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Molecular Monitoring of Lymphomas

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Abstract

Molecular monitoring of tumor-derived alterations has an established role in the surveillance of leukemias, and emerging nucleic acid sequencing technologies are likely to similarly transform the clinical management of lymphomas. Lymphomas are well suited for molecular surveillance due to relatively high cell-free DNA and circulating tumor DNA concentrations, high somatic mutational burden, and the existence of stereotyped variants enabling focused interrogation of recurrently altered regions. Here, we review the clinical scenarios and key technologies applicable for the molecular monitoring of lymphomas, summarizing current evidence in the literature regarding molecular subtyping and classification, evaluation of treatment response, the surveillance of active cellular therapies, and emerging clinical trial strategies.

INTRODUCTION

Sensitive monitoring has transformed the clinical care of hematologic malignancies, with flow cytometry and polymerase chain reaction (PCR)-based techniques in routine use for response assessment and measurable residual disease (MRD) detection in leukemias and plasma cell dyscrasias (1, 2). The application of such approaches in lymphomas has been limited by low circulating cellular disease burden in most subtypes (3). However, the combination of improved molecular and informatic workflows with lower sequencing costs has now enabled sensitive and specific molecular monitoring of lymphomas via cell-free nucleic acids.

Cellular turnover throughout the body is accompanied by the release of short (<200 bp), largely double-stranded DNA fragments termed cell-free DNA (cfDNA) into the peripheral blood (4). While most cfDNA molecules are of hematopoietic origin, in patients with cancer the increased proliferative rate, apoptosis, and necrosis associated with malignancy are accompanied by the release of cancer-specific circulating tumor DNA (ctDNA) (5). While total cfDNA concentrations are variable and dynamic, lymphoma patients typically have elevated total cfDNA levels as compared with healthy adults (6). Although detection of tumor-derived variants in plasma via PCR was demonstrated in the 1990s, the development of accurate genotyping and monitoring has required ultradeep sequencing depths and error correction approaches addressing molecular biology, sequencing, and bioinformatic sources of noise (7).

Several excellent recent review articles address cfDNA assays across cancers (8, 9). In this article, we focus on clinical scenarios and tumor biology informing specific strategies for molecular monitoring of lymphomas, considering clinical data across lymphoma subtypes and assessing opportunities for clinical deployment and technical development of these promising assays.

CLINICAL SCENARIOS FOR MOLECULAR MONITORING

The clinical management of lymphoma patients presents multiple opportunities for molecular assessment from diagnosis through treatment and surveillance (**Figure 1**). In this section we review established and emerging applications of molecular techniques throughout the clinical course.

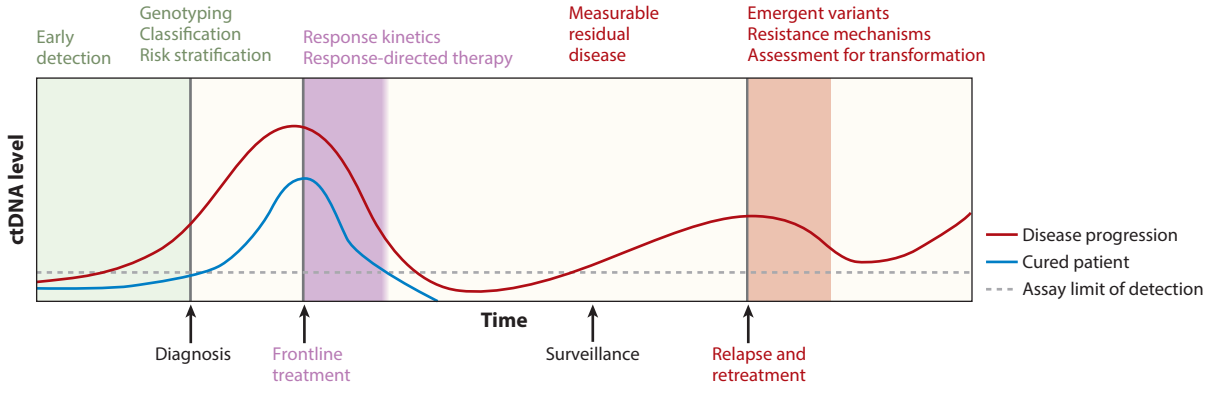

Figure 1

Schematic of the clinical time course of representative lymphoma patients, with clinical time points annotated with potential opportunities for molecular assessment. During surveillance time points, molecular assessment may be negative due to either cure or residual disease present below the assay limit of detection. Abbreviation: ctDNA, circulating tumor DNA.

Pretreatment Genotyping and Classification of Molecular Subtypes

Large-scale tumor sequencing has revealed that the clinical heterogeneity of lymphomas is mirrored by a diversity of genomic alterations (10–12). In addition to the established prognostic significance of cell of origin (COO) and *MYC* gene translocations in diffuse large B cell lymphoma (DLBCL), the risk contribution of mutations in individual genes such as *TP53* has been defined in several subtypes (13). More recently, two novel molecular classifications in DLBCL have systematized co-occurring pathogenic alterations into genomic subgroups with distinct prognostic implications (11, 12). Beyond risk stratification, these proposed subtypes are likely to spark trials of genome-directed therapeutic approaches such as combined enhancer of zeste homologue 2 (EZH2)/B cell lymphoma 2 (BCL2) inhibition for EZB subtype tumors (14).

While these classifications were developed via tumor biopsies, noninvasive genotyping has a high concordance with paired biopsy for both single-nucleotide variants (SNVs) and chromosomal translocations, with highest accuracy in patients with total ctDNA levels exceeding 5 haploid genome equivalents (hGE)/mL (15). Accordingly, an analysis of cfDNA-based classification in DLBCL demonstrated a similar distribution of molecular subtypes to the original cohorts (16).

Importantly, lymphomas demonstrate spatial heterogeneity both within primary tumors and across disease sites (17, 18). Clonal selection and evolution over time and under therapeutic pressure can result in additional clinically relevant heterogeneity (19). A single biopsy may therefore not reflect the full range of disease biology including targetable alterations. As cfDNA comprises molecules shed from all disease sites, noninvasive genotyping could provide a more accurate depiction of relevant mutations (15). Moreover, biopsy may not be possible in anatomically difficult sites, central nervous system (CNS) lymphoma, or radiographically occult disease (20, 21). While excisional biopsy remains the gold standard, liquid biopsy methods are likely to assume an increasingly important role in genotyping at diagnosis and relapse.

