Review Article

Novel markers for determining risk and evaluation of minimal residual disease in diffuse large B-cell lymphoma

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Abstract: Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease that is usually curable with frontline immunochemotherapy and whose prognosis is directly impacted by the usual prognostic factors grouped within the International Prognostic Index (IPI). Despite the overall improvement in the outcome of patients with DLBCL since the introduction of rituximab, 40% of patients are primarily refractory or experience short-term relapse and have an extremely poor prognosis. The prognostic impact of the response to treatment as assessed by intermediate PET could be increased by combining it with the results of the modern molecular biology techniques, which include measuring the VDJ rearrangement in blood, or Next generation sequencing (NGS) of a dedicated somatic mutation panel in circulating tumor DNA, according to the concept of liquid biopsy. Emerging data has also suggested that these methods would be a powerful tool in the assessment of minimal residual disease (MRD) in DLBCL. Important questions remain about the most appropriate timing and the most efficient technique for evaluating the MRD throughout the course of the disease. We conducted a review of the recent literature to present, summarize and explain the major pilot studies that have established novel markers for determining baseline risk in DLBCL, early assessment of disease response and post-therapeutic MRD surveillance. Indeed, in the coming years, the evaluation of the MRD will be a secondary objective of all major clinical trials in this pathology, in order to confirm its clinical relevance and reproducibility, before setting up clinical trials based on the evaluation of the MRD as the primary decision-making criterion for the adaptation of patient treatment.

Keywords: Minimal residual disease; diffuse large B-cell lymphoma (DLBCL); next generation sequencing (NGS); high-throughput sequencing; somatic mutation; ctDNA

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Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most prevalent form of aggressive non-Hodgkin lymphoma (NHL) and represents 30–58% of newly diagnosed lymphoma cases (1). Molecular techniques such as gene expression profiling (GEP) and next generation sequencing (NGS) have described unique molecular signatures and pathways that distinguish three major molecular categories of DLBCL: the activated B-cell-like (ABC) subtype, the germinal center B-cell-like (GCB) subtype, and the primary mediastinal BCL (PMBL) (2-4). All these subtypes are associated with distinct somatic mutational profiles that have been very well described during the past decade (4). More recently, Schmitz *et al.* (5) have identified four prominent genetic subtypes (termed MCD, BN2, N1 and EZB) based on the co-occurrence of genetic alterations with distinct phenotypes and different clinical characteristics. However, the transfer of this classification to clinical practice is so far not feasible due to the difficulty to obtain fresh

histological material in routine clinical practice, especially at each relapse event, and then perform expensive and time consuming molecular biology analyses. The limited ability to collect appropriate fresh tumoral DNA from DLBCL tissue biopsies has hindered the active adoption of somatic gene mutation results in prognostic or theranostic (i.e., a form of diagnostic testing employed for selecting targeted therapy) strategies in routine practice.

Front-line cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) combined with anti-CD20 rituximab (R-CHOP) regimen is the current standard treatment (6,7) for patients with DLBCL and has greatly improved long-term disease control, with complete response (CR) rates ranging from 75% to 80% (8,9), and more than half of both elderly and younger patients are still in CR 5 years after initial treatment (6,9,10). However, despite the improved accuracy of the molecular/histopathological classifications and risk-modified chemo-immunotherapies, there are important clinical heterogeneities among DLBCL patients: 20% of the patients are refractory to the primary R-CHOP treatment, and 30% experience disease relapse after achieving complete remission (11-13). To date, there is no standard procedure for the prompt and accurate detection of relapses, even using interim positron emission tomography (PET). Indeed, PET has not proved its utility in the systematic follow-up of patients in remission after the first-line treatment because the majority of DLBCL relapses are detected outside of the planned follow-up period and the outcomes are not affected (14,15). In addition, a large retrospective study including 680 DLBCL patients noticed that the radiological CT scans surveyed after the end of the treatment did not modify patient outcome compared with standard clinical evaluations (16).

Emerging data describing the use of "liquid biopsy" in which molecular tumor characterization is performed using a simple peripheral blood sample is currently increasing (17,18). This technique represents a major technological leap in the non-invasive management of cancers and may represent a MRD tool, in addition to PET results.

