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Rituximab-Based Therapy in Newly Diagnosed Diffuse Large B-Cell Lymphoma Patients: Individualized Risk-Adapted Therapy Approach Using Molecular Subtypes

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Abstract

Rituximab (R) with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) is the current standard of care as first-line treatment for diffuse large B-cell lymphoma (DLBCL), the most common lymphoma subtype. Patients who fail R-CHOP have a poor outcome with relapse or refractory disease resulting in fatality in majority of patients. This review focuses on novel therapies which are currently being assessed as first-line treatment in combination with R-CHOP in patients with DLBCL. Targeted drug development is a possibility with recent developments like gene expression profiling, RNA interference screening, DNA sequencing, identification of new biomarkers and signaling pathways. Newer drugs such as bortezomib, lenalidomide, and ibrutinib are being investigated as first-line therapy in combination with R-CHOP (XR-CHOP) in the activated B-cell (ABC) subtype of DLBCL. Additionally, inhibitors of BCL6, EZH2, and PI3K/Akt/mTOR are being considered for treatment of germinal center B-cell (GCB) subtype of DLBCL in patients with probable survival of less than 5 years. Double- or triple-hit lymphomas and double-expressor lymphomas also have poor prognosis and research to identify effective first-line therapy in these patients remains an unmet need. Presently, individualized approach that includes effective therapeutic combinations with acceptable safety profiles for use in routine practice, especially in patients likely to have poor outcomes such as relapsed/refractory DLBCL remains a distant possibility. Current evidence shows that untreated high risk patients do not have the greater benefit with use of newer drugs compared with R-CHOP. Therefore, R-CHOP remains the first-line treatment for newly diagnosed DLBCL patients.

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Introduction

Diffuse large B-cell lymphoma (DLBCL), the most common lymphoma subtype, constitutes 30-40% of all non-Hodgkin lymphoma (NHL) cases globally [1-3]. Though the median age at diagnosis of DLBCL is > 60 years, patients of all ages may be affected [1, 2].

The disease is usually aggressive and can be fatal, if untreated [4], with median survival of less than a year [5]. However, the prognosis drastically improves with progress of treatment. The 5-year overall survival (OS) rate is 60% with immunochemotherapy [6] and cure is possible in advanced cases as well [7].

Prior to 2000, cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP) chemotherapy was considered as the standard of practice for DLBCL, but the new millennium saw huge advances in the treatment landscape with promising results from rituximab (R)-based regimens. Initial results showed better efficacy (better response, lesser risk of disease progression and longer OS) and similar safety with R-CHOP than CHOP in patients with aggressive B-cell lymphomas and DLBCL [8, 9]. Food and Drug Administration of United States (FDA) [10], European Medical Agency (EMA) [11], and other agencies have approved use of rituximab in the first-line treatment of CD20 positive patients with DLBCL in combination with CHOP or other anthracycline-based chemotherapy regimens. Since 2006, it has been established as a current standard of care in newly diagnosed DLBCL patients and is recommended by European Society of Medical Oncology (ESMO) [12] as well as National Comprehensive Cancer Network (NCCN) [13].

The Groupe d'Etude des Lymphomes de l'Adulte (GELA) study is one of the early phase 3 randomized controlled trials with long-term follow-up results [9]. The significantly better efficacy of R-CHOP versus CHOP (the 10-year progression-free survival (PFS): 36.5% versus 20.1%, and the 10-year OS: 43.5% versus 27.6%) was confirmed over a median follow-up of 10 years [14]. R-CHOP did not substantially increase long-term toxicity compared to CHOP alone [15]. The East-

ern Cooperative Oncology Group (ECOG) 4494/Cancer and Leukemia Group B (CALGB) 9793 (E4494/C9793) phase 3 trial conducted in DLBCL patients ≥ 60 years old showed that rituximab as administered during induction or maintenance phases in addition to CHOP significantly improved FFS [16]. The MabThera International Trial (MInT) Group study was the first to demonstrate improved efficacy and comparable tolerability of R-CHOP-like treatment compared with CHOP-like chemotherapy in young patients (aged 18 - 60 years) with good-prognosis DLBCL [17, 18].