Clinical Risk Stratification

Pretreatment ctDNA levels in diverse lymphomas have been shown to correlate with clinical measures of disease burden including stage, total metabolic tumor volume (MTV) on body imaging, serum lactate dehydrogenase levels, and risk scores such as the International Prognostic Index (IPI) (22–24). Beyond correlation, ctDNA levels at baseline appear to be independently prognostic of event-free survival (EFS) and overall survival (OS) in DLBCL and follicular lymphoma (FL) (24, 25). More recently, pretreatment ctDNA methylation profile has been examined as a predictor of treatment failure after frontline chemoimmunotherapy (26). ctDNA may therefore complement established clinical factors in upfront risk assessment.

Response Assessment and Measurable Residual Disease Detection

Molecular assessment of treatment response typically incorporates both kinetics (frequently expressed as log reduction in concentration) and absolute posttreatment level or nondetection. In DLBCL, where patients are treated with curative intent, the clinical need for such an approach is underscored by frequent false positives in mid- and posttreatment positron emission tomography (PET) and computed tomography (CT) scans (27). Likewise, in Hodgkin lymphomas (HLs), response-adapted therapy via interim PET/CT imaging could be expanded to incorporate ctDNA dynamics, with emerging evidence associating detectable MRD in patients with incomplete radiographic response (28).

Evaluation of Emergent Mechanisms of Resistance

For patients on long-term therapy including oral targeted agents, detection of emerging resistant subclonal populations could be of clinical utility. Recurrent resistance mutations such as Bruton tyrosine kinase (*BTK*) C481S associated with ibrutinib failure may be detectable via ctDNA surveillance, and two emergent subclones bearing independent *BTK* C481S variants were observed in cfDNA from a patient with relapsed/refractory (R/R) FL receiving ibrutinib (15, 29). This approach is likely applicable to other stereotyped resistance mechanisms including *CD19* alterations in patients receiving chimeric antigen receptor T cell (CAR-T) therapy (30).

Assessment for Transformation

Morbidity in indolent lymphoma patients often occurs via histologic transformation to a more aggressive entity. Several studies comparing transformed FL with its indolent precursor suggest that transformation is accompanied by the acquisition of new genetic alterations (15, 31). However, although emergent variants are detectable via ctDNA, the specificity of any individual lesion for transformation is hindered by substantial overlap with the mutational landscape of the underlying disease (15). Potential strategies to increase specificity in distinguishing transformed lymphomas include consideration of degree of clonal divergence, elevated or increasing ctDNA levels, and emergent epigenetic changes such as distinctive ctDNA methylation and fragmentation profiles (16, 32).

Early Diagnosis and Evaluation of Premalignant Lesions

While cfDNA has been extensively studied in the context of established lymphoma diagnoses, premalignant or subclinical lymphoid lesions are less well characterized. Clonal disorders including monoclonal gammopathy of undetermined significance and monoclonal B cell lymphocytosis are common in healthy older adults. Likewise, the characteristic genomic lesion of FL, t(14;18)(q32;q21), is detectable in most healthy older adults with incidence increasing with age (33). Such precursor populations bear a low but quantifiable risk of transformation to malignancy. Increasing levels of such premalignant clones, or the detection of emergent cooperating mutations, may be of utility in stratifying patients at risk of clinical malignancy.

VARIANTS OF INTEREST IN THE MOLECULAR MONITORING OF LYMPHOMAS

Building on PCR assays targeting recurrent break-point regions and variants, sequencing-based techniques have enabled the detection of a broader range of genomic lesions (**Figure 2**). In this section, we review shared and unique variant classes relevant to the molecular monitoring of lymphomas.

Somatic Mutations

Somatically acquired SNVs and short insertions/deletions (indels) are of broad interest for genotyping and as tumor-specific reporters for disease monitoring. While prior studies have largely focused on coding alterations in the exome space, both coding and noncoding mutations can serve as high-specificity biomarkers to track lymphomas (34). The detection of each individual variant is limited by total evaluable nucleic acid molecules and the technical limits of the sequencing assay. Sensitive detection can therefore be facilitated by increasing DNA input, increasing the number of evaluated loci, or improving technical noise. One strategy to overcome technical noise is to focus on mutations observed on both strands of double-stranded DNA molecules (in *trans*), or so-called duplex support (35).

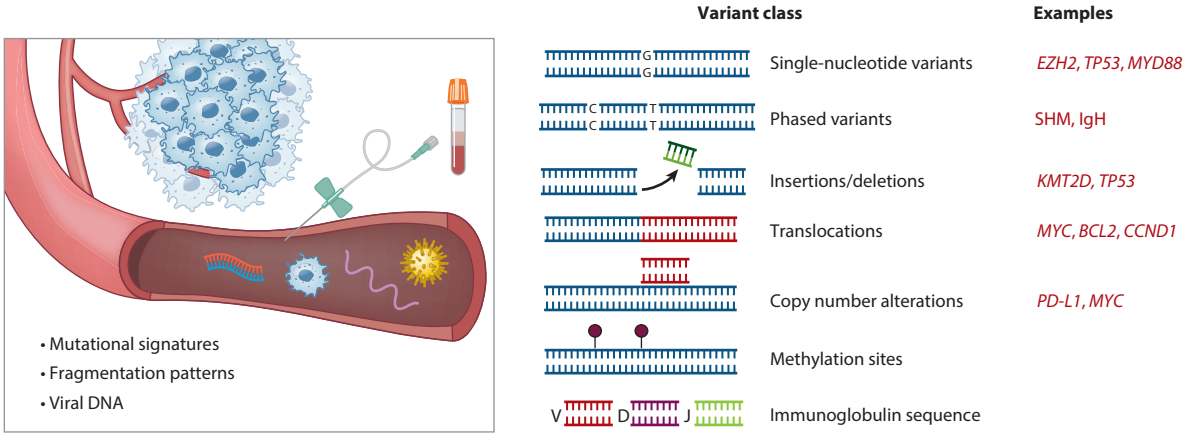

Figure 2

Overview of molecular variants of interest in the surveillance of lymphomas. The diagram depicts a lymphoma tumor shedding DNA, RNA, and circulating tumor cells into the peripheral blood. In virus-associated lymphomas, viral DNA may also be of diagnostic interest. The selection of molecular technique for a given lymphoma subtype is driven by the variant classes targeted and the required limit of detection for the application, with more sensitive techniques required for MRD. Abbreviations: IgH, immunoglobulin heavy chain; MRD, measurable residual disease; SHM, somatic hypermutation.