In this review, we present the currently available new markers, with a special focus on PET metabolic imaging, to determine the baseline prognostic risk of DLBCL patients, and we also summarize the different molecular biology tools at our disposal for measuring early molecular response and MRD in DLBCL with the latest work published in the literature on this emerging and innovative topic, their respective advantages and disadvantages, their potential practical applications but also their limitations. We also propose strategies for the systematic implementation of these techniques in all therapeutic clinical trials around DLBCL, alongside PET analysis.

Novel markers for determining high risk of refractory/relapse DLBCL

Biological factors

The International Prognostic Index (IPI), which was developed in the pre-rituximab era, emerged as the most powerful prognostic tool for DLBCL, remained accurate in the R-CHOP era and has been applied in all clinical trials (19). In particular, an age-adjusted IPI (aaIPI) of 2 or 3 is associated with a significantly lower 65% CR rate after first line anthracycline-based chemotherapy. However, this index currently has a limited ability to anticipate patients who will experience a particularly aggressive course in the R-CHOP era of chemotherapies (20). Recently described novel markers of early relapse in DLBCL include *MYC* rearrangements, double-hit lymphomas and CD5 expression. First, the adverse prognostic impact of patients with DLBCL with *MYC* (8q24) rearrangements was proved in three large studies (21-23) that described undoubtedly inferior 5-year PFS (31% versus 66%, P=0.006) and OS (33% versus 72%, P=0.016) in *MYC*-rearranged versus non-rearranged DLBCL. Secondly, the inferior prognostic of *MYC* rearrangement is amplified in the presence of an additional chromosomal breakpoint affecting the BCL2 or BCL6 loci. These double-hit (DH) lymphomas have a very aggressive clinical history and poor response rate to standard chemotherapy (24-26). Thirdly, MYC and BCL2 protein co-expression using standard immunohistochemistry ("double-expressor" DLBCL) was established as an independent prognostic factor of unfavorable survival in patients treated with both conventional front-line rituximab-containing therapy (27) and dose-intensified immunochemotherapy (28) or after R-ICE-based salvage therapy (29).

Fourthly, CD30 is expressed in approximately 10–15% of DLBCL but the prognostic and biological role of CD30 expression remains undetermined with conflicting results in the past years. Hu *et al.* (30) reported a favorable prognosis of the CD30+ subgroup by immunohistochemistry in a large series of 903 *de novo* DLBCL patients treated with standard R-CHOP. The retrospective data reported by Hao *et al.* (31) including 146 DLBCL patients suggest that CD30 is expressed predominantly in Non-GCB DLBCL and associated with worse OS and PFS. Two other reports

(32,33) established CD30 expression was not associated with prognosis and that CD30 expression and MYC rearrangement were mutually exclusive in *de novo* DLBCL. However, CD30 appears to be a valuable therapeutic target in this subgroup of DLBCL and several clinical trials are underway or recently completed to establish the relevance of brentuximab vedotin in the management of *de novo* and refractory/relapsed CD30+ DLBCL (NCT02594163, NCT03356054, NCT01994850). The results of these trials are highly awaited.

Finally, *de novo* CD5+ DLBCL is a distinct subgroup with adverse prognosis that was highlighted in a large multicenter cohort of patients (34). Despite initial standard immunochemotherapy, the disease course of these patients remains high-risk, and stem cell transplantation is unsuccessful to treat the majority of these patients.

PET measurements for prognostic risk assessment in DLBCL

Another important prognostic factor for determining the risk of DLBCL relapse is the slow responder characteristic at intermediate PET after two cycles of chemotherapy (PET2). In the phase III prospective GAINED trial, Casasnovas *et al.* (35) demonstrated that 69% of PET2–/PET4– (so called "early responders") patients have a particularly good outcome (2y-PFS =90%, 2y-OS =94%) after ACVBP plus rituximab or obinutuzumab induction, and that 15% of PET2+/PET4– (so called "slow responders") benefited from autologous stem cell transplant (ASCT) consolidation with an outcome similar to early responders. Nevertheless, in the PETAL trial (36), PET2+ patients that received six blocks of an intensive Burkitt's lymphoma protocol have similar survival compared to patients that continued standard R-CHOP. In this trial, iPET predicted prognosis, but therapeutic intensification was associated with more toxicity. Thus, with those conflicting results, it appears that no strict recommendations can be drafted for therapeutic intensification in front of a non-response to PET after two cycles, and additional prospective trials are needed before orienting "at risk" patients towards early ASCT consolidation.

