Received: 18 January 2019 | Revised: 6 March 2019 | Accepted: 8 March 2019

DOI: 10.1002/ajh.25460

AJH WILEY

Diffuse large B-cell lymphoma: 2019 update on diagnosis, risk stratification, and treatment

Yang Liu[1] | Stefan Klaus Barta[2]

[1]Fox Chase Cancer Center, Department of Hematology and Oncology, Philadelphia, Pennsylvania

[2]Perelman Center for Advanced Medicine, University of Pennsylvania, Division, Philadelphia, Pennsylvania

Correspondence
Stefan Klaus Barta, Perelman Center for Advanced Medicine, West Pavilion, 4th Floor, 3400 Civic Center Boulevard, Philadelphia, PA 19104.
Email: stefan.barta@uphs.upenn.edu

Abstract

Disease Overview: Diffuse large B-cell lymphoma (DLBCL) is the most common type of aggressive non-Hodgkin lymphoma originating from the germinal center, and it represents a heterogeneous group of diseases with variable outcomes that are differentially characterized by clinical features, cell of origin (COO), molecular features, and most recently, frequently recurring mutations.

Diagnosis: DLBCL is ideally diagnosed from an excisional biopsy of a suspicious lymph node, which shows sheets of large cells that disrupt the underlying structural integrity of the follicle center and stain positive for pan-B-cell antigens, such as CD20 and CD79a. COO is determined by immunohistochemical stains, while molecular features such as double-hit or triple-hit disease are determined by fluorescent in situ hybridization analysis. Commercial tests for frequently recurring mutations are currently not routinely used to inform treatment.

Risk Stratification: Clinical prognostic systems for DLBCL, including the rituximab International Prognostic Index, age-adjusted IPI, and NCCN-IPI, use clinical factors for the risk stratification of patients, although this does not affect the treatment approach. Furthermore, DLBCL patients with non-germinal center B-cell (GCB)-like DLBCL (activated B-cell like and unclassifiable) have a poorer response to up-front chemoimmunotherapy (CI) compared to patients with GCB-like DLBCL. Those with c-MYC-altered disease alone and in combination with translocations in BCL2 and/or BCL6 (particularly when the MYC translocation partner is immunoglobulin) respond poorly to up-front CI and salvage autologous stem cell transplant at relapse.

Risk-Adapted Therapy: This review will focus on differential treatment of DLBCL up-front and at the time of relapse by COO and molecular features.

1 | DISEASE OVERVIEW

Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin lymphoma (NHL) in the United States, representing approximately 24% of new cases of NHL each year.[1,2] The disease is aggressive, and patients typically present with rapidly enlarging lymphadenopathy and constitutional symptoms, necessitating immediate treatment. Although most patients present with lymphadenopathy, there is a high frequency of extranodal disease. The most common up-front treatment is chemoimmunotherapy (CI) with R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone), which leads to cure in approximately 50%-60% of patients. Unfortunately, for those who develop disease that is refractory to up-front treatment, or relapse after achieving remission, outcomes are particularly poor, with high-dose chemotherapy and autologous stem cell transplant (ASCT) achieving long-term remissions in only a minority of patients in the era of rituximab.[3]

Since 1993, clinicians have used the International Prognostic Index (IPI)[4] to characterize prognosis in aggressive NHL based on five clinical factors: age, stage, the number of extranodal sites, performance status, and LDH. However, in the past two decades, tremendous effort has been made to identify unique DLBCL subtypes by cell of origin (COO) and molecular features, which can be used independently of the IPI to identify high-risk disease and predict failure of up-front R-CHOP and/or ASCT at the time of relapse. In a parallel timeframe, multiple clinical studies have explored differential approaches to up-front treatment and treatment of relapsed disease based on disease subtype and our evolving understanding of the underlying disease pathogenesis of each subtype. Most recently, next-generation sequencing and comprehensive genomic analysis has allowed us to further subclassify this disease by recurrent, high-frequency mutations, which provides a solid foundation for the development of novel targeted approaches.[5–7]

2 | DIAGNOSIS

The diagnosis of DLBCL is ideally made from an excisional biopsy of an abnormally enlarged, suspicious appearing lymph node upon clinical examination and radiographic imaging. This allows for the largest amount of tissue to be reviewed by pathology and avoids sampling error and false negatives, which can happen with fine needle aspiration or core biopsy in a highly heterogeneous lymph node tissue environment. DLBCL can frequently involve extranodal sites, including the kidneys, adrenal gland, brain, bones, and other soft tissues. Careful requirements must be made in each patient to obtain a biopsy that is the least invasive and yet provides sufficient tissue. Positron emission tomography-computed tomography can be used to determine the sites of disease with the highest standardized uptake value (SUV) and possibly the most aggressive disease and to notify the preferred site of biopsy. Morphologically, DLBCL is characterized by a diffuse infiltration of medium-to-large cells with large nucleoli and abundant cytoplasm, which disrupt and efface the underlying architecture of the involved lymph node. The cells typically express pan–B-cell antigens, including CD19, CD20, CD22, CD79a, and CD45. The majority of the cells also express surface immunoglobulin (IG).[8] Approximately 14% of cases express CD30, which can portend to a favorable prognosis.[9,10]

2.1 | Cell of origin

In 2000, Alizadeh and colleagues used gene expression profiling (GEP) of 96 normal and DLBCL lymphocytes to identify three unique genetic signatures that portended to three different subtypes of disease based on COO. These include the germinal center B-cell (GCB)-like subtype, which resembles the GEP of normal GCBs, the activated B-cell (ABC)-like subtype, which resemble normal ABCs, and unclassifiable disease in the remaining 10%-15% of samples.[11] Although originally identified by GEP, this assay has had limited clinical adoption as yet because of high cost and the need for fresh frozen tissue. In clinical practice, immunohistochemistry (IHC) algorithms such as the Hans and Tally methods are used to identify COO, with variable concordance to GEP.[12,13] Recently, more novel platforms such as Lymph2Cx allow for digital GEP on fixed, paraffin-embedded tissue and have shown a greater concordance with GEP than IHC.[14,15] This platform currently remains useful in the research setting, and it has not yet been adapted for clinical use. Given the wide adoption of IHC algorithms to assess COO, DLBCL is most commonly classified into GCB DLBCL and non-GCB DLBCL. Although being a misnomer, the non-GCB DLBCL contains ABC DLBCL (which do originate from the germinal center) and the previously unclassifiable disease per GEP. When disease is assessed by GEP, these three distinct subcategories remain.

2.2 | Molecular features

In parallel to COO studies, the subtypes of DLBCL based on molecular features have also been found to have prognostic impacts. c-MYC is a proto-oncogene in chromosome 8q24 and encodes a transcription factor, which when dysregulated leads to downstream effects of cellular survival and proliferation. BCL2 is an oncogene on chromosome 18q21 with antiapoptotic properties, while BCL6 is a transcriptional repressor on chromosome 3q27. Patients with DLBCL and overexpression of the c-MYC oncogene and BCL2 (≥40% and >50% by IHC, respectively) have double expressor lymphoma (DEL), which is associated with an intermediate prognosis to up-front R-CHOP. DELs account for approximately one-third of de novo disease and up to 50% of relapsed/refractory (RR) DLBCL.[16,17] Patients with genetic rearrangements in c-MYC in addition to BCL2 and/or BCL6 have high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 according to the WHO 2016 classification of hematologic malignancies, and are also called double-hit or triple-hit lymphomas (DH/THLs). DH/THLs represent 6%-14% of patients with DLBCL.[18,19] These genetic rearrangements are identified by fluorescent in situ hybridization (FISH). Both IHC and FISH studies should be done at the time of diagnosis, and ideally again at the time of recurrence for prognostic and treatment implications.

When both COO and molecular studies are performed in parallel in de novo DLBCL, DELs are more often associated within the ABC subtype, while DH/THLs tend to occur within the GCB subtype. The International DLBCL R-CHOP Consortium performed an analysis of 893 patients with de novo DLBCL and found that 66% of DELs were ABC DLBCL, while only 39% of non-DELs were ABC DLBCL. The same group found that 7 of 8 patients in a cohort of 327 with de novo DH/THLs had the GCB subtype[9,20] (Figure 1). In a larger study of 1228 patients with DLBCL from three clinical trials, Scott and colleagues performed FISH, COO, and IHC testing of DLBCL samples and found a 1.7% prevalence of DH/THL in ABC DLBCL, compared to a 13.3% prevalence in GCB DLBCL (17.7% with MYC rearrangement).[21] Within GCB DLBCL, a 104-gene double-hit signature has been developed to predict poor response to up-front R-CHOP regardless of the DH/THL status.[22] Research on appropriate disease classification and prognostic implications is ongoing.