Not all somatic variants detected in cfDNA derive from the malignancy under molecular surveillance. Genes altered in age-related clonal hematopoiesis overlap broadly with lymphoid malignancies, and the risk of false positivity is particularly high in patients in clinical remission (36). Strategies for minimizing this issue include surveillance based on tumor-derived variants and sequencing of matched leukocytes, although the latter may be less reliable in lymphomas with a leukemic component (36).

Chromosomal Translocations

Recurrent pathogenic chromosomal translocations have been explored as molecular biomarkers in several B cell lymphoma subtypes. Mantle cell lymphoma (MCL) characteristically harbors the recurrent translocation t(11;14)(q13;q32), and 90% or more FL cases incur the t(14;18)(q32;q21) translocation between the *BCL2* gene and *IGH* loci (33, 37). In both diseases, detectable translocation by PCR-based methods after frontline therapy has been shown to be prognostic of inferior progression-free survival (PFS) (38, 39). While this review is primarily focused on sequencing-based techniques and cfDNA sources, a summary of critical earlier MRD-focused studies from the relevant subtypes is presented in **Table 1**.

However, even within each disease, such assays are limited by break-point heterogeneity across patients. For example, nearly half of all FL cases involve translocations outside of the major break-point and minor cluster regions covered by typical primer sets (40). Similarly, while ~50% of *CCND1* translocations in MCL occur within 100–200 bp of the major translocation cluster (MTC), the remainder of cases scatter across a nearly 300-kb genomic region surrounding the MTC (41). Broader strategies are therefore required for universal applicability.

V(D)J Rearrangement and Somatic Hypermutation

While the COO varies across lymphoma subtypes, precursor lymphocytes have typically already undergone variable (V), diversity (D), and joining (J) gene rearrangements prior to lymphomagenesis. These rearranged regions serve as a molecular signature for clonal B or T cell populations

Table 1 PCR-based studies in lymphoma measurable residual disease

Study	Year	Subtype	Molecular target	Molecular technique	Evaluable patients	Analyte	DOI
Rambaldi et al. (147)	2002	FL	BCL2/IgH	Nested PCR	128	PB, BM	10.1182/blood.V99.3.856
Rambaldi et al. (39)	2005	FL	BCL2/IgH	qPCR	86	BM	10.1182/blood-2004-06-2490
Hirt et al. (148)	2008	FL	BCL2/IgH, IgH	qPCR	43	PB	10.1111/j.1365-2141.2008.07101.x
Goff et al. (149)	2009	FL	BCL2/IgH	qPCR	414	PB	10.1200/JCO.2009.22.6258
Morschhauser et al. (150)	2012	FL	BCL2/IgH	Nested PCR	39	PB, BM	10.1093/annonc/mds202
Ladetto et al. (151)	2013	FL	BCL2/IgH	Nested PCR, qPCR	227	BM	10.1182/blood-2013-06-507319
Pott et al. (152)	2006	MCL	IgH	qPCR	29	PB, BM	10.1182/blood-2005-07-2845
Geisler et al. (153)	2008	MCL	CCND1/IgH, IgH	Nested PCR	79	PB, BM	10.1182/blood-2008-03-147025
Pott et al. (38)	2010	MCL	CCND1/IgH, IgH	qPCR	190	PB, BM	10.1182/blood-2009-06-230250
Cheminant et al. (154)	2016	MCL	IgH (92), CCND1/IgH (5), both (11)	Flow cytometry, qPCR	61	PB, BM	10.3324/haematol.2015.134957

Abbreviations: BM, bone marrow; FL, follicular lymphoma; MCL, mantle cell lymphoma; PB, peripheral blood; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction.

demonstrating concordance with diagnostic lymphoma biopsies (42). Associated clonal mutations are also incurred during affinity maturation and somatic hypermutation, generating a lymphoma-specific ctDNA biomarker (43).

Copy Number Variation and Loss of Heterozygosity

The recurrent loss or amplification of chromosomal regions has been described in diverse lymphoma subtypes. In addition to the clinically relevant amplification of 9p24 in HL and primary mediastinal B cell lymphomas (PMBCLs), characteristic alterations include COO-specific lesions such as trisomy 3 in activated B cell (ABC)-DLBCL and gains of 12q12 in germinal center B cell–like (GCB)-DLBCL (44, 45). Although such lesions involve larger genomic regions than any individual somatic variant, current sequencing methods for the detection of somatic copy number variants (CNVs) are generally considered less sensitive and specific than those used for small lesions such as SNVs and indels. Tumor-informed strategies leveraging genotypes from tissue biopsies increase specificity and may allow clinically useful noninvasive detection and response monitoring (46).

Mutational Signatures

Somatic mutations in cancers are incurred via exogenous (e.g., UV light, smoking-related toxins) and endogenous biochemical processes in stereotyped nucleotide contexts termed mutational signatures (47). The overall repertoire of somatic variants in a tumor or cfDNA specimen may be deconvolved to establish the relative contribution of different signatures, reflecting underlying disease biology. Specifically, in lymphoid malignancies the base substitution signatures and

genomic motifs associated with activation-induced cytidine deaminase (AID)-induced somatic hypermutation (e.g., SBS84/85) may clarify the clonal B cell origin of otherwise nonpathogenic variants (43, 47).