Pivotal studies such as GELA, E4494/C9793, and MInT were followed by various RCTs and observational studies in DLBCL patients across different populations. Overall, there is sufficient evidence in literature, from clinical trials and real-world data, to show that R-CHOP or its variations (like R-miniCHOP), leads to an unequivocal improvement in efficacy (survival) with acceptable tolerability in patients with DLBCL [6, 8, 9, 14-16, 18-22]. R-CHOP has been successfully used to treat different populations and patients of all age groups, including the elderly [9, 14-16] as well as the young adults [18] and is a cost-effective alternative to CHOP [23].

Approximately 40% of patients who fail R-CHOP have a dismal outcome with relapse or refractory disease resulting in fatality in majority of patients [6, 24]. Although different strategies, including intensification of chemotherapy with dose-dense regimens like dose-dense rituximab or dose-adjusted etoposide, prednisone, oncovin (vincristine), cyclophosphamide, and hydroxydaunorubicin (doxorubicin) + R) (DA-EPOCH-R) [25, 26], autologous stem cell transplant (ASCT) consolidation [27], and use of maintenance therapy [28], have been used, the improvement over R-CHOP in patients with high risk of relapse and refractory disease is limited. More therapeutic options to improve outcomes in patients with primary treatment failure or those who are highly likely to fail primary treatment are needed.

Favorable long-term results depend on clinical and/or biological characteristics of the patient and the disease [5]. Thus, optimization of first-line therapy in patients likely to fail R-CHOP and development of better salvage strategies in relapsed and refractory disease remains a key challenge. Furthermore, the results in R-CHOP pre-treated patients, especially in those relapsing early (< 1 year) after first-line treatment are disappointing. Improving current treatment strategies and the development of novel therapeutic approaches remain imperative [29]. The current review aims to discuss major advances in the development of newer drugs which may further improve the efficacy of first-line therapy for DLBCL in various DLBCL subtypes.

Biological/Molecular Understanding of DLBCL

Our understanding of the molecular complexity of DLBCL has evolved in recent years. With the advent of newer techniques, such as gene expression profiling (GEP), ribonucleic acid (RNA) interference screening, and deoxyribonucleic acid (DNA) sequencing, several new signaling pathways have been identified leading to possible therapeutic targets for drug development [30, 31].

DLBCL was earlier considered as a single disease but GEP has identified at least two major distinct DLBCL subtypes based on the cell of origin (COO): an activated B-cell (ABC) and a germinal center B-cell (GCB) [7, 30]. Although histologically undistinguishable, these subtypes can be diagnosed using DNA microarrays and the results are reproducible. There is also evidence of a third subtype, primary mediastinal B-cell lymphoma (PMBL) [5, 30]. Each subtype follows a distinct molecular mechanism and oncogenic signaling pathway and hence, may differ in response to conventional treatment. Chronic active B-cell receptor (BCR) signaling, constitutive myeloid differentiation primary response gene 88 (MYD88) signaling, and subsequent antiapoptotic nuclear factor-kappa B (NF-kappaB) pathway, phosphatidylinositol-3-kinase/serine-threonine kinase (PI3K/Akt/mTOR) pathway, and interferon pathway activation are characteristics of the ABC DLBCL [7, 24, 32]. On the other hand, BCL6 and EZH2 together are extensively studied in the GCB subtype of DLBCL [33].

Various immunohistochemistry (IHC) algorithms are being used at present for identifying DLBCL subtypes based on COO. These IHC algorithms are limited by lack of standardization as well as concordance [34] and GEP is considered as gold standard.

Biomarker-Driven Clinical Trials in DLBCL

With development of COO-based treatment of DLBCL subtypes, COO classifications have become more relevant clinically, particularly for treating ABC DLBCL [7]. However, for implementing an individualized risk-adapted therapy approach, molecular characterization of DLBCL along with the development of relevant biomarkers and targeted drugs is needed.

Biomarkers BCL2, BCL6, Myc and pathways like NF-kappaB and PI3K/Akt/mTOR are mainly being targeted in recent clinical trials. Studies comparing R-CHOP versus CHOP showed that the addition of R to CHOP significantly benefitted BCL2-positive [35, 36], BCL6-negative [37], p21-positive [38], and positive regulatory domain 1 (PRDM1) β-positive [39] patients (Table 1) [35-39].