2.3 | Recurrent mutations by whole exome sequencing

Two recently published studies have used whole exome sequencing to characterize new genetic subtypes of disease based on the presence

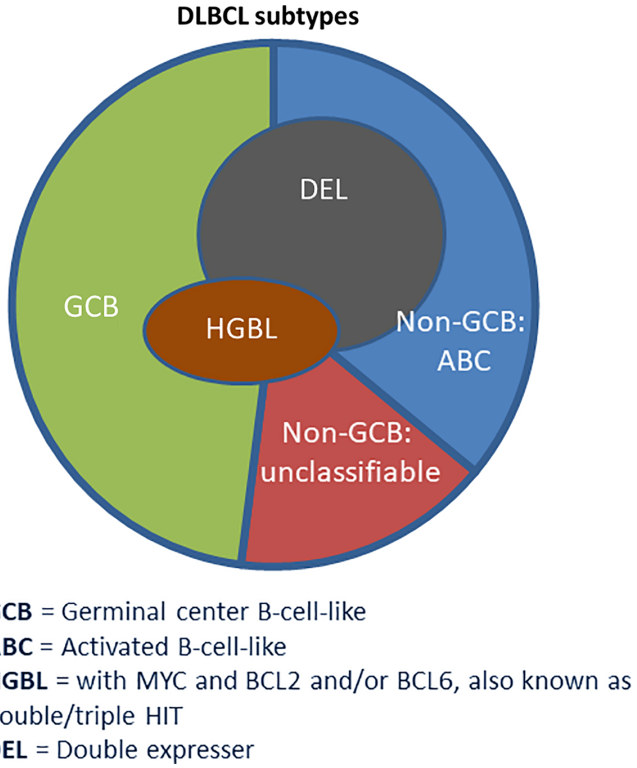

FIGURE 1 Overlap of DLBCL subtypes by COO and molecular features

of recurrent mutations. These categories were mapped onto the ABC and GCB subtypes of DLBCL, as originally identified by GEP. Schmitz and colleagues used whole exome and transcriptome sequencing, array-based DNA copy-number analysis, and targeted amplicon resequencing on 574 primarily pretreatment DLBCL biopsy samples to identify four distinct genetic subtypes of disease with different recurring mutations portending to differential clinical outcomes. These categories include the MCD, BN2, N1, and EZB subtypes.[6] The MCD subtype was characterized by the co-occurrence of MYD88 (L265P) and CD79 mutations, the BN2 subtype by BCL6 fusions and NOTCH2 mutations, the N1 subtype had frequent NOTCH1 mutations, and the EZB subtype had EZH2 and BCL2 translocations. The BN2 and EZB subtypes conferred good prognosis to first-line CI, while the other subtypes conferred a poor prognosis.

In parallel, Chapuy and colleagues classified 304 primary, previously untreated DLBCLs based on low-frequency genetic alterations, recurrent mutations, somatic copy number alterations, structural variants, and coordinate signatures to identify five different DLBCL subsets with differing high-frequency recurrent mutations. These include two distinct subsets of GCB DLBCL with good and poor risk, a low-risk ABC-DLBCL, and an ABC/GCB-independent group with marginal zone/extrafollicular origin.[5]

Whole exome sequencing and the associated next-generation sequencing modalities have not yet been adopted into clinical practice, and tailored therapeutic approaches to these different subtypes have yet to be defined. Ongoing translational work and collaborations with bioinformatics will be necessary to further differentiate these high-frequency mutations into driver mutations (which are necessary and sufficient for lymphomagenesis) and passenger mutations. Driver mutations can then be used to identify therapeutically relevant targets. Some potential therapeutic targets include mutations in MYD88; CD79a/b in the ABC subtype of disease; and EZH2, BCL2, and CREBBP in the GCB subtype of disease.

3 | RISK STRATIFICATION

A combination of clinical factors, COO, and molecular studies are used to predict prognosis in DLBCL.

3.1 | Clinical factors

The IPI has been used since 1993 to predict prognosis in aggressive NHL treated with doxorubicin-containing regimens.[4] This has been validated in the rituximab era (R-IPI) and in patients <60 years of age (age-adjusted IPI).[23] It has also been expanded to include the more granular information about each of these variables in the recent NCCN-IPI.[24] In the most commonly used IPI, patients with a score of 0-1, 2, 3, and 4-5 had a 3-year overall survival (OS) of 91%, 81%, 65%, and 59%, respectively.[25]

3.2 | Cell of origin

Multiple studies have shown that patients with the ABC disease subtype have significantly poorer outcomes to standard up-front rituximab-containing CI compared to GCB disease.[12,14,26] In a study of 157 de novo DLBCL cases treated with up-front rituximab containing CI, patients with the ABC subtype as identified by GEP had a 5-year progression-free survival (PFS) of 31% compared to 76% in GCB disease, which translated to an inferior 5-year OS (45% vs 80%).[12] Similarly, in a study of 344 patients with de novo DLBCL treated with R-CHOP that used the Lymph2Cx assay on the paraffin-embedded tissue to identify COO, the 5-year PFS and 5-year OS was 48% and 56%, respectively, in ABC disease, compared to 73% and 78% in GCB disease.[14] In relapsed disease, the prognostic impact of COO remains less clear. Although the Bio-CORAL study suggested that GCB DLBCL treated with R-DHAP had an improved 3-year PFS compared those treated with R-ICE,[27] multiple other studies have failed to reproduce these results, including patients who went on to receive consolidative ASCT.[28–30]

3.3 | Molecular features

The presence of gene rearrangements in MYC is associated with a poorer response to up-front R-CHOP compared with MYC-counterparts.[31,32] Further research has shown that the MYC translocation partner matters, and MYC rearrangements with the IG partner (~50% of cases) portend to a poorer OS compared to MYC- and non-IG partnered MYC gene translocations.[33,34] The co-occurrence of either a BCL2 or BCL6 rearrangement in DH/THL has a particularly aggressive clinical phenotype with high rates of advanced disease, and extra-nodal disease

leading to a higher IPI at presentation.[31,35–37] This translates into a poor response to standard up-front R-CHOP,[38,39] with one retrospective study reporting a 5-year PFS and 5-year OS of 27% and 18%, respectively.[38]

MYC rearrangement (MYC+) and DH/THL remains significantly prognostic at the time of relapse. In the Bio-CORAL study, patients with RR MYC+ had a higher LDH and age-adjusted IPI than their MYC-RR counterparts. All were treated with salvage chemotherapy and ASCT, and MYC+ patients had low rates of complete response (CR) to R-ICE and R-DHAP (23% and 26% vs 35% and 54% in MYC- disease, and a 4-year PFS of less than 20% and OS of 26% (R-ICE) and 31% (R-DHAP).[40]

DELs are also associated with lower rates of CR and lower PFS and OS to up-front R-CHOP compared to those without DEL[9,39] with one study reporting a 5-year OS and PFS of <30%. These outcomes appear to be intermediate to DLBCL without molecular alterations and the more aggressive DH/THL.

4 | RISK-ADAPTED THERAPY

4.1 | Up-front therapy

In previously untreated DLBCL, R-CHOP remains the backbone of therapy, with the total number of cycles and addition of radiation dependent on stage at presentation and tumor bulk. This approach can achieve durable remissions in approximately 60% of patients (Coiffer 2010). Numerous efforts to improve R-CHOP, including increased dose density with 14-day cycles, the use obinutuzumab in place of rituximab, or intensification of therapy to, for example, dose-adjusted (DA) etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin (EPOCH) have been made, but have generally failed to show significant clinical benefits.[41–44]

4.1.1 | Treatment options in DLBCL by COO—up-front therapy

Efforts to improve up-front therapy CI in non-GCB DLBCL have combined biologic agents, including ibrutinib, bortezomib, or lenalidomide with R-CHOP with varying success. These agents were chosen based on the developing understanding that ABC disease is driven by dysregulation and constitutive activation of B-cell receptor (BCR) signaling, leading to downstream activation of the nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-kB) pathway and uncontrolled gene transcription and cellular survival and proliferation.[45] Ibrutinib, a first-in-class, irreversible Bruton's Tyrosine Kinase (BTK) inhibitor, targets BTK in the BCR signaling pathway, where activating mutations have been found. Bortezomib, a proteasome inhibitor, is thought to prevent proteosomal degradation of IkB kinase, an inhibitory kinase that puts the brakes on NF-kB. The exact role of lenalidomide, an immunomodulatory agent, in the pathogenesis of ABC disease is unclear.

Although a phase Ib study of ibrutinib + R-CHOP showed that the combination was tolerable and had high responses in both GCB (5/7) and ABC disease (2/2 patients),[46] the phase III PHOENIX study of this combination for stage I-IV non-GCB DLBCL as identified by IHC and ABC DLBCL as identified by GEP was stopped early by Jannsen in July 2018 because it did not meet its primary PFS endpoint [NCT01855750]. These data were presented at the American Society of Hematology (ASH) 2018 meeting, while in the whole study, the population outcomes of R-CHOP with and without ibrutinib were not significantly different, an unplanned subset analysis suggested benefits in PFS, event-free survival (EFS), and OS in patients <60. It appeared that increased anti-lymphoma activity was offset by a higher frequency of toxicities abrogating benefits particularly in older patients.

Similarly, in an early phase trial of bortezomib + R-CHOP, the combination was tolerable and suggested improvements in PFS and OS in non-GCB disease (as identified by IHC), but these results could not be confirmed in larger phase II (PYRAMID) and III (ReMoDL-B) studies.[47–49]

A phase II study of lenalidomide in addition to R-CHOP in untreated DLBCL showed an impressive overall response rate (ORR) of 98% (80% CR) and similar response rates between GCB and non-GCB disease (as identified by IHC), suggesting that lenalidomide can overcome the negative prognostic impact of non-GCB disease.[50] A larger phase III ROBUST randomized trial of lenalidomide + R-CHOP vs placebo + R-CHOP in ABC DLBCL (as identified by GEP) has completed accrual, and results are expected in the next 1-2 years [NCT02285062]. A similar study conducted by the Eastern Cooperative Oncology Group for patients with stage II-IV DLBCL has finished recruiting and is awaiting read-outs [NCT01856192], and will have preplanned PFS analysis by COO (by GEP and/or IHC).