Epigenetic Markers

Aberrant DNA cytosine methylation in lymphomas is associated with distinct malignant transcriptional phenotypes, with recurrently altered sites including the *CDKN2A* promoter associated with aggressive disease (48). Indeed, an emerging class of therapeutics including inhibitors of histone deacetylases, *EZH2*, and DNA methyltransferases targets epigenetic dysregulation in lymphoma, suggesting that methylation status may also be informative for clinical management (49). As such epigenetic programs involve dozens to hundreds of genes, consideration of methylation state may yield a greater number of altered molecules for a given noninvasive sample than mutations alone. Recent multicancer early detection efforts have focused on the noninvasive detection of distinctive 5mC marks for common solid tumors and may prove valuable for molecular monitoring of lymphomas (50).

Viral Nucleic Acids

Several viral species have been associated with various lymphoma subtypes, including Epstein-Barr virus (EBV), human T cell leukemia virus type 1, human immunodeficiency virus (HIV), Kaposi sarcoma-associated herpesvirus/human herpesvirus 8, and hepatitis C virus (51). We focus primarily on EBV as the most well-studied circulating viral biomarker for lymphoma monitoring, typically via quantitative PCR (qPCR) techniques applied to whole blood or plasma (52).

PREANALYTIC CONSIDERATIONS FOR MOLECULAR MONITORING

Nucleic Acid Sources

Molecular monitoring assays in lymphoma have focused on circulating DNA from blood analytes including isolated peripheral blood mononuclear cells (PBMCs), serum, or plasma. Benefits of this approach include ease of integration into clinical laboratory workflows and the ability to noninvasively collect specimens at multiple time points. However, appropriate sample handling and choice of analyte are crucial (53).

When considering molecular disease burden, in blood collections from DLBCL patients where ctDNA was detectable in both leukocytes and plasma, the relative concentration in plasma has been reported as a median 150-fold higher (3). Even when accounting for lower total DNA levels in plasma specimens, the absolute level of tumor-derived DNA remained ~2.3-fold higher (3). Similarly, while serum specimens yield a higher total DNA concentration when compared with matched plasma, tumor-derived molecules comprise a lower relative concentration (3, 54). Plasma is therefore the preferred analyte for most monitoring strategies, although PBMCs may be complementary in lymphomas such as MCL with a substantial leukemic component.

Alternative nucleic acid sources under active exploration include cerebrospinal fluid (CSF) in primary or secondary CNS lymphoma. Given the inherent risk and technical challenges of brain biopsies, along with the difficulty of serial sampling, CSF represents an exciting opportunity for genotyping and monitoring. In a study of paired plasma and CSF from 12 patients with primary CNS malignancies, CSF was found to have both higher ctDNA concentration and a more accurate representation of tumor-derived variants than plasma specimens (55).

The extracellular nucleic acid fraction of plasma contains RNA as well as DNA. Although the fragmentation and relative instability of these molecules present a technical challenge, recent work has utilized this analyte in posttransplant and cancer detection applications (56, 57). In

DLBCL patients, the overexpression in cell-free mRNA of individual oncogenes including *MYC* and *BCL2* has been demonstrated by real-time quantitative monitoring of PCR reactions (RT-PCR) (58). Sequencing-based approaches present several potential advantages including the broader characterization of transcriptional alterations in unmutated tissue-specific genes (56). As the majority of cfRNA is of hematopoietic origin, strategies will be required to define the relative contribution of malignant versus normal lymphoid transcripts (56).

Sample Acquisition and Storage

The low concentration and rapid systemic clearance of cfDNA requires careful sample collection and processing. Important considerations include choice of anticoagulant for PCR compatibility and prompt plasma extraction to avoid nucleated cell lysis or endonuclease-mediated cfDNA degradation (9). While standard K2 or K3 EDTA tubes maintain ctDNA yield for refrigerated samples processed within 4–6 h of collection, over longer periods such samples display a time-dependent increase in DNA concentration resulting from lysis of nucleated leukocytes (9). Several commercial cfDNA collection tubes have been developed incorporating fixatives to prevent lysis for up to 7 days after collection at room temperature (59). Such dedicated products should be considered in multisite studies or other clinical situations where sample handling could be heterogenous (60).

TECHNICAL APPROACHES TO MOLECULAR MONITORING OF LYMPHOMAS

Lymphoma-associated variants of interest may be assessed via several competing molecular biology approaches (**Table 2**). Here we review the relative benefits and drawbacks of established and emerging methods.

Table 2 Molecular techniques for lymphoma surveillance

Technique	Assessed genomic space (bp)	Technical variants	Evaluable somatic lesions	Approximate LOD
PCR-based	10^2–10^3	Nested PCR Allele-specific Digital PCR	SNVs Translocations	10^{-4}–10^{-5}
Amplicon sequencing	10^3–10^4	Immunoglobulin sequencing	SNVs Indels Translocations Copy number variants	10^{-4}–10^{-5}
Targeted capture sequencing	10^4–10^6	CAPP-Seq PhasED-Seq	SNVs Indels Translocations Copy number variants	10^{-5}–10^{-6}
Low-pass WGS	Whole genome	Genome amplification	Copy number variants Fragmentation profile	10^{-3}–10^{-5}
Methylation profiling	Variable	Targeted capture MeDIP-Seq Low-pass WGS	Methylation sites Copy number variants	10^{-3}–10^{-4}

Abbreviations: CAPP-Seq, cancer personalized profiling by deep sequencing; indels, insertions/deletions; LOD, limit of detection; MeDIP-Seq, methylated DNA immunoprecipitation sequencing; PCR, polymerase chain reaction; PhasED-Seq, phased variant enrichment and detection sequencing; SNV, single-nucleotide variant; WGS, whole-genome sequencing.

Nonsequencing PCR-Based Approaches

The use of PCR-based diagnostics that do not require sequencing is well established in laboratory medicine and offers advantages including cost-effectiveness, clinical standardization, and characterized analytic performance. An important requirement for most approaches is genetic homogeneity allowing for the interrogation of small defined regions. In such scenarios, regions of interest can be resolved using end-point products of PCR in allele-specific oligonucleotide assays, RT-PCR, or droplet digital PCR (ddPCR). Recurrent chromosomal translocation break points and point mutations are particularly amenable to PCR-based assessment. Examples include the *MYD88* L265P hot-spot mutation occurring in 90% of Waldenström macroglobulinemia patients and more than 70% of primary central nervous system lymphomas (PCNSLs) and *XPO1* mutations in PMBCLs (61–63). However, the heterogenous mutational profile of most lymphomas has limited the applicability of single-locus PCR approaches. Separately, the presence of some of these lesions in healthy adults can limit their clinical specificity for molecular monitoring (33).

