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T-CELL AND OTHER LYMPHOPROLIFERATIVE MALIGNANCIES (J ZAIN, SECTION EDITOR)

The Emerging Role of Liquid Biopsies in Lymphoproliferative Disorders

Jennifer Crombie[1] · Philippe Armand[1]

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Abstract

Purpose of the Review Lymphomas represent clinically and molecularly heterogeneous diseases with variable presentations, treatment algorithms, and outcomes. As treatment options continue to expand, more sophisticated prognostic and predictive biomarkers are needed to guide personalized treatment approaches.

Recent Findings Liquid biopsies, in which the sequencing of circulating tumor DNA (ctDNA) in peripheral blood serves as a surrogate for a tumor biopsy, are now being studied across cancer subtypes, including in lymphoid malignancies. Recent studies have demonstrated the potential of these techniques to improve prognostication and guide individualized treatment strategies, providing a significant advance in the field of precision medicine.

Summary In this review, we describe the sequencing platforms currently available for analysis of ctDNA in lymphoma and their potential applications in clinical practice, which seem poised to refine treatment paradigms across lymphoma subtypes.

Keywords Liquid biopsy · Cell-free DNA · Lymphoma · Lymphoproliferative disorders · Minimal residual disease

Introduction

As treatment options for lymphoma continue to evolve, there is a need for improved prognostic biomarkers that can guide individualized treatment strategies. Current strategies rely on clinical prognostic scoring systems or response assessment by imaging, which have significant limitations in guiding treatment selection. More recently, "liquid biopsies," in which circulating DNA shed from tumors into the blood is used to identify and track disease, have emerged as a powerful new tool to improve prognostication, guide risk-adaptive treatment strategies, and detect early disease relapse.

Furthermore, in the evolving era of targeted therapies, tumor biopsies have become increasingly important for the identification of genomic alterations that can drive treatment choice. Traditional tumor biopsies, while fundamental to diagnosis, are associated with procedural risks, cost, sampling error, and the potential inability to capture spatial heterogeneity in the setting of multifocal disease [1]. Given these challenges and risks, it is rare to be able to obtain serial samples during or after therapy, which limits the ability to monitor for clonal evolution or the development of resistance mutations. In addition, the optimal stratification of patients for treatment duration or intensity depends on the detection of tumor burdens that are below the sensitivity of current radiographic techniques, requiring techniques capable of detecting and quantifying minimal residual disease (MRD).

Liquid biopsies rely on the identification of circulating tumor cells (CTCs) or circulating tumor-specific DNA fragments (cell-free DNA [cfDNA]). cfDNA is shed from both normal and malignant cells, primarily through apoptosis and necrosis, but also through secretion [2–4]. DNA fragments, which are stable in circulation, are present in increased amounts in patients with malignancy [5]. Given their relatively short half-life, they are thought to serve as a reflection of the current state of disease [6]. Since the initial discovery of CTCs and cfDNA, there have been extensive efforts to identify their utility. With the advent of next-generation sequencing (NGS) and the growing knowledge of tumor-specific genomic abnormalities, there has been an increasing interest in the use of liquid biopsies across many types of malignancies. The role of liquid biopsies and cfDNA has been widely studied in solid tumors and has

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✉ Jennifer Crombie
jennifer_crombie@dfci.harvard.edu

Philippe Armand
philippe_armand@dfci.harvard.edu

[1] Dana-Farber Cancer Institute, Boston, MA, USA

resulted in the first liquid biopsy test to be approved by the U.S. Food and Drug Administration (FDA) [7].

Lymphomas represent a heterogeneous group of malignancies with a broad range of treatment approaches and outcomes. While some lymphomas often have a circulating component, lending themselves to straightforward application of liquid biopsies, other subtypes, such as diffuse large B-cell lymphoma (DLBCL) and Hodgkin lymphoma (HL), are typically limited to nodal or solid organ involvement. In these cases, analysis of CTCs and cfDNA is fundamental to the successful implementation of liquid biopsies. In this review, we briefly describe the current methods for performing liquid biopsies and some of the possible applications of this technology across a range of lymphoma subtypes.

