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B-CELL NHL, T-CELL NHL, AND HODGKIN LYMPHOMA (J AMENGUAL, SECTION EDITOR)

Updates on Circulating Tumor DNA Assessment in Lymphoma

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Abstract

Purpose of Review The use of circulating tumor DNA (ctDNA) for the purposes of diagnosis, prognosis, assessment of treatment response, and monitoring for relapse is a new and developing field in lymphoma. This review aims to summarize many of the most recent advances in ctDNA applications.

Recent Findings Recent studies have demonstrated the use of ctDNA assessment across many lymphoma subtypes including diffuse large B-cell lymphoma, follicular lymphoma, Hodgkin lymphoma, and T-cell lymphoma. In addition, many novel applications of ctDNA assessment have been described such as the development of new prognostic models, investigation of clonal evolution and heterogeneity, early assessment of treatment response, and prediction of response to targeted therapy as a form of personalized medicine.

Summary The use of ctDNA has been shown to be feasible across many lymphoma subtypes and has shown significant promise for several new applications. Additional studies will be needed to validate these findings prior to routine use in clinical practice.

Keywords Circulating tumor DNA · Minimal residual disease · Non-Hodgkin lymphoma · Hodgkin lymphoma

Introduction

An accurate assessment of lymphoma tumor burden is essential to providing the best care for patients with lymphoma. However, despite recent advances in treatment of lymphoma, our methods for assessing the disease have remained largely unchanged in several decades. Current methods include biopsies, computed tomography (CT) scans, and positron emission tomography (PET) scans, but these all have limitations. Tumor biopsies often require invasive procedures that expose patients to risk of harm and complications. Furthermore, tumor biopsies only offer information about the lymphoma at one specific time point and only about the small area that was biopsied. If more tumor information is needed during the course of treatment, additional invasive procedures are needed. PET-CT is often used to assess disease burden and to evaluate treatment response. However, this relies on the presence of macroscopic tumor burden in order to detect areas of lymphoma involvement. Due to this limitation, patients can be incorrectly labeled as having a complete remission even though microscopic disease may still be present. Although an improvement over historical techniques, PET-CT has suboptimal specificity, can be associated with high false positive rates in certain settings [1–3], and is subject to variability in interpretation by the reading radiologist [4]. In addition, techniques like PET-CT have relatively high costs and expose patients to radiation that can result in downstream negative health consequences [5–7]. In order to improve upon our current ability to detect and measure lymphoma, the development of novel disease assessment techniques is necessary.

One such novel technique is the detection and measurement of lymphoma in the peripheral blood. This is accomplished by measuring either intact tumor cells or circulating free DNA from the tumor. The term circulating tumor cells (CTCs) refers to intact lymphoma cells that are shed from primary tumors into the bloodstream. Circulating tumor DNA (ctDNA) is fragmented DNA that originates from tumor cells and is released into the bloodstream after the cells have undergone apoptosis or necrosis. Although both CTCs and ctDNA have been

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demonstrated to have potential applications in lymphoma, the majority of recent studies have focused primarily on ctDNA assessment. We have previously reviewed the data regarding CTC and ctDNA assessment in patients with diffuse large B-cell lymphoma (DLBCL) and mantle cell lymphoma (MCL), including the use of minimal residual disease assessment as a means of diagnosis, to determine prognosis, to assess treatment response, and to detect relapse [8•]. Briefly, prior studies have shown that quantitative levels of ctDNA have prognostic value in DLBCL and correlate with clinical stage [9•], LDH [9•, 10, 11], international prognostic index (IPI) score [9•], CT [12], and PET-CT [13]. Following treatment, ctDNA was shown to be more sensitive for residual disease than CT or LDH [14]. After treatment of DLBCL, the presence or absence of ctDNA in patients with imaging-based remission at the end of treatment was associated with a high positive and negative predictive value for lymphoma recurrence [15]. In one study, the presence of ctDNA was more specific for relapse than PET-CT [13]. Here, we aim to highlight more recent advances and applications of ctDNA in patients with DLBCL and other lymphoma subtypes.

Methods

Here, we provide a brief overview of the methods used to assess lymphoma burden in the blood. We have previously reviewed the methods for ctDNA assessment of lymphoma in greater detail, including advantages and limitations of these methods [8•].

Polymerase Chain Reaction-Based Methods

Polymerase chain reaction(PCR)-based minimal residual disease assessment uses PCR primers designed to detect the presence of tumor-specific genomic regions of interest. In lymphoma, the regions of interest typically involve breakpoint fusion regions, fusion-gene transcripts, aberrant genes, immunoglobulin or T-cell receptor gene rearrangements, or allele-specific oligonucleotides. Through the PCR reaction, the presence of the tumor-specific DNA sequence can be detected and amplified, and ultimately quantified. For allele-specific oligonucleotide PCR, specific primers for the region of interest are generated for each individual patient. Real-time quantitative PCR (RQ-PCR) incorporates fluorescent signals during the amplification phase which can be measured in "real-time" with each PCR cycle, eliminating the need for post-PCR processing [16]. This method has a detection limit of approximately 10^{-5} or detection of 1 lymphoma cell in 100,000 white blood cells. Droplet digital PCR (ddPCR) is a variation of PCR in which the sample is separated into an emulsion with many compartments within oil droplets, where individual reactions carried out separately within each droplet. This allows for more precise quantification of DNA and decreases error rates [17].