Further research for DLBCL-related biomarkers is underway. Many clinical trials evaluating efficacy/safety of drugs in DLBCL also aim to assess effect of biomarkers on the outcomes (NCT02530125 [40], NCT02391116 [41], NCT01414855 [42]) or predict outcomes (NCT00450385 [43]) or characterize biomarkers (NCT02530125 [40], NCT01278615 [44]) though majority of these studies are being conducted in those with relapsed or refractory disease. Some clinical trials aim to assess biomarkers in patients with aggressive DLBCL (NCT01287923 [45]). Some retrospective studies also evaluate genes in samples from DLBCL patients to find COO and clinical correlates (NCT01563861 [46]), or understand response to treatment in previously treated patients (NCT00898157 [47]).

Though individualized first-line therapy with biomarker identification remains a distant possibility at present, the COO classification along with identification of major biomarkers and pathways involved have boosted research of suitable drugs based on these characteristics. Emerging first-line therapy op-

Table 1. Clinical Trials of DLBCL Treatment Evaluating Role of Specific Biomarkers

Reference	Biomarker	Study/patient population	Intervention	Main results
Mounier et al, 2003[35]	BCL2	Patients aged between 60-80 years with DLBCL Stage II or higher	R-CHOP versus CHOP	Rituximab can prevent chemotherapy failure in patients with BCL2 protein overexpression.
Wilson et al, 2008 [36]	BCL2	Patients aged ≥ 18 years with untreated DLBCL of stage II or higher	DA-EPOCH-R versus DA-EPOCH	Addition of rituximab only benefited patients with BCL2 positive tumors.
Winter et al, 2006 [37]	BCL6	DLBCL patients aged ≥ 60 years included in E4494, C9793, S4494 trial and with adequate pathology sample	R-CHOP versus CHOP	The addition of R to CHOP reduced treatment failures and death in BCL6-DLBCL cases only. BCL2 protein expression was not predictive of outcome in both groups.
Wilson et al, 2008 [36]	BCL6	Patients aged ≥ 18 years with untreated DLBCL of stage II or higher	DA-EPOCH-R versus DA-EPOCH	BCL6 expression was associated with higher PFS.
Winter et al, 2010 [38]	p21	DLBCL patients aged ≥ 18 years included in E4494 trial and with adequate pathology sample trial	R-CHOP versus CHOP	p21 expression was a favorable independent prognostic indicator in patients treated with R-CHOP but not with CHOP alone. The addition of R to CHOP selectively benefited the p21 positive patients.
Liu et al, 2007 [39]	PRDM1: PRDM1α and PRDM1β	DLBCL patients aged ≥ 18 years with adequate pathology sample	R-CHOP versus CHOP	In the non-GCB patients, PRDM1β gene expression was correlated with short survival time in CHOP versus R-CHOP.

BCL: B-cell leukemia/lymphoma; CHOP: cyclophosphamide, doxorubicin, vincristine and prednisone; DA-EPOCH: dose-adjusted etoposide, prednisone, oncovin (vincristine), cyclophosphamide, and hydroxydaunorubicin (doxorubicin); DLBCL: diffuse large B-cell lymphoma; GCB: germinal center B-cell; PRDM1: positive regulatory domain 1; R: rituximab.

tions for different DLBCL subtypes such as the addition of novel agents (X) to R-CHOP (XR-CHOP combinations) that target specific oncogenic pathways are discussed below. We have focused on studies for first-line DLBCL treatment with published results comparing XR-CHOP with R-CHOP or R-CHOP-like therapies.

Treatment Based on DLBCL Subtypes

ABC or non-GCB subtype of DLBCL

ABC, also referred to as non-GCB subtype of DLBCL, is characterized by constitutive activation of the NF-kappaB pathway, which can negatively impact the chemotherapeutic effect [48]. Patients with this subtype have worse outcomes as compared to those with the GCB subtype even after standard R-CHOP treatment [24, 49]. Therefore, majority of recent and ongoing clinical trials investigating the use of XR-CHOP as front-line treatment specifically target the ABC subtype of DLBCL.