4.1.2 | Treatment options in DLBCL by molecular features—up-front therapy

Despite knowledge that up-front R-CHOP produces poor long-term outcomes in DHL/THL, the rarity of this entity has precluded robust prospective data to help establish an optimal induction regimen. Multiple retrospective studies have suggested that intensification of up-front CI may be superior to R-CHOP, which was confirmed in a systemic review and meta-analysis of 394 patients from 11 studies.[36,51,52] Overall, a multicenter retrospective study of 311 patients with previously untreated DHL who were treated with induction therapy ± ASCT, at a median follow-up of 23 months, the median PFS of those receiving R-CHOP was 7.8 months compared to 21.6 months with more intensive up-front CI strategies, such as R-EPOCH, R-CODOX-M/IVAC, and R-HyperCVAD.[36] The meta-analysis by Howlett et al. suggested a superior PFS to R-EPOCH compared to R-CHOP and other intensified regimens, which has led to widespread adoption of intensified frontline CI in this population, but this needs to be validated in a prospective manner. The CALGB/Alliance 50303 phase III trial of DA-EPOCH showed no differences in EFS or OS in the DLBCL population as a whole, but subset analysis by FISH and IHC has yet to be reported, and will unlikely be able to answer this question due to low populations of DH/THL in the study.

Retrospective data suggest that there is no role of consolidative ASCT in MYC-positive disease in the first remission who were treated with intensive induction regimens. In a recently published multi-center retrospective study of 159 patients with DHL, there was no difference in relapse-free survival and OS between patients who received ASCT

consolidation in first CR compared to those who did not.[53] However, in the subset analysis of patients who received R-CHOP, consolidative transplant was associated with improved survival compared to no transplant, likely due to inferior outcomes with up-front R-CHOP. Several protocols are actively enrolling patients with DH/THL, including a study of R-EPOCH with lenalidomide [NCT02213913] and R-EPOCH with the Bcl2-inhibitor venetoclax [NCT03036904].

Similarly, in DEL, there is lack of robust prospective data validating a role in more intensive up-front CI. Small retrospective, single-center studies also suggest improved outcomes with R-EPOCH, while unplanned subset analysis from the CALGB 50303 trial suggest no difference.[54,55] Prospective data are needed to inform an optimal induction regimen.

5 | RR DISEASE

At the time of progression or disease relapse, the standard treatment for transplant eligible patients remains salvage chemotherapy followed by consolidative ASCT in chemotherapy-sensitive disease (Vose 2001). The benefit of high-dose chemotherapy and ASCT rescue in relapsed disease was originally demonstrated in the pre-rituximab era (PARMA study), where Thierry et al. demonstrated a 5-year EFS of 46% in chemotherapy-sensitive patients to chemoradiation + ASCT compared to 12% in those who received chemoradiation alone without transplant.[56] In the rituximab era, these numbers are less favorable, with the CORAL study reporting a 3-year PFS of only 37% in patients who received either R-ICE or R-DHAP prior to ASCT and only 21% in patients with prior exposure to rituximab as part of up-front CI.[3]

Several specific populations with RR disease have particularly poor outcomes. Less than 20% of patients with primary progressive disease achieve long-term remissions.[30,57] Patients who do not qualify for transplant, either after failing salvage or due to poor performance status or inadequate stem cell collection had a median OS of 3.3 months in the CORAL study.[58] In addition, patients with relapse <12 months after ASCT have a median OS of approximately 10 months.[59]

Recently, the SCHOLAR-1 study combined data from two phase III clinical trials and two observational cohorts to describe the outcomes in the refractory DLBCL population. In over 636 patients with progressive disease or stable disease as the best response to at least 4 cycles of up-front CI or 2 cycles of salvage, respectively, or relapse at or within 12 months of treatment, the median OS was 6.3 months from the start of salvage treatment, with only 28% alive at 1 year.[60]

With a better understanding of the heterogeneity of DLBCL, ongoing efforts are being made to refine treatment in RR disease based on disease subtypes, particularly for patients who are not eligible for transplant or who have relapsed after transplant.

5.1 | Treatment options in DLBCL by COO—for RR disease

5.1.1 | Ibrutinib

In a phase I/II study of 80 patients with RR DLBCL, treatment with the single agent ibrutinib resulted in a 40% ORR in patients with the ABC subtype (14/38), compared to an only 5% ORR in those with the GCB subtype as identified by GEP.[61] Those with ABC disease and mutations in BCR signaling (with a gain of functional mutations in the BCR subunit CD79b) had a higher rate of response (55.5%); responses were also higher in patients with concomitant myeloid differentiation primary response 88 (MYD88) mutations (4/5; 80%). These results have not yet been confirmed in a larger prospective study. A recently published multi-institutional retrospective study of 54 patients with RR DLBCL (36 de novo, 18 transformed) treated with ibrutinib found an ORR of 28% (5 CRs, 10 PRs) and no difference in ORR or median PFS between the GCB and non-GCB subtypes as identified by IHC.[62] With a median PFS of 1.7 and 3.0 months in the GCB and non-GCB subtypes, single-agent ibrutinib may have limited utility, regardless of the COO subtype in RR DLBCL.

Interestingly, a recently published phase I clinical trial of ibrutinib + ICE in RR DLBCL was found to be tolerable without any dose-limiting toxicities at doses up to 840 mg of ibrutinib and reported an ORR of 90% (11 CR, 7 partial response (PR) to 20 patients).[63] In this study, all patients with non-GCB disease who completed at least 1 cycle of therapy achieved a CR. These results need to be confirmed in larger, randomized studies with longer follow-ups. Ongoing studies of ibrutinib in RR DLBCL are listed in Tables 1 and 2. Notably, one study is investigating the role of ibrutinib in RR non-GCB DLBCL who are not candidates for ASCT [NCT02692248], while another is investigating the benefit of adding ibrutinib during and after ASCT in the ABC subtype [NCT02443077].

As in chronic lymphocytic leukemia and mantle cell lymphoma, patients with DLBCL can develop resistance to BTK inhibitors after a period of response. The mechanisms for resistance have been described in the ABC subtype of disease, including activating mutations in CARD-11, deleterious mutations in the NF-kB regulator NFKBIE, and translocations between (immunoglobulin heavy chain (IgH) and interferon regulatory factor (IRF), which bring the IRF8 transcription factor under the IgHV heavy chain promoter.[64,65] Ongoing research is needed to identify methods of overcoming BTK inhibitor resistance.

5.1.2 | Lenalidomide

Early phase studies of lenalidomide, an immunomodulatory agent, as a single agent have shown moderate clinical benefits in RR DLBCL. A study of 108 patients reported an ORR of 28% and a median duration of response (DOR) of 3.7 months, with a median DOR of 10.6 months in responders.[66,67] However, a small study of 44 patients with available histologic materials found a significantly higher ORR in the non-GCB subtype as determined by the Hans algorithm compared to the GCB subtype (52.9% vs 8.7%, =0.006), with no difference in OS. This led to a phase 2/3 clinical trial of lenalidomide vs investigator's choice in 102 patients with RR DLBCL, with patients stratified by COO as determined by the Hans algorithm.[68] The results of this study were recently published, and has also suggested a greater benefit in the non-GCB subtype [ORR (27.5% vs 11.8%) and PFS (13.6 vs 7.8 weeks)] with lenalidomide.

TABLE 1 Selected ongoing studies in RR DLBCL evaluating response by COO

Drug	Title	Inclusion criteria	COO subtype-specific endpoints
Lenalidomide			
Phase I/II NCT02077166	Ibrutinib in combination with lenalidomide and rituximab in participants with relapsed or refractory diffuse large b-cell lymphoma	RR DLBCL	Secondary endpoint (phase II) safety and tolerability in RR non-GCB DLBCL
Phase I/II NCT02628405	R-ICE and lenalidomide in treating patients with first-relapse/primary refractory diffuse large b-cell lymphoma	Phase I: CD20+ B-cell lymphomas Phase II: RR DLBCL	Tertiary objective: To evaluate ORR based on GCB vs non-GCB subtypes
Phase I/II NCT03558750	Rituximab, lenalidomide, and nivolumab in treating participants with relapsed or refractory non-germinal center type diffuse large B cell lymphoma or primary central nervous system lymphoma	RR non-GCB DLBCL and RR primary CNS lymphoma	Primary objective (phase II): Evaluate the efficacy of lenalidomide in combination with standard doses of rituximab and nivolumab in R/R non-GCB DLBCL and PCNSL
Phase I/II NCT03015896	Nivolumab and lenalidomide in treating patients with relapsed or refractory non-Hodgkin or Hodgkin lymphoma	RR NHL and HL	Tertiary objective: To explore the relationship between ABC or GCB DLBCL with ORR to the combination of lenalidomide and nivolumab in patients with relapsed/refractory FL and DLBCL
Ibrutinib			
Phase III NCT02443077	A randomized double-blind phase III study of ibrutinib during and following autologous stem cell transplantation vs placebo in patients with relapsed or refractory diffuse large B-cell lymphoma of the activated B-cell subtype	RR non-GCB DLBCL	All study endpoints are in the ABC subtype
Phase II NCT02692248	Ibrutinib in patients with refractory/relapsed non-GCB Diffuse large B-cell lymphoma non-candidates to autologous stem cell transplantation	RR non-GCB DLBCL	All study endpoints are in the non-GCB subtype
Phase I/IIb NCT02950220	Pembrolizumab and ibrutinib in treating patients with relapsed or refractory non-Hodgkin lymphoma	RR DLBCL	Tertiary objective: To explore the relationship between COO and ORR in ABC vs GCB subtype

