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The Emerging Role of Minimal Residual Disease Testing in Diffuse Large B-Cell Lymphoma

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Abstract

Purpose of Review Diffuse large B cell lymphoma (DLBCL) is characterized by clinical heterogeneity that is not fully accounted for by pathologic features. Furthermore, real-time treatment modifications and detection of relapse are typically guided by radiographic imaging modalities which are imperfect. Here, we review the potential utility of minimal residual disease (MRD) assessment for informing treatment decisions and detecting relapse.

Recent Findings The most promising method of MRD detection is based on analysis of circulating tumor DNA in the peripheral blood of patients with DLBCL. This approach can predict outcomes and response to treatment as well as detect relapse prior to clinical signs of recurrent disease. While some studies of MRD in DLBCL have been in the prospective setting, the ability of this technology to alter clinical outcomes is currently unknown.

Summary MRD detection provides a non-invasive way to gather information about DLBCL at various time points throughout the disease course. Its role is evolving and should be incorporated into prospective studies in order to demonstrate an impact on patient outcomes.

Keywords Diffuse large B cell lymphoma · Circulating tumor DNA · Minimal residual disease · Immunoglobulin next-generation sequencing · Next generation sequencing

Introduction

Diffuse large B cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma (NHL), accounting for approximately 30% of all lymphoma cases [1]. It is a clinically and biologically heterogeneous disease. Initial response rates are high with 75–80% of patients achieving a compete response to treatment. However, up to 40% of patients will have disease recurrence or are refractory to frontline therapy [2, 3]. Standard therapy for DLBCL involves R-CHOP or more intensive regimens such as R-EPOCH or R-hyperCVAD for patients with high-risk features such as concurrent translocations of *MYC* and *BCL*-2/*BCL*-6 (double/triple hit lymphoma) [4–6]. Subsequent treatment for relapsed or refractory disease (r/r DLBCL) unfortunately does not yield durable disease control in the majority of patients. Treatment for r/r DLBCL historically relied on intensive therapy with high-dose chemotherapy followed by autologous stem cell transplant (ASCT) [4] but—more recently—is increasingly involving treatment with anti-CD19 chimeric antigen receptor (CAR) T-cells [6, 7]. Future directions will likely include targeted therapies and/or immune-modulating agents that are rapidly being developed. Therefore, there is a significant interest in personalizing therapeutic regimens and detecting minimal residual disease (MRD) to optimize treatment outcomes.

Treatment response and surveillance for DLBCL is typically monitored with serial CT and/or PET scans yet there are long-term risks of radiation exposure, and use of surveillance imaging has never been shown to improve survival [8–11]. In patients that relapse, the revised International Prognostic Index assessed at relapse (IPI-R) has been shown to predict overall survival undergoing ASCT. Because stage of disease is

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a component of the IPI-R, this may indicate that early detection when disease burden is low may be important in determining outcomes in the relapsed setting [12]. Given the difficulty in detecting disease via imaging, MRD could help identify early relapses or treatment failure.

Additionally, MRD may have a role in predicting response to therapy. MRD levels correlate with disease burden [13••], and a study from the National Cancer Institute found that patients with newly diagnosed DLBCL who had undetectable MRD after two cycles of therapy were significantly more likely to be progression-free at 5 years compared to patients with detectible MRD [14••].

Here, we review the methods of detecting MRD, clinical applications, and potential settings for using this emerging technology for future use in management of DLBCL.

Methods of Detecting Minimal Residual Disease

The two primary approaches of measuring MRD in the blood or bone marrow are (1) detecting circulating tumor cells (CTC) by flow cytometry or (2) circulating tumor DNA (ctDNA) using PCR-based amplification or next-generation sequencing (NGS) [15, 16]. Currently for DLBCL, ctDNA is the most well studied and offers greater possibilities than CTC technologies [13••].

Circulating Tumor Cells

Flow Cytometry

Multicolor flow cytometry uses fluorescently labeled antibodies to identify cells of interest. The detection limit for CTC is 10^{-4} to 10^{-5}. This is the method used to detect MRD in acute lymphoblastic leukemia and has been studied in mantle cell lymphoma [16–18]. Flow cytometry is not the primary means of MRD detection in DLBCL as studies in mantle cell lymphoma have shown it is less sensitive than other methods and lacks standardizations [17, 19]. However, it is more widely available than other methods [16].

