Jaeger, Vienna (Austria)
12:00 – 13:30	Channel 4	ADC Therapeutics
		ELEVATE YOUR KNOWLEDGE ON NOVEL ANTIBODY-BASED THERAPIES TO TREAT R/R DLBCL
		Chair: Carmelo Carlo-Stella, Milan (Italy)
14:00 – 15:30	Channel 1	Bristol Myers Squibb
		ARE WE GOING TOWARDS A NEW CARE PARADIGM IN FL AND CLL?
		Chair: Stefano Luminari, Reggio Emilia (Italy)
14:00 – 15:30	Channel 2	Medscape (supported by an independent educational grant from Takeda)
		SPOTLIGHT ON LYMPHOMA: ADDRESSING REAL WORLD CLINICAL CHALLENGES FOR HL AND PTCL
		Moderator: Timothy Illidge, Manchester (UK)
14:00 – 15:30	Channel 3	AbbVie
		NOT ALL BISPECIFIC ANTIBODIES ARE CREATED EQUAL
		Chair: Gilles Salles, New York, NY (USA)
14:00 – 15:30	Channel 4	Pfizer Oncology
		COVID-19 IN CLL AND B CELL MALIGNANCIES
		Chair: Anthony Mato, New York, NY (USA)
16:00 - 17:30	Channel 1	Kite, a Gilead Company
		BUILDING ON SURVIVAL IN CELL THERAPY: FOCUS ON THE FUTURE
		Chair: John Gribben, London (UK)
16:00 - 17:30	Channel 2	Incyte
		PEARLS OF KNOWLEDGE: EXPERT PERSPECTIVES IN THE TREATMENT OF R/R DLBCL
16:00 - 17:30	Channel 3	Regeneron
		EVOLVING TREATMENT PARADIGM FOR RELAPSED/REFRACTORY DIFFUSE LARGE B-CELL LYMPHOMA
		Chair: Pier Luigi Zinzani, Bologna (Italy)
16:00 - 17:30	Channel 4	Bayer
		THE EVOLVING TREATMENT LANDSCAPE AND EMERGING NOVEL THERAPIES IN INDOLENT NON-HODGKIN'S LYMPHOMA (iNHL)
		Chair: Martin Dreyling, Munich (Germany)

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Friday, June 18, 2021		
18:00 – 19:30	Channel 1	Bristol Myers Squibb
		ADVANCEMENTS OF CAR T CELL THERAPIES IN B-CELL MALIGNANCIES
		Chair: David Maloney, Seattle WA (USA)
18:00 – 19:30	Channel 2	F. Hoffmann-La Roche
		UNLOCKING THE FUTURE: SEARCHING FOR A CURE IN DLBCL
		Chair: Georg Hess, Mainz (Germany)
18:00 – 19:30	Channel 3	Lymphoma Hub
		SEQUENCING OF THERAPIES IN HIGH-RISK RELAPSED/REFRACTORY LYMPHOMA AND CLL
		Chair: Gilles Salles, New York, NY (USA)
18:00 – 19:30	Channel 2	Novartis
		EXPERT PERSPECTIVES IN CAR-T: IDENTIFICATION, REFERRAL AND MANAGEMENT OF PATIENTS WITH LYMPHOMA
		Chair: Anna Sureda, Barcelona (Spain)

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SUPPLEMENT ARTICLE

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Langerhans cell histiocytosis: Version 2021

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Abstract

Children with Langerhnans cell histiocytosis (LCH) develop granulomatous lesions with characteristic clonal CD207+ dendritic cells that can arise as single lesions or life-threatening disseminated disease. Despite the wide range of clinical presentations, LCH lesions are histologically indistinguishable based on severity of disease, and uncertain classification as an immune versus neoplastic disorder has historically challenged the development of optimal clinical strategies for patients with LCH. Recently, activating somatic mutations in MAPK pathway genes, most notably *BRAF*V600E, have been discovered in almost all cases of LCH. Further, the stage of myeloid differentiation in which the mutation arises defines the extent of disease and risk of developing LCH-associated neurodegeneration. MAPK activation in LCH precursor cells drives myeloid differentiation, inhibits migration, and inhibits apoptosis, resulting in accumulation of resilient pathologic dendritic cells that recruit and activate T cells. Recurrent somatic mutations in MAPK pathway genes have also been identified in related histiocytic disorders: juvenile xanthogranuloma, Erdheim–Chester disease, and Rosai–Dorfman disease. New insights into pathogenesis support reclassification of these conditions as a myeloid neoplastic disorders. Continued research will uncover opportunities to identify novel targets and inform personalized therapeutic strategies based on cell of origin, somatic mutation, inherited risk factors, and residual disease.

KEYWORDS
Erdheim–Chester disease, histiocytic disorder, juvenile xanthogranuloma, Langerhans cell histiocytosis, Rosai–Dorfman disease

1 | INTRODUCTION

Histiocytic disorders comprise a heterogeneous group of hematologic and immunologic conditions historically classified based on histologic similarities to cells of the mononuclear phagocyte system.[1] However, with the rapidly increasing understanding of mechanisms of pathogenesis and ontogeny, a revised classification is proposed that includes cellular origins, tissue distribution, and molecular lesions along with histologic features (Tables 1 and 2).[2] Langerhans cell histiocytosis (LCH), the most common histiocytic disorder in children, is the focus of this article. We will also briefly describe feature-related disorders: juvenile xanthogranuloma (JXG), Erdheim–Chester disease (ECD), and sinus histiocytosis with massive lymphadenopathy (SHML), also called Rosai–Dorfman disease (RDD).

2 | LANGERHANS CELL HISTIOCYTOSIS

2.1 | Pathophysiology

LCH has captured the attention of physicians and scientists for more than 100 years. Clinical cases initially recognized in the early 1900s in children with unusual constellations of bone and pituitary lesions (Hand–Schüller–Christian disease), aggressive disseminated

TABLE 1 Historical classification of histiocytic disorders

Dendritic cell related
Langerhans cell histiocytosis
Juvenile xanthogranuloma/Erdheim–Chester disease
Macrophage related
Hemophagocytic syndromes
Primary hemophagocytic lymphohistiocytosis
Secondary hemophagocytic syndromes
Rosai–Dorfman disease
Malignant diseases
Monocyte-related leukemias
Extramedullary monocytic tumor (myeloid sarcoma)
Macrophage-related histiocytic sarcoma
Dendritic cell malignancy (malignant histiocytosis)

Note: Adapted from Favara et al.[1]

disease (Letterer–Siwe disease), or isolated or multifocal bone lesions (eosinophilic granuloma). Pathologists in the 1950s noted histologic similarity of biopsies from patients with these conditions and proposed a unifying hypothesis that these clinically distinct syndromes represent a common pathological entity, "Histiocytosis X". Subsequently, Nezelof and colleagues identified Birbeck granules' pathologic histiocytes of LCH lesions (Figure 1), a feature at that time was thought to be shared only with epidermal Langerhans cells.

LCH lesions are granulomatous lesions consisting of pathologic "Langerhans cells" (LCs), lymphocytes (primarily T-cells), eosinophils, and macrophages. Like physiologic epidermal LCs (eLC), LCH lesion LCs express CD1a and CD207 (langerin) surface markers (Figure 1 and Table 3). Common features among LCH and epidermal LC supported hypotheses of LCH as a reactive immune disorder, neoplastic disorder, or some combination of both (reviewed in Allen et al.[4]). In the 1990s, studies of X-inactivation hinted at the clonal nature of LCH lesion LCs. In 2010, Rollins and colleagues made a breakthrough discovery of recurrent somatic *BRAF*V600E mutations in over 50% of LCH lesions.[5] Subsequently, alternative *BRAF* mutations (indels and fusions) and mutations in *MAP2K1* (encoding MEK1) were also been described. Mutually exclusive somatic activating mutations in MAPK pathway genes have now been identified in approximately 85% LCH lesions (Figure 2).[6]

As discussed above, shared histology between epidermal LC and LCH lesion histiocytes prompted updated branding from "Histiocytosis X" to "Langerhans cell histiocytosis". However, gene expression studies comparing LCH lesion CD207+ cells to eLC revealed LCH cells to be relatively less differentiated. Subsequently, high-sensitivity *BRAF*V600E PCR assays identified the mutation in hematopoietic stem cells from bone marrow aspirate and myeloid precursors from peripheral blood of patients with disseminated LCH. Notably, *BRAF*V600E was not identified in peripheral blood mononuclear cells from patients with single *BRAF*V600E+ lesions. Enforced expression of BRAFV600E in langerin + cells in mice resulted in the formation of some limited LCH-like lesions with minimal impact on overall health, but enforced expression in CD11c + myeloid cells drove rapid formation of severe lesions in bone marrow, lung, liver, and spleen resembling high-risk LCH. Pathologic MAPK activation in LCH lesion cells results in up-regulation of anti-apoptotic program (Bcl-xL) and down-regulation of CCR7, which renders the cells trapped in lesions, unable to migrate to draining lymph nodes.[7]

Addressing the decade-long debate of LCH pathogenesis arising from immune dysregulation versus transformation of eLC, findings over the past decade reframe LCH as a myeloid neoplastic disorder arising from myeloid precursors.[4] Together, observations in LCH patients and mouse experiments support a model of "Misguided Myeloid Differentiation," where state of differentiation of myeloid precursor in which activating MAPK mutation arises determines the extent and severity of disease (Figure 3).[8]

TABLE 2 Proposed revised classification of histiocytocytic disorders

L group	LCH
	Intermediate-cell histiocytosis (ICH)
	Erdheim–Chester disease (ECD)
	Mixed LCH/ECD
C group	Cutaneous non-LCH
	-Xanthomatous granuloma (XG) family: includes JXG
	-Non-XG family includes cutaneous RDD
	Cutaneous non-LCH with major systemic component
	-XG family: xanthoma disseminatum
	-Non-XG: multicentric reticulohistiocytosis
R group	Familial RDD
	Sporadic RDD
	-Classic RDD
	-Extranodal RDD
	-RDD with neoplasia or immune disease
	-Unclassified
M group	Primary malignant histiocytosis
	Secondary malignant histiocytosis
H group	Primary HLH
	Secondary HLH (non-Mendelian)
	HLH of unknown/uncertain origin

Note: Adapted from Emile et al.[2]

Abbreviations: ECD, Erdheim–Chester disease; HLH, hemophagocytic lymphohistiocytosis; JXG, juvenile xanthogranuloma; LCH, Langerhans cell histiocytosis; RDD, Rosai–Dorfman disease.

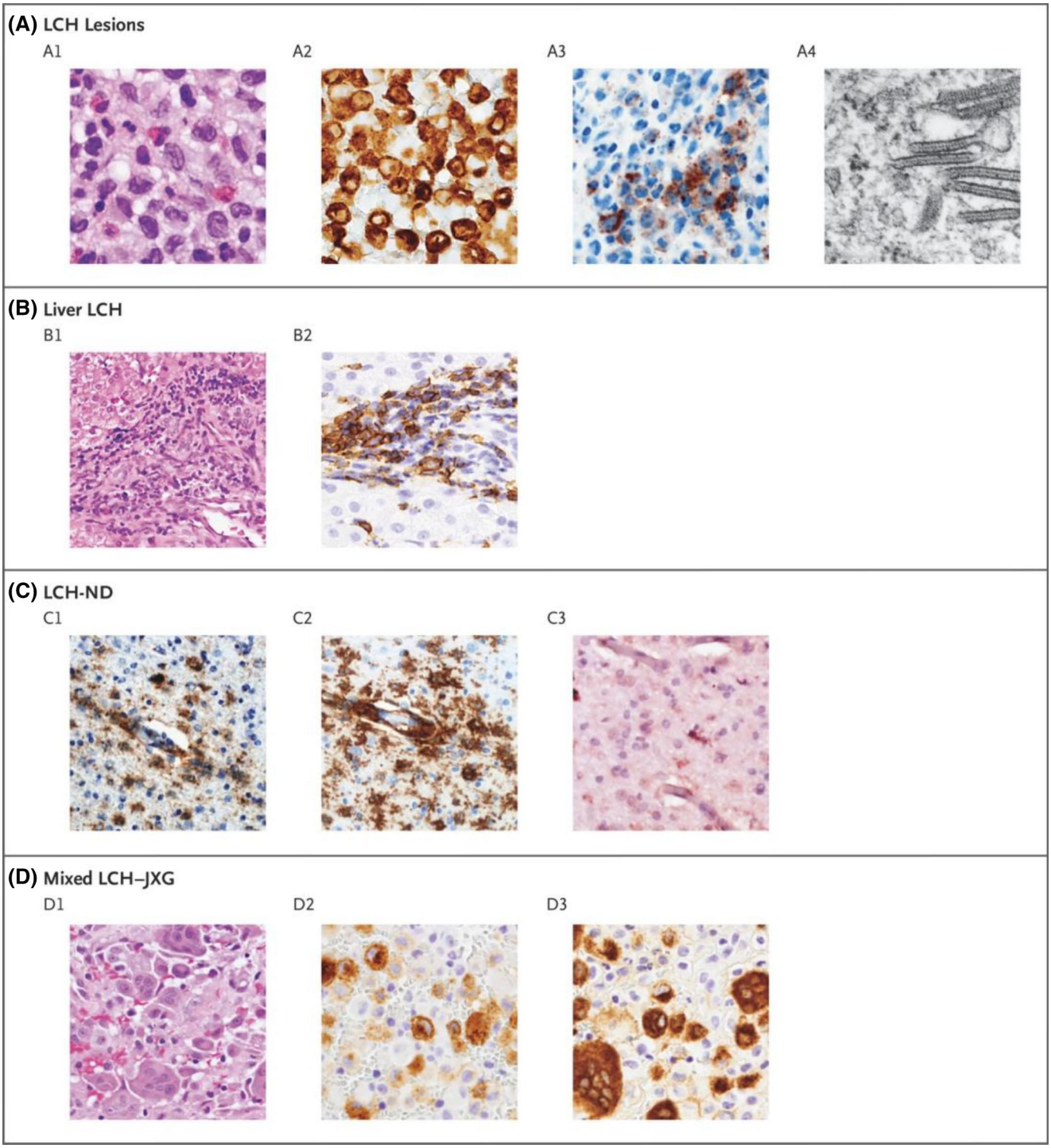

FIGURE 1 Histologic features of Langerhans cell histiocytosis (LCH). Panel A shows typical LCH lesions with large cells, pale cytoplasm, and reniform nuclei on hematoxylin and eosin staining (A1); CD207-positive immunostaining (A2); VE1-positive immunostaining for BRAF V600E protein (A3); and Birbeck granules visualized with electron microscopy (A4). Panel B shows liver involvement, which is frequently characterized by periportal infiltration by histiocytes (B1) and variable CD207-positive staining (B2). Panel C shows biopsy specimens from a patient with severe LCH-associated neurodegeneration (LCH-ND), characterized by perivascular VE1-positive staining (C1), CD163-positive staining (C2), and a P2RY12 infiltrate with occasional P2RY12-positive, tissue-resident microglia (C3). Panel D shows histiocytic lesions that are characteristic of both LCH and juvenile xanthogranuloma (JXG), with heterogeneous histologic features on hematoxylin and eosin staining (D1), including distinct cell populations that are CD207-positive (D2) and CD68-positive (D3) (from *NEJM*, LCH, 379:856–868[3]; Copyright ©2018 Massachusetts Medical Society. Reprinted with permission). JXG, juvenile xanthogranuloma

TABLE 3 Histologic features of histiocytic disorders

	LCH	ECD/JXG	RDD
HLA-DR	++	−	+
CD1a	++	−	−
CD14	+/−	++	++
CD68	+/−	++	++
CD163	−	+	++
CD 207 (Langerin)	+++	−	−
Factor XIIIa	−	++	−
Fascin	−	++	+
Birbeck granules	+	−	−
Hemophagocytosis	+/−	−	−
Emperiopolesis	−	−	+

Note: Adapted from Jaffe.[3]

Abbreviations: ECD, Erdheim–Chester disease; HLH, hemophagocytic lymphohistiocytosis; JXG, juvenile xanthogranuloma; LCH, Langerhans cell histiocytosis; RDD, Rosai–Dorfman disease.

2.2 | Epidemiology

The incidence of LCH is estimated to be approximately 5–10 cases per million children per year and 1–2 cases per million adults per year with male-to-female ratio 1.2:1. Registry studies report an increased incidence in Hispanic populations and rare occurrence of LCH in children with African ancestry.[9] Notably, a GWAS trio study that identified an increased risk of LCH in patients with a germline *SMAD6* variant, which is enriched in Hispanic populations.[10]

2.3 | Clinical presentation and diagnostic workup

LCH has a wide range of clinical manifestations that can be difficult to recognize due to overlap with more common conditions (Figure 4). However, once LCH is considered, diagnosis is fairly straightforward with biopsy. A stepwise approach to diagnosis and staging is outlined in Table 4. Characteristic presentations include lytic bone lesions (~80% of cases), rash (~20%–40% of cases), soft tissue swelling (often in proximity to bony lesions), external ear drainage, lymph node or thymic enlargement, and gum hypertrophy with premature eruption of teeth. More severe systemic involvement reflected by cytopenias, heptasplenomegaly, and/or impaired liver function portends a higher risk of morbidity and mortality. Based on the findings from Histiocyte Society trials, LCH is clinically divided into "high risk" (liver, spleen, and/or bone marrow involvement) and "low risk" (lesions anywhere else), reflecting relative risk of death.[11] The significance of "risk sites" in adults remains uncertain.[12] Historical nomenclature (e.g., Letterer–Siwe) has been replaced with a more generalized attribution of "LCH" along with description of extent of disease (e.g., low-risk single system; low-risk multisystem; high-risk multisystem).

Beyond risk attribution, some characteristic sites merit discussion. Skin lesions in infants may represent isolated skin disease with potential to spontaneously resolve or a component of more extensive systemic disease requiring chemotherapy. Gastrointestinal tract involvement is rare but can present with severe diarrhea, hematochezia, malabsorption, and hypoproteinemia. Isolated pulmonary involvement is more common in young adults with a history of chronic smoking in the third or fourth decades of life, but is occasionally seen in children and adolescents. Pulmonary involvement may lead to a severe, chronic debilitating course and often presents with spontaneous pneumothorax. Central diabetes insipidus (DI) affects approximately 25% of patients, most commonly seen in children with systemic disease and the orbit and skull involvement. Most cases of DI present with initial systemic disease, but can also present as isolated pituitary disease, or arise as a site of relapse. While posterior pituitary involvement is more common, other endocrine manifestations associated with LCH may include growth hormone deficiency, adrenal insufficiency, hyperprolactinemia, or hypogonadism caused by hypothalamic infiltration of the anterior pituitary gland.[13]

LCH-associated neurodegeneration (LCH-ND) is one of the most severe complications of LCH. LCH-ND may develop with the onset of LCH or several years after the patient has completed therapy and is presumed to be in remission. The presence of "central nervous system (CNS)-risk" bone lesions (orbit, mastoid, maxilla, temporal, sphenoid, zygomatic, and clivus) or pituitary lesions at the time of initial diagnosis is thought to increase the risk of developing LCH-ND. While the true risks of "CNS-risk" require additional investigation, current practice is generally to treat isolated CNS risk lesions with systemic chemotherapy. *BRAF*V600E mutation is also associated with increased risk of LCH-ND. Patients with LCH-ND typically present with prolonged decline in cognitive abilities, worsening school performance, and/or development of cerebellar symptoms. Characteristic magnetic resonance imaging (MRI) findings include T2 hyperintense diffuse or polymorphic lesions involving the white matter of the cerebellum, pons, basal ganglia, and less often the cerebral hemispheres (Figure 4).[14] The etiology of LCH-ND remained uncertain for years, with limited biopsy studies demonstrating lymphocytic infiltration and activated microglia interpreted as a paraneoplastic or autoimmune phenomenon. More recent studies demonstrate microglia-like mononuclear cells at sites of neurodegeneration with BRAFV600E, supporting clonal origin with systemic LCH lesions (Figure 1).[15]

2.4 | Therapy

2.4.1 | Local LCH

Treatment options depend on the site and extent of the disease. Isolated skin lesions sometimes resolve spontaneously, with topical steroids, or with oral immune suppression (e.g., methotrexate or hydroxyurea). Single bone lesions in readily accessible and non-CNS-

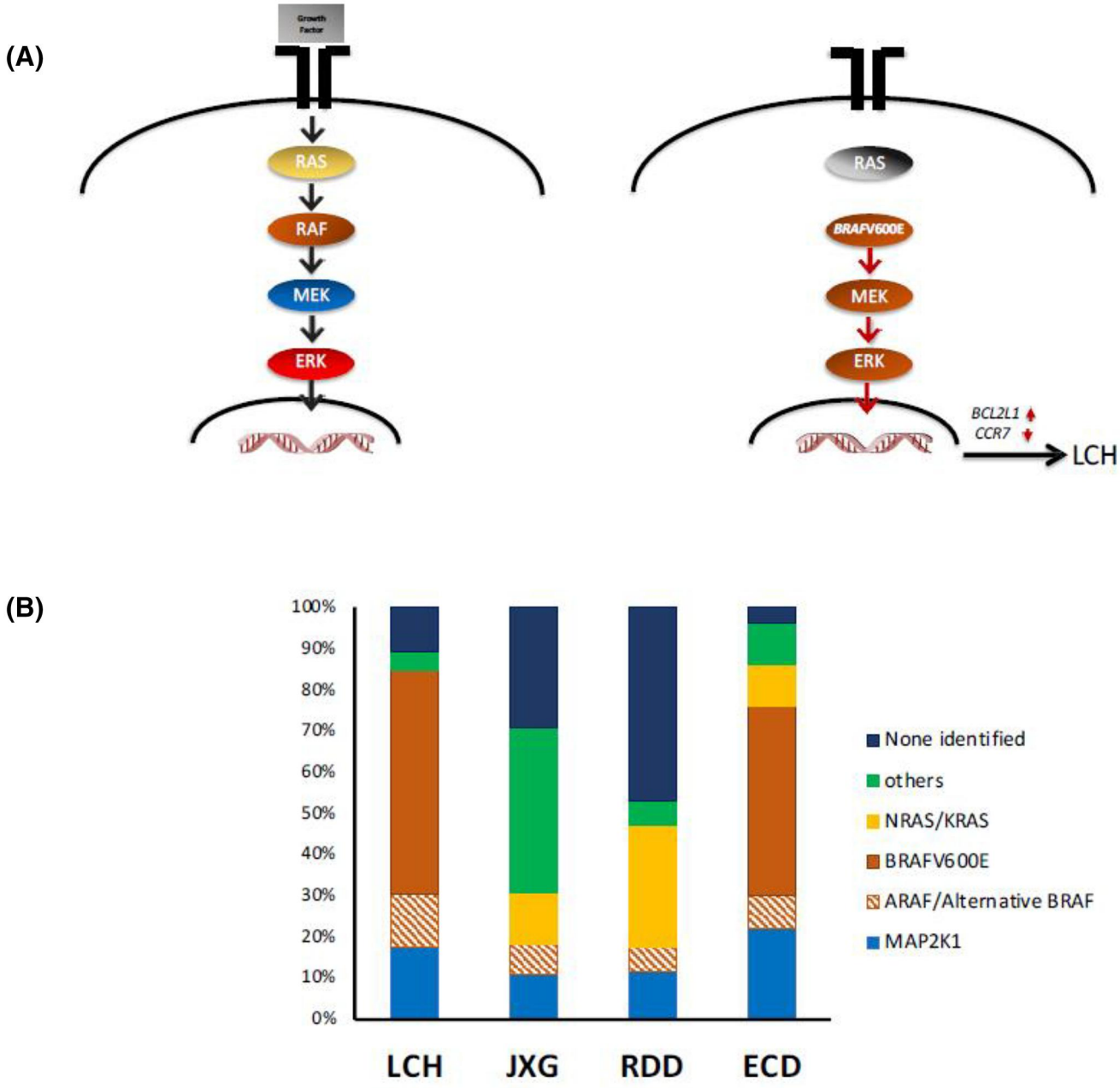

FIGURE 2 MAPK pathway mutations in histiocytic disorders. (A) (left) Schematic of MAPK pathway. Under physiologic conditions, the growth factor (gray box) engages the tyrosine kinase receptor that transduces the signal to the nucleus. (right) Activating mutations (such as BRAF-V600E) drive constitutive ERK activation. In the case of LCH, this drives the expression of anti-apoptosis BCL2L1 (BCL-xL) and inhibits CCR7. (B) Stacked bar graphs represent percentages of MAPK pathway mutations in each histiocytic histologic subtype.

risks sites may be treated with curettage and/or steroid injection. Notably, unlike other pediatric "cancer", LCH does not require complete excision with "clean" margins—in fact, extensive bone resection can impair bone remodeling.

2.4.2 | Frontline therapy

Multiple lesions indicate potential for remote clonal progenitors. Patients with multiple lesions therefore typically require systemic chemotherapy. The current standard of care for initial therapy is vinblastine/prednisone for 1 year (with mercaptopurine added for high-risk LCH), based on the Histiocyte Society LCH-III trial. LCH-III study demonstrated higher rates of progression-free survival (PFS) in patients treated for 1 year versus 6 months (5-year PFS 54% vs. 37%; $p = 0.03$) and no benefit of adding methotrexate for patients with high-risk LCH.[16] Notably, fewer than 50% of patients with high-risk LCH were cured with vinblastine/prednisone. While vinblastine/prednisone may be the current standard, improved strategies are clearly needed. The Histiocyte Society is

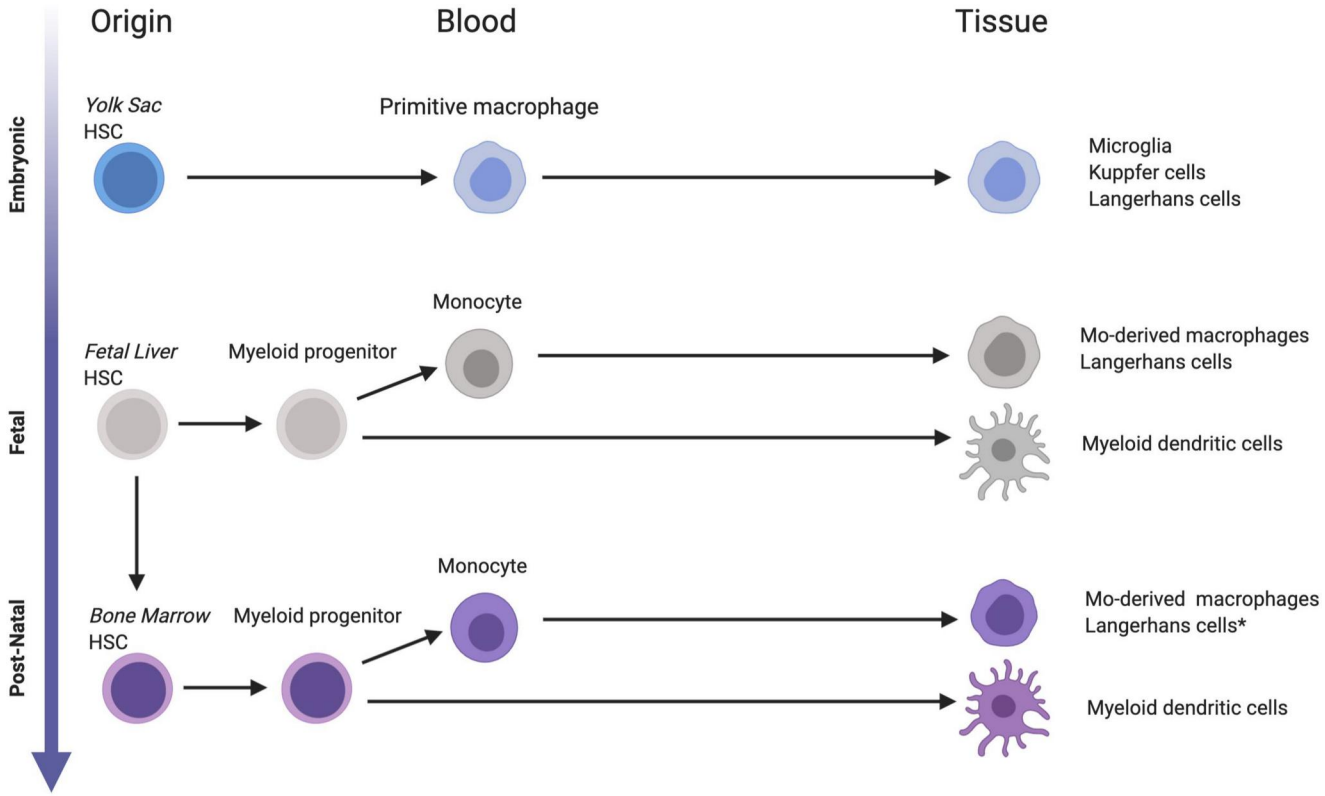

FIGURE 3 Ontogeny of tissue "histiocytes." Tissue macrophages and dendritic cells seed tissues at various stages of development. Microglia, Küpffer cells, and Langerhans cells (LCs) arise from yolk-sac-derived progenitors, followed by fetal liver, then adult hematopoiesis. *Note.* Langerhans cells may be generated from adult HSC monocyte-derived LCs following inflammation or tissue damage. The arrows denotes the sites of LCH lesions

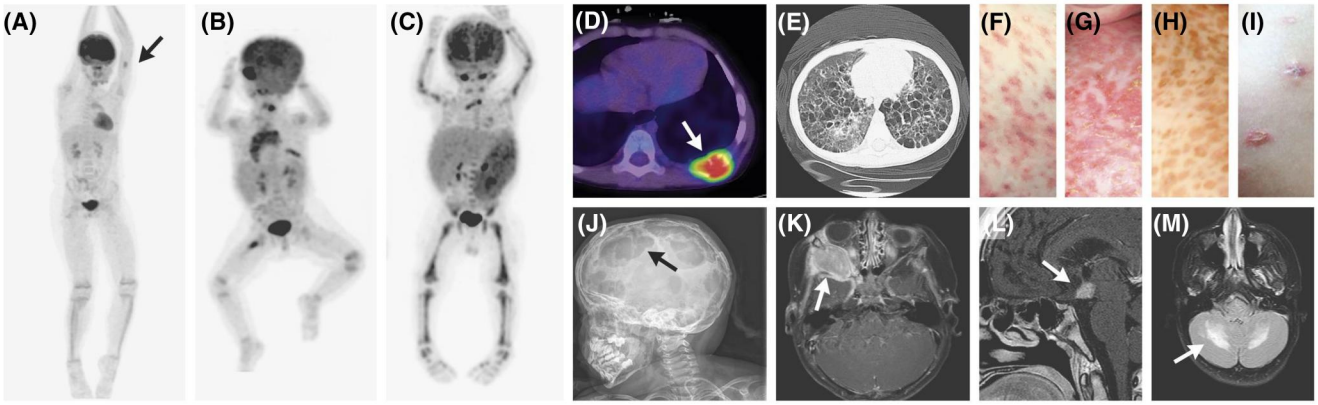

FIGURE 4 Clinical presentations of Langerhans cell histiocytosis (LCH). Positron-emission tomographic (PET) images show a single bone lesion involving the humerus (Panel A, arrow); low-risk lesions involving the orbit, lymph nodes, bone (multifocal lesion), and thymus (Panel B); and high-risk lesions involving the liver, spleen, and bone marrow (Panel C). Other classic presentations include a lytic bone lesion (Panel D, arrow), cystic lung lesions (Panel E), and various skin lesions (Panels F through I). Examples of LCH lesions involving the skull and brain include multifocal skull lesions (Panel J, arrow), an orbital lesion (Panel K, arrow), a pituitary lesion (Panel L, arrow), and LCH-associated neurodegeneration (Panel M, arrow). (from NEJM, LCH, 379:856–8683; Copyright ©2018 Massachusetts Medical Society. Reprinted with permission)

currently testing the impact of further treatment prolongation (2 vs. 1 year with vinblastine/prednisone/(mercaptopurine) for frontline therapy (NCT02205762). Another phase 3 trial is currently randomizing 1 year of vinblastine/prednisone/mercaptopurine versus 1 year of cytarabine monotherapy for frontline LCH (NCT02670707).

TABLE 4 Clinical evaluations for newly diagnosed LCH

Initial evaluation
• History, physical examination
• Laboratory studies a. Complete blood count b. Serum chemistry c. Liver function test d. Sedimentation rate (ESR) e. Lactate dehydrogenase (LDH) f. Serum ferritin g. Immunoglobulin profile h. PT/INR, aPTT (with evidence of liver dysfunction)
• Imaging a. Skeletal survey with two views of chest and four views of the skull (if PET/CT not obtained).
Confirmation of LCH and extent of disease evaluation
• PET/CT • Diagnostic biopsy-excisional biopsy is preferred. Curettage of bone lesions is optimal and complete excision is not required. The presence of abnormal clusters of CD1a+/CD207+ histiocytes are diagnostic (*Note: normal skin and lymph node biopsies may include scattered physiologic CD207+ Langerhans cells*). CD163, fascin, and factor XII help identify mixed histiocytic lesions (such as JXG/LCH, ECD/LCH)
Additional studies(based on lab and/or clinical features)
• All patients <2 years of age, any patients with cytopenias, liver and spleen involvement a. Bilateral bone marrow aspirate and biopsy • Any skull lesions (based on physical exam or lytic lesions on skull x-rays)—CT skull/maxillofacial scans • CNS-risk lesions a. MRI brain with and without contrast for patients with CNS-risk lesions b. For auditory canal or temporal bone involvement, also perform a hearing evaluation c. For clinical suspicion of DI, pituitary dysfunction, or thickened pituitary stalk on MRI brain–urine specific gravity, urine and serum osmolality/water deprivation test. (*Note: If there is an isolated DI and thickened pituitary stalk without any other features suggestive of LCH, perform diagnostic LP for cytology and AFP/B-HCG to rule out germ cell tumor*). d. Other endocrine evaluation as indicated e. Baseline neurocognitive evaluation for patients with DI or evidence of LCH-ND • Spinal cord or vertebral involvement-MRI spine with and without contrast • Pulmonary involvement-CT chest (*chest x-rays and PET/CT may miss small pulmonary nodules, cysts, or thymic involvement*). • Elevated transaminases, elevated direct bilirubin or decreased albumin-abdominal US or MRI • History of malabsorption or hypoalbuminemia-lower GI endoscopy

Abbreviations: DI, diabetes insipidus; ECD, Erdheim–Chester disease; HLH, hemophagocytic lymphohistiocytosis; JXG, juvenile xanthogranuloma; LCH, Langerhans cell histiocytosis; LCH-NH, LCH-associated neurodegeneration; RDD, Rosai–Dorfman disease.

2.4.3 | Salvage therapy

Nucleoside analogs

Optimal approaches for patients who have relapsed or refractory LCH following frontline therapy have not been established. In two phase 2 studies, high-dose cytarabine/cladribine was effective, but associated with significant treatment-related morbidity and mortality. By comparison, lower-dose cladribine monotherapy induced high response rates, but only 3% of patients were cured after 6 months of therapy. Institutional series support the potential efficacy of intermediate-dose cytarabine or clofarabine monotherapy for relapsed and refractory LCH. While nucleoside analogs are promising, prospective trials are needed to determine optimal agent(s), dose, and duration. Allogeneic hematopoietic cell may also be curative, but associated with >25% mortality (reviewed in Rodriguez-Galindo and Allen[13]).

Targeted therapy

New concepts of LCH pathogenesis offer an opportunity to move the treatment for LCH beyond empiricism to rational strategies. Early adult trials have reported extremely high response rates to MAPK pathway-directed targeted therapy with BRAFV600E or MEK inhibition for LCH and ECD.[17,18] Retrospective series report similar findings for children with LCH. However, despite high response rates, MAPK pathway inhibition does not appear to be curative.[19,20] Additionally, duration of therapy, durability of response, and potential for re-response after stopping the medication, and patterns of response/resistance are unknown. In LCH patients treated with MAPK pathway inhibitors, PBMCs with *BRAF*-V600E (presumed precursors) do not consistently clear from circulation, even in patients with complete clinical responses, and patient almost universally relapsed with cessation of therapy.[19,20] Thus, there is an urgent need to improve therapeutic strategies (e.g., MAPK inhibitors with

chemotherapy or anti-apoptotic agents or epigenetic modifiers) to safely cure patients with LCH and related disorders.

2.5 | Adult LCH

The natural history of LCH in adults is understudied. Except for the predominance of lung disease, LCH appears to involve the same potential organ distribution as seen in children, though the incidence may be different. For example, pulmonary LCH usually occurs as a single-system disease in patients, 90% of the cases in adults who are heavy chronic smokers. Other differences include a higher incidence of oral and genital mucosa involvement in adults. Whole exome sequencing studies demonstrate higher somatic mutation burden in adult versus pediatric LCH (where median exome mutation is ~1). When LCH arises de novo in adults, it may reflect acquisition of mutation(s) through clonal hematopoiesis, reflected by mixed-phenotype myeloproliferative neoplastic disorders in some patients.

For adults with single LCH lesions, management strategies similar to the pediatric population include curettage (clean margins are not required) with or without intralesional corticosteroids for single-bone lesions. However, there is no standard of care for the management of multisystem disease. Vinblastine/prednisone may have higher toxicity in adult patients, favoring alternatives such as cytarabine monotherapy. Other options include cladribine, clofarabine, hydroxyurea, methotrexate, 6MP, and MAPK pathway inhibitors.[12]

2.6 | Non-Langerhans cell histiocytic disorders

Juvenile xanthogranuloma (JXG) is a histiocytic disorder that shares many features with macrophage histology, and is histologically indistinguishable from ECD (Table 3). It most commonly affects infants and young children with a slight male predominance and presents as one or more "fleshy skin nodules." However, in some patients, it may be systemic (<5% pediatric cases), involving multiple organs including deeper soft tissue, CNS, bone, lung, liver, spleen, pancreas, adrenal glands, intestines, kidneys, lymph nodes, bone marrow, orbit, and heart. The etiology and basis for prevalence in children are not well defined. An association between JXG and neurofibromatosis (types 1 and 2) and juvenile myelomonocytic leukemia and other molecular alterations such as *CSF1R*, *KRAS*, *NRAS*, and *MAP2K1* (rarely *BRAF*V600E) implicates constitutive MAPK activation in pathogenesis (Figure 2).[6]

Erdheim–Chester disease (ECD) typically arises in patients between the ages of 40 and 70, with a male predominance. Typical presentations include xanthelasma on the upper eyelids, skin rash, and bilateral lower limb bone pain. Consensus diagnostic criteria for ECD require the presence of (1) foamy CD68+/CD1a−histiocytes (Table 3), often with admixed inflammation and fibrosis, and (2) radiographic findings of bilateral and symmetric abnormalities in the diaphyseal and metaphyseal regions of the long bones of the legs. More severe manifestations can include cardiopulmonary insufficiency, renal failure due to retroperitoneal/perinephric infiltration, or CNS symptoms such as cerebellar signs, DI, or cognitive dysfunction. Like LCH, ECD is characterized by activating somatic MAPK pathway gene mutations (*BRAF*V600E being the most common in approximately 50% of patients) (Figure 2). ECD historically carried a dismal prognosis that has been significantly improved with MAPK inhibitors. In 2017 vemurafenib was approved for BRAFV600E + ECD; and MEK inhibition has also been reported with high response rates (reviewed in Goyal et al.[21]).

RDD or SHML likely represents a variety of conditions that share pathologic CD68+/CD1a− cells with emperipolesis (trafficking of viable lymphocytes through histiocytes) (Table 3). It can occur either as an isolated disorder or in conjunction with another autoimmune, malignant, or hereditary disease. Chronic, painless, massive cervical lymphadenopathy is the most common presentation. Other nodal areas and extranodal sites (such as skin, upper respiratory mucosa, ocular structures, bones, and CNS) may also be involved. Treatment is variable and depends on the number of involved sites and can range from observation to systemic chemotherapy. MAPK mutations have been reported in RDD lesions, but less reliably than the other histiocytic disorders discussed above (Figure 2) (reviewed in Abla et al.[22]).

3 | CONCLUSIONS

Clinical advances for patients with LCH and related disorders have historically been stalled by undefined mechanisms of pathogenesis. However, accelerated advances over the past decade have defined LCH as an inflammatory myeloid neoplastic disorder with extent of disease determined by the cell of origin in which activating MAPK somatic mutations arise. The challenge we now face is how to translate biological discovery into improved outcomes for children and adults with histiocytic disorders. Continued research on LCH, JXG, ECD, and RDD will uncover opportunities to identify novel targets and inform personalized therapeutic strategies based on cell of origin, somatic mutation, and inherited risk factors.

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CONFLICT OF INTEREST

The authors have no conflicts to report with respect to this manuscript.

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SUPPLEMENT ARTICLE

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Molecular classification of aggressive lymphomas—past, present, future

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Abstract

Aggressive large B-cell lymphomas (LBCLs) represent a frequent but clinically and molecularly heterogeneous group of tumors. Technological advances over the last decades prompted the development of different classification schemas to either sharpen diagnoses, dissect molecular heterogeneity, predict outcome, or identify rational treatment targets. Despite increased diagnostic precision and a noticeably improved molecular understanding of these lymphomas, clinical perspectives of patients largely remain unchanged. Recently, finished comprehensive genomic studies discovered genetically defined LBCL subtypes that predict outcome, provide insight into lymphomagenesis, and suggest rational therapies with the hope of generating patient-tailored treatments with increased perspective for patients in greatest need. Current and future efforts integrate multiomics studies and/or leverage single-cell technologies and will provide us with an even more fine-grained picture of LBCL biology. Here, we highlight examples of how high-throughput technologies aided in a better molecular understanding of LBCLs and provide examples of how to select rationally designed targeted treatment approaches that might personalize LBCL treatment and eventually improve patients' perspective in the near future.

KEYWORDS
biomarker, diffuse large B-cell lymphoma, high-grade B-cell lymphoma, immune escape, molecular classification, precision medicine, primary central nervous system, primary mediastinal large B-cell lymphoma, primary testicular lymphoma

1 | CAPTURING MOLECULAR HETEROGENEITY OF AGGRESSIVE B-CELL LYMPHOMA

Large B-cell lymphomas (LBCLs) represents a clinically and molecularly heterogeneous group of aggressive non-Hodgkin lymphomas that largely arise from antigen exposed B cells and include primary mediastinal large B-cell lymphoma (PMBL), primary central nervous system lymphoma (PCNSL), primary testicular lymphoma (PTL), and diffuse large B-cell lymphoma (DLBCL).[1] Of those, DLBCL often involves multiple nodal and extranodal sites, while PMBL, PCNSL, and PTL present as localized masses in extranodal sites (Figure 1). Over the last decade, the molecular heterogeneity of these tumors was captured in various classification schemes that (i) improved the accuracy of diagnosis; (ii) identified relevant molecular subtypes; (iii) allowed the development of prognostic and/or predictive markers; and (iv) provided insight into the associated biology.

Technological advances in recent years have the allowed detection and prioritization of all genetic alterations types—recurrent mutations, somatic copy number alterations (SCNAs) and structural variants (SVs)—followed by integration and assessment of

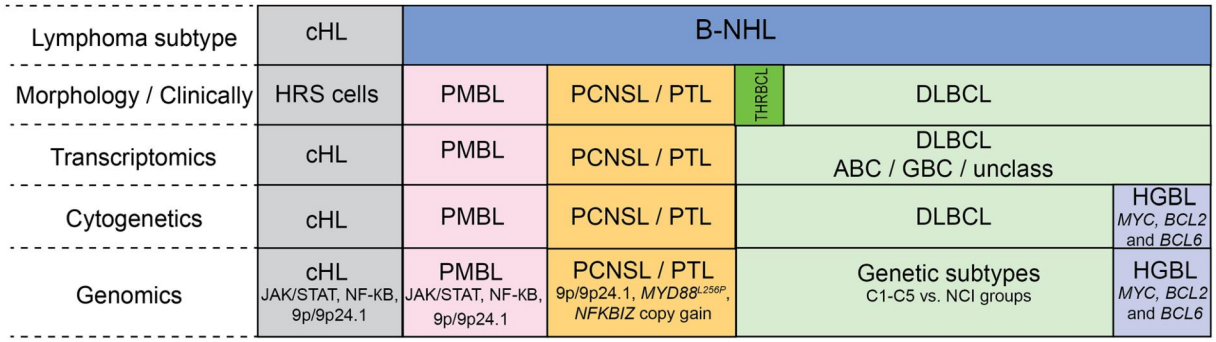


FIGURE 1 Aggressive lymphoma is classified into different entities and subtypes based on morphology/clinically, transcriptomic, cytogenetic, and genomic features

their temporal ordering and associated transcriptional profiles. These comprehensive genomic profiles at base-pair resolution of hundreds of patients diagnosed with primary LBCL has captured the complex intrinsic genetic heterogeneity of these lymphomas, highlighted similarities and differences across entities, identified previously not defined molecular subtypes, provided better insight into the underlying biology, led to the discovery of rational-targeted therapies and might be used to stratify patients for treatments (Figure 1).