Bortezomib plus R-CHOP (VR-CHOP)

Inhibition of NF-kappaB pathway can sensitize DLBCL-subtype ABC cells to chemotherapy and improve outcomes. Bortezomib, a proteasome inhibitor, can inhibit NF-kappaB and thus enhance the activity of chemotherapy in the ABC, but not GCB, subtype of DLBCL [50].

The LYM-2034, a phase 2 study that substituted bortezomib for vincristine (VR-CAP) in first-line R-CHOP therapy of previously untreated non-GCB DLBCL patients [51], showed that response rates or long-term outcomes with VR-CAP versus R-CHOP were not significantly different. Similarly, PYRAMID, a prospective open-label, randomized, phase 2 study that evaluated the efficacy and safety of first-line R-CHOP versus VR-CHOP in previously untreated non-GCB DLBCL, demonstrated no significant differences in efficacy with R-CHOP versus VR-CHOP [52]. However, while interpreting the results of LYM-2034 and PYRAMID phase 2 trials, it must be considered that the non-GCB DLBCL patients in these trials were identified based on IHC (Hans method) [34], and not GEP, which is the standard reproducible method for COO assignment.

Preliminary results of a randomized, double-blind, phase 3 trial (REMoDL-B trial) comparing VR-CHOP versus R-CHOP in newly diagnosed ABC subtype DLBCL defined by central GEP assay (cDNA-mediated annealing, selection, extension and ligation assay, DASL®) showed similar PFS in both ABC and GCB subtype patients (2-year PFS: 71% [53]). Results of the 30-month follow-up of the REMoDL-B trial are awaited. Another study assessing efficacy and safety of bortezomib as maintenance therapy after R-CHOP treatment is currently recruiting high risk non-GCB DLBCL patients (NCT01965977).

Lenalidomide plus R-CHOP

Lenalidomide, an immunomodulatory drug, acts on the NF-kappaB pathway and alters the tumor microenvironment and potentiates the activity of T and natural-killer cells. It is highly efficacious when combined with monoclonal antibodies like

rituximab [54, 55]. Currently, clinical trials are investigating its role in relapsed or recurrent DLBCL and as first-line therapy in ABC DLBCL.

Combination of lenalidomide with R-CHOP (NCT 00670358) showed no difference in 24-month PFS or OS in patients on the basis of IHC-identified non-GCB or GCB subtype (60% versus 59% (P = 0.83) and 83% versus 75% (P = 0.61), respectively) in a recent phase 2 clinical trial [55]. These results suggest that lenalidomide concomitantly given with R-CHOP can attenuate the negative prognosis in non-GCB phenotype.

A phase 3 trial (REMARC study, NCT01122472) evaluated the benefit of lenalidomide maintenance after response to R-CHOP in patients aged 60 - 80 years including those with untreated DLBCL [56]. In patients responding to R-CHOP, lenalidomide maintenance for 2 years significantly improved PFS (hazard ratio (HR): 0.71, 95% CI: 0.54 - 0.93; P = 0.0135) without significant effect on OS. The results of COO analysis are awaited.

Recently, a randomized, double-blind, phase 3 trial (ROBUST, NCT02285062) comparing lenalidomide plus R-CHOP versus placebo plus R-CHOP in newly diagnosed ABC subtype DLBCL, defined by the GEP assay, has been initiated, with PFS as the primary endpoint [57]. Recruitment is ongoing for a study (NCT02128061) that will compare OS of CD20-positive DLBCL patients ≥ 80 years old with R-miniCHOP plus lenalidomide (subcutaneous rituximab-miniCHOP plus lenalidomide) versus R-miniCHOP. The OS among GCB and non-GCB patients will be compared.

Ibrutinib plus R-CHOP (ibru-R-CHOP)

Constitutively activated signaling through BCR and its associated protein tyrosine kinases (such as BTK) play a crucial role in the development and survival of malignant B cells, including pathogenesis of the ABC subtype of DLBCL. Ibrutinib is a small molecule that permanently binds to BTK and acts as a kinase inhibitor [58]. Majority of trials using ibrutinib are being conducted in relapsed or refractory DLBCL patients.

