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Review

REVIEW

Beyond RCHOP: A Blueprint for Diffuse Large B Cell Lymphoma Research

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Abstract

Diffuse large B cell lymphoma (DLBCL) comprises multiple molecular and biological subtypes, resulting in a broad range of clinical outcomes. With standard chemoimmunotherapy, there remains an unacceptably high treatment failure rate in certain DLBCL subsets: activated B cell (ABC) DLBCL, double-hit lymphoma defined by the dual translocation of *MYC* and *BCL2*, dual protein–expressing lymphomas defined by the overexpression of MYC and BCL2, and older patients and those with central nervous system involvement. The main research challenges for DLBCL are to accurately identify molecular subsets and to determine if specific chemotherapy platforms and targeted agents offer differential benefit. The ultimate goal should be to maximize initial cure rates to improve long-term survival while minimizing toxicity. In particular, a frontline trial should focus on biologically defined risk groups not likely to be cured with cyclophosphamide, doxorubicin, vincristine, and prednisone plus rituximab (R-CHOP). An additional challenge is to develop effective and personalized strategies in the relapsed setting, for which there is no current standard other than autologous stem cell transplantation, which benefits a progressively smaller proportion of patients. Relapsed/refractory DLBCL is the ideal setting for testing novel agents and new biomarker tools and will require a national call for biopsies to optimize discovery in this setting. Accordingly, the development of tools with both prognostic and predictive utility and the individualized application of new therapies should be the main priorities. This report identifies clinical research priorities for critical areas of unmet need in this disease.

Background

Overview

In 2015, over 71 000 patients will be diagnosed with non-Hodgkin lymphoma (NHL) and nearly 20 000 patients will die of their disease, thus ranking NHL among the top 10 causes of cancer mortality (1). Diffuse large B cell lymphoma (DLBCL) is the most common NHL in the United States, accounting for 40% of the global lymphoma burden (2). Although the incidence increases with age, DLBCL affects a broad segment of society, occurring in every decade of life, both genders, and all races and manifesting in nearly any organ or body part. While frontline management has improved survival for many patients, DLBCL harbors a number of histologic, clinical, and molecular variants. This heterogeneity leads to variable patient outcome and

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Table 1. Diffuse large B cell lymphoma biomarkers*

Recommendations	Standard of care	Integral	Integrated	Exploratory	Validated	Identifies high-/low-risk groups	Identifies therapeutic target
Histopathology							
BCL2, MYC, and BCL6 protein expression by IHC	Yes	Yes	Yes	No	Yes	Yes	Yes
Target-specific IHC (eg, CD20, CD30, etc.)	Yes	Yes	Yes	No	Sometimes	No	Yes
Genetics							
Fluorescent in situ hybridization for *BCL2*, *MYC*, and *BCL6* abnormalities	Yes	Yes	No	No	Yes	Yes	No
Molecular analysis							
Cell-of-origin using Nanostring Lymph2CX assay GCB vs ABC vs unclassified	Yes by various methods	Yes	Yes	No	Yes	Yes	Yes
Pre- and post-treatment MRD analyses by DNA sequencing of blood or bone marrow V(D)J	No	No	Yes	Yes	No	Yes	No
Targeted resequencing of oncogenic driver genes	No	No	Yes	Yes	No	Yes	No
Whole-exome sequencing and clonality	No	No	No	Yes	No	No	Maybe
Additional							
Absolute lymphocyte count	Yes	No	Yes	No	Yes	Yes	No
Serum immunoglobulin–free light chains	No	No	Yes	Yes	Yes	Yes	No
Vitamin D levels	No	No	Yes	Yes	Yes	Yes	No
Serum cytokines/chemokines	No	No	Yes	Yes	No	Yes	No
Serum metabolomics	No	No	No	Yes	No	Yes	No

ABC = activated B cell subtype of diffuse large B cell lymphoma;
BCL2 = B cell lymphoma 2; BCL6 = B cell lymphoma 6; GCB = germinal center B subtype of diffuse large B cell lymphoma;
IHC = immunohistochemistry;
MRD = minimal residual disease; V(D)J = recombination of immunoglobulin variable, diversity, and joining gene segments.

complicates the ability to use a standard approach. Relapsed or refractory DLBCL after cyclophosphamide, doxorubicin, vincristine, and prednisone plus rituximab (R-CHOP) therapy is increasingly difficult to salvage. Traditional treatments, such as high-dose chemotherapy and autologous stem cell transplant, benefit a relatively smaller proportion of relapsed patients, and there is an urgent need for more targeted and efficacious approaches.

