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Integration of molecular testing for the personalized management of patients with diffuse large B-cell lymphoma and follicular lymphoma

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Abstract

Diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) are the most common forms of aggressive and indolent lymphoma, respectively. The majority of patients are cured by standard R-CHOP immunochemotherapy, but 30%–40% of DLBCL and 20% of FL patients relapse or are refractory (R/R). DLBCL and FL are phenotypically and genetically hereterogenous B-cell neoplasms. To date, the diagnosis of DLBCL and FL has been based on morphology, immunophenotyping and cytogenetics. However, next-generation sequencing (NGS) is widening our understanding of the genetic basis of the B-cell lymphomas. In this review we will discuss how integrating the NGS-based characterization of somatic gene mutations with diagnostic or prognostic value in DLBCL and FL could help refine B-cell lymphoma classification as part of a multidisciplinary pathology work-up. We will also discuss how molecular testing can identify candidates for clinical trials with targeted therapies and help predict therapeutic outcome to currently available treatments, including chimeric antigen receptor T-cell (CAR-T), as well as explore the application of circulating cell-free DNA, a non-invasive method for patient monitoring. We conclude that molecular analyses can drive improvements in patient outcomes due to an increased understanding of the different pathogenic pathways affected by each DLBCL subtype and indolent FL *vs.* R/R FL.

INTRODUCTION

B-cell lymphomas are classified into over 19 distinct entities, as defined by the 2022 World Health Organization (WHO) classification[1]. Diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkin lymphoma (NHL), representing approximately 30% of lymphomas of mature B-cells[2], while follicular lymphoma (FL) is the second most common NHL. However, both DLBCL and FL are phenotypically and genetically hereterogenous B-cell neoplasms. For example, the majority of DLBCL patients are cured by standard rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone (R-CHOP) immunochemotherapy, but 30%–40% relapse or are refractory (R/R), while for FL, approximately 20% of patients treated with chemoimmunotherapy will progress within the first two years of diagnosis (POD24)[3]. Thus, improvements in patient outcomes will rely on an increased understanding of the different pathogenic DLBCL and FL pathways that lead to treatment failure and/or progression.

Next-generation sequencing (NGS) studies together with copy-number analysis are determining genes with recurrent alterations in DLBCL and FL, some of which can refining diagnosis and prognostic stratification. In this minireview, we will describe how molecular analyses are revealing differences in somatic mutations according to disease subtypes, helping with differential diagnosis, as well as to determine new targets for the development of directed therapies. We will also explore the application of circulating cell-free DNA, a non-invasive method for patient monitoring. Finally, we will discuss how the incorporation of precision medicine can identify candidates for clinical trials with targeted therapies and help predict therapeutic outcome to currently available treatments in a drive towards a more personalized treatment approach.

We aim to convince the reader that the incorporation of molecular testing for somatic gene mutations can improve the diagnosis and prognosis of patients with DLBCL and FL as part of a multidisciplinary pathology work-up.

CONVENTIONAL CLASSIFICATION OF DLBCL

FL is characterized by the t(14;18)(q32;q21) translocation, present in 90% of FL patients, resulting in overexpression of *BCL2* under the *IGH* promoter. In cases lacking t(14;18), BCL6 and CD10 expression patterns confirm FL diagnosis[10]. Rearrangement of *BCL6* (3q27) may also be found in grade 3 FL, with or without t(14;18)[11].

Prognostic biological factors include age > 60 years and hemoglobin < 12 g/dL, as well as other biomarkers, such as LDH or β2-microglobin above normal, according to the FLIPI and FLIPI-2 scores, respectively[12,13].

Several first-lines of immunochemotherapy exist, including bendamustine + rituximab (BR), rituximab alone, or R-CHOP, with choice largely down to the clinician's preference. Treatment improvements are a necessity, given that POD24 is a predictor of overall survival (OS), with rates of just 50% for patients with POD24 *vs.* 90% in those with no POD24 following R-CHOP treatment[3]. As highlighted in the recent editorial by J.P. Leonard, there is currently "no reliable way" to determine at diagnosis whether a patient with FL is likely to respond optimally to immunochemotherapy[14]. The hope is that molecular analyses could help identify a subgroup of at-risk patients who would benefit from upfront treatment with a specific targeted therapy.