Circulating Tumor DNA

Circulating tumor DNA is the fraction of cell-free DNA that originates from tumor cells. Physiologic levels of cell-free DNA are present in the plasma after it is released from healthy tissue into the bloodstream through normal cell turnover—via apoptosis or, in the case of stress or injury, via necrosis. ctDNA is released into the bloodstream in the same manner by tumor cells during disease presence. Cell-free DNA is often double-stranded DNA with fragment lengths of 150–200 base pairs and has a short half-life of less than an hour [15, 20]. Given the short half-life of ctDNA and the need to isolate ctDNA from background of normal cell-free DNA, detection is technically challenging and has limited its use until recently.

Polymerase Chain Reaction

Polymerase chain reaction (PCR) is the method of using specific primers to amplify, detect, and quantify, pre-specified DNA sequences. In DLBCL, each tumor has a clonal immunoglobulin gene product which is often the tumor-specific sequence used for PCR. The detection limit is 10^{-5} [15, 16]. The two commonly used techniques are real-time quantitative PCR (RQ-PCR), which utilizes florescent signals and allows for "real-time" quantification, and droplet digital PCR (ddPRC), which separates the sample into an emulsion and increases the precision while decreasing error rates [16, 21]. Pyrosequencing is also being used to detect epigenetic aberrations as well as sequence information. This technique utilizes PCR to amplify promoter genes and then detect methylation [22••, 23•]. Pyrosequencing, as opposed to Sanger sequencing, relies on synthesis rather than termination. It utilizes light generation which occurs when nucleotides are incorporated into DNA and pyrophosphate is released [24]. Pyrosequencing is technically simple; however, it provides shorter DNA reads than Sanger sequencing, is costly, and not universal [25]. While the advantages and limitations differ slightly for each type of PCR method, in general PCR-based methods are less sensitive and more cumbersome than next-generation sequencing [16].

Next-Generation Sequencing

Next-generation sequencing (NGS) targets either a whole-genome or whole-exome so coverage includes many nucleotides and is broader than PCR which targets certain DNA sequences and thus covers fewer nucleotides [15]. Two platforms that detect different genes have been developed and studied in DLBCL. Detection of immunoglobulin genes via ClonoSEQ developed by Adaptive Biotechnologies (referred to as IgNGS) and detection of somatic genetic alterations with ultrasensitive, panel-directed NGS via cancer personalized profiling by deep sequencing (CAPP-Seq) [13••, 14••, 16]. NGS methods are the most sensitive with a detection rate of 10^{-6} and panel-directed NGS appears to be the most sensitive platform [16]. IgNGS can identify an immunoglobulin clone, preferentially from a baseline tumor sample, for subsequent MRD assessment in > 70–80% of patients with DLBCL [13••]. Panel-directed NGS is better at detecting alterations in plasma with somatic alterations detected in 67–87% of plasma samples at diagnosis [16]. IgNGS is a commercially available test for multiple myeloma and ALL with the potential to expand to NHL in the future.

Panel-directed NGS is still limited to research laboratories but will likely soon be commercially available.

Clinical Applications

Early Detection and Diagnosis of Cancer

The diagnosis of DLBCL is established based on histologic characteristics of tissue biopsy and immunophenotyping which cannot be provided by ctDNA. There is however increasing interest to use cell-free DNA as tumor screening and thus early diagnosis. A clinical trial is underway studying the use of cell-free DNA testing in patients with newly diagnosed malignancies of all types and normal controls to develop a cell-free DNA test with high specificity to use as a screening tool (NCT02889978). Preliminary results show whole-genome bisulfite sequencing for methylation is the most sensitive test for identifying several tumor types including lymphoma [26•]. A group in China used genome-wide sequencing of plasma DNA and methylation profiles of different tissues to study the major tissue contributors to the cell-free DNA pool. A patient eventually diagnosed with follicular lymphoma had cell-free DNA with an elevated contribution of DNA from B-cells [27]. This same finding was seen in patients with hepatocellular carcinoma having an elevated DNA contribution from the liver [27]. Amat et al. used parallel sequencing of maternal plasma cell-free DNA in over 4000 pregnant women and found 3 patients with aberrant genome profiles. These three patients were eventually diagnosed with ovarian cancer, follicular lymphoma, and Hodgkin lymphoma [28]. Despite these interesting reports, using cell-free DNA as an early diagnostic tool is the furthest clinical application from reality.