Immunoglobulin High-Throughput Sequencing

Immunoglobulin high-throughput sequencing (IgHTS) leverages clonotypic rearrangements of the IgH, IgL, or IgK loci as disease-specific markers. From a technical standpoint, rearrangements are identified in pretreatment tumor specimens through multiplex PCR of the V, D, and J regions followed by sequencing of the resulting amplicons. The dominant clonotype can then be tracked in blood or bone marrow samples to assess for MRD. Typically, $\geq$500 ng of cellular DNA are utilized for input, reflecting hundreds of thousands of genomes and allowing for an analytic limit of detection (LOD) of $\sim 10^{-6}$ after successful calibration (64). This impressive sensitivity has led to clinical approval in B-precursor acute lymphoblastic leukemia, chronic lymphocytic leukemia, and multiple myeloma (65–67).

While IgHTS can be applied to plasma, the practical sensitivity in this setting is much lower and is limited by DNA input mass typically ~1,000-fold lower than leukocyte-derived DNA from the same phlebotomy volume (3). Accordingly, assays tracking many independent variants rather than a single rearrangement are more sensitive with limited amounts of available DNA (3, 68).

Amplicon Sequencing

Another established approach involves sequencing of end-point PCR products (amplicons), with benefits including simplicity, efficient target enrichment, and easy personalization of targeted regions. However, amplicon size is limited by the parameters of the PCR reaction, and only a limited number of amplicons can be multiplexed in a single reaction without additional techniques such as droplet emulsions. This can be an effective approach in combination with tumor sequencing confirming patient-specific variants but is less suited for broad genotyping. Traditional amplicon workflows have also precluded error correction via unique molecular barcoding, although this deficit is addressed in contemporary approaches (69). Separately, amplicon approaches eliminate secondary characteristics of cfDNA molecules such as length and fragmentation pattern (16).

Targeted Capture Sequencing

Genomic enrichment via hybrid capture with biotinylated DNA oligonucleotides offers several important advantages, including a broader genomic space up to megabases and the preservation of source molecules for unique barcoding and fragment-based analyses. A workflow based on hybrid capture enrichment followed by molecular barcoding and error correction, termed CAPP-Seq (cancer personalized profiling by deep sequencing), has been extensively evaluated in lymphoma

subtypes including FL, DLBCL, and HL (3, 7, 15). In a workshop at the 15th International Conference on Malignant Lymphoma (15-ICML), an international working group selected CAPP-Seq as the preferred method due to the favorable balance of genomic breadth, sequencing depth, and error rate (70).

Recently, a related capture-based method called PhasED-Seq (phased variant enrichment and detection sequencing) has been developed leveraging the favorable error profile of clustered mutations to improve technical sensitivity (34). Rather than evaluating individual point mutations, PhasED-Seq tracks multiple SNVs located in close genomic proximity and segregating in *cis* on a given strand of cfDNA (phased variants). When such clusters occur within a single sequencing read, the technical error rate is lowered via joint conditional probability. For example, if the chance of a sequencing error at a given position is 1:1,000, then the theoretical chance of observing two such events at defined positions within the same read will be 1:1,000,000. This improved error rate has been demonstrated to allow more sensitive MRD detection in both lymphomas and solid cancers (34). The high mutation density in regions aberrantly targeted by AID makes GCB lymphomas especially suited to this method, as hundreds of phased variants can be detected with standardized capture panels, avoiding the need for personalized approaches.

Low-Pass Whole-Genome Sequencing

While some cancers are characterized by recurrently mutated genomic regions or high overall tumor mutational burden, other malignancies may carry few or uniquely distributed mutations. On the basis of this observation and the high frequency of CNVs and aneuploidies in diverse cancer types, several groups have proposed low-pass whole-genome sequencing (LP-WGS) as an approach for sensitive cancer detection. In plasma samples from patients with advanced and metastatic cancers with very high ctDNA burden, the ichorCNA technique has demonstrated concordance of clonal copy number alterations with paired tumor sequencing (71). LP-WGS has been primarily explored for CNV due to very low sensitivity for any given point variant. However, recent approaches assessing many thousands of patient-specific mutations at genome scale have achieved high analytic sensitivity (46).

Epigenetic Techniques

Several recent techniques have focused on the noninvasive evaluation of epigenetic alterations in cancer, targeting either methylated cfDNA or fragmentation patterns associated with transcriptional dysregulation. As traditional whole-genome bisulfite sequencing is associated with DNA loss during conversion, the low input mass of cfDNA presents a technical challenge. Utilizing targeted capture of differentially methylated sites, Liu et al. (50) assessed a pan-cancer detection approach that demonstrated sensitivity >75% in stage II–IV lymphoid malignancies. An alternative approach to enrichment of differentially methylated sites incorporates immunoprecipitation of cfDNA with anti-5mC monoclonal antibodies and has been evaluated for detection of several cancers (72).

Additionally, over the last several years, evidence has emerged that tissue-specific nucleosome configurations are imprinted in peripheral cfDNA fragmentation patterns. Specifically, regions with closed chromatin are better protected against intra- and extracellular endonucleases, while open chromatin regions are prone to degradation. Metrics developed to quantify this phenomenon include depth of sequencing [at the nucleosome-depleted region (NDR)] (73), disruption of nucleosome positioning (74), and increased fragment length diversity [using promoter fragmentation entropy (PFE)] at sites of open chromatin (16). As chromatin accessibility at transcription start sites (TSS) is associated with active gene expression, EPIC-Seq (epigenetic expression inference

from cfDNA sequencing) has recently been described as a novel hybrid capture method combining PFE at TSS regions with decreased sequencing coverage at the NDR to infer expression of individual genes from plasma sequencing (16). This approach has demonstrated utility for noninvasive cancer detection and classification of cancer subtypes in DLBCL, highlighting the potential of epigenetic features in liquid biopsies (16).