2 | PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMAS

PMBL is a rare LBCL that predominantly occurs in young women and was initially categorized as a morphological subtype of DLBCL.[1] Transcriptional profiling of PMBL revealed reduced expression of B-cell receptor (BCR) signaling components, including surface immunoglobulin, and constitutive activation of Janus kinase (JAK)/signal transducer and activator of transcription (STAT) and nuclear factor-κB (NF-κB) signaling cascades, molecular features reminiscent of classical Hodgkin lymphoma (cHL) (Figure 1).[2–4] The shared clinical, pathomorphological, and molecular features of PMBL and cHL, and differences between PMBL and DLBCL led to the recognition of PMBL as a distinct lymphoma entity.[1]

Subsequent studies have defined genetic mechanisms underlying the activation of NF-κB and JAK/STAT signaling pathways, including the identification of the recurrently amplified region on 9p/9p24.1, which includes *JAK2* and increases JAK/STAT signaling in PMBL.[5–8] Interestingly, the 9p24.1 amplified region also affected the PD-1 ligands, namely *PD-L1 (CD274)* and *PD-L2 (PDCD1LG2)*, providing a genetic mechanism of immune escape in PMBL.[4,5,8] Notably, the coamplification of *PD-L1/PD-L2* and *JAK2* increases the expression of the PD-1 ligands both directly, by increasing their copy number, and indirectly, increases the ligands' expression via JAK/STAT signaling and thereby result in an effective immune escape.[5,9] SVs deregulating *PD-L1* and *PD-L2* by translocation, inversion, or deletion of the 3′-untranslated region of *PD-L1* have also been reported in PMBL.[10] The success of immune checkpoint blockade with PD-1 blocking agents in cHL[11–14] and these striking genetic similarities between PMBL and cHL prompted the clinical evaluation of PD-1 blockade in PMBL. The high response rate of single PD-1 blockade with pembrolizumab in relapsed/refractory PMBL led to rapid FDA approval in 2018 and underscored the importance of characterizing targetable genetic vulnerabilities in this disease and related lymphoma.[15,16] Interestingly not all patients with genetic bases of PD-1 deregulation responded to PD-1 blockade[15,16] which resulted in additional clinical trials that combined PD-1 blockade with other rational targets, such as the anti-CD30 antibody–drug conjugate, brentuximab vedotin, with promising responses and duration.[15]

The clinical heterogeneity in responses to PD-1 blockade of PMBL warranted further molecular understanding and resulted in comprehensive genomic studies that captured somatic mutations, SCNAs and SVs and prioritized candidate cancer genes (CCGs).[17,18] These studies highlighted that CCGs can be perturbed by more than one genetic mechanism and that multiple genetic alterations converge on specific signaling pathways, including the NF-κB and JAK/STAT signaling pathways (Figure 1).[17,18] One of these studies highlighted previously unappreciated aspects of the PMBL genetic signature that was shared with those in cHL, and could be associated with an increased response rate to PD-1-blockade, including frequent genetic alterations affecting the ligands of the PD-1 (*PD-L1/PD-L2*) with increased PD-1 expression, and a relatively high mutational burden, compared to that in other lymphoid and solid cancers.[17,19] Additionally, both PMBLs and cHLs exhibit microsatellite instability and APOBEC mutational signatures that have been associated with a more favorable response to PD-1 blockade.[20,21] On the other side, PMBLs, like cHLs, exhibited multiple genetic bases of perturbed major histocompatibility complex (MHC) class I expression and less frequently decreased MHC class II expression that also impacted the cellular composition of the tumor microenvironment[17] and likely could be associated with reduced response to PD-1 blockade. Importantly, these studies provided a genomic framework to comprehensively assess molecular mechanisms of response and resistance to PD-1 blockade in subsequent studies and provide the basis for additional rational combination therapies.

3 | PRIMARY CENTRAL NERVOUS SYSTEM LYMPHOMA AND PRIMARY TESTICULAR LYMPHOMA

PCNSL and PTL, that both arise in sites previously described as immune sanctuaries, are primary extranodal LBCLs with inferior responses to current empiric treatment regimens.[22,23] Prior studies had focused on individual epigenetic or genetic features and transcriptional profiling had suggested that PCNSL/PTL share similarities to the activated B-cell (ABC) subtype of systemic DLBCL (Figure 1).[24] To gain insight into potential actionable genetic alterations a comprehensive study characterized the genomic landscape of PCNSL and PTL and compared the derived genetic alterations to those in DLBCL and PMBL. That study identified unique combinations of genetic alterations (i.e., genetic signatures) for discrete LBCL subtypes and putative bases for targeted therapy.[8,25] For instance, like PMBL, PCNSL, and PTL exhibited frequent 9p24.1/*PD-L1/PD-L2* genetic alteration,[8] providing a genetic basis of affecting the immune checkpoint pathway (Figure 1). Encouraged by the high response rates in lymphomas with genetic bases of PD-1 deregulation (cHL and PMBL), checkpoint inhibition with PD-1 blocking antibodies is currently being tested clinically.[26] PCNSL and PTL, like DLBCL, frequently exhibited genomic instability, but the molecular mechanisms operating in PTL and PCNSL are distinct from DLBCL and largely caused by high-level copy losses of the tumor-suppressor *CDKN2A*,[8] while in DLBCL several monoallelic alterations in p53 modifiers were observed.[27] Additionally, PCNSL and PTL harbored frequent *MYD88*^{L265P} mutations and *NFKBIZ* copy gains leading to near-uniform oncogenic Toll-like receptor (TLR) activation.[8] Notably, most of the PCNSLs had mutations in the proximal BCR-associated gene, *CD79B*[8,25] similarly to a subset of ABC–DLBCLs (Figure 1).[28–30] This mutational pattern is reminiscent of a discrete molecular subtype of DLBCL that is depending on oncogenic TLR signaling involving an endosomal multiprotein super complex (i.e., My-T-BCR complex).[29–31] The importance of this oncogenic pathway in PCNSL is further substantiated by recent insights that highlighted the role of autoantigens and associated chronic BCR stimulation for the brain tropism of malignant PCNSL B cells.[32,33] Consistently, the clinical evaluation of targeted inhibitors against the BCR- and TLR-signaling pathways, including BTK inhibitors, such as ibrutinib alone or in conjunction with intensive chemoimmunotherapy,[34] or lenalidomide and derivatives are under clinical evaluation.[35]

4 | HIGH GRADE B-CELL LYMPHOMA WITH *MYC* TRANSLOCATION AND/OR *BCL2* TRANSLOCATION AND/OR *BCL6* TRANSLOCATION

In 2016, the World Health Organization (WHO) has recognized a rare but high-grade B-cell lymphoma with *MYC* and *BCL2* and/or *BCL6* rearrangements to be included in a single category (high grade B-cell lymphoma), also known under the prior clinical acronym "double hit"/ "triple hit" lymphoma, and suggested a provisional entity (Figure 1).[1,36] According to WHO guidelines[1,36], all aggressive B-cell tumors with a germinal center B-cell (GCB) phenotype or high-grade morphological features or to cases with more than 40% MYC-expressing cells should be tested by Fluorescence in situ hybridization (FISH) for translocations in *MYC*, *BCL2*, and *BCL6* and in case of double positivity grouped in this categories. Due to different techniques, cut offs and less standardization these tumors are less well defined. Currently, this is an active area of research in which several groups attempt to define standards and capture some of the biology by gene-expression-based or genetic platforms. However, while initial reports suggested an dismal clinical outcome,[37–39] more recent reports suggests that with more frequent testing the outcome becomes less dismal.[29,40,41] A recent multi-institutional intranational retrospective study reanalyzed all patients with this genotype using FISH and highlighted that the negative prognostic impact is only present when *MYC* was juxtaposed to the *IgH* enhancer.[41] This data suggests that in the initial reports were probably based on some selection bias and that a comprehensive characterization of these lymphomas is still needed as it is likely that this category itself reflects a heterogenous group of tumors.

5 | DIFFUSE LARGE B-CELL LYMPHOMA

DLBCL is the most common aggressive LBCL accounting for up to 35% of all lymph node cancers. Although the majority of patients with DLBCL are curable with combination immunochemotherapy consisting of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone (R-CHOP), a substantial fraction of patients develop recurrent or progressive disease that is often fatal. This clinical heterogeneity had inspired decades of researchers to develop clinical and/or molecular classifiers capturing the biology, of which several provide some prognostic guidance and others help to dissect the molecular underpinnings of the disease. Some of the new next generation sequencing (NGS)-based classifications might even pave the way to become the first molecular predictive markers in this disease.

5.1 | Clinical heterogeneity

Clinically, the most widely used risk model is the international prognostic index (IPI), that has been established in the last century,[42] and with minimal changes, that is until today the most robust clinical model used in daily routine.[43] Key criticism of the IPI is the missing link to molecular features that might be amenable to targeted therapy.

5.1.1 | Morphological heterogeneity

Initial attempts to leverage morphological subtypes and explore their power for classification is not extensively used with the exception of recognizing T-cell/histiocyte-rich large B-cell lymphoma (THRBCL).

THRBCL is a morphological subtype, in which the rare malignant DLBCL cell is surrounded by a brisk but ineffective inflammatory background (Figure 1).[1] For that reason, earlier studies explored the genetic similarities between TCRLBCL and nodular lymphocyte predominant Hodgkin lymphoma.[44] Recent works further support a genetic relationship to cHL and PMBL, as TCRLBCL also exhibits frequent PD-1 ligand expression and a genetic bases of PD-1-mediated immune escape.[45,46]

5.1.2 | Transcriptional heterogeneity

Besides recognized morphological subtypes,[1] the heterogeneity of DLBCL is also partially captured in transcriptionally defined subtypes that provide insights into disease pathogenesis and candidate treatment targets.[28,47–51] The cell-of-origin (COO) classification identifies ABC- and GCB-type DLBCLs,[47,52] based on whole transcriptome gene expression profiling and similarities to normal B-cell counterparts (Figure 1).[53] An alternative transcriptional classification subdivides primary DLBCLs into BCR signaling, oxidative phosphorylation, and host response DLBCLs based on unsupervised consensus clustering solely in the space of DLBCLs without comparison to normal tissue (consensus cluster classification; Figure 1).[48] Also this transcriptional classification prompted in subsequent studies the discovery of BCR and metabolic dependencies of the respective DLBCL subsets.[50,51,54] The widely used ABC and GCB distinction had also provided insights into the biological differences with increase baseline NF-κB signaling and an differentiation block towards plasma cells in ABC-type DLBCLs and an epigenetic locking into the germinal center program in GCB DLBCLs (for detailed biology reviews on ABC/GCBs see References 52 and 56). In particular, driven by outcome differences between the two transcriptional phenotypes in some studies (ABC, inferior clinical outcome), a plethora of parsimonious assays including immunohistochemistry (IHC),[55] digital gene expression such as nanostring-based assays[56] or NGS approaches have been developed. Despite numerous issues regarding accuracy and reproducibility of IHC-based assays, it is extensively used in practice and biomarker-driven studies. Thus far, patient stratification for treatment based on transcriptional subtypes either by IHC or gene expression-based stratifications have been unsuccessful. Moreover, although patients with ABC DLBCLs are reported to have less favorable responses to standard therapy than those with GCB DLBCLs,[40,47,57] targeted analyses of select alterations have indicated that there is additional undefined genetic complexity[27,49,58–60] and thus prompted further comprehensive genetic studies.

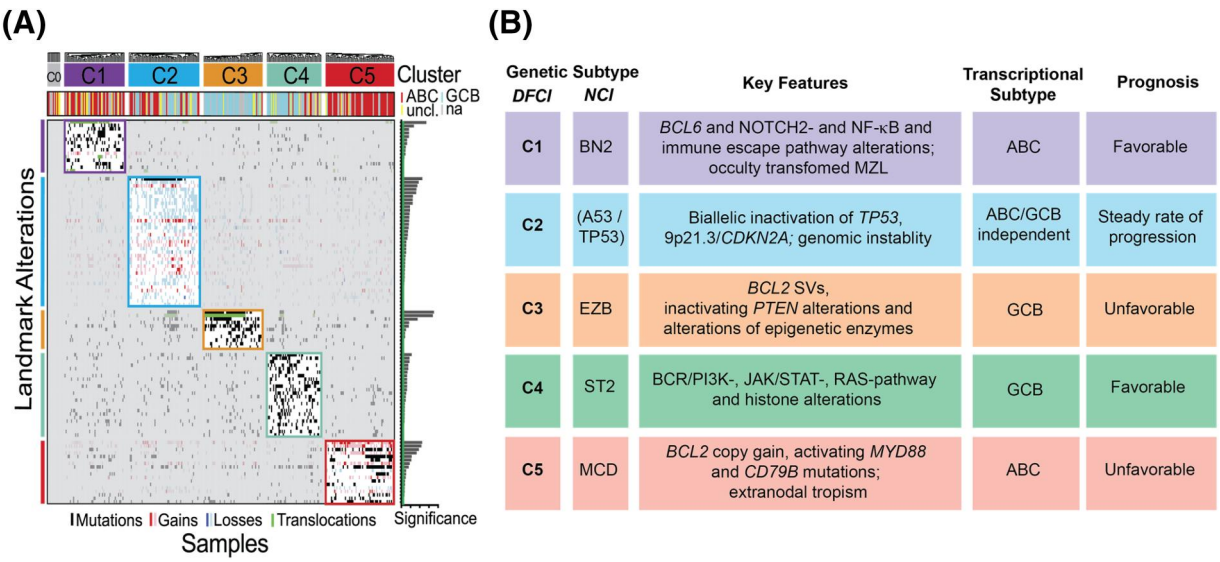
5.1.3 | Genetic heterogeneity of DLBCL

Initial genetic studies on DLBCL were focused on the discovery of single genetic alterations, often in the context of COO-defined transcriptional subtypes.[1,27,61–65] Advances in genome-wide technologies and integrative genomic analysis has allowed the characterization of a more precise picture of the DLBCL genome, but were either limited by focusing on single types of alterations, sample size, clinical annotation, or the lack of data integration.[29,30,40]

To overcome these limitations, we integrated significant genetic alterations composed of recurrent somatic mutations, SCNAs and SVs, and discovered five genetically distinct DLBCL subsets[29] that predicted outcome to state-of-the-art frontline treatment, suggested new insights into the lymphomagenesis of DLBCLs and suggested rational combination treatment (Figure 2). These five subsets included: (1) a high-risk ABC DLBCLs with near-uniform *BCL2* copy gain, frequent activating *MYD88* and *CD79B* mutations and extranodal tropism (C5 DLBCLs); (2) previously unappreciated favorable-risk ABC DLBCLs with genetic features of an extrafollicular, possibly marginal zone origin (C1 DLBCLs); (3) poor-risk GCB-DLBCLs with *BCL2* SVs, inactivating mutations and/or copy loss of *PTEN* and alterations of epigenetic enzymes (C3 DLBCLs); (4) a newly defined group of good-risk GCB DLBCLs with distinct alterations in BCR/phosphoinositide 3-kinase (PI3K), JAK/STAT and BRAF pathway components and multiple histones (C4 DLBCLs); and (5) an ABC/GCB-independent group of tumors with biallelic inactivation of *TP53*, *9p21.3/CDKN2A* and associated genomic instability (C2 DLBCLs).

The biology of these five DLBCL subsets was largely confirmed by an independent nonoverlapping large-scale study that followed a completely different analytical approach and discovered similar groups with shared pathogenetic mechanisms.[30] In particular, C1 DLBCLs[29] were similar to BN2 (*BCL6/NOTCH2*),[30] C3 DLBCLs[29] were similar to EZB (*EZH2/BCL2*)[30] and C5 DLBCLs[29] were similar to MCD (*MYD88/CD79B*) (Figure 2).[30] In subsequent work, the biology of the remaining DLBCL subtypes (C2 and C4 DLBLCs) have recently been also independently validated by two other groups (C4 ~ ST2; C2 ~ A53 |C4 ~ SGK1; C2 ~ TP53) (Figure 2)[66,67]. These studies underscore that DLBCL is a genetically heterogenous disease with at least 5 different molecular subtypes. At the same time, it also highlights, that despite having several hundreds of tumors, we still do not capture the full spectrum of DLBCL subsets and in particular for low-frequency subtypes larger sample sizes are needed.

Importantly, the genetic DLBCL subtypes has provided new insights into the pathogenesis of these tumors with suggestions for novel rational combination therapies and predict outcome.[29] In a proof-of-concept study, it has been demonstrated that in cell line models of C3 DLBCLs the combination of BCL-2 and PI3Kα/δ inhibitors cured 75% of the xenotransplanted mice, underscoring that the new classification is a roadmap to genotype-informed targeted combination therapies.[29,68] More work is needed, to link genetically defined subtypes to actionable vulnerabilities, and eventually build molecular classifiers that link genetics to dependencies with the hope of using them for precision medical trials.



Genetic Subtype *DFCI*	Genetic Subtype *NCI*	Key Features	Transcriptional Subtype	Prognosis
C1	BN2	*BCL6* and NOTCH2- and NF-κB and immune escape pathway alterations; occulty transfomed MZL	ABC	Favorable
C2	(A53 / TP53)	Biallelic inactivation of *TP53*, 9p21.3/*CDKN2A;* genomic instablity	ABC/GCB independent	Steady rate of progression
C3	EZB	*BCL2* SVs, inactivating *PTEN* alterations and alterations of epigenetic enzymes	GCB	Unfavorable
C4	ST2	BCR/PI3K-, JAK/STAT-, RAS-pathway and histone alterations	GCB	Favorable
C5	MCD	*BCL2* copy gain, activating *MYD88* and *CD79B* mutations; extranodal tropism	ABC	Unfavorable

FIGURE 2 Discovery of genetic DLBCL subtypes (A) with associated genetic, transcriptomic and prognostic features (B). DLBCL, diffuse large B-cell lymphoma

6 | OUTLOOK/PERSPECTIVE

After several decades of empirical clinical trial design, we are reaching a ceiling for clinical improvements in unselected patients.[69] For that reason, it is important that technological advances lead to comprehensive genomic studies in informative and large LBCL cohorts, that identify unique genetic signatures for discrete LBCL subtypes. The derived genetic signatures, their temporal ordering and the associated mutational signatures and transcriptional profiles have aided in better understanding of the associated biology and lymphomagenesis, identified prognostic markers of response to standard induction treatment and suggested rationally treatment combinations suited for individual lymphoma subtypes. Importantly, new treatment modalities are quickly moving into the clinics, including molecular subtype agnostic therapies, such as CAR-T cells, antibody–drug conjugates and bi-specific antibodies, but also other subtype-specific (precision medicine) approaches. Comprehensive genomic studies suggest that within one lymphoma entity there are subtypes, which differ genetically as largely as two unrelated cancer types, while at the same time there are shared genetic signatures across entities. This information needs to be appreciated in next-generation clinical trial designs (i.e., basket vs. umbrella trials). Many driver alterations of LBCL subtypes encode for proteins that are readily druggable with small molecule inhibitors or monoclonal antibodies and for LBCL subtypes that are still poorly understood we need to create model systems, perform functional screens (both genetic and pharmacological) and integrate this information with other omics technologies, such as recently suggested with microenvironmental immune signatures.[70] While the last years were focused on bulk genomics, recent studies shed light on the tremendous intratumoral heterogeneity within DLBCL[71] and pave the way for the coming years. Eventually, molecular classifiers have the potential to perform patient stratification in real-time and in clinical settings. We believe that the integration of bulk and single-cell omics technologies with large-scale functional screens will help to select more specific therapies for patients with high-risk subtypes of B-cell lymphoma and thereby hopefully, bring precision medicine for DLBCL to its full potential.

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CONFLICT OF INTERESTS

The authors declare no potential conflict of interests.

PEER REVIEW

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Not relevant.

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SUPPLEMENT ARTICLE

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Mantle cell lymphoma—Advances in molecular biology, prognostication and treatment approaches

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Abstract

Mantle cell lymphoma (MCL) is clinically characterized by its heterogenous behavior with courses ranging from indolent cases that do not require therapy for years to highly aggressive MCL with very limited prognosis. A better understanding of the complex biology of MCL has already led to the approval of several innovative agents, expanding the landscape of MCL therapies and improving therapeutic options especially for refractory or relapsed disease. Nevertheless, to further optimize MCL treatment, early identification of individual risk profile and risk-adapted, patient-tailored choice of therapeutic strategy needs to be prospectively incorporated in clinical patient management. This review highlights recent advances in deciphering the molecular background of MCL, the definition of prognostically relevant factors and the identification of potential druggable targets and summarizes current treatment recommendations for primary and relapsed/refractory MCL including novel targeted therapies.

KEYWORDS
genetics, mantle cell lymphoma, pathogenesis, prognostication, therapy

1 | INTRODUCTION

Mantle cell lymphoma (MCL) is clinically characterized by its heterogenous behavior with courses ranging from indolent cases that do not require therapy for years to highly aggressive MCL with very limited prognosis.[1] Patients typically present with lymphadenopathy of several sites, most of the patients are diagnosed with advanced stage disease (Ann Arbor stage III, IV). Extranodal manifestations occur in 90% of patients, including infiltration of bone marrow (53%–82%), blood (50%), liver (25%), and the gastrointestinal tract (20%–60%).[1,2] The spleen is enlarged in 40% of patients.[1] In some cases, leukemic manifestation in combination with massive splenomegaly is clinically prominent. These nonnodal, leukemic cases are often characterized by a more indolent clinical course. Accordingly, in the WHO 2016 update of lymphoid malignancies, MCL now consists of two distinct categories.[3] Nodal MCL (80%–90% of cases) is characterized by unmutated immunoglobulin heavy chain variable region genes (IGHV), sex-determining region Y-box 11 (SOX11) overexpression and a generally more aggressive clinical behavior. Nonnodal leukemic MCL (10%–20% of cases) typically displays mutated IGHV, SOX11 negativity and presents with indolent biological behavior. Histologically, besides "classical" MCL, pleomorphic and blastoid variants can be distinguished.[3] MCL with blastoid morphology often features high proliferation rates, displaying a more aggressive clinical course.[3,4]

Traditionally, MCL was associated with a poor prognosis with a median overall survival (OS) of 3–5 years. However, major advances in the treatment of MCL patients have been achieved over the last

years, especially by the development of an induction immunochemotherapy including cytarabine and anti-CD20 antibodies and by introducing a consolidation high-dose therapy with autologous stem cell transplantation (ASCT).[5] Moreover, introduction of rituximab as maintenance therapy, especially for those patients not eligible for high-dose therapy, significantly improved survival rates in this group of patients.[6] Yet, long-term prognosis is still limited and patients with relapsed/refractory disease usually have a dismal outcome. Therefore, improved understanding of cellular and molecular biology of MCL and identification of relevant factors determining prognosis to optimally use risk-adapted treatment approaches will be critical to further improve outcomes in this disease.

2 | PATHOGENESIS AND MOLECULAR BIOLOGY

The development of MCL is the result of a complex pathogenetic interplay between cellular and microenvironmental processes. Genetic hallmark of MCL and considered the primary oncogenic event in the pathogenesis is the chromosomal *t*(11;14)(q13;q32) translocation, leading to overexpression of cyclin D1 and dysregulation of cell cycle at the G1–S phase transition.[7] Cyclin D1 negative MCLs usually carry CCND2/CCND3 rearrangements with immunoglobulin genes instead. A subset of cyclin $D1^{-}/D2^{-}/D3^{-}$ MCL with aggressive features has cyclin E dysregulation. In these rare cyclin D1-negative cases, immunohistochemistry for SOX-11 is especially helpful to confirm the diagnosis.

The transcription factor SOX-11 is overexpressed in more than 90% of MCL cases, whereas a leukemic nonnodal variant, resembling chronic lymphocytic leukemia (CLL), lacks SOX-11 expression and is associated with a more indolent course.[8] In this subset of patients with leukemic, nonnodal presentation, SOX-11 expression proved to be prognostically relevant, identifying a favorable outcome in patients with negative SOX-11 with mutated IGHV.

The constitutive activation of the B-cell receptor and its multiple downstream signaling pathways also plays an important role in the development of the disease.[7]

Furthermore, genomic profiling revealed a high number of secondary genetic alterations and recurrent mutations affecting for example regulation of cell cycle, DNA damage response and apoptosis pathways that contribute to the pathogenesis and aggressiveness of MCL.[7] In recent years, next generation sequencing approaches to unravel the genetic background of MCL led to the identification of numerous recurrent somatic mutations including genes involved in genotoxic stress pathways (*ATM*, *TP53*, *CDKN2A*), epigenetic regulators (*WHSC1*, *KMT2D*, *MEF2B*, *KMT2C*, *SMARCA4*, *SMARCB1*) and genes regulating DNA replication (*SAMHD1*), RNA processing (*HNRNPH1*) as well as cell homeostasis, cell growth and cell death (*CCND1*, *TP53*, *CDKN2A*, *CDKN1B*, *BIRC3*, *CARD11*, *TRAF2*, *RB1*, *POT1*, *NOTCH1/2*).[9] Yet, functional relevance remains unclear for most of the mutations and is currently under further investigation.

3 | PROGNOSTIC FACTORS

Important clinical and serological factors, associated with a worse clinical outcome include age, poor general condition, advanced stage of disease (Ann Arbor stage III or IV), splenomegaly and anemia, the serum level of β2-microglobulin and lactate dehydrogenase (LDH), blastoid cytology, extranodal presentation, and constitutional symptoms.

A prognostic score that has been confirmed in numerous series, the MCL International Prognostic Index (MIPI), was established implementing four independent prognostic factors: age, performance status, LDH, and leukocyte count.[10]

Yet, the most important prognostic markers independent of clinical features are the proliferation rate as measured by Ki-67 expression and the expression of p53. These two (p53 high and Ki67 > 30%), together with blastoid morphology, were recently reported to define a high-risk biology with significantly shorter failure-free and OS. Immunohistochemical determination of Ki-67 expression has been prospectively confirmed as a reliable prognostic marker and is, in combination with the MIPI (MIPI-c), a highly recommended tool to estimate individual risk profile and to identify high-risk patients (Ki-67 > 30%) who may qualify for more aggressive therapeutic approaches.[11] Furthermore, a cell proliferation gene signature (MCL35) that distinguishes patient subsets that differ by more than 5 years in median survival has been identified.

Deletions of 17p13 or mutations of *TP53* as well as deletions of *CDKN2A* were reported to be associated with worse clinical outcome in the majority of the studies published. Despite treated with high-dose cytarabine and ASCT, younger MCL patients with deletions of *CDKN2A* (p16) and *TP53* show an unfavorable prognosis. Furthermore, *TP53* mutations were significantly associated with high Ki67 (>30%), blastoid morphology, MIPI high-risk, and inferior responses to both induction- and high-dose chemotherapy.[12]

Other genetic lesions with inferior outcomes include mutations in the *NOTCH* genes and in *KMT2D* as well as MYC alterations and mutations in *WHSC1* and *CCND1*.

Prognostic markers - current and future - are summarized in Table 1.

Concerning the prognostic impact of minimal residual disease (MRD) status, several studies have been published, providing evidence of the strong prognostic potential of MRD status predicting improved subsequent progression-free survival (PFS) for MRD-negative patients at the end of induction and before high-dose consolidation.[5] In addition, lack of molecular remission after end of currently recommended standard treatment was shown to be strongly predictive for early clinical relapse within 1–2 years.[5,13] However, so far use of MRD analysis is restricted to clinical trials. Furthermore, the impact of MRD monitoring in the context of the new-targeted treatments, such as the *Bruton's tyrosine kinase* (BTK) inhibitor ibrutinib, remains unclear.

TABLE 1 Prognostic markers—current and future

In clinical routine	Potential for future use
Age	"MCL35" RNA expression analysis
Performance status	SOX11 expression
Central nervous system involvement at diagnosis	*TP53* mutations/deletions by sequencing analysis
Stage of disease (I and II vs. III and IV)	MRD testing
Serum level of β2-microglobulin and LDH	
Morphology (classic vs. blastoid)	
MIPI	
Ki67 (<30% vs. >30%)	
TP53 expression by immunohistochemistry	

Abbreviations: LDH, lactate dehydrogenase; MIPI, MCL International Prognostic Index; MRD, minimal residual disease.

3.1 | Therapy in patients ≤65 years

3.1.1 | Induction: Dose-intensified, cytarabine-containing regimen

The administration of the R-CHOP/DHAP regimen compared to administration of R-CHOP alone prior to myelo-ablative consolidation with ASCT more than doubled time to treatment failure (TTF) (109 vs. 47 months).[5] We do not recommend an additional routine central nervous system prophylaxis.

Another commonly used treatment approach, predominantly applied in the USA, is the intensive chemo-immunotherapy regimen rituximab in combination with hyper-CVAD. This regimen achieved high complete response (CR) rates and long-term remissions and does not require consolidation with ASCT. However, this regimen is hampered by significant therapy-associated toxicity, including secondary malignancies, and should only be considered in young, fit patients.

3.1.2 | Consolidation: ASCT

In several studies, the addition of high-dose consolidation followed by ASCT resulted in impressive survival rates.[5,14] A large randomized trial proved that consolidation by myeloablative radiochemotherapy followed by ASCT in first remission significantly prolonged PFS (3.3 vs. 1.5 years) and OS[15] independently of the addition of rituximab. Unfortunately, even after such intensive consolidation regimen, a majority of patient's relapse.

3.1.3 | Maintenance

Rituximab maintenance after ASCT is currently considered standard of care for younger patients with MCL based on the results of a large phase III trial showing a significant optimization of PFS (83% vs. 64% after 4 years) and OS (89% vs. 80% after 4 years) after 3 years of rituximab maintenance compared to observation only.[14]

Recently, another phase III trial revealed a benefit from a lenalidomide maintenance after autologous transplantation with improved PFS (80% vs. 64% after 3 years) compared to observation.[16] However, due to the elevated toxicity profile (especially hematotoxicity), lenalidomide maintenance should be only applied to patients not suitable to receive rituximab.

Figure 1 suggests a risk-adapted treatment strategy for patients ≤65 years.

3.2 | Therapy in patients more than 65 years

3.2.1 | Induction

The group of the over 65 year olds ineligible for transplantation presents very heterogenous regarding physical and cognitive performance. A suggested therapeutic algorithm is depicted in Figure 2. Fit patients greater than 65 years should receive conventional immunochemotherapy followed by rituximab maintenance.[6] The VR-CAP regimen recently proved to be superior over R-CHOP in a large International phase III trial with a doubled OS after 82 months (90.7 vs. 45.7 months). However, hematologic toxicity (especially >Grade 3 thrombopenia) was significantly increased in the experimental arm (57% vs. 6%).[17] The R-BAC scheme offers another useful option. Yet, this regimen was accompanied by severe hepatotoxicities and should therefore only be administered to very fit older patients with high-risk features (e.g., blastoid variant, high LDH count).[18] Alternatively, for patients not qualifying for such intensive therapy regimens, R-Bendamustine offers an appropriate alternative. This combination resulted in similar response rates (93% vs. 91%) compared to R-CHOP and was even superior in PFS (35 vs. 21 months) with a more favorable toxicity profile observed.[19] Taken

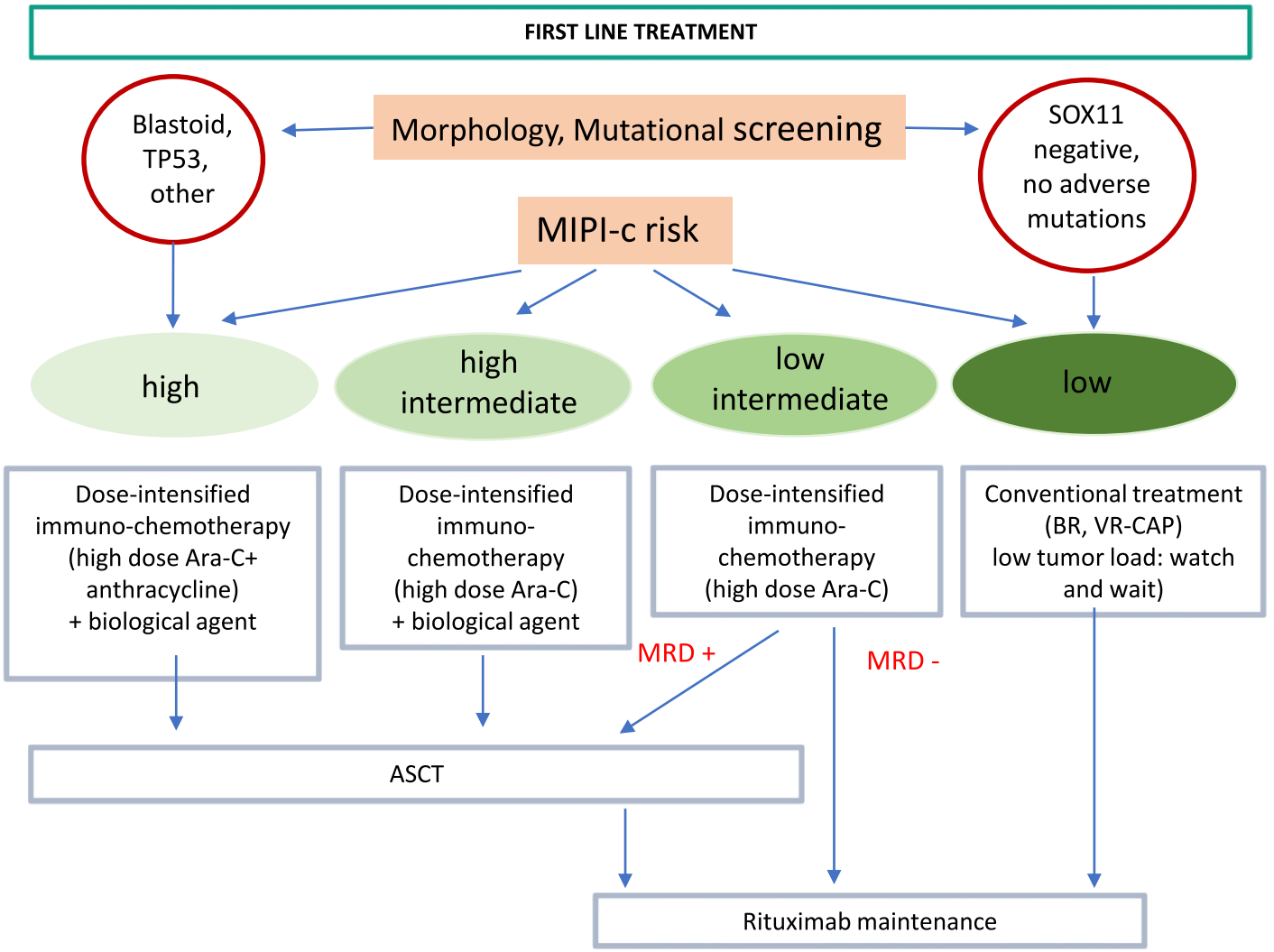

FIGURE 1 Suggested therapeutic algorithm

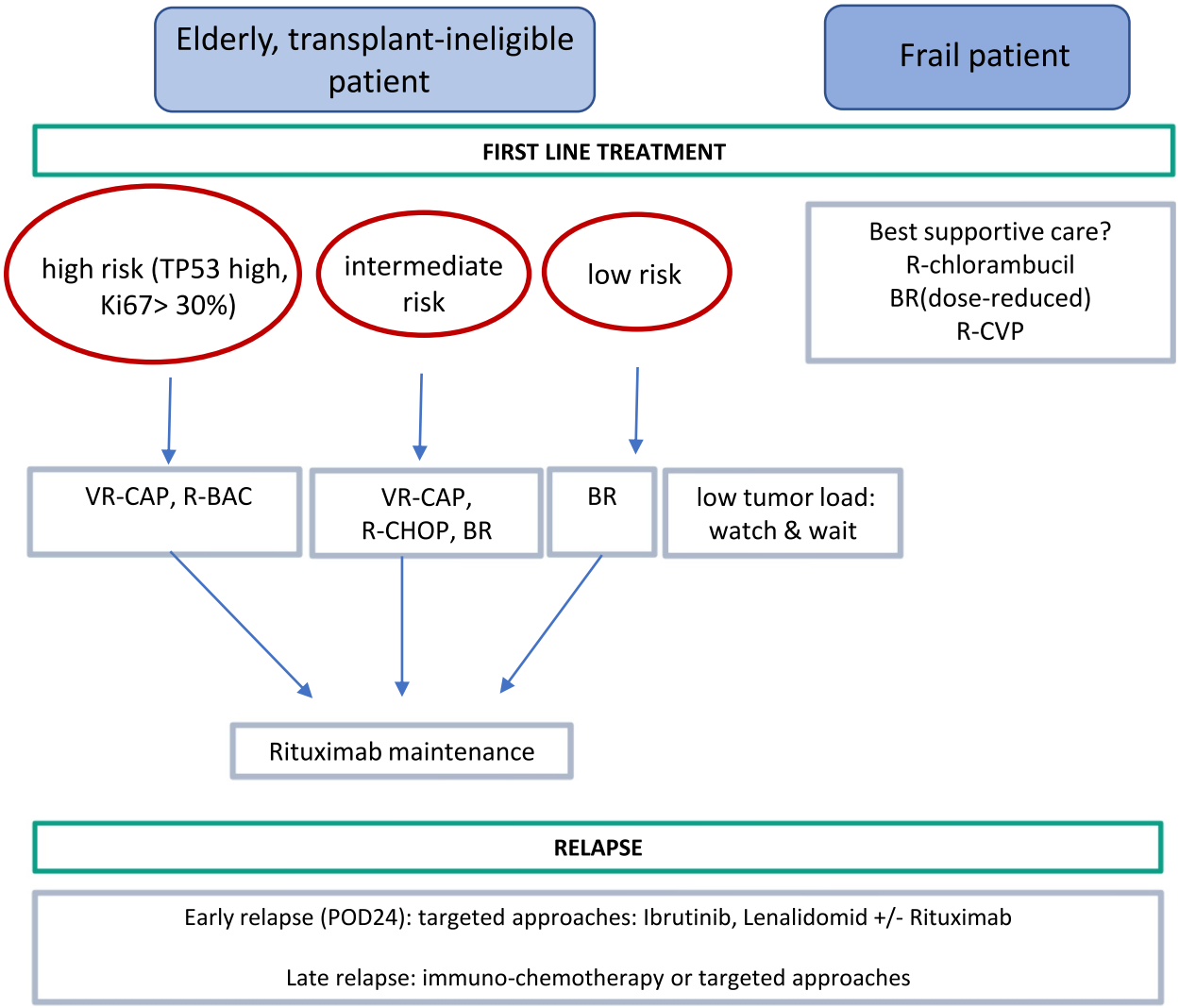

FIGURE 2 Suggested therapeutic algorithm for patients greater than 65 years

together, VR-CAP and bendamustine–rituximab (BR) represent the current standard approaches in older patients not eligible for high-dose therapy, who represent the majority of MCL patients. VR-CAP should be preferably considered for patients with a higher risk-profile such as high Ki-67 expression or blastoid morphology. BR may be preferable especially in patients with a more indolent CLL-like presentation.

3.2.2 | Maintenance

A large, randomized, European phase III trial compared rituximab maintenance to interferon (IFN) maintenance, confirming superiority of rituximab as maintenance therapy. In this study, after four years, 58% of the patients receiving rituximab after induction therapy with R-CHOP were in remission, compared to 29% in the IFN arm ($p = 0.01$). PFS and OS were also significantly improved in the Rituximab arm (5-years PFS R vs. IFN 51% vs. 22%, 5 years OS R vs. IFN 79% vs. 59%).[20] Based on these results, rituximab maintenance after R-CHOP is now generally recommended. Although investigation of the additional benefits of rituximab maintenance therapy after BR chemotherapy is pending, we recommend a similar approach.

4 | RECURRENT AND REFRACTORY DISEASE

4.1 | Molecular-targeted therapies

Several targeted therapy approaches have been investigated in different studies as single agents or in combination with immunochemotherapies or other targeted therapies (Table 2).

Targeting the *B-cell receptor pathway* with the BTK inhibitor ibrutinib resulted in the highest response rates of all targeted approaches so far leading to its approval in relapsed MCL. In a large International phase II study, response rates of 68% were achieved with ibrutinib in patients with relapsed disease.[21] The combination

TABLE 2 Molecular targeted therapies in MCL

Regimen	Phase	Number of patients	ORR (CR) %	Median PFS (months)
Lenalidomide	Phase II	134	28 (7.5)	4
Lenalidomide	Phase II	57	35[12]	8.8
Lenalidomide versus.	Phase II	170	46[11]	8.7
Monochemotherapy		84	23[8]	5.2
Lenalidomid + rituximab	Phase II	44	57 (36)	11.1
Lenalidomid + rituximab	Phase II	38		64% (After 5 years)
Ibrutinib	Phase II	111	68[21]	13.9
Ibrutinib vs. temsirolimus	Phase III	280	72[19]	14.6
			40[1]	6.2
Ibrutinib + rituximab	Phase II	50	88 (44)	
Ibrutinib + lenalidomid + rituximab	Phase II	50	76 (56)	
Idelalisib	Phase I	40	40[5]	3.7
Abt-199 (venetoclax)	Phase I	28	75[21]	14
Abt-199 (venetoclax) + ibrutinib	Phase II	24	71	
Acalabrutinib	Phase II	124	81 (40)	
Zanubrutinib	Phase II	86	84 (68.6)	22.1

Abbreviations: CR, complete response; MCL, mantle cell lymphoma; ORR, overall response rate; OS, overall survival; PFS, progression-free survival.

with rituximab was effective in all cases with low Ki-67, whereas in highly proliferating disease, only half of the patients responded to this approach.[22] A pooled analysis of the results of three different trials testing ibrutinib as monotherapy revealed overall response rates of 66% with median PFS and OS of 12.8, respectively 25 months.[23] Ibrutinib given in combination with bendamustine and rituximab in patients 65 years of age or older with newly diagnosed MCL is currently being evaluated in the phase 3 SHINE trial (NCT01776840). However, interindividual responsiveness is heterogenous and primary and secondary resistance has been reported with poor clinical outcome.[24] In patients with mutations in the *P53* gene, median PFS was shown to be significantly worse. Patients suffering early relapses after ibrutinib therapy demonstrated very aggressive clinical courses.[24]

Second generation BTK inhibitor acalabrutinib was approved in October 2017 by the Food and Drug Administration (FDA) for patients with relapsed/refractory MCL who had received at least one prior therapy as promising results, especially regarding tolerability, were observed in an open-label phase 2 study.[25] Acalabrutinib in combination with BR compared to BR alone in previously untreated MCL patients more than 65 years of age is currently evaluated in an ongoing phase-3 study (NCT02972840).

Next-generation BTK-inhibitor zanubrutinib is a highly potent, selective, bioavailable, and irreversible BTK inhibitor with maximized BTK occupancy. It was approved in 2019 in the United States and China for the treatment of patients with R/R MCL based on results from a phase II study in Chinese patients with R/R MCL reporting high overall response rates with durable CRs and improved safety and tolerability over existing treatments. The potential for use of zanubrutinib in the first-line setting is currently under evaluation in the randomized phase III MANGROVE study (NCT04002297) in which patients with treatment-naive MCL will receive zanubrutinib + rituximab or bendamustine + rituximab.

For patients suffering early relapses after ibrutinib therapy, a monotherapy with the *B-cell-lymphoma 2-inhibitor* Abt-199 (venetoclax) might be a promising alternative, as a phase I trial showed response rates of 75% in patients with relapsed MCL and 60% in patients having received prior ibrutinib therapy. Recently, the combination of ibrutinib and venetoclax proved to be highly effective in a small study cohort.[26] The potential advantage of ibrutinib combined with venetoclax over ibrutinib alone is currently being examined in an ongoing phase-3 study (SYMPATICO) (NCT03112174).

Various studies confirmed a benefit of the orally available *immunomodulatory drug lenalidomide* in relapsed MCL, with response rates of 35%–50%. In a randomized phase II trial, this approach was superior to monochemotherapy (response rate 46% vs. 23%). Based on an in vitro synergism, lenalidomide in combination with rituximab resulted in long lasting remissions in first-line therapy of a rather low risk patient cohort.[27] This approach may be applied especially in patients with contraindications against BTK inhibitors.