Methods for Liquid Biopsies in Lymphoma

Flow Cytometry

Traditionally, liquid biopsies were limited to lymphoid malignancies with circulating disease, such as chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL). In these cases, multicolor flow cytometry (MCFC) has been used to detect CTCs in the peripheral blood, marked by fluorescently labeled antibodies, with a detection limit of 10^{-4} or 10^{-5} (Table 1) [10–12]. MCFC is a widely available method for MRD detection and response assessment and thus remains a commonly used tool especially in CLL; however, it is not sensitive enough to be used for liquid biopsies of other lymphoid cancers in which the circulating component is too small or non-existent.

PCR-Based Methods

Polymerase chain reaction (PCR)-based methods, including quantitative real-time quantitative PCR (qPCR) and digital droplet PCR (ddPCR), provide another means to identify circulating tumor from peripheral blood, either from DNA obtained from CTCs or cfDNA (Table 1) [13]. In these approaches, primers are utilized for amplification of specific genomic regions of interest, including disease-defining or patient-specific genomic abnormalities. MCL and follicular lymphoma (FL) often harbor canonical chromosomal rearrangements, t(11;14) and t(14;18), respectively [14]. In these cases, using qPCR, primers targeting these known rearrangements can detect circulating disease with a limit of detection to $\sim 10^{-5}$ [8]. In cases where there is not a cancer-defining genomic abnormality, as in DLBCL or variants of MCL or FL, primers against patient-specific DNA from the clonal rearrangement of the immunoglobulin heavy (*IGH*) gene locus can be used with a limit of detection as low as 10^{-6}. It should be noted that *IGH* clonal rearrangements are unique to each B-cell clone; thus, patient-specific primers targeting allele-specific oligonucleotides (ASOs) must be designed using biopsy samples or peripheral blood, which has heretofore limited the use of this method to specialized laboratories [15•]. ddPCR, which is less labor intensive than qPCR and does necessarily rely on patient-specific primers, can also be used to identify specific somatic mutations of interest [16, 17]. In general, these methods can be used for MRD detection and potentially to identify the presence of specific targetable mutations. However, the necessity to construct patient-specific primers (for ASO-PCR) and the limited breadth of genomic sampling (for ddPCR) have hampered their broad use in clinical research and practice.

NGS-Based Methods

The advent of NGS, which involves massive parallel sequencing of DNA molecules to read through millions of concurrent sequences, has dramatically improved the ability to analyze ctDNA. This can range from the identification of single nucleotide variants (SNVs) to whole genome or exome sequencing, depending on the method used. Incorporation of NGS into liquid biopsy testing provides several advantages as compared to alternative approaches, expanding its application beyond MRD monitoring to also allow sophisticated genomic evaluation at the time of diagnosis, assessment of clonal evolution, and identification of resistance mutations.

One of the most widely used assays in lymphoma involves NGS of the immunoglobulin heavy chain (IgNGS) or T cell receptor (TCR) genes, such as the ClonoSEQ® assay (Adaptive Biotechnologies, Seattle, WA) (Table 1). As with qPCR, in B-cell malignancies, this allows for tracking of specific immunoglobulin genes within a B-cell clone. Rather than using patient-specific primers, PCR is performed with universal primers that target all possible VDJ, DJ, or Igκ rearrangements in primary tumor tissue or blood samples [8]. Subsequent deep sequencing allows determination of the clonotype(s), or unique nucleotide sequence of the immunoglobulin heavy chain gene for a given patient's malignant clone. PCR amplification and NGS can subsequently be performed using CTCs (in peripheral blood mononuclear cells [PBMCs]) or cfDNA (in plasma) to detect and quantify the pre-determined clonotype(s) [8]. IgNGS is able to detect ctDNA with a detection limit of $\sim 10^{-6}$.

The panel-specific NGS-based assay, cancer personalized profiling by deep sequencing (CAPPSeq), is another emerging assay that can be successfully used in lymphoid malignancies (Table 1). This technique utilizes a sequencing panel that can be tailored for the disease of interest, targeting recurrent SNVs, insertions/deletions, and breakpoints of genes that participate in canonical fusions, which in the case of DLBCL, includes *BCL-2*, *BCL-6*, *MYC*, and *IGH* [18••]. Targeted sequencing approaches using a hybrid capture approach is then applied to identify and enrich genetic regions of interest [2]. Deep sequencing of these loci can be used to compare variant and normal

Table 1 Methods available for liquid biopsies use in lymphoid malignancies [8, 9]