CLINICAL APPLICATIONS OF MOLECULAR TECHNIQUES BY LYMPHOMA SUBTYPE

Diffuse Large B Cell Lymphoma

The routine genomic characterization of DLBCL has found its way into many academic centers. Here we review clinical evidence for molecular strategies in the most common aggressive B cell lymphoma.

Genotyping. While genotyping does not yet impact routine clinical care, there is evidence that genetic properties bear prognostic and predictive information (11, 12, 75). Recent phase III randomized trials have attempted to guide therapy using molecular COO subtypes, frequently by selecting patients with ABC-DLBCL given their high clinical risk (76, 77). While no such study has yet achieved a significant clinical end point, there is emerging evidence that more granular subtyping may identify cases sensitive to targeted therapies such as ibrutinib (78).

While studies to date have utilized tumor tissue, molecular profiling from liquid biopsies could facilitate genotype-driven protocols, given the ease of clinical implementation and potentially better representation of tumor heterogeneity. Several studies have evaluated DLBCL genotyping from plasma ctDNA (15, 79–83). Sensitivity is a function of ctDNA levels and disease burden and is typically high in DLBCL. In 41 tumor–plasma pairs analyzed by CAPP-Seq, 91% of tumor-identified driver mutations could be recovered in plasma genotyping, with higher accuracy in advanced stage disease (15). Similarly, in a study analyzing 36 tumor–plasma pairs, the sensitivity of plasma genotyping was 83% compared with that of tumor genotyping (79).

Plasma genotyping can also infer both COO and novel genetic subtypes (15, 16). While prior studies have relied on mutations associated with ABC and GCB tumors to infer transcriptionally defined COO subtypes, it has more recently been demonstrated that EPIC-Seq analysis of cfDNA fragmentation patterns enables accurate COO subtyping based on inferred gene expression with high correlations to RNA and mutation-based methods (16).

Pretreatment risk stratification. Beyond genotyping, pretreatment ctDNA levels are prognostic of EFS, PFS, and OS (22, 24, 81, 83). Importantly, in several studies, including the largest study of 267 newly diagnosed DLBCL patients, independent prognostic significance was maintained when adjusting for the IPI (22). The latter study also demonstrated that a short interval between diagnosis and treatment was associated with higher pretreatment ctDNA levels, suggesting that ctDNA may be an objective measure of treatment urgency with additional utility to avoid selection biases in clinical trials (22).

Response assessment and measurable residual disease. Several techniques have been evaluated for MRD detection in DLBCL, including VDJ rearrangements (3, 60, 84), somatic mutations (24, 34, 79, 81, 82), and copy number abnormalities (85). As previously discussed, sensitivity for disease detection appears to be higher when using plasma cfDNA rather than cellular DNA (3). Two large studies have explored the role of early response assessment after one to two cycles of therapy in previously untreated DLBCL cases. The first study tracked clonotypic rearrangements in cfDNA for 108 cases, and patients without detectable disease after two cycles had a superior 5-year PFS compared with patients who remained ctDNA positive (80.2% versus 41.7%,

$p < 0.0001$) (84). The second study used CAPP-Seq for ctDNA detection and demonstrated that patients with a 100-fold (2-log) drop in ctDNA levels after one cycle of therapy [early molecular response (EMR)] or a 2.5-log drop after two cycles [major molecular response (MMR)] had superior outcomes compared with patients without these molecular milestones (24). When these patients were analyzed utilizing the more sensitive PhasED-Seq technique, patients who were ctDNA negative after two cycles of therapy by conventional CAPP-Seq could be further stratified for EFS using PhasED-Seq, confirming the clinical relevance of increased analytic sensitivity (34).

Other studies have confirmed the prognostic significance of posttreatment MRD negativity: A recent publication using a capture sequencing approach in 71 high-risk large-cell lymphoma patients found that failure-free survival was poor in patients with detectable disease at a posttreatment time point (14% of patients, $p = 0.00011$) (81). While most studies to date have evaluated treatment with cyclophosphamide, hydroxydaunorubicin, vincristine (Oncovin), and prednisone (CHOP)-like chemoimmunotherapy, a study of patients receiving ibrutinib/nivolumab observed improved PFS with a >2-log reduction in ctDNA levels after 28 days of treatment, confirming similar utility of ctDNA kinetics with novel therapeutics (86).

Relapsed/Refractory Diffuse Large B Cell Lymphoma

As in pretreatment DLBCL, elevated ctDNA levels before treatment of R/R DLBCL have been demonstrated to be prognostic for shorter PFS [hazard ratio (HR) 0.18, 0.05–0.65] and OS (HR 0.02, 0.06–0.68), with clinical complete response (CR) accompanied by a larger decrease in ctDNA (87). With the emergence of CAR-T therapy as a preferred option in R/R DLBCL, molecular monitoring has also been evaluated in this context. In a study analyzing 72 patients using IgHTS, higher pretreatment ctDNA levels were associated with both progression after axicabtagene ciloleucel infusion and the development of immune-mediated toxicities related to treatment (60). Cellular therapy also presents the opportunity for specialized applications including tracking chimeric T cell–derived cfDNA and emergent CAR-T–specific resistance mechanisms (30).

Hodgkin Lymphoma

While the low malignant cell fraction of HL tumors has complicated sequencing of traditional biopsies, patients with advanced HL have elevated ctDNA levels comparable with those of other aggressive lymphomas, facilitating noninvasive genotyping (23). This paradoxical finding may be driven by high cellular turnover and frequent polyploidy in Hodgkin/Reed-Sternberg (HRS) cells and presents a unique opportunity for noninvasive molecular techniques.

Genotyping. In a cohort of 96 patients with paired biopsy and plasma samples, ctDNA demonstrated an 87.5% sensitivity in reference to tumor genotype as established by microdissection of HRS cells (23). More recently, a larger cohort demonstrated comprehensive characterization of somatic mutations, CNV, and viral DNA, confirming cfDNA as a preferred genotyping analyte in HL (88). Similar performance has been demonstrated in the pediatric HL population, which may particularly benefit from noninvasive approaches (89).