4.2 | Allogeneic transplantation versus CAR T-cells

For younger high-risk patients with TP53—mutated and relapsed MCL, who are transplant—eligible, the option of allogeneic transplantation

could be considered. Reduced-intensity allogeneic stem cell transplantation resulted in long-term disease-free survival in about 30% of the patients and may be applicable also in patients older than age 60 years. Yet, transplantation-associated severe acute and delayed toxicities, including chronic graft versus host disease and 20%–25% treatment-related mortality are frequent. Therefore, allogeneic transplantation is not recommended in the first-line setting and should only be discussed in relapsed disease.[1]

Recently, the FDA has approved the autologous CD19 CAR T-cell construct brexucabtagene autoleucel (formerly KTE-X19; Tecartus), based on results of the ZUMA-2 trial, evaluating safety and efficacy of this CD19 CAR T-cell construct in patients with R/R MCL. Treatment with brexucabtagene autoleucel resulted in the induction of durable remissions, although serious and life-threatening toxic events were reported.[28] Another CD19-directed CAR T-cell product (Lisocabtagene Maraleucel) for relapsed/refractory MCL is currently being evaluated in the ongoing Phase 1 study TRANSCEND NHL 001 (NCT02631044). Overall, results are promising and CAR T-cell constructs may have also the potential to cure MCL patients. Yet, much longer follow-up is needed.

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CONFLICT OF INTEREST

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SUPPLEMENT ARTICLE

WILEY

Personalized medicine for Hodgkin lymphoma: Mitigating toxicity while preserving cure

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Abstract

The treatment of classical Hodgkin lymphoma in young patients is one of the success stories of modern medicine. The use of risk- and response-adapted approaches to guide treatment decisions has led to impressive cure rates while reducing the long-term toxicity associated with more intensive therapies. Tissue biomarkers have not yet proven more effective than clinical characteristics for risk stratification of patients at presentation, but functional imaging features such as metabolic tumor volume may be used to predict response, if early observations can be validated. The success of treatment in younger patients has unfortunately not been mirrored in those over 60, where complex decision-making is often required, with a paucity of data from clinical trials. The use of PD1 blocking antibodies and brentuximab vedotin in this cohort, either alone or in combination with chemotherapy, may provide attractive options. The incorporation of frailty assessment, quality-of-life outcomes, and specialist geriatric input is also important to ensure the best outcomes for this diverse group.

KEYWORDS
antibody–drug conjugate, checkpoint blocking antibody, FDG-PET, Hodgkin lymphoma

1 | INTRODUCTION

Classical Hodgkin lymphoma (cHL) is a malignancy of germinal center B cells, characterized by the presence of the Hodgkin Reed–Sternberg (HRS) cell. It is the most common lymphoid malignancy diagnosed in children and young adults in developed countries, with a bimodal distribution peaking in the 2nd and 7th decades. Treatment with radiotherapy, multimodality chemotherapy regimens, newer antibody–drug conjugates, and immunotherapy checkpoint inhibitors (ICI), using 2-(^{18}F)-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (FDG-PET) to direct therapy has translated into 10-year overall survival (OS) rates above 80%.[1] However, the ongoing challenge remains of identifying patients with high-risk disease who will benefit the most from intensified therapy, while de-escalating treatment in those likely to be cured by less toxic regimens, to minimize the long-term morbidity and mortality seen in a minority of survivors, without comprising outcomes. This article will outline the emergence of new biomarkers to aid risk stratification and guide treatment decisions at diagnosis, the use of different response-adapted approaches, and the incorporation of new targeted agents in the treatment of both younger and older patients, in a more personalized approach to therapy.

2 | APPROACHES IN YOUNGER PATIENTS WITH HODGKIN LYMPHOMA

The initial treatment of advanced-stage HL with either bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine and prednisolone (escalated BEACOPP) or doxorubicin, vinblastine,

bleomycin, and dacarbazine (ABVD) is guided by risk stratification at diagnosis, comorbidity, and patient preference. Excellent disease control is achieved with more intensive BEACOPP regimens; however, this is at the price of increased acute toxicity and long-term morbidity, including second malignancies, infertility and cardiovascular disease among survivors, when compared to less intensive regimens. The use of six cycles of ABVD compared to four cycles of escBEACOPP plus two cycles of standard BEACOPP in the Italian HD2000 study showed no difference in 10-year OS, despite a significant difference in progression-free survival (PFS) in favor of the BEACOPP group at 5 years.[1] This is perhaps explained by the significantly lower rates of second malignancy in patients treated with ABVD had when compared to escBEACOPP (0.7% vs. 6.6%) and the success of autologous stem cell transplant (ASCT) in the salvage of relapsed disease. In the modern era, morbidity is predicted to be lower as the number of BEACOPP cycles has been reduced using a PET-directed approach[2]; however, identification of patients with higher risk disease at diagnosis seems important for choosing the correct intensity of therapy, to optimize the chance of cure.

Risk stratification of patients' disease by the International Prognostic Score (IPS) has previously been used to guide clinicians with initial treatment decisions; however, compared to the dynamic assessment of response by PET, it is less able to identify those patients with high-risk disease that have a poorer outlook.[3] The incorporation of biologic features such as gene expression profiles in addition to IPS has so far not yielded any prospectively validated biomarkers, but measurement of metabolic tumor volume (MTV) and total lesional glycolysis (TLG) at the baseline PET may provide a more quantifiable assessment of tumor burden, a known predictor of poor outcome.[4] The European collaborative group retrospectively analyzed baseline total MTV (TMTV) in 258 patients with early-stage HL in the standard combined modality arm of the H10 trial and showed that both TMVT and interim PET (iPET) following two cycles of ABVD were independently prognostic of response to treatment, and when combined, allowed identification of a high-risk patient group with a 5-year PFS of only 25% (TMVT >148 cm^3 and iPET positive—Deauville Score [DS] 4–5).[5] In this study, the TMTV was calculated by summing all the extranodal and nodal lesions using the 41% maximum standardized uptake value threshold (SUVmax) method. In advanced-stage disease (stage IIB–IV), 848 patients enrolled in the RATHL trial had baseline total/bulk MTV and TLG measured using SUV $\geq$ 2.5 when compared to the liver (the 41% SUVmax method was found not to be associated with PFS or 3-year HL events in this patient cohort).[6] Patients with a positive iPET following two cycles of ABVD had a significantly higher total/bulk MTV and TLG when compared to iPET-negative patients ($p = 0.0002$); however, in a multivariate analysis, only total TLG, B symptoms, and age were significantly associated with PFS. Patients with a negative iPET and high-volume TLG at baseline (defined as >3318 g) had a 5-year treatment failure rate of 31%, compared with 13.1% in low-volume TLG.[6] A study which retrospectively analyzed a total of 392 patients enrolled in both arms of the AHL 2011 LYSA trial identified a small number of patients with a high-baseline PET TMVT (set at a threshold of 350 ml using the 41% SUVmax method) who had a positive iPET (DS 4–5) following two cycles of escBEACOPP, with a 2-year PFS of 61% compared to 88% and 96% in patients with a low TMTV/positive iPET and a low TMTV/negative iPET, respectively.[7] The rate of progression among patients with stage IV disease and a negative iPET in the RATHL trial was 20% compared with less than 10% of patients enrolled in the GHSG H18 trial and LYSA study, suggesting a more reliable negative predictive value of iPET after more intensive regimens such as escalated BEACOPP in patients with high-risk disease.[3] Thus, baseline total MTV and TLG may prove useful in the context of guiding initial intensity of treatment, by identifying those at risk of treatment failure despite a negative iPET. Measurement of total MTV/TLG will require standardization; however, similar to the Deauville scoring system that was developed for iPET assessment to allow reproducibility and consistency when stratifying patients into different risk groups and setting consistent threshold values.[4] Prospective validation of this potential biomarker in a large clinical trial is needed to ascertain its true prognostic value.

Patients with advanced-stage HL and a positive iPET after two cycles of ABVD in the RATHL trial went on to receive escalated treatment with more intensive BEACOPP regimes (four cycles of escBEACOPP or six cycles of BEACOPP-14), with a 5-year PFS of 65.7% and OS of 85.1%. This compares favorably to continuation of ABVD following iPET in previous studies, where the PFS was consistently less than 40%.[3] The South West Oncology Group (SWOG) 0816 trial showed at 5-year follow-up, 59 patients with advanced-stage HL (here defined as stage III–IV) and a positive iPET (DS 4–5) escalated to escBEACOPP after two cycles of ABVD had a similar PFS of 66%, but the rate of second malignancy was 14% with a short median onset of 4.2 years. In this study, six cycles of escBEACOPP were given compared to four cycles in RATHL, which may partly explain the high rate of secondary malignancy.[8] The GHSG H18 trial showed that patients with iPET-positive disease following two cycles of escBEACOPP who were treated with a total of six cycles of escBEACOPP had a secondary malignancy rate of 9% at 5.5 years of follow-up.[2]

In the RATHL study, the treatment failed despite escalation to BEACOPP regimens in 20 out of 37 patients with a DS of 5 on iPET, and this group almost certainly requires a different approach to improve their survival. The use of salvage therapy with high-dose chemotherapy (HDT) followed by ASCT is an option for patients with initial chemorefractory disease, and was investigated by the Italian HD0801 trial.[9] Here a positive iPET was defined as a DS of 3–5, and therefore included a more favorable patient group when compared to outcomes from RATHL and LYSA trials. Following two cycles of ABVD, 81 (19%) patients remained iPET positive and received HDT ASCT, with a 2-year PFS of 75% suggesting that early intensification might improve outcomes for this group.[9] The use of newer agents such as brentuximab vedotin (BV) and anti-PD1 antibodies in the frontline treatment of patients with high-risk iPET-positive disease may provide an alternative to ASCT, given their activity in the relapsed/refractory disease; however, there is as yet little data to support their use in a PET-driven approach for this selected group of patients. The Phase III ECHELON-1 trial

incorporated six cycles of brentuximab with AVD chemotherapy (A + AVD) and showed a 3-year modified PFS (including a DS 3–5 at the end of treatment as an event) of 83.1% compared with 76.2% in patients' receiving six cycles of ABVD (7.1% difference $p = 0.005$), with a beneficial trend observed in iPET-positive patients <60 years receiving A+ AVD (3-year PFS 69.2% vs. 54.7%, respectively).[10] Therefore, A+ AVD may be an attractive option for those patients with high-risk disease who wish to reduce the risk of long-term toxicity associated with BEACOPP regimes or who are unable to tolerate escalation of therapy following a positive iPET.

In early stage unfavorable disease, the addition of BV to four cycles of AVD within in a phase II PET-directed pilot study in the United States allowed the reduction of dose and intensity of radiotherapy without apparently compromising treatment efficacy, with a 2-year PFS of 97% among 29 patients who did not receive any consolidation radiotherapy.[11] The phase III GHSG H17 trial in a similar patient cohort also showed that the omission of radiotherapy in those with a negative PET following two cycles of ABVD plus two cycles of escBEACOPP was noninferior in terms of 5-year PFS (2.2% difference in favor of the radiotherapy group).[12] An initial high-intensity approach in the early-stage disease thus appears to maximize cure rates without the need for consolidation radiotherapy in those patients with a negative PET at the end of the treatment, showing an improvement in the negative predictive value of iPET when compared to the use of less intensive regimens.

The RATHL trial showed that the omission of bleomycin in patients with a complete metabolic response at iPET did not compromise survival outcomes, and resulted in a lower incidence of pulmonary toxicity (5-year PFS and OS 84% and 98% vs. 86% and 97%, respectively).[3] Similarly in the AHL 2011 LYSA trial, 5-year PFS was not significantly different between patients treated with continued escBEACOPP or de-escalated to ABVD (86.2% standard arm vs. 85.7% PET-driven arm) leading to the conclusion that therapy can be reduced in those patients whose disease responds to initial therapy without compromising survival outcomes.[13] The optimal number of escBEACOPP cycles was investigated by the GHSG H18 trial in this context, and showed that in patients with a negative iPET (DS 1–2) following two cycles of escBEACOPP, the duration could be safely reduced to two further cycles, with a small but statistically significant improvement in 5-year survival outcomes when compared to four cycles (PFS 92.2% vs. 90.8% OS 97.7 vs. 95.4%, respectively).[2] For patients with an IPS score of 1–2 and favorable baseline characteristics, an initial two cycles of ABVD with de-escalation to AVD if iPET negative and escalation to four cycles of escBEACOPP if iPET positive has a high probability of cure while minimizing the number of patients exposed to the acute and long-term toxicity of BEACOPP regimes. The omission of radiotherapy in those patients with a complete metabolic response did not affect survival outcomes in the GHSG H15 study[14] and only 6.5% of patients received consolidation radiotherapy without loss of disease control in the RATHL trial.[3] There may be a role for radiotherapy in single-site iPET-positive disease to reduce the number of patients escalated to more intensive chemotherapy regimens; however, there is currently a lack of prospective data supporting this approach.

3 | IMMUNE CHECKPOINT INHIBITORS AND EMERGING BIOMARKERS

The use of immune checkpoint inhibitors (ICI) in relapsed Hodgkin lymphoma is well established, and the use of anti-PD1 antibodies combined with multi-agent chemotherapy is being explored in the first-line setting. A study of affected nodes in those treated with anti-PD1 antibodies showed modification of the HL microenvironment in response to anti-PD1 therapy, with rapid depletion of HRS cells and a reduction in PDL1-expressing tumor-associated macrophages and regulatory T cells.[15] There was no clonal expansion and activation of cytotoxic T cells as is seen in solid tumors, suggesting a mechanism of action that is particular to HL, involving interruption of T cell–B cell signaling pathways. Combination of nivolumab with AVD chemotherapy (N + AVD) for advanced-stage HL (stage IIB–IV) was investigated by Ramchandren et al. who first gave nivolumab monotherapy for 4 doses, followed by combination therapy (N + AVD) for 12 doses every 2 weeks, with response assessment at the end of monotherapy, after two combination cycles and at the end of the therapy.[16] Interestingly, at the end of monotherapy, the complete response rate was 21%, with all patients in the highest quartile for expression of PDL1 on HRS cells achieving a CR after combination therapy, maintained at 32 weeks of follow-up. Discontinuation rates were low (10%) with a febrile neutropenia rate of 10%. The most common endocrine immune-mediated adverse event (IMAE) was hypothyroidism, and the main nonendocrine IMAE was rash (grade 1–2). Generally, the regimen was well tolerated, but there was one treatment-related death in an older patient, in CR after two cycles of combination therapy who experienced four grade 3–4 adverse events.[16] The use of pembrolizumab monotherapy prior to 4–6 cycles of AVD in 30 patients with early unfavorable and advanced disease showed an impressive 100% CR rate by the end of two cycles of AVD. Responses were durable, with no progression or death at 22 months of follow-up, with no consolidation radiotherapy given at the end of the treatment.[17] Phase III trial data comparing N + AVD versus BV + AVD in the first-line setting are awaited.

The pattern of disease response in the context of anti-PD1 therapy has prompted revision of the Lugano Classification lymphoma response criteria, to include immunomodulatory therapy (LYRIC), due to early imaging suggestive of progressive disease (PD) in patients who later gained clinical benefit.[18] The phenomenon of tumor flare or pseudo-progression is well documented in patients with solid tumors treated with ICI, as a result of immune cell infiltration or the delayed effect of these drugs allowing early tumor growth. The use of early iPET to guide response-adapted treatment in HL may be particularly difficult to interpret in this context, with the risk of tumor flare interpreted as a positive iPET and patients subsequently escalated to more intensive regimes and exposed to unnecessary toxicity whose disease may have responded at a later time point. This resulted in the addition of indeterminate response (IR) to CR PR and PD and allows the flexibility for patients to continue treatment with further imaging at 12 weeks to confirm either PD or response (Table 1). There may be a role for anti-PD1

TABLE 1 Lymphoma response to immunomodulatory therapy criteria

	IR criteria
IR1	Increase in overall tumor burden ≥50% of up to six measurable lesions within the first 12 weeks of treatment without clinical deterioration
IR2	Appearance of new lesions or increase in one or more existing lesions ≥50% at any time during treatment in the context of <50% increase in overall tumor burden
IR3	Increase in FDG update of ≥1 lesion(s) without an increase in size or number of lesions

Abbreviations: FDG, fluoro-2-deoxy-D-glucose; IR, indeterminate response.

TABLE 2 List of instrumental activities of daily living

1	Planning, preparing and cooking a meal
2	Housekeeping and laundry
3	Ability to use the telephone
4	Managing finances
5	Shopping for personal items and food
6	Ability to drive or use public transportation

therapy in those patients with a DS of 5 on iPET as an alternative to escalation of therapy to more intensive regimes, whose disease is refractory to traditional chemotherapy.

4 | APPROACHES FOR OLDER PATIENTS WITH HODGKIN LYMPHOMA

The use of BV and ICI in the elderly may be an attractive option as monotherapy, or in combination with less toxic chemotherapy regimens, to improve the poorer survival outcomes when compared to the younger population. The problems of comorbidity, poor performance status (PS), increased adverse events, and low tolerance of chemotherapy regimens at full dose in this heterogeneous population have resulted in the reported 3-year PFS and OS rates of 55% and 78%, respectively.[19] Evens et al. investigated BV in sequential combination with AVD in 48 patients over 60 with untreated HL (stage II–IV) in a Phase II trial, which showed encouraging 2-year PFS and OS of 84% and 93%, respectively.[20] Patients were given two cycles of lead-in and consolidation BV, based on previous studies which have shown poor durability of responses in older patients treated with BV monotherapy or BV plus dacarbazine.[21] Only 52% of patients completed the full course

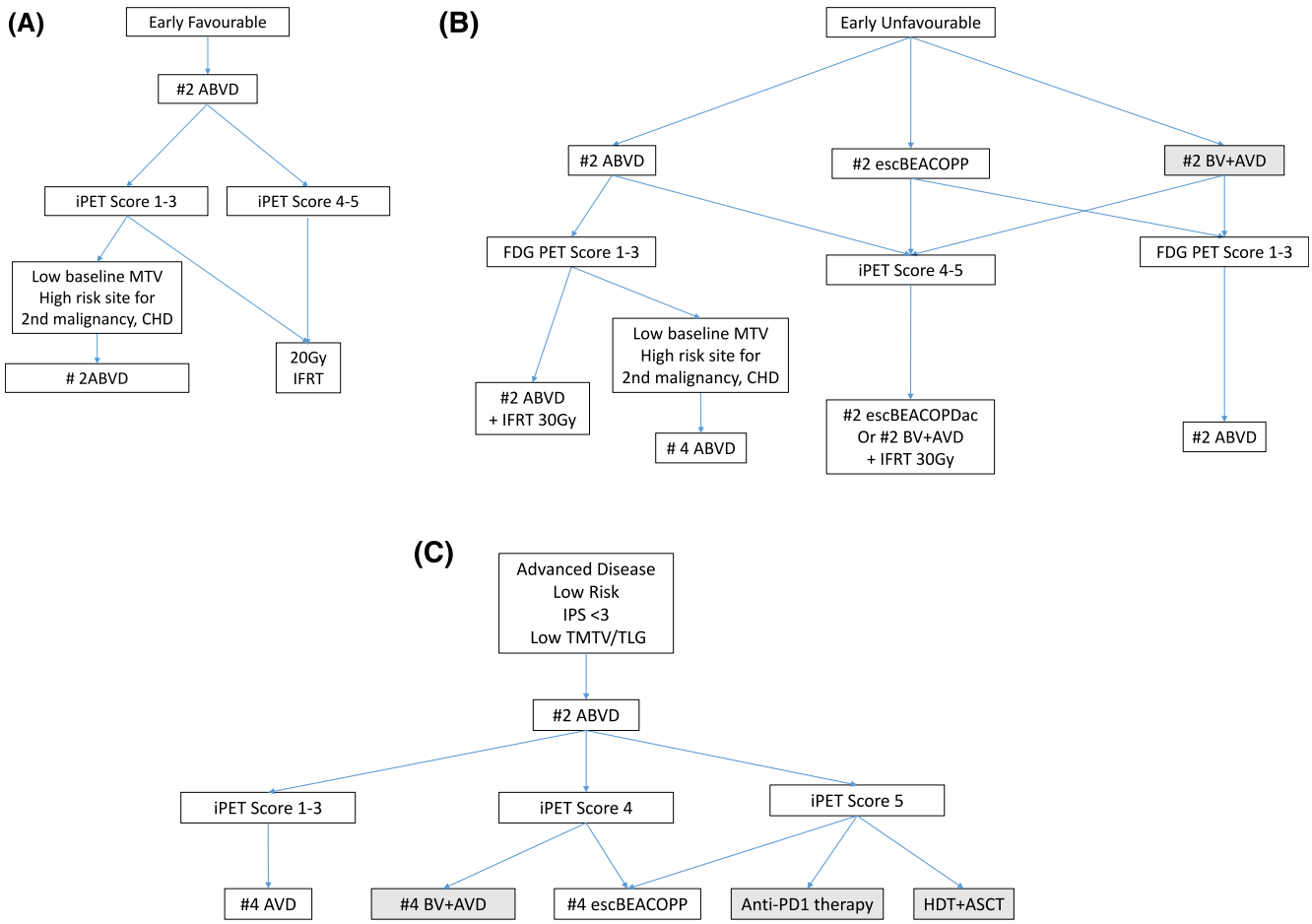

FIGURE 1 Flow charts of proposed treatment approaches for different presentations of Hodgkin lymphoma. Potential experimental approaches for which the evidence is not yet mature are shown shaded

of therapy owing to side effects, but 75% of patients who completed less than six cycles of AVD remained in CR at 2 years.[20]. Longer follow-up is needed to ascertain if these responses are durable and to assess the long-term cardiotoxicity associated with receiving anthracycline chemotherapy in this patient cohort which included eight patients >80 years and 9 patients with a PS of 2.

The combination of N + AVD may prove to be a promising strategy in fit older patients able to tolerate anthracycline chemotherapy plus ICI therapy as initial treatment. The Phase II trial by Ramachandren et al. detailed above included six patients over 60, five of whom obtained a CR, while one died from toxicity related to treatment.[16] Data from a larger Phase II trial combining nivolumab with AVD in older patients with high-risk disease (IPS score ≥3) and a positive iPET (DS 4–5) are awaited (NCT03033914) to assess the activity and safety of this regimen in a larger cohort of patients.

A chemotherapy-free approach in older patients is an attractive one in those with poor performance status. A Phase II study combining nivolumab and BV (N + BV) in elderly patients with a PS of 0–1 by Yasenchak et al. showed a CR rate of 72% following 16 cycles of treatment that was well tolerated.[22] A subsequent interim analysis of a similar phase II trial which included a patient population with poorer PS (0–2) showed a more modest ORR of 61% with CR of 48% following eight cycles of treatment, which unfortunately did not meet the predefined ORR of 68% and was therefore terminated early.[23] The frequency of adverse events was reduced compared to the smaller trial (26% vs. 37% sensory neuropathy [all grades], respectively) which may be explained by the difference in the number of cycles received. The strategies to reduce the number of treatment cycles without compromising survival should be explored in future, to minimize toxicity and allow the majority of patients to complete therapy with minimal dose reductions and treatment interruptions.

The use of comprehensive geriatric assessment (GCA) and quality-of-life (QOL) outcomes in older patients are recommended, but data on their feasibility are lacking in the prospective clinical trial setting. Evens et al. used the Cumulative Illness Geriatric (CIRS-G)

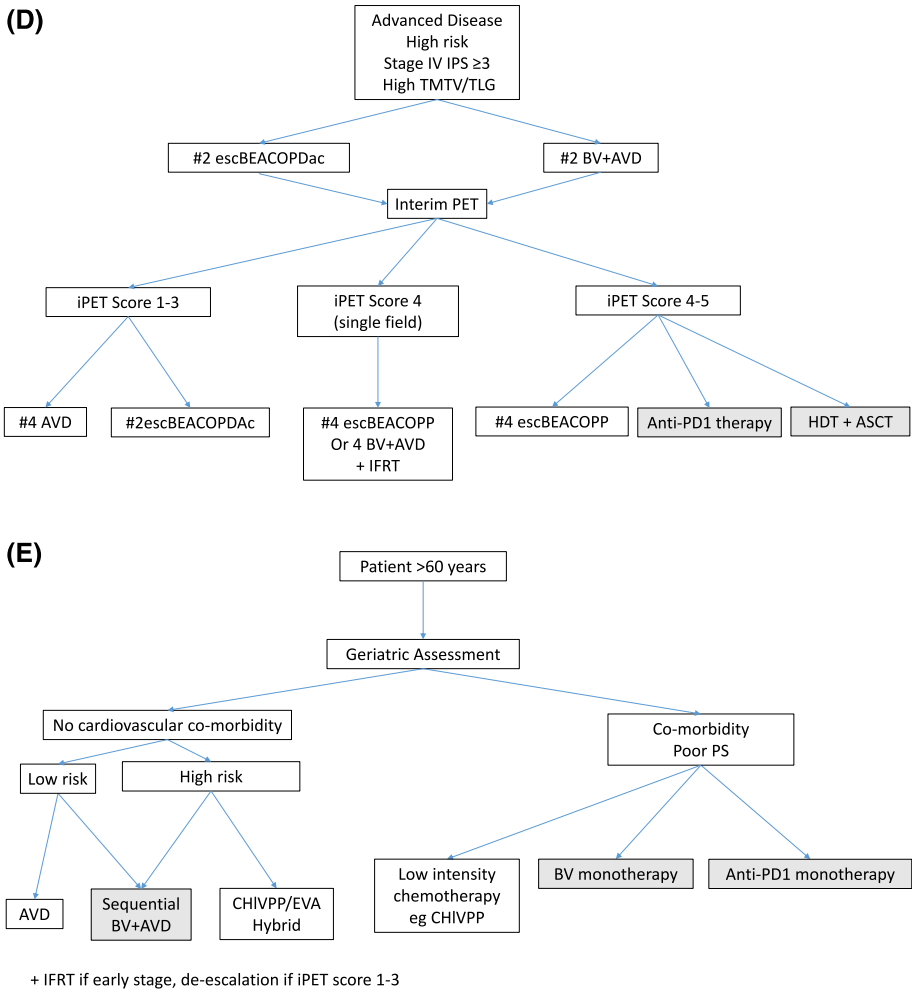

FIGURE 1 (Continued)

comorbidity score (https://www.mdcalc.com/cumulative-illness-rating-scale-geriatric-cirs-g) to stratify patients into high or low groups at diagnosis (CIRS-G score cut-off 10); however, in a multivariable analysis, this was not significant in predicting PFS, while the loss of instrumental activities of daily living (IADLs) were predictive of both OS and PFS, with 2-year PFS of 94% versus 25% in patients with no IADL loss versus IADL loss and 2-year OS of 97% versus 67%, respectively.[20] This assessment is quick and convenient for the busy oncologist to use in the clinic, to inform the initial treatment decisions, and help tailor initial therapy to each individual patient's circumstances (Table 2).

5 | CONCLUSIONS

The use of PET-directed therapy in younger patients with advanced HL has allowed safe de-escalation of treatment for those with responsive disease at iPET, sparing the acute and long-term toxicity of more intensive chemotherapy regimens and consolidation radiotherapy. Figure 1 summarises the approaches currently in use, and some of the areas of emerging evidence for new treatments. Further information regarding high-risk features at diagnosis in the PET-directed era is required, however, and measurement of baseline PET characteristics may prove a valuable predictive biomarker. The use of ICI therapy as part of initial therapy, guided by prospective trials incorporating biomarkers related to the tumor microenvironment is awaited, although the use of PET-directed therapy in this context is likely to be more complex, with the phenomenon of tumor flare and delayed response making interpretation harder. The use of BV and ICIs in older patients appears promising; however, prospective incorporation of geriatric and QOL assessment into clinical trials is currently lacking. The use of IADL assessment may provide oncologists with a tool to quickly assess patients in the clinic and help with decision-making in this complex patient population.

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CONFLICT OF INTEREST

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SUPPLEMENT ARTICLE

WILEY

Cutaneous T-cell lymphomas—An update 2021

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Abstract

Cutaneous T-cell lymphomas (CTCL) represent the majority of primary cutaneous lymphomas (CL). Mycosis fungoides (MF) and cutaneous CD30+ lymphoproliferative disorders account for 80% of all CTCL. CTCL show overlapping histological features. Thus clinical-pathological correlation is of importance to achieve final diagnosis. MF shows a characteristic evolution with patches, plaques, and in a subset of patients (10%–20%) with tumors. Therapy is stage-adapted with skin-directed therapies such as UV-light therapies and corticosteroids in early disease stage (i.e., patch and limited plaque stage) and systemic therapies (retinoids, interferon, mono chemotherapy, targeted therapy) and/or radiation therapy (local or total skin beam electron) in advanced stages. Novel therapies include targeted therapy such as mogamulizumab (anti-CCR4) or brentuximab vedotin (anti-CD30) and histone deacetylase inhibitors. Considering the impact of targeted therapies, biomarkers such as CD30 are not only crucial for the diagnosis and correct classification of an individual lymphoma case, but also for therapy as they may represent therapeutic targets. In the recently revised WHO classification 2017 and the updated WHO-EORTC classification for CL 2018, primary cutaneous CD8+ acral T-cell lymphoma has been introduced as a new still provisional entity. It displays characteristic clinical, histological, and phenotypic features and exhibits an excellent prognosis. Rare, but aggressive CTCL include cutaneous primary cutaneous aggressive epidermotropic CD8-positive T-cell lymphoma and cutaneous gamma/delta T-cell lymphoma, which present with rapid onset of necrotic or ulcerated plaques and tumors. As they have a poor prognosis, treatment includes multiagent chemotherapy and hematopoietic stem cell transplantation.

KEYWORDS
classification, cutaneous, dermatopathology, diagnosis, lymphoma, skin, T-cell, therapy

1 | INTRODUCTION

The group of primary cutaneous lymphomas (CL) are the second most common group of extranodal lymphomas. Among CL, cutaneous T-cell lymphomas (CTCL) represent the majority accounting for approximately 65%–75% of all CL.[1] All other CTCL forms are rare with each entity accounting for less than 1% of all CTCL. Primary CL present in the skin without extracutaneous disease at the time of diagnosis and often remain limited to the skin over long periods of disease evolution. Progression with extracutaneous spread occurs usually in advanced late stages.

CTCL exhibit a wide spectrum of clinical, histological, immunophenotypic features and genetic alterations. As these histological and phenotypic features overlap within the various forms of CTCL, correlation of the clinical features with the histological and immunophenotypic findings is an essential element of the diagnostic work-up

and implies that clinical images and dermatologic examination are indispensable to achieve the final diagnosis. The prognosis and treatment significantly differ among the entities within the CTCL group and also from systemic lymphomas with similar histological features.

Recent developments such as targeted therapy have expanded the therapeutic spectrum in CTCL and contribute to a significant change in the prognosis, particularly in advanced forms of several CTCL forms. The therapeutic response to a distinct targeted therapy, however, varies among different CTCL entities. The exact classification of CTCL entities is important to compare results of clinical trials and to assess the value of targeted therapy. CTCL without further specification should not be used as a diagnostic term in therapeutic trials as this designation does not allow to identify differences in the responses to therapy among the distinct CTCL forms. The classification and terminology of CL should follow the current revised WHO classification 2017 (Table 1) and the updated WHO-EORTC classification for CL 2018.[1,2]

An example for the efficacy of targeted therapy in CTCL is brentuximab vedotin (BV), which targets CD30 expressed on tumor cells. It is not only a new and efficient treatment for relapsed or therapy refractory CD30 lymphoproliferative disorders (LPDs) such as multifocal primary cutaneous anaplastic large cell lymphoma (PC-ALCL) but also expanded the therapeutic strategies for advanced mycosis fungoides (MF) and Sézary syndrome.[3] Moreover, BV was even effective in some patients in whom rare and aggressive CTCL forms such as cutaneous gamma/delta T-cell lymphoma showed exceptional expression of CD30.

Considering the impact of targeted therapies, biomarkers such as CD30 are not only crucial for the diagnosis and correct classification of an individual lymphoma case, but also for therapy as they may represent therapeutic targets. In consequence, the detection of biomarker expression by immunohistochemistry or other in situ techniques became an important aspect in the diagnostic work-up of CTCL. Inter-observer variability and differences in the technical aspects, however, are critical factors in the assessment of biomarkers. Thus, detection methods and expression level need to be standardized especially in situations, in which the expression level of a target is decisive for the selection of therapy. Digital image analysis tools are currently developed for the automatized evaluation of immunohistochemical slides with the aim to reduce the interinstitutional and interrater variability. Nevertheless, the results should always be interpreted in a synoptic way taking into account the context of clinical and histopathological context, especially since CD30 expression is found in a broad range of CTCL.[4]

2 | CTCL ENTITIES

MF is the most common form of CTCL and accounts for nearly 50% of all primary CL.[1] MF shows a characteristic disease evolution with erythematous patches (patch stage) which may evolve into more infiltrated plaques (plaque stage). The patches and

TABLE 1 Spectrum of primary cutaneous T-cell lymphomas according to revised WHO classification of hematopoietic tumors (2017) and the updated WHO-EORTC classification for primary cutaneous lymphomas (2018)

Mycosis fungoides (MF)
MF variants • Folliculotropic MF • Granulomatous slack skin • Pagetoid reticulosis
Sézary syndrome
Adult T-cell leukemia/lymphoma
Primary cutaneous CD30-positive lymphoproliferative disorders • Lymphomatoid papulosis • Primary cutaneous anaplastic large cell lymphoma
Subcutaneous panniculitis-like T-cell lymphoma
Primary cutaneous gamma/delta T-cell lymphoma
Primary cutaneous aggressive epidermotropic CD8-positive T-cell lymphoma (provisional)
Primary cutaneous CD4+ small/medium-sized pleomorphic T-cell lymphoproliferative disorder (provisional)
Primary cutaneous acral CD8+ T-cell lymphoma (provisional)
Primary cutaneous peripheral T-cell lymphoma, not otherwise specified (NOS)
Extranodal NK/T-cell lymphoma, nasal type
Hydroa vacciniforme-like lymphoproliferative disorder

Abbreviation: MF, mycosis fungoides.

plaques usually persist over long time, that is, months or years. In a subset of patients, large and often ulcerated tumors (tumor stage) develop.

As the histological findings may be subtle especially in patch stage of MF, distinction from benign inflammatory skin diseases such as chronic eczema may not always be possible at this disease stage and require repeated biopsies from different lesions. This may also explain the delay of several months to years in the diagnosis of early MF.

Enlarged lymph nodes with clinical and/or radiologic suspicion for lymphoma involvement should be either biopsied. Bone marrow biopsy is only indicated in advanced disease stage or if circulating atypical lymphocytes in the peripheral blood are present.

Histologic work-up is not only important to identify MF and to distinguish MF from inflammatory skin diseases, but it also allows to identify variants and subtypes of MF which may differ in their course from classic MF. Folliculotropic MF (FMF) is a distinct variant of MF accounting for approximately 10% of all MF cases and characterized by folliculotropic infiltrates, that is, exocytosis of tumor cells into the hair follicle epithelia resulting in alopecic patches and plaques.[5] Recently, two prognostic subsets of FMF could be identified. The early form of FMF manifests with follicular papules, acneiform lesions with comedones and cysts and histologically with subtle lymphocytic infiltrates. The advanced form of FMF is characterized by more extensively infiltrated thicker alopecic plaques and deep, dense lymphocytic infiltrates. The course and prognosis of advanced form is similar to tumor stage of MF, whereas the early form displays the same indolent course as the classic form of MF (patch stage).

The prognosis in the patch and limited plaque stage is favorable with 5- and 10-year survival rates over 90% reflecting the slowly progressive and indolent course of the disease in most patients. In stage IB the 10-year-survival rate is 80% but drops to 40% in stage IIB.[6] whereas the prognosis in the patch and limited plaque stage is favorable. MF in tumor stage is aggressive and associated with the risk for extracutaneous spread which often first involves lymph nodes. During disease progression, visceral or other organs may become involved and sepsis originating from bacterial colonization of ulcerated tumors. Remarkably, the majority of patients (80%) will not progress to tumor stage. Thus, it is important to emphasize this point in counseling MF patients as they will get often terrified when looking to images of advanced MF seen during their Internet searches to retrieve more information about the disease and the treatment modalities.

Treatment of MF is stage-adapted which is also reflected in international and national guidelines (Table 2).

Skin-directed therapies (UV light, topical corticosteroids, nitrogen mustard) are the main strategies for early stage of MF and systemic therapies (retinoids, chemotherapy, targeted therapy) for advanced disease (extensive plaque and tumor stage).[7,8]

It should be noted that in selected patients with stage IA (limited patch stage) a watch-and-wait strategy may be justified. In early MF (patch and limited plaque stage), skin-directed therapies comprise treatment by UV-light (UVB narrow band, psoralen UVA [PUVA]), topical corticosteroids (CS) and the newly introduced topical mechlorethamine (nitrogen mustard) representing a topically applied cytotoxic agent. Topical CS and UV-light approaches result in complete clinical response in up to 60% and 80%–90% of the patients, respectively. These therapies should be intermittently applied to prevent or reduce the long-term complications of UV-light treatment (epithelial skin cancers) and toxicity of potent topical corticosteroids (skin atrophy and fragility). Topical mechlorethamine (nitrogen mustard) represents a novel therapy for MF in patch and limited plaque stage with response rates up to 60% of the patients and represents an alternative, but more cost-intense treatment for patients not responding to other skin-directed therapies. Irritative and allergic dermatitis are seen as adverse effects.

In patients with relapses or therapy refractory course and development of plaques, skin-directed treatment is combined with systemic treatment including retinoids and retinoid analogues (acitretin; bexarotene) or low-dose methotrexate. Interferon-alpha (IFN), which was an effective alternative to retinoids and reached response

TABLE 2 Treatment of MF

Topical therapies:
Corticosteroids ointments
Bexarotene gel (US)
Chlormethine gel
UV-light therapies:
UVB narrow band (if patches only)
PUVA (alone or in combination with other therapies)
Systemic therapies:
Interferon alpha[a]
Oral bexarotene
Mogamulizumab (anti-CCR4)
Brentuximab vedotin (anti-CD30 coupled with monomethyl auristatin E)
Histon deacetylase inhibitors
Chemotherapy:
Methotrexate (low dose) or pralatrexate (US)
Gemcitabine (low-dose)
Pegylated liposomal doxorubicin
CHOP (extracutaneous spread)
Radiotherapy:
Local radiotherapy (solitary or few tumors)
Total skin electron beam (generalized thick plaques and tumors).
Extracorporeal photopheresis (erythrodermic MF)
Allogeneic hematopoietic stem cell transplantation

Abbreviation: MF, mycosis fungoides.

[a]Production of non-pegylated INF alpha 2a stopped, alternatively pegylated interferon alpha (limitation: not approved for MF).

rates of more than 50% in combination with PUVA, is not any longer produced. The non-pegylated INF alpha is not any longer available, but pegylated INF alpha might be an alternative. Similar response rates can be achieved by the combination of PUVA and retinoids (acitretin or bexarotene).

As an alternative to systemic retinoids, topical retinoids such as alitretinoin, bexarotene, and tazarotene can be used. As in mechlorethamine, irritation is a common adverse effect. In patients with single tumors in otherwise patch and plaque stage, local radiotherapy for tumors is effective.

Recently, new treatment options became available such as mogamulizumab, a humanized antibody against CCR4 (CC-chemokine receptor 4) as targeted therapy which leads to a prolonged progression-free survival. Histone deacetylase inhibitors (HDACi), such as vorinostat, romidepsin, and resminostat, interact with various pathways involved in tumor cell growth. Clinical response to HDACi therapy is observed in approximately one-third of patients. Resminostat is tested in clinical trials as a maintenance therapy and seems to enhance response to other therapies.

In more advanced disease (thick plaques, tumor stage), monochemotherapy with gemcitabine or pegylated liposomal doxorubicin are effective. CHOP-based chemotherapy regimens should be restricted to patients with extracutaneous spread and usually result in only short-lived response. Moreover, they seem to increase the risk for sepsis. Neoplastic cells in MF tumors may express CD30 which renders them to candidates for a targeted therapy with BV, an anti-CD30-based antibody conjugated to monomethyl auristatin E as a cytostatic drug. In cases with more than 5% of the tumor cells expressing CD30, clinical response to BV was observed and could induce regression of even large, ulcerated tumors. Alternatively, total body skin electron beam irradiation is effective for patients with multiple MF tumors. This treatment modality is, however, only available in certain centers. Allogeneic hematopoietic stem cell transplantation is a potentially curative treatment option and is effective in up to 60% of patients with advanced MF and Sézary syndrome but is limited by the high relapse rate and the high mortality associated with this therapy.[9] Primary refractory disease, relapse, or progression in patients that had received three systemic treatments prior to transplant were identified as independent adverse prognostic factors. Pretreatment with total skin electron beam therapy may be beneficial before stem cell transplantation.[7]

Bacterial toxins, especially those of *Staphylococcus aureus*, are able to activate STAT3 and stimulate proliferation of tumor cells, and suppress the activity of tumor-infiltrating CD8+ cells against the tumor cells. Antibiotic treatment resulted in clinical improvement in MF as well as in Sézary syndrome.[10]

Sézary syndrome (SES) is an aggressive form of CTCL, which is derived from central memory T-cells. Clinically, SES manifests with generalized skin involvement with erythroderma accompanied by intense pruritus, enlarged lymph nodes (with or without specific involvement by the neoplastic cells), and a leukemic spread of tumor clone. Diagnosis requires demonstration of the same tumor clone in the skin lesions and the peripheral blood as well as additional hematologic findings (absolute Sézary cell count of >1000 cells/μl or an expanded CD4+ T-cell population leading to a CD4/CD8 ratio ≥ 10, CD4+/CD7-cells ≥ 40% or CD4+/CD26-cells ≥ 30%). Histological diagnosis may be challenging as nonspecific findings are common. Immunohistochemical (bio-)markers such as PD-1, TOX, CD7, KIRDL2, and the proliferation rate were shown to be useful markers in the histopathological diagnosis of SES and the distinction from benign inflammatory erythrodermic skin diseases such as atopic dermatitis or drug eruption.[11] Other T-cell non-Hodgkin lymphomas present with a leukemic phase and may involve the skin such as T-cell large granular lymphocytosis (T-LGL), T-cell prolymphocytic leukemia (T-PLL), and adult T-cell lymphoma/leukemia (ATLL). The tumor cells in T-LGL are large with abundant cytoplasm and azurophilic granula. In contrast to SES, the course of T-LGL is usually indolent. Expression of TCL-1 detected by immunohistochemistry or flow cytometry is helpful in the diagnosis of T-PLL. ATLL is linked to human T-cell lymphotropic virus 1 which differs from SES, T-LGL, and T-PLL. Treatment of SES includes extracorporeal photopheresis (ECP) with a wide range of response rates up to 80% and complete response rates up to 25%.[7] ECP is often combined with systemic therapies used in MF, for example, bexarotene. Alemtuzumab and chemotherapy (gemcitabine, pegylated liposomal doxorubicin) are used as second-line treatment. Recently mogamulizumab was reported as an effective treatment in SES.

Primary cutaneous CD30-positive lymphoproliferative disorders (CD30+ LPD) are the second most common group of CTCL (25% of all CTCL) and include PC-ALCL, lymphomatoid papulosis (LYP) and borderline lesions.[4,12] CD30+ LPD exhibit an indolent course with a good prognosis despite recurrences are frequent. PC-ALCL manifests with a solitary or grouped, rapidly growing, and often ulcerated large tumor(s). Histologically, dense cohesive infiltrates of predominantly large pleomorphic or anaplastic tumor cells which express CD30 and show a variable loss of T-cell markers. PC-ALCL does not have a t(2;5) and thus is negative for anaplastic lymphoma kinase (ALK). Rearrangements of the *DUSP22-IRF4* locus are found in approximately 25-30% of PC-ALCL and rearrangements of TP63 are very rare in PC-ALCL. In contrast to systemic ALCL, DUSP22-IRF4 rearrangemnt does not have a prognostic impact in PC-ALCL. CD30 expression is not restricted to CD30+ LPD, in which the vast majority of tumor cells express this marker. Therefore, other CTCL such as MF, particular in tumor stage, need to be distinguished from PC-ALCL and LYP by correlation of the histological findings with clinical presentation to achieve correct diagnosis within the group of CTCL. Despite relapses are common, PC-ALCL has a favorable prognosis with 5-year survival rate of 90%. Patients with extensive limb disease and multifocal PC-ALCL are at risk to develop extracutaneous spread and show impaired prognosis.

