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Large B-cell lymphomas - Section 12

Molecular classification of aggressive B-cell lymphoma

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Take home messages

- Sequencing-based approaches are readily used for molecular classifications of aggressive B-cell lymphomas.
- Genetic classifiers identify novel vulnerabilities and inform clinical trial designs.
- Genetically-distinct DLBCLs provide insights into unique lymphomagenesis, prognosis prediction and combination of targeted treatments.

Introduction

Large B-cell lymphomas (LBCLs) represent a molecularly and clinically heterogenous group of tumors that are thought to arise from antigen exposed B-cells which includes Burkitt Lymphoma (BL), Diffuse Large-B-cell Lymphoma (DLBCL), Primary Mediastinal Lymphoma (PMBL) and Primary Central Nervous System Lymphoma (PCNSL).[1] The intrinsic heterogeneity of these tumors prompted the development of various classification schemes over the last decades and included molecular classifiers that (i) improved the accuracy of diagnosis; (ii) identified relevant molecular subtypes (iii) developed prognostic models for relevant clinical endpoints; and (iv) more recently stratified patients for disease management. Technical advantages paved the way for next generation sequencing (NGS)-based techniques to be included in clinical and/or low throughput-based molecular classifiers. Here, we summarize recent advances and highlight notable examples.

Current state-of-the art

Primary mediastinal lymphoma (PMBL)

PMBL was initially classified as a morphological subtype of DLBCL. Comparative whole transcriptome gene expression profiling highlighted similarities of PMBL with classical Hodgkin Lymphoma (cHL) and defined PMBL as a distinct WHO recognized entity.[2,3] Subsequent genomic studies revealed also striking genetic similarities between PMBL and cHL, including 2p/*REL* and 9p24/*JAK2/PD-L1/PD-L2* amplifications.[4,*5] Given the success of immune checkpoint blockade in cHL, the frequent 9p24/*JAK2/PD-L1/PD-L2* gain inspired evaluation PD-1 blockade in PMBL with very good and durable responses that eventually led to FDA approval in 2018.[6]

Primary central nervous system lymphoma (PCNSL) and primary testicular lymphoma (PTLs)

PCNSL and PTL are primary extranodal LBCLs with inferior responses to current therapies. To identify targetable genetic lesions in these rare entities, we characterized somatic mutations, somatic copy number alterations (SCNAs) and structural variants (SVs), and compared them to genetic signatures of DLBCL and PMBL.[*5] These studies identified unique combinations of genetic alterations in discrete LBCL subtypes and subtype-selective bases for targeted therapy. PCNSLs and PTLs exhibited multiple genetic mechanisms ($MYD88^{L265P}$ mutations and $NFKBIZ^{gain}$) that led to near-uniform oncogenic Toll-like receptor (TLR) activation.[*5] Most PCNSLs also harbored co-occurring activating mutations in the proximal B-cell receptor (BCR) molecule, *CD79B*, suggesting clinical evaluation of targeted inhibitors of the BCR- and TLR-signaling pathways. Notably, PCNSLs and PTLs also shared frequent 9p24.1 genetic alteration with PMBLs but not with DLBCLs.[*5] This genetic basis of PD-1 mediated immune escape suggests that these tumors might be sensitive to checkpoint inhibition, which is currently being tested.[7]

Transcriptional heterogeneity in DLBCL

DLBCL is the most common aggressive LBCL with cure rates in up to 65% of patients. The remainder eventually succumbs to their disease, highlighting the unmet clinical need to develop new treatment approaches for relapsed patients and to identify those patients that benefit/not benefit from current treatments.

Besides recognized morphological subtypes, DLBCLs is transcriptionally divided into activated B-cell (ABC) and germinal

The authors have indicated they have no potential conflicts of interest to disclose.