Tumor genotyping and cell-of-origin (COO) classification for DLBCL are two applications for ctDNA in the diagnostic setting. While we obtain molecular profiling and COO from tumor tissue, ctDNA may be a complementary test. Tissue biopsies only identify clones in the biopsy site while ctDNA can capture multiple clones and thus give information regarding genomic heterogeneity [15]. In a prospective, observational study that evaluated ctDNA in 30 newly diagnosed DLBCL patients, CAPP-seq was used for tumor genotyping. This study found that pre-treatment ctDNA correctly discovered DLBCL associated mutations with > 20% variant allele frequency in tumor biopsies with high sensitivity (> 90%) and specificity (100%) [29••]. Another study found that 95% of FISH confirmed translocations in *BCL2*, *BCL6*, and *MYC* were also detected in ctDNA [30••]. Importantly, we have seen disease-associated mutations detected in plasma samples that were not found in the tumor biopsy suggesting that ctDNA may provide more information than tumor biopsies alone [29••, 30••].

Prognosis and Disease Monitoring

DLBCL is a diverse disease with inadequate prognostic tools. Clinically, the IPI is still used to risk stratify patients, while biologically COO and presence/absence of double/triple hit lymphoma are used. Genomic complexity has been further subclassified by two independent and seminal papers that identify additional subgroups of DLBCL [31, 32]. COO is a known prognostic marker but can be challenging to elicit correctly [33–35]. Although COO was originally developed using gene expression profiling, the current use of COO for DLBCL in routine clinical practice is most commonly determined by immunohistochemistry algorithms, with the most common being the Hans algorithm [36]. Scherer and Kurtz et al. were able to show that NGS of pretreatment plasma samples was 80% concordant with the currently used Hans algorithm. Additionally, the authors found that using DNA genotyping to classify COO was more accurate at predicting patient outcomes than the Hans algorithm [30••]. For now, tumor biopsies remain the gold standard for tumor genotyping and COO classification; however, if ctDNA can provide more accurate and reliable information, it may become a complementary test.

ctDNA can also be used to monitor the disease course of DLBCL. Kurtz et al. prospectively evaluated immunoglobulin high-throughput sequencing (Ig-HTS) from peripheral blood samples in 75 patients with DLBCL throughout the course of their disease. The authors compared plasma Ig-HTS to lactate dehydrogenase (LDH) as LDH has been show to correlate with disease burden and predict adverse clinical outcomes. When compared to PET/CT at diagnosis or relapse, plasma Ig-HTS had a sensitivity of 88% versus LDH which had a sensitivity of 59% for disease detection ($P = 0.01$). Plasma Ig-HTS also correlated with metabolic tumor burden on PET/CT which correlates with outcomes [13••]. This same group proceeded to utilize deeper sequencing via CAPP-Seq and found similar results. In addition, they found ctDNA levels at time of diagnosis independently correlated with a worse progression-free survival in a multivariate analysis [30••, 37••].

A few studies have investigated the role of methylation through pyrosequencing with provocative results. Kristensen et al. evaluated plasma samples from 74 patients with DLBCL at the time of diagnosis. They found that the 5-year overall survival was significantly lower for patients carrying aberrant methylation of promoter genes *DAPK1* and *DBC1*. Additionally, *DAPK1* methylation status correlated with stage. Interestingly, patients who had methylated *DAPK1*, lost methylation status with treatment, and then regained methylation status, died shortly thereafter [22••]. In another study, Wedge et al. found that global hypomethylation detected in cfDNA was the strongest risk factor for survival in a multivariate analysis with a hazard ratio 11.87 (CI 2.80–50.20) [23•].

Guiding Therapy and Monitoring Response

Using ctDNA to guide initial therapy selection, duration of therapy, and adjustments to therapy is an active area of research. In regard to initial therapy selection, success of several targeted therapies hinges on mutational status, e.g., *MEF2B* mutations predicting response to panobinostat, *MYD88* mutations predicting response to ibrutinib, *XPO1* alterations predicting sensitivity to selective inhibitors of nuclear exports, and *EZH2* activating mutations predicting response to *EZH2* inhibitors [38••, 39–41]. Using digital PCR, Camus et al. were able to show that detecting *XPO1*, *EZH2*, and *MYD88* mutations in plasma ctDNA could be achieved with a high sensitivity [42]. Prospective trials are needed to show that these data could be used to individualize DLBCL treatment.