Diagnosis

The diagnosis of de novo DLBCL requires adequate tissue specimens and expert hematopathology review. An adequate biopsy specimen allowing full clinical interrogation and research investigation is at least 1 cubic cm or multiple core biopsies. The rate of pathology ineligibility due to alternative diagnosis can be as high as 15% to 20% of patients because of composite histologies or difficulty in recognizing rarer subtypes. Hence, for the purpose of clinical trials, central pathology review remains essential. Re-biopsy at the time of first relapse should be strongly encouraged or required because of high rates of false findings on positron emission tomography (PET) and the possibility of low-grade lymphoma relapse.

Biology

DLBCL originates from malignant transformation of B cells undergoing clonal expansion in the germinal center. The pathogenesis of DLBCL is complex, with multiple underlying subtypes with common histology but unique biology that likely explains differences in clinical outcome (3,4). The first landmark observation, using gene expression profiling (GEP), identified two molecular subgroups (5). These two categories, termed “germinal center” (GC) DLBCL and “activated B cell” (ABC, or non-GC) DLBCL utilize separate oncogenic pathways and have gene expression patterns reminiscent of their cell of origin (COO). GC DLBCL is characterized by ongoing somatic hypermutation, upregulation of *BCL6*, and near-universal CD10 expression (4,6). In contrast, ABC DLBCL is associated with chronic active B cell receptor (BCR) signaling and NF-kB deregulation (4,6). A third category, termed “unclassifiable,” was also identified and includes up to 15% of cases. The clinical significance of COO is evident in both CHOP- and R-CHOP-treated groups, whereby ABC DLBCL has a substantially inferior outcome, and is discussed below. Given the clinical implications, the new World Health Organization (WHO) classification now recognizes DLBCL subtypes as separate diagnostic categories: ABC (non-GC DBCL) and GCB DLBCL (7). Determination of COO initially used cDNA microarray technology and hierarchical clustering in frozen tumor material. However, this is not feasible in daily practice, prompting a number of immunohistochemical (IHC) algorithms to be developed using paraffin-embedded tissue (8). There are at least four proposed IHC models, but all are hampered by variable reproducibility and accuracy when tested against GEP as the gold standard determination of COO (9). Newer platforms capable of testing gene expression on RNA from formalin-fixed, paraffin embedded tissue, including NanoString (Lymph2CX) and Illumina platforms, are emerging (Table 1). A strong

recommendation from the Clinical Trials Planning Meeting (CTPM) is that prospective determination of COO, preferably via molecular methods, be included as either an integral or integrated biomarker in DLBCL trials. However, COO classification is not fully applicable to uncommon DLBCL variants like histiocyte/T cell–rich DLBCL or primary mediastinal DLBCL because these patients were generally excluded from studies of the role of COO in DLBCL. Also, COO classification cannot replace pathology review.

In addition to COO designation, which compares DLBCL with normal B cells, an alternative analysis of transcriptional profiling evaluated DLBCL cases based on relative signatures (10). Shipp and colleagues identified three distinct biologic subsets, or "consensus clusters," termed "oxidative phosphorylation," "B cell receptor/proliferation," and "host response" (10). While the clinical impact of the consensus clusters is less clear than COO, this and subsequent studies emphasize the contribution of the lymphoma microenvironment and host immune status on lymphoma pathogenesis. In light of a number of new agents targeting components of host immunity, such as checkpoint inhibitors and others, the information gleaned from consensus clusters is increasingly relevant.

A number of genetic and epigenetic lesions also contribute to pathobiology of DLBCL, likely related to the normal pressures on B cells as they undergo somatic hypermutation and class switch rearrangement as part of their normal development. Of interest, DLBCL ranks in the top third of cancers with the highest mutation frequency (11). The broad spectrum of mutations involves histone modification genes, *BCL6* deregulation, altered genes preventing immune surveillance, *TP53* mutations, BCR signaling, NF-kB pathway mutations, and others (reviewed in [4, 12, 13]).