TABLE 2 Selected ongoing studies in RR DLBCL targeting MYC-altered disease

Drug	Title	Inclusion criteria	Molecular subtype specific endpoints
BET inhibitors			
Phase I/II NCT01943851	A dose escalation study to investigate the safety, pharmacokinetics (PK), pharmacodynamics (PD) and clinical activity of GSK525762 in subjects with relapsed, refractory hematologic malignancies	Phase I: RR AML, MM, or NHL Phase 2: DHL and THL	All study endpoints are in MYC-altered disease
Phase I NCT01949883	A phase 1 study evaluating CPI-0610 in patients with progressive lymphoma	NHL or Hodgkin's lymphoma	Secondary outcome: Changes in the expression of MYC and other genes in tumor tissue
Phase 2 NCT02674750	Study to evaluate the efficacy and safety of CUDC-907 in patients with RR DLBCL, including patients with MYC alterations	RR DLBCL, including DHT and THL and transformed DLBCL	All study endpoints are in MYC-altered disease

Lenalidomide has been studied in combination with rituximab in RR DLBCL, although responses have not been reported by COO subtype. The mechanism of action of lenalidomide in RR DLBCL is not entirely clear, but is thought to be immunomodulatory by promoting the recruitment of Aiolos and Ikaros, both transcriptional repressors of IL-2 secretion, to the cereblon–E3 ubiquitin ligase complex. This leads to increased ubiquitination and degradation of both factors, which results in increased IL-2 and IL-2 derived apoptosis.[69,70]

In a phase II trial of lenalidomide with rituximab, 45 patients with RR DLBCL, follicular lymphoma, and transformed follicular lymphoma

received lenalidomide 20 mg daily on days 1-21 of a 28-day cycle and rituximab 375 mg/m^2 weekly during cycle 1.[71] The study found an ORR of 33% with a median response duration of 10.2 months and a median PFS and OS of 3.7 and 10.7 months, respectively. The regimen was tolerable, and the majority of patients (9/15) could proceed to ASCT, which translated to improved outcomes. A variation of this regimen was studied in a small series of 23 heavily pretreated patients with RR DLBCL >70 years of age, and reported an ORR of 35% at the end of treatment, with high rates of CR (8 of 10 responders) and a median DOR of 32 months.[72]

In addition, patients with RR DLBCL who were responsive to rituximab-based salvage chemotherapy but are not candidates for ASCT may benefit for maintenance of lenalidomide. In a multicenter phase II trial, 48 patients were treated with oral lenalidomide 25 mg daily on days 1-21 of a 28-day cycle until disease progression or intolerance, and reported a 1-year PFS of 70%.[73] Benefits were observed in all subgroups including GCB and non-GCB disease. Ongoing studies of lenalidomide in RR DLBCL are listed in Tables 1 and 2.

5.1.3 | Bortezomib

Bortezomib has also been studied in the RR population. In a small study of 25 RR DLBCL patients (19 GCB subtype, 6 ABC subtype), single agents such as bortezomib had no activity, but when combined with chemotherapy had an ORR of 93% with a median OS of 10.8 months in the ABC subtype, compared to 3.4 months in the GCB subtype.[74] Confirmation in larger studies are pending. The addition of bortezomib to a salvage regimen in the relapsed setting may be beneficial for virally driven aggressive lymphomas.[75]

5.2 | Treatment options in DLBCL by molecular features—for RR disease

5.2.1 | Autologous stem cell transplant

The role of ASCT in the salvage setting for MYC+ RR disease is controversial. Patients with DHL who undergo transplant have poorer outcomes than their non-DHL counterparts. In a large retrospective study of 117 patients with chemotherapy-sensitive RR DLBCL who underwent ASCT, those with DHL had an inferior 4-year PFS and OS (28% and 25%) compared to those without DHL (57% and 61%, respectively).[16] This study also looked at DELs and reported a similarly inferior 4-year PFS (48% vs 59%, P = .049) compared to non-DEL patients, but no significant difference in the 4-year OS. In a multicenter retrospective study of 175 patients who underwent salvage chemotherapy with an intention to transplant, patients without MYC translocation had a 30% 2-year OS rate, compared to 0% in patients with MYC translocation, and 9.9% in patients with DH/THL.[76] It is unlikely that high-dose chemotherapy alone can overcome the chemoresistance conferred by unfavorable genetic translocations in the relapsed setting.

5.2.2 | Chimeric antigen receptor T-cell therapy

For patients with RR disease, cellular therapy with chimeric antigen receptor T-cell therapy (CAR-T) plays an increasingly important and evolving role in the treatment. In this form of cellular therapy, pheresed autologous T cells are genetically modified with cloned DNA plasmids carrying a gamma retroviral or lentiviral recombinant vector as well as genes expressing a chimeric T cell receptor targeting a cell surface antigen of interest. Early phase studies of different second-generation CART-19 constructs targeting CD19 in the RR setting have led to the FDA approval of Kymriah (tisagenlecleucel) and Yescarta (axicabtagene ciloleucel) in RR DLBCL after two prior lines of therapy. In the phase IIa JULIET study of tisagenlecleucel, a CART-19 construct with the 4-IBB co-stimulatory domain, the best ORR was reported at 52%, with a CR of 40% in 93 patients. In DHL/THL (n = 19), the ORR was 50% with a CR of 25%. The updated results at a median follow-up of 14 months from infusion showed a median OS and PFS of not being reached in patients who achieved a CR, with some remissions lasting at least 2 years.[77] Recently updated 2-year follow-up data from the single-arm multicenter ZUMA-1 trial of axicabtagene ciloleucel (CD28 costimulatory domain) reported a 74% best ORR (54% CR) and median DOR and OS of not being reached. The study did not include detailed preplanned analysis of molecular features.[78] Recently presented abstracts at the ASH 2018 annual meeting describe comparable outcomes and toxicities in patients treated outside of clinical trials.[79,80] A trial of a third second-generation CART-19 construct, JCAR017, is ongoing and will include patients with MYC translocation [NCT02631044]. Early phase trials of third- and fourth-generation CARs, including those with more than one target, and multiple costimulatory domains are currently ongoing. CAR-T presents a viable and very promising therapy in RR DLBCL, including MYC-altered disease, and is an area of active research. A table of selected ongoing studies of CAR-T and other novel therapies in RR DLBCL is listed in Table 3.

5.2.3 | Allogeneic stem cell transplant

Allogeneic stem cell transplant remains a potential curative option in the treatment of RR DLBCL, and carries the benefit of a tumor-free allograft and potential graft vs lymphoma effect. However, many patients will not qualify for transplant due to age, significant comorbidities, and poor performance status from aggressive disease and/or multiple lines of prior therapy. Historic studies have reported the long-term survival of up to 40%-50%, but in the setting of significant treatment-related mortality (30%-40%).[81] Over the past two decades, efforts to improve these outcomes have focused on reducing the intensity of conditioning regimens. A recent retrospective study from the CIMBTR of 396 patients who received allotransplant for DLBCL between 2000 and 2009 found that myeloablative regimens were associated with lower rates of relapse/progression at 1, 3, and 5 years,[82] but were associated with a higher rates of nonrelapse mortality. The study found no difference in PFS or OS (OS 26% vs 20 vs 18% at 5 years) between patients receiving myeloablative, nonmyeloablative, or reduced intensity-conditioning regimens at any of the timepoints.