Several studies have prospectively followed ctDNA throughout treatment, and some clinical trials have started to add ctDNA as a correlative study which has provided informative but not yet practice changing data. Rossi et al. used panel-directed NGS to analyze 127 plasma samples longitudinally in 50 DLBCL patients receiving R-CHOP. They obtained plasma samples at diagnosis, prior to each R-CHOP cycle, at the end of treatment, and during remission. The investigators found rapid clearance of mutations in responders but lack of mutation disappearance in resistant patients [29••]. The Stanford group analyzed ctDNA via deep sequencing NGS in 127 patients before and during therapy. They found that patients achieving an early molecular response (2 log decrease in ctDNA after 1 cycle of therapy) or a major molecular response (2.5 log decrease in ctDNA after 2 cycles of therapy) had superior event-free survival at 24 months compared to those that did not achieve these milestones (83% vs 50%, $P = 0.0015$; 82% vs 46%, $P < 0.001$, respectively) [37••]. Similar results were seen by Roschewski et al. when they monitored ctDNA via IgNGS in 108 patients with newly diagnosed DLBCL. Five-year time to progression was 41.7% in patients with MRD positivity and 80.2% in patients with MRD negativity after two cycles of therapy [14••]. A phase 2 study evaluating panobinostat with or without rituximab in relapsed DLBCL found that ctDNA levels at day 15 were strongly associated with lack of response with a sensitivity of 71.4% and specificity of 100% [38••]. These studies raise the possibility of monitoring ctDNA throughout treatment and switching therapy if ctDNA levels are not decreasing or mutations are not being cleared.

Tracking Clonal Evolution

The role for ctDNA in tracking clonal evolution will be important in the era of targeted therapy and mutation-driven resistance mechanisms. As mentioned above, Rossi et al. used panel-directed NGS to analyze 127 plasma samples longitudinally in patients receiving R-CHOP. In addition to mutational status during treatment being predictive of response, they were able to identify new mutations in treatment-resistant patients [29••]. The Stanford group also showed the ability to monitor clonal evolution via panel-directed NGS. They followed plasma samples in patients with indolent lymphomas experiencing transformation, those without transformation, and with relapsed/refractory DLBCL. There was a higher fraction of emergent variants in transformed follicular lymphoma patients compared to the other groups [30••]. These results suggest that assessment of clonal evolution is a promising tool for the future, but additional information is still needed to fully understand how to utilize this information in clinical practice.

Relapse Detection

To monitor for disease recurrence, DLBCL patients typically undergo regularly scheduled surveillance imaging with CT and/or PET scans as well as clinical assessment. The reliability of imaging is of unproven benefit and has never been shown to improve survival. Due to the costs, anxiety, and radiation exposure, there is increasing recognition that surveillance imaging may be suboptimal in patients with DLBCL, particularly beyond the first year after completion of therapy [8–11].

ctDNA may be a better tool to monitor for relapse as it is non-invasive and can detect low level of disease well before radiographic of clinical relapse. Investigators at the National Cancer Institute utilized Ig-HTS to monitor ctDNA in 107 DLBCL patients who achieved a complete remission after initial therapy. For patients who developed detectable ctDNA compared to those who did not, the hazard ratio for clinical disease progression was 228 (CI: 51–1002). Circulating tumor DNA detection during surveillance had a positive predictive value of 88.2% and a negative predictive value of 97.8%. Importantly, ctDNA detected disease recurrence at a median of 3.5 months prior to clinical evidence of disease [14••]. Scherer and Kurtz et al. monitored ctDNA via panel-directed NGS in 92 patients with DLBCL of which 11 achieved a complete response to initial therapy and eventually relapsed. MRD positivity was detected in all 11 patients and prior to relapse in 8 patients (73%). Interestingly, 2 patients had isolated CNS disease at relapse and ctDNA was still detected. ctDNA detected relapse at a mean of > 6 months before imaging detection [30••]. In the previously discussed study by Rossi et al., 1 of 2 patients who initially responded to R-CHOP but had an early relapse had detectable tumor DNA present in plasma despite being in complete remission [29••]. Overall, ctDNA shows great promise as a novel way to detect relapse earlier than currently used modalities and we envision it being used in conjunction with or in place of imaging in the near future.