HemaSphere (2019) 3:S2
Received: 20 May 2019
Citation: Bojarczuk K, Chapuy B. Molecular classification of aggressive B-cell lymphoma. *HemaSphere*, 2019;3:S2. http://dx.doi.org/10.1097/HS9.0000000000000273

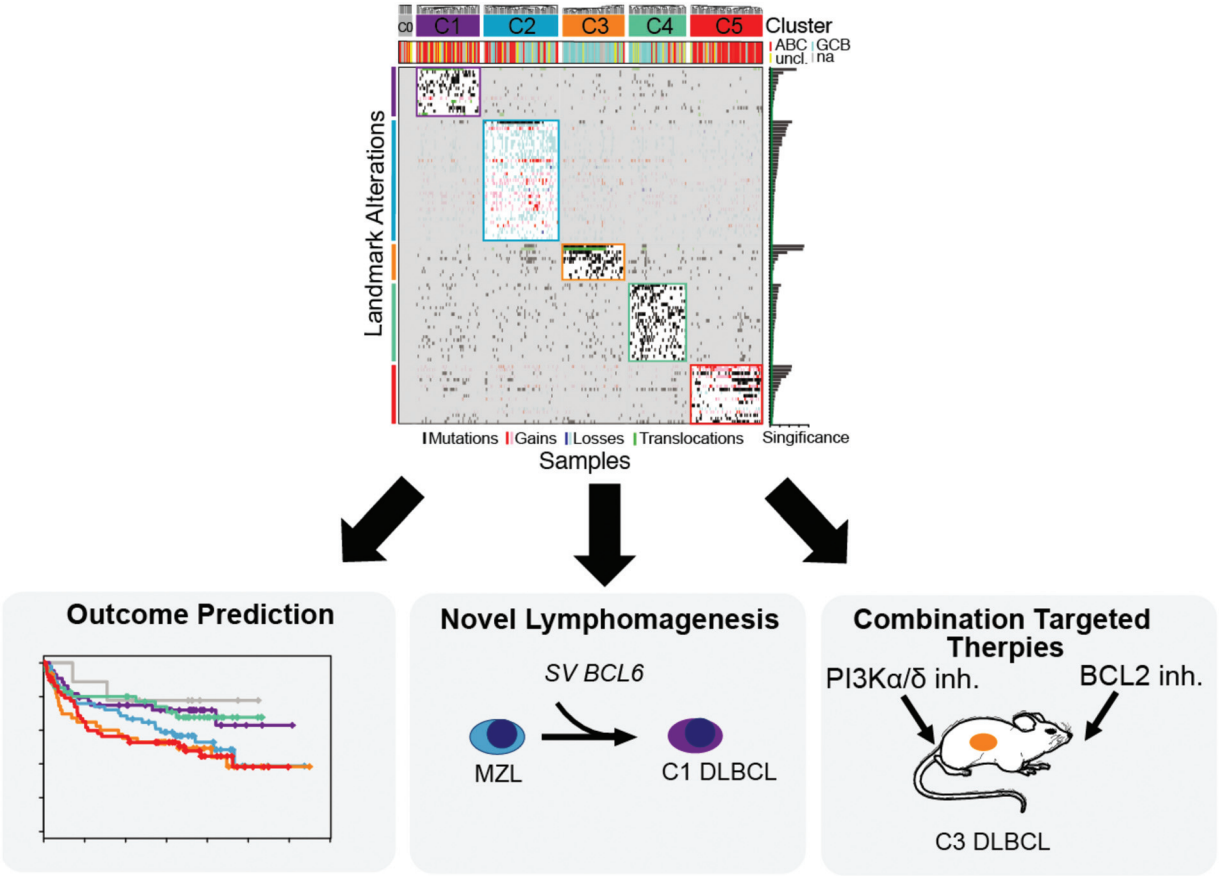

Figure 1. Genetically-defined DLBCLs. Coordinate genetic signatures of C1-C5 DLBCLs (top). Types of genetic alterations are color-coded – mutations (black), copy number gains or losses (red or blue), structural variants (green). Transcriptionally defined cell-of-origin classification at top (ABC, red; GCB, blue; unclassified, yellow). C1-C5 DLBCLs are predictive for outcome following standard induction therapy (lower panel on the left), provide new insights into previously unappreciated pathogenetic mechanisms of lymphomagenesis (lower panel in the middle), and help to identify targetable genetic features for therapeutic intervention (lower panel on the right).