TABLE 3 Additional novel therapies in RR DLBCL

Drug	Clinical trial	Title	Primary objectives in RR DLBCL
CAR-T therapy			
Axicabtagene ciloleucel	NCT02348216 Phase III	Efficacy of axicabtagene ciloleucel compared to standard of care therapy in subjects with RR DLBCL (ZUMA-7)	To evaluate whether axicabtagene ciloleucel therapy improves the clinical outcome compared with standard-of-care second-line therapy in RR DLBCL
Tisagenlecleucel	NCT03570892 Phase III	Tisagenlecleucel in adult patients with aggressive B-cell NHL (BELINDA)	To evaluate the efficacy, safety, and tolerability of tisagenlecleucel compared to standard of care in RR aggressive B-cell NHL
	NCT03630159b Phase I	Study of tisagenlecleucel in combination with pembrolizumab in RR DLBCL (PORTIA)	To evaluate the safety and efficacy of the administration of tisagenlecleucel in combination with pembrolizumab in RR DLBCL
JCAR014	NCT02631044 Phase I	Study evaluating the safety and pharmacokinetics of JCAR017 in B-Cell NHL (TRANSCEND-NHL-001)	To evaluate the safety, PK, and antitumor activity of modified T cells (JCAR017) administered to adult patients RR B-cell NHL
	NCT02706405 Phase I	JCAR014 and durvalumab in treating patients with RR B-Cell NHL	To evaluate the safety of JCAR014 in combination with durvalumab in adult patients with RR B-cell NHL
huJCAR014 (Anti-CD19CAR-4-1BB-CD3zeta-EGFRt)	NCT03103971 Phase I	huJCAR014 CAR-T cells in treating adult patients with RR B-Cell NHL or acute lymphoblastic leukemia	To evaluate preliminary safety of huJCAR014 in adult patients with CD19+ RR B-cell NHL or ALL
CD19/CD22	NCT03233854 Phase 1	CD19/CD22 chimeric antigen receptor T cells and chemotherapy in treating patients with RR CD19 positive DLBCL or B acute lymphoblastic leukemia	To determine the feasibility of producing CD19/22-CAR T cells and assessing the safety of escalating doses of cells
CD19/22 (AUTO3)	NCT03287817 Phase I/II	CD19/22 CAR T cells followed by anti-PD1 antibody consolidation (AUTO3) for the treatment of DLBCL (ALEXANDER)	To determine the safety and ORR of AUTO3 in RR DLBCL
Polatuzumab	NCT02600897 Phase Ib/II	A study of obinutuzumab, polatuzumab vedotin, and lenalidomide in RR follicular lymphoma (FL) and rituximab in combination with polatuzumab vedotin and lenalidomide in RR DLBCL	To evaluate the safety, efficacy, and pharmacokinetics of rituximab, polatuzumab vedotin, and venetoclax in RR DLBCL
	NCT02729896 Phase Ib/II	A study evaluating safety and efficacy of obinutuzumab, polatuzumab vedotin (Pola), and atezolizumab (Atezo) in participants with RR follicular lymphoma (FL) and rituximab, atezo, and pola in participants with RR DLBCL	To evaluate the safety, efficacy, pharmacokinetics, and immunogenicity of obinutuzumab + atezolizumab + polatuzumab in RR DLBCL
	NCT02257567 Phase Ib/II	A study of polatuzumab vedotin (DCDS4501A) in combination with rituximab or obinutuzumab plus bendamustine in participants with RR FL or DLBCL	To determine safety and tolerability of polatuzumab vedotin (DCDS4501A) in combination with rituximab or obinutuzumab plus bendamustine in RR DLBCL
Blinatumomab	NCT02568553 Phase I	Lenalidomide and blinatumomab in treating patients with relapsed NHL	To determine the MTD of lenalidomide when given in combination with blinatumomab in relapsed NHL
	NCT03340766 Phase Ib	Open label study investigating the safety and efficacy of blinatumomab in combination with pembrolizumab (KEYNOTE-348)	To determine the maximum tolerated dose (MTD) of blinatumomab in combination with pembrolizumab in adult subjects with RR DLBCL
	NCT02568553 Phase I	Lenalidomide and blinatumomab in treating patients with relapsed NHL	To determine the side effects and best dose of lenalidomide and blinatumomab when given together in relapsed NHL
MOR208	NCT02399085 Phase I	A study to evaluate the safety and efficacy of lenalidomide with MOR00208 in patients with RR DLBCL (L-MIND)	To characterize the safety and efficacy of the human-anti-CD19 antibody MOR00208 in combination with lenalidomide in adult subjects with RR DLBCL

(Continues)

TABLE 3 (Continued)

Drug	Clinical trial	Title	Primary objectives in RR DLBCL
	NCT02763319 Phase I	A trial to evaluate the efficacy and safety of MOR208 with bendamustine (BEN) vs rituximab with BEN in adult patients with RR DLBCL (B-MIND)	To compare the safety and efficacy of MOR208 with BEN vs RTX with BEN in adult patients with RR DLBCL
Nivolumab	NCT03484819 Phase II	Copanlisib and nivolumab in treating participants with RR DLBCL or primary mediastinal large B-cell lymphoma	To study the efficacy of copanlisib and nivolumab work in treating participants with RR DLBCL
	NCT03015896 Phase I/II	Nivolumab and lenalidomide in treating patients with RR NHL or HL	To determine the safety and tolerability of the combination of lenalidomide and nivolumab in RR NHL or HL
	NCT03038672 Phase II	Nivolumab with or without varlilumab in treating patients with RR aggressive B-cell lymphomas	To determine the anti-tumor activity of combination therapy with CDX-1127 (varlilumab) and nivolumab as compared to nivolumab alone in patients with advanced aggressive B-cell non-Hodgkin lymphomas
Pembrolizumab	NCT03340766 Phase I	Open label study investigating the safety and efficacy of blinatumomab in combination with pembrolizumab (KEYNOTE-348)	To determine the maximum tolerated dose (MTD) of blinatumomab in combination with pembrolizumab in adult subjects with RR DLBCL
	NCT03150329 Phase I	Pembrolizumab and vorinostat in treating patients with RR DLBCL, FL, or HL	To determine side effects and best dose of vorinostat when given together with pembrolizumab in RR DLBCL
	NCT03309878 Phase I/II	Mogamulizumab and pembrolizumab in treating patients with RR lymphomas	To determine the best dose and side effects of mogamulizumab in combination with pembrolizumab in RR DLBCL
Venetoclax	NCT02992522 Phase Ib/II	Obinutuzumab, venetoclax, and lenalidomide in treating patients with relapsed or refractory B-cell non-Hodgkin lymphoma	To determine the dose-limiting toxicity and the recommended phase 2 dose of the combination of obinutuzumab, venetoclax, and lenalidomide in patients with RR B-cell NHL
	NCT03136497 Phase I	A study of ABT-199 plus ibrutinib and rituximab in patients with RR DLBCL	To determine the safety and MTD of venetoclax + ibrutinib in RR DLBCL
	NCT03223610 Phase I	Venetoclax, ibrutinib, prednisone, obinutuzumab, and revlimid (ViPOR) in RR B-cell lymphoma	To study the safety of ViPOR for people with B-cell lymphoma

Abbreviations: RR, relapsed and/or refractory; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma; HL, Hodgkin lymphoma; MTD, maximum tolerated dose.

Recently, Herrera et al. performed a multicenter retrospective analysis of 78 patients with DHL (13%) and DEL (47%) and found no differences in the 4-year PFS and 4-year OS between patients with and without DEL, and between patients with and without DHL.[83] Patients with DEL had a 4-year PFS and 4-year OS of 30% and 31% (P = .24), while those with DHL had a 4-year PFS and 4-year OS of 40% and 50% (P = .46), respectively. These data are retrospective in nature but suggest that allogeneic transplant may overcome the poor prognostic implications of these aggressive disease subtypes, especially in patients with an excellent performance status and few comorbidities. As such, these numbers may not reflect real-world experience, as they are vulnerable to selection bias for young and fit patients.

5.2.4 | Bromodomain and extraterminal motif inhibitors

Bromodomains are proteins that read the acetylation signature on histone tails and process the extent that genes will be transcribed. Once an acetyl group is found on a histone tail, the bromodomain recruits other transcriptional proteins, such as the transcription factor c-MYC. Bromodomain and extraterminal motif (BET) inhibitors prevent bromodomains from reading the acetyl-group signature and prevent recruitment of transcription factors.[84] in vitro studies have confirmed inhibition of DLBCL cell line proliferation in a dose-dependent manner, as well as reductions in the levels of MYC expression.[85] Additional in vitro studies have shown synergy with the B-cell leukemia/lymphoma-2 (BCL-2) inhibitor venetoclax in double-hit cell lines.[86] Early-phase clinical trials of BET inhibitors that include patients with RR DLBCL with MYC translocations are listed in Tables 1 and 2 [NCT02543879, NCT01943851].

5.2.5 | PI3K inhibitors

CUDC-907 (fimepinostat) is a first-in class small molecular combined histone deacetylase (classes I and II) inhibitor and a PI3K (classes Iα, β, and δ) inhibitor. In MYC-driven cell lines, CUDC-907 has shown downregulation of MYC mRNA and protein levels. It has also shown

antitumor activity in MYC-driven animal models.[87] A recently published phase I clinical trial of CUDC-907 in RR DLBCL enrolled 37 patients. Twenty-five patients received CUDC-907 alone, while 12 received CUDC-907 in combination with rituximab. Therapy was tolerable and reported an ORR of 37% with a median DOR of 11.2 months. The study included 14 patients with MYC alterations (including MYC copy number gains and MYC expression ≥40% by IHC, with 2 DELs and no DH/THLs), and reported a response rate of 64%.[51] A phase II study is ongoing. Other PI3K inhibitors have been studied in RR DLBCL. A small phase II study of copanlisib, a pan-class I PI3K inhibitor in RR DLBCL patients (n = 40 in the per-protocol analysis) showed an ORR of 25%, with a 13.6% response rate in the GCB subtype and 25% in the ABC subtype.[88] A phase II trial of idelalisib in RR DLBCL is ongoing [NCT03576443].

5.2.6 | BCL2 inhibitors

A phase I study of the BCL-2 inhibitor venetoclax was completed in 106 patients with RR NHL, and with 34 patients with RR DLBCL. The response rates for single-agent DLBCL were 18%, with a medial PFS of 1 month.[89] Venetoclax is currently being studied in combination with DA-EPOCH-R in a phase I study patients with DH/THL [NCT03036904]. In the RR setting, there are ongoing studies in combination with other biologic agents [NCT02992522, NCT03223610].