In addition to GEP and molecular signatures, *MYC* deregulation and concurrent *BCL2* deregulation are clinically relevant in DLBCL. *MYC*, a proto-oncogene and transcription factor classically associated with Burkitt lymphoma (BL), is rearranged in 6% to 16% of DLBCL cases, thus more common than BL (14). Unlike BL, *MYC* rearrangement in DLBCL confers an adverse prognosis, likely due to a distinct set of target genes (15). While initial retrospective studies focused primarily on the role of *MYC* in DLBCL (16,17), it is increasingly clear that the concurrent rearrangement of the *BCL2* gene substantially contributes to poor outcomes. Furthermore, in comparison with BL, where *MYC* rearrangement is often the sole abnormality ("MYC-simple"), non-BL histologies harboring *MYC* rearrangements almost always have secondary and tertiary genetic aberrations ("MYC-complex") (18). The WHO defines concomitant *MYC* and *BCL2* (or *BCL6*) rearrangements as a biologic entity termed "double-hit lymphoma (DHL)" (2). Although uncommon (comprising approximately 5% of DLBCL), DHL is associated with dismal outcomes and rare long-term survivors (19). The majority of DHLs occur in the context of GC DLBCL and fare poorly with R-CHOP therapy. Reflecting these clinical and biologic distinctions, the 2016 WHO classification now defines an entity of high-grade lymphoma with *MYC* and *BCL2* or/and *BCL6* translocation (7). As such, DHL morphologically consistent with DLBCL is no longer referred to as DLBCL but is instead termed "high-grade lymphoma with *MYC* and *BCL2* or/and *BCL6* translocation."

However, recent results with IHC analysis of MYC expression complicate understanding of the roles of MYC and BCL2 in DLBCL. Up to one-third of unselected DLBCL cases have protein overexpression of both MYC and BCL2. The clinical implication of these "dual protein"–expressing lymphomas is inferior outcomes (19–22). Most series, all retrospective, show less than 30% long-term disease control. Of interest, and consistent with the DHL subtype, it is the coincidence of MYC and BCL2 protein overexpression that seems to confer the adverse prognosis (20,22). The largest series to evaluate dual protein–expressing lymphomas included 893 DLBCL cases, with both a training and validation set; multivariable analysis provocatively found that MYC/BCL2 protein overexpression was the main driver of adverse prognosis in both GC-DLBCL and ABC-DLBCL and, in this analysis, was more prognostic than COO designation. There are currently no prospective data to guide the management of MYC and BCL2 protein–overexpressing lymphomas.

One additional comment regarding pathobiology is the overlap between Burkitt lymphoma and DLBCL, more properly termed "B cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL" (BCLU) (23). The true frequency of BCLU is unknown, and older reports have referred to this as atypical Burkitt lymphoma or Burkitt-like lymphoma. BCLU is more likely to harbor cytologic pleomorphism, a complex karyotype that often includes *MYC* rearrangements, dual or triple translocations of *MYC* and *BCL2* or *BCL6* (ie, "double hit" and "triple hit"), and a high proliferation rate. Given the high frequency of dual MYC and BCL2 overexpression, clinical trials focusing on DHL will need to include and/or otherwise address BCLU, which remains distinct from the category of high-grade lymphoma with *MYC* and *BCL2* or/and *BCL6* translocation.

Newly Diagnosed DLBCL

The addition of rituximab to CHOP chemotherapy in the early 2000s represented the first major advance in DLBCL management since the advent of combination chemotherapy. There are now a number of pivotal trials showing not simply a progression-free survival benefit, but an overall survival benefit of approximately 15% in both younger and older populations (24–28). Consequently, the development of novel combinations for upfront treatment of DLBCL requires the incorporation of chemoimmunotherapy as its backbone, until there is sufficient evidence that novel agents are able to cure the disease.