NGS APPLICATION IN LYMPHOMAS

According to the 2022 WHO and ICC classifications and the European Society for Medical Oncology's 2021 clinical guidelines, no molecular analyses are currently recommended at diagnosis for DLBCL or FL[1,15,16]. To date, only a few entities of lymphoid neoplasms are defined by genomic criteria. This is in stark contrast to other hematological malignancies, in particular myeloid neoplasms, where the use of NGS is well-established in diagnosis and risk-stratification[1,16,17]. For example, for acute myeloid leukemia a complete genomic evaluation, including NGS panel, is obligatory at diagnosis to define disease subtypes and to direct therapies[1,16,17]. Nevertheless, both international consortiums acknowledge that molecular analyses in B-cell lymphomas have identified genomic alterations "with diagnostic, prognostic, and predictive impact

in different entities"[18] and explicitly state that it is highly probable that more entities will be defined by genomic criteria in the near future[1,16,18].

MOLECULAR ANALYSES IN DLBCL

In recent years, advances in next-generation sequencing (NGS) techniques are redefining our understanding of the genetic basis of lymphomas. Molecular studies are revealing recurrent genetic events and thus are helping to identify the key pathways that are important in DLBCL pathogenicity and evolution, and may even have prognostic impact[19].

Mutations in the genes *MYD88, CARD11, EZH2* and *CD79A/CD79B* have been identified in approximately 40% of DLBCL and are considered drivers of lymphomagenesis[20]. Moreover, NGS studies have revealed that GCB and ABC have a distinct profile of somatic mutations. For instance, mutations in *GNA13* are found in GCB but are rare in other B-cell lymphoma subtypes[21], whereas the *MYD88 L265P* mutation is found in ABC but is rarely identified in GCB DLBCL[21]. Thus, mutational information can assist in providing an accurate diagnosis, for example for the differential diagnosis of DLBCL from primary mediastinal large B-cell lymphoma (PMBCL, a relatively rare NHL with large B-cell morphology)[22], mantle cell lymphoma (MCL), or grade 3 FL. In addition, relapse has been associated with mutations in certain genes, such as the *B2M* and *CD58* immune surveillance genes[23].

Recent findings suggest that tumor genotype also influences treatment response. For example, whole genome sequencing analysis of 20 patients with high-risk GCB DLBCL revealed that those with cryptic rearrangements of *MYC* or *BCL2* (not detectable by FISH) had worse outcomes to R-CHOP[24]. Moreover, ABC tumors that harboured both a mutation in *CD79B* and the *MYD88* L265P mutation were more sensitive to the BTK inhibitor ibrutinib, whereas NOS subtype tumors with the *MYD88* L265P mutation and *CD79B* wild-type showed a poor response to ibrutinib[25].

Although NGS of DLBCL is not currently recommended in routine clinical practice[1,16], huge efforts are underway to characterize the prognostic value and thus

functional impact of driver mutations, for instance *via* the whole-exome sequencing of 1001 DLBCL samples[26].

Such large-scale studies using NGS techniques together with copy-number analysis, to identify genes with recurrent alterations with prognostic value, have led to the proposition of new DLBCL classifications. After studying 574 DLBCL biopsy samples Schmitz *et al* proposed four genetic subtypes: termed MCD (based on the co-occurrence of *MYD88* and *CD79B* mutations), BN2 (based on *BCL6* fusions and *NOTCH2* mutations), N1 (based on *NOTCH1* mutations), and EZB (based on *EZH2* mutations and *BCL2* translocations)[27]. These subtypes differed in their responses to immunochemotherapy, with favorable survival in the BN2 and EZB subtypes and inferior outcomes in the MCD and N1 subtypes[27], whereas MCD and N1 subtypes responded well to R-CHOP with ibrutinib[28]. Importantly, data on the BN2 subtype, with overlap with the NOS subgroup, revealed that patients are likely to be responsive to antagonists of B-cell receptor signaling such as the BTK inhibitors. Similarly, Chapuy *et al* studied 304 DLBCL biopsies and identified six genetic subgroups[29]. Of note, mutations in *CD79B* were associated with relapse independently of the subtype or International Prognostic Index (IPI) risk group[30].