6 | IMMUNODIRECTED THERAPIES IN RR DISEASE

6.1 | Checkpoint inhibitors

Studies of checkpoint inhibitors in RR DLBCL have thus far been disappointing, with low response rates and short remissions. A phase I clinical trial of nivolumab in RR DLBCL had an ORR of 36% with a median PFS of only 7 weeks.[90] Checkmate 036, the combination of nivolumab plus ipilimumab yielded an ORR of 20% in RR NHL (10/15 with DLBCL), with a median PFS of only 1.5 months.[91] In a recently published multicenter phase II trial of 121 patients with RR DLBCL who had failed or were noneligible for ASCT, ORRs were 10% and 3%, respectively, at 9 and 6 months of the follow-up.[92] However, certain subtypes of extranodal DLBCL, including DLBCL leg-type, primary mediastinal, and primary CNS lymphoma may have higher PD1/PDL-1 expression, and respond better to checkpoint inhibitors than others. Ongoing efforts in RR DLBCL are focused on combining checkpoint inhibitors with various other antibodies [NCT03038672, NCT02951156], anti-CD20 antibodies, or in combination with CAR-T therapy [NCT02926833, NCT02706405].

Polatuzumab-vedotin is a humanized anti-CD79b monoclonal antibody conjugated to the cytotoxic agent monomethyl auristatin E. CD79b is expressed on the surface of B cells including malignant lymphocytes in NHL. At the 2017 ASH meeting, a phase II trial randomized patients with RR de novo DLBCL after at least one prior line of therapy to either polatuzumab-vedotin in addition to rituximab-bendamustine (BR) or BR alone. At a median follow-up of 11.1 months, the study reported an ORR rate of 45% compared to 17.5% in the BR arm alone. The majority of these responses were complete (40% vs 15%) with a median DOR of 8.8 months vs 3.7 months in the BR group. Early phase studies of polatuzumab-vedotin with lenalidomide and obinutuzumab [NCT02600897], or lenalidomide, obinutuzumab, and venetoclax [NCT02611323] are ongoing in RR DLBCL.

MOR208 is an Fc-enhanced monoclonal antibody against CD19, which can lead to potentiation of antibody-dependent cell-mediated toxicity and phagocytosis, as well as direct cytotoxicity. It was initially studied in RR NHL as a single agent and showed a 26% response rate in heavily pretreated, mostly rituximab refractory DLBCL patients.[93] The most common adverse events were infusion reactions and neutropenia. Most recently, data from the L-MIND study, an ongoing phase II study of MOR208 in combination with lenalidomide in RR DLBCL, were presented at the ASH 2018 annual meeting.[94] At a median follow-up of 12 months, the ORR was 58%, with 33% CR and 20% PR. In addition, 15% of patients had SD. Median DOR was not reached. Toxicities of the combination were most commonly neutropenia, thrombocytopenia, and anemia, followed by diarrhea and fatigue. A phase II/III study of MOR208 with bendamustine compared to rituximab and bendamustine in RR DLBCL is actively recruiting patients [NCT02763319].

Blinatumomab, a bispecific T-cell antibody, that transiently links CD3-positive T cells to CD19-positive B cells currently approved for the treatment of adult and pediatric adult lymphocytic leukemia, has been studied in the phase II setting in heavily pretreated RR DLBCL.[95] The study reported a 43% ORR after 1 cycle of therapy, with a 19% CR. However, treatment continuation beyond one cycle was limited by toxicity, with 9% of patients experiencing grade 3 or higher encephalopathy and aphasia. A phase Ib study of blinatumomab with pembrolizumab is ongoing [NCT03340766].

7 | CONCLUSIONS

DLBCL remains a heterogeneous disease at many levels. Of the 40% of patients with RR disease, the vast majority of these patients are not salvaged by high-dose chemotherapy followed by ASCT and will succumb to their disease. Novel therapeutics in RR DLBCL are needed. Tremendous progress has been made over the past 20 years to identify the subtypes of this disease based on either COO or molecular and immunophenotypic features, which carry poor prognostic impacts. Refinements in our understanding of the complex pathogenesis of these DLBCL subtypes have led to multiple efforts to target therapy with varying success, as illustrated by this review. As of now, however, there is limited data to inform differential therapy by disease subtype.

Efforts are ongoing, and two recent landmark studies led by the NCI and the Dana Farber Cancer Institute have harnessed next-generation sequencing technologies to identify the subtypes of disease by the presence of highly recurring mutations, which portend to different clinical phenotypes of disease. These new subtypes of disease are overlaid on the COO subtypes. A better understanding of the

downstream effects of these recurring mutations can help us understand the unique disease pathogenesis of each subtype. Although much more work needs to be done to distinguish driver mutations from passenger mutations and identify actionable mutations which can be translated into therapeutic targets, advancements in bioinformatics and gene-editing technologies in translational medicine will lead to significant process in this area in the next decade. All of this runs in parallel to the development of cellular therapy and bispecific T-cell engager therapy, which may be agnostic of COO and molecular features. Nevertheless, targeted therapy to DLBCL subtypes will surely be a large part of how to successfully tackle the heterogeneity of RR DLBCL.

ORCID

Yang Liu https://orcid.org/0000-0001-6606-9201
Stefan Klaus Barta https://orcid.org/0000-0002-3280-5271

REFERENCES

1. SEER. *SEER Database: All Lymphoid Neoplasms with Detailed Non-Hodgkin Lymphoma Subtypes; SEERb Incidence Rates and Annual Percent Change by Age at Diagnosis*. NCI; 2002-2011.
2. Siegel R, Miller K, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin*. 2019;69(1):7-34.
3. Gisselbrecht C, Glass B, Mounier N, et al. Salvage regimens with autologous transplantation for relapsed large B-cell lymphoma in the rituximab era. *J Clin Oncol*. 2010;28(27):4184-4190.
4. The International Non-Hodgkin's Lymphoma Prognostic Factors. A predictive model for aggressive non-Hodgkin's lymphoma. *N Engl J Med*. 1993;329:987-994.
5. Chapuy B, Stewart C, Dunford AJ, et al. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nature*. 2018;(5):679-669.
6. Schmitz R, Wright GW, Huang DW, et al. Genetics and pathogenesis of Diffuse large B-cell lymphoma. *N Engl J Med*. 2018;378:1396-1407.
7. Reddy A, Zhang J, Davis NS, et al. Genetic and functional drivers of diffuse large B cell lymphoma. *Cell*. 2017;171(2):481-494.
8. Swerdlow, SH, Campo, E, Pileri, SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neoplasms. *Blood*. 2016;127:2375-2390.
9. Hu S, Xu-Monette ZY, Balasubramanyam A, et al. CD30 expression defines a novel subgroup of diffuse large B-cell lymphoma with favorable prognosis and distinct gene expression signature: a report from the International DLBCL Rituximab-CHOP Consortium Program Study. *Blood*. 2013;121:2715-2724.
10. McPhail et al. Inferior survival in high-grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements is not associated with MYC/IG gene rearrangements. *Haematologica*. 2018;103(11):1899-1907.
11. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature*. 2000;403(6769):503-511.
12. Gutiérrez-García, G, Cardesa-Salzmann, T, Climent, F, et al. Gene-expression profiling and not immunophenotypic algorithms predicts prognosis in patients with diffuse large B-cell lymphoma treated with immunochemotherapy. *Blood*. 2011;117(18):4836-4843.
13. Ott G, Ziepert M, Klapper W, et al. Immunoblastic morphology but not the immunohistochemical GCB/nonGCB classifier predicts outcome in diffuse large B-cell lymphoma in the RICOVER-60 trial of the DSHNHL. *Blood*. 2010;116:4916-4925.
14. Scott DW, Mottok A, Ennishi D, et al. Prognostic significance of diffuse large B-cell lymphoma cell of origin determined by digital gene expression in formalin-fixed paraffin-embedded tissue biopsies. *J Clin Oncol*. 2015;33(26):2848-2856.
15. Staiger AM, Ziepert M, Horn H, et al. Clinical impact of the cell-of-origin classification and the MYC/BCL2 dual expresser status in diffuse large B-cell lymphoma treated within prospective clinical trials of the German high-grade non-Hodgkin's lymphoma study group. *J Clin Oncol*. 2017;22:2515-2526.
16. Herrera A, Mei M, Low L, et al. Relapsed or refractory double-expressor and double-hit lymphomas have inferior progression-free survival after autologous stem-cell transplantation. *J Clin Oncol*. 2017;35(1):24-31.
17. Miura K, Takahashi H, Nakagawa M, et al. Clinical significance of co-expression of MYC and BCL2 protein in aggressive B-cell lymphomas treated with a second line immunochemotherapy. *Leuk Lymphoma*. 2016;57(6):1335-1341.
18. Aukema SM, Siebert R, Schuuring E, et al. Double-hit B-cell lymphomas. *Blood*. 2011;117(8):2319-2331.
19. Friedberg JW. Double-hit diffuse large B-cell lymphoma. *J Clin Oncol*. 2012;30(28):3439-3443.
20. Hu S, Xu-Monette ZY, Tzankov A, et al. MYC/BCL2 protein coexpression contributes to the inferior survival of activated B-cell subtype of diffuse large B-cell lymphoma and demonstrates high-risk gene expression signatures: a report from The International DLBCL Rituximab-CHOP Consortium Program. *Blood*. 2013b;121(20):4021-4031.
21. Scott DW, King RL, Staiger AM, et al. High grade B-cell lymphoma with MYC and BCL2 and/or BCL6 rearrangements with diffuse large B-cell lymphoma morphology. *Blood*. 2018;131(18):2060-2064.
22. Ennishi D, Jiang A, Boyle M, et al. Double-hit gene expression signature defines a distinct subgroup of germinal center B-cell-like diffuse large B-cell lymphoma. *J Clin Oncol*. 2019;37(3):190-201.
23. Ziepert M, Hasenclever D, Kuhnt E, et al. Standard International prognostic index remains a valid predictor of outcome for patients with aggressive CD20+ B-cell lymphoma in the rituximab era. *J Clin Oncol*. 2010;28(14):2373-2380.
24. Zhou Z, Sehn LH, Rademaker AW, et al. An enhanced International Prognostic Index (NCCN-IPI) for patients with diffuse large B-cell lymphoma treated in the rituximab era. *Blood*. 2014;123:837-842.
25. Ziepert M, Hasenclever D, Kuhnt E, et al. Standard International prognostic index remains a valid predictor of outcome for patients with aggressive CD20+ B-cell lymphoma in the rituximab era. *J Clin Oncol*. 2010;28(14):2373-2380.
26. Rosenwald A, Wright G, Chan WC, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med*. 2002;346:1937-1947.
27. Thieblemont C, Briere J, Mounier N, et al. The germinal center/-activated B-cell subclassification has a prognostic impact for response to salvage therapy in relapsed/refractory diffuse large B-cell lymphoma: a bio-CORAL study. *J Clin Oncol*. 2011;29:4079-4087.
28. Gu K, Weisenburger DD, Fu K, et al. Cell of origin fails to predict survival in patients with diffuse large B-cell lymphoma treated with autologous hematopoietic stem cell transplantation. *Hematol Oncol*. 2012;30(3):143-149.
29. Moskowitz CH, Zelenetz AD, Kewalramani T, et al. Cell of origin, germinal center versus nongerminal center, determined by immunohistochemistry on tissue microarray, does not correlate with outcome in patients with relapsed and refractory DLBCL. *Blood*. 2005;106(10):3383-3385.
30. Costa LJ, Maddocks K, Epperla N, et al. Diffuse large B-cell lymphoma with primary treatment failure: ultra-high risk features and

