Aggressive B-cell lymphoma: chasing the target

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ABSTRACT

One of the first achievements of molecular biology in lymphoma science was a discovery of cell of origin (COO) classification around 20 years ago with defining activated B-cell like (ABC) and germinal center B-cell like subtypes of diffuse large B-cell lymphoma (DLBCL) with the use of gene expression profiling. These categories were considered important as seemed to present different biology, response to treatment, and prognosis. Immunochemotherapy R-CHOP21 has been a standard of care for 2 decades, and it results in long-term disease-free survival or cure of 60% of patients with DLBCL but efficacy in an individual patient depends on age and other International Prognostic Index clinical risk factors and is within a range of 30% to more than 90%. Clinical attempts to enhance activity of immunochemotherapy in high-risk DLBCL like ABC or others included adding targeted agents to the R-CHOP backbone: bortezomib, lenalidomide, ibrutinib. Unfortunately, randomized clinical trials did not confirm the expected benefit. Recently, advanced molecular techniques were used to classify B-cell lymphomas beyond COO or MYC alterations and correlated with clinical outcome as illustrated by 2 recently published influential studies from the National Institutes of Health and from Dana Farber Cancer Center, USA. Advanced molecular pathogenesis descriptions of DLBCL provide a framework for actionable classifications that should be used for designing future clinical trials and hopefully bring success to treatment of high-risk aggressive lymphoma.

Current classification of lymphoid neoplasms updated in 2016[1] identifies 22 clinical and pathological disease entities of B-cell origin that are clinically aggressive. They constitute around one-quarter of the new cases of the lymphoid neoplasms,[2] including chronic lymphocytic leukemia, Hodgkin's lymphoma and plasma cell myeloma. The most frequent is diffuse large B-cell lymphoma-not otherwise specified (DLBCL-NOS), and treatment recommendations for aggressive lymphoma are based on data derived from this entity and extrapolated to other disease subtypes except for a few that were specifically investigated including primary central nervous system lymphoma, primary mediastinal large B-cell lymphoma, and Burkitt lymphoma. New entities of particular interest are defined based on accumulated genetic and clinical data justifying their distinction: high-grade B-cell lymphoma (HGBL) with *MYC* and *BCL2* and/or *BCL6* rearrangement,[3 4] and HGBL-NOS, as well as DLBCL-NOS subtypes based on cell of origin (COO): germinal center B-cell like (GCB) and activated B-cell like (ABC). Growing clinical evidence suggests a need for individualized approach to these entities.[1 3 4]

Immunochemotherapy R-CHOP21 has been a standard of care for 2 decades, and it results in long-term disease-free survival or cure of 60% of patients with DLBCL but efficacy in an individual patient depends on age and other International Prognostic Index (IPI) clinical risk factors and is within a range of 30% to more than 90%. National Comprehensive Cancer Network and British Columbia Cancer Agency recently validated the prognostic value of the IPI in patients with DLBCL treated with R-CHOP in 2000–2010 period.[5] The prognostic value of all 5 factors including age, performance score, disease stage, elevation of lactate dehydrogenase (LDH), and extranodal involvement was confirmed and age and LDH level were subdivided into ranges to account for a continuous negative influence of these variables on survival. In addition, confirmed negative prognostic influence of particular extranodal sites (E) including bone marrow, central nervous system (CNS), gastrointestinal tract and liver, and lung but not the E number itself.

One of the first achievements of molecular biology in lymphoma science was a discovery of COO classification around 20 years ago with defining ABC and GCB subtypes with the use of gene expression profiling (GEP). These categories were considered important as seemed to present different biology, response to treatment, and prognosis. Given that GEP is not generally available for routine diagnostics, it is acceptable to use one of several immunohistochemical (IHC) algorithms, mostly Hans algorithm, for discriminating GCB and non-GCB subtypes: GCB=CD10+ or CD10− and BCL6+; non-GCB=CD10 and BCL6− or BCL6+ and MUM-1+. Accuracy of the IHC methods is however only around 80%.

Some retrospective data indicated worse outcome of patients with ABC/non-GCB DLBCL treated with R-CHOP compared with patients with GCB lymphoma.

ABC-DLBCL subtype is believed to originate from the B-cell that underwent germinal center reaction and is committed to plasmablast differentiation.[1] ABC lymphomas demonstrate

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increased activity of NF-κB, genetic alterations of NF-κB modifiers and B-cell receptor (BCR) signaling pathway elements, as well as disturbed terminal differentiation.

GCB lymphomas likely originate from the light zone of the germinal center, may have alterations of the chromatin-modifying enzymes, PI3K signaling disturbances, and BCL2 structural variants.