	Technique details	Detection limit	Advantages	Disadvantages
Multicolor flow cytometry	-Use of 3 to 8 fluorescently labeled antibodies on flow cytometry	~ 10^{-4}	-Widely available -No individualization required	-Decreased sensitivity compared to other methods -Requires circulating disease
qPCR	-PCR using primers targeting known gene rearrangements -PCR performed using patient-specific primers	~ 10^{-5}	-Well validated in some lymphoma subtypes (e.g., mantle cell lymphoma, follicular lymphoma)	-Time intensive -Typically requires development of individual patient primers
ddPCR	-Wild type and mutant fluorescent probes applied -Individual PCR reactions performed within partitioned droplets	~ 10^{-5}	-Does not require patient-specific primers -Quantitative -Can identify somatic mutations of interest	-Requires individual optimization -Limited breadth of genomic sampling as compared to NGS -Limited to research settings
IgNGS	-Tumor clonotype determined from tumor or in some cases blood -PCR performed using consensus primers	~ 10^{-6}	-Quantitative -High sensitivity -Commercial assay available	-Typically requires access to biopsy sample
Targeted NGS	-NGS performed using a panel of preselected genes of interest	~ 10^{-6}	-Allows for profiling of comprehensive gene panels -No individualization required -Applicable across lymphoma subtypes -Ability to identify clonal evolution and de novo resistance mutations	-Limited to research setting -Only assesses for mutations in pre-determined sequencing panel -Limited detection of mutations with low allele frequencies
WGS/WES	-WGS performed to estimate tumor content -WES performed if adequate tumor fraction	> 10% cfDNA content	-Full characterization of genomic landscape -Has ability to detect structural variants and copy number alterations	-Expensive -Time consuming -Requires adequate circulating tumor fraction

qPCR quantitative polymerase chain reaction (PCR), *ddPCR* digital droplet PCR, *IgNGS* immunoglobulin heavy chain next generation sequencing, *WGS* whole genome sequencing, *WES* whole exome sequencing, *cfDNA* cell-free DNA

reads, allowing for quantification of cfDNA [8]. Like IgNGS, CAPPSeq has a limit of detection of cfDNA of 10^{-6} [8]. CAPPSeq has several unique advantages over other tools for liquid biopsy because it can capture comprehensive genetic alterations. This provides molecular information that can be used to refine treatment choice, as well as a way to dynamically monitor for clonal evolution and the emergence of resistance mutations. It also provides a more robust way of identifying tumor ctDNA when a primary tissue biopsy is not available, which is less often successful with IgNGS [8]. However, while targeted NGS approaches can capture a range of mutations, prior knowledge of the abnormalities of interest is required. In addition, targeted NGS is limited in its ability to capture other aspects of genetic diversity, including somatic copy number alterations (SCNAs). Whole genome- and whole exome-based sequencing methods have the potential to overcome these limitations, though require at least 5–10% tumor content in the plasma in order to achieve reasonable sensitivity with standard depth sequencing (Table 1) [19]. Whole genome sequencing (WGS) performed at ultralow coverage (0.1×) has recently been applied in solid tumors as a means to estimate tumor fraction and identify patients suitable for whole exome sequencing (WES) and has potential to be applied in lymphoid diseases [19].

Application of Liquid Biopsies in Lymphoma

DLBCL

DLBCL is the most common subtype of non-Hodgkin lymphoma (NHL), with approximately 27,000 new diagnoses in the USA each year [20]. Defining features of DLBCL include clinical and molecular heterogeneity. While treatment with rituximab and multi-agent chemotherapy results in cure for many patients, approximately a third of patients have relapsed or refractory disease [21, 22]. A variety of prognostic tools predict response to frontline therapy, including the international prognostic index (IPI) and cell-of-origin (COO) classification, though none have yet definitively impacted therapeutic management [23]. While efforts to intensify therapy for high-risk groups have been explored, these trials have not demonstrated improvements in survival [24–26]. Interim positron emission tomography (PET) has similarly been utilized to identify patients for treatment intensification but so far without success [27, 28]. Furthermore, the use of CT or PET scan for post-treatment disease monitoring, which is associated with significant cost and radiation exposure, has been shown to be of limited if any utility in this disease [29, 30].