Other PET parameters, such as pre-therapy total metabolic tumor volume (TMTV), are able to predict "high-risk" patients. In 2014, Sasanelli *et al.* (37) described a series of 114 newly diagnosed DLBCL and established that high metabolic burden (TMTV >550 cm^3) was an independent prognostic factor for OS. In another study, Zhou *et al.* (38) depicted the strong prognostic impact of baseline total lesion glycolysis (TLG), which represents the sum of the products of MTV and mean SUV in all measured lesions, in a series of 91 DLBCL patients homogeneously treated with first line R-CHOP. In this study, TLG of baseline PET was the only quantitative parameter which accurately depicted tumor burden and high TLG was a more powerful poor prognosis factor than MTV. In addition, Malek *et al.* reported a large series of 197 DLBCL patients treated with R-CHOP-like regimen and retrospectively established that the combination of a delta-SUVmax >72% and a delta-MTV >52% on interim PET was clearly associated with a better PFS. This finding was also confirmed by Mikhaeel *et al.* (39) in their series of 147 DLBCL patients who underwent PET measurements (MTV, TLG, Deauville Scale) before and after two cycles of R-CHOP. The authors established that combination of baseline MTV and early PET2 response improves the predictive power of interim PET and defines a poor-prognosis group (baseline MTV ≥400 cm^3 and Deauville 4–5) in whom most of the events occur. Nevertheless, prognostic TMTV thresholds are not the same in all studies (i.e., 261 or 300 or 550 cm^3) and the measurement techniques of TMTV and TLG may differ between teams, including both "threshold-based" (fixed percentage of SUVmax) or "gradient-based" methods (40,41), and interobserver variability limit the reproducibility of these results, which have yet to be confirmed in large-scale prospective clinical trials.

Combination of biology and PET for risk definition

A recently published interesting initiative has been to combine the molecular profiling of DLBCLs (ABC/GCB subtype, MYC and/or BCL2 overexpression, i.e., "dual expressors") with baseline PET data. Cottereau *et al.* (42) have thus demonstrated that the combination of a high MTV (≥300 cm^3) with the molecular risk data (BCL2/MYC overexpression or dual expressors) makes it possible to better define more accurately select high-risk DLBCL patients. This was also confirmed in another retrospective study including 114 DLBCL patients with available RT-MLPA GEP data and PET results (43), in which the authors were able to integrate a new risk model and delineate three distinct risk groups : low TMTV (<261 cm^3) regardless of the GCB/ABC subtype, high TMTV and GCB phenotype and high TMTV with ABC phenotype. The outcome after R-CHOP was the most unfavorable in this last "high risk" subgroup (5-y PFS 17%). In addition, Tout *et al.* (44)

Table 1 Minimal residual disease (MRD) assessment methods in patients with diffuse large B cell lymphoma (DLBCL)

MRD techniques	References	Baseline MRD positive	Detection limit	Advantages	Disadvantages
High-throughput Ig-VDJ rearrangement sequencing (ClonoSEQ)	Roschewski *et al.* (45)	92%	10^{-6}	Real-time dynamic assessment; anticipates relapse 7 months earlier than conventional imaging; commercially available and FDA approved	Cannot detect Ig-negative phenotype of PMBL; calibration failures (frozen tissue *vs.* FFPE); not suitable to tailor targeted therapy; no detection of drug-resistant clone during therapy
Panel-directed HTS (CAPP-seq and Lymphopanel)	Bohers *et al.* (17); Kurtz *et al.* (46); Rossi *et al.* (18)	Lymphopanel: 96%; CAPP-seq: 88–97%	10^{-6}	Tumor-specific; identify targetable activating mutations; tracking emergence of treatment-resistant clones; liquid biopsy; tumor burden assessment	No standardized technique; not commercially available; insufficient sensitivity for subclonal mutations VAF <20%; discrepancy between tissue and ctDNA testing
Droplet digital PCR	Alcaide *et al.* (47); Camus *et al.* (48)	Unknown	10^{-5}	Short turnaround time; low cost; detection of "hotspot" targetable activating mutations; easy serial testing	Not commercially available; insufficient data to verify the reproducibility; false-positive and detection limit concerns

Ig, immunoglobulin; PMBL, primary mediastinal B cell lymphoma; HTS, high-throughput sequencing; CAPP-seq, Cancer Personalized Profiling by deep Sequencing; ctDNA, circulating tumor DNA; VAF, variant allele frequency; FDA, Food and Drug Administration.

established that rituximab exposure is directly influenced by baseline MTV and predicts outcome of DLBCL patients included in the LNH2007-3B and GELAMS 02.03 programs, with exposure to rituximab decreasing as baseline TMTV increases, suggesting the need for prospective clinical trials using nomogram for rituximab dose individualization according to baseline TMTV.