Data on predominant molecular alterations and deteriorated response to treatment of ABC compared with GCB lymphoma were the major indications for prospective clinical trials of agents targeted to these alterations.[6] There were a number of reasons to believe that adding proteasome inhibitor bortezomib, which is able to inhibit nuclear translocation and transcriptional activity of NF-κB, to R-CHOP backbone, will likely improve outcome in ABC lymphoma subtype.

A randomized phase II study PYRAMID[7] involving 206 patients with a diagnosis of non-GCB DLBCL established by the use of Hans algorithm compared R-CHOP with VR-CHOP (bortezomib added at 1.3 mg/m^2 intravenous days 1 and 4) failed to show statistically significant improvement of progression-free survival (PFS). In addition, the outcome in a reference arm R-CHOP was markedly better than assumed in this patient population (2-year PFS 77.6% after R-CHOP and 82.0% after VR-CHOP).

A randomized phase III REMoDL-B study[8] involved 1128 previously untreated patients with DLBCL in need of systemic therapy. All patients received 1 initial cycle of R-CHOP and diagnostic tissue sample was submitted to central pathology review with whole genome expression analysis for determining of the COO and classifying the case as either ABC, GCB, or unclassifiable DLBCL.

Patients were randomized 1:1 to R-CHOP or RB-CHOP arm (added bortezomib at 1.3 mg/m^2 intravenous or 1.6 mg/m^2 subcutaneous days 1 and 8 of cycles 2–6) and stratified by the IPI risk factors and COO subtype. If the quality or quantity of RNA in diagnostic sample was suboptimal (14.7% of cases), the patient was given standard treatment with R-CHOP. It was the first major study in patients with DLBCL using real-time molecular characterization for prospective screening, stratifying, randomization, and final analysis of data in biologically defined patient subgroups. However, adding bortezomib did not improve PFS. Contrary to expectations, bortezomib proved not to be an effective inhibitor of NF-κB pathway in ABC-DLBCL. Even in cases with identified somatic mutations of genes related to NF-κB activation like *CARD11*, *CD79A/B*, *MYD88*, *TNFAIP3*, *TNFRSF11A*, PFS was identical in both study arms. On the other hand, explorative subgroup analysis suggests that proteasome inhibitor may positively influence the outcome in other high-risk subsets of DLBCL like *double hit* and *double expressor* what needs to be addressed by appropriate clinical study. Design of the REMoDL-B study is a useful model for future investigations of new targeted agents for patients with DLBCL. Utility of R-CHOP regimen as a backbone for new protocols was also confirmed by this study.

Another rational approach to improving activity of immunochemotherapy was developing of more active anti-CD20 antibody. Obinutuzumab is a type II antibody with a glycosyl moiety engineered by means of fructose deletion that demonstrates increased ability to induce antibody-dependent cellular cytotoxicity and lysosome-dependent cell death with attenuated activation of complement-dependent cytotoxicity.

A recently published randomized study GOYA[9] in patients with advanced DLBCL with 2 or more IPI risk factors and/or presence of bulky disease directly comparing PFS of patients treated with obinutuzumab or rituximab both combined with CHOP showed no difference: 3-year PFS of 70% and 67% for G-CHOP and R-CHOP, respectively.

A concept of a prolonged 96 hours' continuous infusion of cytotoxic agents with a dose adjustment to the degree of hematologic toxicity had been explored for decades at the *National Cancer Institute*, USA, with a variable success and recently considered potentially active in high-risk aggressive lymphoma did not prove significantly superior to R-CHOP in recently published randomized study[10] with 2-year PFS of 78.9% and 75.5% for DA-EPOCH-R and R-CHOP, respectively, and with markedly increased toxicity of the infusional regimen including infection, neutropenic fever, mucositis, and neuropathy.

Another promise was brought with Bruton's tyrosine kinase inhibitor ibrutinib suppressing BCR signaling that was shown preferentially active against ABC-DLBCL in monotherapy in a phase I/II study[11] in recurrent/refractory disease (overall response rate 37%) as well as safe in a combination with R-CHOP in a phase I study[12] for previously untreated patients. However, a double-blind, randomized study PHOENIX[13] designed to compare event-free survival (EFS) in patients with DLBCL of non-GCB subtype centrally determined by Hans-based immunohistochemistry and IPI score of 1 or more treated with R-CHOP plus ibrutinib or R-CHOP plus placebo did not show expected improvement with ibrutinib. Interestingly, statistically significant interaction between treatment and age was found. In patients younger than 60, ibrutinib significantly improved EFS, PFS, and overall survival (OS) (HR 0.579, 0.556, and 0.330, respectively) and increased incidence of serious adverse events (35.7% vs 28.6%). In patients aged 60 years or older, adding ibrutinib to R-CHOP decreased EFS, PFS, and OS, increased incidence of serious adverse events (63.4% vs 38.2%), and decreased treatment compliance. The authors conclude that the study did not meet its primary endpoint in the intent-to-treat (ie, non-GCB) or ABC patients (as determined by the retrospective GEP analysis) but the interaction of age with treatment needs further investigation.