MOLECULAR ANALYSES IN FL

Mutations in genes encoding epigenetic modifiers (and the resultant pattern of aberrant DNA methylation) are a molecular hallmark of FL (Table 1)[31,32]. Moreover, such mutations are likely to be early driver events[33].

NGS studies have revealed that the acquisition of additional mutations contributes to disease progression and the risk of transformation of FL to DLBCL. For example, *TP53* mutations have been associated with shorter progression-free survival and OS[34,35], while the gain of mutations in genes such as *EBF1, MYD88* and *TNFAIP3* are associated with progression to a more aggressive disease[32]. Additionally, expression studies have revealed chromosome regions, such as 1p36 and 6q21 deletion

associated with transformation[36]. Thus, genetic analyses can improve the prognostication of patients with FL.

In the case of FL, the mutational status of seven genes (***EZH2***, ***ARID1A***, ***MEF2B***, ***EP300***, ***FOXO1***, ***CREBBP*** and ***CARD11***) was added to the preexisting ECOG performance status, FL International Prognostic Index (ECOG PS and FLIPI) risk stratification algorithms to develop the m7-FLIPI risk score[38]. Application of the m7-FLIPI risk score defined a high-risk group with a significantly shorter failure-free survival after receiving first-line R-CHOP.

Specifically, mutations in *EZH2* were associated with the low-risk m7-FLIPI group and with higher OS[38]. As such, the presence or absence of the *EZH2* Y646 point mutation can help decide the chemotherapy regime in a patient-specific manner, since patients with such a mutation were shown to respond well to R-CHOP[38] (higher OS and lower relapse rate) while patients without this mutation responded better to bendamustine[39].

Although the m7-FLIPI was not prognostic for FL patients who received rituximab, patients with *EZH2* mutations had longer time to treatment failure while *EP300* mutations were associated with shorter time to treatment failure[40]. Therefore, it remains to be determined if the m7-FLIPI risk score is prognostic for FL patients treated with other chemotherapy regimes other than R-CHOP. Furthermore, the use of such risk scores in the routine clinical practice is not common, partly due to lack of availability of mutational studies in some centers[41].

TARGETED THERAPIES IN DLBCL

It is clear that improvements in DLBCL outcomes will rely on an increased understanding of the different pathogenic pathways affected by each DLBCL subtype. Indeed, an *in silico* drug discovery analysis showed that 46% of cases harbored at least one genomic alteration considered to be a potential drug response target (according to early clinical trials or preclinical assays in DLBCL or other B-cell lymphomas)[30].

But, to date, only one targeted therapy against a molecular driver has been approved for DLBCL – selinexor – although many others are in development. Selinexor is a specific inhibitor of the XPO1 nuclear export transporter protein that was approved by the FDA in June 2020 for the treatment of adults with R/R DLBCL NOS, including DLBCL progressed from FL, after at least two previous lines of therapy[42].

Besides targeted agents, many immunotherapy strategies are in development, with the aim to promote the immune recognition and cytotoxic attack of T cells or macrophages. Some have achieved approval, such as rituximab, a monoclonal antibody against the common surface antigen CD20, and polatuzumab vedotin (Pola; Polivy™), an antibody-drug conjugate. Pola includes an anti-CD79B monoclonal antibody for cell targeting, which upon binding allows the antineoplastic agent monomethyl auristatin E (MMAE) to enter the cell and inhibit microtubule assembly, preventing cell mitosis and ultimately causing apoptosis[43]. Pola was approved by the FDA in June 2019 in combination with rituximab and bendamustine for the treatment of adults with R/R DLBCL after at least two previous lines of therapy. Such strategies have the advantage that the surface antigens they target are universally expressed on all DLBCL subtypes.