In summary, the definition of "high-risk" patients according to the latest data from the literature could be patients with ABC phenotype, MYC/BCL2 dual-expression, high TMVT and slow response to immunochemotherapy (PET2 positive Deauville 4–5, deltaSUVmax <66%). These patients would benefit from dedicated clinical trials with new therapeutic approaches to hopefully improve their poor prognosis. Nevertheless, the use of MRD evaluation could strengthen the prognostic impact, the precision and the clinical utility of these intermediate biomarkers and PET data, leading to therapeutic changes in the individual management of DLBCL patients.

Major biology techniques for molecular response assessment in DLBCL

Table 1 summarizes MRD assessment methods in patients with DLBCL.

Pre-analytical requirements

Current data establish that the optimal specimen type for non-invasive liquid biopsy and MRD evaluation is circulating tumor DNA (ctDNA) extracted from plasma obtained from peripheral blood collected in EDTA tubes and then processed as expediently as possible (within 6 hours of collection) with sequential low and high speed (49) centrifugations at in order to minimize leukocyte lysis. The influence of storage temperature (–20 or –80 ℃) is still unclear and the use of leukocyte stabilization tubes may grant greater flexibility (50). Failure to comply with these pre-analytical recommendations may alter the quality of specimen and its suitability for cell-free DNA extraction and ctDNA experiments (51). The extraction of plasma ctDNA is easier for the vast majority of academic laboratories, as it can be achieved using commercially available kits for plasma (48,52).

Nevertheless, various ctDNA purification methods exist and insufficient data remain on biological factors that may contribute to the release of cell-free DNA, such as smoking, heart disease, auto-immune disorders and inflammatory conditions. We encourage researchers to precisely document and describe pre-analytical variables and patient factors in future publications on MRD in DLBCL in order to improve our knowledge of these criteria.

Non-invasive VDJ rearrangement monitoring

The first MRD molecular biology technique in DLBCL to be published uses the principle of immunoglobulin gene rearrangements. Each DLBCL tumor contains a singular clonotypic variable-diversity-joining (VDJ) immunoglobulin rearrangement that can be used as a "barcode" for the instantaneous detection of disease recurrence in the peripheral blood. Deep-sequencing analysis of the rearrangement of the immunoglobulin heavy chain gene (VDJ-seq) may trace the clonal evolution models of DLBCL relapse (53). NGS-based tests have a proven capacity to recognize and measure ctDNA that encodes the VDJ junctions of the immunoglobulin (Ig) receptors in the patients' plasma before treatment in 69% of DLBCL patients (54). Two retrospective studies have shown that VDJ rearrangements in the plasma may serve as a MRD tool in 82% of patients and suggest that these Ig-based NGS tests (ClonoSEQ® Process, Adaptive Biotechnologies, CA, USA) can be broadly used to foresee relapse in DLBCL patients several months earlier than standard imaging (55,56). However, this detection of occult disease was not universal, as each of the eight patients who eventually progressed had at least one negative ctDNA assay, which underlines the need for serial testing (55). In addition, these high-throughput VDJ sequencing techniques have several limitations: (I) there is a need for tumor biopsy to be able to identify the specific clonal rearrangement of the lymphoma at diagnosis, which can then be followed in the patient's blood and the success rate of tumor clonotype identification is higher in fresh/frozen tissue than in routine FFPE biopsies (93% versus 53% respectively, P=0.007); (II) VDJ rearrangements are not detectable in all patients (for instance, in the immunoglobulin-negative phenotype of PMBL as well as in some *de novo* DLBCL with unproductive VDJ rearrangements) (57); (III) the technology is not useful as a means of tailoring a targeted therapy or detecting the rise of resistant clones during treatment (58). Nevertheless, it is important to underline that ClonoSEQ® is the only DLBCL MRD technique currently marketed, FDA-approved and commercially available in the world.