center B-cell (GCB) subtypes.[8] The distinction between GCB-, ABC-type and unclassifiable DLBCLs is based on whole transcriptome gene expression profiling.[8] Subsequent efforts focused on capturing the complex transcriptional phenotype by more parsimonious assays, including easy to implement immunohistochemistry (IHC)[9] or the more recently reported nanostring-based assays.[10] IHC-based assays are extensively used in practice to date and consequently adopted for biomarker-driven studies, despite numerous issues that have been raised regarding its accuracy and reproducibility. Thus far, patient stratification for treatment based on transcriptional subtypes has been unsuccessful.

High grade lymphoma and double hit lymphoma

Recently, the WHO recognized a new provisional entity called "high grade lymphoma" that showed a blastoid morphology and/or is phenotypically an intermediate between BL and DLBCL. Many of these tumors exhibit juxtaposition of either *BCL2*, *BCL6*, or *MYC* to strong enhancer elements such as the Ig-enhancers (ie, "double or triple hits"). Notably, this genetic constellation also occurs in the absence of above morphological/phenotypical markers in DLBCL NOS underscoring that these category is a heterogenous group of lymphomas of which some have unfavorable outcome following standard chemoimmunotherapy or after high-dose chemotherapy with autologous transplantation.[11] Further work on larger cohorts is needed to clarify the biology of these lymphomas.

Genetic heterogeneity of DLBCL

The recognized clinical heterogeneity in DLBCL inspired the search for an underlying genetic heterogeneity. While initial studies were focused on the discovery of single genetic alterations, genome-wide technologies allowed for a more precise picture of the DLBCL genome.[12–16,*17] However, these studies were either limited by focusing on single alterations, low sample size or clinical annotation.

In order to overcome these limitations, we recently integrated recurrent mutations, SCNAs and SVs and discovered 5 distinct DLBCL subsets[*18] (Fig. 1), including: (1) a high-risk ABC DLBCLs with near-uniform *BCL2* copy gain, frequent activating *MYD88* and *CD79B* mutations and extranodal tropism (C5 DLBCLs); (2) previously unappreciated favorable-risk ABC-DLBCLs with genetic features of an extrafollicular, possibly marginal zone origin (C1 DLBCLs); (3) poor-risk GCB-DLBCLs with *BCL2* SVs, inactivating mutations and/or copy loss of *PTEN* and alterations of epigenetic enzymes (C3 DLBCLs); (4) a newly defined group of good-risk GCB-DLBCLs with distinct alterations in BCR/PI3K, JAK/STAT and BRAF pathway components and multiple histones (C4 DLBCLs); and (5) an ABC/GCB-independent group of tumors with biallelic inactivation of *TP53, 9p21.3/CDKN2A* and

associated genomic instability (C2 DLBCLs). Importantly, these genetically defined subtypes provided new insights into the pathogenesis of these tumors, suggest novel rational combination therapies and predict outcome (Fig. 1). These results were largely confirmed by an independent non-overlapping large-scale study following a completely different analytical approach and that found similar groups with shared pathogenetic mechanisms.[*19]

Future perspectives

Current strategies for the treatment of DLBCL do not reflect the genomic complexity of the disease. At present, novel targeted agents are tested in unselected patients or those defined solely by clinical prognostic categories, such as the International Prognostic Index (IPI), or tumor transcriptional subgroups, GCB vs ABC. This approach has led to failed phase III trials of multiple targeted agents and uncertain prospects for others. We anticipate that the provided genetic framework will guide rational study design as recently exemplified in a preclinical model of C3 DLBCLs, that had long-term remission through a rational combination of BCL-2 and PI3Kα/δ inhibitors.[*20]

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