Immunomodulatory agent lenalidomide has been tested in several B-cell lymphoma types based on mechanistic rationale including reduction of interferon regulating factor-4 needed for plasmablastic differentiation and cell survival as well as derepression of interleukin-2 synthesis. In addition, some phase II data suggested that lenalidomide may reverse the negative prognostic impact of the ABC phenotype.

A randomized phase III study ROBUST[14] included untreated patients with ABC DLBCL (CD20+) subtype prospectively determined with the use of NanoString Lymphoma Subtyping test based on gene expression analysis technique Lymph2Cx (Scott, Blood 2014). Eligible were patients with IPI score of 2 or more and were randomized to the standard treatment R-CHOP21 plus placebo or lenalidomide 15 mg oral days 1–14 to 6 cycles and 2 additional doses of rituximab according to local practice. PFS, the primary endpoint, was similar in both arms (HR 0.85; 95% CI 0.63 to 1.14; p=0.29),

although R2CHOP showed tendency to better outcome in patients with more advanced clinical stage and IPI score ≥3. ORR was 91% in both arms, complete response (CR) 69% and 65% in R2CHOP and R-CHOP arms, respectively. All 6 cycles of treatment completed 74% and 84% of patients in both arms, respectively. The most frequent cause of treatment discontinuation was neutropenia.

A similar randomized but phase II study with lenalidomide ECOG-ACRIN1412[15] involved patients with DLBCL regardless of COO ABC or GCB with similar to ROBUST study clinical risk factors (IPI≥2, Eastern Cooperative Oncology Group Performance Status ≤2). COO classification was performed with the use of the same method of gene expression analysis NanoString Lymph2Cx with the aim of evaluating patient outcome in ABC DLBCL subtype. Patients were randomized to standard R-CHOP21 treatment or lenalidomide added to R-CHOP21 at 25 mg oral days 1–10 to 6 cycles. The primary endpoint was PFS. In this study, adding lenalidomide to R-CHOP was associated with 33% reduction of PFS risk and was statistically significant (HR 0.67; 95% CI 0.44 to 1.03; p=0.03 (one sided)). Based on COO, PFS HR for R2CHOP was: 0.68 for ABC, p=0.15, 0.86 for GCB, 0.83 for unclassified, and 0.61 for unknown cases. Objective response and CR rate was similar in R-CHOP and R2CHOP arms of 92% and 67%, and 97% and 72%, respectively (p NS). The 2-year OS was 87% and 80%, respectively. Toxicity was as expected for R-CHOP with significantly different rates of grade 3 or more adverse events for diarrhea (6% vs 0.6%, p=0.005), febrile neutropenia (25% vs 12%, p=0.003), and thrombocytopenia (36% vs 12%, p<0.0001) in R2CHOP versus R-CHOP arm, respectively. In conclusion, contrary to the ROBUST phase III study, the addition of lenalidomide to R-CHOP in this phase II study improved PFS in newly diagnosed DLBCL.

It is intriguing that these 2 similar studies resulted in a different conclusion. However, there were differences between the studies: phase II versus phase III, target patient cohort non-GCB versus DLBCL unspecified, lenalidomide dosing 25 mg/10 days vs 15 mg/14 days. Taken together, it should be stated that R2CHOP regimen is not yet ready as alternative for patients with DLBCL. In addition, COO classification of ABC and GCB subtypes based on GEP techniques may not be precise enough to guide treatment choice, and thus explain why the clinical trials with agents targeting COO subtypes failed.

Among new aggressive lymphoma cases, around one-third show alterations of *c-MYC* oncogene including rearrangements, gain of copy number or increase of MYC protein expression. *MYC* rearrangement can be found in 12% of patients, and in around 8% of cases it is associated with *BCL2* and/or *BCL6* rearrangements. These double-hit and triple-hit lymphomas are now classified as HGBL with *MYC* and *BCL2* and/or *BCL6* rearrangements. It is generally believed that these lymphomas are associated with poor prognosis if the patients are treated with R-CHOP, present more frequently with poor risk factors, and are at higher risk of CNS involvement. Reports from single centers suggested better outcome if the patients received intensive induction treatment. A role of high-dose chemotherapy and autologous hematopoietic cell transplantation as a consolidation of remission is not clear. Cases of protein MYC and BCL2 overexpression referred to as 'double expressor DLBC' are believed to be associated with poor prognosis as well.[6]

Recently, advanced molecular techniques were used to classify B-cell lymphomas beyond COO or *MYC* alterations and correlated with clinical outcome as illustrated by 2 recently published influential studies. A group from the National Institutes of Health, USA,[16] reported results of exome and transcriptome sequencing, targeted amplicon resequencing, and array-based DNA copy number analysis of 372 genes from the fresh biopsy samples of 574 patients with DLBCL to identify genes with recurrent aberrations.[16] The authors developed and implemented an algorithm that was able to assign 47% of cases to 4 prominent genetic subtypes in DLBCL based on the co-occurrence of genetic alterations. The subtypes were termed:

- MCD (co-occurrence of *MYD88*^{L265P} and *CD79B* mutations).
- BN2 (*BCL6* fusions and *NOTCH2* mutations).
- N1 (*NOTCH1* mutations)/.
- EZB (*EZH2* mutations and *BCL2* translocations).