Immunoglobulin or T-Cell Receptor Gene Next-Generation Sequencing

Immunoglobulin next-generation sequencing (IgNGS) and T-cell receptor gene next-generation sequencing (TCR-NGS) involve a two-step process: first, the clonal tumor sequences must be determined from a tumor-containing tissue or blood sample, and then, subsequent blood samples can be tested to determine whether there are CTCs or ctDNA present. The number of CTCs or ctDNA molecules present can be quantified in relation to the total number of sequencing reads in a specimen. Ig or TCR-NGS utilizes a PCR reaction with universal primers to amplify the *Ig* or *TCR* genes. The amplified gene products are then sequenced with NGS and any sequence that constitutes 5% or greater of the Ig or TCR sequences is considered a tumor "clonotype." Then the burden of tumor-specific clones present in a blood sample can be assessed using the same two-step process with a sensitivity of $\sim 10^{-6}$ or 1 lymphoma molecule in 1,000,000 diploid genomes sequenced.

Cancer Personalized Profiling by Deep Sequencing

Like PCR-based methods and Ig/TCR-NGS, cancer personalized profiling by deep sequencing (CAPP-Seq) is used to identify tumor-specific genomic regions of interest in a tissue sample. The differentiating feature of CAPP-Seq is the application of NGS using a panel of single-nucleotide variants, insertions or deletions, and translocations that are recurrently altered in a disease of interest to allow for the concurrent evaluation of several genes (as compared to only Ig or TCR). Normal and mutant sequencing reads are compared to allow for quantification of the burden of tumor-associated sequences present. There is a high concordance rate between genomic abnormalities detected in tumor samples and blood drawn when the malignancy is active; therefore, it may be possible for CAPP-Seq to be applied directly to blood samples for liquid genotyping [18, 19•].

Circulating Tumor DNA Levels Across Lymphoma Subtypes

The amount of ctDNA shed by a lymphoma appears to vary depending on lymphoma subtype [11]. In one study evaluating ctDNA levels across a range of lymphomas, mediastinal gray zone lymphomas and primary mediastinal B-cell lymphomas had the highest levels of ctDNA. Other aggressive lymphomas, including diffuse large B-cell lymphoma and transformed follicular lymphoma, had lower but still high levels of ctDNA. Classical Hodgkin lymphoma and follicular lymphoma had the lowest levels. Renal function did not affect ctDNA levels, indicating that accurate ctDNA detection is possible in patients with renal failure. A previous study evaluating overall levels of

cell-free DNA among patients with different lymphoma subtypes compared with healthy controls similarly showed that cell-free DNA levels were lowest in FL [20].

Diffuse Large B-Cell Lymphoma

Kurtz et al. [10] demonstrated that ctDNA can be an accurate predictor of tumor burden and treatment outcome in newly diagnosed patients with DLBCL. In this study, ctDNA was assessed in 183 patients from six centers using CAPP-Seq. Pre-treatment ctDNA was detected in 97% of patients. Patients with higher levels of pre-treatment ctDNA had correspondingly higher stage disease ($P < 0.0001$). CtDNA levels also varied between patients in different IPI risk groups with higher IPI groups having a higher concentration of ctDNA (one-way ANOVA, $P < 0.0001$). There was also a significant correlation between metabolic tumor volume on PET scan and ctDNA level ($P < 0.0001$). CtDNA concentration significantly correlated with both event-free survival (EFS) and overall survival (OS). Patients with a pre-treatment ctDNA level above the median had a significantly worse EFS compared to those below the median ($P = 0.005$, HR 2.7, 95% CI 1.4–4.6). Given the strong correlation that was found between ctDNA and traditional clinical prognostic factors, this study demonstrates that ctDNA can be an effective tool for prognostication in the initial treatment of DLBCL.

In another study, serial ctDNA measurements were used to develop a dynamic model for personalized disease risk assessment. A total of 125 patients were evaluated with serial ctDNA monitoring using CAPP-Seq during the first three cycles of treatment. Prior to treatment, ctDNA was detected in 98% of the patients. Similar to the previous study, pre-treatment ctDNA level was again found to correlate with EFS. After one cycle of treatment, patients who achieved a 2-log or greater reduction in ctDNA had a significantly better EFS at 24 months than those who did not (HR 24, 95% CI 6.6–89, $P < 0.0001$). Likewise, patients who achieved a 2.5-log or greater reduction in ctDNA after two cycles also had a significantly better EFS (HR 8.6, 95% CI 2.2–33, $P = 0.002$). Serial ctDNA measurements were then used to develop a model for personalized risk assessment, termed the continuous individualized risk index (CIRI). CIRI was better than the IPI at predicting 24-month EFS (AUC 0.64 vs 0.79; net reclassification improvement 0.47, $P = 0.02$) and OS (AUC 0.56 vs 0.84; net reclassification improvement 0.74, $P = 0.004$) [21]. This study demonstrates an important advantage of ctDNA assessment compared to traditional disease assessment techniques—the ability to easily perform repeated testing for serial monitoring, which can be applied to dynamically assess response to therapy and the risk of adverse outcome throughout the course of treatment.