A randomized, double-blind, phase 3 trial (PHOENIX, NCT01855750) comparing ibru-R-CHOP with placebo plus R-CHOP in newly diagnosed non-GCB subtype DLBCL, with event-free survival (EFS) as the primary endpoint, has completed patient recruitment and is currently ongoing [59].

Histopathologically confirmed treatment-naive non-GCB DLBCL patients are being recruited in a clinical trial assessing the efficacy and safety of the combination of rituximab, lenalidomide, and ibrutinib, when given alone and with standard chemotherapy (EPOCH). The primary endpoint of this trial is overall response rate (ORR) (NCT02636322 [60]). Efficacy and safety profiles of XR-CHOP in ABC DLBCL in pivotal clinical trials are summarized in Table 2 [32, 51, 52, 55, 61].

GCB subtype of DLBCL

Though GCB subtype has better prognosis than ABC subtype of DLBCL, approximately 30-40% of patients with GCB subtype do not survive beyond 5 years [62, 63].

BCL6 inhibitors and topoisomerase II inhibitors

The transcriptional repressor BCL6 can be used as a selective target as it is highly expressed in the GCB subtype but rarely in the ABC subtype of DLBCL and thus can be used as a selective target in GCB DLBCL [64]. BCL6 represses many target genes involved in lymphocyte activation, apoptosis, and the DNA damage response. Chromosomal translocations/mutations can lead to constitutive expression of BCL6 resulting in tumor proliferation and treatment failure. Preclinical studies with BCL6 inhibitors have shown promising results [65, 66]. Furthermore, topoisomerase II inhibition with drugs like etoposide also results in downregulation of BCL6 expression, and may further prolong survival in GCB subtype of DLBCL patients [48]. Indeed, the DA-EPOCH-R regimen that includes two topoisomerase II inhibitors (etoposide and doxorubicin) has demonstrated very high efficacy in patients with GCB DLBCL with 5-year PFS and EFS as high as 100% and 94%, respectively (significantly higher than non-GCB DLBCL, P = 0.008 for both) [67]. However, a phase 3 RCT of R-CHOP versus DA-EPOCH-R (262 patients registered in each arm) demonstrated no difference in EFS (HR: 1.02, P = 0.89 at a median follow-up of 4.9 years) or OS (HR: 1.19, P = 0.40 at median 5.0 years). DA-EPOCH-R showed increased toxicity though grade 5 toxicity was not increased. Analyses of results of GCB versus ABC subtypes are ongoing [25].

EZH2 inhibitors

More than 20% patients with GCB subtype of DLBCL display mutations of the histone methyltransferase EZH2 that plays a role in the development of a number of cancers, including DLBCL [68, 69]. BCL6 and EZH2 together accelerate lymphomagenesis in the GCB subtype of DLBCL [33]. With encouraging pre-clinical results, EZH2 inhibitors are currently being investigated in phase 1 and 2 clinical trials [70-73] and have the potential for greater tumor specificity and lower generalized toxicity. The FDA has granted fast track designation for the investigation of tazemetostat, a first-in-class EZH2 inhibitor, for the treatment of patients with relapsed or refractory DLBCL whose tumors carry an EZH2 activating mutation [74]. Phase 1/2 studies of tazemetostat as a monotherapy are currently ongoing. A phase 1b/2 trial of tazemetostat in combination with R-CHOP as a first-line treatment for DLBCL patients is ongoing [75]. However, specific studies involving first-line therapy in GCB subtype patients have not yet commenced.