The identification of ctDNA in DLBCL has emerged as a potential means to improve prognostication, guide risk-adaptive therapy, and detect patients at highest risk for disease relapse (Fig. 1a) (Table 2). Studies applying IgNGS technology and targeted NGS panels have shown cfDNA detection rates in 80 to 100% of patients with DLBCL, with baseline levels correlating with known prognostic features, such as IPI [15•, 18••, 31•, 32]. In one study using CAPPSeq, cfDNA levels at diagnosis continuously and independently correlated with inferior progression-free survival (PFS) in a multivariate analysis incorporating key clinical parameters, suggesting that cfDNA measurements serve as an independent prognostic biomarker [18••]. A similar study using CAPPSeq demonstrated that ctDNA could be detected in 98% of patients and that levels were continuously associated with event-free survival (EFS) and overall survival (OS) in patients receiving either frontline or salvage therapy [33••].

cfDNA has also been utilized for response monitoring (Table 2). In one study, IgNGS after two cycles of chemotherapy provided significant prognostic information [31•]. Sixty-three percent of patients with detection of MRD at that time point clinically progressed, as compared to only 20% in patients with undetectable cfDNA [31•]. At 5 years, this translated to time to progression of 41.7% versus 80.2% in patients with and without detectable interim cfDNA and an OS of 65% as compared to 83% [31•]. A recent study using CAPPSeq has also demonstrated a prognostic role of ctDNA dynamics in response to therapy [33••]. This study demonstrated that an early molecular response (EMR), as defined as a 2-log decrease in ctDNA after one cycle of chemotherapy, or a major molecular response (MMR), as defined as a 2.5-log decrease after two cycles, was associated with improvements in EFS at 24 months in patients receiving both frontline and salvage therapies [33••]. While the role of interim response to guide therapy remains an active area of research, this data suggests that cfDNA monitoring may be an alternative or complementary risk-adaptive tool. Additionally, surveillance cfDNA at the end of treatment using IgNGS has a positive predictive and negative predictive value of 88% and 98%, respectively, with detection of molecular disease in the plasma often preceding the detection of relapse by either CT or PET among patients who initially achieved remission [31•]. CAPPSeq has been used to demonstrate that cfDNA was detectable in 73% of patients prior to disease relapse, with a mean time from cfDNA detection to clinical relapse of 188 days [18••]. Similarly, cfDNA detection as determined