Preliminary data demonstrates that ctDNA assessment may be useful in the detection of a rare subtype of DLBCL, intravascular large B-cell lymphoma (IVLBCL). In IVLBCL, lymphoma cells are demonstrated to be infiltrating into blood vessels on a tissue biopsy. Because patients with this disease often lack a focal tumor mass, diagnosis, and monitoring of this entity can be difficult. Therefore, the prospect of detecting IVLBCL via the peripheral blood is appealing. In 9 patients with IVLBCL, targeted NGS sequencing for *CD79B*, *MYD88*, *PIM1*, *PRDM1*, *BTG2*, *B2M*, *CARD11*, and *TNFAIP3* was performed on blood samples using the Ion Torrent Personal Genome Machine (Thermo Fisher Scientific). Analysis of c.794T>C, p.Leu265Pro, (*L265P MYD88*) *MYD88* mutation was performed using ddPCR. At least one mutation in *CD79B*, *MYD88*, *PIM1*, *PRDM1*, or *BTG2* was found in all 9 patients via ctDNA compared with 5 of the patients via tumor-derived DNA (tdDNA) such as from bone marrow or random skin biopsies. Variant allele frequencies were also significantly higher in ctDNA than for tdDNA (mean, 39.8% vs 2.6%; $P < 0.001$) [22]. These findings suggest that ctDNA may be a useful method for monitoring and genotyping IVLBCL and thus warrants further investigation.

Somatic copy number alterations (SCNAs) have been shown to have prognostic significance in DLBCL [15, 23]. However, detection of SCNAs can be difficult as it often requires invasive tissue biopsy. Therefore, Jin et al. [24] evaluated the use of CAPP-Seq to detect and monitor SCNAs in the peripheral blood. Pre-treatment blood samples from 209 patients with DLBCL ($n = 168$), primary mediastinal B-cell lymphoma ($n = 21$), and classical Hodgkin lymphoma ($n = 20$) were analyzed and compared to 139 sequenced tumor biopsies. The sensitivity for detecting SCNAs was 77% when ~ 1% of the plasma contained circulating tumor, and 95% when ~ 5% of the plasma had circulating tumor. In DLBCL, copy number gains were detected in *BCL2* (24%), *PD-L1* (23%), *BCL6* (19%), and *MYC* (12%), and copy number losses were detected in *TP53* (27%) and *CDKN2A* (19%). These were 85% concordant with matching tumor biopsies. Similar to other previously reported studies, it was found that changes in copy number had prognostic significance. The copy number gains in *MYC* and *BCL2*, and loss of *TP53* predicted inferior survival ($P < 0.0001$, $P = 0.0013$, $P < 0.0001$, respectively). These findings demonstrate the feasibility of detecting SCNAs in the peripheral blood as well as the predictive value of SCNAs in regard to survival. Given the ease of obtaining peripheral blood samples, as opposed to tissue biopsy, this could become a useful method for determining tumor biology and guiding treatment decisions.

Follicular Lymphoma

Multiple recent studies have validated the ability to perform ctDNA assessment in follicular lymphoma (FL) as well as the prognostic value of ctDNA. The utility of IgNGS to measure minimal residual disease (MRD) in follicular lymphoma was

assessed in a study of 113 patients. IgNGS was used to sequence IGH clonotypes of peripheral blood and bone marrow samples. An IGH clonotype was detected in 105/113 patients (93%) with similar frequency between peripheral blood and bone marrow samples. Results were validated by comparison with either *t*(14;18) RQ-PCR ($n = 38$) or IGH ASO RQ-PCR ($n = 32$). Post-induction MRD assessment by IgNGS correlated well with RQ-PCR ($r^2 = 0.599$, $P < 0.00001$). In a subset of 43 patients, MRD response measured by IgNGS was a good predictor of PFS at a median follow-up of 6.2 years, with patients deemed to be MRD negative having a median PFS of 11 years compared to 2.2 years for those who were MRD positive ($P = 0.0085$). These results suggest that IgNGS of peripheral blood and bone marrow may be a feasible method of determining MRD in follicular lymphoma [25].