Surgical excision or radiation therapy is the first-line treatment for solitary or localized PC-ALCL. For patients with multifocal lesions, low-dose methotrexate (MTX; up to 25 mg/kg per week) or local radiotherapy in case of only a few lesions is recommended. BV is an

effective treatment with high response rates in patients who do not respond to MTX or develop extracutaneous spread to locoregional lymph nodes.[3,8] Multiagent chemotherapy is indicated only in patients with extracutaneous spread to visceral organs or CNS or lack of response to BV.

LYP presents with grouped or generalized papules and small nodules which show the characteristic spontaneous regression of the individual lesions within a few weeks. Histology shows a broad spectrum with five different subtypes (referred to as type A–E) and a subtype with chromosomal rearrangements involving the *DUSP-IRF4* locus on 6p25.3 listed in the revised WHO and updated WHO-EORTC classification.[1,2,4] Some of these histological types pose diagnostic difficulties as they simulate aggressive CTCL (e.g., type D and E). Thus clinical-pathological correlation is crucial for the diagnosis as LYP displays overlapping histological features with other CTCL forms. LYP carries an excellent prognosis with 5 and 10-year-survival rate of almost 100%. Nevertheless, LYP patients are at risk to develop a second lymphoma, especially MF and cutaneous or nodal ALCL which occurs in 15% of the patients.

In regard to the excellent prognosis, a watch-and-wait strategy for LYP can be justified. UV-light (UVB narrow band or PUVA) and low-dose methotrexate (5–20 mg per week) are the most common therapies, but relapses after withdrawal of treatment are common.[13] Thus, the effects of these therapies need to be balanced against long-term adverse effects. For patients with disseminated lesions and lack of response to MTX or UV-light therapies, BV represents a new therapeutic strategy and has been used with lower dose than in other CD30+ T-cell lymphomas. For larger lesions (i.e., 2–3 cm in diameter) which do not regress within 3 months, surgical excision or local radiotherapy can be applied.

Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a cytotoxic T-cell lymphoma defined by infiltrates of mostly CD8+ pleomorphic lymphocytes in the lobuli of subcutaneous fat tissue.[1,2] The neoplastic cells express TCR alpha/beta which distinguishes them from expression of TCR gamma/delta by the tumor cells in the subcutaneous form of gamma/delta T-cell lymphoma. SPTCL may affect children as well as adults and presents with subcutaneous plaques or nodules which involve preferentially the extremities. Regression of lesions results in focal lipatrophy. In addition to the skin lesions, systemic symptoms including fever, fatigue and weight loss, cytopenia, and elevated liver enzymes can be present. Some patients suffer from systemic lupus erythematosus. There are overlapping features between lupus panniculitis to SPTCL.

In most patients, SPTCL shows an indolent course with a favorable prognosis (5-year-SR: 80%).[1] In 15% of the patients, SPCTL is complicated by hemophagocytic lymphohistiocytosis (HLH), which is linked to an aggressive course with high mortality. Recently, germline mutations in HAVCR2 were found in 60% of SPTCL which result in loss of function or missense variants of T-cell immunoglobulin mucin 3 as a modulator of immune responses expressed on subgroups of T and innate immune cells.[14] This results in persistent immune activation and increased production of inflammatory cytokines which promote HLH and SPTCL. The hyperactive immune reaction in SPTCL may explain the fact that immunosuppressive therapies are effective in these patients. Immunosuppressive drugs such as steroids, MTX, and cyclosporine have replaced chemotherapy as first-line therapy for SPTCL, independent of the presence of HLH.[8]

CD8+ acral T-cell lymphoma (CD8+ ATCL) is a new provisional CTCL entity introduced for the first time in the revised WHO classification 2017 and updated WHO-EORTC classification 2018.[1,2] This lymphoproliferation manifests with a solitary or rarely bilateral nodule(s) at acral sites, that is, ears, face, and feet. Histology is characteristic and shows a dense dermal infiltrate of small to medium-sized atypical lymphocytes with moderate nuclear atypia.[15] There is no epidermotropism of neoplastic cells in CD8+ ATCL which differs from the epidermotropic infiltrates in CD8+ MF and CD8+ AECTCL. The tumor cells in CD8+ ACTL exhibit a characteristic phenotype with expression of CD3+, CD8+, and TIA-1+ in the absence of other cytotoxic markers such as granzyme B and perforin. In the CD68 stain, a characteristic perinuclear dot-like pattern is observed. The course is indolent with an excellent prognosis. Staging is not necessary in cases with typical clinical presentation and histology. CD8+ ACTL can be treated with surgical excision or radiotherapy.

3 | CONCLUSIONS

CTCL are characterized by distinct clinical features but show significant overlap in histological and phenotypic features. Thus clinical-pathologic correlation is of outmost importance in the diagnostic work-up of CTCL. MF and cutaneous CD30+ LPD represent the most common form of CTCL. The therapeutic approach for MF depends on disease stage and evolution. CD30 LPD exhibit a favorable prognosis. Therefore, unnecessary aggressive treatment should be avoided. Targeted therapy are novel and effective treatment modalities for advanced MF and in patients with therapy refractory CD30 LPD.

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

PEER REVIEW

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SUPPLEMENT ARTICLE

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What's new in peripheral T-cell lymphomas

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Abstract

Peripheral T-cell lymphomas (PTCLs) are a rare, heterogeneous group of hematological malignancies with extremely poor prognosis for almost all subtypes. The diverse clinicopathological features of PTCLs make accurate diagnosis, prognosis, and choice of optimal treatment strategies difficult. Moreover, the best therapeutic algorithms are still under debate due to the extrapolated approaches developed for B-cell lymphomas and to the absence of few treatment protocol specifically developed for PTCLs. Some advances have been made with CD30 monoclonal antibody, mainly for anaplastic large-cell lymphomas, with improvements in progression-free survival and overall survival. Several new drugs are under evaluation in clinical trials, although not all the results are as encouraging as expected. In this review, we briefly present the most updated information on diagnosis, prognostication, and treatment strategies in PTCLs.

KEYWORDS
chemotherapy, novel agents, peripheral T-cell lymphoma, prognostic factors

1 | INTRODUCTION

Peripheral T-cell lymphomas (PTCLs) comprise a heterogeneous subgroup of rare hematological malignancies originating from post-thymic lymphocytes. From different available data, PTCLs account for approximately 5%–15% of all lymphomas in Western countries, with an incidence of 0.5–2 per 100,000 people per year.[1,2] Four main clinical subtypes have been identified: nodal, leukemic, disseminated, and cutaneous PTCLs. From a pathologic point of view, the most recent edition of the World Health Organization (WHO) Classification of lymphomas identifies around 30 subtypes of PTCLs, now also defined as mature T-cell lymphomas (MTCLs), some of which are extremely rare. The most common MTCL subtypes are PTCL not otherwise specified (NOS), angioimmunoblastic T-cell lymphoma (AITL), anaplastic large-cell lymphoma (ALCL), and natural killer (NK)/T-cell lymphoma (NKTCL).[1,3,4] With the exception of cutaneous T-cell lymphomas (CTCL), which are usually characterized by an indolent course, and of anaplastic lymphoma kinase (ALK) positive ALCL, all other MTCLs are associated with an aggressive course and poor outcomes, with 5-year survival rates across subtypes of 30%.[3]

Improvement in the approach to MTCL is proceeding slowly, with advances in recent years seen in improving the classification of MTCL, patient management and prognostication, and treatment.

In the 2016 revision of the WHO of mature T-cell neoplasms, nodal lymphomas of T follicular helper (TFH) cell origin were introduced. AITL is the most studied TFH subtype, but an additional 40% of cases of PTCL-NOS have been shown to share some of the clinical and pathological features of the TFH phenotype, which requires the expression of at least two of three TFH-related antigens, including PD1, CD10, BCL6, CXCL13, ICOS, SAP, and CCR5. Recurrent genetic abnormalities associated with TFH phenotype include mutations of in epigenetic modifiers (TET2, IDH2, DNMT3A), RHOA, and T-cell receptor associated genes (PLCG1, CD28, VAV1, FYN).[3,5] Among the non-TFH PTCL-NOS, gene expression profiling and microRNA profiling studies have delineated two additional subgroups: those with an increased

expression of GATA3 and those with an increased expression of TBX21.[3,5] The GATA3 group is associated with poor outcomes and has more loss or mutation of tumor suppressor genes including TP53, PTEN, PRDM1, and CDKN2A/B, and gains in STAT3, REL, and MYC oncogenes.[6] The TBX21 group is enriched with alterations of genes involved in DNA methylation.[7] These three groups are added to other previously characterized subtypes with specific cell of origin, which include ALCL, adult T-cell leukemia/lymphoma, and gamma-delta PTCL. The better characterization of the cell of origin in PTCL is advantageous in the classification of these lymphomas as it reduces the undefined basket of PTCL-NOS and provides a strong rationale for determining the most effective therapies in these lymphomas. Immunomodulatory agents and epigenetic modifiers are more suitable for TFH subtypes, while phosphatidylinositol 3-kinase (PI3K) inhibitors, hypomethylating agents, and Janus kinase/signal transducer and activator of transcription (JAK/STAT) inhibitors might find a better role in GATA3 or TBX21 subtypes.[8]

1.1 | Prognosis and staging

In the last two decades, many studies have been conducted to identify and validate clinical and biological factors that can be used to predict the heterogenous outcome of PTCL patients. Several of these studies have confirmed that a poor Eastern Cooperative Oncology Group Scale of Performance Status score, extranodal involvement, advanced disease, bulky, Ki67, and a high lactate dehydrogenase rate correlate with shorter overall survival (OS).[9–11] The International Prognostic Index (IPI) was formally validated in PTCL[1,12] but the lack of clearly defined risk groups prompted the search for PTCL-specific prognostic indexes.[9,11] The Fondazione Italiana Linfomi defined the first Prognostic Index for PTCL-NOS (PIT), which stratifies patients into four distinct groups; the PIT showed an independent correlation with OS. Subsequently, PIT was updated to modified PIT by replacing bone marrow involvement with Ki67 rate expression.[10] A new model, the T-cell score, has recently been defined by using the prospectively collected data registered in the T-cell Project.[13] More recently, novel prognostic indexes have been identified and validated for specific PTCL subtypes, including enteropathy-associated T-cell lymphoma (EATCL) and nasal-type extranodal NKTCL. As shown in Table 1, all available prognostic indexes share the same structure of categorical scores based on simple laboratory and clinical features. Although all of these indexes have been formally validated, the accuracy of prognostication in PTCL remains suboptimal; thus, more prognostic studies that take into account novel biomarkers and novel prognostic features are warranted. Among recent advances, a better definition of response by means of ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) may play an important role in PTCL management and decision making.[14] PTCLs are listed among FDG-avid diseases, and several studies have already confirmed the role of metabolic tumor volume and of interim and end-of-treatment FDG-PET to predict outcomes.[15,16] Although promising, data regarding the role of metabolic response in PTCLs are very preliminary and thus need to be confirmed by larger studies.

1.2 | Treatment

The optimal management of patients with PTCL, which is disputed, is in any case limited to few options, all with unsatisfactory efficacy. None of the currently available recommendations are based on high-quality evidence, and few well-designed randomized clinical trials (RCTs) have been conducted to support therapeutic choices. The currently recommended treatment strategy for PTCLs derives mostly from B-cell lymphoma treatment strategies, with the recommended use of an aggressive approach with anthracycline-based polychemotherapy (i.e., CHOP or CHOEP) and with autologous stem cell transplant (autoSCT) to consolidate response to first-line therapy or to manage relapsed patients.[17]

Regarding the role of anthracyclines in PTCL, while their role is still debated, anthracycline void regimens have so far failed to demonstrate their superiority to CHOP.[17] Based on a recent meta-analysis, the 5-year OS achieved with this approach was 36.6%.[18]

Several attempts have been made to improve the poor results achieved with CHOP. These include the addition of novel agents and the intensification of therapy. Some clinical studies on etoposide intensification of standard CHOP have shown conflicting results. However, the addition of etoposide has shown better progression-free survival (PFS), especially in patients with ALCL, in those with favorable risk factors, and in patients age $\leq$ 60 years.[12,19–21]

The results of three randomized trials that evaluated the efficacy of adding a novel agent to the CHOP backbone are available.

One prospective trial combined alemtuzumab, an anti-CD52 monoclonal antibody, with CHOP; it failed to show improved outcomes compared to chemotherapy alone.[22] Another randomized trial compared standard CHOP with CHP (cyclophosphamide, vincristine, prednisone) combined with the anti-CD30 antibody-drug conjugate brentuximab vedotin (BV-CHP; ECHELON-2 trial).[23] This trial, which enrolled 452 treatment naïve CD30-positive PTCLs, demonstrated improved PFS and OS rates for the BV-CHP combination.[23] Most of the patients included in this trial (about 75%) had ALCL, with clearly positive results for this subtype. However, the scientific community was left without any clear demonstration of the efficacy of BV-CHP in non-ALCL CD30-positive subtypes.

A third trial compared CHOP with CHOP + the histone deacetylase inhibitor romidepsin, showing promising single-agent activity in relapsed refractory patients (ROCHOP trial).[24] The ROCHOP RCT conducted by the LYSA group enrolled 421 patients with PTCL who were not planned to receive autoSCT or allogeneic SCT (alloSCT). The median PFS (mPFS) for patients in the experimental arm was 12 months (9–25.8), without significant difference compared to the

TABLE 1 Prognostic models in PTCL

Variable	IPI International NHL Prognostic Factors Project (1993)	PIT Gallamini et al. (2004)	IPTCLP Vose et al. (2008)	mPIT Went et al. (2006)	TCS Federico et al. (2018)	AITL Hong et al. (2018)	PINK Kim et al. (2016)	EPI de Baaij et al. (2015)
Age > 60	X	X	X	X		X		X
ECOG ≥ 1	X	X	X	X	x			X
LDH (abn. values)	X	X		X			X	X
Stage III–IV	X				x	X	X	X
ENS > 2	X					X		X
BM+		X						
Plt < 150 K/mmc			X			X		
S-Alb					x			
Neutrophils					x			
Ki67 ≥ 80%				X				
Anemia (M < 13, F < 11g/dl)						X		
Serum IgA (>400 mg/dl)						X		
B symptoms							X	X
Regional lymph nodes							X	
PTCL subset	*All*	*PTCL-NOS*	*PTCL-NOS*	*PTCL-NOS*	*PTCL-NOS*	*AITL*	*NK-TCL*	*EATL*

Abbreviations: AITL, angioimmunoblastic T-cell lymphoma; BM, bone marrow; EATL, entheropathy-associated T-cell lymphoma; ENS, extranodal sites; EPI, EATL prognostic index; IPI, International Prognostic Index; ECOG, Eastern Cooperative Oncology Group; IPTCLP, International Peripheral T-cell Lymphoma Project; LDH, lactate dehydrogenase; mPIT, modified Prognostic Index for T-cell lymphoma; NKTCL, natural killer/T-cell lymphoma; PINK, Prognostic Index of natural killer lymphoma; PLT, platelet count, PTCL-NOS, peripheral T-cell lymphoma not otherwise specified; S-Alb, serum albumin; TCS, T-cell score.

reference arm (mPFS 10.2 months [7.4–13.2]; hazard ratio: 0.81; 95% confidence interval: 0.63–1.04). Although this study was not able to confirm the initial hypothesis of the superiority of Ro-CHOP, the subgroup analysis seems to suggest that the novel combination acts differently in different PTCL subtypes, with relatively higher activity observed for AITLs.[24]

In summary, even if associated with unsatisfactory results, CHOP chemotherapy should still be considered as the reference therapy for most PTCL subtypes with the main exception of ALCL for which BV-CHP is the preferred recommended option and of NKTCL. The use of CHOEP is supported by low quality of evidence but can be considered as a reasonable option in young and fit subjects with non-ALCL PTCLs.

The use of high-dose chemotherapy followed by autoSCT in first complete remission (CR1) is recommended by most of the available guidelines[17,25] (Table 2). Several groups have reported that achieving CR before autoSCT is a significant independent predictor of improved survival in patients with PTCL receiving upfront autoSCT.[26–28] However, there have been no RCTs specifically designed to evaluate upfront autoSCT in comparison with observation in CR1 for PTCL.[29–31] Several retrospective studies and prospective single-arm phase II trials have reported encouraging results with this approach (Table 3). The largest prospective phase II study, published by the Nordic Group (NLG-T-01), included 160 patients with PTCLs; 72% of patients underwent autoSCT in first remission after six courses of CHOEP chemotherapy.[32] All nodal PTCL subtypes were included, with the exception of A.

1.2.1 | ALK-positive ALCLs

One hundred thirty patients achieved CR (63%) or partial response (PR; 37%), and 115 (88.5%) underwent ASCT. Overall, the 5-year OS and PFS for the intention-to-treat population were 51% and 44%, respectively. Considering subtype distribution, better outcomes were observed for ALK-negative ALCL than for other subtypes.[32] In a second study by Reimer et al.,[33] 83 patients with PTCL were enrolled, with the exclusion of CTCL and of ALK-positive ALCL. Fifty-nine patients (71%) completed stem cell mobilization after CR (66%) or PR (34%) and 55 underwent autoSCT. The 3-year OS was 48%.

TABLE 2 ESMO and NCCN clinical practice guidelines for auto-alloSCT in PTCLs

	Primary diagnosed PTCLs		Relapsed/refractory PTCLs	
PTCLs subtype	***ESMO***	***NCCN***	***ESMO***	***NCCN***
PTCL-NOS	PR, CR, transplant eligible—autoSCT	Clinical trials, or ASCT, or observation if CR, or if PR—see rel/ref settings	PR, CR, transplant eligible—alloSCT (or ASCT)	PR, CR, transplant eligible—alloSCT (or ASCT)
AITL	PR, CR, transplant eligible—autoSCT	Clinical trials or ASCT or observation if CR, or if PR—see rel/ref settings	PR, CR, transplant eligible—alloSCT (or ASCT)	PR, CR, transplant eligible—alloSCT (or ASCT)
ALK-negative ALCL	PR, CR, transplant eligible—autoSCT	Clinical trials, or ASCT or observation if CR, or if PR—see rel/ref settings	PR, CR, transplant eligible—alloSCT (or ASCT)	PR, CR, transplant eligible—alloSCT (or ASCT)
ALK-positive ALCL	No further treatment, Or autoSCT if high-risk profile	Only chemotherapy ± ISRT	PR, CR, transplant eligible—alloSCT (or ASCT)	PR, CR, transplant eligible—alloSCT (or ASCT)
EATL	autoSCT	Clinical trials, or ASCT, or observation if CR, or if PR—see rel/ref settings	PR, CR, transplant eligible—alloSCT (or ASCT)	PR, CR, transplant eligible—alloSCT (or ASCT)
HSTCL	ASCT or allo if donor available	CR or PR—preferred alloSCT	PR, CR, transplant eligible—alloSCT (or ASCT)	Preferred alloSCT if eligible
ENKTCL	ASCT	Stage IV if CR—allo or ASCT	PR, CR, transplant eligible—alloSCT (or ASCT)	AlloSCT (or ASCT)

Abbreviations: AITL angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; alloSCT, allogeneic stem cell transplant; ASCT, autologous stem cell transplant; CR, Complete remission; EATL, entheropathy-associated T-cell lymphoma; ENKTCL, entranodal T-cell lymphoma; HSTCL, hepatosplenic T-cell lymphoma; ISRT, involved site radiotherapy; PR, partial remission; PTCL, peripheral T-cell lymphoma.

Some recently published studies provide additional insights into the role of autoSCT in CR1. A real-world data analysis from the Swedish Lymphoma Registry found prolonged OS and PFS for transplanted patients with PTCL-NOS, AITL, ALK-negative ALCL and EATCL after adjustment for confounding factors.[34] However, the selection of non-ASCT patients used as the control group may have been biased by early progressing patients after induction therapy. Another study was a large multicenter analysis conducted by the LYSA group. Among the 527 studied cases, a final cohort of 269 patients age less than 65 years with a CR or PR after induction chemotherapy was identified: 78 cases of PTCL-NOS, 123 cases of AITL, and 68 cases of ALK-negative ALCL. Overall, 81% were in CR and 19% in PR; 50% of the final cohort was allocated to autoSCT (134 patients). Neither the Cox multivariate model nor the propensity score analysis found any survival advantage in favor of autoSCT as a consolidation procedure for patients in response after induction therapy. Subgroup analyses did not reveal any further difference in terms of response status, disease stage, or risk category.[35] Recently, Park et al.[36] published their first report of the large prospective observational COMPLETE study, conducted by 56 U.S. academic centers. This paper described the outcomes of 119 patients who achieved CR after induction therapy, including 54 PTCL-NOS, 35 AITL, and 30 ALK-negative ALCL. Thirty-six patients underwent autoSCT; patients with AITL had significantly improved OS and PFS but patients with other PTCL subtypes did not. Finally, an exploratory of the ECHELON-2 trial was conducted, for the 82 patients with a declared intention to transplant out of the 177 patients randomized to BV-CHP arm (ALK-positive ALCL were excluded). SCT was in fact performed in 38 patients (27 ALK-negative ALCL and 11 non-ALCL patients), most of whom were from non-Asian centers, suggesting regional practice differences. Despite the fact that the ECHELON-2 study was not designed to evaluate the role of upfront consolidation with ASCT, numerical PFS estimates favored the use of consolidative SCT in patients with ALK-negative ALCL and with non-ALCL who achieved a CR at the end of induction after frontline BV-CHP.[23]

Interpreting the results from these studies on the role of autoSCT consolidation is complicated by the diverse eligibility criteria adopted, the suboptimal rates of transplantation among PTCL subtypes, and the differing rates of CR before autoSCT. The decision to proceed to autoSCT in a subject with PTCL who responds to first-line chemotherapy is difficult and should always be discussed with the individual patient. Researchers are strongly encouraged to run well-designed clinical trials that adopt the same up-to-date criteria for response definition (i.e., FDG-PET). These trials, which would necessarily require considerable international cooperation, would hopefully provide data by PTCL subtype.

alloSCT could be identified as alternative option to autoSCT as consolidation of CR1 patients. Schmitz et al.[37] recently published data from the first randomized phase 3 trial of auto versus alloSCT as part of first-line therapy in poor-risk PTCLs excluding ALK-positive

TABLE 3 Available prospective and retrospective studies of ASCT in PTCLs

Study	N	PTCLs subtype	Time of transplant	Response (%)	PFS (years)	OS (years)
Reimer et al. (2004) Prospective	83	39% PTCL-NOS 16% ALCL (ALK-negative) 33% AITL	Upfront	CR: 47 PR: 24	36% (4)	48% (4)
Corradini et al. (2006) Prospective	62	45% PTCL-NOS 30% ALCL (ALK-positive) 16% AITL	Upfront	CR: 56 PR: 16	EFS reported: 30% (12)	34% (2)
Rodriguez et al. (2007) Prospective	26	42% PTCL-NOS 31% ALCL (ALK-positive) 27% AITL	Upfront	CR: 65 PR: 8	53% (3)	86% (3)
Mercadal et al. (2008) Prospective	41	49% PTCL-NOS 29% AITL 5% HSTCL 5% T/NK	Upfront	CR: 49 PR: 10	30% (4)	39% (4)
d'Amore et al. (2012) Prospective	160	39% PTCL-NOS 19% ALCL (ALK-negative) 19% AITL 13% EATL 4% panniculitis-like 3% T/NK	Upfront	CR: 83 PR: 31	44% (5)	51% (5)
Fossard et al. (2018) Retrospective	134	34% PTCL-NOS 23% ALCL (ALK-negative) 43% AITL	Upfront	CR: 75 PR: 25	46.3% (5)	59.2% (5)
Roerden et al. (2019) Retrospective	58	25.9% AITL 22.4% EATCL 20.7% PTCL-NOS 19% ALCL (ALK-negative) 8.6% ALCL (ALK-positive) 3.4% T/NK	Upfront (40 pts) Relapse/refractory (18 pts)	CR: 75 PR: 25	Upfront ASCT 44% (5) ASCT in first relapse 60.6% (5)	Upfront ASCT 53% (5) ASCT in first relapse 77.4% (5)
Park et al. (2019) Prospective	36	42% PTCL-NOS 11% ALCL (ALK-negative) 47% AITL	Upfront	CR: 63 PR: 37	44% (5)	51% (5)

Abbreviations: AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large cell lymphoma; ALK, anaplastic lymphoma kinase negative; ASCT, auto stem cell transplant; CR complete response; EATCL, enteropathy-associated T-cell lymphoma; EATL, entheropathy-associated T-cell lymphoma; EFS, event-free survival; HSTCL, hepatosplenic T-cell lymphomas; OS overall survival; PFS, progression-free survival; PR, partial response; PTCLs, peripheral T-cell lymphomas; PTCL-NOS, peripheral T-cell lymphomas not otherwise specified; T/NK, T-cell lymphoma/natural killer.

ALCLs. The trial enrolled 18–60-year-old patients of all stages and IPI and was planned to detect an improvement of event-free survival at 3 years from 35% achieved with autoSCT to 60% by alloSCT in the intent-to-treat population. After the enrollment of 104 patient randomization and recruitment was prematurely stopped because a planned interim analysis had shown that it was highly unlikely to meet the primary endpoint. The transplant-related mortality observed contributed to this result. In conclusion, alloSCT cannot be recommended as consolidation therapy for CR1 PTCL patients due to the lack of evidence and because of it toxicity profile. The only

TABLE 4 Activity of novel agents from clinical trials in relapsed refractory PTCLs

Drug	PTCL subtype	No. of patients	Study phase	ORR; CR	Reference
Pralatrexate	PTCL	111	2	29%; 11%	O'Connor et al. (2011)
Romidepsin	PTCL	131	2	25%; 15%	Coiffier et al. (2012)
Brentuximab vedotin	CD30 + PTCL	34	2	41%; 24%	Horwitz et al. (2012); Pro et al. (2012)
	ALCL	58		86%; 57%	
Belinostat	PTCL	129	2	25.8%; 10.8%	O'Connor et al. (2015)
Bendamustine	PTCL	60	2	50%; 28%	Damal et al. (2013)
Mogamulizumab	CCR4 + PTCL/CTCL	29	1	34%; 17%	Ogura et al. (2014)
Lenalidomide	PTCL	39	2	26%; 8%	Tournishey et al. (2015)
Copanlisib	NHL	17	2	21%; 14%	Dreyling et al. (2017)
Cerdulatinib	PTCL	18	2a	43%	Horwitz et al. (2018)
Duvelisib	PTCL + CTCL	16	1	50%; 19%	Horwitz et al. (2018)
Alisertib	PTCL	271	3	33%; 18%	O'Connor et al. (2019)
Tipifarnib	AITL, CXCL12 + TCL	43	Interim analysis	45%	Witzig et al. (2019)
Pembrolizumab	PTCL	18		33%; 27%	Barta et al. (2019)
Panobinostat + bortezomib	PTCL	25	1	43%; 22%	Tan et al. (2015)
Gemcitabine + romidepin	PTCL	20	1	30%; 15%	Pellegrini et al. (2016)
Pralatrexate + romidepsin	TCL	14	1	71%; 29%	Amengual et al. (2018)
5-Azacytidine + romidepsin	PTCL	31	1	73%; 55%	O'Connor et al. (2019)

Abbreviations: AITL, angioimmunoblastic T-cell lymphoma; ALCL, anaplastic large-cell lymphoma; CCR4, chemokine receptor-4; CR, complete response; CTCL, cutaneous T-cell lymphoma; CXCL12, C-X-C motif chemokine 12; NHL, non-Hodgkin lymphoma; ORR, overall response rate; PTCL, peripheral T-cell lymphoma; TCL, T-cell lymphoma.

exception to this general statement might be represented by hepatosplenic T-cell lymphomas for whom a systematic review of 44 cases treated with alloSCT at first or second relapse demonstrated a 3-year relapse-free survival of 42% and OS of 56%.[38]

1.3 | Relapsed/refractory disease

Approximately 70% of patients with PTCL are expected to develop relapsed or refractory disease after first-line therapy.[39,40] A dismal outcome can be expected for these patients, with median OS of a few months, even for those who are able to proceed to salvage therapy.[39] Among the options available, the effectiveness of autoSCT in relapsed disease is uncertain due to the frequent use of autoSCT in CR1 in eligible patients, and because the salvage therapies available for relapsed PTCLs have very limited activity, thereby further reducing the feasibility of an autoSCT program when planned. Available salvage regimens were previously developed as a first-line strategies mostly for B-cell malignancies, from them well-known ICE (ifosfamide, carboplatin, etoposide), DHAP (high-dose cytarabine, cisplatin, dexamethasone), GDP (gemcitabine, cisplatin, dexamethasone), and ESHAP (etoposide, cytarabine, cisplatin, and methylprednisolone). Despite the fact that these regimens were previously studied for aggressive lymphomas, due to the rarity of PTCLs a small number of patients were included without independent subset analysis.[41–44] Even if limited by a low power due, a subset analysis of the Canadian Cancer Trials Group LY.12 randomized phase 3 study was not able to confirm DHAP superiority over GDP in PTCLs.[45] In relapsed/refractory PTCLs, alloSCT is also a feasible option in almost all subtypes after failing prior autoSCT.[17,25,46] However, nonrelapse mortality varies from 8.2% to 40%.[47–49] These scatter data suggest that it is necessary to carefully select possible candidates for alloSCT.

Several new agents have been tested in the relapsed refractory setting, and, while some have already received formal approval for clinical use, approval is not uniform across countries. These include the anti-CD30 antibody-drug conjugate BV, pralatrexate (approved in the United States only), and four histone deacetylase inhibitors (HDAC): romidepsin (United States only), belinostat (United States only), vorinostat (United States only), and chidamide (China only). Results achieved with these agents are very similar with CR rates of 10%–25% and with a median PFS of less than 1 year.

1.4 | Novel agents

Pharmacology research is very active in PTCL, and therapeutic development is mainly driven by advances in the understanding of the biology of the disease (Table 4).

The frequent alteration of the epigenetic machinery in PTCL, mainly of the TFH phenotype, justifies strong rationale for the search of novel HDAC inhibitors and to test the efficacy of combining more than one epigenetic modifier. A recent phase 1 combination trial of 5-azacitidine and romidepsin reported very interesting OR and CR rates of 73% and 55%, respectively.[50]

Inhibition of spleen tyrosine kinase (SYK) signaling and of PI3K pathways have also been investigated, with promising results from phase I and II studies. Cerdulatinib is an oral SYK, JAK1, JAK3, and Tyk2 inhibitor; in a phase IIa study on 41 PTCL patients, it was able to produce an overall response rate of 34%, with 27% CR rates.[51] Among PI3K inhibitors, the oral duvelisib was used in a phase I study with 16 PTCL and 19 CTCL patients. The overall response rate was 50% for the PTCL patients and the median PFS was 8.4 months.[45] The same agent has been evaluated in combination with romidepsin, showing greater activity in AITL and PTCL-NOS (overall response rates 74% and 64%, respectively, CR rates 63% and 36%, respectively).[52]

A promising therapeutic strategy in PTCL is represented by targeting of tumor microenvironment. Blocking the PD1 interaction with its ligand is justified by the finding of an increased expression of PD-L1 in both malignant and stromal cells of several PTCL subtypes. Indeed, some activity of anti-PD1 agents in PTCL has been described by phase I studies,[53] and more convincing results have been achieved with NKTCL. The use of PD1 blockers, however, has also been associated with cases of hyperprogression, thus making further clarification of PD1 inhibition in PTCL urgently needed.

Finally, cellular therapy based on the concept of chimeric antigen receptor T cells is also being developed for T-cell lymphomas,[54] as is the use of bispecific antibodies targeting both CD30 and CD16A.[55]

2 | CONCLUSIONS

Management of PTCL patients continues to be a real challenge for hematologist–oncologists. The oversimplified approach that has been used for many years, replicating the rules of B-cell lymphoma management, is clearly unsatisfactory and requires radical reassessment. New insights into the biology of the disease and a renewed interest on the part of the scientific community in the management of PTCL have led to the identification of new targets and to confirming the activity of new agents, which are now moving PTCLs into the era of targeted therapy. Moreover, taking into account the different biology and unique behavior of PTCL subtypes, each with a different response to therapy, has become indispensable; these differences result in more difficulties in interpreting the available data and in designing future trials. PTCL remains a challenging disease which requires massive international cooperation.

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CONFLICT OF INTERESTS

Luminari served as advisor for Roche, BMS, Jannsen, Regeneron, Genmab, Gilead. Skrypets has no conflict of interest to declare.

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SUPPLEMENT ARTICLE

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Recognizing but not harming. Borderline B-cell lymphoid proliferations

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Abstract

In the last years several borderline B-cell lymphoid proliferations have been recognized that lie at the interface between benign and malignant. These lesions can be divided in two groups; those considered precursor lesions of well recognized lymphoid malignancies and the group of indolent lymphomas with limited potential for progression. Precursor lesions are monoclonal and share many genetic features of their malignant counterpart. The first recognized precursor lesion was monoclonal gammopathy of undetermined significance. Thereafter, the widespread use of immunohistochemistry, fluorescence-activated cell sorting analysis and molecular techniques in lymphoid samples led to the recognition of other precursor lesions such as monoclonal B-cell lymphocytosis, in situ follicular neoplasia and in situ mantle cell neoplasia. The second group of disorders comprises monoclonal lymphoid proliferations with limited malignant potential without a counterpart among the currently recognized lymphoma entities; these include pediatric-type follicular lymphoma, pediatric nodal marginal zone lymphoma, and duodenal-type follicular lymphoma. Despite their clonal nature, a conservative treatment has been shown to be sufficient in most cases. The diagnostic criteria of precursor/indolent B-cell proliferations, recent advances in the understanding of progression and lymphomagenesis and current recommendations for treatment will be discussed. In order to avoid unnecessary and potentially harmful therapy, these lesions need to be recognized and diagnosed correctly.

KEYWORDS
B-cell lymphoma, early lesion, indolent lymphoma, precursor lesion

1 | INTRODUCTION

The paradigm that invasive cancer is frequently preceded by pre-malignant lesions has been proven in solid tumors. However, this concept is difficult to apply to lymphoid neoplasms because lymphocytes due to their innate properties recirculate through different lymphoid tissues and organs based on the patterns of normal lymphocyte homing.[1]

The increasing use of immunohistochemistry, fluorescence-activated cell sorting (FACS) analysis and molecular techniques on lymphoid tissue samples has led to the identification of a number of borderline B-cell lymphoid proliferations between benign and

TABLE 1 Precursor/indolent lesions in lymphoid neoplasias

Precursor lesions phenotypically and genotypically similar to malignant counterpart
Monoclonal gammopathy of undetermined significance
Monoclonal B-cell lymphocytosis
- Chronic lymphocytic leukemia (CLL) type
- Atypical CLL type
- Non-CLL type
In situ follicular neoplasia
In situ mantle cell neoplasia
Clonal lymphoid proliferations of limited malignant potential (B and T)
Duodenal-type follicular lymphoma
Pediatric-type follicular lymphoma
Pediatric nodal marginal zone lymphoma
Primary cutaneous marginal zone lymphoma
Primary cutaneous acral CD8+ T-cell lymphoma
Primary cutaneous CD4+ T-cell lymphoproliferative disorder (LPD)
Indolent T-cell LPD of the gastrointestinal tract
Lymphomatoid papulosis

malignant.[1] Some of these lesions represent precursor lesions and some clonal lymphoid proliferations with limited malignant potential (Table 1). The precursor lymphoid proliferations are often incidental findings in asymptomatic individuals with mostly indolent biological behavior. Importantly, these lymphoid proliferations are monoclonal and carry many of the molecular features of their malignant counterpart such as the *t*(14;18) and *t*(11;14) translocations associated to in situ follicular neoplasia (ISFN) and in situ mantle cell neoplasia (ISMCN), respectively. Other B-cell proliferations with low risk of progression include monoclonal B-cell lymphocytosis (MBL) of chronic lymphocytic leukemia (CLL)-type and the non-CLL type. In pediatric and young populations two clonal B-cell proliferations with apparently no risk of progression are recognized including pediatric-type follicular lymphoma (PTFL) and pediatric nodal marginal zone lymphoma (PMZL). There are some lymphoid proliferations that home to specific anatomic sites and remain localized such as duodenal-type follicular lymphoma (DFL).

2 | PRECURSOR LESIONS IN B-CELL LYMPHOMAGENESIS

2.1 | Monoclonal gammopathy of undetermined significance

Monoclonal gammopathy of undetermined significance (MGUS) is the paradigmatic example for the clonal evolution of lymphoid neoplasms.[2] It is defined as a serum monoclonal protein less than 3 g/dl, less than 10% monoclonal plasma cells in the bone marrow (BM) and absence of SLiM-CRAB criteria (≥60% clonal plasma cells, free light chain (FLC) ratio ≥100, >1 focal lesion on magnetic resonance imaging, hypercalcemia, renal insufficiency, anemia and lytic bone lesions). MGUS is a premalignant clonal disorder that progresses at a rate of 1% per year. The incidence of MGUS increases with age affecting 3%–4% individuals ≥50 years and 5% at ≥70 years. It is classified based on the involved immunoglobulin (IG) in IGM, non-IGM and light chain types. Non-IGM MGUS might progress to multiple myeloma (MM), solitary plasmacytoma or amyloid light chain disease, whereas IGM MGUS has an increased risk to progress to Waldeström macroglobulinemia (WM) or CLL. Interestingly, MGUS, smoldering MM (SMM) and MM share cytogenetic alterations including major translocations involving the IGH gene and numerical chromosomal abnormalities (CNAs). Nevertheless, there seems to be a temporal acquisition of CNAs, some of which are more prevalent at later stages such as del13q, indicating that they may be secondary events. Similarly, although *t*(11;14) is observed in MGUS and MM, *t*(4;14) and *t*(14;16) are more common in SMM and MM. MGUS has a less complex genomic landscape carrying rarely MM-associated somatic mutations. Furthermore, *MYC* translocations and *TP53* deletions and mutations are not observed in MGUS, suggesting that these are drivers of progression. The most common CNAs for IGM MGUS/WM are del6q, +18q, trisomy 4, 5, 12, and monosomy 8. The most frequent mutation is *MYD88* L265P, followed by mutations in *CXCR4* and *KMT2D*, akin to manifest WM.

A major advance in the understanding of MM pathogenesis was the recognition of the dynamic interaction between the tumor cells

and the surrounding microenvironment. The permissive BM microenvironment favors clonal selection and progression from MGUS to MM. The BM microenvironment seems to exert a selective pressure that promotes the expansion of subclones with selective advantage and facilitates progression to symptomatic disease. Additionally, the immunosuppressive BM microenvironment through expansion of regulatory T-cells and T-helper 17 cells determines whether MGUS remains stable or progresses to MM.

There are several risk stratification models to predict MGUS progression. The most widely used is the "Mayo Clinic model": (1) M-protein greater than 1.5 g/dl, (2) non-IgG isotype, and (3) FLC ratio less than 0.26 or more than 1.65, which stratifies patients from low to high risk. High risk patients have all three risk factors and a 20-years progression risk of 58%. Currently, there is no recommended treatment for MGUS except for careful monitoring for progression to enable early detection and intervention.[3] An exception is what has been called "MGCS." This refers to patients with MGUS criteria but experiencing symptoms and signs related to the nerves, skin and kidney causing serious disease. Treatment recommendations include IV IG, rituximab, and plasma cell-directed therapy.[4] There are promising clinical trials investigating different therapeutic strategies to prevent progression from high-risk MGUS to symptomatic disease including a phase II trial with daratumumab.[3] Introduction of novel therapies that seem to be both safe and effective are challenging the belief that MM should only be treated when symptomatic.

2.2 | Monoclonal B-cell lymphocytosis

MBL is defined as a monoclonal B-cell count less than 5000/μl in peripheral blood (PB), persisting for at least 3 months in otherwise asymptomatic individuals.[5] Based on its clinical significance, MBL is divided into high-count (500–5000/μl) and low-count MBL (<500/μl); the latter without risk to develop CLL, and therefore, no follow-up is required. MBL is classified into three categories based on its phenotype: (1) CLL type, (2) atypical-CLL type, and (3) non-CLL type.

(1) *MBL of CLL type* is the most common (75%), and has an identical phenotype to CLL (CD19+, CD20dim, CD23+, CD5+, LEF1+). Among healthy individuals, MBL has a reported incidence between 3% and 12%, depending on the age. CLL is always preceded by MBL, which is considered a premalignant condition with a progression rate to CLL of 1%–2% per year; however, only a minority of MBL cases will eventually progress into CLL.[1] The number of MBL cells at presentation seems to be the highest risk factor for progression. It is now well established that progression from MBL to CLL is due to the combined effect of genetic defects and its interaction with the microenvironment.[1] MBL cases usually have mutated IGHV, and carry the same genetic alterations as seen in CLL including del13q, trisomy 12, del11q, and del17p, although at lower levels suggesting that these changes occur early in clonal evolution. Point mutations in *TP53*, *NOTCH1*, *ATM*, and *SF3B1* genes are also detected in MBL but mostly as subclones.[6,7] Current recommendations include yearly monitoring for early intervention if clinically indicated.

(2) *MBL with atypical CLL phenotype* shows CD20 bright by FACS analysis and often is CD23 negative but express CD5. The possibility of a mantle cell lymphoma (MCL) should be excluded.

(3) *MBL of non-CLL type* is characterized by CD20+, CD19+ B cells with moderate to bright surface IG expression but CD5 negativity (or CD5 dim in 20% of cases), a phenotype that has been related to marginal zone (MZ) lymphoma. In PB the lymphocytes show a broad cytological spectrum with variable numbers of villous lymphocytes and lymphocytes with plasmacytoid differentiation. BM biopsy shows always B-cell infiltrations of various degrees. Most patients remain stable without progression but around 17% will develop splenomegaly and laboratory findings suggestive of splenic MZ lymphoma or splenic B-cell lymphoma unclassifiable. The term CBL-MZ origin has been proposed.[8] The immunogenetic signature of these cases is strongly indicative of antigen selection with highly mutated IG genes (76%) and predominance of the IGHV4-34 gene (23%). Up to 23% of the cases show complex karyotype with frequent del7q and isochromosome 17q. Mutational analysis is limited to few cases; however, *NOTCH2* (13%), *TNFAIP3* (6%), and *CD79B* (6%) gene mutations have been described.[9] Interestingly, *MYD88* mutations were identified in 25% of cases, especially those cases secreting IGM; the differential diagnosis with WM is mandatory. Accordingly, it is recommended to evaluate individuals at diagnosis with detailed immunophenotype, cytogenetics (fluorescence in situ hybridization), *MYD88* gene mutation screening and imaging. The role of BM biopsy at diagnosis is debatable since there is no correlation between the extent of BM and PB infiltration. Long follow-up is recommended but treatment should only be considered in cases with evidence of progression.