PI3K/Akt/mTOR inhibitors

The PI3K/Akt/mTOR pathway plays a critical role in cell survival and proliferation [76]. Abnormally overexpressed phosphorylated AKT (p-AKT) may have poor prognostic impact in

Table 2. Efficacy and Safety of XR-CHOP in ABC DLBCL in Clinical Trials

Trial	Intervention	Patients	Efficacy	Safety
Bortezomib				
LYM-2034, NCT01040871 [51]	VR-CAP versus R-CHOP	Previously untreated non-GCB DLBCL patients (identified by Hans method)	No significant differences between VR-CAP and R-CHOP. VR-CAP did not improve efficacy versus R-CHOP in non-GCB DLBCL: CR rate (64.5%, 66.2%; OR, 0.91; P = 0.80); ORR, (93.4%, 98.6%; OR, 0.21; P = 0.11); PFS: HR, 1.12; P = 0.76); OS: HR, 0.89; P = 0.75).	Rates of AEs were similar with VR-CAP and R-CHOP: AEs with grade ≥ 3 (88%, 89%), serious AEs (38%, 34%), discontinuations due to AEs (7%, 3%), and deaths due to AEs (2%, 5%). Grade ≥ 3 peripheral neuropathy rates were 6% and 3%, respectively.
PYRAMID, NCT00931918 [52]	R-CHOP versus VR-CHOP	Non-GCB DLBCL patients (identified by Hans method)	VR-CHOP did not have significant efficacy advantage over R-CHOP in previously untreated non-GCB DLBCL patients. Two-year PFS (R-CHOP versus VR-CHOP) was 77% versus 82% (HR: 0.77; 90% CI: 0.45-1.30; P = 0.70). At data cut-off, 15% and 11% of patients in the R-CHOP and VR-CHOP arms had died (HR: 0.65; 90% CI: 0.32 - 1.29); 2-year OS rates were 80% versus 82%. In 86 R-CHOP and 90 VR-CHOP response-evaluable patients, ORRs were 98% versus 92% and CRs were 52% versus 54%.	In the R-CHOP (n = 100) versus VR-CHOP (n = 101) safety populations, the proportion of AEs was as follows: Grade ≥ 3 AE: 71% versus 79%; serious AEs: 31% versus 34%; drug-related AEs with grade ≥ 3: 55% versus 68%, the most common being neutropenia (34% versus 28%) and thrombocytopenia (8% versus 20%). Peripheral neuropathies grade ≥ 3 were 1% versus 5%.
REMoDL-B, NCT01324596 [32, 61]	R-CHOP versus VR-CHOP	Newly diagnosed ABC subtype DLBCL defined by central GEP assay	No difference in PFS of ABC versus GCB subtype patients (2-year PFS: 71%)	
Lenalidomide				
NCT00670358 [55]	Lenalidomide plus R-CHOP	Adults with newly diagnosed untreated stages II-IV CD20-positive DLBCL. Non-GCB versus GCB analyses were conducted.	Of 64 enrolled patients, 60 were evaluable for response. The ORR was 98% with 80% CR. EFS and OS at 24 months were 59% (95% CI: 48-74%) and 78% (95% CI: 68-90%), respectively. In contemporary cohort of non-GCB versus GCB DLBCL patients treated with R-CHOP, 24-month PFS and OS were 28% versus 64% (P < 0.001) and 46% versus 78% (P < 0.001), respectively. Contrastingly, there was no difference in 24-month PFS or OS for the study patients on the basis of non-GCB and GCB subtype (60% versus 59% (P = 0.83) and 83% versus 75% (P = 0.61) at 2 years, respectively).	Following toxicities were seen in the patients: grade ≥ 3 non-hematologic toxicities, 25%; grade ≥ 3 hematologic toxicities, 94%; grade 4 hematologic toxicities, 77%; grade 3 neutropenia, 13%; grade 4 neutropenia, 75%; grade 3 febrile neutropenia, 9%; grade 3 thrombocytopenia, 27%; grade 4 thrombocytopenia, 17%; and thrombocytopenia leading to bleeding complications, 1.6%.

ABC: activated B-cell; AE: adverse event; CHOP: cyclophosphamide, doxorubicin, vincristine and prednisone; CR: complete response; DLBCL: diffuse large B-cell lymphoma; EFS: event-free survival; GCB: germinal center B-cell; HR: hazard ratio; OR: odds ratio; ORR: overall response rate; OS: overall survival; PFS: progression-free survival; R: rituximab; V: bortezomib; VR-CAP: bortezomib, rituximab, cyclophosphamide, doxorubicin, and prednisone.