Many recent studies assessing the use of ctDNA in follicular lymphoma have focused on the assessment of ctDNA as a prognostic tool prior to treatment. Soo et al. [26] evaluated the use of ctDNA in comparison to tumor biopsies in the application of the m7-FLIPI risk score by the use of CAPP-Seq to detect mutations in *CREBBP*, *EZH2*, *ARID1A*, *MEF2B*, *FOXO1*, *EP300*, and *CARD11*. They found a 100% concordance of m7-FLIPI risk category when comparing two separate lymph node biopsies obtained at diagnosis ($n = 5$). There was also a 100% concordance of m7-FLIPI risk category when comparing tumor biopsy samples from the time of diagnosis and at the first relapse ($n = 36$). However, concordance of the risk categories between tumor biopsy and plasma samples obtained at the time of diagnosis was 78.6% ($n = 14$). They reported no significant difference in concordance of the individual m7 genes, however when evaluating mutation pairs, concordance of paired samples was lowest with *EZH2* ($P = 0.028$). The assessment of the m7-FLIPI through ctDNA in place of a tumor biopsy would be helpful in practice, but this study demonstrates that further study is necessary.

A retrospective study of 133 patients evaluated the prognostic value of pre-treatment CTCs and ctDNA as compared with baseline total metabolic tumor volume (TMTV) measured by PET-CT. DNA was analyzed using PCR with 5′ primers for *BCL2* mixed with 3′ primers for the Jak homology (JH) domains *JH1*, *JH2*, *JH3*, *JH4*, *JH5*, and *JH6*. Both CTCs and ctDNA were found to correlate with TMTV ($P < 0.0001$ and $P < 0.0001$, respectively), suggesting that both may be effective means of assessing tumor volume. Furthermore, the authors were able to identify threshold values for each of the methods that predicted poorer outcomes. At a median 48-month follow-up, patients with pre-treatment TMTV > 510 cm^3 ($P = 0.0004$), CTCs > 0.0018 PB cells ($P = 0.03$), or circulating free DNA (cfDNA) > 2550 equivalent-genome/mL ($P = 0.04$) had a lower 4-year PFS. Interestingly, when these values were applied to 82 patients who received maintenance therapy, high ctDNA and TMTV were predictive of lower PFS but CTCs did not have prognostic value [27]. A similar observation was made in 92 patients from the PRIMA study where detection of pre-treatment CTCs predicted decreased PFS and time to next treatment in patients who did not receive maintenance rituximab, but this observation was not present in patients who were given maintenance rituximab [28].

Sarkozy et al. [29] aimed to characterize the heterogeneity of follicular lymphoma in a retrospective evaluation of 34 patients registered in the PRIMA study. Using IgNGS to detect unique rearrangements of *IGH* and *IGK* genes, they were able to identify tumor clonotypes for tracking in 29 patients out of 34 from diagnostic tumor samples. Prior to treatment, ctDNA was detected in 25 out of the 29 patients (86%) who had a clonotype identified in the diagnostic tumor sample. However, IgNGS failed to detect ctDNA in 4 of 29 patients (14%) who had an adequate clonotype detected in the tumor sample, suggesting that IgNGS may require further refinement in order to be applicable to all patients with FL. Patients who had a ctDNA level above the median of 39,720 clonotypes per million diploid genome at the time of diagnosis had a significantly worse PFS after chemoimmunotherapy compared to those below the median (median 15.3 months vs. not reached, $P = 0.004$). However, in the patients who received rituximab maintenance, this prognostic impact of pre-treatment ctDNA disappeared, as no significant difference was seen between the 8 patients with high ctDNA level and the 4 patients with low ctDNA levels ($P = 0.18$). Interestingly, in 15 patients who had more than one lymphoma clonotype in their tumor biopsy at presentation (range 2 to 71 subclones), it was found that those with a high number of subclones detected (> the median of 9) trended towards a higher 6-year PFS than those with a low number of subclones (< 9) (83% vs 44%; $P = 0.12$). The authors postulated that the higher number of subclones could reflect more occult disease that developed a higher mutation rate over a longer period of time, possibly resulting in greater immune response to the tumor cells. However, it was found that in 16 patients who had multiple subclones detected in ctDNA (range 2 to 20 subclones), there was no difference in PFS based on the number of subclones (more or less than 2 subclones) ($P = 0.85$). Given the small number of patients in each of these groups, further exploration of the association between degree of clonality and treatment of outcome is warranted.

One promising application of ctDNA is to utilize non-invasive genotype detection to develop personalized treatment strategies. Daigle et al. [30] used IgNGS on pre-treatment ctDNA and archived tumor DNA to assess for mutations in DLBCL ($n = 122$) and FL ($n = 63$) that could predict treatment response to tazemetostat, an *EZH2* inhibitor in phase 2 clinical development for relapsed or refractory non-Hodgkin lymphoma. The most common mutations identified in the samples were *KMT2D* (65%), *BCL2* (55%), *CREBBP* (36%), *HIST1H1E* (23%), *EZH2* (14%), and *EP300* (11%). In

DLBCL, mutations in *CREBBP* had the greatest association with treatment response ($P < 0.10$), and *EZH2* had the greatest association in FL ($P < 0.07$), although neither were statistically significant. Conversely, mutations in 10 genes including *PIM1*, *BCL6*, *TP53*, and *HIST1H1E* were associated with the lack of response to tazemetostat and progression of disease (all $P < 0.06$) in both DLBCL and FL. However, only variations in *BCL2* demonstrated statistical significance through multiple test corrections (adjusted P value = 0.04). These findings demonstrate a novel application of ctDNA in the treatment of lymphoma. With further validation, ctDNA assessment could be utilized to non-invasively determine the mutation profile of a tumor to allow for personalized medicine using targeted therapies or immunotherapies that are known to be effective in patients with certain genomic profiles.

