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Large B-cell lymphomas - Section 12

Aggressive B-cell lymphoma subtyping: a pathologists viewpoint

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Take home messages

- Prognostic and predictive biomarkers should be biologically meaningful, robust, reproducible, widely available and affordable to serve as meaningful companion diagnostics.
- Multimodality profiles will be needed for successful biomarker-driven introduction of personalized and targeted treatments; single markers will not suffice.

Introduction

All hematological malignancies should be classified according to the most recent update of the WHO Classification, now the 2016/2017 edition, and all patients have the right to a complete classifying diagnosis at first presentation of their disease.[1] The major reason for this is that the classifying diagnosis inherently encompasses information on the expected clinical course of the disease. Until recently, expectations on outcome for large B-cell lymphoma (LBCL) were largely restricted to standard R-CHOP treatment. In the past few years, however, the insights in the biological variation of LBCL and its impact on prognosis have massively increased and strategies to translate this knowledge to adapted treatment are currently a focus of international efforts. Therefore, implementation of molecular prognostic and predictive biomarker information has increasingly found its way into the WHO classification, especially for LBCL.

State-of-the-art

MYC, BCL2 and BCL6 fluorescent in situ hybridization analysis in LBCL

Immunohistochemistry (IHC) and fluorescent in situ hybridization (FISH) are classical techniques that are familiar to pathology practice and directly related to the morphological frame of reference of pathologists. In the current WHO classification, FISH is used as a classifying parameter for various lymphoma classes and indeed the diagnosis of "aggressive B-cell lymphoma with MYC and BCL2 and/or BCL6 translocation" (double/triple hit lymphoma, D/TH) cannot be made without this information. Various studies have shown that D/TH lymphomas have a significantly worse prognosis than LBCL without this genotype, serving as an argument for dedicated treatment protocols in various countries.[2,*3] In that specific setting, MYC/BCL2/BCL6-FISH may be considered mandatory. It should be realized that this decision then also implies a joint effort of pathologists and hematologists to make the technique available for all patients and covered in the medical financial system. If dedicated treatment protocols are not offered or available, mandatory implementation of FISH by pathology labs is far less obvious.

Virtually all MYC-FISH positive lymphomas express MYC protein that can be demonstrated using IHC, while MYC-IHC positive LBCL bear a MYC translocation in approximately 30% of the cases.[*3] This means that MYC-IHC cannot serve as a surrogate for FISH. MYC-FISH positive lymphomas are most often CD10 positive and pre-screening strategies by MYC and/or CD10 IHC prior to FISH to limit expenses has been proposed. Albeit that this pre-screening may help to enrich for FISH-positive cases (good positive predictive value), it is generally thought that the negative predictive value is too high and not advised.

Cell-of origin classification in diffuse large B-cell lymphoma

In 2000, the "cell-of-origin" (COO) concept was introduced for DLBCL.[4,5] Outcome differences between so-called germinal center cell (GCB)-like and activated B-cell (ABC)-like as determined by gene expression profiling (GEP) has largely stood firm, but are far from absolute. The underlying genetic differences and inferred oncogenetic pathways have been shown to differ between the COO-classes, albeit with considerable overlap.[5] Various clinical trials using novel compounds have been based on COO-classification with the aim to specifically target characteristic and relevant pathways in these tumors.[6,7,8] Consequently, pressure has been put on pathologists to implement COO-classification in daily practice. GEP for routinely formalin-fixed/

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paraffin-embedded material is very well feasible and reproducible, but not widely available.[9] Alternative approaches using relatively cheap and widely available IHC have been proposed. As a downside, these all depend in part on poorly reproducibly staining antibodies (eg, BCL6, MUM1, FOXP1) and arbitrary cut points for scoring that together result in poorly reproducible classification.[10] Moreover, correlation to the gold standard of GEP is suboptimal. Results of clinical trials aimed at COO-classes are unfortunately rather disappointing; several trials using proteasome inhibition aiming at NFkB signaling in ABC/non-GCB patients have come out negative.[6,7,8] For both pathological and clinical reasons, the inclusion of (mandatory) COO-classification for DLBCL in the current WHO classification might, therefore, be considered rather premature and should, in any case, be dealt with in a critical manner.