DLBCL [32, 77, 78]. In a clinical trial of 262 DLBCL patients with both GCB and non-GCB subtypes, high p-AKT group had higher proportion of advanced stage disease, two or more extranodal involvement, lactic dehydrogenase elevation, higher international prognostic index (IPI) risk groups, and the presence of B symptoms [32]. The disease deteriorated at a faster rate in high p-AKT group versus the low p-AKT group (median OS, 115.0 months versus not reached, P = 0.004; median PFS, 25.5 versus 105.8 months, P = 0.019). For some cases of GCB subtype of DLBCL involving this signaling pathway, inhibition of this cascade is an obvious goal. Various PI3K inhibitors are being evaluated in ongoing clinical trials [79-81] and may lead to development of effective targeted therapies in the GCB subtype of DLBCL with activated PI3K/Akt/mTOR

pathways.

Other newer treatment options

Obinutuzumab

Obinutuzumab (GA101, G) is a glycoengineered, type II anti-CD20 monoclonal antibody with greater direct cell death induction and antibody-dependent cellular cytotoxicity and phagocytosis activity than rituximab. The GOYA study assessed G-CHOP versus R-CHOP (1:1) as first-line treatment in 1,418 CD20-positive DLBCL patients [82]. The results showed that G-CHOP had comparable safety profile but did not significantly improve investigator-assessed PFS compared with R-CHOP in these patients. Results of subgroup analyses are awaited.

Polatuzumab vedotin

Polatuzumab vedotin is an anti-CD79b antibody-drug conjugate. Preliminary results of a phase 1b dose-escalation clinical trial of polatuzumab vedotin combined with R-CHOP demonstrated an acceptable safety profile in previously untreated DLBCL patients. Of the 10 DLBCL patients enrolled, seven were assessed for response at end of treatment: five CR (one at 1.0 mg/kg, three at 1.4 mg/kg, and one at 1.8 mg/kg), one partial response (PR, at 1.0 mg/kg), and one unevaluable (at 1.8 mg/kg) [83].

Brentuximab vedotin (BV)

BV is an anti-CD30 monoclonal antibody linked to the microtubule-disrupting agent monomethyl auristatin E (MMAE). In a phase 2 clinical trial, BV (1.2 or 1.8 mg/kg) was administered concomitantly with R-CHOP as first-line therapy in DLBCL patients and showed a manageable safety profile [84, 85]. The interim results showed high CR (60% overall) with estimated 1-year PFS of 82% (95% CI: 58-93%) [85].

High risk DLBCL

Double-hit lymphomas (DHLs), triple-hit lymphomas (THLs), and double-expressor lymphomas (DELs) are aggressive subtypes of DLBCL [24]. DHLs are seen in approximately 5-10% of DLBCL patients and usually involve rearrangement of *Myc* oncogene with BCL2 (or sometimes BCL6) protein [24, 86, 87]. Using immunophenotypical data from larger studies on DHL, Aukema et al found that coexpression of CD10, BCL6, BCL2, and a high Ki67 proliferation index might be used to select potential DHLs in morphologically diagnosed DLBCL, though it may not be specific [88]. However, a histopathological study of 492 mature aggressive B-cell neoplasms concluded that the proliferation fraction by Ki67 immunostaining is not useful to prescreen B-cell lymphomas as DHL or THL [89]. DEL overexpress *Myc* and *BCL2/BCL6* but typically without rearrangements of these genes [24, 87], though cut-off of double expression is not universally defined so far. THL is associated with rearrangement of *Myc*, *BCL2* and also *BCL6* [24]. These tumors have poor prognosis with current therapy and represent the highest unmet clinical need in lymphoma management. Further, the oncogene rearrangement patterns in these tumors may have prognostic impact on outcomes [90], with the worst prognosis in THL.

DHL is mostly like the GCB subtype [24] and usually responsible for the poorer outcomes in GCB tumors otherwise known to have good prognosis. On the other hand, DEL mostly belongs to the ABC subtype [24] and may be responsible for the prognostic difference between ABC and GCB DLBCL [91].