TARGETED THERAPIES IN FL

Genetic studies have identified epigenetic mechanisms in the pathogenesis of both DLBCL and FL, such as acetylation/deacetylation affected by *CREBBP* and *EP300* mutations, or histone methylation changes affected by *EZH2* mutations. Indeed, Tazemetostat (Tazverik™), an EZH2 inhibitor, was the first directed therapy to be approved by the FDA (in June 2020) for the treatment of R/R FL after two lines of previous therapy[44]. The *EZH2* mutation is predictive of Tazemetostat response but, interestingly, this targeted agent was also shown to improve the outcome of patients without an *EZH2* mutation[44].

Other FDA-approved agents for R/R FL include four PI3K signaling inhibitors: Idelalisib (July 2014), copanlisib (September 2017), duvelisib (September 2018), and

umbralisib (February 2021)[45-48]. Further information on FL therapies in development can be found in this recent review[37].

CAR-T

Besides targeted therapies, an improved understanding of the genetic and immune biology of DLBCL and FL has led to the development of chimeric antigen receptor T-cell (CAR-T) therapies, considered a major scientific breakthrough and offering an alternative treatment option for patients with R/R B-cell lymphomas[49,50].

In 2020, our center obtained the license to provide the European Medicine Agency-approved anti-CD19 CAR-T axicabtagene ciloleucel (Yescarta™) and Tisagenlecleucel (Kymriah™) for the treatment of adult patients with R/R DLBCL or PMBCL after two or more previous lines of treatment. As of February 2021, the third CAR-T lisocabtagene maraleucel (Breyanzi™) also obtained FDA approval for the treatment of R/R DLBCL[51].

CAR-T is also an option for the treatment of adult R/R FL patients after two or more previous lines of treatment[52,53], following the FDA approval of axicabtagene ciloleucel in March 2021.

The proliferation and persistence of CAR-T cells in the body is an important factor influencing therapy durability, with the loss of a CAR-T signal associated with progression of the disease[54]. A quantitative TaqMan PCR (qPCR) assay can be used to monitor the number of CAR-T cells circulating in peripheral blood *via* detection of the quimeric CD19 recognition domain (FMC63)[55]. Flow cytometry is an alternative method for CAR-T cell monitoring, but has the disadvantage that it needs to be carried out on fresh samples and has lower sensitivity. Future studies are required to explore the correlation between the expansion/persistence of CAR-T cells and clinical outcomes including treatment efficacy and clinical symptoms.

CIRCULATING CELL-FREE TUMOR DNA

Surgical excision biopsies are the gold-standard technique used in the diagnosis and follow-up of patients with lymphomas, although core needle biopsies are a useful and viable alternative under certain conditions[56]. However, both surgical excision and core needle biopsies are resource intensive, can be painful, and impact negatively on patients, and surgical excision biopsies, in particular, have an associated risk of morbidity due to bleeding and infection. Additionally, some lymphomas may not be easily accessible which can limit the availability of tissue for genomic studies. Moreover, the extraction of genomic DNA from formalin-fixed, paraffin-embedded (FFPE) biopsies for downstream NGS applications is not ideal since chemicals used in the fixation can degrade nucleic acids, thus decreasing NGS sensitivity.

Liquid biopsy techniques are currently being explored as non-invasive methods for tumor diagnosis and disease monitoring[57]. Circulating cell-free DNA (ctDNA), consisting of highly fragmented DNA in plasma that is released by normal or tumor cells that undergo apoptosis or necrosis[58], may better reflect intratumoral heterogeneity than can be obtained from a single tissue biopsy. Indeed, in comparison with the sequencing of genomic DNA extacted from the diagnostic tissue biopsy, the sequencing of ctDNA can identify somatic mutations with a similar accuracy and identified additional clinically relevant mutations that were not detected in the diagnostic tissue biopsy[59]. Moreover, the analysis of ctDNA could overcome some other limitations of biopsies. For example, in the case of a biopsy at an extranodal site, it is not uncommon for the paraffin block to also contain other non-tumoral tissue.

Due to their easy accessibility through non-invasive procedures (such as a simple peripheral blood draw), ctDNA analyses can be repeated regularly to track lymphomas over time, such as to monitor treatment response. Indeed, studies have shown that changes in ctDNA quantification correlated with positive responses to chemotherapy and could even detect relapse, months earlier than conventional CT scan monitoring[60]. Thus they may also be useful as "surveillance" methods in patients who have completed treatment but may be at risk of relapse, *e.g.* those with mutations in *CD79B*

or and those with a high pretreatment ctDNA quantitative burden for early relapse detection[32,59].