Response assessment. Contemporary HL therapy has focused on minimizing therapeutic burden for the majority of patients with excellent responses to therapy, including response-adapted strategies utilizing interim PET/CT (90). However, interim PET/CT is complicated by a relatively high rate of false positivity reflecting inflammation rather than active lymphoma. Although baseline ctDNA levels in classic HL (cHL) demonstrate only moderate correlation with baseline MTV, early ctDNA kinetics after as little as 1 week on treatment are predictive of interim PET response (88). Likewise, patients achieving a 2-log or greater drop in ctDNA with treatment have

favorable outcomes in individual cases regardless of interim PET status (23, 88, 91). While these findings should be validated in larger prospective cohorts, they suggest a potentially complementary role for noninvasive response assessment in addition to established interim PET/CT time points.

Copy number alterations and immunotherapy. High response rates to checkpoint inhibition immunotherapy in HL are associated with recurrent gains of chromosome 9p24 encompassing the immunoregulatory ligand gene *PD-L1* (92). Noninvasive evaluation of copy number aberrations via LP-WGS in 10 HL patients confirmed recurrent gains of chromosomes 2p and 9p (93). More recently, a larger prospective study evaluating 177 patients with early-stage HL detected CNAs in more than 90% of patients, and targeted capture approaches have also been evaluated for this application (88, 94). As higher-level alterations at this locus appear to be associated with superior PFS, noninvasive assessment could be of clinical utility in HL patients identified as candidates for immunotherapy (92).

Mantle Cell Lymphoma

Molecular evaluation of MCL is facilitated by the frequent involvement of malignant cells in the peripheral blood or bone marrow. The ease and utility of leukocyte-based assessments has resulted in established clinical strategies reviewed below.

PCR-based measurable residual disease strategies. Utilizing qPCR-based methods targeting the stereotyped translocation t(11;14)q(13;32) or clonal IgH rearrangements, detectable disease after induction chemotherapy has been repeatedly associated with shorter PFS with both conventional treatments and intensive regimens (38, 95). Notably, this approach paved the way for the incorporation of high-dose cytarabine into the frontline treatment of MCL when higher MRD negativity rates were observed in the R-DHAP (rituximab, dexamethasone, cytarabine, cisplatin)/R-CHOP (rituximab plus CHOP) arm of the MCL Younger trial compared with R-CHOP induction alone (61% versus 26%) (96). The favorable EFS associated with MRD negativity suggests that in MCL, beyond being a prognostic tool, MRD detection could drive preemptive therapeutic strategies such as on-demand anti-CD20 retreatment for MRD+ patients after autologous stem cell transplantation but without biochemical or clinical relapse (97). A summary of major studies is included in **Table 1**.

Other approaches utilizing *SOX11* and *CCND1* expression as MRD markers have demonstrated lower sensitivity that may not reach clinical significance (98). qPCR has therefore remained the gold standard in MCL due to good performance and extensive clinical validation. However, some drawbacks limit the usage of this technique: the complexity of multiple or personalized PCR probes, sensitivity limited to at most 10^{-5}, and a technical design that excludes ~15% of patients with atypical break points (99).

Emerging sequencing techniques. Sequencing-based assays offer an appealing alternative due to increased sensitivity and the potential benefit of direct and/or broader genomic characterization (100). IgHTS utilizing the commercial clonoSeq assay demonstrated 82% concordance with qPCR in a cohort of 158 MCL samples, and a head-to-head comparison of these methods in the US-Canadian Intergroup trial E1411 showed better sensitivity for the next-generation sequencing (NGS)-based method under optimal conditions (100, 101). ClonoSeq positivity during treatment has also proven to be an effective predictor of failure of induction therapy (102).

Although other sequencing techniques have been less explored in MCL, a correlative analysis of the AIM (ABT-199 & Ibrutinib in Mantle Cell Lymphoma) study of ibrutinib/venetoclax utilized amplicon-based NGS of tumor-confirmed variants to demonstrate ctDNA dynamics in response

to therapy, as well as plasma LP-WGS for the detection of emergent copy number alterations at progression (103).

Potential clinical applications. A significant subset of patients with newly diagnosed MCL can benefit from a delayed initiation of treatment. In retrospective data, patients with low blood involvement, low Ki67, and good performance status can delay treatment initiation for months to several years without jeopardizing their survival (104). While deferred patients are under expectant management, clonal surveillance could help determine treatment initiation timing and modality by detecting shifts in mutational loads, copy number abnormalities, or signals of epigenetic dysregulation. In particular, the emergence of high-risk lesions including *TP53* mutations could be a trigger for treatment (37). The same rationale also applies to indolent/nonnodal MCL patients, as more than 30% of such patients will eventually undergo treatment (105).

Follicular Lymphoma

Noninvasive molecular techniques in FL represent a challenge due to relatively low ctDNA concentrations as compared with other lymphoma subtypes (106). In contrast to DLBCL, the tumor-derived fraction rarely exceeds 10%, reducing sensitivity (25). For 20 patients evaluated via paired cfDNA and tumor specimens, only 42% of detected variants were concordant, predominantly due to nondetection in plasma (107). However, this challenge is accompanied by several important opportunities.

Genotyping and targeted therapy selection. Genomic characterization has an established role in FL, with the m7-FLIPI clinicogenetic risk score used to identify patients at high risk of poor outcomes (108, 109). As FL is an indolent lymphoma requiring longitudinal care, cfDNA surveillance may help spare repeated invasive biopsies and/or surveillance imaging. Total cfDNA levels assessed by ddPCR have been shown to correlate with MTV, with elevated levels associated with shorter PFS (25). The marked spatial heterogeneity seen among FL disease sites suggests that noninvasive genotyping could provide more comprehensive clonal characterization than an individual biopsy, with substantial clonal heterogeneity also seen on plasma immunoglobulin sequencing (17, 110). Given improved responses to tazemetostat in the presence of *EZH2* hot-spot mutations, sensitive genotyping via cfDNA may also improve choice of targeted therapy (111).