Hodgkin Lymphoma

In Hodgkin lymphoma (HL), due to the relatively small number of tumor cells present compared to the number of surrounding inflammatory cells, tumor genotyping has proven to be difficult. Initial studies appeared to show that ctDNA assessment in HL might be plagued by the same difficulty. In a study by Oki et al. [31], a clonotype was identified in just 12 of 17 (71%) of tumor biopsy specimens and ctDNA was detected in 8 of 11 (73%) peripheral blood specimens by IgNGS. Similarly, in another study, a clonotype was detected in tumor biopsy specimens using IgNGS in just 5 of 9 patients (55%) with HL compared to 63 of 69 (91%) of patients with NHL or CLL [32]. However, using CAPP-Seq, Schroers-Martin et al. [11] were able to detect ctDNA in 97.4% of all patients, with a similar rate of detection for HL compared to other lymphoma types.

In another retrospective study of 80 newly diagnosed and 32 relapsed/refractory classical Hodgkin lymphoma (cHL) patients, CAPP-Seq was used to assess ctDNA [33••]. Liquid genotyping using ctDNA identified a total of 106 mutations, compared to 96 mutations identified in micro-dissected tumor biopsy specimens. Mutations that were identified most frequently in the biopsy specimens were *STAT6* (37.5%), *TNFAIP3* (35%), and *ITPKB* (27.5%). Notably, prior studies describing findings of tumor genotyping in HL by other methods (e.g., flow-sorting of Hodgkin Reed Sternberg cells) had not described STAT6 as the most common recurrently mutated gene in HL. Eighty-four of the 96 mutations (87.5%) identified in the tumor biopsy specimen were also identified in the ctDNA. A subset of 24 patients with advanced stage cHL had serial plasma samples evaluated during therapy. In these patients, a 2-log decrease in ctDNA after two cycles of ABVD was found to be associated with complete response whereas a decrease of less than 2-logs was associated with progression of disease and inferior survival. This association of a 2-log decrease in ctDNA after two cycles and favorable outcome has also been reported for DLBCL [34]. If validated, interim ctDNA level could identify an important dynamic biomarker of response in patients during treatment and in the future, could help guide decisions regarding escalation or de-escalation of therapy in real time. In this small patient sample, it was demonstrated that ctDNA was a better predictor for risk of relapse than interim PET-CT after two cycles of ABVD. Six out of the 24 advanced stage cHL patients ultimately had progression of disease after initiation of treatment. In these 6 patients, 4 had a negative interim PET-CT (Deauville 3 or less). Thus, 4 out of the 6 patients (67%) had falsely negative scans in light of their subsequent disease progression. However, all 6 of the patients who had progression of disease failed to achieve a 2-log reduction in ctDNA. On the other hand, 18 of the 24 patients did not experience lymphoma progression after treatment. Four out of these 18 patients (22%) had a positive interim PET-CT but achieved a complete response on the end of treatment PET-CT. However, all 18 patients achieved a 2-log reduction in ctDNA [33••]. Although this was a small patient population, the findings suggest that liquid genotyping using ctDNA may simplify genotyping of HL and may provide complementary genotypic information compared to traditional genotyping techniques. Using dynamic ctDNA information (in this study, a threshold of 2-log reduction in ctDNA level) may have a better predictive value of subsequent treatment outcome than interim PET-CT. These findings require further validation but suggest that ctDNA assessment may overcome some of the traditional challenges of genotyping HL and may have important applications in the treatment of the disease.

Peripheral T-Cell Lymphoma

Although much of the focus to date in evaluating ctDNA assessment in lymphoma has centered on B-cell lymphomas and HL, preliminary evidence suggests that ctDNA assessment may be a useful tool in peripheral T-cell lymphomas (TCL). In a study of 88 patients with a variety of lymphoma subtypes who underwent allogeneic stem cell transplant, 10 patients had a TCL. A tumor clonotype was identified in the blood of 83.3% of patients with TCL using TCR-NGS, demonstrating the feasibility of ctDNA in TCL [32].