The International Prognostic Index (IPI; comprised of age, lactate dehydrogenase [LDH], stage, more than one extranodal site, and performance status) retains its prognostic significance in R-CHOP-treated patients and is a useful tool in cross-trial evaluation of outcomes (29). Refinements of the IPI, such as the Revised IPI (R-IPI) and National Comprehensive Cancer Network IPI (NCCN-IPI) have been proposed and tested (30,31). The R-IPI reassessed clinical prognostic variables in a population-based cohort of DLBCL patients treated almost uniformly with rituximab; the authors found that the original components of the IPI remain prognostically significant, but three groups, rather than five, emerged (30). Most encouraging in their data is the identification of a very low-risk patient population with an IPI of 0 that achieved long-term disease control and survival of over 94%. The NCCN-IPI, based on an analysis of 1650 adults with DLBCL and validated using a Canadian cohort of more than 1100 patients, identified age, LDH, sites of involvement, Ann Arbor stage, and Eastern Cooperative Oncology Group (ECOG) performance status as the key prognostic variables (31); compared with the original IPI, the NCCN-IPI was better able to distinguish high-risk and low-risk subgroups. Although there are clearly limitations to clinical prognostic indices, which by design do not incorporate biologic risk factors, ongoing and future clinical trials should prospectively include all components of the IPI and the level of LDH elevation.

Table 2 Results of phase III studies of firstline treatment of diffuse large B cell lymphoma

Regimen	No.	CR/Cru (95% CI), %	OS (95% CI), %	EFS/PFS (95% CI), %	Reference
CHOP vs RCHOP*	197	63	57 at 2 y (50 to 64)*	EFS 38 at 2 y (32 to 45)	25
	202	75	70 at 2 y (63 to 77)	EFS 57 at 2 y (50 to 64)	
CHOP-like vs RCHOP like†	411	68 (63 to 73)	84 at 3 y (80 to 88)	EFS 59 at 3 y (54 to 64)	26
	413	86 (82 to 89)	93 at 3 y (90 to 95)	EFS 79 at 3 y (75 to 83)	
CHOP vs RCHOP	140	NR	52 at 2 y	PFS 51 at 2 y	24
	152	NR	78 at 2 y	PFS 69 at 2 y	
CHOP RCHOP‡	314	NR	NS§	EFS 46 at 3 y	28
	318	NR		EFS 53 at 3 y	

*Patients age 60 years and older. CHOP = cyclophosphamide, doxorubicin, vincristine, and prednisone; CR = complete response; CRu = complete response unconfirmed;
EFS = event-free survival;
NR = not reported;
NS = not significant; OS = overall survival; PFS = progression-free survival;
R-CHOP = CHOP + rituximab.
†Patients age 18–60 years.
‡RCHOP plus CHOP with R maintenance.
§Difference not statistically significant; however, secondary random assignment to maintenance was performed.

Current Landscape of Clinical Trials

Frontline Treatments

The current landscape of trials has taken several approaches, including the addition of new agents onto an R-CHOP backbone, testing dose-dense delivery of R-CHOP with growth factor support, evaluating infusional delivery of cytotoxic agents (ie, DA-EPOCH-R), or testing alternative anthracycline-based combinations (ie, R-ACVBP) (Table 2). The majority of trials have combined all patients with treatment-naïve DLBCL, sometimes with stratifications based on either clinical (ie, IPI) or molecular (ie, cell of origin) features. Only one trial to date has demonstrated a survival advantage. The R-ACVBP regimen had a three-year overall survival (OS) of 92% (95% confidence interval [CI] = 87% to 95%) vs 84% (95% CI = 77% to 89%) for R-CHOP ($P = .007$, two-sided), but widespread use is likely to be limited by increased toxicity in older patients and limited global availability of vindesine. Infusional chemoimmunotherapy as used in the DA-EPOCH-R regimen has impressive activity in phase II settings, and a large United States intergroup study (CALGB 50303) comparing DA-EPOCH-R to R-CHOP is complete, with results expected in 2016.