IHC is also used as predictive marker for other, selected purposes. Specifically, pathologists have been asked to provide minimum positivity scores for CD30 expression as a marker to select patients for Brentuximab-Vedotin. Various arbitrary cut points have been proposed, which may be as suboptimally reproducible as COO-markers described above.[*11] In daily practice, this has not given much problems for CD30, however, since even extremely low membranous CD30 protein density has been showing to suffice for Brentuximab-Vedotin to effectively bind and deliver its toxin to tumor cells.[12] IHC scoring of PDL1 expression as a selection criterion for PDL/PD1-checkpoint inhibition is difficult for other reasons. Up to now, the mode of action of PDL/PD1-checkpoint inhibition is largely unknown and especially the impact of expression of PDL1 on tumor cells versus histiocytes and the nature of the effector cells (CD8, CD4) is unclear.[13,14] These biomarkers, therefore, do not meet all of the required criteria for biomarkers: biologically meaningful, robust, reproducible, widely available and affordable. This does not necessarily mean that there is no place for these assays, but a critical attitude is required.

Next generation sequencing in diffuse large B-cell lymphomas

A burst of next generation sequencing (NGS) activities around 2011 to 2013 resulted in a comprehensive inventory of the mutational spectrum of DLBCL.[15,16,17] Translation to clinical decision making proved to be more stubborn, however and as in COO-directed targeted treatment, direct "specific mutation-targeted compound-based" clinical trials have unfortunately largely come out negative. Only very recently, 3 large NGS based studies have demonstrated that the genetic landscape of DLBCL is actually far more complex than was initially anticipated.[18,*19,*20] On the one hand this likely explains why previous COO-directed clinical trials have so far been disappointing, and on the other hand this opens new doors and provides new challenges for multi-modality genome-based classification and risk-stratification of patients at first diagnosis. One of these studies provides a comprehensive catalog of gene-expression and mutation information in a very large series of DLBCL and had primarily a purpose to answer biological questions.[18] Two other studies had a more clinical approach and, while using a very different starting point and design, resulted in roughly similar subclasses remarkably arrived at a similar dissection of 5 to 6 biologically defined classes with differential prognostic impact.[*19,*20] Both combine gene-expression, mutation and FISH-translocations data and underpin the separation of good- and poor prognosis groups within the original COO-classes and define novel molecular classes. This means that in the near future, biomarkers will be more complex multimodality profiles rather than single markers or even single modalities.

Other approaches for subtyping DLBCL

Various other approaches can be taken to substratify DLBCL. These include immunophenotypic features such as expression of targetable proteins (eg, CD30, PDL1) and prognostic protein markers such as MYC and BCL2 protein (either alone or in combination) using immunohistochemistry. Moreover, some classes of DLBCL are marked by a specific crosstalk with their immune microenvironment which infers specific therapeutical options. Once better defined, these parameters may also find their way into diagnostic practice.

Future perspectives

Stimulated by the clinical need to provide more effective treatment with less unwanted side effects, enabled by the rapid accumulation of biological insights and supported by technological advances, more successful personalized and targeted treatment programs can be expected in the coming years. Pathologists will be challenged to provide companion prognostic and predictive biomarkers that are biologically meaningful, robust, reproducible, widely available as well as affordable. By providing knowledge to bridge biology to clinical applications, pathologists may play a pivotal role in multidisciplinary meetings and complement hematologists/oncologists and radiation oncologists, nuclear medicine physicians and (intervention) radiologists to achieve optimal clinical management of lymphoma patients.

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There is more to DLBCL biology than cell-of-origin dichotomy.

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