More recently, ctDNA assessment was evaluated in TCL patients treated with EPOCH chemotherapy. Fifty TCL patients were included in the study, including patients with anaplastic large-cell lymphoma ($n = 21$), peripheral T-cell lymphoma—not otherwise specified ($n = 14$), angioimmunoblastic T-cell lymphoma ($n = 6$), hepatosplenic T-cell lymphoma ($n = 5$), primary cutaneous gamma-delta T-cell lymphoma ($n = 2$), enteropathy-associated T-cell lymphoma ($n = 1$), and subcutaneous panniculitis-like T-cell lymphoma ($n = 1$). Patients had tumor clonotypes detected using a pre-treatment formalin-fixed paraffin-embedded (FFPE)

tissue or serum sample using TCR-NGS to sequence the *CDR3* region of *TCRβ/TCRγ* genes. Tumor-specific clonotypes were detected in 19 of the 23 patients (83%) with available tumor biopsy specimens, and in 14 of 27 (52%) patients with available serum samples. The use of ctDNA as a means of detecting MRD was evaluated in a subset of 28 patients within 1 month of completion of treatment. There was a trend towards improved PFS in patients who were MRD negative at the end of treatment compared to patients who were MRD positive (median, NR vs. 208 days, $P = 0.07$). Of the 20 patients who achieved remission after treatment, 10 eventually developed progression of disease. In 6 of the patients who progressed, ctDNA was detected prior to clinical relapse with a median lead time of 13.2 months (range 2.7 to 88.5 months). Interestingly, in the 10 patients who did not have progression of disease, 5 had low level ctDNA detected without overt clinical progression [35].

Sakata-Yanagimoto et al. [36] applied ctDNA detection in angioimmunoblastic T-cell lymphoma and PTCL-NOS using NGS to sequence *TET2*, *RHOA*, *DNMT3A*, and *IDH2*. In the 14 patients studied, 24 out of the 29 (83%) mutations identified in a biopsy of the primary tumor were also detected via pre-treatment ctDNA. Patients who had *RHOA* ($n = 6$) or *IDH2* ($n = 2$) mutations in the tumor biopsy had 100% concordance with ctDNA, although these represented a small number of patients. In patients who had *TET2* ($n = 10$) or *DNMT3A* ($n = 5$) mutations, ctDNA had an 80% correlation with the biopsy specimen. Of interest, one patient had mutations in *TET2*, *DNMT3A*, and *RHOA* detected by ctDNA at the time of diagnosis. But upon progression of disease, *RHOA* was no longer detected by ctDNA while *TET2* and *DNMT3A* mutations remained detectable. This may represent clearing of one of many tumor clones present at the time of diagnosis, with the tumor clone containing the *RHOA* mutation responding to treatment while *TET2*- and *DNMT3A*-mutated clones continued to be released from tumor cells that were resistant to the chemotherapy. This finding demonstrates the potential limitations of tracking a single mutation, which may provide information about a specific clone but lead to a falsely negative assessment of disease status and highlights the potential advantages of methods that assess multiple mutations.

Conclusion

Circulating tumor DNA allows for the non-invasive assessment of lymphoma genotype and tumor burden. An increasing number of studies have demonstrated the feasibility of ctDNA assessment across lymphoma subtypes, including DLBCL, FL, HL, and TCL. An expanding body of work has shown the ability of ctDNA to be used as a method of liquid genotyping, as a prognostic tool prior to treatment, as a dynamic marker of tumor burden during treatment, and as a method to detect recurrence of lymphoma after treatment. In addition, the simplicity of assessing ctDNA through a blood draw can potentially increase the ease with which repeated lymphoma assessments can be performed. Although ctDNA assessment has not yet been sufficiently validated to justify its use in routine clinical practice, the incorporation of ctDNA assessment into ongoing and future clinical trials will evaluate the utility of ctDNA-guided therapeutic decision-making. The findings of these studies will be critical in determining whether the promise of increased sensitivity and specificity for detecting lymphoma will translate into improved treatment outcomes when ctDNA-based decision-making is applied.

Compliance with Ethical Standards

Conflicts 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.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance •• Of major Importance

1. Han HS, Escalon MP, Hsiao B, Serafini A, Lossos IS. High incidence of false-positive PET scans in patients with aggressive non-Hodgkin's lymphoma treated with rituximab-containing regimens. Ann Oncol. 2009;20(2):309–18. https://doi.org/10.1093/annonc/mdn629.
2. El-Galaly T, Prakash V, Christiansen I, Madsen J, Johansen P, Boegsted M, et al. Efficacy of routine surveillance with positron emission tomography/computed tomography in aggressive non-Hodgkin lymphoma in complete remission: status in a single center. Leuk Lymphoma. 2011;52(4):597–603. https://doi.org/10.3109/10428194.2010.547642.
3. Moskowitz CH, Schoder H, Teruya-Feldstein J, Sima C, Iasonos A, Portlock CS, et al. Risk-adapted dose-dense immunochemotherapy determined by interim FDG-PET in advanced-stage diffuse large B-cell lymphoma. J Clin Oncol. 2010;28(11):1896–903. https://doi.org/10.1200/JCO.2009.26.5942.
4. Ansell SM, Armitage JO. Positron emission tomographic scans in lymphoma: convention and controversy. Mayo Clin Proc. 2012;87(6):571–80. https://doi.org/10.1016/j.mayocp.2012.03.006.
5. Berrington de Gonzalez A, Mahesh M, Kim KP, Bhargavan M, Lewis R, Mettler F, et al. Projected cancer risks from computed tomographic scans performed in the United States in 2007. Arch Intern Med. 2009;169(22):2071–7. https://doi.org/10.1001/archinternmed.2009.440.
6. Brenner DJ, Hall EJ. Computed tomography–an increasing source of radiation exposure. N Engl J Med. 2007;357(22):2277–84. https://doi.org/10.1056/NEJMra072149.
7. Huntington SF, Svoboda J, Doshi JA. Cost-effectiveness analysis of routine surveillance imaging of patients with diffuse large B-cell lymphoma in first remission. J Clin Oncol. 2015;33(13):1467–74. https://doi.org/10.1200/JCO.2014.58.5729.