There are several ongoing phase II and III subtype-specific trials that will be informative. Based on retrospective observations that lenalidomide and ibrutinib appear to have selective activity in relapsed ABC-DLBCL as compared with GC-DLBCL, there are currently prospective randomized trials designed to assess activity in treatment-naïve DLBCL, enriched for ABC-DLBCL, with an R-CHOP backbone. As an example, lenalidomide has an overall response rate of 53% in relapsed/refractory ABC-DLBCL vs 9% in GC-DLBCL (two-sided $P = .006$), with COO designation via an IHC algorithm (32). Addition of lenalidomide to R-CHOP in two phase II trials showed improved progression-free survival (PFS) compared with historical controls specifically for the ABC-DLBCL subtype (33,34). This result has prompted a US intergroup randomized phase II trial powered to have sufficient ABC-DLBCL patients on both arms and a pharmaceutical trial, both of which compare R-CHOP to R-CHOP plus lenalidomide. Given the role of proteasome inhibition in ABC-DLBCL, bortezomib plus R-CHOP has been studied in two randomized phase II studies with no apparent benefit (35,36). A large phase III trial comparing bortezomib-R-CHOP vs R-CHOP alone has been completed; while no difference in response rates was reported, the study is maturing in regards to long-term outcomes data (37). The negative results to date in trials have sparked considerable debate; the use of IHC to determine COO (vs GEP) and the impact of excluding "sicker" patients in need of urgent treatment from clinical trials on the outcome of the control arm have been proposed to explain the negative findings (38).

Postinduction approaches in DLBCL have used either autologous hematopoietic stem cell transplant (autoHCT) or "maintenance/consolidation" with targeted agents or monoclonal antibodies. The PRELUDE trial, which randomly assigned more than 700 high IPI DLBCL patients 2:1 to either oral enzastaurin or placebo, is instructive despite being a negative trial (39). Although the trial focused on high-risk patients, all patients had to have a complete response in order to be randomly assigned. There was no difference in disease-free survival, and overall outcomes were better than expected in both groups, with 70% long-term disease-free survival and OS despite high IPI at baseline. This trial suggests that chemosensitivity, documented by either a CT or PET complete remission, may have greater clinical significance than initial risk factors and underscores the complexity in identifying a high-risk group. The use of postinduction rituximab was initially thought to be noncontributory in patients treated with R-CHOP (28), but more recent studies show that gender and rituximab dose intensification may influence outcomes in some patient subgroups (40). Consolidative autoHCT has been tested in a key US intergroup study following CHOP/R-CHOP in aggressive lymphomas (41). The overall trial was negative, but patients with very high IPI had improved PFS, suggesting that some high-risk patients may benefit from intensive consolidation. Overall, the current landscape of trials does not support the routine use of postinduction approaches in DLBCL. However, newer techniques of determining minimal

residual disease, more standardized PET assessments, and improved biologic definition of risk may help to identify patients who are most likely to benefit from this type of approach.

As discussed, there is broad agreement that both DLBCL and BCLU harboring dual *MYC* and *BCL2* rearrangements are rarely cured with R-CHOP. A single-center review of 129 patients reported a two-year disease-free survival of 33% following R-CHOP. More intensive regimens were variably effective, and consolidative autologous stem cell transplantation (ASCT; performed only in patients achieving complete remission) did not provide a statistically significant benefit. A multicenter retrospective analysis similarly showed that R-CHOP was inferior to all intensive regimens, with a hazard ratio of 0.53 and less than 20% long-term survival; again, ASCT consolidation was not significantly beneficial (42). Dual MYC and BCL2 protein overexpression (DPL), while not a distinct entity, is more common than DHL and confers a poor prognosis. Approximately 25% to 30% of unselected retrospective series identify dual protein overexpression that may or may not have underlying DHL. Several points regarding DHL and DPL are worth noting. First, over 90% of DHLs occur in GC DLBCL, whereas over 60% of DPLs occur in non-GC DLBCL, reflecting distinct pathogenetic mechanisms underlying the overexpression (43). Additionally, DPL patients are older (median age = 71 years) and more likely to have a poor performance status, more advanced stage disease, more B symptoms, higher IPI, and more chemoresistance compared with DLBCL without dual protein overexpression. A promising feature of both DHL and DPL is that MYC and BCL2 are potentially druggable, and there are a number of targeted agents that should be added to chemoimmunotherapy backbones. To date, there are no large prospective trials powered to study either DHL or dual protein–expressing lymphomas, and this is an area of clear unmet need. Preliminary results of a phase II National Cancer Institute study of DA-EPOCH-R in MYC-associated lymphomas are promising, with a PFS of 87% with very short follow-up (44). Of note, the presence of BCL2 protein overexpression assessed by IHC was an important negative prognostic factor, supporting BCL2 as a biologically rational target.