2.3 | In situ follicular neoplasia

FL is the second most common B-cell lymphoma in adults and accounts for about 20% of all lymphomas. Most patients have widespread disease at diagnosis with BM involvement in 40%–70% of the cases. FL cells acquire resistance against apoptosis through the chromosomal translocation *t*(14;18)/*BCL2*/IGH present in around 85% of the cases. Using immunohistochemistry and highly sensitive molecular techniques two putative FL precursor forms have been identified.[10] (1) FL-like B cells carrying the *t*(14;18) translocation can be identified in the PB in up to 55% of healthy individuals. These cells increase with age, smoking, and exposure to pesticides. Most *t*(14;18)-positive clones are long-lived and can persist for several years, indicating that the deregulated BCL2 expression is not sufficient for the development of manifest (m)FL. FL-like B cells have high levels of somatic hypermutation (SHM) and class switch recombination (CSR) suggestive of intense trafficking in the germinal center (GC). Importantly, patients who develop mFL seem to have a higher

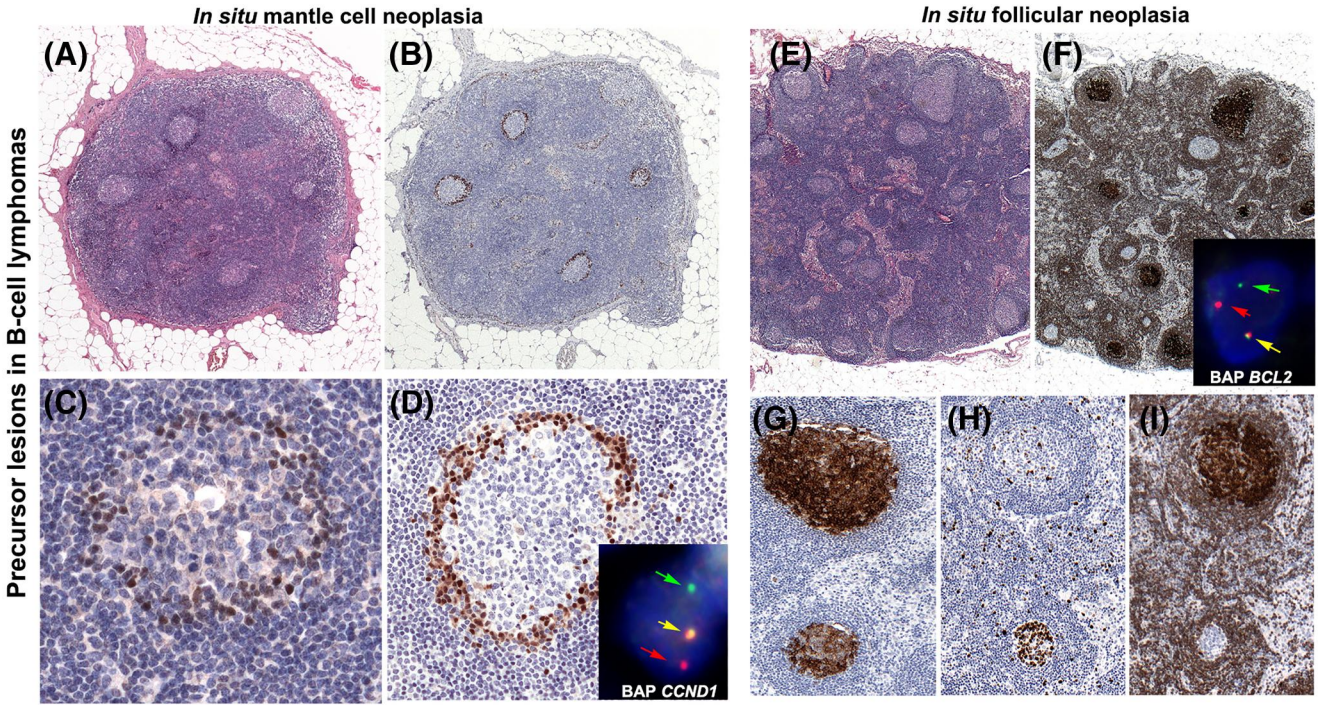

FIGURE 1 Precursor lesions in B-cell lymphomas. *In situ mantle cell neoplasia*. (A) Panoramic view of a normal-appearing lymph node (hematoxylin and eosin [H&E], ×25). (B) Cyclin D1 highlights the presence of cyclin D1+ cells in the inner cuff of the mantle zone (immunohistochemistry, ×25). (C) The cells are also positive for the transcription factor SOX11 (immunohistochemistry, ×200). (D) Higher magnification demonstrates a specific nuclear staining for cyclin D1 (immunohistochemistry, ×200). Insert: Fluorescence in situ hybridization (FISH) analysis using a *CCND1* break-apart probe (BAP) demonstrates one normal colocalized signal (yellow arrow) and two separate signals (red and green arrows) indicative of a translocation. *In situ follicular neoplasia*. (E) Panoramic view of a lymph node stain with H&E showing normal architecture (original magnification, ×25). (F) BCL2 stain shows that several germinal centers are replaced by lymphoid cells with strong BCL2 expression (immunohistochemistry, ×25). Insert: FISH analysis using a *BCL2* BAP demonstrates one normal colocalized signal (yellow arrow) and two separate signals (red and green arrows) indicative of a translocation. (G) The cells are strongly positive for CD10. (H) MIB1 stain shows an abnormally low proliferation in the upper follicle with a normal polarized proliferation in the lower follicle. (I) BCL2 stain shows the abnormal expression of BCL2 in the upper follicle, stronger than the normal B cells of the mantle zone and reactive T cells. In contrast in the lower follicle the germinal center remains BCL2 negative (G–I, immunohistochemistry, ×200)

number of circulating FL-like B-cells many years before the diagnosis of mFL compared with controls.

(2) ISFN is a precursor lesion considered to be a tissue manifestation of FL-like B cells, which show the genetic and immunophenotypic features of FL, but localized to the GC of reactive-appearing lymph nodes (LN) (Figure 1). ISFN can only be identified by BCL2 immunostain, is monoclonal and carries the *t*(14;18) translocation. The incidence of ISFN is unknown, but studies of unselected LNs from adults revealed a prevalence of 2%, and the rate for progression to mFL has been estimated to be between 2% and 3%. Nevertheless, because 15%–20% of ISFN cases are diagnosed in a LN involved by another B-cell lymphoma (not only FL), it is recommended to have complete staging workup, including imaging studies and BM biopsy to rule out concurrent lymphoma. No treatment is needed.[11]

ISFN represents a FL precursor lesion with no or few CNAs.[11] Nevertheless ISFN shares many genetic features with mFL including early introduction of N-glycosylation sites substituting conventional antigen binding favoring the generation of long-lived clones and increasing the chances of secondary hits. Accordingly, ISFN carry mutations in *CREBBP*, *EZH2*, *TNFRSF14*, and *KMT2D* but at lower frequencies when compared to paired mFL.[12] The putative model of FL lymphomagenesis suggests that the *t*(14;18) is the first hit resulting from a repair error during the IGH variable diversity joining region recombination process in the BM leading to the accumulation of cells that will undergo repetitive rounds of GC reaction, acquiring additional hits by SHM and CSR (FL-like B cells). These cells acquire functional N-glycosylation sites that will interact with M2-polarized macrophages in the GC that retain the cells in the GC (ISFN), demonstrating the importance of the interaction of activated B-cell receptor (BCR) and its microenvironment. These cells will undergo clonal expansion and evolution with acquisition of CNAs and chromatin modifier gene mutations necessary for final transformation into mFL. In contrast to MGUS and MBL, there are no predictive biomarkers available to know which individuals will progress from ISFN to mFL.

2.4 | In situ mantle cell neoplasia

MCL represents around 6%–9% of all B-cell lymphomas. At diagnosis most patients present with disseminated disease with frequent

infiltration of the gastrointestinal tract and BM. MCL is characterized by the *t*(11;14)/*CCND1*/IGH translocation in more than 90% of cases with the residual 10% showing mostly cryptic *CCND2/IG* or *CCND3*/IG translocations.[6] The phenotype includes the expression of B-cell markers (CD19, CD20) together with expression of CD5 and SOX11 in most nodal cases. Rare cells with *t*(11;14) translocation have been detected in up to 7% of healthy individuals in PB confirming that the *CCND1*-IGH translocation is an early event in mantle cell lymphomagenesis. MCL-like cells and ISMCN are seen at a significantly lower frequency than precursor lesions in FL. ISMCN is a rare incidental finding in lymphoid tissue (<1%) characterized by the presence of atypical cyclin D1+ cells located within the 3–5 inner layers of the mantle cuffs in an otherwise normal LN (Figure 1). ISMCN is less characterized than ISFN; however, these two precursor lesions seem to have in common the dependency on activated BCR and the interaction with the microenvironment crucial for homing to their natural niche.[1] The prolonged and repeated antigen stimulation is believed to trigger the accumulation of genetic and epigenetic aberrations in the MCL precursor cells, which eventually progress to an overt lymphoma. Current recommendations include staging with imaging and BM biopsy to rule out a concurrent overt lymphoma. No treatment is required and follow-up is advised. The risk to progression is unknown.

3 | INDOLENT LOCALIZED B-CELL LYMPHOMAS

3.1 | Duodenal-type follicular lymphoma

DFL is a FL variant with unique clinical features. Patients present with small polypoid lesions confined to the mucosa and submucosa often in the region of the ampulla of Vater but can also involve jejunum, ileum, and even colon. The clinical course is indolent with a risk of progression and dissemination of about 3%. The overall survival at 10 years is 100% with limited or no treatment. The diagnosis of DFL is often incidental and detected upon endoscopy for other unrelated causes.[13] Histologically, the lesion is characterized by well circumscribed follicles composed almost entirely by centrocytes that also infiltrate the lamina propria with the same phenotype as FL (CD10+, BCL2+, and BCL6+) (Figure 2). Some studies have shown disrupted follicular dendritic cell meshworks and reduced or lack activation-induced deaminase expression compared to nodal FL. DFL is monoclonal and carries the *t*(14;18) translocation. The expression of α4β7 integrin by the tumor cells seem to be responsible for the peculiar homing of these cells to the intestinal mucosa, a homing receptor observed in mucosa-associated lymphoid tissue lymphomas. Similar to ISFN, DFL has low genomic complexity with frequent mutations in *CREBBP*, *TNFRSF14*, *KMT2D*, and *EZH2*. Multiple *KMT2D* mutations as seen in advanced FL are absent. Gene expression profiling suggested that the immune microenvironment of DFL is distinct from nodal FL and characterized by a chronic inflammatory signature relevant to the pathophysiology of this disease. DFL and ISFN are considered early/precursor lesions, clinically indolent with low risk of progression. In fact DFL most probably represents a form of in situ FL of the gastrointestinal tract. The Lugano staging system is recommended to evaluate the potential of progression. If nodal involvement is present (Stage II) there is a higher rate of progression. DFL should be distinguished from conventional FL with intestinal involvement. In such cases, mesenteric LN involvement is often present. There is no significant difference in survival or time to progression between treated and untreated patients, which supports the view that "watch and wait" strategy is preferred to therapeutic intervention.[14]

3.2 | Pediatric-type follicular lymphoma

PTFL is a FL variant that differs clinically, morphologically, and genetically from the adult counterpart. Although this lymphoma predominates in children and adolescents, similar cases have been described in adults.[15] PTFL is recognized as an indolent disease affecting predominantly young males (M:F ratio of 20:1) with a median age of 15 years, and presenting with isolated lymphadenopathy in the head and neck regions.[16] Morphologically, it is characterized by partial or total LN effacement by expansile, serpiginous follicles with a prominent "starry-sky" pattern but without polarization. In some cases, evidence of MZ differentiation may be seen in the periphery of the neoplastic follicles. The cells within the follicles show high-grade cytology (Grade 3) and express strongly CD10 and BCL6. BCL2 expression is usually absent, but around 20% of the cases show weak positivity. PTFL is always monoclonal, which is required for the diagnosis to exclude "atypical" follicular hyperplasia. The tumor cells lack the characteristic *t*(14;18)/*BCL2*/IGH translocation of conventional FL. Overall, PTFL lacks mutations of histone modifying genes frequently found in FL and shows low levels of genomic complexity.[17] Recurrent genetic alterations of potential importance for its pathogenesis that disrupt pathways associated with the GC reaction (*TNFRSF14*, *IRF8*), immune escape (*TNFRSF14*) and anti-apoptosis (*MAP2K1*) have been described.[15,18] Complete staging involving BM biopsy and lumbar puncture is standard care for children. Most patients presenting with localized disease have an excellent response with surgery alone (completed resected lesion) without further therapy. The actual recommendation is "watch and wait" after resection. For patients with limited stage disease without complete resection two to four courses chemotherapy according to protocols for children with mature B-cell lymphomas is the standard of care. Relapses are very rare. The overall survival of children and adolescents with PTFL is 100% in all published series.[19]

3.3 | Pediatric nodal MZ lymphoma

PMZL is uncommon and shows remarkably overlapping clinical and pathological features with PTFL. It is more common in males under 18 years of age and presents as asymptomatic isolated lymphadenopathy commonly in the head and neck region. Histologically,

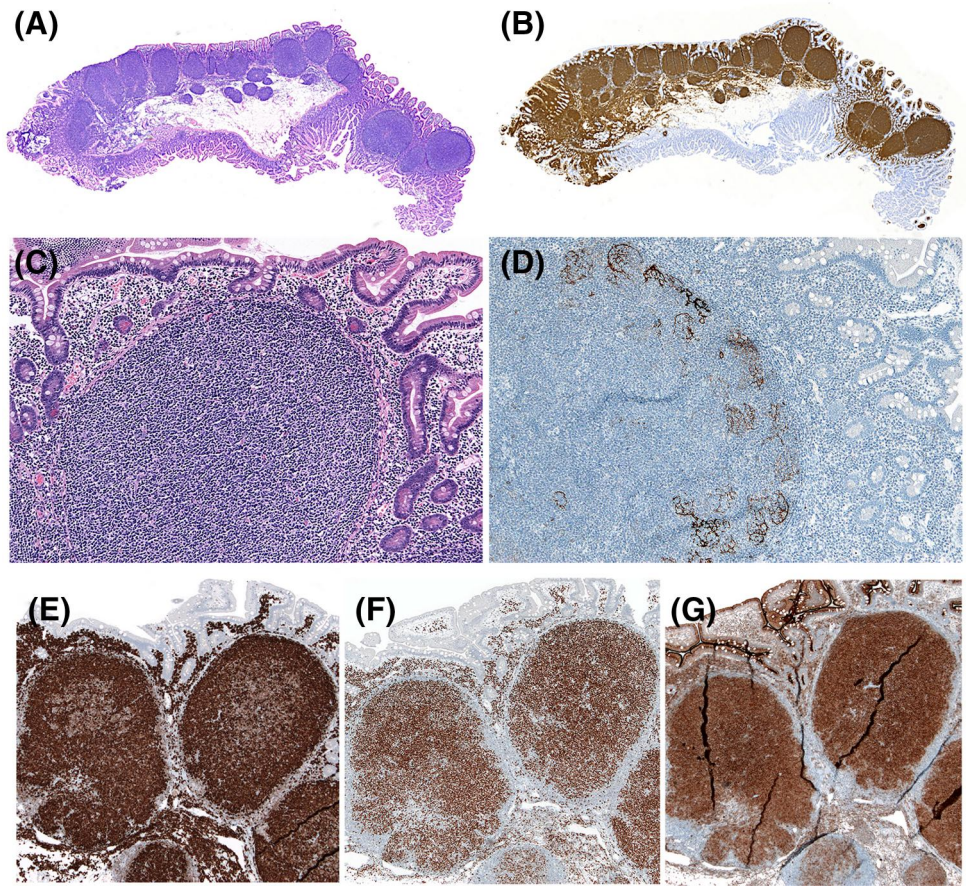

FIGURE 2 Duodenal-type follicular lymphoma. (A) Aggregates of neoplastic follicles mainly restricted to the duodenal mucosa (hematoxylin and eosin [H&E], snap-shot of a scanned slide). (B) Strong and homogeneous CD20 positivity in the follicular structures and in the lamina propria (immunohistochemistry, snap-shot of a scanned slide). (C) At higher magnification the follicles are replaced by mainly centrocyte-like cells (Grade 1) (H&E, ×100). (D) CD23 stain highlights the abnormal distribution of follicular dendritic cells in the periphery of the neoplastic follicles (immunohistochemistry, ×100) The tumor cells are positive for (E) BCL2, (F) BCL6, (G) and CD10 (immunohistochemistry, ×50)

residual follicles in PMZL are often present and show fragmentation due to infiltration of the mantle cells into the remaining GC resembling progressive transformation of germinal centers (PTGC). The mantle zone is expanded and can be highlighted by IgD staining, which in addition, emphasizes the PTGC-like changes. In contrast to PTFL, there is interfollicular infiltration of CD10-, BCL6-tumor cells and diffuse areas. Staining for CD279/PD1 shows numerous cells in the reactive GCs, a feature that is useful in the differential diagnosis with PTFL. Monoclonality is detected in all cases. No recurrent mutations have been identified. These cases show low levels of genomic complexity with few genetic aberrations such as trisomy 18 and trisomy 3. Similar to PTFL a "watch and wait" strategy is recommended after resection.[19]

4 | CONCLUSIONS

The precursor lesions discussed here have unique clinical, morphological, immunophenotypical, and genetic features. Nevertheless, MBL, ISFN, and ISMCN share some biological features, such as dependency on BCR activation and the important interaction with the microenvironment. These precursor lesions can be detected in healthy individuals and in general have a low risk of progression to a malignant lymphoma. The complex interplay between genetic alterations, BCR activation and microenvironment seems to be responsible for progression to an overt lymphoma. Patients with these precursor lesions should be staged at diagnosis but treatment is recommended only if progression occurs, although this dogma might change in the future. Indolent lymphomas such as DFL, PTFL, and PMZL represent challenging diagnoses sometimes difficult to separate from reactive conditions. The understanding and definition of these disorders have increased tremendously in the last 5 years. For PTFL and PMZL complete surgical resection followed by "watch and wait" strategy seems an adequate therapy with low-intensity chemotherapy restricted to incompletely resected disease. Finally, the most important take home message is to recognize these lesions and avoid unnecessary and potentially harmful therapy.

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CONFLICT OF INTERESTS

The author declares that there is no conflict of interests.

PEER REVIEW

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SUPPLEMENT ARTICLE

Sequencing of myeloma therapy: Finding the right path among many standards

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In the last decade, major changes have occurred in the diagnostic criteria, staging system, and response criteria for multiple myeloma (MM).[1,2] These changes have been accompanied by several advances in treatment of the MM, including many new drugs (carfilzomib, pomalidomide, daratumumab, elotuzumab, panobinostat, ixazomib, selinexor, isatuximab, and belantamab). Numerous clinical trials provide data on best practices along the spectrum of the disease. The purpose of this article is to describe the complexity of MM therapy in a landscape where many parallel standards exist for the same indications, and to provide an outline selecting the first line and subsequent sequencing of therapy according to dynamic changes in the disease and patient features.

1 | INTIAL THERAPY

Approach to initial therapy in myeloma is affected by host factors (age, performance status, renal function), eligibility for stem cell transplantation, and presence or absence of high risk features. High-risk MM is defined by the presence of *t*(4;14), *t*(14;16), *t*(14;20), deletion 17p, gain 1q, or p53 mutation.[3] Double-hit MM refers to the presence of any two or more high-risk abnormalities. Triple-hit MM refers to the presence of three or more high-risk abnormalities. Although minimal residual disease (MRD) negative status is associated with improved progression-free survival (PFS) and overall survival (OS), there are no data from randomized trials that modifying therapy in MRD positive patients in an attempt to make them MRD negative will lead to better outcomes.

The current algorithms for the treatment of symptomatic newly diagnosed MM is shown in Figure 1. Patients who candidates for autologous stem cell transplantation (ASCT) are treated with three to four cycles of induction therapy followed by stem cell harvest. After stem cell harvest, the standard of care has been ASCT followed by maintenance. However, in selected patients who have standard risk MM, ASCT can be delayed until relapse, and randomized trials show no detrimental effect on OS with such an approach.

The preferred initial therapy is bortezomib, lenalidomide, and dexamethasone (VRd).[4] The 4-year OS rate with VRd is greater than 80% with or without early ASCT.[5] An important alternative to VRd in newly diagnosed MM is daratumumab, lenalidomide, and dexamethasone (DRd). DRd has shown significant efficacy in a randomized trial conducted in transplant ineligible patients, with improved PFS compared with Rd.[6] However, there are important differences between the two approaches, particularly in transplant ineligible patients where VRd consists of triplet therapy for 6 months followed by maintenance, while DRd requires continuous triplet therapy until disease progression. Thus due to overall cost, and strength of long-term data, I prefer VRd over DRd for most patients.[7] However, DRd is a suitable alternative for patients with preexisting neuropathy or for patients who have intolerance to VRd. In high-risk transplant eligible patients with double hit MM or triple hit MM, I recommend addition of daratumumab to the standard VRd regimen (Dara-VRd), or the daratumumab, bortezomib, thalidomide, and dexamethasone (Dara-VTd) regimen.[8] There is no significant benefit with carfilzomib, lenalidomide, and dexamethasone (KRd) over VRd in newly diagnosed MM.[9] KRd is more expensive, and is associated with a higher risk of serious cardiac, renal, and pulmonary toxicity than VRd.

In certain settings, the treatment regimens for newly diagnosed MM must be modified. Thus, bortezomib, cyclophosphamide, and dexamethasone (VCd) is the preferred regimen in patients presenting with acute renal failure due to light chain cast nephropathy. In patients with primary plasma-cell leukemia multiagent combination chemotherapy such as bortezomib/dexamethasone/thalidomide/cisplatin/doxorubicin/cyclophosphamide/etoposide (VDT-PACE) is usually needed initially to achieve rapid disease control. In elderly frail patients who are unable to travel to receive parenteral therapy, the all oral regimen of ixazomib, lenalidomide, and dexamethasone

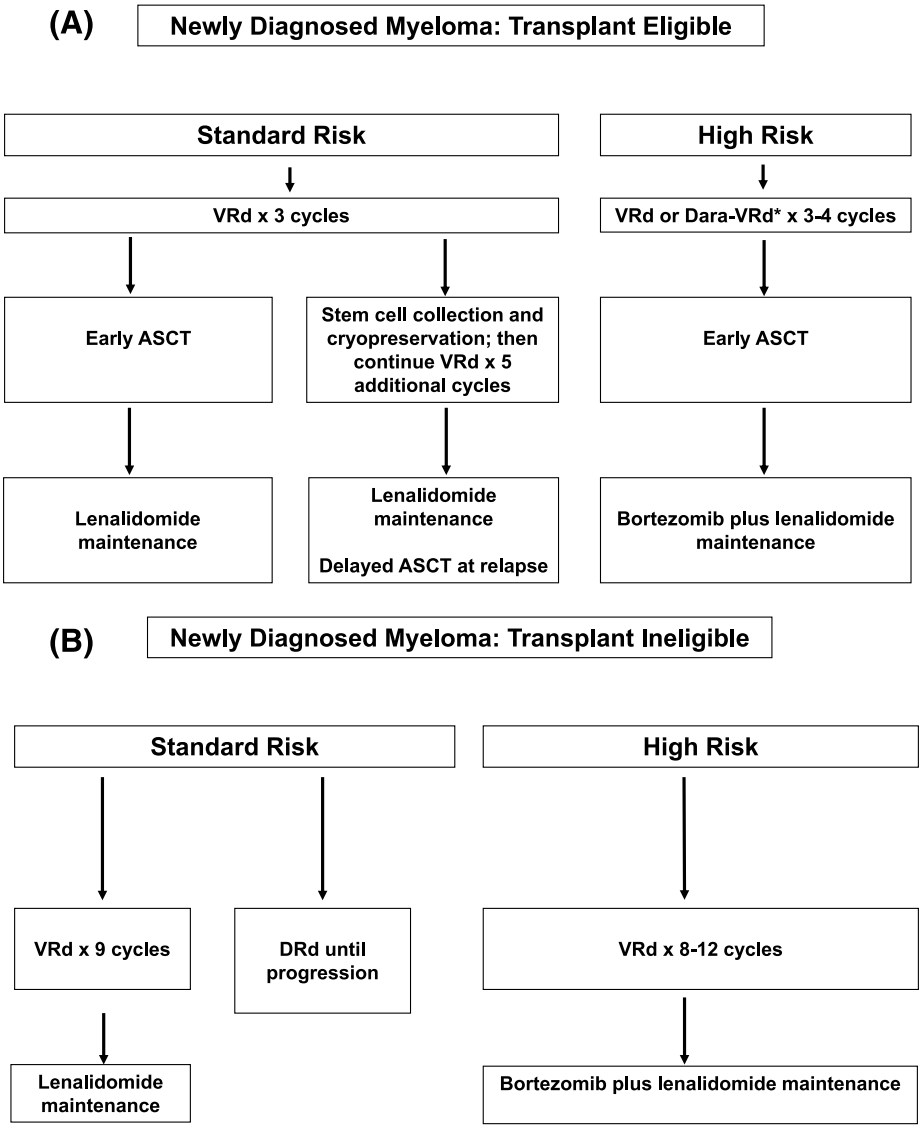

FIGURE 1 Approach to the treatment of newly diagnosed myeloma in transplant eligible (A) and transplant ineligible (B) patients. ASCT, autologous stem cell transplantation; Dara-VRd, daratumumab, bortezomib, lenalidomide, dexamethasone; DRd, daratumumab, lenalidomide, dexamethasone; VRd, bortezomib, lenalidomide, dexamethasone. Modified from Rajkumar and Kumar[2]

(Ixa-Rd) is a reasonable alternative to VRd and DRd. Melphalan-based regimens are no longer recommended for newly diagnosed MM due to concerns about stem cell damage, secondary myelodysplastic syndrome, and acute leukemia.

1.1 | Timing of stem cell transplantation

One of the main controversies in initial therapy is concerning the sequencing of ASCT. Older randomized trials have found similar OS with early ASCT (immediately following four cycles of induction therapy) versus delayed ASCT (at the time of relapse as salvage therapy). More recently, the Intergroupe Francophone du Myelome (IFM) trial found no significant OS difference between early versus delayed ASCT in patients treated with VRd as initial therapy and lenalidomide maintenance.[10] No difference has emerged even after 8 years of follow-up. There is a significant improvement in PFS as expected with early ASCT, and there are other logistical benefits to ASCT. In general, early ASCT is preferred, but based on the IFM results it is reasonable to consider a delayed ASCT in patients with standard-risk multiple MM who prefer such an approach for personal reasons.

The role of consolidation therapy and tandem (double) ASCT is limited. Results of randomized trials are contradictory and likely reflect the availability of new treatment options in the salvage setting. In the United States, where multiple options for salvage therapy are available, there seems to be no benefit with tandem ASCT. At present, outside of a clinical trial setting, we consider tandem ASCT only in selected young patients with del 17p.

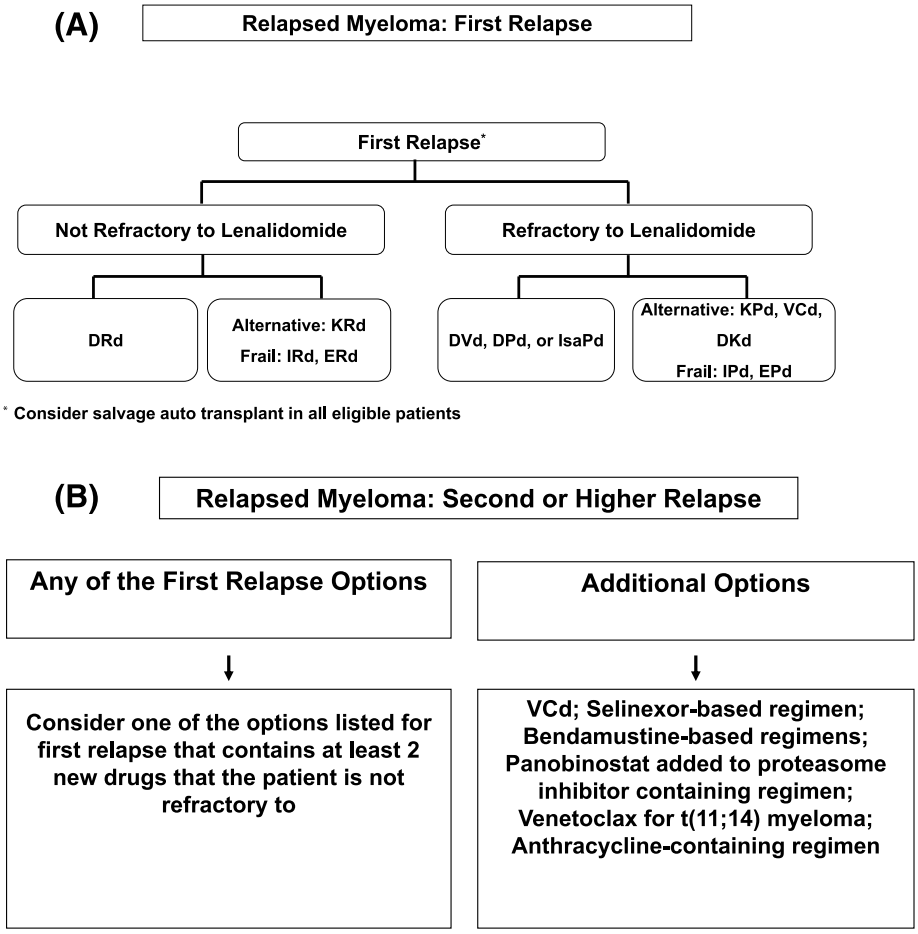

FIGURE 2 Approach to the treatment of relapsed multiple myeloma in first relapse (A) and second or higher relapse (B). DKd, daratumumab, carfilzomib, and dexamethasone; DPd, daratumumab, pomalidomide, and dexamethasone; DRd, daratumumab, lenalidomide, and dexamethasone; DVd, daratumumab, bortezomib, and dexamethasone; ERd, elotuzumab, lenalidomide, and dexamethasone; IPd, ixazomib, pomalidomide, and dexamethasone; IRd, ixazomib, lenalidomide, and dexamethasone; KPd, carfilzomib, pomalidomide, and dexamethasone; KRd, carfilozomib, lenalidomide, and dexamethasone; VCd, bortezomib, cyclophosphamide. Modified from Rajkumar and Kumar[2]

1.2 | Maintenance therapy

The standard of care following initial therapy MM is lenalidomide maintenance. In high-risk patients, bortezomib plus lenalidomide, or VRd maintenance is preferable. There are limited data on optimal duration of maintenance. Many patients can benefit from a drug-free interval, and trials are examining if the duration of maintenance can be modified based on MRD results.

1.3 | Relapsed MM

MM is characterized by multiple remissions and relapses. The sequencing of therapies appropriately is critical in achieving the best long-term survival. Many patients with MM receive five or more lines of therapy in a sequential manner over several years. The choice of treatment at each relapse is affected by the Timing of the relapse, Response to prior therapy, Aggressiveness of the relapse, and Performance status. Patients are eligible for ASCT should be considered for transplantation if they had elected to delay the procedure, or if they achieved excellent remission duration with the first ASCT, defined as a remission of 36 months or longer with maintenance.

In order to provide the best sequential therapy, we need to select the most active regimen early on. A triplet regimen is preferred at first relapse. At each subsequent relapse, a triplet, quadruplet, or multidrug regimen that contains at least two new drugs that the patient is not refractory to should be used. The algorithm for the treatment of relapsed MM is given in Figure 2.

Treatment is typically continued until disease progression. However, based on tolerability and response, increasing the interval between cycles, as well as treatment-free intervals should be considered.

At first relapse, my preferred option is DRd for patients who are not refractory to lenalidomide. If patients are refractory to lenalidomide, the choices are daratumumab, bortezomib dexamethasone

(DVd), daratumumab pomalidomide, dexamethasone, or isatuximab, pomalidomide, dexamethasone. In patients who are refractory to daratumumab at first relapse, carfilzomib-based regimens such as KRd or carfilzomib, pomalidomide, dexamethasone (KPd) are excellent options. For patients who are frail, Ixa-Rd would be a reasonable first choice for relapse.

There are numerous other alternatives, and these can be used in second and subsequent relapses. They include elotuzumab, pomalidomide, dexamethasone (EPd), bortezomib, cyclophosphamide, dexamethasone (VCd), bortezomib, pomalidomide, dexamethasone (VPd), and daratumumab, carfilzomib, and dexamethasone (DKd). Unfortunately, none of the triplet regimens used in relapsed MM have been compared head-to-head in randomized trials.

When sequencing therapy, there are a few other important considerations. At each relapse, any of the regimens that were mentioned for use in first relapse can be considered, with the goal of having at least two new drugs that the patient is not refractory two, and preferably from a different drug class. In many instances, this may mean the necessity of adding a monoclonal antibody to one of the triplets to create a quadruplet regimen. Although it is in the same drug class, pomalidomide has clinical activity in patients who are refractory to lenalidomide. Similarly, carfilzomib has activity in patients who are refractory to bortezomib. Carfilzomib is typically administered twice-weekly at a dose of 27 mg/m^2, but a once-weekly schedule of 56–70 mg/m^2 may be equally effective and safe, and more convenient.[11] Carfilzomib has a lower risk of neurotoxicity than bortezomib, but approximately 5% of patients can experience serious cardiac side effects.

1.4 | Refractory myeloma

There are several additional options for patients with MM refractory to immunomodulatory drugs, proteasome inhibitors, alkylators, CD38 antibodies, and elotuzumab. One option is to add panobinostat to a proteasome-inhibitor containing regimen. A second option is to use a selinexor-containing regimen such as selinexor, bortezomib, dexamethasone (SVd). A third options is treatment with belantamab mafodotin, a humanized anti-BCMA antibody that is conjugated to monomethyl auristatin-F, a microtubule disrupting agent.[12] Other options for refractory disease include bendamustine-containing regimens or anthracycline-containing regimens.

Venetoclax is not approved for use in MM, but has single-agent activity in patients with *t*(11;14) subtype of MM.[13] A recent randomized trial found significantly higher mortality with venetoclax in relapsed MM despite producing deeper responses and better PFS.[14] Therefore, venetoclax is considered investigational, and its use should be restricted to patients with *t*(11;14) who have relapsed disease.

With careful analysis of the various options and combinations possible, we can induce remissions multiple times with creative strategies. At each step opportunities for clinical trials may open up and should be considered.

1.5 | Investigational treatments

One of the most exciting options being studied is chimeric antigen receptor T cells targeting B cell maturation antigen such as bb2121.[15] In studies so far, more than 80% of patients appear to respond, with median response duration of approximately 12 months. Another promising new strategy is the use of bispecific T-cell engager, such as AMG 701, talquetamab, or cevostamab. Iberdomide and other cereblon inhibitors are also showing promise.

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CONFLICT OF INTERESTS

The author declares that there is no conflict of interest.

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SUPPLEMENT ARTICLE

WILEY

Molecular diagnostics and reporting in lymphoid malignancies: Current status and beyond

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1 | INTRODUCTION

Thanks to the advent of next-generation sequencing (NGS) technologies more than 10 years ago, the genomic landscape of most larger cancer types has been unraveled. Hematological and lymphoid malignancies were among the first cancer types to be sequenced,[1] probably as they are more easily accessible. Based on these studies, the number of clinically relevant genetic aberrations with diagnostic, prognostic, and/or predictive impact has increased rapidly.[2] This increases demands on molecular diagnostics to ensure that different types of genetic alterations can be readily detected in a diagnostic setting and within a reasonable time frame.

For hematological malignancies, genetics have been an integral part of diagnostics since decades,[3] including methods such as cytogenetics, fluorescence in situ hybridization (FISH) and targeted mutational analysis, and/or Sanger sequencing (Figure 1). With the more powerful NGS technologies, targeted gene panels have been added to the arsenal of methods in the diagnostic laboratory. Regardless of the type of diagnostic test, it is essential to harmonize methodology according to international standards and guidelines. To ensure comparable results, especially in multicenter studies, it is equally important that the interpretation of the results and the clinical reporting follow established guidelines. This is particularly relevant as genetic test results are becoming more complex with different NGS assays covering a range of different genetic aberrations.

This paper gives an overview of different *state-of-the-art* molecular technologies that are applied in clinical diagnostics of lymphoid malignancies, their advantages, and limitations. It also discusses aspects that need to be considered to harmonize clinical interpretation and reporting of NGS data.

2 | TECHNOLOGIES APPLIED FOR DIAGNOSTICS OF LYMPHOID MALIGNANCIES

2.1 | Molecular cytogenetics

Cytogenetics, or chromosome banding analysis, has long been instrumental to define recurrent chromosomal aberrations observed in different lymphoid malignancies. While cytogenetics has remained central in leukemias (i.e., acute myeloid leukemia [AML], acute lymphoblastic leukemia [ALL], and chronic myeloid leukemia), molecular cytogenetics or FISH is preferred for mature lymphoid malignancies. The introduction of the FISH technology enabled analysis not only on metaphase chromosomes, but also on interphase chromosomes (i.e., nondividing cells). FISH is therefore more rapid than cytogenetics and can be used to screen for recurrent diagnostic or risk stratifying genomic aberrations. Moreover, FISH can be applied to various tissue types, such as blood smears, imprints as well as formalin-fixed, paraffin-embedded (FFPE) tissue. Robust, commercial FISH probes are available for most recurrent aberrations, although they are relatively expensive. It is commonly used to detect translocations in lymphomas; for example, t(11;14) in mantle cell lymphoma and *MYC* and *BCL2* translocations in diffuse large B-cell lymphoma (DLBCL).[3] In chronic lymphocytic leukemia (CLL), a panel of probes is usually applied to detect risk-stratifying aberrations, that is, 11q-deletion, 13q-deletion, 17p-deletion, and trisomy 12, whereas

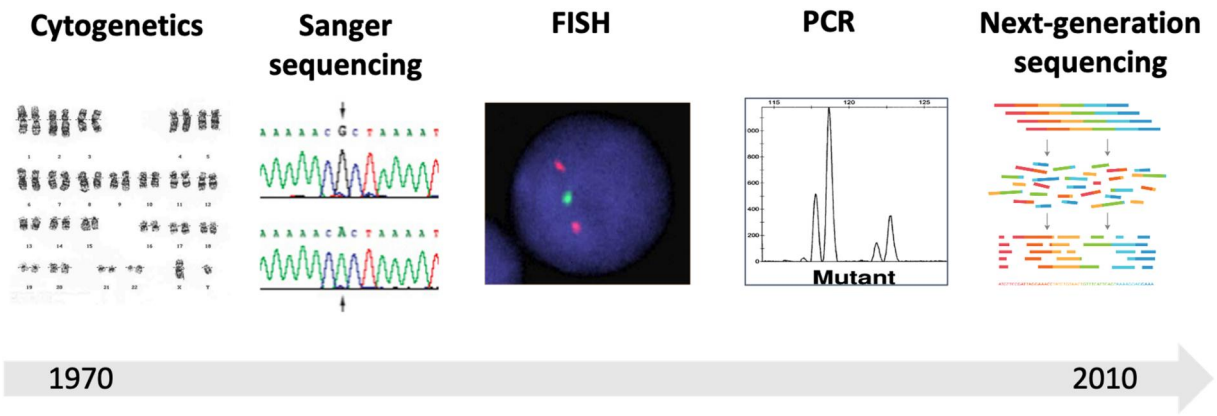

FIGURE 1 Evolution of genetic diagnostics. FISH, fluorescence in situ hybridization

in multiple myeloma, probes are used to identify t(4;14), t(11;14), t(14;16), 1q gain, and 17p deletion. However, while FISH on interphase cells can give a quick answer as to whether a specific genetic aberration is present or not, it is not a particularly sensitive technique; the cutoff level to detect an aberration has to be determined by each laboratory and is usually around 5%.

2.2 | Targeted mutation analysis

Before the NGS era, most clinical laboratories applied Sanger sequencing for targeted mutation analysis. Sanger sequencing has been the gold standard since the 1980s and is a very robust technology. However, it rapidly becomes expensive and time consuming if several genes or large genes are investigated. Another limitation with Sanger sequencing is its sensitivity which is usually between 10% and 20%.

In CLL, *TP53* mutations are linked to poor response to chemo-immunotherapy and poor overall survival, and *TP53* gene screening is nowadays mandatory before start of any line of treatment.[4] *TP53* gene analysis (encompassing exons 2-11) has typically been performed by Sanger sequencing, although more recently many laboratories have shifted to NGS.

Another sequenced-based molecular test is the immunoglobulin heavy variable (IGHV) gene mutational status that defines CLL with unmutated IGHV genes and an inferior outcome, and CLL with mutated IGHV genes and an expected favorable prognosis. This analysis is mainly performed by polymerase chain reaction (PCR) amplification of the clonal IGH rearrangement followed by Sanger sequencing,[5] although new NGS protocols have been developed.[6]

For both *TP53* analysis and IGHV gene analysis, the European Research Initiative on CLL (ERIC) has provided technical recommendations and guidance for interpretation.[4,5] It has also implemented dedicated certification systems that enable clinical laboratories to regularly certify their method (Sanger sequencing or NGS) against standard operation procedures.

For hotspot mutation detection, such as *BRAF* (V600E) in hairy cell leukemia and *MYD88* mutations (L265P) in Waldenström's macroglobulinemia, specific assays have been established. These use allele-specific PCR or quantitative PCR to detect the mutant allele. More recently, digital droplet PCR has been developed that enables a very sensitive detection (down to 0.01%) and can be applied to detect recurrent mutations or to follow patients over time. It is, for instance, used to detect *BTK/PLCG2* mutations in CLL patients progressing on ibrutinib treatment.

2.3 | Next-generation sequencing

Since more than 10 years, we have had access to new types of sequencing instruments that provide massive parallel sequencing or NGS. Using this technology, we can analyze the entire genome (whole-genome sequencing [WGS]), the exome (whole-exome sequencing), or selected regions of particular interest, that is, targeted NGS or gene panels. Depending on the detail of sequencing, the number of obtained sequence reads differs. Using WGS, the recommendation for tumor samples is to sequence to a sequence depth of 90× (and 30× for the matched normal sample). For targeted gene panels, depending on the size of the panel, one aims for a high sequence depth of at least 500× but preferably above 1000×.

Until recently, the majority of gene panels used within clinical diagnostics were amplicon-based, meaning they use PCR to amplify selected amplicons covering the genes/exons of interest. These amplicon-based panels can be implemented relatively easily using either a panel that is designed in-house, or commercially available panels. However, the limitations with amplicon-based technology are potential biases in amplification, particularly in difficult to sequence regions (i.e., GC rich and/or repetitive regions), that can cause drop-out of amplicons, as well as an increased risk of low-frequency, false-positive mutations; this can happen in particular when FFPE material is used as source of input.

In an attempt to study the robustness of amplicon-based gene panels, we within ERIC recently conducted a multicenter study using three different technologies (Multiplicom, HaloPlex, and TruSeq), targeting 11 genes recurrently mutated in CLL.[7] All six centers analyzed the same 48 CLL samples, and each technique was analyzed

by two centers. A very high concordance was achieved between technologies and centers for gene mutations with a variant allele frequency (VAF) above 5%, while discrepancies started to appear for variants with a VAF <5%. Hence, we conclude that amplicon-based sequencing can be safely adopted for somatic mutation detection with VAFs >5%.

We also tested a high-sensitivity assay containing unique molecular identifiers (UMIs) which could confirm subclonal mutations with a VAF <5%.[7] In this approach, duplicate reads are removed which improved sensitivity. Therefore, inclusion of UMIs should be considered when new panels are designed.

More recently, capture-based enrichment panels, using baits (probes) to hybridize to the region of interest, have been developed. These panels are usually larger in size (hundreds of genes) and produce more even sequence reads. This means that more "problematic" regions can be sequenced. Another advantage is that they enable simultaneous detection of different types of genomic aberrations (as they are larger in size). For instance, in addition to investigating a selected number of genes for single nucleotide variants (SNVs) and insertions/deletions (indels), it is also possible to analyze copy-number changes (i.e., deletions and amplifications) and structural variants (e.g., translocations). He et al.[8] combined a DNA and RNA-based broad, capture-based gene panel for diagnostics of a large number of hematological malignances (n = 3696) including different lymphoid malignancies. Using either bone marrow or FFPE samples, they could detect all aforementioned types of genomic aberrations with high accuracy and reproducibility.

Gene panels have rapidly been introduced into routine diagnostics of myeloid malignancies. In Sweden, we recently shifted from a 54 gene amplicon-based gene panel to a national capture-based myeloid panel including 195 genes. In lymphoid malignancies, smaller amplicon-based gene panels are currently in clinical use. As mentioned, in CLL, we test for *TP53* mutations before start of treatment and many centers have switched to amplicon-based NGS-gene panels. There are also additional genes of diagnostic, prognostic, and predictive impact in lymphomas that can be captured with these smaller panels. For instance, detection of certain mutations has diagnostic utility, for example, *MYD88* mutations in Waldenström's macroglobulinemia, *BRAF* mutations in hairy cell leukemia, *KLF2* mutations in splenic marginal zone lymphomas, and *STAT3/STAT5B* mutations in T-cell lymphomas, while other gene mutations are associated with response to treatment (e.g., *MYD88/CXCR4* mutations in Waldenström's macroglobulinemia patients treated with ibrutinib) or treatment resistance (e.g., *BTK/PLCG2* mutations in ibrutinib-treated CLL).[2]

Considering that a broad spectrum of genetic aberrations is involved in ontology and evolution of lymphoid malignancies,[1] the introduction of capture-based panels will be particularly useful in this patient group to capture not only SNV/indels, but also copy-number aberrations (CNAs) and translocations[9] as well as more complex markers such as IG/T-cell receptor gene analysis. In Sweden, we have recently developed a (national) capture-based lymphoid panel including 252 genes that is currently under validation and will soon be implemented into routine diagnostics.