benchmarking for experimental therapies. *Am J Hematol*. 2017;(2): 161-170.
31. Barrans S, Crouch S, Smith A, et al. Rearrangement of MYC is associated with poor prognosis in patients with diffuse large B-cell lymphoma treated in the era of rituximab. *J Clin Oncol*. 2010;28(20):3360-3365.
32. Savage KJ, Johnson NA, Ben-Neriah S, et al. MYC gene rearrangements are associated with a poor prognosis in diffuse large B-cell lymphoma patients treated with R-CHOP chemotherapy. *Blood*. 2009;114(17): 3533-3537.
33. Copie-Bergman C, Cuillière-Dartigues P2, Baia M, et al. MYC-IG rearrangements are negative predictors of survival in DLBCL patients treated with immunochemotherapy: a GELA/LYSA study. *Blood*. 2015;126:2466-2474.
34. Rosenwald, A, Sehn, L, Maucort-Boulch, D., et al. Prognostic significance of MYC single, double, triple hit and MYC-translocation partner status in diffuse large B-cell lymphoma - a study by the Lunenburg lymphoma biomarker consortium (LLBC); ASH Annual Meeting; 2018; San Deigo.
35. Oki et al. Double hit lymphoma: the MD Anderson Cancer Center clinical experience. *Br J Haematol*. 2014;166:891-901.
36. Petrich AM, Gandhi M, Jovanovic B, et al. Impact of induction regimen and stem cell transplantation on outcomes in double-hit lymphoma: a multicenter retrospective analysis. *Blood*. 2014;124(15):2354-2361.
37. Tomita N, Tokunaka M, Nakamura N, et al. Clinicopathological features of lymphoma/leukemia patients carrying both BCL2 and MYC translocations. *Haematologica*. 2009;94:935-943.
38. Johnson NA, Slack GW, Savage KJ, et al. Concurrent expression of MYC and BCL2 in diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. *J Clin Oncol*. 2012;30(28):3452-3459.
39. Green TM, Young KH, Visco C, et al. Immunohistochemical double-hit score is a strong predictor of outcome in patients with diffuse large B-cell lymphoma treated with rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone. *J Clin Oncol*. 2012;30(28):3460-3467.
40. Cuccuini W, Briere J, Mounier N, et al. MYC+ diffuse large B-cell lymphoma is not salvaged by classical R-ICE or R-DHAP followed by BEAM plus autologous stem cell transplantation. *Blood*. 2012;119 (20):4619-4624.
41. Cunningham D, Hawkes EA, Jack A, et al. Rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisolone in patients with newly diagnosed diffuse large B-cell non-Hodgkin lymphoma: a phase 3 comparison of dose intensification with 14-day versus 21-day cycles. *Lancet*. 2013;381:1817-1826.
42. Delarue R, Tilly H, Mounier N, et al. Dose-dense rituximab-CHOP compared with standard rituximab-CHOP in elderly patients with diffuse large B-cell lymphoma (the LNH03-6B study): a randomised phase 3 trial. *Lancet Oncol*. 2013;14:525-533.
43. Vitolo U, Trněný M, Belada D, et al. Obinutuzumab or rituximab plus CHOP in patients with previously untreated diffuse large B-cell lymphoma: final results from an open-label, randomized phase 3 study (GOYA). *Blood*. 2016;128:470.
44. Wilson WH, sin-Ho J, Pitcher BN, et al. Phase III randomized study of R-CHOP versus DA-EPOCH-R and molecular analysis of untreated diffuse large B-cell lymphoma: CALGB/Alliance 50303. *Blood*. 2016;128:469.
45. Dunleavy K, Roschewski M, Wilson WH, et al. Precision treatment of distinct molecular subtypes of diffuse large B-cell lymphoma: ascribing treatment based on the molecular phenotype. *Clin Cancer Res*. 2014;20:5182-5193.
46. Younes A, Thieblemont C, Morschhauser F, et al. Combination of ibrutinib with rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) for treatment-naive patients with CD20-positive B-cell non-Hodgkin lymphoma: a non-randomised, phase 1b study. *Lancet Oncol*. 2014;15(9):1019-1026.
47. Ruan J, Martin P, Furman RR, et al. Bortezomib plus CHOP-rituximab for previously untreated diffuse large B-cell lymphoma and mantle cell lymphoma. *J Clin Oncol*. 2011;29(6):690-697.
48. Leonard JP, Kolibaba KS, Reeves JA, et al. Randomized phase II study of R-CHOP with or without Bortezomib in previously untreated patients with non-germinal center B-cell-like diffuse large B-cell lymphoma. *J Clin Oncol*. 2017;35(31):3538-3546.
49. Davies AJ, Barrans S, Maishman T, et al. Differential efficacy of bortezomib in subtypes of diffuse large B-cell lymphoma (DLBL): a prospective randomized study stratified by transcriptome profiling: REMoDL-B. Paper presented at: 14th International Conference on Malignant Lymphoma Palazzo dei Congressi, Abstract 121; 2017.
50. Nowakowski GS, LaPlant B, Macon WR, et al. Lenalidomide combined with R-CHOP overcomes negative prognostic impact of non–germinal center B-cell phenotype in newly diagnosed diffuse large B-cell lymphoma: a phase II study. *J Clin Oncol*. 2015;33:251-257.
51. Oki Y, Kelly KR, Flinn I, et al. CUDC-907 in relapsed/refractory diffuse large B-cell lymphoma, including patients with MYC-alterations: results from an expanded phase I trial. *Haematologica*. 2017;102(11):1923-1930.
52. Howlett C, Snedecor SJ, Landsburg DJ, et al. Front-line, dose-escalated immunochemotherapy is associated with a significant progression-free survival advantage in patients with double-hit lymphomas: a systematic review and meta-analysis. *Br J Haematol*. 2015;170(4):504-514.
53. Landsburg DJ, Falkiewicz MK, Maly J, et al. Outcomes of patients with double-hit lymphoma who achieve first complete remission. *J Clin Oncol*. 2017;35(20):2260-2267.
54. Aggarwal A, Rafei H, Alakeel F, et al. Outcome of patients with double-expressor lymphomas (DELs) treated with R-CHOP or R-EPOCH. *Blood*. 2016;128:5396.
55. Sathyanarayanan V, Oki Y, Issa A, et al. High risk diffuse large B cell lymphoma: a comparison of aggressive subtypes treated with dose adjusted chemotherapy—the University of Texas MD Anderson experience. *Blood*. 2016;128:106.
56. Philip T, Guglielmi C, Hagenbeek A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. *N Engl J Med*. 1995;333(23):1540-1545.
57. Vardhana et al. Outcomes of primary refractory diffuse large B-cell lymphoma (DLBCL) treated with salvage chemotherapy and intention to transplant in the rituximab era. *Br J Hematol*. 2017;176:591-599.
58. Van Den Neste et al. Outcome of patients with relapsed diffuse large B-cell lymphoma who fail second-line salvage regimens in the International CORAL study. *Bone Marrow Transplant*. 2016;51:51-57.
59. Van Den Neste E, Schmitz N, Mounier N, et al. Outcomes of diffuse large B-cell lymphoma patients relapsing after autologous stem cell transplantation: an analysis of patients included in the CORAL study. *Bone Marrow Transplant*. 2017;52:216-221.
60. Crump M, Neelapu SS, Farooq U, et al. Outcomes in refractory diffuse large B-cell lymphoma: results from the international SCHOLAR-1 study. *Blood*. 2017;130:1800-1808.
61. Wilson WH, Young RM, Schmitz R, et al. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. *Nat Med*. 2015; 21(8):922-926.
62. Winter AM, Landsburg DJ, Mato AR, et al. A multi-institutional outcomes analysis of patients with relapsed or refractory DLBCL treated with ibrutinib. *Blood*. 2017;130:1676-1679.
63. Sauter CS, Matasar MJ, Schoder H, et al. A phase I study of Ibrutinib in combination with R-ICE in patients with relapsed or primary refractory DLBCL. *Blood*. 2018;131(16):1805-1808.
64. Fox LC, Yannakou CK, Ryland G, et al. Molecular mechanisms of disease progression in primary cutaneous diffuse large B-cell lymphoma, leg type during Ibrutinib therapy. *Int J Mol Sci*. 2018;19:1758.
65. Chen JG, Liu X, Munshi M1, et al. BTKCys481Ser drives ibrutinib resistance via ERK1/2 and protects BTKwild-type MYD88-mutated cells by a paracrine mechanism. *Blood*. 2018;131:2047-2059.
66. Wiernik PH, Lossos IS, Tuscano JM, et al. Lenalidomide monotherapy in relapsed or refractory aggressive non-Hodgkin's lymphoma. *J Clin Oncol*. 2008;26(30):4952-4957.