Relapsed/Refractory DLBCL

The historical approach to recurrent or refractory DLBCL based on the Parma trial (45) has been salvage chemotherapy followed by high-dose chemotherapy and autologous bone marrow transplant in patients with chemosensitive disease, leading to long-term disease control in approximately half of patients (46). However, the Collaborative Trial in Relapsed Aggressive Lymphoma (CORAL) study demonstrated that, on an intent-to-treat basis, less than 20% of patients treated with a rituximab-containing frontline regimen and relapsing within one year benefited from salvage autoHCT (47). Among responding patients able to proceed to autoHCT, three-year PFS was 39% vs 14% ($P < .001$) for patients not receiving transplant. Of note, there was no difference in efficacy between the two salvage regimens studied, R-ICE and R-DHAP, although a post hoc review suggested higher activity of R-DHAP in patients with GC-DLBCL (48). A second randomized trial in the rituximab era compared R-DHAP with R-GDP in 619 patients with relapsed aggressive lymphomas (49). Again, the choice of salvage regimen did not impact outcomes, and four-year event-free survival (EFS) was 26% in each group (hazard ratio [HR] = 0.99, 95% CI = 0.82 to 1.21). In contrast to these studies, a Center for International Blood and Marrow Transplant Research (CIBMTR) registry study suggests that early relapse is not invariably associated with poor outcomes because three-year PFS was 44% (95% CI = 38% to 50%) for patients with chemosensitive relapse, even within 12 months of initial treatment (50). Overall, these studies suggest an ongoing role for autoHCT at relapse, but for an arguably smaller patient population. An important, as yet unanswered, question is whether or not biologic and/or targeted agents can improve upon the 60% response rate achieved by current standard salvage regimens. In addition, as the major reason for treatment failure post-transplant is disease progression, consolidation and/or maintenance with immunotherapy approaches or checkpoint inhibitors may be worth exploring.

There is a striking paucity of data regarding prognosis or the impact of disease biology at relapse, perhaps reflecting the lack of banked tissue biopsies in this setting. Some groups have evaluated prognosis in the context of autoHCT, for example, second-line IPI and PET negativity impact outcomes following autoHCT. A recent evaluation of 129 patients with relapsed DLBCL found that the only factor at relapse impacting outcomes after autologous stem cell transplant was a metabolic response to salvage chemotherapy reflected by a Deauville score of 1–3 (51). Importantly, this study found that cell of origin did not impact outcomes. Outside of transplant settings, there are limited reports evaluating prognosis and survival. The only prognostic variable that may impact trial design is the time to relapse from initial treatment. A large registry study of almost 1600 DLBCL patients found that patients remaining in remission at 24 months had excellent outcomes equivalent to age-matched controls (38). Others have shown that patients progressing less than one year after autoHCT have an inferior outcome compared with those who relapse beyond one year (OS = 8 months, 95% CI = 5 to 13 months, vs OS = 27 months, 95% CI = 4 months to not achieved) (52). While prognostic information is lacking, several groups have shown predictive value of cell of origin for specific regimens (ie, R-DHAP) or agents (ie, ibrutinib and lenalidomide). These observations of differential activity need to be expanded and validated.

Patients ineligible for autoHCT or relapsing despite autoHCT have a dismal outcome. A population-based study from Canada reported a median OS of less than four months for 326 relapsed/refractory DLBCL patients unable to undergo autoHCT (53). Despite a growing number of biologic targets and a large number of phase I and phase II trials, no single agent or regimen results in long-term disease control, and there are no current phase III trials. Thus the management of relapsed and refractory DLBCL remains a dire unmet need.

Clinical Trial Questions: Areas of Controversy and Consensus

1. Definition of High Risk in the Frontline Setting

This is a controversial area because it involves both clinical and molecular risks, without clear scientific distinctions. High risk can be defined by molecular subtype (GCB vs ABC), specific genetic aberrations (*MYC* and *BCL2* translocations, P53 dysfunction), viral etiology (EBV, HHV8, HIV), phenotypic distinctions (MYC, BCL2, BCL6, KI-67 protein expression), and/or clinical parameters (IPI and other clinical features). Overarching risks such as advanced age and CNS spread (extranodal disease) add complexity to the definition of high risk.