3 | REPORTING AND INTERPRETATION OF RESULTS

For all types of molecular reports, it is important to include a number of key parameters so that the results can be easily understood by other laboratories. The report should include general information, such as personal id, referring doctor, and more specific information, such as tissue type investigated, method applied, and genetic aberrations assessed (Table 1). For FISH analysis, it is important to state the probes used and number of cells investigated as well as the cutoff applied. For more targeted analyses, such as hotspot mutation detection, the technology, and sensitivity should also be provided.

For NGS-based analysis, it is important to state gene coverage and sequence depth as well as cutoffs for variant calling. The sequence variants should be listed and include variant description at cDNA/protein level, following the HGVS nomenclature, the number of variant reads versus total number of sequence reads and the VAF (Table 1). It should also be noted if the variant has been deemed as pathogenic using locus-specific databases or international guidelines (e.g., following the American College of Medical Genetics (ACMG) criteria[10]). Furthermore, in the concluding remark of the report, it should be stated if a somatic variant has been detected before in this disease entity that has diagnostic, prognostic, or predictive impact, based on the WHO classification,[3] clinical consensus guidelines, or available published literature. More complex data that includes CNAs and/or structural aberrations will require continued development of bioinformatics tools to visualize the reported data, for example, in the format of copy-number plots or circus plots.

As we enter the precision medicine era, more and more targeted therapies have become available in oncology. Some academic centers and commercial companies have developed new support systems for clinical decision-making that assist in defining if a mutation is considered "actionable" or not.[11] Based on large databases, these tools can, for a certain variant, provide an alert if a clinically relevant finding has been made and provide links to available potential targeted drugs and/or clinical trials (e.g., FDA-approved drugs, ongoing clinical trials, or if a drug has been used for another malignancy). Thus far, these support systems have been mainly used for solid cancer and less frequently in hematological malignancies.

4 | CONCLUDING REMARKS

Genetic diagnostics within hematological malignancies has over the years evolved dramatically from cytogenetics to targeted NGS strategies (Figure 1). For lymphoid malignancies, we apply different FISH analysis as well as targeted sequencing/NGS for diagnostic and risk-stratifying purposes.[3] However, with the ever-increasing

TABLE 1 Data to be included in the molecular report based on sequencing

Basic data:	Patient data (name, date of birth and/or id number)
	Diagnosis
	Type of material (peripheral blood, bone marrow, lymph node)
	Date of sample collection/delivery
	Requested by (department/hospital)
Method:	Sequencing strategy: Sanger sequencing, amplicon-based NGS or capture-based NGS
	Genes/exons analyzed, minimum/mean sequence depth, detection limit, reference sequence
Results:	Variant description (cDNA/protein level)
	Variant type (missense/non-sense/frameshift)
	Variant allele frequency (number of variant reads/total number of reads)
	Variant pathogenicity
Conclusion:	Clinical interpretation of the variant detected and summary of its clinical impact (diagnostic, prognostic and/or predictive) according to current knowledge

Abbreviation: NGS, next-generation sequencing.

number of clinically relevant genetic variants that are detected in lymphoid malignancies, we need to continue developing "dynamic" NGS-based strategies, such a capture-based sequencing, that include large numbers of genes and types of genetic aberrations. In this regard, a recent study identified seven novel distinct subgroups of DLBCL based on broad genetic characterization.[12] This illustrates the need to develop more comprehensive analyses for diagnostic purposes, also to carry out future precision medicine studies. Furthermore, it is important to develop decision support tools that can inform clinicians on "actionability" for the different genetic events detected.

Within rare disease diagnostics, WGS has increasingly replaced multigene testing.[13] Could this be a way forward also for hematological malignancies where we usually perform multiple testing? Some national and/or regional programs are now testing if WGS combined with RNA-sequencing could replace the "old" technologies in acute leukemia (ALL and AML). In a proof-of-concept study, Klintman et al. demonstrated that WGS and targeted NGS had a high concordance for SNVs/indels in CLL, while the concordance between FISH and WGS was lower.[14] Hence, before we can start to use whole-genome techniques, we have to be certain that all mandatory genomic aberrations can be detected and provided within a reasonable time frame.

Another rapidly evolving areas of interest are liquid biopsies and the detection of circulating tumor DNA, which have the potential to enable sensitive NGS-based follow-up of lymphoma patients and can detect genetic aberrations in cases where it is difficult to take a biopsy.[15] These analyses have not yet entered clinical use in lymphoid malignancies, but they are foreseen to be an important part of future diagnostics.

Finally, although there are a few ongoing precision medicine studies in lymphoid malignancies world-wide, it will be very important to initiate future clinical trials based on targeted drugs/immune therapy to realize the full potential of precision medicine.

CONFLICT OF INTEREST

Richard Rosenquist has received honoraria from Abbvie, AstraZeneca, Illumina, Janssen and Roche.

PEER REVIEW

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SUPPLEMENT ARTICLE

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New drugs and pharmacological interactions in real life

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Abstract

A high number of new drugs have entered clinical development and many of them have recently been approved for patients with lymphoid malignancies. The availability of new drugs offers additional treatment options, but it also requires particular attention for the emergence of adverse events. In addition, new drugs may also have interactions with other drugs, which could further increase the risk of toxicities or result in decreased efficacy. Here we review potential drug interactions for nonchemotherapy new drugs approved for patients with lymphoid malignancies.

KEYWORDS
drug interactions, lymphoid malignancies, new drugs

1 | INTRODUCTION

Over the last years, several new drugs including small molecules, monoclonal antibodies (either naked or conjugated), and more recently adoptive cell therapies have been approved for the treatment of patients with lymphoid malignancies.[1–8] With the exception of chimeric antigen receptor-T cells, most of the new drugs are administered chronically (i.e., up to disease progression or relapse or up to the occurrence of adverse events) aiming to achieve long-term disease control. However, long-term drug administration can result in adverse events and may also increase the risk of interactions with other drugs.

While recognized as a potential risk, the frequency and severity of drug–drug interactions (DDIs) in oncology is not clear. Here, we review current knowledge regarding potential interactions that may involve drugs that have become recently available for patients with lymphoid malignancies and may affect every day clinical practice.

2 | DDIs IN ONCOLOGY

Interactions among concomitantly administered drugs can result in changes in the way one drug acts in the body and thus to altered efficacy or toxicity. DDIs can derive from pharmacokinetic, pharmacodynamic, or pharmaceutical interactions among two drugs (or among a drug and alternative medications, herbs, or food).[9] A pharmacokinetic interaction may affect any of the pharmacokinetic properties (absorption, distribution, metabolism, and/or excretion [ADME]) of one drug by another. The best-characterized is based on cytochrome P450 (CYP) hepatic enzymes and occurs when drugs that reduce (CYP inhibitors) or increase (CYP inducers) CYP activity are concomitantly administered with CYP substrates resulting respectively in decreases or increases in the metabolism of the substrate drug. Interestingly, not only drugs but also food or herbs can have an effect on CYP and therefore interfere with the metabolism of CYP substrates, like grapefruit juice and Seville oranges that can act respectively as strong or moderate CYP3A inhibitors and St. John's wort that can induce CYP3A. Other pharmacokinetic interactions can also occur, including interactions with the P glycoprotein 1 (P-gp) drug transporter that can result in altered drug exposure and finally altered pharmacokinetic properties.[10]

Pharmacodynamic and pharmaceutical interactions occur, respectively, when two drugs have similar mechanism of action (and therefore can result in additive, synergistic, or antagonistic effects) or when there are physical or chemical incompatibilities.[9]

Drug interactions represent an important issue in oncology given the older age of patients with cancer and the frequent use of several medications (so-called polypharmacy) used to treat cancer-related symptoms or concomitant diseases.[11] Older series including mainly patients with solid tumors reported that up to one-third of cancer patients are exposed to potential DDIs.[12] Major potential DDIs were identified in 16% of cancer patients in a large

retrospective cohort[13] and up to 25% of patients on anticancer treatments were found to have a potentially clinically significant DDI in another study performed in one center.[14] A prospective trial including also patients with hematological malignancies, reported potential clinically relevant DDIs in 81 of 302 included patients (27%).[15] Data from patients enrolled in phase II–IV clinical trials with approved medications (mainly tyrosine kinases and monoclonal antibodies) have been also published. DDIs that had to be avoided or drugs to be used with caution were detected by protocol guidance in 10% of patients, although the majority of subjects did not have clinical relevant interactions based on pharmacist review. In the same study, the use of the Lexicomp database detected moderate to major DDIs in 24% of patients with 9.4% having a clinically relevant DDI.[16]

However, despite these data, the real frequency of DDIs in oncology is unclear and there is lack of standardized criteria with regards to clinical consequences and assessment of their severity.[17] For patients with lymphoid malignancies and especially those on treatment with new drugs, data on DDIs are even scarcer. A recent study performed in 118 patients with chronic lymphocytic leukemia (CLL) on treatment with the Bruton's tyrosine kinase (BTK) inhibitor ibrutinib showed that 64% of patients were on medications that could increase ibrutinib toxicity and 3% on medications that could decrease its efficacy.[18]

Over the last years, improvements in our understanding of the biology of lymphomas and advances in antibody technology have permitted the development of many new compounds that have become available for patients with lymphoma and CLL. These new compounds comprise mainly small molecules and monoclonal antibodies which have different mechanisms of action, different toxicities, and potential for DDIs. Taking as a referral Food and Drug Administration approvals of new drugs for lymphomas and CLL over the last 5 years (Table 1), potential interactions, and recommendations for their management will be presented in the following paragraphs.

3 | BTK INHIBITORS

The development of BTK inhibitors has represented one of greatest recent therapeutic achievements in the treatment of lymphoid malignancies. Following the approval of ibrutinib, two other compounds, acalabrutinib and zanubrutinib, have been recently approved, while others are in clinical development and may be added in the list of available therapies for the treatment of lymphoid malignancies responding to BTK inhibitors currently including CLL, mantle-cell lymphoma, marginal lymphoma, and Waldenström's macroglobulinemia.

BTK inhibitors represent a class of compounds with known potential of pharmacokinetic DDIs. The first-in-class inhibitor ibrutinib is primarily metabolized by the cytochrome P450 CYP3A.[19] Although clinical trials in patients with CLL and lymphoma have excluded concomitant use of strong CYP3A inhibitors or inducers, pharmacokinetic studies in healthy volunteers and physiologically based pharmacokinetic models have revealed changes in ibrutinib exposure when administered concomitantly with CYP3A inhibitors or inducers that may be clinically relevant.[20,21] Accordingly, it is recommended to avoid concomitant administration of ibrutinib with strong CYP3A inhibitors or inducers and to consider a reduction of its dose if a moderate CYP3A inhibitor must be used.[22] In addition to the abovementioned CYP3A-mediated interactions which may have a clinical consequence, there is in vitro evidence that ibrutinib may also interact with rituximab antagonizing its antibody-dependent cell-mediated cytotoxicity though inhibition of interleukin-2 inducible tyrosine kinase which is necessary for natural killer cell function.[23] However, the clinical significance of this possible interaction is not known.

Similarly to ibrutinib, the second generation BTK inhibitors acalabrutinib and zunabrutinb are also primarily metabolized by CYP3A, and they have the same recommendation of avoiding coadministration with strong CYP3A inhibitors or inducers. Adaptations of their dose should be considered when CYP3A moderate inducers or inhibitors must be used.[24,25] In addition, acalabrutinib solubility decreases with increasing gastric pH resulting in significant decreases in exposer when administered with antacids and proton-pump inhibitors. Therefore, the recommendation is that acalabrutinb should not be coadministered with proton-pump inhibitors (due to their long-lasting effect), while antacids and H2-receptor antagonists may be administered but at least 2 h after the administration of acalabrutinib.

While the above reported interactions are based on pharmacokinetic mechanism and modulation of CYP3A, there are other potential interactions of BTK inhibitors that should be taken in consideration. In particular, attention should be given to the concomitant administration of anticoagulants which can lead to increased risk of bleeding events. Concomitant administration of warfarin is contraindicated. On the other hand, apixaban and rivaroxaban undergo CYP3A4-mediated metabolism.[26,27]

4 | PHOSPHOINOSITIDE 3-KINASE INHIBITORS

Another class of compounds that have entered clinical development and have beshown activity mainly in CLL and some indolent lymphomas (follicular lymphoma in particular) is represented by phosphoinositide 3-kinase (PI3K) inhibitors. Following the first approval of idelalisib, other compounds targeting PI3K have been developed more recently and two of them, copanlisib and duvelisib, have been approved for follicular lymphoma.

Copanlisib is an intravenous, pan-class I phosphatidylinositol-3-kinase (PI3K inhibitor) with predominant PI3K-α and PI3K-δ inhibitory activity. Approximately more than 90% of copanlisib metabolism is mediated by CYP3A. Strong CYP3A inducers result in significant decreases of copanlisib AUC and Cmax and should not be given concomitantly. On the other hand, CYP3A strong inhibitors cause a significant increase of copanlisib AUC and again should not be

TABLE 1 Selected recently approved drugs for lymphoid malignancies with known CYP3A and/or P-gp interactions. Refer to the prescribing information of each drug.

Drug	Recommendation	Effects on other drugs
Ibrutinib	Avoid concomitant use with strong CYP3A inhibitors. If these inhibitors are used as short-term consider interrupting ibrutinib	May increase the concentration of oral P-gp or BCRP substrates with narrow therapeutic index (e.g. digoxin, methotrexte).
	Dose adjustments (70 mg OD or 140 mg OD) if voriconazole or posaconazole must be given concomitantly	
	Dose adjustment to 280 mg OD if a moderate inhibitor must be administered concomitantly	
	Strong CYP3A inducers should be avoided	
Acalabrutinib	Coadministration with strong CYP3A inhibitors should be avoided or, if the inhibitor will be used short-term, acalabrutinib should be interrupted	Not reported
	Dose adjustment at 100 mg OD if moderate CYP3A inhibitors are used	
	Coadministration with strong CYP3A inducers should be avoided. If they must be used consider increasing the dose of acalabrutinib at 200 mg BID	
	Proton-pump inhibitors should be avoided	
	Antacids and H2-receptor antagonists to be taken at least 2 h after acalabrutnib	
Zanubrutinib	Zanubrutinib dose to be reduced in case of concomitant administration with moderate or strong CYP3A inhibitors	Not reported
	Avoid coadministration with moderate or strong CYP3A inducers	
Copanlisib	Concomitant use with strong CYP3A inhibitors should be avoided. Copanlisib dose to be reduced at 45 mg if a strong CYP3A inhibitor must be used	Not reported
	Strong CYP3A inducers should be avoided	
Duvelisib	Concomitant use with strong CYP3A inhibitors should be avoided. Duvelisib dose to be reduced at 15 mg BID if a strong CYP3A inhibitor must be used	May increase AUC of sensitive CYP3A4 substrates
	Strong CYP3A inducers should be avoided	
Venetoclax	Strong CYP3A inhibitors should not be used during ramp-up. Venetoclax dose to be reduced when strong inhibitors are used at steady state	May increase warfarin Cmax and AUCinf resulting in increased risk of bleeding. Venetoclax increases Cmax and AUCinf of P-gp substrates
	If moderate CYP3A or P-gp inhibitors are used during ramp-up or at steady state, dose of venetoclax must be reduced	
	Concomitant use with strong or moderate CYP3A inducers should be avoided	

Abbreviations: CYP3A, cytochrome P450 3A; P-gp, P glycoprotein 1.

administered concomitantly, or the dose of copanlisib should be reduced in case concomitant use with strong inhibitors cannot be avoided.[28]

Finally, duvelisib, an inhibitor of PI3K with inhibitory activity predominantly against PI3K-δ and PI3K-γ isoforms, is also primary metabolized by CYP3A cytochrome and has the same indications as with copanlisib for strong inducers or inhibitors. In addition, duvelisib can lead to increase AUC of CYP3A substrates and therefore to increased toxicity of these drugs which may require adaptation of their dose.[29]

5 | VENETOCLAX

Another small molecule-targeted agent that has been approved for the treatment of CLL is the bcl-2 inhibitor venetcolax. As with the compounds previously reported, venetoclax is mainly metabolized by the cytochrome CYP3A4.[30] Concomitant use with a strong or moderate CYP3A inhibitor or a P-gp inhibitor increases venetoclax plasma concentration and exposure which may increase the risk of adverse events, including tumor lysis syndrome, a well-known adverse event of venetoclax in CLL and reason for a particular

ramp-up dosing scheme. Therefore, the recommendation is to avoid concomitant use with a strong CYP3A inhibitor at initiation and during the ramp-up phase. During treatment at the steady dose, alternative medications or dose adaptation of venetoclax and frequent monitoring for adverse events should be considered. Strong or moderate CP3A inducers can also result in changes and in particular in decreased exposure to venetoclax and therefore current recommendation is to avoid concomitant administration.

Finally, venetoclax may also alter the exposure to other drugs and in particular it can increase warfarin levels and thus increase the risk of bleeding. International normalized ratio should therefore be regularly checked in patients taking warfarin with venetoclax.

6 | CONCLUSION

Drug interactions can involve recently approved new drugs for patients with lymphoid malignancies. In particular, small molecules-targeted agents are primary metabolized by CAP3A and their coadministration with strong or moderate CYP3A inhibitors or inducers can result respectively in increased or decreased plasma concentrations and therefore in risks of toxicity or decreased efficacy. Awareness of this problem and a regular check of the medications of the patients and consultation with pharmacists in case of any doubts for potential DDIs could help to prevent these interactions and especially those that could result in clinically significant consequences for patients.

CONFLICT OF INTERESTS

Institutional grants for clinical trials: Merck, Bayer, Roche, Novartis, Pfizer, ADC Therapeutics, MEW Pharma, Eli Lilly; advisory board: Roche; consultant: Bayer, Eli Lilly; travel grant: PharmaMar, Abbvie.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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SUPPLEMENT ARTICLE

WILEY

Vulnerabilities in the tumor and microenvironment in follicular lymphoma

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Abstract

Follicular lymphoma (FL) is a paradigm of tumors that require the interaction between tumor and microenvironment cells to foster their development from initial steps to progression. Recent large-scale genome studies have uncovered multiple genetic alterations of FL that influence the microenvironment in two main directions, promoting tumor cell survival and proliferation and facilitating their evasion from immune antitumor signals. Understanding the crosstalk between tumor B-cells and the microenvironment will facilitate the identification of vulnerabilities that may offer novel targets for treatment of the patients. This review highlights recent findings showing the effect of common genetic mutations modulating the cell composition of the tumor microenvironment and the novel therapeutic perspectives to target these interactions.

KEYWORDS
follicular lymphoma, genomic alterations, microenvironment, target therapies

1 | INTRODUCTION

Follicular lymphoma (FL) develops in the germinal center (GC) of lymphoid follicles due, in most of the cases, to the acquisition of the *t* (14;18) translocation in precursor cells of the bone marrow which leads to the overexpression of the antiapoptotic protein BCL2. A second early oncogenic event in FL is the introduction of somatic mutations in the variable regions of immunoglobulin genes creating novel N-glycosylation sites for highly mannosylated glycans that directly interact with endogenous lectins found in cells of the tumor microenvironment (TME). These interactions activate B-cell receptor signals required for tumor development. The third pillar in early steps of FL is recurrent mutations in epigenetic regulator genes that confer selective growth advantages to the B-cell and promote favorable interactions with the microenvironment.[1,2] Further, development and progression of FL are associated with subsequent acquisition of additional genomic alterations that target different pathways related to cell differentiation, survival, proliferation, dissemination, and metabolic advantages among others. In addition to the genomic and epigenomic alterations, FL cells modulate the microenvironment to promote the tumor cell growth.[3] Understanding these complex phenomena and the crosstalk between tumor and stromal cells may facilitate the identification of vulnerabilities that will offer novel targets for treatment of the patients.

2 | FL GENOME AND NEW VULNERABILITIES

2.1 | Epigenetic modulators

Large-scale genomic studies combined with functional analysis have elucidated the mutational profile of FL and defined the several altered pathways involved in the pathogenesis of these tumors. The most common aberrations are mutations in the epigenetic regulators *KMT2D* (60%–90%), *CREBBP/EP300* (50%–70%/10%–20%), and *EZH2* (10%–30%). The high frequency of these lesions, their findings in "in situ" follicular neoplasia and acquisition in later steps in the evolution indicate that they are very early events but also favor the progression of the tumors.[4,5] The loss of function mutations in the histone H3K4 methyltransferase *KMT2D* and in the H3K27

TABLE 1 Genomic and microenvironment alterations in follicular lymphoma as potential targets for novel treatment

Target	Type of alteration	Biological consequence	Targeted drug	Combinations	Status
Genomic aberrations					
CREBBP	Loss-of-function mutations	Downregulation of MHC class II	BRD3308	Anti-PDL1	Preclinical
EZH2	Gain-of-function mutations	Increased dependency on FDC	Tazemetostat	None	Approved
TNFRSF14	Inactivating mutations	Increased T_{FH} recruitment	CAR-T	None	Preclinical
RRAGC	Activating mutations	Insensitivity to nutrient deprivation	Temsirolimus	Bendamustine rituximab	Clinical
		Increased B cell responses			
FL-microenvironment crosstalk					
B-cell receptor	FL-FDC	BCR activation	Ibrutinib	Rituximab	Clinical
	FL-DC-SIGN$^+$ Mϕ				
PI3Kδ	FL-T_{FH}	CD40-CD40L activation, T_{reg} recruitment	Idelalisib	None	Approved
	FL-T_{reg}				
PI3Ky	FL-monocyte/Mϕ	Myeloid cell recruitment, M2 polarization	Duvelisib	None	Approved
CSF-1R	FL-Mϕ	Myeloid cell recruitment and differentiation, M2 polarization	Pexidartinib	Rituximab	Preclinical
CD47- SIRP-α	FL-Mϕ	CD47$^+$ FL cells inhibit phagocytosis by Mϕ and neutrophils	Anti-CD47	Rituximab	Clinical
	FL-neutrophils				

Abbreviations: BCR, B-cell receptor; FDC,follicular dendritic cell; FL, follicular lymphoma; MHC, major histocompatibility complex; T_{reg}, T-regulatory cell; T_{FH}, T-follicular helper.

acetyltransferase *CREBBP/EP300* together with the gain of function in the H3K27 methyltransferase *EZH2* tend to confer a repressive functional state of the genes targeted by these chromatin modifiers related to B-cell differentiation and cell cycle regulation that maintain tumor cells in a GC stage (Table 1).[1,5] Particularly, *CREBBP* mutations silence genes that are direct targets of the BCL6-HDAC3 onco-repressor complex, including those that regulate B-cell signaling and immune responses, such as class II major histocompatibility complex (class II MHC). Other B-cell neoplasias, such as Hodgkin's lymphoma and primary mediastinal large B-cell lymphoma show class II MHC deregulation, but the mechanism is different mainly associated with *CIITA* alterations.[6] *CREBBP*-mutated tumors also seem to have less helper and cytotoxic T-cells in the microenvironment suggesting that these mutations favor tumor cell immune evasion.[5,6] These crucial alterations in FL pathogenesis support the idea that *CREBBP* may be a gene with high therapeutic potential. In this sense, HDAC3 inhibition restores in part the immune responses and therefore may represent a new therapeutic approach in FL.[7] Recent studies have also linked *EZH2* mutations to the reprograming of the tumor and microenvironment interactions. Mutant cells in the light zone of the GC seem to be less dependent on T-follicular helper (T_{FH}) cells while potentiate their interaction and dependence on follicular dendritic cells (FDCs).[8] On the other hand, EZH2 also seems to play a role in the development of T and natural killer (NK) cells. The potential benefit of EZH2 inhibitors independently of its mutational status may be related to this extra tumor cell activity. Interestingly, Food and Drug Administration has approved the use of the selective EZH2 inhibitor tazemetostat for adult relapse/refractory (R/R) patients with EZH2-mutated tumors and patients with R/R FL who have no satisfactory alternative treatment options.

2.2 | Immune evasion

Mutations and deletions of *TNFRSF14* in 1p36, also known as herpesvirus entry mediator A are a common event in FL (~50%).[1] TNFRSF14 is the ligand for BTLA expressed in TFH cells and induces inhibitory signals on these cells. The disruption of these interactions results in increased recruitment of the tumor supportive T_{FH} and release of cytokines that also favor a pro-TME. In addition, TNFRSF14 generates inhibitory signals on the B-cell receptor (BCR) of the B-cells that are released by the oncogenic inactivation. A preclinical CAR-T construct has been developed to continuously produce soluble TNFRSF14 in the microenvironment that restores its inhibitory function.[9]

Ephrin receptor A7 (EPHA7) is a soluble tumor suppressor inactivated in approximately 70% of FL by mutations and deletions. Loss of expression avoids its binding to EPHA2 receptor, which inhibits extracellular regulated MAP kinase (ERK) and SRC proto-oncogene, non-receptor tyrosine kinase oncogenic signals.

Therapies that restore EPHA7 function or inhibit downstream oncogenic signals activated due to EPHA7 loss might be useful to treat FL-mutated patients.[10]

Recent studies have identified mutations in mammalian target of rapamycin complex 1 (mTORC1) pathway. Particularly, *RRAGC* activating mutations are present in 17% of FL patients. These mutations activate mTORC1 bypassing amino acid deprivation and also confer an independent requirement of T_{FH} cells for tumor survival. Concordantly, these tumors have less T_{FH} cells in their stroma.[11] Thus, the identification of patients carrying mutations in this metabolic pathway may benefit of selective inhibitors.

Cathepsin S (CTSS) has been found to be mutated (6%) or overexpressed (13%) in FL leading to its hyperactivation.[12,13] CTSS cleaves CD74, among other targets, which is fundamental for MHC II assembly and antigen (Ag) presentation. Hyperactive CTSS yields a more efficient Ag-specific CD4+ T activation, increased CD4+ T-cell infiltration, and proinflammatory cytokine perturbation in FL mouse models and human FL samples. Interestingly, the subversion of TME by this alteration correlates with high PDL2 expression that yielded FL patients more responsive to anti-PD1 regimens, and also associates with better outcomes to immunochemotherapy (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone [R-CHOP]).

Other mutated genes in FL that also target the interactions between tumor cells and microenvironment are B2-microglobulin and CD58 that seem to be more common in transformed FL than in the early steps of the disease.[1] Thus, reactivating their function may improve cytotoxic response via $CD8^+$ T cells.

3 | THERAPEUTIC OPPORTUNITIES FROM TME

Interaction of FL cells with nontumor cells constitutes a key feature in the pathophysiology of the tumor. A precise characterization of the TME could uncover new vulnerabilities to treat these patients. The tumor niche of this lymphoma is composed of different cells types, which create a tumor supportive environment and facilitate the scape from the host antitumor immune responses. The main actors in this TME are different subpopulations of T-cells, myeloid derived cells, mainly tumor-associated macrophages (TAMs), and stroma cells, mainly FDCs and cancer-associated fibroblasts, among others. These cells communicate among them and with tumor cells through a network of cytokines and cell-to-cell interactions.

3.1 | T-cells

T-cells are a heterogeneous group of cells that interplay with FL cells at different levels mediating antitumor responses or, contrarily, providing supportive protumoral signals. $CD8^+$ cytotoxic cells, together with NK cells and probably Tγ/δ cells, mediate antitumor responses. However, FL cells may counteract this antitumor effect by secreting interleukin 12 (IL-12) that leads to an exhaustion of $CD8^+$ cells and by recruiting T-regulatory cells (Tregs) that inhibit degranulation and cytotoxic activity of $CD8^+$ cells.[14] On the other side of the balance, two subpopulations of $CD4^+$ cell, T_{FH}, and Treg play key roles in providing tumor support and facilitating immune evasion. In this way, T_{FH} stimulate tumor B-cells mediated by CD40L/CD40 and MHC class II interactions.[15] These cells secrete IL-4, which triggers activation of B-cells mediated by ERK and STAT6, and the chemokine CCL22 that recruits immunomodulatory Treg cells. Treg cells are a subset of $CD4^+$ cells characterized by the expression of the transcription factor FOXP3. These cells seem to play a pro-tumor role due to their immunosuppressive activity on CD4 and CD8 cells. However, a subset of T follicular regulatory cells (Tfr) has been also recognized by the additional expression of BCL6, CXCR5, ICOS, and PD1. These cells limit the expansion of the GC reaction and downregulate the effects of T_{FH} cells. However, there is still some controversy regarding the impact of Tfr FOXP3+ expression patterns on FL survival.[16,17]

The relevance of T_{FH} and Treg cells in FL has been highlighted by the effect of PI3Kδ inhibitors disrupting the crosstalk between FL cells and T_{FH} cells. In vitro studies have shown that the PI3Kδ inhibitor idelalisib, diminishes tumor cell proliferation, and reshapes immune microenvironment inhibiting the recruitment of classical Treg cells by downregulating CCL22.[18]

Finally, in this context of B–T-cell crosstalk, FL cells present immunoglobulin neoantigens that may play a determinant role in host immune responses, and constitute potential immunotherapeutic targets.[19]

3.2 | TAMs and other stromal cells

The role of TAMs in FL has been controversial. In the prerituximab era, some studies suggested that macrophage infiltration correlated with lower survival. Nevertheless, the addition of rituximab to chemotherapeutical drugs modified their prognostic impact. The number of infiltrating macrophages in patients treated with standard immunochemoterapeutic regimens such as R-CHOP was associated with improved overall survival.[20]

In addition to the influence of new therapies in the possible role of TAM, different subpopulations of these cells may also play different roles in the pathogenesis of FL. Macrophages polarized to a M1 phenotype may exert antitumor properties by producing proinflammatory such as IL-1, IL-6, IL-12, and tumor necrosis factor α, whereas M2-polarized macrophages are protumor since they are able to downregulate MHC and IL-12, as well as expressing anti-inflammatory molecules such as arginase and IL-10, and the scavenger receptor CD163. Moreover, M2 macrophages are also involved in tumor angiogenesis. The protumor effect of TAMs, particularly with an M2 polarization, makes these cells and their interactions with tumor cells an attractive target for therapies.

In is noteworthy, that contrary to other B-cell lymphomas such as diffuse large B-cell lymphoma (DLBCL) or Hodgkin lymphoma, FL cells do not express PD-L1, and PD-L2 is moderately expressed in a

high proportion of FL cases.[21] However, PD1 ligands are present in the immune infiltrates where PD-L1$^+$ histiocytes have been detected in the T-cell-rich zone of the neoplastic follicles,[22] justifying the therapeutic targeting of this pathway.

A target to interfere the FL-TAMs crosstalk is the colony-stimulating factor 1 receptor (CSF-1R), also known as macrophage colony-stimulating factor receptor, as it is a relevant element in the differentiation and survival of macrophages. Noteworthy, high levels of CSF-1R expression in FL have been associated with higher histological grade and risk of transformation suggesting that targeting CSF-1R may be relevant in high-risk patients. Although CSF-1R is expressed in both M1 and M2 subtypes, its inhibition by pexidartinib preferentially diminish the viability of M2 macrophages and repolarize them to M1 macrophages suggesting that it may reeducated TAMs toward an antitumor phenotype.

CD47 is a receptor usually expressed in cancer cells that prevents phagocytosis forming a complex with signal-regulatory protein α (SIRP-*α*). Among other phagocytes (e.g., neutrophils), macrophages may express SIRP-α in their membrane compromising their antitumor phagocytic function when interacting with CD47-positive lymphoma cells (Table 1). By administrating a therapeutic antibody that blocks CD47, phagocytosis of tumor cells is increased and adaptive immunity is enhanced.[23] This effect has been explained by the Ag-presenting function of macrophages. After phagocytizing tumor cells, macrophages may present tumor Ag to CD4$^+$ T-helper cells triggering an antitumor response. Altogether, anti-CD47 antibodies are now in clinical trials as promising drug candidates to activate the immune system in FL, especially in combined immunotherapeutic regimens including rituximab.

PI3Kγ is expressed by microenvironment cells that support tumor growth such as CD4 and M2 macrophages. The dual PI3Kγδ inhibitor duvelisib exerts antitumor effects by targeting both the tumor and microenvironment cells (Table 1). This compound inhibits tumor cell proliferation and survival whereas also promotes the differentiation of M2-like TAMs to a M1 phenotype and inhibits the antitumor effect of T-cells interfering with the tumor supporting properties of the TME. Other drugs that interfere with BCR pathway such as ibrutinib may also affect the crosstalk of tumor cells and macrophages. While BTK inhibitors (BTKi) have not showed optimal results as monotherapy, the combination of the BTKi ibrutinib with the anti-CD20 rituximab has improved clinical trials results.[24]

FDCs are from mesenchymal origin and build a network to support the GC reaction. They are able to present antibody—Ag complexes on their cell surface engaging BCR activation, survival of malignant cells and recruitment of T_{FH} cells.[18] Moreover, we have recently demonstrated that FL-FDC crosstalk induces monocyte recruitment and their differentiation to M2-protumoral macrophages.[25]

Other cell types that may play an important role in the disease are different subsets of T-cells, TAMs, mesenchymal stem cells, or follicular reticular cells, among others. Interestingly, the evolution of this TME might be determinant in the transformation of FL to DLBCL.

4 | CONCLUDING REMARKS

Although in most cases FL initially presents as an indolent disease, a significant proportion of cases are primary refractory to standard treatment (R-CHOP or derivatives) and a high percentage of those who initially respond to treatment will eventually relapse. Once the relapse occurs, the prognosis of the patients worsens, especially in those who suffer an early relapse, within the first 24 months of treatment progression of disease within 2 years. Furthermore, those patients are in higher risk of histological transformation to an aggressive lymphoma, mainly DLBCL. The evolution of the patients cannot be precisely predicted exclusively based on single genetic alterations. Recent studies have shown that assays that include multiple genetic aberrations or combining both tumor B-cell biology and tumor microenvironment alterations may be better predictive models. These findings reinforce the need to integrate the mutational profile of the tumor cells together with the complex interactions of the TME to predict the biological risk of the patients. This comprehensive perspective should assist in identifying tumor and microenvironment vulnerabilities that will allow treating them effectively and avoiding early clinical progression.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

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SUPPLEMENT ARTICLE

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Upfront identification of high-risk follicular lymphoma

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Abstract

Follicular lymphoma (FL) is a common disease with clinically indolent behavior, and a long natural history for the majority of patients. Despite excellent therapeutic strategies currently available for FL, approximately 10%–20% of patients will experience early disease progression, defined as occurring within two years of diagnosis. These patients have poor outcomes, with overall survival at 5 years ranging between 37% and 50%. Much of the biology driving early progression and inferior survival is attributed to early transformation events; however, transformation alone does not account for all the observed clinical heterogeneity and survival differences among patients. Several clinical, genetic, and molecular alterations in FL have been discovered that help define subsets of patients at risk for multiply relapses and refractory disease, and are slowly making their way into risk calculators to be used in daily practice. Additionally, the role of functional imaging with PET scan, as well as circulating and cell free tumor DNA are being evaluated as tools to define high-risk subsets of patients with FL. This review seeks to provide an over view of current and evolving biomarkers that define high-risk FL at diagnosis. The goal is for these tools to assist clinicians in integrating these rapidly evolving prognosis models into clinical practice, in the hopes of risk-stratifying treatments and improving outcomes for patients.

KEYWORDS
follicular lymphoma, high-risk disease, relapse

1 | INTRODUCTION

Follicular lymphoma (FL) arises from precursor B cells that acquire the t(14;18), undergo additional genetic alterations, and develop malignant transformation within the germinal center of the lymph node.[1] Affecting approximately 2–4 people per 100,000 person-years, FL is the most common indolent lymphoma diagnosed in the United States and Europe.[2] On a global scale, the incidence of FL appears to be rising in developed countries, suggesting possible environmental contributions affecting pathobiology and genetic susceptibility. FL is described as clinically and biologically heterogenous, owing to rare morphologic FL subtypes including pediatric type and duodenal type FL variants, and distinct clinical behavior leading to divergent outcomes in ~20% of cases.[3]

FL clinical behavior is generally indolent, where 80% of patients experience long-term disease control following standard chemo-immunotherapy combinations. However, 20% or so of cases experiencing early disease progression have poor outcomes (5 years overall survival [OS] of 35%–50%).[4] An additional subset of patients are refractory to both anti-CD20 antibodies and alkylators (so called "double refractory"), have continuous relapses over time, or undergo transformation to aggressive histology. These patients do not enjoy the long natural history of FL with median OS of nearly 2 decades and are often designated as "high risk," owing to a more aggressive clinical phenotype and less favorable outcomes.[5]

Identifying patients with high-risk FL upfront is a global research effort aimed at minimizing risk of death, histologic transformation, and optimizing duration of treatment response to ease suffering and

morbidity from this disease. The subject of this review is to discuss current biomarkers and those in development that can aide in identifying high-risk FL.

1.1 | Current biomarkers identifying high-risk patients

Over the last several years, the discovery of novel clinical and biologic markers has revolutionized FL disease prognostication and risk precision.[6] However, despite well-established clinical calculators and molecular classifiers, the precise utilization of these tools remains in question as it relates to selection of therapy at diagnosis or relapse. When discussing biomarkers and their utilization to identify subsets of patients with high-risk disease, it is relevant to define the differences between prognostic and predictive markers.[7] Prognostic biomarkers appraise risk of a particular outcome such as disease progression or death. Prognostic markers can assist in developing risk-adapted treatments. In contrast, predictive biomarkers are reserved to associate a specific therapy with a particular clinical response, or lack thereof. As such, identifying disease response to specific therapies is enhanced with predictive biomarkers. Complicating matters is that in many cases, some biomarkers are both prognostic and predictive. In FL, the most well established prognostic markers of outcome include clinical calculators such as the FLIPI or FLIPI-2, histologic variations such as grade, disease-specific markers such as tumor burden and stage, and surrogates for tumor kinetics such as early progression of disease (POD24). Molecular markers such as the presence of somatic mutations, and differential expression of genes within and outside the FL tumor microenvironment, are gaining widespread attention as critical determinants of disease outcome. Many studies have established that functional imaging with PET at the end of therapy also predicts both progression free (PFS) and overall survival (OS) irrespective of treatment.

1.2 | Clinical risk calculators

The FL International Prognostic Index (FLIPI) is the most commonly used clinical calculator to determine FL prognosis.[8] The FLIPI's five pretreatment patient characteristics (nodal sites, lactate dehydrogenase, age > 60 years, Stage III-IV, and hemoglobin <12 g/dl) were strongly prognostic of OS in over 900 patients with low-grade FL treated with chemotherapy (no immunotherapy) strongly associated with 5- and 10-year OS based on low, intermediate risk, or high-risk designation (Table 1). In a retrospective analysis of rituximab treated patients by the German Low Grade Lymphoma Study Group (GLSG), patients with high-risk FLIPI had inferior time to treatment failure (67% at 2 years) compared to those with intermediate risk and low-risk FLIPI groups (90% and 92%, respectively).[9] Similarly, The National LymphoCare Study, a prospective, multicenter observational cohort of patients with FL in the United States including nearly 70% of rituximab treated patients, observed a hazard ratio for OS higher in the high-risk FLIPI group compared to the intermediate risk FLIPI groups.[10]

The FLIPI 2 calculator was another method used to predict PFS upfront using additional parameters such as increased beta 2 microglobulin (B2M) and bone marrow involvement, and had comparable predictive ability as the FLIPI.[11] When attempting to specifically identify patients with POD24, data using the FLIPI and FLIP2 are more limited; however, a study by Jurinovic et al.[12] reported a sensitivity between 70% and 78%, and a specificity of 56%–58%, suggesting a heterogeneity among the POD24 patients that is inadequately captured by this prognostic index.

Other clinical indices have attempted to capture high-risk patients, by using a parsimonious mode incorporating only bone marrow involvement and B2M level.[13] The PRIMA-PI initially included patients from the randomized PRIMA study of patients treated with chemoimmunotherapy, with or without rituximab maintenance. High-risk patients had elevated B2M greater than 3 mg/L and had 5-year PFS of only 37%. Other groups validated the utility of the PRIMA -PI in chemotherapy-free approaches using immunomodulator lenalidomide on the RELVANCE study, and using rituximab and interferon in the Nordic Lymphoma Group studies. The PRIMA-PI was not able to sufficiently discriminate patients treated with lenalidomide, but did identify a high-risk group of patients with FL experiencing shortened OS and time to treatment failure. While authors did not explicitly evaluate the PRIMA to assess POD24, there was no difference in transformation rates between both groups, and they did find improved risk stratification compared to the FLIPI, especially in patients older than 60 years.[14]

The FLEX calculator was developed from patients treated on the phase 3 GALLIUM trial (NCT01332968) using Obinutuzumab based chemoimmunotherapy combinations in previously untreated advanced FL. This novel prognostic model sough to identify high-risk patients compared to FLIPI, FLIPI-2, and PRIMA-PI. The primary endpoint was PFS but OS and risk of POD24 were evaluated. It included nine clinical factors (male sex, sum of the product diameter [SPD] in the highest quartile, Grade 3A histology, >2 extranodal sites, ECOG PS > 1, hemoglobin <12 g/dl, elevated B2M, elevated LDH, and peripheral blood absolute natural killer cell count <100/μl).[15]

The model was validated in the SABRINA trial (randomized Phase 3 trial of subcutaneous rituximab vs. intravenous rituximab for first-line FL treatment; NCT01200758). Low-risk patients had zero to two factors; and high-risk patients had three to nine factors. PFS at 3 years was 86% for high-risk patients and 68% for low-risk patients. Using FLEX, the sensitivity for a high-risk score to predict POD24 was 60%, versus 53% for FLIPI and FLIPI-2, and 69% for PRIMA-PI. Specificity for POD24 was 68% with FLEX compared with 59% for FLIPI and FLIPI-2, and 47% for PRIMA-PI (Table 1).

Compared to other frequently used clinical prognostic calculators, the FLEX appeared to have greater accuracy for predicting POD24, particularly with novel immunotherapy combinations, and the first prognostic score in FL developed in patients treated with bendamustine- and obinutuzumab-based regimens.

TABLE 1 Prognostic calculators in follicular lymphoma

Risk model	Factors	Risk groups	Prognostic impact	Sensitivity of high-risk group for predicting POD24	Specificity of high-risk group for predicting POD24
FLIPI	- Age >60 - Stage III-IV - Hg < 12 g/dl - Elevated LDH - > Four nodal sites	Low risk(0–1 points) Intermediate risk(2 points) High risk(3 or more points)	91% 5-year OS 78% 5-year OS 53% 5-year OS	53%–78%	56%–62%
FLIPI2	- Age >60 - Bone marrow involvement - Hg < 12 g/dl - Elevated β2 microglobulin - Mass > 6 cm	Low risk(0–1 points) Intermediate risk(2 points) High risk(3 or more points)	80% 5-year OS 51% 5-year OS 19% 5- year OS	53%	59%–76%
PRIMA-PI	- β2 microglobulin > 3g/L - Bone marrow involvement	Low risk(0 points) Intermediate risk(1 point) High risk(2 points)	69% 5-year PFS 55% 5-year PFS 37% 5- year PFS	69%	48%
FLEX	- Male sex - SPD in the highest quartile - Histologic grade 3A - > 2 extranodal sites - ECOG PS > 1 - Hg < 12 g/dl - Elevated β2 microglobulin - NK cell count >100/μl - Elevated LDH	Low risk(0–2 points) High risk(3–9 points)	86% 3-year PFS 68% 3-year PFS	60%	68%
M7-FLIPI	- ECOG PS > 1 - FLIPI high risk - Mutations in EP300, CREBBP, CARD11, MEF2B, EZH2, ARID1A, FOXO1	Low risk High risk	68%–77% 5-year FFS 22%–38% 5-year FFS	43%–61%	77%–86%
POD24-PI	- ECOG PS > 1 - FLIPI high risk - Mutations in EP300, EZH2, FOXO1	Low risk High risk	72%–77% 5-year FFS 36%–50% 5-year FFS	54%–78%	67%–73%
Tumor microenvironment	PDL-2 expression	Immune infiltration^{LO} Immune infiltration^{HI}	– –	Not reported	Not reported

1.3 | Impact of the tumor microenvironment on identifying high-risk FL

The microenvironment surrounding FL tumor cells (tumor microenvironment [TME]) is a critically influential component of FL tumorigenesis and clinical behavior. In 2004, the Leukemia and Lymphoma Molecular Profiling Project described pivotal findings using gene expression profiling on biopsy samples from patients treated in the prerituximab era.[16] Two combinations of gene expression were assessed for ability to predict survival, where increased expression of genes in T-cells and macrophages had a more favorable prognosis (relative risk of death 0.15). In comparison, increased expression of genes from macrophages or dendritic cells had higher risk disease, with an unfavorable prognosis (relative risk of death 9.35). Subsequent studies have been unable to consistently reproduce this same impact of the immune system's effect of FL prognosis, largely due to heterogeneously treated patient populations and study small sizes. Kridel et al.[17] reported on the adverse prognostic impact of CD163 + macrophages, only in patients treated with rituximab and cyclophosphamide, vincristine, prednisone (R-CVP), but not rituximab with cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). Similarly, other studies assessing the impact of number, distribution, and frequency of tumor-associated macrophages and tumor-associated T cells have resulted in inconsistent conclusions on FL prognosis.