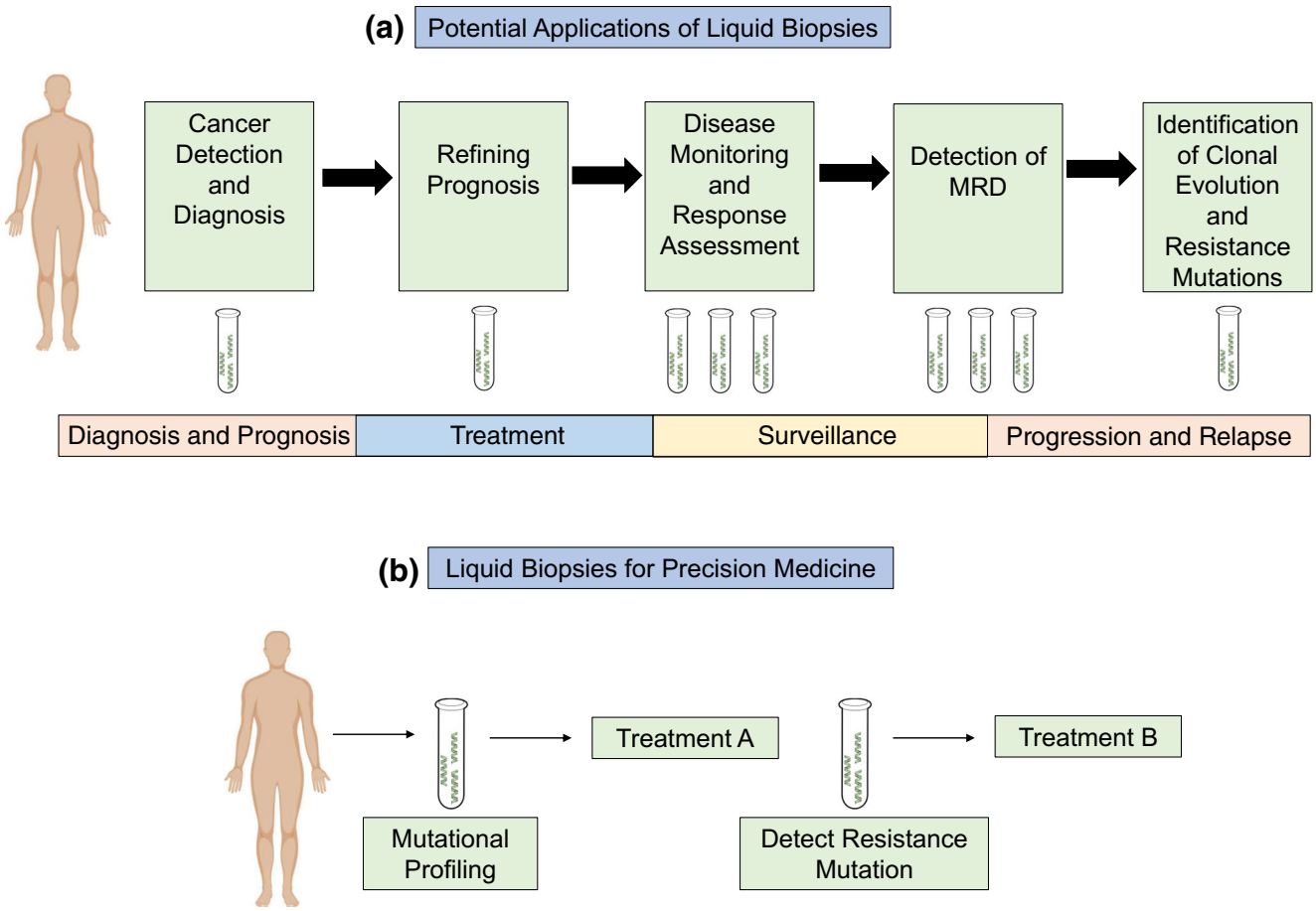

Fig. 1 **a** Possible applications of liquid biopsies. **b** Application of liquid biopsies for precision medicine

Table 2 Studies incorporating the use of liquid biopsies in lymphoma

	Role of liquid biopsy	Type of sequencing	Findings
DLBCL	Prognostication	IgNGS CAPPSeq	-IgNGS measurements reflected metabolic tumor burden by PET (Kurtz et al. [15•]) -IgNGS measurements correlated with LDH and IPI (Roschewski et al. [31•]) -ctDNA by CAPPSeq correlated with LDH, metabolic tumor burden, Ann Arbor stage, and IPI (Scherer et al. [18••]) -Pretreatment ctDNA levels by CAPPSeq were continuously associated with EFS and OS in patients receiving frontline or salvage therapy (Kurtz et al. [33••])
	Response assessment	IgNGS CAPPSeq	-MRD by IgNGS after two cycles of chemotherapy-stratified patients (5-year OS of 65% versus 83% in MRD-negative patients) (Roschewski et al. [31•]) -EMR (2-log decrease in ctDNA after one cycle of chemotherapy) or MMR (2.5-log decrease in ctDNA after two cycles) using CAPPSeq associated with improvements in EFS at 24-months in patients receiving frontline and salvage therapy. Following frontline therapy, EMR: EFS, 83% vs. 50% and MMR: EFS, 82% vs.46%. Following salvage therapy, EMR: EFS, 100% vs.13% (Kurtz et al. [33••])
	MRD evaluation	IgNGS CAPPSeq	-MRD by IgNGS preceded clinical relapse by a median of 88 days (Kurtz et al. [15•]) -80% of patients had detectable ctDNA by IgNGS prior to clinical relapse. 98% of patients without progression remained MRD negative. (Roschewski et al. [31•]) -Using CAPPSeq, mean of 188 days from ctDNA positive result to clinical relapse (Scherer et al. [18••])
	Identification of mutational landscape, evaluation of clonal evolution	ddPCR CAPPSeq	-ddPCR detected XPO1, EZH2, and MYD88 mutations in cfDNA, with a sensitivity of 0.05% (Camus et al. [17]) -CAPPSeq detected a median of 134 somatic variants, assigned cell-of-origin classification, monitored clonal evolution associated with transformation, and identified resistance mutations (Scherer et al. [18••]) -CAPPSeq accurately identified somatic mutations of > 20% allelic abundance and tracked emergence of treatment-resistant clones (Rossi et al. [35••])
Mantle cell lymphoma	MRD evaluation	qPCR	-PFS of 20 months if MRD-positive by qPCR post-ASCT, compared with 142 months in the MRD-negative group. OS was 75% at 10 years in the MRD-negative group, compared with only 35 months in the MRD-positive group (Kolstad et al. [51•]) -Pre-emptive rituximab converts patients to MRD negativity and possibly postponing clinical relapse (Kolstad et al. [51•]) -MRD positivity at end of induction in PB and BM associated with a HR for PFS of 2.26 and 1.40, respectively. Similar findings based on MRD after ACST (Hermine et al. [52]) -End of induction MRD-positivity associated with shorter OS and PFS. Median OS for MRD-negative patients not reached with 82% survival at 5 years versus 3.01 years for MRD-positive patients (Cowan et al. [53])
Follicular lymphoma	Prognostication	qPCR ddPCR IgNGS	-Baseline undetectable ctDNA by qPCR or low molecular tumor burden showed higher CR rate and longer PFS with a 3-year PFS of 80% vs. 59% (Galimberti et al. [56]) -PFS was shorter for patients with high baseline cfDNA using ddPCR (Delfau-Larue et al. [59]) -High levels of ctDNA by IgNGS independent predictor of PFS (Sarkozy et al. [62])
	MRD evaluation	qPCR	-3-year PFS was 66% for MRD-negative disease versus 41% for MRD-positive disease by qPCR at 12 months, and 84% versus 50% at 24 months (Galimberti et al. [56])
Hodgkin lymphoma	MRD	CAPPSeq	-Patients with CR had a larger drop in ctDNA load after two cycles of chemotherapy (Spina et al. [74••]) -Quantification of ctDNA complemented PET in determining residual disease (Spina et al. [74••])
	Identification of mutational landscape, evaluation of clonal evolution	CAPPSeq	-CAPPSeq detected nonsynonymous mutations in 81.2% of patients and able to monitor clonal evolution (Spina et al. [74••])