The one exception to the controversy is double-hit lymphomas, where there was uniform agreement regarding poor outcomes; however, the low frequency of true double-hit

lymphoma and the rapid disease course make this a more challenging group to study. Although dual expression of MYC and BCL2 likely includes heterogeneous entities, there was majority consensus that given their poor outcome with standard treatment, this subgroup would be appropriate for testing new therapies that target either MYC and BCL2 or the pathogenetic lesions underlying their overexpression.

2. Inclusion of Molecular Diagnostics

Trials in the frontline setting should include state-of-the-art companion diagnostics to define molecular subsets of DLBCL. Currently, Lymph2Cx utilizing the Nanostring platform is furthest along in development and has a turnaround time of two to five days, which could allow for subtype designation prior to treatment initiation. The other well established high-risk group with short biomarker time to results is DHL, as defined by fluorescence in situ hybridization (FISH) and dual expression of MYC and BCL2. Importantly, DHL and dual expressers can be targeted by novel agents including BCL2 inhibitors and agents modifying MYC expression and function. Other equally efficient approaches utilizing different platforms may emerge, but they will need to be adequately validated.

Given the various definitions of high-risk disease, there remains controversy over whether a frontline DLBCL trial should be biomarker driven or be broadly inclusive. It may be more informative to include wide eligibility, but to stratify patients based on known high-risk features (ie, cell of origin, dual protein expression) and to appropriately power the arms to determine differential treatment effects. In addition, there is further need to standardize hematopathology and diagnostic interpretation of double-hit and dual protein expression (eg, establishing thresholds for FISH and immunohistochemical positivity). Importantly, if a trial is assessing an agent known from preclinical and early clinical evaluation to be selectively beneficial in specific molecular or biologic subgroups, the clinical trial should aim to enrich this subgroup.

3. Timing of Initiation of an Investigational Agent/Regimen in a Trial

The requirement for performing molecular diagnostics in a biomarker-driven trial may cause delays in treatment initiation and result in selection bias. There are varied opinions over the timing of the addition of a novel agent to upfront treatment. Most investigators believe that the experimental agent/regimen should preferably begin on the first cycle, whereas others suggest that adding an agent to the second cycle may be a reasonable compromise to allow time for molecular diagnostics without delaying start of treatment. Overall, there was consensus that the use of a prephase such as steroids would be acceptable and should allow for the experimental agents to be introduced with the first cycle.

Trial Design Considerations in the Frontline Setting

End Points

Although response rate (RR) assessed by CT or PET is useful, it is an insufficient surrogate marker for PFS, EFS, or OS. Recently, it was reported that landmarked PFS and EFS, particularly as assessed at 24 months post-therapy, may better predict outcome in terms of overall survival (38). Consequently, at present we recommend time-based or continuous PFS or EFS as a primary end point in randomized phase II and phase III studies.

Additionally, end points are dependent on the molecular subtype and the clinical features of included patients. PFS is an important end point as it reflects the curative potential of the treatment. OS is also important but can be confounded by secondary treatments and unrelated deaths. Complete remission by routine imaging is not considered a strong end point and is not recommended as a primary end point for upfront trials. Given the emerging data regarding limitations of routine surveillance imaging in detecting early relapse, there is a need to develop biomarkers to establish depth of remission and detect early relapse. Currently, promising approaches include detection of circulating tumor DNA, but other platforms may emerge.

Given the large number of novel agents in early development, the relatively small size of molecularly defined treatment cohorts, and the absence of well-validated biomarkers at present, we concluded that the National Clinical Trials Network (NCTN) groups should not focus on phase III trials at this time. Multiple pharmaceutical companies are performing trials of R-CHOP +/- novel agents, which should inform the next generation of phase III studies. The goal of the NCTN should not be to confirm these registration-based trials, but to consider novel study designs such as randomized or adaptive phase II trials with multiple arms, based on molecular and risk-defined subtypes, that will provide leads for future phase III studies.

Biomarkers

DLBCL is a heterogeneous disease with variable clinical outcomes. A number of prognostic factors have been identified in this disease. Although clinical factors including age, performance status, and derivatives like the International Prognostic Index remain a cornerstone of prognostication and may be useful in stratification, novel biomarkers based on tumor biology play an increasingly important role in the selection of therapy. Table 1 is a limited list of biomarkers in DLBCL that currently can be considered in development in clinical trials for this disease.