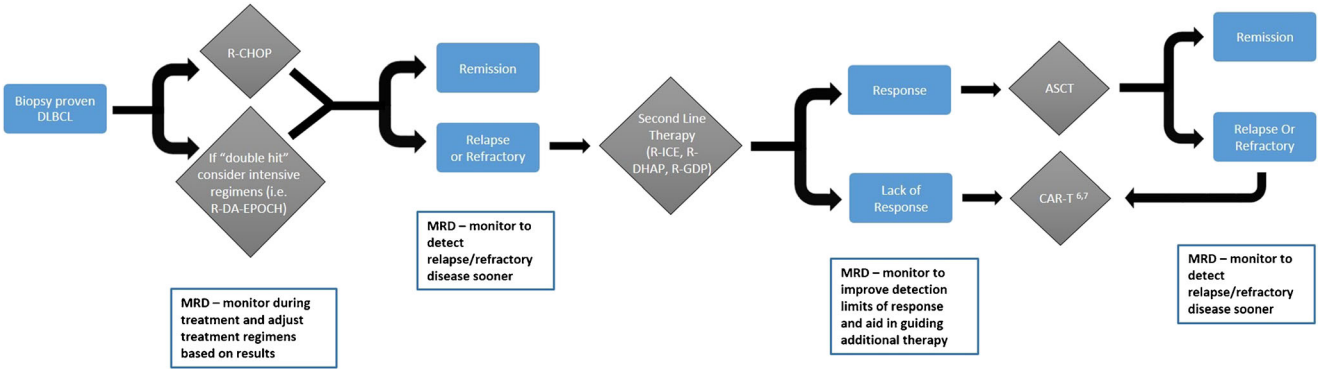

Fig. 1 Treatment schema in advanced stage DLBCL and proposed role of MRD. Abbreviations: ASCT, autologous stem cell transplantation; CAR-T, chimeric antigen receptor T-cells; DLBCL, diffuse large B-cell lymphoma; MRD, minimal residual disease; R-CHOP, rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone; R-DA-EPOCH, rituximab plus etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin; R-DHAP, rituximab plus dexamethasone, high-dose cytarabine, and cisplatin; R-GDP, rituximab plus gemcitabine, cisplatin, and dexamethasone; R-ICE, rituximab plus ifosfamide, carboplatin, and etoposide.

Future Directions

While there are several informative studies with exciting results, the role of ctDNA in DLBCL diagnosis, treatment, and surveillance is early in development. It remains unclear if using MRD to tailor treatment will affect outcomes. The most impactful role may be in tailoring therapy. In Fig. 1, we show the typical disease course and treatment paradigm of advanced stage DLBCL and highlight the areas where ctDNA could be practice changing. Monitoring ctDNA during upfront treatment may direct us to alter treatment. For example, if MRD is not achieved after 3 cycles of R-CHOP, perhaps changing treatment to more intensive chemotherapy or alternative approaches such a CAR-T cell therapy may be more effective than continuing R-CHOP. Alternatively, if MRD is achieved, de-escalation of therapy may be a viable treatment approach, particularly in elderly patients who are at high risk for treatment-related complications.

ctDNA may help detect relapsed disease sooner in both the upfront and relapsed setting allowing subsequent therapy to be initiated before clinical progression. In the relapsed setting, response to therapy may be improved when tumor burden is low; thus, early detection is important [12]. Additionally, ctDNA could be monitored after second-line therapy is given to determine if patients with r/r DLBCL should proceed with transplant. For example, if MRD is achieved after second-line therapy, patients could proceed with ASCT, but if it is not achieved, they instead proceed with CAR-T cell therapy. For patients who receive CAR-T cells, MRD has been used to detect relapse and could potentially guide early intervention such as use of immunomodulatory agents [43•]. As different types of PCR- and NGS-based assays are currently being studied, we need to further optimize ctDNA analysis [15, 16, 18]. Ultimately, MRD needs to be incorporated into clinical trials and studied in a prospective setting.

Conclusions

DLBCL is a curable disease yet many patients relapse after initial therapy. Because the clinical heterogeneity of the disease course is not predictable based solely on clinical or pathologic grounds, MRD-guided assessments may allow for response-adapted therapy and may aid in monitoring patients in remission for relapse. The data reviewed here suggest ctDNA may play a role in diagnosis, treatment decisions, and disease monitoring in the near future.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- • Of importance
- •• Of major importance

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30.•• Scherer F, Kurtz DM, Newman AM, Stehr H, Craig AF, Esfahani MS, et al. Distinct biological subtypes and patterns of genome evolution in lymphoma revealed by circulating tumor DNA. Sci Transl Med. 2016;8(364):364ra155. https://doi.org/10.1126/scitranslmed.aai8545 **Utilizing CAPP-Seq the Stanford group evaluated ctDNA in 92 lymphoma patients and 24 healthy patients. ctDNA levels in plasma at diangosis were predictive of outcomes, could idenify cell of origin, and could be used to track clonal evolution**.
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