PET positron emission tomography, *LDH* lactate dehydrogenase, *IPI* international prognostic index, *EFS* event-free survival, *OS* overall survival, *MRD* minimal residual disease, *EMR* early molecular response, *MMR* major molecular response, *PFS* progression-free survival, *ASCT* autologous stem cell transplantation, *CR* complete response

by IgNGS was often detected prior to clinical disease relapse in patients with lymphoma, including DLBCL, after allogeneic transplant [34]. Further investigation will be required to determine whether intervention on earlier detection can translate to improved outcomes, though the use of cfDNA remains a promising strategy in this regard. Surveillance monitoring of cfDNA specifically has the potential to guide the use of maintenance therapy or early therapeutic intervention.

Use of cfDNA has also been utilized to identify specific mutations that have therapeutic implications, thus serving as a true "liquid biopsy" (Fig. 1a) (Table 2). Camus et al. used ddPCR to detect PO1, EZH2, and MYD88 mutations in plasma cfDNA, with a sensitivity of 0.05% [17]. CAPPSeq studies have also demonstrated that sequencing of cfDNA is as accurate as genotyping of diagnostic biopsy samples for somatic mutations with > 20% allelic abundance [35••]. In one study, sequencing of cfDNA also identified mutations that were not detected from tumor biopsies, presumably due to broader tumor sampling than an actual tissue biopsy [35••]. Lastly, CAPPSeq is able to detect the emergence of new acquired mutations, marking clonal evolution and potential mechanisms of resistance [18••, 35••].

Recent comprehensive genomic analyses in DLBCL have shed further light into the genomic complexities of DLBCL [36–38]. One study specifically revealed that a range of aberrations, including SCNAs and SVs, contribute to distinct genomic subtyping [36]. The use of WES of cfDNA has yet to be studied in lymphoid malignancies, though it holds great promise as a means to identify personalized therapeutic strategies for DLBCL (Fig. 1b).

Mantle Cell Lymphoma

MCL is an aggressive, small B cell lymphoma that comprises 4–9% of NHLs [39, 40]. It is often characterized by the molecular hallmark, t(11;14), which leads to dysregulation of the gene encoding cyclin D1. In the absence of allogenic stem cell transplantation, MCL is thought to be an incurable disease. It is also known to be a heterogeneous disease, with variable clinical and biologic features [41]. While there is no standard frontline therapy, younger patients are typically treated with intensive cytotoxic chemotherapy, followed by consolidation with autologous stem cell transplantation and maintenance rituximab [42–46]. More indolent presentations, in which active surveillance may be appropriate, have also been well-described [47, 48].