Trial Designs in Relapsed/Refractory DLBCL Patients

General Considerations and End Points

Relapsed/refractory DLBCL is the ideal setting for rapidly assessing new agents, new combinations, and new biomarkers. Trials of novel agents should be inclusive of all relapsed/refractory patients to help determine if molecular subgroups might have a differential response to selected agents. Appropriate trial design would incorporate a uniform profiling platform at trial entry, treatment arms tailored to the results of the profiling/biomarker tool, multi-arm trials with ability to cross-over at progression, and adaptive designs. An exception to this approach would be if there were a strong preclinical rationale for an agent to be tested in a specific subgroup (eg, highly targeted and selective agents such as BCL2 inhibitors, monoclonal antibodies) to enrich for likely responders. The trial end points should incorporate response rate, duration of response, PFS, and overall survival. We suggest that trials in relapsed/refractory patients not be limited to patients who are “transplant ineligible”

because this limits the ability for less heavily pretreated patients to enter nontransplant trials of novel agents and implies that transplant is the default standard of care, which is a point of some controversy. An unresolved issue is how to manage the confounding effect on PFS and duration of response end points, as inevitably some patients would proceed to autologous stem cell transplantation, which is still considered a preferable option by many investigators.

To rapidly advance the field, a multi-arm trial with one control arm design should be considered, where treatment arms are assigned based on biomarkers (Table 1). If there are not enough promising agents for a multi-arm study, a two-arm study can be considered. Because of the aggressive nature of relapsed disease, response rate is a reasonable primary end point in the relapsed setting, with secondary end points of PFS, EFS, or OS. With novel agents, many patients may benefit clinically but not formally meet the criteria for an objective response. Therefore, routinely including a secondary end point reflecting freedom from disease progression (EFS or PFS) should be strongly encouraged.

Biomarkers

There are no well-validated prognostic biomarkers in the relapsed/refractory setting, and the current lack of biological information hampers the ability to study subgroups. The committee agreed that patients with longer time to relapse have a more favorable outcome, as shown in the CORAL study and in nontransplant studies (38,54). For transplant-eligible patients, second-line IPI is prognostic. COO (ABC vs GCB) is not prognostic in the relapsed setting, although it may be predictive of response to specific agents. A concerted effort to collect tissue at relapse (see below) is urgently needed to address this population with an individualized approach. Trials that require collection of tissue at relapse to develop useful biomarkers are strongly recommended.

Stem Cell Transplantation

The DLBCL committee agreed that autologous stem cell transplantation continues to benefit some patients with relapsed/refractory DLBCL, but the overall percentage of patients benefiting from this approach is diminished in the modern era. Furthermore, the benefit of transplantation following dose-escalated regimens such as ACVBP-R and DA-EPOCH-R remains unproven. However, it is not practical to remove autologous stem cell transplantation from disease management as it remains a potentially curative strategy for some patients with relapsed/refractory DLBCL and will continue to be used by many investigators. Research questions in the transplant setting should incorporate evaluation of minimal residual disease (MRD) when investigating new platforms. PET response could also be an appropriate measure of MRD, but should be an exploratory and not a primary end point. All phases of transplantation (pretransplant salvage, preparative regimens, post-transplant) could be points of entry for study of novel agents. In general, studies evaluating single-agent maintenance therapies post-transplantation as the sole focus of investigation were not deemed to have a worthwhile approach, with the exception of innovative immunotherapy approaches such as CAR-T cells and checkpoint inhibitors.

Summary

In summary, classification of diffuse large B cell lymphoma has evolved to encompass a number of clinical and biologic variants. Although R-CHOP is a substantial improvement over past regimens, a number of high-risk subgroups of patients will not be cured. The focus of trials in the frontline setting should be an improved cure rate based on trials that are powered to test efficacy in these high-risk populations. Given the smaller patient numbers within subgroups and the need to explore a large number of regimens, frontline trials should currently be randomized phase II trials with an EFS or PFS end point. In the relapsed setting, there is no standard of care, particularly for transplant-ineligible patients or those who relapse despite transplant. The focus for this group of patients should be based on repeated biopsies of sufficient size to allow genomic and biologic interrogation and multi-arm studies with either a response-based or survival-based end point. Once this generation of trials is complete, we should be moving beyond R-CHOP and toward more individualized treatment with higher cure rates and effective salvage options.

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