67. Witzig TE, Vose JM, Zinzani PL, et al. An international phase II trial of single-agent lenalidomide for relapsed or refractory aggressive B-cell non-Hodgkin's lymphoma. *Ann Oncol*. 2011;22(7):1622-1627.
68. Czuczman MS, Trněný M, Davies A, et al. A phase 2/3 multicenter, randomized, open-label study to compare the efficacy and safety of Lenalidomide versus Investigator's choice in patients with relapsed or refractory diffuse large B-cell lymphoma. *Clin Cancer Res*. 2017;23(15):4127-4137.
69. Gandhi AK, Kang J, Havens CG, et al. Immunomodulatory agents lenalidomide and pomalidomide co-stimulate T cells by inducing degradation of T cell repressors Ikaros and Aiolos via modulation of the E3 ubiquitin ligase complex CRL4(CRBN). *Br J Haematol*. 2014;164(6):811-821.
70. Lopez-Girona A, Mendy D, Ito T, et al. Cereblon is a direct protein target for immunomodulatory and antiproliferative activities of lenalidomide and pomalidomide. *Leukemia*. 2012;26(11):2326-2335.
71. Wang M, Fowler N, Wagner-Bartak N, et al. Oral lenalidomide with rituximab in relapsed or refractory diffuse large cell, follicular and transformed lymphoma: a phase II clinical trial. *Leukemia*. 2013;27(27):1902-1909.
72. Zinzani PL, Pellegrini C, Derenzini E, et al. Long-term efficacy of the combination of lenalidomide and rituximab in elderly relapsed/-refractory diffuse large B-cell lymphoma patients. *Hematol Oncol*. 2013;31(4):223-224.
73. Ferreri AJ, Sassone M, Zaja F, et al. Lenalidomide maintenance in patients with relapsed diffuse large B-cell lymphoma who are not eligible for autologous stem cell transplantation: an open label, single-arm, multicentre phase 2 trial. *Lancet*. 2017;4(3):e137-e146.
74. Dunleavy K, Pittaluga S, Czuczman MS, et al. Differential efficacy of bortezomib plus chemotherapy within molecular subtypes. *Blood*. 2009;113(24):6069-6076.
75. Reid E, Looney D, Maldarelli F, et al. AMC-053: pilot study of an oncolytic viral strategy using Bortezomib with ICE +/− rituximab for relapsed/refractory HIV+ lymphomas. *Blood*. 2016;128:786.
76. Epperla et al. C-MYC-positive relapsed and refractory, diffuse large B-cell lymphoma: impact of additional "hits" and outcomes with subsequent therapy. *Cancer*. 2017;123(22):4411-4418.
77. Schuster S, Bishop M, Tam S, et al. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med*. 2019;380:45-56.
78. Neelapu SS, Locke FL, Bartlett NL, et al. Axicabtagene Ciloleucel CAR T-cell therapy in refractory large B-cell lymphoma. *N Engl J Med*. 2017;377(26):2531-2544.
79. Nastoupil L, Jain M, Spiegel J, et al. *Axicabtagene Ciloleucel (Axi-Cel) CD19 Chimeric Antigen Receptor (CAR) T-Cell Therapy for Relapsed/-Refractory Large B-Cell Lymphoma: Real World Experience*. San Diego: American Society of Hematology; 2018.
80. Jacobson C, Hunter B, Armand P, et al. *Axicabtagene Ciloleucel in the Real World: Outcomes and Predictors of Response, Resistance and Toxicity*. San Diego: American Society of Hematology; 2018.
81. Doocey RT, Toze CL, Connors JM, et al. Allogeneic haematopoietic stem-cell transplantation for relapsed and refractory aggressive histology non-Hodgkin lymphoma. *Br J Haematol*. 2015;131(2):223.
82. Bacher U, Klyuchnikov E, Le-Rademacher J, et al. Conditioning regimens for allotransplants for diffuse large B-cell lymphoma: myeloablative or reduced intensity? *Blood*. 2012;120:4256-4262.
83. Herrera AF, Rodig SJ, Song JY, et al. Outcomes after allogeneic stem cell transplantation in patients with double-hit and double-expressor lymphoma. *Biol Blood Marrow Transplant*. 2018;24(3):514-520.
84. Pérez-Salvia M, Esteller M, et al. Bromodomain inhibitors and cancer therapy: from structures to applications. *Epigenetics*. 2017;12(5):323-339.
85. Trabucco SE, Gerstein RM, Evens AM, et al. Inhibition of bromodomain proteins for the treatment of human diffuse large B-cell lymphoma. *Clin Care Res*. 2015;21(1):113-122.
86. Johnson-Farley N, Veliz J, Bhagavathi S, et al. ABT-199, a BH3 mimetic that specifically targets Bcl-2, enhances the antitumor activity of chemotherapy, bortezomib and JQ1 in "double hit" lymphoma cells. *Leuk Lymphoma*. 2015;56(7):2146-2152.
87. Sun K, Atoyan R, Borek MA, et al. Dual HDAC and PI3K inhibitor CUDC-907 downregulates MYC and suppresses growth of MYC-dependent cancers. *Mol Cancer Ther*. 2017;16(2):285-299.
88. Lenz G, Hawkes E, Verhoef G, et al. Phase II study of single-agent copanlisib in patients with relapsed or refractory diffuse large B-cell lymphoma (DLBCL). *J Clin Oncol*. 2017;35:7536-7536.
89. Davids MS, Roberts AW, Seymour JF, et al. Phase I first-in-human study of Venetoclax in patients with relapsed or refractory non-Hodgkin lymphoma. *J Clin Oncol*. 2017;35(8):826-833.
90. Lesokhin AM, Ansell SM, Armand P, et al. Nivolumab in patients with relapsed or refractory hematologic malignancy: preliminary results of a phase Ib study. *J Clin Oncol*. 2016;34(23):2698-2704.
91. Ansell A, Gutierrez M, Shipp M, et al. A phase 1 study of Nivolumab in combination with Ipilimumab for relapsed or refractory hematologic malignancies (CheckMate 039). *Am Soc Hematol*. 2016;128(22):183.
92. Ansell S, Minnema M, Johnson P, et al. Nivolumab for relapsed/-refractory diffuse large B-cell lymphoma in patients ineligible for or having failed autologous transplantation: a single-arm, phase II study. *J Clin Oncol*. 2019;37(6):481-489.
93. Jurczak W, Zinzani PL, Gaidano G, et al. Phase IIa study of the CD19 antibody MOR208 in patients with relapsed or refractory B-cell non-Hodgkin's lymphoma. *Ann Oncol*. 2018;29:1266-1272.
94. Salles G, Callet-Bauchu E, Besson H, et al. Single-arm phase II study of MOR208 combined with lenalidomide in patients with relapsed or refractory diffuse large B-cell lymphoma: L-Mind. *Blood*. 2018;130:4123.
95. Viardot A, Goebeler ME, Hess G, et al. Phase 2 study of the bispecific T-cell engager (BiTE) antibody blinatumomab in relapsed/refractory diffuse large B-cell lymphoma. *Blood*. 2016;127:1410-1416.

How to cite this article: Liu Y, Barta SK. Diffuse large B-cell lymphoma: 2019 update on diagnosis, risk stratification, and treatment. *Am J Hematol*. 2019;94:604–616. https://doi.org/10.1002/ajh.25460