To identify the role of the TME in predicting the risk of POD24, Tobin et al.[18] recently targeted gene sequencing using NanoString technology from paraffin embedded tissue and multispectral immunofluorescence on a tissue microarray, and applied them to two groups: a discovery cohort of 132 patients from Princess Alexandria Hospital with early and advanced staged follicular lymphoma who received either chemotherapy or observation, and two independent validation cohorts of 198 patients with advanced stage disease treated with RCHOP and RCVP from the German low-grade lymphoma study group and the British Columbia Cancer Agency. They also performed T cell repertoire analysis, flow cytometry, immunofluorescence, and next generation sequencing. Gene expression profiling revealed distinct clustering of follicular lymphoma samples based on high or low expression of immune infiltrating cells. *Low* expression of certain immune markers (including PDL-2, TNF-α, CD4, and CD68) were all associated with poor outcome; however, the most specific marker with the highest specificity and sensitivity was PD-L2. They then dichotomized PD-L2 expression into immune infiltration "high" and "immune infiltration low" in subsequent analyses. PD-L2 is an immune checkpoint present broadly on both tumor cells and the tumor microenvironment. To localize its distribution, they performed flow cytometry in fresh FL samples, and quantified PDL2 expression by PCR. They identified that PD-L2 gene expression was distributed in both CD20+ tumor cells as well as non CD20+ cells in the tumor microenvironment. However, the proportion of PDL2 was *lower* in the *CD20+* cells. Overall there was lower expression of *all* immune cells in the immune infiltration low phenotype compare to the immune infiltration high phenotype. When testing the relevance of immune infiltration and POD24, they found that POD24 events in the Princess Alexandria discovery set were more enriched for the immune infiltration low phenotype. These findings were also validated in the British Columbia cancer agency and German lymphoma study group populations. Nearly 50% of patients with low PD-L2 had POD24 events compared to 16% in those with high PD-L2, concluding that low PD-L2 identifies a subset of patients enriched for POD24.[18]

1.4 | Somatic mutations, gene expression profiling, and clinic-pathologic models

Toward a precision approach, investigators from the GLSG harmonized clinical and pathologic data to create a clinico-genetic risk model aimed at more accurate risk prognostication in patients receiving front line chemoimmunotherapy.[19] They performed deep DNA sequencing from formalin fixed pretreatment biopsies to analyze the mutational status of genes in 151 patients with follicular lymphoma tumor samples. The resulting prognostic tool, called the m7-FLIPI, distilled down 74 genes into seven genes with nonsilent mutations occurring at a variant allele frequency of 10% or greater, and combined these with high-risk FLIPI status and ECOG performance status. These included genes that increased risk of progression, including EP300, FOX01, CREBBP, CARD11, and those that decreased risk of progression, including EZH2, ARID1A, and MEF2B. The cumulative risk score was calculated by combining relative weights of these genes in a multivariate analysis predicting failure-free survival. This m7-FLIPI score was tested to identify POD24 but only captured about 50% of patients as high risk. Jurinovic et al.[12] later developed the POD24-PI, designed to augment the sensitivity and specificity of the m7-FLIPI in early progressing patients. This model included only three genes, including EP300, FOX01, and EZH2, performance status, and FLIPI score. The POD24-PI was more sensitive at identifying POD24 patients but did not outperform other metrics due to lower specificity.[12]

A recently developed prognostic model, termed the Bio-FLIPI, integrated intrafollicular CD4 expression with FLIPI to improves identification of FL patients at risk for early treatment failure. Mondello et al.[20] evaluated 496 patients using tissue microarrays for CD4, CD8, FOXP3, CD32b, CD14, CD68, CD70, SIRPa, TIM3, PD-1, and PDL1. They found CD4, CD8, FOXP3, PD-1, and SIRPα were associated with risk of early failure and were subsequently evaluated in the validation set; however, only intrafollicular CD4 expression remained significant in multivariate analysis. Lack of intrafollicular CD4 expression was associated with higher risk of early failure in both the discovery and validation cohorts, with a pooled OR = 2.29 (95% CI 1.47–3.58; $p < 0.001$). The BioFLIPI then combined CD4 intrafollicular expression and FLIPI into a one-to-four scale and was robustly associated with risk of early failure.[20]

Gene expression signatures beyond those identified in the TME have clinical utility on the upfront identification of patients with poor outcomes in FL. While not yet ready for standard use, these molecular signatures are highly promising for further investigation. Huet et al.[21] performed a large-scale gene expression profiling study using samples from 160 untreated FL patients from the PRIMA study in FL to establish define correlates of PFS and early treatment failure. The authors identified 23 genes associated with the TME and B-cell biology. These were strongly associated with disease progression based on a score of low risk or high risk. Further adjusting for clinical prognostic tools such as the FLIPI, use of maintenance rituximab, the gene signature sustained its predictive utility.[21] This method predicted POD24 with a sensitivity of 43% and a specificity of 79%.

1.5 | PET-based functional imaging biomarkers

Metabolic assessment of FL disease burden is considered standard of care following first-line chemoimmunotherapy. It is well established that attainment of complete metabolic response on a five-point scale (ref) is highly prognostic of outcome, regardless of treatment used. A pooled analysis of European patients treated on three prospective clinical trials established that a positive PET scan (defined as Deauville score of 4 or 5) following front-line treatment was highly prognostic of both PFS and OS.[22] A novel method of metabolic activity has gaining attention is total metabolic tumor volume (TMTV). Defined as a cut off of 510 cm^3 or greater, high TMTV at diagnosis is associated with a poor prognosis.[23] When combined with circulating

tumor cells and cell free DNA, TMTV at diagnosis has also been strongly correlated with inferior PFS.[24]

2 | WHAT ARE HOPES FOR THE FUTURE TO IDENTIFY HIGH-RISK FL UP FRONT?

2.1 | Implementation of biomarkers into clinical practice

There are currently a multitude of clinical, molecular, clinicopathologic, and functional imaging based biomarkers that can group patients with FL into several risk categories at the time of diagnosis. These tools do not yet inform on how best to treat patients, which remains a significant limitation to day-to-day practical implementation. Additional research is needed to integrate these important developments in clinically meaningful ways. A promising strategy may involve use minimal residual disease (MRD) testing with circulating tumor- or cell-free DNA. This has been used by Delfaue-Larue and colleagues in combination with TMTV to identify groups of patients with FL at risk of poor outcomes (PFS of 65% at 4 years). Several other studies demonstrate value in MRD assessment after chemoimmunotherapy as high prognostic of end of treatment outcome, and others also determine high levels of circulating tumor DNA as associated with poor PFS.[24]

Use of predictive markers will be especially relevant as the design of precision, risk adapted therapies are developed. Gain of function mutations in enhancer of zeste homolog 2 (*EZH2*) are associated with favorable outcome in FL and occur in approximately 20% of individuals. The first in class oral inhibitor of EZH2 tazemetostat was recently approved by the United States Food and Drug Administration for patients with FL having an *EZH2* mutation or those with no other treatment options available. *EZH2* mutation in Fl diagnostic samples has been shown to be a favorable prognostic marker; and also appears to be correlate with response to therapy. In the pivotal Phase 2 study, patients harboring mutation in *EZH2* had higher objective response rates compared to patients with wild type *EZH2* (69% vs. 35%).[25] Moreover, a recent publication by Jurinovic et al.[26] suggested that patients with an EZH2 mutation had a longer PFS if they were treated with CHOP or CVP in the frontline setting versus bendamustine-based therapy.

We look forward to the opportunity to risk adapt therapy by using precision approaches for patient with FL. These and other studies support the optimism that the ability to do so is on the horizon.

CONFLICT OF INTEREST

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SUPPLEMENT ARTICLE

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High-risk follicular lymphoma: Treatment options

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Abstract

Follicular lymphoma (FL) is the most common indolent non-Hodgkin lymphoma in the Western hemisphere. The natural history of FL appears to have been favorably impacted by the introduction of rituximab. Randomized clinical trials have demonstrated that the addition of rituximab to standard chemotherapy induction has improved the overall survival. Maintenance rituximab strategies can improve progression-free survival (PFS). Obinutuzumab was superior to rituximab for PFS in the GALLIUM study, although the benefit was small and required more drug. Chemotherapy platforms have changed in the past decade, as bendamustine combined with rituximab has become commonly utilized frontline strategy in North America and parts of Europe, although there is certainly no one standard treatment. However, several unmet needs remain, including a better ability to identify high-risk patients at diagnosis, the development of predictive biomarkers for targeted agents, the development of novel combinations, and strategies to reduce the risk of transformation. A multitude of novel therapies are under investigation in both the frontline and relapsed/refractory settings. It will be critical to identify the most appropriate populations for new agents and to develop validated surrogate endpoints, so that novel agents can be tested (and adopted, if appropriate) efficiently.

KEYWORDS
follicular lymphoma, high-risk, therapy

1 | THERAPY OF SYMPTOMATIC, HIGH-TUMOR BURDEN FOLLICULAR LYMPHOMA

The addition of rituximab to conventional chemotherapy, has improved outcomes in follicular lymphoma (FL), including overall and complete response rates, progression-free survival (PFS) and overall survival (OS). The results were remarkably consistent across four randomized clinical trials. Clearly, rituximab added to chemotherapy has a therapeutic advance in FL. However, newer data raises questions regarding the optimal anti-CD20 monoclonal antibody and the optimal chemotherapy backbone remains unsettled.

2 | WHICH ANTI-CD20 MONOCLONAL ANTIBODY IS BEST?

The landmark GALLIUM trial compared obinutuzumab, a glyco-engineered type II anti-CD20 monoclonal antibody against rituximab in the frontline management of high-tumor burden FL.[1] The study randomly assigned more than 1200 patients across the globe to either six cycles of either obinutuzumab + chemotherapy or rituximab + chemotherapy, followed by maintenance anti-CD20 for 2 years. Each participating center selected a chemotherapy backbone, with the option of bendamustine, cyclophosphamide, adriamycin, vincristine, prednisone (CHOP), or CVP. The rates of chemotherapy

use were approximately 60% for bendamustine, 30% for CHOP, and 10% for CVP. The trial demonstrated a statistically significant reduction in the risk for progression or death in the patients assigned to obinutuzumab + chemotherapy. The hazard ratio was 0.66, which equates to a 34% risk reduction. At the landmark of 3 years, 80% of the obinutuzumab + chemotherapy patients were in remission versus 73% of the rituximab + chemotherapy patients. No OS differences were observed. The obinutuzumab-treated patients had slightly more infusion reactions, episodes of cytopenia, and infections than the rituximab-treated patients. So is obinutuzumab a superior anti-CD20 monoclonal antibody? Certainly, the outcome of PFS was certainly better in the obinutuzumab-treated patients. However, the results must be interpreted in the context of the dose and schedule. Patients received higher doses of obinutuzumab and more doses of obinutuzumab. A patient who is 2 m^2 would receive 36% more obinutuzumab than rituximab on this study. Note the ~35% risk reduction is very similar to the ~35% difference in dosing. As a result of the design issues with GALLIUM, it is impossible to know if obinutuzumab is truly a better monoclonal antibody than rituximab in FL or whether the results observed are simply of function of dosing differences. As a result, obinutuzumab has not been universally accepted as the new standard of care and some investigators continue to administer rituximab.

3 | WHICH CHEMOTHERAPY IS THE BEST?

Before the introduction of bendamustine, the most commonly used regimens in the United States were R-CHOP (rituximab, cyclophosphamide, vincristine, prednisone) (60%), R-CVP (rituximab, cyclophosphamide, prednisone) (27%), and R-fludarabine-based regimen (13%). A randomized comparison of these regimens indicated R-CHOP had the best risk-benefit profile of the three, as it was more active than R-CVP and less toxic than R-FM.[2]

The alkylating agent bendamustine has gained widespread, although not universal, adoption as the chemotherapy platform of choice in FL. A phase III trial from the StIL group comparing bendamustine, rituximab (BR) to R-CHOP demonstrated BR had better efficacy and reduced toxicity. In this multicenter phase III study, 549 patients with high-tumor burden indolent non Hodgkin lymphoma (NHL) and mantle cell lymphoma (MCL) (median age 64 years) were randomized to receive bendamustine 90 mg/m^2 on days 1 and 2, with rituximab 375 mg/m^2 on day 1, every 28 days (the BR group) or to receive standard R-CHOP chemotherapy every 21 days. The overall response rates (ORRs) were similar in the BR versus R-CHOP groups (92.7% vs. 91.3%, respectively), but the complete response (CR) rate was significantly higher in the BR group (39.8%) compared with the R-CHOP group (30.0%) ($p = 0.03$). When evaluating just the FL patients, with a median follow-up of 45 months, the median PFS was significantly longer after BR compared with R-CHOP (median PFS, not reached vs. 40.9 months, $p = 0.007$). OS did not differ between both groups. There were less hematologic toxicity, alopecia, infections, peripheral neuropathy, and stomatitis with BR. Drug-associated erythematous skin reactions were seen more frequently in the BR group. These data suggest that BR is a better option for untreated high-tumor burden FL. Nine-year updates from this trial were presented at the 2017 American Society of Clinical Oncology Meetings.[3] The long-term follow-up indicated the PFS benefit persisted over time but no OS benefit emerged.

A confirmatory randomized phase III trial (BRIGHT study) was conducted in North America. Previously untreated indolent NHL patients with high-tumor burden were randomized to BR or R-CHOP/R-CVP. Control arm patients were identified as an R-CHOP or R-CVP candidate prior to randomization. The primary endpoint was to show noninferiority of BR in the CR rate. Seventy percent of the 447 enrolled patients had FL, and in these patients, BR therapy was found to be noninferior to the R-CHOP/R-CVP control arm for CR rate (30% vs. 25%) and the ORR (99% vs. 94%). Side-effect profiles were distinct, with more GI toxicity and rash with BR, and more neuropathy and alopecia with R-CHOP/R-CVP. Long-term follow-up was reported in 2019.[4] When one separates out the mantle cell lymphoma patients and limits the analysis to indolent lymphoma, BR was not statistically superior to R-CHOP/R-CVP for PFS, although there was trend toward improvement in PFS with BR with a hazard ratio of 0.70 (95% CI: 0.49–1.01). Although, the BRIGHT data do not exactly replicate the StIL data for BR, they do suggest that BR remains an attractive alternative to R-CHOP or R-CVP in FL.

4 | WHO SHOULD RECEIVE MAINTENANCE ANTI-CD20 THERAPY?

The question of whether to administer maintenance rituximab after frontline R-chemotherapy was addressed in the phase III PRIMA trial. The study evaluated the efficacy and safety profile of maintenance rituximab in newly diagnosed FL patients who responded to initial treatment with rituximab plus chemotherapy. Induction treatment was selected by center; R-CHOP (75%), R-CVP (22%), or R-FCM (3%). Patients were randomized to either observation or a single dose of rituximab every 2 months for 2 years. At a median follow-up of 36 months from randomization, the 2-year PFS in the maintenance rituximab arm was 75% versus 58% in the observation arm ($p < 0.0001$). The beneficial effect of maintenance rituximab was seen irrespective of the induction chemotherapy backbone and in both CR and partial remission (PR) patients. Grade 3–4 adverse events were slightly higher in the maintenance rituximab arm (24% vs. 17%). Long-term follow-up from this dataset was published recently.[5] With a median follow-up of 9 years, the 10-year PFS estimates were 51.1% in the rituximab maintenance arm and 35.0% in the no maintenance arm. No difference in OS was observed, with both groups exhibiting an OS of 80% at 10 years. No new safety signals emerged. Given the lack of OS benefit, the decision regarding the use of maintenance rituximab can be individualized, but a 50% likelihood of remaining disease free at 10 years is highly appealing and begs the question of whether a proportion of FL patients are actually being cured with frontline therapy.

5 | WHAT ABOUT MAINTENANCE ANTI-CD20 THERAPY AFTER BENDAMUSTINE?

A highly interesting and informative subgroup analysis on chemotherapy selection arose from the GALLIUM study.[6] The chemotherapy choice (bendamustine vs. CHOP vs. CVP) was center dependent and not the result of a randomization process. As a result, these data must be interpreted with caution, but several aspects are noteworthy. The 3-year PFS rates were highest in the bendamustine group and lowest in the CVP group. The risk of grade 3–5 adverse events was higher with CHOP than with CVP or bendamustine, but most of these events were transient cytopenias. Grade 3–5 infections were more likely with bendamustine than with CHOP or CVP and fatal adverse event (AEs) were more common with bendamustine than with CHOP or CVP. The difference in infections was particularly notable during the maintenance and follow-up phase. A potential explanation is the significant difference in CD4+ T-cell suppression following treatment. Patients treated with bendamustine experienced profound decreases in CD4+ T-cell counts during bendamustine treatment, with the nadir occurring at end of induction and failing to recover to baseline even 3 years after treatment, whereas the T-cell counts were impacted negligibly by CHOP and CVP therapy. It should be noted that the bendamustine-treated patients were slightly older on average than the CHOP-treated patients, with more patients over age 70 and 80. However, the age distribution of the bendamustine and CVP-treated patient was quite similar. Given the significant T-cell depletion experienced after bendamustine-based treatment, it is quite possible that maintenance therapy with anti-CD20 therapy (and its associate prolonged B-cell depletion) tips the risk–benefit ratio unfavorably in FL. Additional data on the risks versus benefits of maintenance rituximab should come from the Stil NHL7-2008 Maintain trial, which prospectively compares 2 versus 4 years of maintenance rituximab following BR induction therapy. Initial results suggested no major benefit for 4 years over 2 years and an acceptable safety profile.[7]

Attempts to improve the outcomes standard immunochemotherapy with the addition of novel agents have been unsuccessful to date. For example, E2408 was a three-arm randomized phase II clinical trial, using BR followed by rituximab maintenance as the control arm, added the proteasome inhibitor bortezomib to standard BR induction therapy in one of the experimental arms (BVR with R maintenance), and added the immunomodulatory agent lenalidomide to maintenance rituximab in the third arm (BR with lenalidomide, rituximab (LR) maintenance). The 3-year PFS was similar in all three arms, suggesting no benefit for these interventions.[8]

6 | IS SINGLE AGENT RITUXIMAB SUFFICIENT IN THIS PATIENT POPULATION?

Most of the data examining the role for single agent rituximab in the frontline management of FL has been generated in patients who were asymptomatic and had low-tumor burden by GELF criteria (see RESORT Trial from Kahl et al., or UK Trial from Ardeshna et al.). For patients with high-risk FL, due to high-tumor burden, there is no question that single agent rituximab is not as potent as rituximab plus chemotherapy. Having stated that, there may be situations where it is reasonable to consider single agent rituximab. Patients may be elderly and frail, or have significant comorbidities, and the risk of incorporating chemotherapy may be unacceptably high in certain circumstances. Or patients may simply refuse treatment with cytotoxic chemotherapy for personal reasons. Single agent rituximab can be adequately efficacious as was demonstrated by Ghielimini et al. in the SAKK 35/98 trial, where patients were not required to have low-tumor burden. The single agent ORR was 67%. Previously untreated patients receiving a maintenance strategy (n = 20) enjoyed a 45% probability is remaining disease-free at 5 years, suggesting this strategy is perfectly reasonable for selected patients. Prior work from Ghielmini suggests and single agent rituximab loses efficacy when lymph nodes exceed 5 cm in size and work from Witzig et al. showed single agent rituximab is quite ineffective in frontline FL if the lactate dehydrogenase (LDH) is elevated. To summarize, single agent rituximab can be considered a reasonable option in high-tumor burden FL, particularly in elderly or frail patients but clinicians should be aware that the efficacy appears to be diminished if the tumor burden is too high or if there is evidence for high proliferation.

7 | ARE THERE NOVEL STRATEGIES WORTHY OF FRONTLINE CONSIDERATION?

A novel strategy, combining the immunomodulatory agent lenalidomide with rituximab, for the initial management of FL was first reported by investigators from the MD Anderson Cancer Center. The promising results led to the launch of the RELEVANCE trial.[9] A total of 1030 patients with previously untreated FL were randomly assigned to the standard arm of rituximab plus chemotherapy (investigators choice of R-CHOP, BR, or R-CVP) or the experimental arm of lenalidomide plus rituximab. For the first six cycles, lenalidomide was administered orally at 20 mg/day for 3 out of every 4 weeks of each 28-day cycle and rituximab was given at 375 mg/m^2 Intravenous (IV) on day 1 of each cycle. Patients responding after cycle 6 could continue on therapy for up to 12 more cycles, receiving lenalidomide at a dose of 10 mg and rituximab every 8 weeks. The efficacy between the two strategies was similar in terms of ORR, complete response rate, PFS and OS. The 3-year PFS was 77% in the lenalidomide rituximab arm compared to 78% in the R-chemotherapy arm. There was more grade 3–4 neutropenia and febrile neutropenia in the chemotherapy arms and more rash and cutaneous reactions in the lenalidomide arm. Despite the excellent results obtained for lenalidomide–rituximab, it was technically a "negative" study since it was designed (and failed) to show superiority for the novel combination, and it has not received a frontline indication in the US or EU. The MD Anderson group has completed a phase II study of obinutuzumab–lenalidomide, with promising initial results, exhibiting an ORR of 100% and CR rate of 75% in 57 patients with high-tumor burden FL.[10]

TABLE 1 Efficacy data in frontline high-tumor burden follicular lymphoma

Trial	*N*	Induction	Maintenance	3-year PFS (%)
PRIMA	513	R-CHOP/R-CVP/R-FCM	None	58
	505	R-CHOP/R-CVP/R-FCM	Rituximab	75
STiL	140	R-CHOP	None	55
	139	BR	None	75
BRIGHT	186	R-CHOP/R-CVP	Rituximab in 45%	75
	187	BR	Rituximab in 43%	80
GALLIUM	341	BR	Rituximab	81
	345	BO	Obinutuzumab	85
	203	R-CHOP	Rituximab	77
	196	O-CHOP	Obinutuzumab	82
	57	R-CVP	Rituximab	77
	60	O-CVP	Obinutuzumab	77
E2408	65	BR	Rituximab	77
	99	BVR	Rituximab	82
	125	BR	R-lenalidomide	76
RELEVANCE	517	R-CHOP/BR/R-CVP	Rituximab	78
	513	R-lenalidomide	R-lenalidomide	77

8 | WHAT ABOUT UNIQUE PATHOBIOLOGIC SITUATIONS?

There are several potential scenarios that warrant special mention. If a patients' biopsy does not demonstrated transformation but the clinical presentation creates a high level of suspicion for transformation, such as significant B symptoms, hypercalcemia, significantly elevated LDH, very high standard uptake values on positron emission tomography (PET) imaging, is prudent to select anthracycline-based immunochemotherapy. If the patients' diagnostic biopsy demonstrates grade 3B FL, the preponderance of data suggests these patients are best managed with anthracycline-based immunochemotherapy. If the patients' diagnostic biopsy demonstrates grade 3A FL, then these patients can be reasonably managed like typically grade 1–2 FL. Finally, the entity of duodenal FL appears to have a unique underlying biology and natural history that is more indolent than typical FL. These patients can often be very successfully managed with prolonged periods of no active treatment or at most, single-agent rituximab, and rarely should chemotherapy be required.

9 | SUMMARY OF FRONTLINE TREATMENT CONSIDERATIONS

The last 20 years of research into the management of high-tumor burden FL has cemented immunochemotherapy as the standard approach. However, there is no one universally standard immunochemotherapy regimen. In fact, there is quite a bit of geographic variation in the standards. The most commonly utilized options include either obinutuzumab or rituximab combined with either bendamustine or CHOP chemotherapy. Given the lack of OS benefit for maintenance therapy with an anti-CD20, it can be considered optional. Maintenance anti-CD20 therapy has a fairly profound impact on PFS after R-CHOP, and thus is often utilized. On the other hand, maintenance anti-CD20 appears to have much less benefit after BR therapy, and the GALLIUM study suggests it may do more harm than good. A summary of outcomes from selected frontline studies is included in Table 1.

9.1 | Therapy for relapsed and refractory follicular lymphoma

Multiple options exist for the treatment of patients who have failed first-line therapy, and the decision of which therapy to use depends on a number of factors, including the prior treatment utilized, duration of prior response, patient age, comorbid illnesses, and goals of therapy. Duration of prior responses has consistently been shown to be a powerful predictor of future outcomes. Patients who experience disease progression within 24 months (POD24) of frontline immunochemotherapy have been shown to have a 50% overall survival at 5 years, compared with over 90% survival for patients who do not experience POD24. How to best manage POD24 patients remains unclear. Retrospective data from stem cell transplant registries have suggested superior outcomes for transplant strategies in this population, and certainly should be considered in young, fit patients. One word of caution, however, investigators from the British Columbia Cancer Agency have reported that up to 50% of the POD24 population

may harbor transformed disease suggesting it is imperative to investigate this possibility before choosing additional therapy in a POD24 patient.

Besides stem cell transplantation, a variety of nonintensive strategies exist for R/R FL. Certainly, one can administer repeat course of immunochemotherapy (e.g., patients relapsing after BR can receive 0-CHOP). The increasing menu of novel agents is providing additional options for management. There are now four PI3 kinase inhibitors available (idelalisib, duvelisib, copanlisib, and umbralisib).[11–13] Response rates for all three agents tend to run in the 45%–60% range and average durability is typically around 11 months. The oral PI3k inhibitors carry the risk of pneumonitis and colitis, although the risk with umbralisib may be less. Copanlisib does not seem to have these risks although transient hypertension and hyperglycemia around administration must be monitored and addressed. More promising than the PI3k inhibitors is the combination of lenalidomide and rituximab, as demonstrated in the AUGMENT study.[14] The ORR for the combination was 78% and the median PFS was 39 months, compared to 53% and 14 months for rituximab plus placebo. Toxicities were increased compared to the rituximab control arm, most notably for infection (63% vs. 49%), neutropenia (58% vs. 23%), and cutaneous reactions (32% vs. 12%). Given the marked improvement in efficacy, the increased toxicity profile is quite acceptable. Finally, a first-in-class EZH2 inhibitor, tazemetostat, received FDA approval in R/R FL in the summer of 2020. Activating mutations of EZH2, an epigenetic regulator, appear to play significant roles in the pathogenesis of FL. Accordingly, agents designed to suppress EZH2 enzymatic activity are being developed. Tazemetostat 15 is an oral EZH2 inhibitor and was tested in 99 patients with R/R FL, 45 of whom had mutated EZH2 genes and 54 with wild-type EZH2.[15] In the EZH2-mutated patients, the ORR was 69% and the median PFS was 13.8 months. In the EZH2 wild-type patients, the ORR was 35% and the median PFS was 11.1 months. The safety profile appears very good, with negligible grade 3–4 toxicities. The regulatory approval includes both mutated and wild-type patient populations. Table 2 summarizes data for the US FDA-approved targeted agents in R/R FL.

There are several promising investigational immunotherapy approaches in development in relapsed FL. Targeting the "don't eat me" CD47 antigen, expressed on many tumor types and preventing macrophage engulfment, with monoclonal antibodies against CD47 appears promising in early studies. Magrolimab demonstrated an ORR of 61% and CR rate of 24%, as reported by Advani et al. and the 2019 ICML meeting. The bispecific monoclonocal antibody mosunetuzumab targets both CD20 and CD3 and is a potent T-cell engager. An ongoing trial has demonstrated an ORR of 68% with an impressive CR rate of 52% in R/R FL. Finally, and perhaps most encouraging, is recent data generated testing CAR-T therapy in R/R FL. Axicabtagene ciloleucel (axi-cel) demonstrated on ORR of 94% with an CR rate of 80% in a population of R/R FL patients. With a median follow-up of 17 months, the 12-month PFS rate is 77%. More study and longer follow-up are needed for these immuno-oncology agents, but the early data are very promising and summarized in Table 3.

10 | SUMMARY

Outcomes are generally very good in FL with median OS exceeding 15 years. As a result, it is sometimes deprioritized as a cancer in need of therapeutic advances. Certainly, there are FL patients who never require therapy or who only require one line of therapy and can be

TABLE 2 Efficacy data for approved, targeted agents in R/R follicular lymphoma

Agent(s)	Class	*N*	ORR (%)	complete response rate (CRR) (%)	median progression free survival (mPFS) (m)
Lenalidomide–rituximab	Immunomodulatory	147	78	34	39
Rituximab	anti-CD20 MoAb	148	53	18	14
Idelalisib	PI3k inhibitor	72	60	15	11
Duvelisib	PI3k inhibitor	83	48	2	10
Copanlisib	PI3k inhibitor	104	60	14	11
Umbralisib	PI3k inhibitor	117	45	5	11
Tazemetostat (mutated)	EZH2 inhibitor	45	69	13	14
Tazemetostat (wild-type)	EZH2 inhibitor	54	35	4	11

Abbreviation: ORR, overall response rate.

TABLE 3 Investigational immunotherapy agents with promising activity in R/R follicular lymphoma

Agent	Class	*N*	ORR (%)	CRR (%)	Presented
Magrolimab	Anti-CD47 MoAb	41	61	24	Advani, Lugano 2019
Mosunetuzumab	Bi-specific MoAb	62	68	52	Assouline, ASH 2020
Axi-cel	CAR-T	84	94	80	Jacobson, ASH 2020

Abbreviation: ORR, overall response rate.

considered functional cures. On the other hand, subsets of FL patients are at high risk of death from their disease. These include patients with high-risk m7-FLIPI scores, patients who experience recurrence within 2 years of R-chemotherapy, patients who experience histologic transformation, and FL patients under the age of 60 at diagnosis. Future research should (a) seek to identify prognostic biomarkers capable of identifying high-risk patients at diagnosis; (b) continue to develop targeted therapies (with predictive biomarkers); (c) test interventions designed to reduce the risk for histologic transformation; and (d) seek to reliably cure FL.

CONFLICT OF INTEREST

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DATA AVAILABILITY STATEMENT

All data provided in this manuscript is publicly available.

PEER REVIEW

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SUPPLEMENT ARTICLE

WILEY

Allogeneic stem cell transplant in non-Hodgkin lymphomas: Still an indication?

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Abstract

Allogeneic hematopoietic cell transplantation (alloHCT) used to play a defined role in the treatment of non-Hodgkin lymphoma (NHL). With the advent of modern targeted molecular therapies and immunotherapies, treatment standards at least for B-cell lymphoma have undergone significant changes, thereby questioning the traditional role of alloHCT in these diseases. This paper attempts to describe the current place and the perspectives of alloHCT in the rapidly evolving treatment landscape of NHL.

KEYWORDS
allogeneic transplantation, CAR-T cells, lymphoma, NHL

1 | INTRODUCTION

Entering the clinical stage more than 50 years ago, allogeneic hematopoietic cell transplantation (alloHCT) was the first immunotherapy successfully applied to patients, and can be considered as the ancestor of modern cellular immunotherapy.[1] Despite its inherent drawbacks of significant non-relapse mortality and morbidity due to graft-versus-host disease, until recently, alloHCT has been playing a defined role in the management algorithms of the main NHL subtypes (i.e., diffuse large B-cell lymphoma [DLBCL]; follicular lymphoma [FL]; mantle cell lymphoma [MCL]; and peripheral T-cell lymphoma [PTCL]), mostly in the salvage setting. With the advent of modern targeted molecular therapies and immunotherapies, treatment standards at least for B-cell lymphoma have undergone substantial changes, thereby questioning the traditional role of alloHCT in these diseases. This paper attempts to describe the current place and the perspectives of alloHCT in the rapidly evolving treatment landscapes of DLBCL, FL, MCL, and PTCL.

2 | DIFFUSE LARGE B-CELL LYMPHOMA

As in almost all neoplastic indications where it is effective, the basis of alloHCT in DLBCL is graft-versus-lymphoma activity (GVL). Circumstantial evidence for GVL efficacy in DLBCL can be derived from the effectiveness of immunomodulation for preventing or treating post-transplant relapse, and from observations showing that non-myeloablative alloHCT can provide long-term disease control in patients having failed autologous hematopoietic cell transplantation (autoHCT). However, compared to the other main NHL entities, DLBCL appears to be less GVL-sensitive resulting in long-term progression-free survival rates of 30%–40%, and even less if calculated by intent-to-treat.[2–4]

While the standard indication for alloHCT in DLBCL used to be chemosensitive disease following failure of autoHCT, chimeric antigen receptor-engineered T-cells (CARTs) have become the preferred cellular immunotherapy in this setting.[3] A preliminary intent-to-treat comparison of alloHCT versus CARTs in large B-cell lymphoma (LBCL) suggested that in patients having failed at least two lines of systemic therapy, survival tended to be better with the CART versus the alloHCT approach.[5] One limitation of this study was the short follow-up of 10 months in the CART group. Figure 1 shows an update of this comparison with a median follow-up of 15 (9–24) months, suggesting that the favorable trend for CARTs was maintained, both measured from treatment indication and start of cellular therapy.

In conclusion, for the time being, alloHCT remains an indication for advanced DLBCL when CARTs have failed or are not feasible.[3]

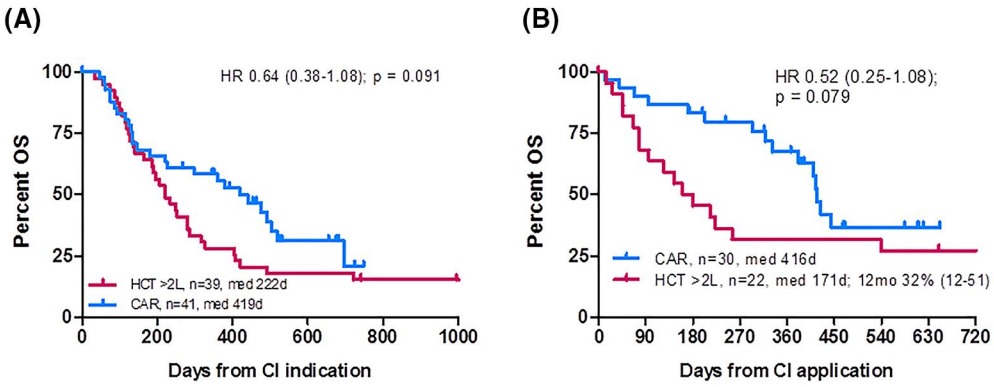

FIGURE 1 Overall survival of patients with diffuse large B-cell lymphoma beyond the second line by cellular immunotherapy intended (Heidelberg, 2005–2020, *N* = 80). (A) Survival from indication; (B) Survival from administration of cellular immunotherapy. CAR, chimeric antigen receptor T-cells; HCT, allogeneic hematopoietic cell transplantation; HCT>2L, HCT intended after having failed two or more lines

However, it is clear that emerging innovations, such as bispecific antibodies and moving CARTs to the second line, have the potential for further modifying the importance of alloHCT for rescuing patients with relapsed/refractory DLBCL.

3 | FOLLICULAR LYMPHOMA

Although FL appears to be the most GVL-sensitive disease among all alloHCT indications currently considered as standard, with a 5-year relapse risk less than 20% in all major studies published,[2,6] the number of allogeneic transplantations for this subtype is decreasing since several years, making FL the least frequent allo indication of the four entities discussed in this review. In 2018, the numbers of allo-transplants registered with the European Society for Blood and Marrow Transplantation (EBMT) for PTCL, DLBCL, MCL, and FL were 401, 320, 161, and 144, respectively (EBMT data on file, PROMISE download 19 February 2020). This has to do with the indolent course of the disease, the high efficacy of standard first-line treatment with chemoimmunotherapies and CD20 antibody maintenance, and the availability of a broad effective toolkit for salvage treatment, including revlimid, targeted therapies, and also autoHCT. Even in high-risk disease, defined by failure of first-line treatment within 2 years ("POD24"), there is no proven benefit of alloHCT over autoHCT.[7]

In contrast to DLBCL and MCL, there is no CART therapy approved for FL available to date, and due to the indolent character of the disease the benefit of CART approaches in terms of durable lymphoma control will be more difficult to assess. Nevertheless, the development of CD19-directed CARTs and other immunotherapies for indolent lymphoma is already quite advanced, suggesting that the alloHCT indication will further narrow in the near future.

For today, alloHCT is still a potentially curative option for those patients with FL who are resistant to less aggressive approaches, that is, who relapse early after salvage autoHCT or a similarly intensive regimen, and for patients with emerging exhaustion of hematopoiesis, or incipient myelodysplasia.

4 | MANTLE CELL LYMPHOMA

Efficacy of donor lymphocyte infusions (DLI) and plateaus in the relapse curves after reduced intensity conditioning (RIC) suggest that there is a biologically relevant contribution of GVL also in MCL.[8,9]

Given the poor prognosis of MCL recurring after state-of-the-art intensive first-line treatment with consolidating autoHCT and rituximab maintenance, the traditional place of (RIC) alloHCT has been consolidation of second-line responses. With the introduction of Bruton's tyrosine kinase inhibitors (BTKi) as standard of care salvage therapy in MCL, and the recent approval of the CART product brexucabtagene autoleucel for relapsed/refractory MCL, the place of alloHCT in the MCL management algorithm needs to be re-evaluated. A recent international consensus project recommended considering alloHCT in MCL only if CARTs have failed or are not feasible (Hamadani et al., manuscript in preparation). This would mean that alloHCT comes into play only on the fourth place after standard induction, BTKi, and CARTs. However, in areas where CARTs are not available, considering alloHCT already for consolidation of second-line responses to BTKi might be worthwhile in high-risk patients, such as those without a complete response to BTKi or early failure after standard induction.[10] Again, the expected advent of novel molecular agents and immunotherapeutics in the clinical routine of MCL management, such as venetoclax, enhanced CD19-directed antibodies, and next-generation phosphatidylinositol 3-kinase delta inhibitors has the potential to further differentiate the alloHCT indication in MCL in the near future.

5 | PERIPHERAL T-CELL LYMPHOMA

In contrast to B-cell lymphoma, there have been no major therapeutic improvements for PTCL in the last decades, with the exception of anaplastic large cell lymphoma (ALCL). Although the value of autoHCT consolidation is ambiguous, accepted standard treatment for the three predominant nodal PTCL subsets (i.e., ALK-negative ALCL, PTCL not other specified [PTCL-NOS], and angioimmunoblastic T-cell lymphoma

- Large B-cell lymphoma: 4th line after failure of induction, autoHCT attempt, and CAR T-cell therapy.
- Follicular lymphoma: early relapse after salvage autoHCT or a similarly intensive regimen, and emerging exhaustion of hematopoiesis/incipient myelodysplasia.
- Mantle cell lymphoma: 4th line after failure of induction, BTKi, and CAR T-cell therapy.
- Peripheral T-cell lymphoma: relapsed/refractory disease.

Abbreviations: alloHCT, allogeneic hematopoietic cell transplantation; autoHCT, autologous hematopoietic cell transplantation; BTKi, Bruton's tyrosine kinase inhibitors; CAR T-cell therapy, chimeric antigen receptor-engineered T-cell therapy.

TABLE 1 Select lymphoma alloHCT standard indications at a glance

[AITL]) consists in CHOP-like induction followed by high-dose intensification.[11] With this strategy, 5-years progression-free survival rates of 35%–45% can be expected.[12] Of particular concern in PTCL is the high rate of primary refractoriness which can affect up to one third of the patients.

Similar to FL and MCL, PTCL appears to be quite susceptible to GVL effects as illustrated by survival plateaus around 50% after alloHCT across numerous studies and efficacy of DLI,[2,4,8,12,13] prompting the exploration of allotransplantation as first-line consolidation. A large randomized trial comparing alloHCT with autoHCT in the first-line setting, however, failed to show a benefit for the alloHCT strategy, largely because the lower relapse risk associated with alloHCT was neutralized by excess non-relapse mortality.[14] Thus, except for selected orphan PTCL subsets such as hepatosplenic T-cell lymphoma, first-line alloHCT should not be performed outside of clinical trials.

In contrast, alloHCT is the preferred option in relapsed/refractory PTCL, ideally after having achieved a state of controlled disease prior to transplant.[11,12] This is because of the lack of therapeutic alternatives with curative perspective. In patients not having undergone autoHCT during first-line treatment, also autotransplantation may be considered though appearing inferior to alloHCT in intent-to-treat comparisons.[13] Similar to the other lymphoma subsets described in this paper, reduced intensity conditioning provides outcomes in PTCL that are at least similar to that observed after myeloablative conditioning, and haplo-identical donors seem to be a valuable alternative if matched related or unrelated donors are not available.[12]

6 | CONCLUSIONS

Although substantial therapeutic innovations in particular for B-cell lymphoma have entered the clinical stage recently, or are at the doorstep, immunotherapy by alloHCT remains an effective and potentially curative option for settings where the medical need unmet by traditional chemotherapy can also not be covered by novel therapeutics. This gap might be bigger than believed as some promises of novel agents given in phase-2 studies have not been fully kept in the real world.[15] Suggestions for current indications for alloHCT are summarized in Table 1. If alloHCT is taken into account according to these suggestions, it has to be kept in mind that the window of opportunity for a successful outcome of transplantation is largest before tumor refractoriness and performance status deterioration have developed through serial palliative or experimental treatment attempts.

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SUPPLEMENT ARTICLE

WILEY

Optimizing CAR T cell therapy in lymphoma

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Abstract

Chimeric antigen receptor (CAR) T cell therapy has significantly improved the outlook for patients with certain types of poor-risk lymphoma. Despite these advances, a majority of patients undergoing CAR T therapy will suffer progression or relapse of disease, and toxicity remains a concern. Additionally, the patients and disease subtypes that are most likely to benefit from CAR T have yet to be fully defined. Many ongoing trials are exploring novel CAR T approaches to address these concerns. In this review, we highlight some of the primary strategies and relevant studies aimed at improving the utility of CAR T therapy in lymphoma.

KEYWORDS
cellular therapy, chimeric antigen receptor T cell, lymphoma

1 | INTRODUCTION

In 2017, the first chimeric antigen receptor (CAR) T cell therapy was approved for use in relapsed or refractory aggressive B cell lymphoma. The initial CAR T studies demonstrated remarkable efficacy in a population of patients with otherwise dismal outcomes. In the 4 years since, there has been a marked expansion in the use of CAR T cell therapy and the development of novel CAR T strategies for the management of lymphoma.

Despite these encouraging results, significant challenges and opportunities for improvement in CAR T therapy remain. While the majority of patients attain responses to treatment, most patients will eventually develop relapsed or refractory disease. Toxicity also remains a major limitation to CAR T therapy; cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS) often require hospitalization and present a major source of cost and morbidity.

With the goal of addressing these shortcomings, over 200 clinical trials investigating CAR T therapy in lymphoma are underway, with more strategies in preclinical development (clinicaltrials.gov). This review aims to highlight some of the most promising strategies for improving the outcomes of lymphoma patients using CAR T therapy. Some of the greatest opportunities for advancement include broadening the eligible patient pool and identifying those most likely to benefit from CAR T, expediting CAR T manufacturing, optimizing lymphodepletion, improving CAR T design, and co-administering agents that may improve the efficacy or reduce the toxicity of CAR T therapy.