8.• Herrera AF, Armand P. Minimal residual disease assessment in lymphoma: methods and applications. J Clin Oncol. 2017;35(34):3877–87. https://doi.org/10.1200/JCO.2017.74.5281. **This article provides a thorough review of prior applications of circulating tumor DNA and serves as a background for this review.**

9.• Roschewski M, Dunleavy K, Pittaluga S, Moorhead M, Pepin F, Kong K, et al. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. Lancet Oncol. 2015;16(5):541–9. https://doi.org/10.1016/S1470-2045(15)70106-3. **This study applied IgNGS to analyze ctDNA in patients with DLBCL and demonstrated the ability to identify patients at risk of recurrence prior to clinical evidence of disease.**

10. Kurtz DM, Jin M, Soo J, Scherer F, Craig A, Chabon JJ, et al. Circulating tumor DNA is a reliable measure of tumor burden at diagnosis of diffuse large B cell lymphoma: an international reproducibility study. ASH 59th Annual Meeting; Atlanta, GA Blood; 2017. p. 310.

11. Schroers-Martin JG, Kurtz DM, Soo J, Jin M, Scherer F, Craig A, et al. Determinants of circulating tumor DNA levels across lymphoma histologic subtypes. ASH 59th Annual Meeting; Atlanta, GA: Blood; 2017. p. 4018.

12. Assouline SE, Nielsen TH, Yu S, Alcaide M, Chong L, MacDonald D, et al. Phase 2 study of panobinostat with or without rituximab in relapsed diffuse large B-cell lymphoma. Blood. 2016;128(2):185–94. https://doi.org/10.1182/blood-2016-02-699520.

13. Kurtz DM, Green MR, Bratman SV, Scherer F, Liu CL, Kunder CA, et al. Noninvasive monitoring of diffuse large B-cell lymphoma by immunoglobulin high-throughput sequencing. Blood. 2015;125(24):3679–87. https://doi.org/10.1182/blood-2015-03-635169.

14. Armand P, Oki Y, Neuberg DS, Faham M, Cummings C, Klinger M, et al. Detection of circulating tumour DNA in patients with aggressive B-cell non-Hodgkin lymphoma. Br J Haematol. 2013;163(1):123–6. https://doi.org/10.1111/bjh.12439.

15. Hartert K, Tadros S, Bouska A, Moore D, Pak C, Heavican T, et al. DNA copy number gains of *TCF4* (E2-2) are associated with poor outcome in diffuse large B-cell lymphoma. ASH 58th Annual Meeting; San Diego, CA: Blood; 2016. p. 2686.

16. van der Velden VH, Hochhaus A, Cazzaniga G, Szczepanski T, Gabert J, van Dongen JJ. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. Leukemia. 2003;17(6):1013–34. https://doi.org/10.1038/sj.leu.2402922.

17. Drandi D, Kubiczkova-Besse L, Ferrero S, Dani N, Passera R, Mantoan B, et al. Minimal residual disease detection by droplet digital PCR in multiple myeloma, mantle cell lymphoma, and follicular lymphoma: a comparison with real-time PCR. J Mol Diagn. 2015;17(6):652–60. https://doi.org/10.1016/j.jmoldx.2015.05.007.

18. Newman AM, Bratman SV, To J, Wynne JF, Eclov NC, Modlin LA, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. Nat Med. 2014;20(5):548–54. https://doi.org/10.1038/nm.3519.

19.• 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. **This study demonstrates the application of CAPP-Seq analysis of ctDNA in patients with DLBCL.**

20. Hohaus S, Giachelia M, Massini G, Mansueto G, Vannata B, Bozzoli V, et al. Cell-free circulating DNA in Hodgkin's and non-Hodgkin's lymphomas. Ann Oncol. 2009;20(8):1408–13. https://doi.org/10.1093/annonc/mdp006.

21. Kurtz DM, Scherer F, Jin M, Soo J, Craig A, Esfahani MS, et al. Development of a dynamic model for personalized risk assessment in large B-cell lymphoma. ASH 59th Annual Meeting; Atlanta, GA: Blood; 2017. p. 826.