Future studies are required to optimize the application of ctDNA analyses in the management of patients with B-cell lymphomas. Nevertheless, ctDNA is currently used in the clinic in some fields of oncology, such as in the molecular profiling of patients with non-squamous non-small cell lung cancer at diagnosis, as recommended by the National Comprehensive Cancer Network (NCCN)[61].

IMPLEMENTATION OF MOLECULAR TESTING IN CLINICAL PRACTICE

The application of NGS, together with other molecular techniques, is key to the integration of personalized medicine approaches into healthcare services. The use of NGS targeted panels, which focus on a limited and relevant set of genes or gene regions that have known associations with a particular pathology, produce large quantities of genetic information with diagnostic, prognostic and theranostic value with a high sensitivity. The simultaneous analysis of an elevated number of genes (15-200) is more resource efficient as it drastically reduces the cost and time required to obtain such genetic information enabling a more precise diagnosis and prognosis. Furthermore, the use of NGS permits the detection of emerging clones which can help inform disease follow-up and may be associated with treatment resistance, thus providing data that can help guide individualized patient therapeutic plans.

In 2016 our team implemented NGS into the routine diagnosis and prognosis of patients with acute myeloid leukemia[62]. Since then, the use of NGS has expanded to include a targeted myeloid panel for the diagnosis of patients with myeloproliferative neoplasms and myelodysplastic syndromes, a chronic lymphocytic leukemia-specific panel, and a panel for the detection of germline hematologic malignancies. However, the molecular analysis of B-cell lymphoma samples in our center is currently limited to the qPCR-based analysis of several individual genes with prognostic value (including *MYD88, TP53,* and *EZH2*) to complement the conventional cytometry, IHC and FISH tests used in routine clinical practice.

Several commercial gene panels are currently available on the market for the detection of mutations with diagnostic, prognostic or theranostic value in DLBCL and FL, given the considerable overlap of genetic alterations between GCB DLBCL and FL[32], including Oncomine™ Lymphoma (ThermoFisher), FusionPlex® Lymphoma (Archer) and Lymphoma Solution® (SOPHiA).

Incorporating a comprehensive NGS-based characterization of somatic gene mutations as a precision medicine strategy for B-cell lymphomas would assist in the daily practice by refining DLBCL and FL classification and prognosis. Importantly, it would also facilitate individualized therapeutic decision-making for patients and increase treatment opportunities by identifying candidates for clinical trials with targeted therapies. However, feasibility studies would be required to determine the clinical utility and added value of incorporating an NGS panel in the multidisciplinary diagnostic work-up, since "while many stakeholders believe that personalized medicine can provide benefits to patients and the healthcare system, payer and providers are often reluctant to change policies and practices without convincing evidence of clinical and economic value"[63].

It is also important to consider the limitations of introducing such molecular analyses for B-cell lymphomas into routine hematology laboratories. Difficulties arise in interpretation of the results generated by extensive NGS panels due to the data's complexity and uncertainty about the biological relevance as not all molecular variants are clinically actionable. For this reason, it is essential to have highly trained staff with experience in the interpretation of the clinical impact of tumor variants. Other limitations include the economic cost of molecular analyses and the turnaround time, which has a large impact on the applicability of genomic tests to clinical decision-making. The potential to multiplex lymphoma samples with other targeted panels in the same sequencing run would help optimize the resources dedicated to library preparation and sequencing, and minimize the time required to analyze patient samples and report results to guide clinical decision-making. This is essential for aggressive B-cell lymphomas where immediate treatment is frequently required.

CONCLUSION

The incorporation of molecular testing into the routine clinical management of patients with B-cell lymphomas *via* the implementation of a targeted NGS panel would help improve disease subtype classification, allow the prediction of therapeutic outcome to currently available treatments, and identify patients for personalized treatment. Moreover, the optimization of non-invasive ctDNA analysis could allow for closer patient monitoring and earlier relapse detection.

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