High-throughput sequencing (HTS) of ctDNA somatic mutations

In 2015, Bohers *et al.* detected non-immunoglobulin somatic mutations in the circulating DNA of 12 DLBCL patients for the first-time using Ion Torrent PGM® (ThermoFisher Scientific, MA, USA) NGS. The 34 gene panel (so called "Lymphopanel") was informative for 96% of the DLBCL patients that displayed similar mutational profiles in tumor and plasma ctDNA (17), in whom it identified the most frequent somatic alterations, some of which could probably be targeted by new pharmacological inhibitors (*EZH2* Y641N, *MYD88* L265P, *XPO1* E571K) and/or serve as molecular biomarkers of residual disease.

CAncer Personalized Profiling by deep Sequencing (CAPP-seq) is a remarkably sensitive new method for quantifying ctDNA that uses high-performance, high-throughput sequencing and grants fast detection of rare circulating somatic variants in various tumors (59). First endorsed in solid tumors, CAPPseq applies a disease-specific "selector", which is a set of exonic and intronic targets selected to include regions of known recurrent mutations for a singular cancer type. In an impressive report, Scherer *et al.* were able to correctly determine the DLBCL cell of origin (COO) subtypes using somatic alterations that were detectable in ctDNA using the CAPP-seq technology (60), with an excellent (88%) concordance between the tumor and plasma COO classification. ctDNA data obtained by this CAPP-seq technique are dynamically equated with the tumor volume assessed using TEP imaging during the treatment and there was an excellent relationship between the plasma ctDNA changes, the therapeutic response after two cycles of chemotherapy and the clinical outcome in DLBCL patients (61).

In 2017, Rossi *et al.* demonstrated in a landmark study, including a prospectively collected consecutive series of 30 newly diagnosed DLBCL patients, that sequential testing of ctDNA under chemotherapy showed a rapid disappearance of DLBCL somatic mutations from the ctDNA of standard R-CHOP responding patients, confirming the clinical validity of ctDNA as a real-time monitoring tool for the surveillance of therapeutic response (18). Moreover, CAPP-seq approach enables the detection of emerging treatment-resistant clones during therapy follow-up in DLBCL patients. Very recently, the impressive results of CAPP-seq were confirmed in an international retrospective study including 183 DLBCL patients from six centers, with plasma ctDNA detectable in 97% before therapy and >90% of patients from each center having detectable ctDNA (46). The authors demonstrated a strong association between high MTV, high ctDNA level and high IPI, with elevated pretreatment ctDNA levels being firmly associated with worse event-free survival (EFS) and OS, attesting to the strong prognostic impact of ctDNA, which reflects tumor burden and anticipates the aggressiveness of the

disease. This study is important because it highlights the internationally reproducible nature of circulating DNA assays for the first time, which were usually performed in an isolated and monocentric manner in each center involved in the study of DLBCL MRD. Nevertheless, the CAPP-seq approach did not reach the sensitivity of typical MRD assay, because of its chemistry-dependent sensitivity limit (~10^{-3}) (18) with half of tumoral low-abundance mutations (variant allele frequency <20%) being missed in the plasma This suggests that ctDNA is currently a complementary source of tumor DNA for DLBCL genotyping compared with the tissue biopsy and that improvements in terms of bioinformatics pipeline and ultra-deep sequencing are necessary to achieve a better technique sensitivity, closer to that of MRD by quantitative PCR techniques (~10^{-5}).

Finally, new results were recently obtained in a prospective and single centre study analysis of ctDNA and tumor DNA (gDNA) in untreated DLBCL and compared with PET imaging (62). Mutational status of the 34-gene Lymphopanel (63) was evaluated in both tumor DNA and ctDNA at diagnosis using Ion Torrent PGM® technology. gDNA mutations from tissue biopsies (11 frozen/14 FFPE) were found in 22/25 (88%) cases. In 3/22 cases for whom gDNA analysis failed, an informative mutational pattern was successfully obtained in matched ctDNA. ctDNA mutations were observed in all patients with elevated LDH and high tumor burden. Very high MTV (>2,000 cm^3) was associated with the highest concentrations of ctDNA and VAF rate. Moreover, 3/4 patients presenting additional mutations in ctDNA as compared to gDNA had a very high MTV, suggesting that ctDNA mutations more accurately reflect tumor heterogeneity than gDNA analysis. ctDNA mutations were identified in 20 patients (67%) and after R-CHOP therapy, a rapid clearance of ctDNA mutations was noticed in 13 cases. Conversely, among the 4 patients whose basal mutations did not disappear at mid-treatment, 3 were in partial metabolic response. This study argues that ctDNA analysis can serve as a complementary method to PET scan imaging at baseline and during follow-up for the management of DLBCL.