While prognostic biomarkers have been identified in MCL, ctDNA has the capacity to provide enhanced prognostication and guide treatment selection and duration (Table 2). The ability to achieve deep molecular remissions, as measured by MRD-negativity, has been identified as an independent predictor of outcome in MCL in a number of studies [49–52]. The Nordic Lymphoma Group, for example, has demonstrated that MRD after autologous transplant, determined by ASO-PCR, was associated with shorter progression-free and OS [51•]. The PFS was 20 months in patients who were MRD-positive after transplant, as compared to 142 months in the MRD-negative group [51•]. Studies have demonstrated that MRD at the end of induction therapy, but before transplantation, is also associated with shorter progression-free and OS as compared with MRD-negative patients [49, 52, 53]. These studies have called into question the role of autologous stem cell transplantation in patients with deep molecular remissions, thus prompting the Eastern Cooperative Oncology Group's (ECOG-ACRIN) phase III, randomized trial, comparing autologous stem cell transplant and maintenance rituximab to maintenance rituximab alone in patients achieving MRD-negative remission after induction therapy (NCT03267433) [41]. Of note, this trial will incorporate an IgNGS approach for MRD assessments, given improved detection of cfDNA as compared to qPCR.

MRD assessments following completion of therapy also have a role in pre-emptive treatment initiation (Table 2). The Nordic Lymphoma Group demonstrated that rituximab could lead to MRD negativity if not initially achieved after transplant, thus deepening responses in high-risk patients [51•]. Currently, standard therapy involves maintenance rituximab for all patients after transplant, though given risks of prolonged therapy, including, immune suppression, it would be valuable to identify patients likely to have maximum benefit. As targeted therapies, such as BTK and BCL2 inhibitors, are active in relapsed/refractory disease, there may also be a role for cfDNA detection using a panel-specific NGS method such as CAPPSeq to guide early discontinuation (in case of emergent resistant mutations) or treatment holidays (for patients achieving MRD clearance) in patients being treated with targeted therapy.

The continued development of prospective clinical trials that incorporate the use of cfDNA will be required to further identify the optimal use of cfDNA in guiding treatment intensification and duration. Additionally, as we gain further insights into the underlying molecular pathogenesis of MCL and resistance mechanisms to chemotherapy and targeted therapy, the role of liquid biopsy is likely to expand.

Follicular Lymphoma

FL is a common, indolent NHL characterized by the frequent (though not universal) chromosomal translocation t(14;18)(q32;q21), which involves the *BCL-2* and *IGH* genes. While a subset of patients with early-stage disease have the potential for cure, advanced-stage FL, like MCL, is thought to be incurable with traditional chemoimmunotherapy. FL is markedly heterogeneous, with some patients experiencing prolonged periods of observation or remission, while others have more aggressive disease that is refractory to or relapses early after therapy. Clinical prognostic scoring systems, such

as the FLIPI score can be used to predict survival, but are unable to identify biologic mechanisms that drive disease heterogeneity or guide risk-adaptive approaches [54]. Prognostic models have incorporated somatic gene mutations, though have found limited utility so far in clinical practice [55].

Baseline levels of cfDNA and detection of MRD after therapy has been shown to have prognostic value in FL in predicting PFS with conventional therapy (Table 2) [56–59]. In one study, 3-year PFS was 66% for MRD-negative disease versus 41% for MRD-positive disease at 12 months, and 84% versus 50% at 24 months [56]. MRD assessments have primarily utilized qPCR-based assays targeting the major breakpoint region or minor cluster region of *BCL-2,* the two most common regions involved in the translocation. While this approach is appealing, this technique has only able to detect cfDNA in approximately half of patients, likely given variability in the translocation locus [56]. Baseline levels of cfDNA have also been shown to correlate with known prognostic markers, such as FLIPI score, though not universally across studies, potentially reflecting the limitations of qPCR in this disease [59–61]. The use of IgNGS appears to increase the yield of detection, with one small study identifying tumor clonotypes from plasma of 74% of patients tested [62]. In this study, high levels of cfDNA were found to be an independent factor associated with PFS. Of note, detection of Ig rearrangements can also be limited in the setting of high rates of somatic hypermutation in FL [9, 63].