2 | CURRENT PRACTICE

At time of writing, three products, axicabtagene ciloleucel (axi-cel), tisagenlecleucel (tisa-cel), and most recently, lisocabtagene maraleucel (liso-cel), are available for commercial use in relapsed or refractory large B cell lymphoma in various countries. Results with each agent in their index clinical trials are summarized in Table 1. Encouraging overall response rates ranging from 52%–82% were reported, with 12-month overall survival of 48%-59%.[1–3] These results compared favorably with historical outcomes in relapsed or refractory DLBCL, where patients undergoing conventional salvage therapy had a median overall survival of 6.3 months.[4] All three agents had characteristic toxicity profiles, with severe (grade ≥ 3) CRS in 1%–22% of patients, and severe (grade ≥ 3) neurotoxicity in 12%–28% of patients. Similar efficacy and toxicity outcomes have been noted in real-world analyses where patients were, on average, older and with more comorbidities. Trial design and outcomes are reviewed in greater detail in Table 1, and more detailed analysis of these three products have recently been published.[5]

Most recently, brexucabtagene autoleucel (brexu-cel) demonstrated promising results in relapsed or refractory mantle cell lymphoma (MCL) and is now commercially available.[6] In the ZUMA-2

TABLE 1 Landmark clinical trials in CAR T cell therapy for lymphoma

	ZUMA-1	JULIET	TRANSCEND	ZUMA-2
CAR T cell product	Axicabtagene ciloleucel (axi-cel, Yescarta)	Tisagenlecleucel (tisa-cel, Kimriyah)	Lisocabtagene maraleucel (liso-cel, JCAR017)	Brexucabtagene autoleucel (brexu-cel, Tecartus)
Bridging therapy	None	93%	72%	37%
Lymphodepletion	Flu/Cy 500/30 × 3d	Flu/Cy 250/25 × 3d versus Benda	Flu/Cy 300/30 × 3d	Flu/Cy 500/30 × 3d
Construct	Anti-CD19 scFV, CD28, CD3ζ	Anti-CD19 scFV, 4-1BB, CD3ζ	Anti-CD19 scFV, 4-1BB, CD3ζ	Anti-CD19 scFV, CD28, CD3ζ
Disease indications	DLBCL, HGBCL, tFL, PMBCL	DLBCL, HGBCL, tFL	DLBCL, HGBCL, tFL, PMBCL, FL grade 3B	MCL
Prior lines of therapy	≥2	≥2	≥2	≥2 (prior anti-CD20, anthracycline, BTKi required)
ORR	82%	52%	80%	93%
12 month PFS	44% (34–53)	N/A	44.1% (37.3–50.7)	61%
12 month OS	59% (49–68)	48.2% (38.6–57.1)	57.9% (51.3–63.8)	83%
CRS (Grade ≥3)	93% (13%)	58% (22%)	35% (1%)	91% (15%)
ICANS (Grade ≥3)	64% (28%)	21% (12%)	19% (13%)	63% (31%)

Note: These studies have resulted in regulatory approval and commercial availability of their respective CAR T products.

Abbreviations: Benda, bendamustine; BTKi, bruton tyrosine kinase inhibitor; CAR, chimeric antigen receptor; CRS, cytokine release syndrome; d, days; DLBCL, diffuse large B-cell lymphoma; Flu/Cy, fludarabine/cyclophosphamide; HGBCL, high grade B-cell lymphoma; ICANS, immune effector cell-associated neurotoxicity syndrome; MCL, mantle cell lymphoma; N/A, not available; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PMBCL, primary mediastinal B-cell lymphoma; scFV, single chain variable fragment; tFL, transformed follicular lymphoma.

trial, brexu-cel demonstrated an overall response rate (ORR) of 93%, and prolonged 12-month progression-free and overall survival at 61% and 83%, respectively. Most patients had CRS and neurotoxicity, with 15% developing grade 3 CRS and 31% having grade ≥3 ICANS.

3 | PATIENT SELECTION

At time of publication, there are no reliable predictive models to anticipate treatment efficacy or toxicity in CAR T recipients, and this remains an active area of investigation. The only readily reproducible determinant to date associated with response is the degree of tumor burden, as reflected by tumor volume or lactate dehydrogenase (LDH). Other data suggest that patients with an altered performance status (Eastern Cooperative Oncology Group (ECOG) > 1) or a heightened inflammatory state (C-reactive protein elevation) have a higher risk of treatment failure.[7,8] Factors classically associated with prognosis with standard chemoimmunotherapy, such as age, prior therapy, International Prognostic Index (IPI), and cell of origin, are not predictive of outcome in CAR T therapy. Other tumor biomarkers, including immune checkpoint molecules such as PD-L1 status, LAG-3 expression, and TIM-3 have been investigated with conflicting results in their predictive capacity.[9]

A major determinant of response may be the quality and composition of the patient's autologous T cells that are engineered into CAR T cells. In one study of chronic lymphocytic leukemia (CLL) patients, sustained remission was seen in patients with higher proportions of a T cell subset with $CD45RO^{-}CD27^{+}CD8^{+}$ immunophenotype, characterized by long-lived memory cells in a resting state, with high capacity for expansion and acquisition of effector functions on antigen re-exposure.[10] $CD27^{+}PD\text{-}1^{-}CD8^{+}$ cells with high expression of IL-6R were also predictive of response, providing potentially intriguing rationale for the interruption of the PD-1/PD-L1 axis via CAR T modification or concomitant checkpoint blockade. T cell polyfunctionality, defined as the capacity of a T cell subset to deploy multiple immune programs, has also been associated with both increased overall response and toxicities.[11] These factors may not only provide prognostic guidance, but also help refine our practices in preservation and collection of an adequate T cell product. T cell fitness is compromised by chemotherapy, particularly regimens incorporating bendamustine, and avoiding such regimens or collecting prior to such treatments may allow for more effective CAR T products.[12]

4 | EXPANDING CAR T INDICATIONS

Commercially available CAR T products are approved for use in relapsed or refractory diffuse large B cell lymphoma, high-grade large B cell lymphoma, transformed follicular lymphoma, and MCL. Intuitively these constructs, all targeting the CD19 cell surface antigen, are likely to have efficacy in other B cell lymphomas, and ongoing

studies have demonstrated encouraging preliminary results in other subtypes, including indolent diseases such as follicular lymphoma, marginal zone lymphoma, and chronic lymphocytic leukemia.

While indolent lymphomas can often be observed or controlled with first-line therapies, many patients will ultimately develop multiply relapsed disease refractory to currently available treatments. Preliminary data (Table 2) from ongoing Phase 2 studies evaluating axi-cel, tisa-cel, and liso-cel have demonstrated promising efficacy, though final results and Food and Drug Administration (FDA) review are pending.[13–16]

Outside the realm of B-cell non-Hodgkin lymphoma, CAR T cell therapy is being explored in Hodgkin lymphoma and T cell lymphoma. An anti-CD30 CAR T cell therapy was recently developed with encouraging preliminary data for Hodgkin lymphoma.[17] While T cell lymphoma is an intriguing target for CAR T therapy, there are a number of unique challenges to this approach. While patients can tolerate the sustained B-cell aplasia associated with anti-CD19 therapy, a similar elimination of the T cell pool would likely cause severe immunosuppression and lead to infectious complications. As such, CAR T cells must target cell surface antigens that are disproportionately expressed on the T cell malignancy as compared to the normal T cell repertoire in order to mitigate "on-target, off-tumor" toxicity. Targets of interest at this time include CD5, CD7, TRBC1, CD37, CD38, and B7-H3, among others. Another challenge is CAR T cell fratricide—the possibility of CAR T cells recognizing a self-antigen, triggering cytotoxic killing of other CAR T cells. One potential solution is to alter expression of the target antigen on the CAR T cells themselves.

Alongside histologic indications, the use of CAR T therapy in CNS lymphoma is gradually expanding. The initial landmark trials for axi-cel and tisa-cel excluded patients with active CNS disease, given unclear efficacy and potential for neurotoxicity. Since then, small studies had shown that commercial CAR T therapy off trial demonstrated efficacy in secondary CNS lymphoma with no evidence of excess neurotoxicity.[18,19] The TRANSCEND phase II study of liso-cel included seven patients (3%) with secondary CNS lymphoma, with three of six evaluable patients achieving complete response.[3] Studies incorporating patients with primary CNS lymphoma are also underway.

5 | INCLUDING CAR T IN EARLIER LINES OF THERAPY

The commercial use of CAR T therapy is currently approved in the setting of multiply relapsed/refractory disease. Patients with aggressive lymphomas who have failed two prior lines of chemoimmunotherapy are more likely to have complications from their disease or prior treatment; as a result, they may have more extensive disease or greater comorbidities at the time of CAR T therapy. Understandably, there is interest in determining whether CAR T therapy in an earlier phase of treatment is appropriate. Three ongoing Phase 3 clinical trials are investigating the three main CAR T products in the second line setting. In these studies, patients with relapsed or refractory DLBCL after first-line chemoimmunotherapy that are transplant eligible are randomized to receive either up-front CAR T cell therapy or the current standard of care, salvage chemoimmunotherapy followed by autologous stem cell transplantation.[20]

Axi-cel CAR T therapy is also being investigated in the first-line setting for those with aggressive B cell lymphoma with initial adverse characteristics in ZUMA-12.[21] In this Phase 2 study, patients with positive interim PET after two cycles chemoimmunotherapy underwent leukapheresis followed by lymphodepletion and CAR T therapy. Interim analysis of 32 patients revealed an ORR of 85% and CR rate of 74%, which compares favorably with historical controls.[21]

6 | EXPEDITING THE COLLECTION, MODIFICATION AND EXPANSION OF CAR T CELL THERAPY

A significant challenge to the implementation of CAR T therapy is the delay required for collection, modification, and expansion of CAR T cells. Median turnaround time from apheresis to infusion in a real-world analysis of commercial therapy was 28 days for axi-cel and 44 days for tisa-cel in the United States.[22] Fourteen (9%) of patients receiving axi-cel and 3 (4%) of those receiving tisa-cel died from progression of disease prior to infusion.

Point-of-care CAR T manufacturing, as opposed to the current model of centralized CAR T manufacturing, has already shown promise. A closed-system manufacturing platform capable of T cell enrichment, CAR vector transduction, washing and expansion at the treatment site has already been utilized in phase I clinical trials, with successful production of effective CAR T cells and turnaround time from apheresis to infusion of 14 days[23,24] Such systems would allow for more rapid administration of CAR T product to patients and may prove more cost-effective over time.

Another approach is the use of an allogeneic CAR T product derived from healthy donors, which would bypass the delays and costs of autologous CAR T cell collection and manufacturing altogether, while also avoiding the use of potentially dysfunctional patient-derived T cells. Challenges to such an approach include the risk of graft versus host disease (GVHD) and host rejection of the allogeneic CAR T cell. The ALPHA study is an ongoing phase I trial investigating ALLO-501, an allogeneic anti-CD19 CAR T product.[25] ALLO-501 is genetically modified to disrupt the TCR alpha constant gene as prevention against GVHD. CD52 is also disrupted to allow administration of an anti-CD52 monoclonal antibody for host-specific lymphodepletion, with the goal of preventing CAR T rejection. Preliminary results of the first nine evaluable patients showed encouraging initial response rates with ORR 78%, though three of seven responders subsequently progressed. While durability of response remains a question, response rates are encouraging and such products may be useful as a bridge to other therapies, such as allogeneic stem cell transplantation. Alongside

TABLE 2 Selected clinical trials investigating expanded indications for CAR T therapies, including indolent lymphoma, CLL, and MCL

	ZUMA-5	TRANSCEND NHL 001 (MCL)	TRANSCEND CLL 004	ELARA
Disease indications	FL grade 1-3A, EN MZL	MCL	CLL	FL grade 1-3A
Prior lines of therapy	≥2 (anti-CD20, alkylating agent required)	≥2 (anti-CD20, alkylating agent, BTKi required)	≥3 (standard-risk disease) ≥2 (high-risk disease)	≥2 (anti-CD20, alkylating agent required)
CAR T cell product	Axi-cel	Liso-cel	Liso-cel	Tisa-cel
Median follow-up, months	17.5	10.9 (DL1), 3.1 (DL2)	18	6.5
Patients enrolled (treated per protocol)	151 (151)	42 (32)	23 (22)	98 (52)
CRR (95% CI)	76%	59%	45%	71.1% (56.5–84.0)
ORR (95% CI)	92% (85–97)	84%	82%	84.8% (71.1–93.7)
% Patients with ongoing response	72% at 12 months	-	50% at 18 months	84.4% at 6 months
CRS (Grade ≥3)	82% (7%)	50% (3%)	N/A (9%)	48% (0%)
ICANS (Grade ≥3)	60% (19%)	28% (9%)	N/A (22%)	10% (2%)

Note: Of note, outcomes of TRANSCEND NHL 001, TRANSCEND CLL 004, and ELARA are preliminary and based on the most recent available published results at time of writing.

Abbreviations: BTKi, Bruton tyrosine kinase inhibitor; CAR, chimeric antigen receptor; CLL, chronic lymphocytic leukemia; CRR, complete response rate; CRS, cytokine release syndrome; DL, dose level; DOR, duration of response; EN MZL, extranodal marginal zone lymphoma; FL, follicular lymphoma; ICANS, immune effector cell-associated neurotoxicity; ITT, intention to treat; NHL, non-hodgkin lymphoma; ORR, overall response rate; PP, per protocol.

allogeneic CAR T cells, CAR-modified allogeneic natural killer (NK) cells represent a promising novel therapy, and have shown activity in non-hodgkin lymphoma (NHL).[26] Potential advantages include the ability to administer HLA-mismatched products, and avoidance of the CRS and neurotoxicity seen in CAR T therapy, though questions remain about NK cell persistence and long-term disease control (Table 3).

7 | OPTIMIZING LYMPHODEPLETION

Lymphodepletion prior to CAR T infusion has been associated with improved CAR T cell expansion and antitumor efficacy in hematologic malignancies.[27] It is thought that lymphodepletion benefits CAR T cell function via several mechanisms, including the clearance of endogenous lymphocytes that act as "cytokine sinks" and improved access to cytokines that facilitate T cell expansion, removal of immunosuppressive cells such as regulatory T cells and myeloid-derived suppressor cells, and improved function of antigen-presenting cells.[28] While lymphodepletion incorporating fludarabine and cyclophosphamide (Flu/Cy) is an established component of CAR T therapy, there is little head-to-head data to guide the choice of agents or dosing.

The role of lymphodepletion in modulating the host immune profile is gaining increasing interest. In one study, an increase in monocyte chemoattractant protein-1 after lymphodepletion, and higher interleukin-7 (IL-7) peak, were associated with improved progression-free survival (PFS) in CAR T recipients. Higher-intensity Flu/Cy conditioning was associated with this favorable cytokine profile, suggesting that modification of the lymphodepletion regimen to achieve an optimal cytokine response may result in better outcomes.[29] Another recent preclinical study investigating CAR T therapy in models of lung cancer found that including oxaliplatin in the lymphodepletion regimen increased intratumor macrophage production of T-cell recruiting cytokines and improved CAR T cell tumor infiltration.[30] Whether a similar strategy of employing immunogenic chemotherapy would be efficacious in B cell malignancies remains to be seen.

8 | BUILDING A BETTER CAR

8.1 | Improving CAR design

CAR T cells are produced by the transduction, via lentivirus or retrovirus, of a genetically engineered CAR into an autologous T cell population. All commercially available products utilize a "second-generation" CAR, which consists of an extracellular antigen-binding single chain variant domain (scFV), which targets the B cell antigen CD19; hinge and transmembrane domains, often derived from CD8α or an extension of CD28; a CD3-derived activation domain; and one or more costimulatory domains, most commonly either CD28 or 4-1BB. Many variations on this CAR T product are being explored in preclinical and early clinical trials, exploring modifications in the number and type of costimulatory domains, the use of alternative targets or dual-targeted CAR products.

TABLE 3 Challenges and strategies for improvement of CAR T therapy for lymphoma

Challenge	Strategies for improvement	Examples
Prolonged time to treatment	Point-of-care CAR T manufacturing	Closed-system platforms
	Off-the-shelf allogeneic CAR T product	ALLO-501
Autologous T cell dysfunction	Off-the-shelf allogeneic CAR T product	ALLO-501
	Avoiding lymphotoxic chemotherapy prior to T cell collection	CAR T therapy in earlier lines of treatment earlier T cell collection in high-risk pts
Improving CAR-T trafficking	Combine with cytokine	TRUCKs co-expressing IL-12, IL-15, or IL-18
	Novel lymphodepletion approaches	Higher dose fludarabine/cyclophosphamide
		Alternative agents (oxaliplatin)
CD19 loss	Novel CAR T targets	scFVs specific for CD20, CD22, CD79b, BAFF-R, kappa light chain, ROR1
	Dual antigen targeting	Bispecific CAR T cells: LV20.19: anti-CD19 + anti-CD20 AUTO-3: anti-CD19 + anti-CD22
CAR T exhaustion	Blocking immune checkpoint signaling	Co-administration of checkpoint inhibitors
		Anti-PD-1 or anti-PD-L1 blocker secretion
		PD-1 gene silencing
		PD-1/CD28 "switch receptors"
	Modulating downstream signaling	Third-generation CAR T cells
		CD3 ITAM modulation (1XX)
	Other immunomodulators	Ibrutinib
		Lenalidomide
Recruiting endogenous immune cells	Local cytokine production	TRUCKS co-expressing IL-12, IL-15, or IL-18
	Antigen presenting cell licensing	Armored CARs secreting CD40L
Toxicity	Anti-inflammatory agents	Anakinra
	Other immunomodulators	Ibrutinib
	Off-switches	Inducible Casp9
		EGFR surface expression
		CD20 expression
	Reversible CAR T inhibition	Dasatinib
	Changes to CAR T design	Hinge/linker changes—CD19-BBz(86)
		GM-CSF gene silencing

Abbreviations: CAR, chimeric antigen receptor; GM-CSF, granulocyte-macrophage colony stimulating factor; ITAM, immunoreceptor tyrosine-based activation motif; scFV, single chain variable fragment.

8.2 | Fine-tuning CAR signaling

After binding target antigen, intracellular domains trigger downstream signals leading to T cell activation, proliferation, and antitumor cytotoxicity. The degree of downstream activation appears to be crucial in determining efficacy. Inadequate signaling leads to insufficient antitumor activity and poor T cell persistence, whereas excessive or redundant T cell activation can drive CAR T exhaustion. Several strategies to optimize CAR signaling are underway investigating various components of the CAR construct (Figure 1).

So-called "third-generation" CARs have been developed which carry both CD28 and 4-1BB costimulatory domains, with unclear benefit to date. In one Phase 1 trial of patients with NHL, patients were simultaneously infused with both second- and third-generation CAR T cells, with the later CAR T cells exhibited greater expansion and persistence than second generation CAR T cells, particularly in patients with lower disease burden.[31]

Reducing the activation potential may also improve CAR T cell persistence and limit exhaustion, allowing for more durable responses. Modification of the CD3ζ activation domain in order to modulate the activation potential of CARs has yielded intriguing

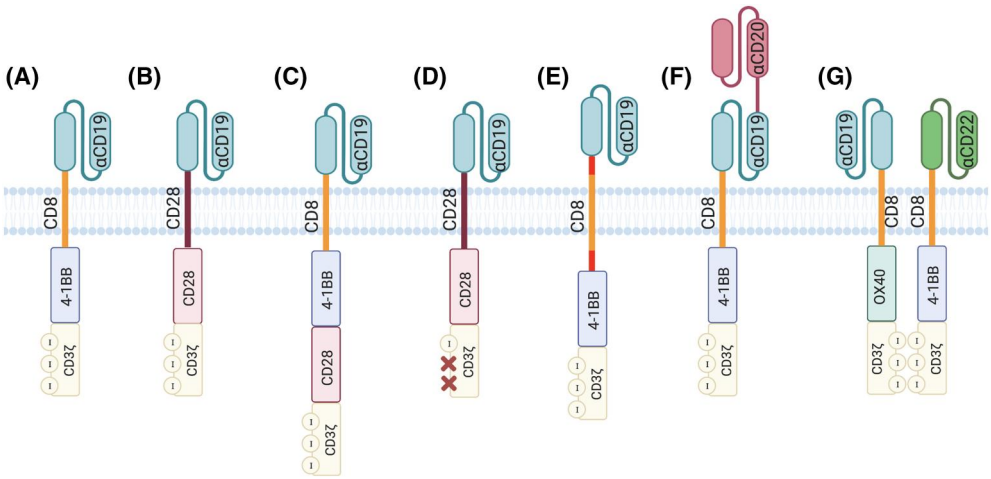

FIGURE 1 Selected CAR T cell constructs currently in use or in development. Figure 1A,B are second-generation constructs consistent with tisa-cel and axi-cel, respectively. Third generation (Figure 1C) CAR T cells contain two costimulatory domains. The 1XX CAR construct (Figure 1D) is genetically modified with activation of the second and third ITAMs, reducing redundant activation and downstream signaling. Modifications to the hinge and transmembrane domains (Figure 1E) have been demonstrated to alter activation potential and cytokine production. Dual-targeted CAR T cells have also been developed either expressing a single CAR with two scFV domains targeting two separate surface antigens (Figure 1F) or expressing two separate CARs with differing targets (Figure 1G). The figure was created with BioRender.com. CAR, chimeric antigen receptor; ITAM, immunoreceptor tyrosine-based activation motif

results in preclinical studies. In one preclinical study, inactivation of the second and third immunoreceptor tyrosine-based activation motifs (ITAMs) on CD3ζ, leaving only the most proximal ITAM, led to increased anti-tumor activity and T cell persistence.[32] This "1XX" CAR construct is currently being investigated in an ongoing clinical trial.

The hinge and transmembrane domains also play a role in CAR signaling. In one such example, the standard CD19-BBz(71) product utilized in tisa-cel was modified with additional amino acids, lengthening the extracellular and intracellular transmembrane domains.[33] The resultant CD19-BBz(86) product retained cytolytic capacity while exhibiting markedly decreased cytokine production as compared to standard CD19-BBz. A Phase I clinical trial of CD19-BBz(86) showed comparable antitumor efficacy with remarkably low toxicity; no patients had greater than grade I CRS/ICANS, and there were no significant elevations in serum cytokine levels after infusion. The underlying mechanism for this functional change remains to be elucidated, but does reinforce the dynamic role of hinge and transmembrane domains, and the potential for less toxic CAR T constructs.

8.3 | Alternative and dual-targeted CAR T cells

One of the primary mechanisms of resistance to CD19-directed CAR T therapy is antigen escape via CD19 surface antigen loss (induced by various molecular alterations in the *CD19* gene). To address this, alternative CARs with scFVs recognizing CD20, CD22, CD79b, BAFF-R, kappa light chain, and ROR1 have been developed and are in various stages of preclinical and clinical development. Bispecific CAR T cells targeting two surface antigens simultaneously may prevent resistance via antigen escape, and clinical studies investigating these therapies are underway. One such study of LV20.19 CAR T cells, with bispecific CD19 and CD20 targeting, showed promising efficacy and safety profile (ORR 82%, CR 64%).[24] Notably, none of the patients who had progression or relapse had loss of CD19. Similarly encouraging results have been reported with AUTO-3, a bispecific CAR T expressing anti-CD19 and anti-CD22 CARs.[34]

8.4 | Armored CARs, TRUCKs, and other co-modifications

In addition to CAR modifications, T cell co-modifications are being investigated to overcome the immunosuppressive tumor microenvironment, stimulate intrinsic immune response and in some cases deliver antitumor drugs in a localized fashion.[35] TRUCKs (T cells Redirected for Universal Cytokine Killing) are CAR T cells engineered to secrete cytokines such as IL-12, IL-15, or IL-18. In preclinical models these modifications have been shown to increase cytotoxicity and CAR T cell expansion, increase resistance to Treg cell-mediated inhibition, and in some cases recruit endogenous immune cells to the tumor microenvironment. CAR T cells expressing CD40 ligand upregulate antigen presentation in B cell malignancies and activate antigen-presenting cells, and may facilitate a sustained endogenous immune response against tumor. Other models have been developed using various strategies to disrupt the PD-1/PD-L1 immune checkpoint axis and disinhibit immune effector cells, including the CAR T cells themselves.

Co-modifications meant to mitigate toxicity have also been developed. Mutations silencing the production of granulocyte-macrophage colony stimulating factor (GM-CSF), which activates

monocytes and macrophages, appear to mitigate CRS while improving efficacy in preclinical models.

In the event of significant CAR T-related toxicity, mechanisms for halting CAR T activity are under investigation. Engineered surface expression of CD20 or EGFR allows monoclonal antibody-mediated killing of CAR T cells via rituximab or cetuximab, respectively. The engineered "kill switch" protein iCasp9 can be activated through administration of a small molecule that facilitates dimerization of iCasp9 and results in CAR T cell apoptosis. Other approaches include the use of small molecule therapy for reversible inhibition of CAR T activity, as recently demonstrated in preclinical models with dasatinib.[36]

9 | COMBINATION THERAPY

A number of clinical studies are underway evaluating the combination of established CAR T therapies with a number of potentially additive or synergistic agents thought to enhance the antitumor activity of CAR T cells, reduce T cell exhaustion, or mitigate toxicity. Phase 1/2 trials investigating CAR T products including checkpoint inhibitors, immunomodulators, and Bruton tyrosine kinase (BTK) inhibitors are ongoing. Other treatments intended to reduce the risk or severity of CRS and neurotoxicity are also underway, with the anti-IL-1R agent anakinra undergoing active clinical investigation.

Disruption of the PD-1/PD-L1 immune checkpoint through PD-1 and PD-L1 inhibitors has shown activity in many malignancies, including Hodgkin lymphoma, though their activity in B-cell NHL is limited. There is interest in using checkpoint inhibitors alongside CAR T therapy to reduce CAR T cell exhaustion and facilitate more robust antitumor responses. One study showed that PD-1 with pembrolizumab used as "rescue" for patients who progressed after CAR T therapy showed CAR T re-expansion in 9 of 12 patients, with an ORR of 27%.[37] While modest, the fact that this therapy generated responses in patients who had otherwise failed CAR T therapy suggests a role for checkpoint inhibitors. Concomitant therapy with checkpoint inhibitors and CAR T is being investigated in a number of clinical trials, with preliminary results showing adequate safety profiles. Larger studies are needed to determine whether such combinations result in superior outcomes.

Concomitant administration of ibrutinib, a BTK inhibitor, has been demonstrated in preclinical models to reduce CART-related cytokine production. A recent Phase I study of ibrutinib given alongside CAR T therapy for ibrutinib-refractory CLL showed an similar response rates and lower CRS severity, with a trend toward superior CAR T expansion, when compared to another cohort that did not receive ibrutinib.[38] Larger prospective clinical trials in CLL and NHL are ongoing to further evaluate these preliminary findings.

Preclinical data also suggest that immunomodulatory agents such as lenalidomide may also enhance the anti-tumor effects of CAR T cells, and ongoing studies are evaluating the safety and efficacy of this and other immunomodulatory agents alongside established CAR T constructs.

10 | CONCLUSIONS

CAR T therapy has revolutionized the management of patients with B-cell lymphoma. The applicability of CAR T is expected to expand to include additional lymphoma subtypes, disease states, and phases of treatment in the coming years. Challenges remain in overcoming barriers to CAR T treatment, improving efficacy, and minimizing toxicity. A number of promising solutions are already in preclinical and early clinical development, with early data suggesting many safe and effective CAR T regimens. Determining which of these CAR T strategies are most effective in improving outcomes will become an increasingly important question, particularly as they are largely developed in parallel with few head-to-head comparisons. Outside of the CAR T spectrum, other promising modalities, including bispecific antibodies, are showing promising results, and determining when to utilize CAR T over these other strategies presents another challenge. Going forward, thoughtfully designed clinical trials comparing these strategies, and further translational studies to understand the biological underpinnings of this novel technology, will be necessary to ensure continued improvement in outcomes for patients with lymphoma.

CONFLICT OF INTEREST

David Qualls has no conflicts of interest to disclose. Gilles Salles has received in the last 12months financial compensations for participating to advisory boards or consulting from Abbvie, Beigene, BMS/Celgene, Debiopharm, Genentech/Roche, Genmab, Incyte, Kite/Gilead, Milteniy, Morphosys, Novartis, Velosbio

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SUPPLEMENT ARTICLE

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Bispecific antibodies for the treatment of lymphomas: Promises and challenges

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Abstract

The potential of bispecific antibodies to direct antigen-specific T cell-mediated cytotoxicity toward malignant cells bearing a target antigen was recognized over 35 years ago. Generally, this is accomplished by combining a T-cell receptor-specific monoclonal antibody or monoclonal antibody-derived fragment that is capable of activating and expanding resting T cells with a second monoclonal antibody or monoclonal antibody fragment directed against a tumor target antigen. Bispecific antibodies induce effector T cells that bind to tumor cells independently of their T-cell receptor specificity and without the requirement of MHC-mediated antigen presentation, focusing effector T-cell cytotoxicity on tumor cells bearing the target antigen. The therapeutic efficacy of this approach for treatment of relapsed or refractory B-cell lymphomas was first demonstrated with blinatumomab, a single molecule comprised of two linked single-chain variable fragments with binding specificities for CD19 and CD3. The recent demonstration that chimeric antigen receptor (CAR) modified T cells can achieve very durable remissions in some patients with relapsed or refractory B-cell lymphomas, as well as the potential efficacy of bispecific antibodies in CAR T cell failures, has rekindled interested in bispecific antibodies as a T cell-mediated therapeutic approach. We review the early results of phase 1 clinical trials of bispecific antibodies targeting CD20 on B cells and engaging T cells via CD3 in 1:1 or 2:1 CD20:CD3 Fab formats for treatment of relapsed or refractory B-cell lymphomas.

KEYWORDS
bispecific antibody, blinatumomab, glofitamab, immunotherapy, mosunetuzumab, odronextumab

The term bispecific antibody (bsAb) refers to an antibody or an antibody-derived protein construct that has binding specificities for two different antigens. In humans and most mammals, all naturally occurring monomeric antibodies are bivalent with respect to number of antigen-binding sites. These two binding sites are almost always monospecific (i.e., having the same antigen-binding specificity at each of an antibody's two antigen-binding regions). In comparison, bispecific antibodies combine two different monospecific antigen-binding regions, or variable regions, from different antibodies to achieve a single antibody or antibody-derived molecule with bispecific antigen binding.

In 1960, nearly simultaneous with Porter's seminal observation that digestion of rabbit antibody with papain yielded two almost identical univalent antigen-binding fragments (Fab') per antibody molecule, Nisonoff and coworkers observed that pepsin digestion produced a single bivalent antigen-binding fragment [F(ab')2] which, after treatment with a disulfide-splitting reagent, yielded two univalent Fab' fragments that could be recombined into an F(ab')2 by

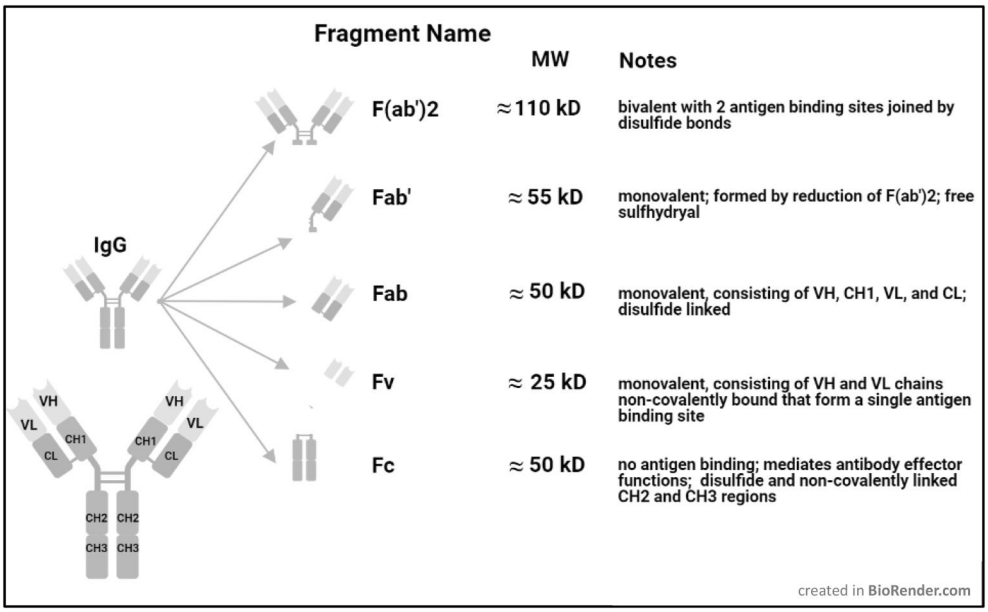
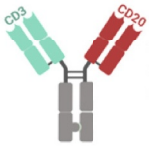
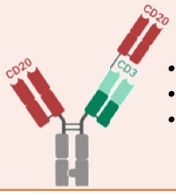
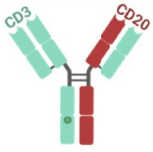


FIGURE 1 Antibody fragments—the building blocks of bispecific antibodies

TABLE 1 Structure of selected bi-specific antibodies

Bi-Specific Antibody	Targets	Design	Ig Fragment Formats	Ref.
blinatumomab	**CD19 x CD3**		• two murine scFv joined by a glycine-serine linker • monovalent CD19 and monovalent CD3 binding • cloned from anti-CD19 (clone HD37) and anti-CD3 (clone L2K-07) murine mAbs	1, 2, 3
mosunetuzumab	**CD20 x CD3**		• humanized mouse heterodimeric IgG1-based antibody • monovalent CD20 and monovalent CD3ϵ binding • modified Fc devoid of FcγR and complement binding	4
glofitamab	**$(CD20)_2$ x CD3**		• humanized mouse IgG1-based antibody • bivalent CD20 and monovalent CD3ϵ binding • modified Fc devoid of FcγR and complement binding	5
odronextamab	**CD20 x CD3**		• fully human IgG4-based heterodimeric antibody • monovalent CD20 and monovalent CD3ϵ binding • Fc-dependent effector function-minimized antibody with Fc of the anti-CD3ϵ heavy chain modified to reduce Protein A binding • common κ light chain from anti-CD3ϵ mAb	6
epcoritamab	**CD20 x CD3**		• humanized mouse IgG1-based heterodimeric antibody • monovalent CD20 and monovalent CD3 binding • IgG1 Fc modified to minimize Fc-dependent effector functions and to control Fab-arm exchange of mAb half-molecules, resulting in high bispecific product yield	7

Ig, immunoglobulin; scFv, single-chain variable fragment; mAb, monoclonal antibody; Fc, fragment crystallizable; FcγR, Fc gamma receptor

[1]Dufner V, *et al*. Blood Adv (2019) 3:2491; [2]Goebeler ME, *et al*. J Clin Oncol (2016) 34:1104; [3]Viardot *et al*. Blood (2016) 127(11):1410; [4]Schuster SJ, *et al*. ASH 2019, Plenary Abstract 6; [5]Hutchings M, *et al*. ASH 2020, Abstract 403; [6]Bannerji R, *et al*. ASH 2020, Abstract 400; [7]Hutchings M, *et al*. ASH 2020, Abstract 406

	blinatumomab[1,2,3]		mosunetuzumab[4]	glofitamab[5]		odronextumab[6]		epcoritamab[7]	
Design	Phase 1 (≥ MTD cohort[1])	Phase 2[3]	Phase 1/1b (dose escalation/expansion)	Phase 1 (dose escalation and expansion)		Phase 1 (dose escalation and expansion)		Phase 1 (dose escalation and expansion)	
Patients	r/r NHL (N=38) (DLBCL, n=5)	r/r DLBCL (N=25) (Evaluable, n=21)	r/r NHL (N=270) (DLBCL/tFL, n=116)	r/r NHL N=52 (DLBCL, n=10)		r/r NHL N=136 (DLBCL, n=78)		r/r NHL N=68 (DLBCL, n=46)	
Dosing	Dose escalation to 90 μg/m²/day	Stepwise escalation (9-28-112 μg/day or flat 112 μg/day)	aggressive NHL: 2.8 - 40.5 mg indolent NHL: 2.8 - 13.5 mg	Two target dose cohorts (C1D1, 2.5 mg; C1D8, 10 mg; C2D1 [target dose], 16 or 30 mg)		Dose range: 0.03-320 mg Weekly x 12, then every 2 weeks		Dose range: 0.0128 - 60 mg SC step-up, then every 28 days RP2D = 48 mg	
ORR	≥ 60 μg/m²/day N=25 (n=1 DLBCL) **64%**	(DLBCL, n=21) **43%**	indolent / aggressive n=124 / n=67 **62.7% / 37.1%**	16+30 mg cohorts combined, n=52 **63.5%**	indolent / aggressive n=24 / n=28 **66.7% / 60.7%**	DLBCL ≥ 80 mg *no CAR T* **55%** (n=6/11)	DLBCL ≥ 80 mg *prior CAR T* **33%** (n=8/24)	DLBCL 12-60 mg: **68%** (n=15/22) 48-60 mg: **91%** (n=10/11)	Follicular NHL 0.76-48 mg: **90%** (n=9/10) 12-48 mg: **80%** (n=4/5)
CR	**36%**	**19%**	indolent / aggressive **43.3% / 19.4%**	all NHL **53.8%**	indolent / aggressive **54.2% / 53.6%**	DLBCL ≥80mg, no CAR **55%** (n=6/11)	DLBCL ≥80mg, CAR **21%** (n=5/24)	DLBCL 12-60 mg: **46%** (n=10/22) 48-60 mg: **55%** (n=6/11)	Follicular NHL 0.76-48 mg: **50%** (n=5/10) 12-48 mg: **60%** (n=3/5)
PFS	median PFS 1.5 yrs. for ≥ 60 μg/m²/day (range, 0-10.3) median follow-up 4.6 years	median PFS 3.7 mos. (95% CI: 1.4-7.7) median follow-up 15 months	not reported 82% indolent ongoing responses to 26 months; 70% aggressive ongoing responses to 16 months	not reported median follow-up 1.8 months for indolent and 3.7 months for aggressive		not reported 83% CR ongoing at 3-21 months	not reported 100% CR ongoing at 3-20 months	not reported DLBCL 12-60 mg: 72% in remission at 6 months	not reported Follicular NHL: 100% CR ongoing at 3-13 months
CRS	not reported (20% ≥ Grade 3 CRP increase; n=76)[2]	not reported (13% ≥ Grade 3 CRP increase)	28.9% (All Grades) 1.1% Grade 3; no ≥ Gr. 4	63.5% (All Grades) 3.8% ≥ Grade 3		61% (All Grades) 7% ≥ Grade 3		59% (All Grades) no events ≥ Grade 3	
ICANS-like	22% Grade 3 (no 4/5)[2]	22% Grade 3	1.1% Grade 3	not reported (< 10%)		3.7% Grade 3 (no Grade 4)		2 (3%) Grade 1; 2 (3%) Grade 3	

[1]Dufner V, *et al*. Blood Adv (2019) 3:2491; [2]Goebeler ME, *et al*. J Clin Oncol (2016) 34:1104; [3]Viardot *et al*. Blood (2016) 127(11):1410; [4]Schuster SJ, *et al*. ASH 2019, Plenary Abstract 6; [5]Hutchings M, *et al*. ASH 2020, Abstract 403; [6]Bannerji R, *et al*. ASH 2020, Abstract 400; [7]Hutchings M, *et al*. ASH 2020, Abstract 406

FIGURE 2 Summary of selected bi-specific antibodies: safety and efficacy

oxidation of sulfhydryl groups.[1,2] (Figure 1). Immediately recognizing the significance of their observations, these investigators speculated, "It should also be of interest to attempt to prepare antibody of mixed specificity."[2] Soon afterwards in 1964, proof of this concept was established in an elegant series of experiments by Fudenberg et al. that demonstrated the successful construction, starting with monospecific rabbit antisera, of a single "bivalent hybrid antibody" or more precisely an F(ab')2 with bispecific binding to both bovine gamma globulin and chicken ovalbumin.[3] Thus began the development of bispecific antibodies and their application for diagnostic and therapeutic purposes.

The confluence of subsequent discoveries in basic and tumor immunology, technical innovations in laboratory immunology, and development of recombinant DNA technology over the next 50 years resulted in a vast variety of approaches for creating antibodies or single-molecule antibody derivatives of "mixed specificity." Using combinations of immunoglobulin-derived proteins or genetic modules designed to modify the specificity, valency, size, flexibility, pharmacokinetic and pharmacodynamic properties, and to enhance large scale production, a panoply of bsAb formats has evolved and continues to evolve. Current bsAb formats include full antibody-sized, antibody fragment-sized, and complex larger than antibody formats, including bivalent and multivalent formats (reviewed in Brinkmann and Kontermann[4]).

In 1985, the feasibility of directing T cells to effect antigen-specific cytotoxicity toward cells bearing the target antigen of choice by using "hybrid antibodies" (in this case, chemically linked monoclonal antibodies) was demonstrated in vitro.[4–6] At that time, it was recognized that these antibody "heteroconjugates" could (1) overcome MHC-restricted antigen presentation to cytotoxic T cells, (2) eliminate the problem of rejection of transplanted effector cells, and (3) focus effector T-cell cytotoxicity on cells bearing the target antigen. This was achieved by combining a T-cell receptor specific monoclonal antibody capable of activating and expanding resting T cells, as well as inducing effector T cells, together with a second monoclonal antibody directed against any chosen target antigen.

In 2008, Bargou et al. reported the first use of a bsAb for treatment of lymphomas, namely, blinatumomab.[7] Using two single-chain variable fragments (Fv, Figure 1; Table 1) with binding specificities for CD19 + CD3 linked by a serine-glycine peptide and administered by continuous intravenous infusion because of its small size and rapid clearance, responses to blinatumomab were reported in patients with relapsed follicular lymphoma, mantle cell lymphoma, and chronic lymphocytic leukemia. Along with these objective responses, the first observations of what are now recognized syndromes of special interest related to T-cell engaging therapies—cytokine release syndrome (CRS) and immune effector cell-associated neurotoxicity syndrome (ICANS)—were reported. Subsequent multicenter phase 1 and phase 2 studies in patients with relapsed or refractory B-cell non-Hodgkin lymphomas of various histologies ($N = 76$) and diffuse large B-cell lymphomas ($N = 25$) were completed (Figure 2).[8–10] These studies confirmed the clinical activity of blinatumomab with overall response rates of 64% and 43% for varied B-cell lymphomas and for diffuse large B-cell lymphoma cohorts, respectively; remarkably, 6 of 25 patients treated at the effective dose of at least 60 μg/m² per day remain in ongoing remissions beyond 5 years (3 with follicular lymphoma, 2 with mantle cell lymphoma, and 1 with diffuse large B-cell lymphoma).[8] The acute toxicity profile of blinatumomab, especially neurologic adverse events, is considerable but manageable, and no late toxicities have

been observed. In 2014, blinatumomab was approved by the US FDA for treatment of Philadelphia chromosome-negative relapsed or refractory B-cell precursor acute lymphoblastic leukemia (ALL); its approval was expanded in 2018 to include minimal residual disease-positive B-cell precursor ALL.

Although the early results for blinatumomab were promising and supported further development of the bsAb approach for treatment of B-cell lymphomas, the emergence of CD19-directed chimeric antigen receptor (CAR) modified T-cell therapies (CAR T) over the last 10 years briefly eclipsed the ongoing development of bsAbs.[11,12] However, the observations that CAR T cells are capable of achieving very durable remissions in some patients with relapsed or refractory B-cell lymphomas, as well as the potential efficacy of bsAb in CAR T cell failures, supported the T cell-mediated approach to therapy, whether by CAR T or bsAb, and ultimately rekindled interested in bsAbs.

Currently, a variety of bsAbs are under development as therapy for B-cell lymphomas. The early results of phase 1 bsAb clinical trials are summarized in Table 1 and Figure 2. These bsAbs target CD20 on B cells and engage T cells via CD3 in a 1:1 or 2:1 CD20:CD3 Fab format. They are full size antibody derivatives and thus have pharmacokinetic profiles that allow intermittent dosing. In general, neurotoxicity is significantly less frequent than observed with CD19-directed CAR T or blinatumomab therapies. Overall response rates range from 60 to 90% with complete response rates from 40 to 60% in relapsed or refractory indolent and follicular lymphomas.[13–15] In relapsed or refractory large B-cell lymphomas, overall response rates range from 37% to 90% with complete response rates from 19% to 55%. Importantly, these bsAbs appear to have activity in patients failing CD19-directed CAR T therapy. However, follow-up for these new bsAbs is short and the durability of responses remains to be established.

While proof of principle for bsAbs has been clearly demonstrated, optimal clinical use of bsAbs in B-cell lymphomas remains to be established. As we wait for the early phase clinical trials to complete accrual and to mature, we can expect bsAb combinations to be explored in the next generation of bsAb clinical trials.

CONFLICT OF INTEREST

Schuster is associated with AlloGene (Ad Board), AstraZeneca (Ad Board), BeiGene (Ad Board) Celgene/BMS (Ad Board, Consulting, Steering committee), Genentech/Roche (Ad Board, Consulting, Steering committee, Research Grant), Incyte (Ad Board), Janssen (Ad Board), Legend Biotech (Ad Board), Loxo Oncology (Ad Board, Consulting), Novartis (Ad Board, Consulting, Steering committee, Research Grant), and Regeneron (Ad Board).

DATA AV1AILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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