The median OS in DHL is usually less than 1 year [92]. A retrospective study of 129 cases of DHL demonstrated that R-EPOCH regimen resulted in better EFS compared to standard R-CHOP or rituximab, hyperfractionated cyclophosphamide, vincristine, doxorubicin, dexamethasone, alternating with cytarabine plus methotrexate (R-HyperCVAD/MA), with 2-year EFS rates of 67%, 25%, and 32%, respectively [93]. A recent meta-analysis synthesized data from 11 studies examining 394 DHL patients in first-line setting [94]. HRs of dose-escalated treatments versus R-CHOP were estimated using a Weibull proportional hazards model within a Bayesian meta-analysis framework. The relative risk of a progression was reduced by 34% with first-line R-EPOCH versus with R-CHOP ($P = 0.032$); however, OS was not significantly different.

A phase 2 study assessing the efficacy and safety of metformin in combination with DA-EPOCH-R for previously untreated double-hit DLBCL is currently recruiting participants (NCT02815397 [95]). Another phase 2 trial is evaluating if ibrutinib improves disease-free survival in DHL patients post-ASCT (NCT02272686 [96]).

DHL usually involves rearrangement of *Myc* oncogene with BCL2; Navitoclax (ABT263) and Venetoclax (ABT199), both BCL2 inhibitors [97-100] and Alisertib, a Myc-targeting aurora A kinase inhibitor [101, 102] have shown promise in preclinical and clinical studies.

Inhibition of the bromodomain and extraterminal (BET) family of proteins leads to suppression of c-Myc expression. BRD4, a member of the BET family increases expression of oncogenes such as *c-Myc* leading to malignant transformation. JQ1 is a small molecule that inhibits BRD4 and other bromodomain proteins. Targeting BRD proteins with drugs such as JQ1 in combination with chemoimmunotherapy or other novel agents may improve outcomes in DHL, THL, and DEL [103].

Future of Biomarker-Driven Clinical Trials in DLBCL

The success of biomarkers and pathway-based therapy is dependent on wide availability of accurate, reproducible and affordable clinical assays to identify and characterize gene mutations activating oncogenic pathways in a timely manner. Furthermore, it is important to remember that lymphomagenesis is not understood by the study of genetics alone. In order to obtain a comprehensive understanding of tumor progression

and response to therapy, the integration of genomic data with proteomics and metabolomics coupled with functional studies defined by a systems biology approach is critical.

Rationally designed biomarker-driven clinical trials with pre-selected patient populations with adequate power are required to establish the role of novel targeted drugs for personalized therapy for DLBCL. This development of new targeted cancer drugs will have to be accompanied by discovery and validation of corresponding biomarker diagnostics. In the near future, this may translate into individualized pathway-based therapy approach in clinical setting resulting in higher response rates and durable remissions in DLBCL patients.

Cost of identifying biomarkers, reliability and validity of biomarker assays, feasibility of drug development against specific biomarkers wherein the drugs have higher efficacy and acceptable tolerability, combined with high heterogeneity of DLBCL form barriers to personalized therapy. At population level, cost-effectiveness analysis of personalized medicine versus first-line R-CHOP for all DLBCL patients and second-line treatment for relapse/refractory (with biomarker identification) may be needed.

Conclusions

R-CHOP is currently first-line treatment for majority of newly diagnosed DLBCL patients. Deeper understanding of the pathophysiology and molecular basis of DLBCL has led to the development of new drugs (X), which in combination with R-CHOP, are currently being assessed as first-line treatment. The treatment landscape may change and prognosis of DLBCL may improve further if superior efficacy and acceptable safety of XR-CHOP are established in larger clinical trials.

An individualized approach in DLBCL requires identification of mutations/translocations that lead to tumor proliferation and progression of disease and cause drug resistance in patients. Translational research is the need of the hour for effective therapeutic combinations with acceptable safety profiles that can be used in routine practice to provide individualized approach, especially to patients likely to have poor outcomes.

Presently, the evidence from numerous clinical trials with emerging new drugs targeted for high risk untreated DLBCL patients do not demonstrate superior benefit than R-CHOP. Therefore, R-CHOP still remains the standard of care for DLBCL patients in first-line setting.

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Lindong Li and Yiqun Zhou are employees of Shanghai Roche Pharmaceuticals Ltd, China. Lei Fan and Jianyong Li declare that no competing or financial interests exist.

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