While data suggests that detection of MRD has the ability to predict PFS, studies have not clearly identified an impact on testing on OS (Table 2). This is consistent with the absence of proven survival advantage with earlier therapy, whether at diagnosis or at relapse [64, 65]. Further investigation will be required to determine whether cfDNA levels (or MRD status) can be used to guide treatment interruption or intensification and ultimately impact OS (Fig. 1a).

Additionally, there is limited data on the use of targeted NGS panels for detection of cfDNA in FL, though whole genome and WES of FL biopsy samples have significantly improved the understanding of the FL mutational landscape [66]. Recurrent mutations in linker histone, JAK-STAT signaling, NF-κB signaling, and B-cell developmental genes have been presumed to play a role in lymphomagenesis [66, 67]. Additionally, early driver mutations appear to be distinct from those seen with transformation [18••, 67]. The application of NGS to cfDNA holds great promise to identify the prognostic and potentially therapeutic implications of these mutations. As in other lymphoma subtypes, there is also utility in monitoring for clonal evolution of the development of resistance mutations in patients on targeted therapy.

Hodgkin Lymphoma

HL is an uncommon lymphoma affecting approximately 8500 people in the USA each year [68]. HL occurs in a bimodal distribution, often occurring in patients between 15 and 30 years of age and in those older than 55 years [69]. The main challenge in the treatment of HL has been balancing the risk of relapse with the late toxicities associated with intensive therapy. There has therefore been a strong focus on devising risk-adaptive treatment algorithms. To date, those are primarily based on the use of interim PET scan to guide treatment intensification or de-escalation [70–72]. While interim PET has been a useful tool to guide therapy, it remains an imperfect method for guiding treatment, as a significant subset of patients with PET positivity experience sustained responses without intensification [73].

Given the rarity of neoplastic cells in HL, detection of cfDNA might have been more elusive than for NHL. Interestingly, pregnant women with HL undergoing non-invasive prenatal testing (NIPT) through massive parallel sequencing of circulating fetal DNA were found to have complex cfDNA profiles, presumably due to circulating tumor DNA. Follow-up prospective analyses of cfDNA in patients with HL revealed specific genomic imbalances, including 2p and 9p gain, which were known to be characteristic of HL [75]. Furthermore, initiation of chemotherapy normalized cfDNA profiles, suggesting the possible utility of cfDNA as a risk-adaptive tool [75]. Another study identified that the presence of XPO1 mutations at the end of therapy, as detected by ddPCR, conferred an inferior prognosis [76]. This study again highlighted the potential prognostic utility of cfDNA in HL.

More recently, CAPPSeq has been used in HL to identify a range of mutations without the need for microdissection or sorting of malignant cells (Table 2). In a recent study, CAPPSeq was used to identify the mutation profile of patients, with high concordance between cfDNA and microdissected tumor genomic DNA. Additionally, CAPPSeq allowed early identification of treatment failure and the longitudinal assessment of clonal evolution throughout treatment [74••]. A reduction in cfDNA by 100-fold after two cycles of chemotherapy was associated with a complete response to therapy, while less than 100-fold reduction was associated with disease progression and inferior survival [74••]. In fact, this assay was superior to interim PET in predicting treatment failure, strongly suggesting the possible benefit of this technique in risk-adaptive treatment algorithms [74••]. cfDNA may also find use in assessing response following treatment with checkpoint blockade, in which PET scans may provide equivocal results [74••].

Conclusions

The past decade has witnessed a dramatic expansion of our knowledge of lymphoma biology and its heterogeneity between and within tumor types, as well as in therapeutic options. With this has come an increased need for biomarkers

that can underpin personalized treatment algorithms. cfDNA has clearly demonstrated prognostic value across several lymphoma subtypes. The next steps, besides validating and extending those findings, and broadening the availability of liquid biopsy, will be to explore whether this can indeed be used to beneficially guide treatment strategies. Clinical trials aimed at determining how to incorporate cfDNA for treatment change, intensification or de-escalation, use and choice of maintenance therapy, and pre-emptive intervention at molecular relapse, may provide the next step in the use of liquid biopsy in lymphoma, and ultimately are likely to fundamentally alter treatment paradigms in all subtypes.

Compliance with Ethical Standards

Conflict of Interest Philippe Armand reports interest from Adaptive, BMS, Merck, Affimed, Pfizer, and Roche, outside the submitted work. Jennifer Crombie declares that she has no conflicts 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 major importance

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