These subtypes differed phenotypically by differences in gene expression signatures and response to treatment with favorable survival in the BN2 and EZB subtypes and inferior outcome in MCD and N1 subtypes. Genetic pathway analysis suggested that MCD and BN2 subtypes depended on chronic active BCR signaling that is theoretically susceptible to therapeutic inhibition. Analyzing cases by COO according to GEP showed that 23.1% and 13.6% of ABC cases were classified as MCD and BN2, respectively, and 37.2% and 11.6% of GCB cases as EZB and BN2, respectively. However, the majority of both ABC and GCB cases were unclassified. Survival analysis of 119 patients treated with R-CHOP or similar regimen in genetic subtypes showed 5-year OS rates of 26%, 36%, 65%, and 68% for MCD, N1, BN2, and EZB subgroups, respectively.

A group from Dana Farber Cancer Institute, USA, analyzed 304 biopsy samples from untreated patients with DLBCL who subsequently received R-CHOP regimen. A study involved a comprehensive genetic analysis, identifying low-frequency alterations, capturing recurrent mutations, somatic copy number alterations, and structural variants, and defining coordinate signatures in patients with available outcome data. The genetic drivers were integrated using consensus clustering, and 5 robust DLBCL subsets called 'clusters' were identified: C1–C5.[17] Around 96% of cases were able to be classified into 5 clusters (C) that were defined based on predominant alterations:

C1—*BCL6* structural variants, *NOTCH2* mutations, mutations of NF-κB pathway elements: *BCL10*, *TNFAIP3(A20)* and *FAS*, alterations responsible for immune escape including inactivating mutations of *B2M*, *CD70*, *FAS* and structural variants of *PD-L1*, *PD-L2*.

C2—biallelic inactivating mutations of *TP53* and *17p* copy loss, copy loss of *9p21.13*/*CDKN2A* and *13q14.2*/*RB1*, which perturb chromosomal stability and cell cycle.

C3—*BCL2* mutations with concordant structural variants that juxtapose *BCL2* to the *IgH* enhancer, frequent mutations in chromatin modifiers, *KMT2D, CREBBP, EZH2*, as well as *PTEN* inactivating alterations.

C4—mutations in histone genes, multiple immune evasion molecules *CD83, CD58, CD70*, BCR/Pi3K signaling intermediates (*RHOA, GNA13, SGK1*), NF-κB modifiers (*CARD11, NFKBIE, NFKBIA*) and RAS/JAK/STAT pathway members (*BRAF, STAT3*).

Table 1 Relation of commercially available therapeutic agents and new molecular subtypes[6]

DLBCL molecular subtype	Potential therapeutic agent
MCD/C5	Ibrutinib, acalabrutinib, venetoclax
BN2/C1	Ibrutinib, bortezomib, carfilzomib
EZB/C3	Venetoclax, idelalisib, copanlisib, duvelisib
C4	Idelalisib, copanlisib, duvelisib, bortezomib, carfilzomib, ruxolitinib

DLBCL, diffuse large B-cell lymphoma.

C5—*18q* gain increasing expression of BCL2 and MALT1, frequent mutations in *CD79B* and *MYD88*^{L265P}, gains of *3q*, *19q13.42* and inactivation of *PRMD1*, *18p* copy gains. These alterations along with additional mutations in *ETV6*, *PIM1*, *GRHPRTBL1XR1*, and *BTG1* are similar to those described in primary CNS and testicular lymphoma, thus implicating that the C5 genetic signature is associated with extranodal tropism.

When correlated with COO, C1 and C5 signatures correlated with ABC, and C3 and C4 with GCB. C5 signature corresponds to the unfavorable part of ABC, and C3 signature to the unfavorable part of GCB.

Comparing these 2 genetic classifications suggests similarities between molecular subtypes, in particular MCD with C5, BN2 with C1, and EZB with C3. Advanced molecular pathogenesis descriptions of DLBCL provide a framework for actionable classifications that should be used for designing future clinical trials and hopefully bring success to treatment of high-risk aggressive lymphoma.

The potential match of potential therapeutic targeted agents and new molecular subtypes is summarised in table 1.

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