Real-time quantitative PCR (RQ-PCR) and digital PCR (dPCR)

RQ-PCR is a well-known and described method of t(11;14) MRD quantification in mantle cell lymphoma (MCL) and is applicable in the great majority (95%) of patients suffering from this disease (64), but in DLBCL most tumors do not display a chromosomal rearrangement that can be monitored by this technique. RQ-PCR of the Ig genes has been explored in DLBCL and was suitable in only 23% of the 155 patients (65), but patient-specific allele-specific oligonucleotide (ASO) primer development requires a time consuming and labor-intensive process and common somatic hypermutation restricted its relevance.

dPCR is a fast, easy and inexpensive technology that requires little DNA and is useful for plasma ctDNA somatic mutation testing. This was established in two studies in DLBCL (47,48), in which the detection of hotspot point mutations was directly quantified in the patients' tumor and plasma, without the need for a calibration range as for RQ-PCR.

Moreover, the use of the droplet dPCR technique could be used as a diagnostic tool in certain forms of rare and difficult-to-biopsy DLBCL, such as Primary Central Nervous System Lymphoma (PCNSL) or PMBL. Specifically, these subtypes include (I) PCNSL with *MYD88* L265P mutation detection (66,67), (II) PMBL with *XPO1* E571K mutation detection (48,68) and (III) intravascular large B cell lymphoma with *MYD88* L265P and *CD79B* Y196 mutations (69). However, these somatic mutations are not specific for these lymphoma types and a moderately conserved histology, at a minimum, will be mandatory to make a suitable first diagnosis in addition to mutational screening.

Nevertheless, the sensitivity threshold and the reproducibility of this technique are not yet well known, and it is not appropriate for determining MRD using single point mutations in cases of sub clonality. This is particularly true in the case of actionable alterations because an effective treatment might eradicate those subclones, while mutation-negative clones could persist yet be undetectable by this assay.

Multicolor flow cytometry (MFC)

Flow cytometry-based evaluation of blood lymphocytes may be useful to clarify the prognosis of newly diagnosed DLBCL patients. Specifically, increased numbers of B cells and decreased levels of T-regs and apoptotic cells after treatment might predict a poor clinical outcome in patients treated with RCHOP (70). To be clinically validated, MFC needs a pattern of cell surface markers unique to the lymphoma at hand, and a high enough level of circulating disease (typically >10^{-5} of the assayed population) which render this technique more appropriate for lymphoid disease with leukemic phase, such as CLL of MCL (71), and not relevant for DLBCL. In addition, there is currently

no inter-laboratory standardization for flow cytometry techniques, which limits applicability in routine practice outside of expert centers.

Which timing for molecular response assessment and MRD follow-up testing?

Numerous studies demonstrating the relevance and feasibility of MRD in DLBCL are now available, but none specify the best timing for analyzing the molecular persistence of VDJ rearrangements in blood or of somatic mutations in ctDNA in order to draw the most clinically relevant conclusion. In addition, the appropriate blood sampling rate for post-therapeutic MRD monitoring of patients in CR, in order to detect relapse before standard imaging, is not yet known. Serial testing is a major advantage of ctDNA compared to other biomarkers but it is unclear as of today how to best utilize these non-invasive serial measurements. Kurtz *et al.* (72) attempted to answer this question by profiling 468 samples from 125 patients obtained during their first three cycles of immunochemotherapy by CAPP-seq method. Before treatment, ctDNA was detectable in 98% of subjects; after treatment start, ctDNA levels changed promptly, with a 2-log (i.e., 100-fold) decrease after one cycle, called "early molecular response" (EMR), firmly associated with positive outcome. ctDNA levels continued to drop during cycle 2, revealing a distinct threshold of a 2.5-log decrease, called "major molecular response" (MMR) that also layered patients for EFS (HR: 8.6, 95% CI: 2.2–33, P=0.002). Next, the authors incorporated serial ctDNA measurements with established risk-factors to propose a pattern to predict a personal's disease risk. This model—the Continuous Individualized Risk Index (CIRI)—supplies a personalized estimate of disease risk over time that can classify single patient relapse and survival probabilities. Dynamic risk assessment of MRD is thus probably broadly appropriate and could lead future personalized therapeutic approaches.