22. Suehara Y, Sakata-Yanagimoto M, Hattori K, Nanmoku T, Itoh T, Kaji D, et al. Liquid biopsy for the identification of intravascular large B-cell lymphoma. Haematologica. 2018;103:e241–4. https://doi.org/10.3324/haematol.2017.178830.

23. Quesada AE, Desai P, Tang G, Lin P, Westin JR, Seegmiller AC, et al. *MYC* copy number aberrancies predict a worse prognosis in patients with diffuse large B-cell lymphoma. ASH 58th Annual Meeting; San Diego, CA: Blood; 2016. p. 154.

24. Jin M, Kurtz DM, Esfahani MS, Soo J, Craig A, Scherer F, et al. Clinical impact of somatic copy number alterations in circulating tumor DNA from diverse lymphoma subtypes. ASH 59th Annual Meeting; Atlanta, GA: Blood; 2017. p. 2747.

25. Pott C, Knecht H, Herzog A, Genuardi E, Unterhalt M, Mantoan B, et al. Standardized IGH-based next-generation sequencing for MRD detection in follicular lymphoma. ASH 59th Annual Meeting; Atlanta, GA: Blood; 2017. p. 1491.

26. Soo J, Scherer F, Kurtz DM, Schroers-Martin JG, Jin M, Craig A, et al. Reproducibility of m7-FLIPI risk scores in follicular lymphoma using tumor biopsies and blood specimens. ASH 59th Annual Meeting; Atlanta, GA: Blood; 2017. p. 1466.

27. Delfau-Larue MH, van der Gucht A, Dupuis J, Jais JP, Nel I, Beldi-Ferchiou A, et al. Total metabolic tumor volume, circulating tumor cells, cell-free DNA: distinct prognostic value in follicular lymphoma. Blood Adv. 2018;2(7):807–16. https://doi.org/10.1182/bloodadvances.2017015164.

28. Sarkozy C, Seymour JF, Ferme C, Caballero D, Ghesquieres H, Leppa S, et al. Rituximab maintenance obviates the poor prognosis associated with circulating lymphoma cells in patients with follicular lymphoma. Blood. 2014;123(17):2740–2. https://doi.org/10.1182/blood-2014-02-553693.

29. Sarkozy C, Huet S, Carlton VE, Fabiani B, Delmer A, Jardin F, et al. The prognostic value of clonal heterogeneity and quantitative assessment of plasma circulating clonal IG-VDJ sequences at diagnosis in patients with follicular lymphoma. Oncotarget. 2017;8(5):8765–74. https://doi.org/10.18632/oncotarget.14448.

30. Daigle S, McDonald AA, Morschhauser F, Salles G, Ribrag V, McKay P, et al. Discovery of candidate predictors of response to tazemetostat in diffuse large B-cell lymphoma and follicular lymphoma using NGS technology on ctDNA samples collected pretreatment. ASH 59th Annual Meeting; Atlanta, GA: Blood; 2017. p. 4013.

31. Oki Y, Neelapu SS, Fanale M, Kwak LW, Fayad L, Rodriguez MA, et al. Detection of classical Hodgkin lymphoma specific sequence in peripheral blood using a next-generation sequencing approach. Br J Haematol. 2015;169(5):689–93. https://doi.org/10.1111/bjh.13349.

32. Herrera AF, Kim HT, Kong KA, Faham M, Sun H, Sohani AR, et al. Next-generation sequencing-based detection of circulating tumour DNA after allogeneic stem cell transplantation for lymphoma. Br J Haematol. 2016;175(5):841–50. https://doi.org/10.1111/bjh.14311.

33.•• Spina V, Bruscaggin A, Cuccaro A, Martini M, Di Trani M, Forestieri G, et al. Circulating tumor DNA reveals genetics, clonal evolution, and residual disease in classical Hodgkin lymphoma. Blood. 2018;131(22):2413–25. https://doi.org/10.1182/blood-2017-11-812073. **While tumor genotyping has been historically difficult in Hodgkin lymphoma, this study demonstrates the feasibility of identifying ctDNA by using the CAPP-Seq sequencing method.**

34. Kurtz DM, Scherer F, Newman AM, Craig AFM, Khodadoust MS, Lovejoy AF, et al. Prediction of therapeutic outcomes in DLBCL from circulating tumor DNA dynamics. J Clin Oncol. 2016;34(15_suppl):7511. https://doi.org/10.1200/JCO.2016.34.15_suppl.7511.

35. Melani C, Pittaluga S, Yee L, Lucas A, Shovlin M, Jacob A, et al. Next-generation sequencing based monitoring of circulating-tumor DNA in untreated peripheral T-cell lymphoma. ASH 59th Annual Meeting; Atlanta, GA Blood; 2017. p. 2728.

36. Sakata-Yanagimoto M, Nakamoto-Matsubara R, Komori D, Nguyen TB, Hattori K, Nanmoku T, et al. Detection of the circulating tumor DNAs in angioimmunoblastic T-cell lymphoma. Ann Hematol. 2017;96(9):1471–5. https://doi.org/10.1007/s00277-017-3038-2.