Attitude of clinicians with MRD results in DLBCL

Currently, MRD is only used in DLBCL for research and prognosis purposes. Nevertheless, recent advances and numerous publications around MRD suggest that the clinician will soon need to consider MRD findings for his or her daily patient management practice. Dynamic changes in MRD will likely soon be useful in guiding therapy escalation or de-escalation and be used to design MRD-driven clinical trials that personalize management decisions. However, the success of such an approach demands both a valid and conclusive test (more sensitive than interim PET) as well as an adequate intervention (chemotherapy intensification or targeted therapy introduction) before its generalization. A recent study used both the CAPP-seq method and droplet dPCR in DLBCL patients treated with panobinostat. Of the 14 patients who showed progression of the disease, 10 subjects displayed increasing ctDNA levels, and it appeared that the ctDNA fluctuations were correlated with the response to treatment (73). This was the first clinical trial using ctDNA monitoring to measure the response rate in DLBCL. In light of these results, researchers may consider clinical trials in which therapy is changed during the trial based on the MRD results. Currently, only one phase II clinical trial studying the effect of bispecific blinatumomab on MRD in DLBCL subjects post ASCT is recruiting patients (NCT03298412). The study will estimate the MRD-negative response rate after treatment with blinatumomab in subjects with high-risk DLBCL who are MRD-positive following ASCT. In addition, MRD-based depth of response might be employed as an alternate end point to help the appraisal of novel treatments. Targeted therapies such as ibrutinib or immunotherapy such as checkpoint inhibitors are administered until progression of disease, but dynamic MRD monitoring during therapy may enable the safe interruption of treatment for selected patients, or help to inform physicians about inadequate response to immunotherapy, which is not easily analyzable by standard PET imaging (74), with frequent "indeterminate response" due to flare/pseudo-progression under PD-1/PDL-1 inhibitors. Nevertheless, we wish to emphasize that although it may be a relevant powerful prognostic factor, we still do not have therapeutic algorithms to improve prognosis in patients who do not achieve MRD negative CR.

An additional critical application of MRD is in post-CR surveillance. It is well established that the results of routine blood tests such as lactate dehydrogenase (LDH) measured during scheduled clinical visits do not reveal DLBCL recurrence before clinical symptoms emerge (75), but these tests are still regularly performed during routine patient follow-up. In addition, PET imaging has not demonstrated its clinical utility in the systematic follow-up of patients in CR after the first-line treatment because the majority of DLBCL relapses occur outside of the planned follow-up period and the outcomes are not affected (14). Furthermore, PET raises concerns regarding its cost and the frequent irradiation of patients (76,77). Consequently, after obtaining

the first complete remission, DLBCL patients who are not included in therapeutic clinical trials are currently monitored simply by a close clinical examination, usually every 3 months for 2 years and then every 6 months for 3 years. Exploratory evidence proposes that MRD techniques in DLBCL may detect relapse ahead of clinical symptoms and before PET imaging, but whether early MRD-based intervention can modify survival of the patients remains to be confirmed. More data are urgently needed regarding the conditions under which MRD most precisely anticipates relapse and this question must be addressed in future clinical trials.

Conclusions

We are approaching an era of precision and personalized medicine and mounting evidence suggests that NGS-based MRD techniques appear to be the most successful at present, with the Ig-based HTS method being the only one currently marketed, making its application widely possible in lymphoma expert centers participating in major international clinical trials. Nevertheless, although the current enthusiasm around MRD techniques is high, it is necessary to remain cautious before integrating MRD measurement into the routine practice of DLBCL patient management. As there is currently very little data available concerning the clinical validity of MRD results, no recommendation can be made for the immediate application of salvage therapy in the case of molecular relapse in DLBCL and there is an urgent need to measure MRD in all major clinical trials in order to gain more experience and knowledge about the applicability of these techniques on a large scale. To accomplish the hope of MRD monitoring in order to improve the management of patients with DLBCL, it is crucial that pre-analytical constraints, as well as blood collection timing and storage be thoroughly studied to guarantee the robustness and accuracy of the subsequently generated MRD data. We believe that, if these guidelines are followed, results of clinical trials based on MRD-guided care might completely change the treatment decision-making for first-line DLBCL patients in the next five years and move us closer to our goal of a transformative approach to DLBCL diagnosis, monitoring and early detection of clonal evolution and relapse, with the possibility of pre-emptive therapy before occurrence of high tumor burden.

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Footnote

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