Risk stratification and measurable residual disease detection. Enabled by the near ubiquity of *BCL2* translocations in FL, PCR-based methods assaying t(14;18) in peripheral blood or bone marrow have been explored extensively; a summary of major studies is included in **Table 1**. MRD negativity at a threshold of $\leq 10^{-4}$ has been shown to be independently predictive of recurrence risk with contemporary therapies (112). However, recent attempts to risk stratify maintenance therapy by PCR-based MRD detection failed to provide a benefit, with decreased PFS among even MRD-negative patients (113). Clinically impactful MRD assessment therefore likely requires more sensitive detection than standard nested or RT-PCR techniques.

Sequencing-based MRD has been evaluated only in preliminary cohorts: For example, utilizing an amplicon-based panel in 13 FL patients receiving chemotherapy and rituximab maintenance, inferior PFS was seen in patients with detectable MRD at the end of treatment, and lower MRD concentrations were seen in patients achieving radiographic CR (114). The relative sensitivity and clinical utility of these techniques in relation to established PCR methods is yet to be established.

Early disease progression and transformation. The histologic transformation of FL to DLBCL presents a clinical challenge, as transformed disease may be radiographically ambiguous or not amenable to biopsy. Molecular features associated with transformation include higher

ctDNA levels and emergent somatic variants, and a logistic regression classifier incorporating these features displayed 83% sensitivity and 89% specificity for transformation in a small cohort comparing FL, transformed FL, and R/R DLBCL patients (15). Methylation profiling has also been explored in discriminating FL from DLBCL, and a four-gene classifier displayed an area under the curve of 88.5% in a small testing cohort (32). Both approaches warrant further exploration in independent cohorts.

T Cell Lymphomas

While this review focuses primarily on B cell lymphomas, similar ctDNA techniques have been evaluated in T cell subtypes. Here we review clinical data including the established role of MRD assessment in anaplastic lymphoma kinase (ALK)-positive disease.

Genotyping. In angioimmunoblastic T cell lymphoma (AITL), a stereotyped mutational repertoire including *RHOA* G17V and recurrent alterations in *TET2*, *DNMT3A*, and *IDH2* has enabled focused noninvasive genotyping. Targeted amplicon sequencing of these four genes in 14 patients [9 AITL, 5 peripheral T cell lymphoma, not otherwise specified (PTCL-NOS)] detected tumor-confirmed variants in 83% of cfDNA specimens (115). A broader hybrid capture approach assaying 41 genes was employed to assess noninvasive genotyping and treatment response dynamics in extranodal natural killer/T cell lymphoma (ENKTL), revealing *KMT2D* mutations as a negative prognostic marker (116).

Measurable residual disease assessment. In ALK-positive ALCL, the *NPM1-ALK* fusion transcript allows specific disease detection, with positive RT-qPCR in peripheral blood or bone marrow at diagnosis reported as a poor prognostic factor (117). In the posttreatment setting, Kalinova et al. (118) reported that five out of nine relapses were preceded or accompanied by increased bone marrow *NPM1-ALK* levels by RT-qPCR. The predictive power of PCR-based methods has been further validated in independent cohorts (119). As with other translocation-based techniques, a limitation is the requirement for primers encompassing a patient's specific translocation site. As an alternative to fusion-specific PCR, Quelen et al. (120) developed a *3′ALK* universal amplification protocol that enables detection of diverse *ALK* fusions with 100% concordance with standard PCR.

Analogous to B cell IgHTS, T cell receptor (TCR) sequencing of the CDR3 regions of TCRβ/TCRγ genes can enable surveillance when primary tumor tissue is available. This approach was initially utilized to evaluate MRD in 10 patients with cutaneous T cell lymphoma after allogeneic hematopoietic cell transplantation, with positivity associated with clinical recurrence (121). In a retrospective analysis of pretreatment PTCL patients receiving frontline EPOCH-based chemotherapy, 9 patients (38%) cleared ctDNA after two cycles of therapy, while 11 patients (46%) had detectable ctDNA at the end of treatment. Although patients with detectable ctDNA showed a trend toward worse survival, specificity was imperfect: Notably, 2 patients with persistently detectable ctDNA remained in remission with 10 years of follow-up (122). Similar performance was seen by Mehta-Shah et al. (123), who evaluated 41 PTCL patients treated with CHOP-based therapies. Among 24 MRD+ subjects, 63% remained in radiographic remission, with no significant difference in OS or PFS seen by MRD status at a median follow-up of 20.7 months (123). Alternative MRD techniques may therefore be more suitable for PTCL.

Central Nervous System Lymphomas

Noninvasive evaluation of CNS lymphomas offers a clear appeal due to the technical challenges of brain biopsies. However, detection of ctDNA in peripheral blood is challenging, potentially due

to lower total tumor volume and the effect of the blood–brain barrier. Here we review emerging evidence for this exciting clinical application.

Noninvasive detection and response assessment. Several technical strategies have been applied to the low ctDNA concentrations typical in PCNSL. An amplicon-based approach with an empiric LOD of 0.5% allelic fraction (AF) detected tumor-concordant variants in only 32% (8/25) of a cohort of PCNSL patients (124). By contrast, a narrower but more sensitive approach utilizing ddPCR for the *MYD88* L265P hot-spot mutation was positive in 57% (8/14) of patients with brain biopsies carrying this variant, suggesting that highly sensitive assays are required for peripheral blood detection (125).

The evaluation of CSF-derived rather than peripheral blood cfDNA may increase sensitivity. In two cohorts, *MYD88* L265P was detected by ddPCR in 73% (8/11) and 71% (10/14) of CSF specimens (126, 127). Although the frequent incidence of *MYD88* L265P in PCNSL makes this a feasible single-gene biomarker, increasing assayed mutations is likely to increase sensitivity. Utilizing variant-specific ddPCR for tumor-derived mutations, ctDNA was detected in the CSF of 100% of 6 patients with CNS-restricted disease but only 33% (2/6) of paired plasma samples, with higher concentrations seen in CSF (128).

Most recently, a larger study assessing 92 pretreatment CNS lymphoma (CNSL) patients via sensitive targeted capture sequencing detected ctDNA with reference to tumor-confirmed variants in 78% of plasma and 100% of CSF specimens, with median AF of 0.01% in plasma and 0.62% in CSF (median CSF volume: 2.5 mL) (21). A noninvasive classifier was developed via comparison with 44 patients with nonlymphoma brain cancers or inflammatory CNS disease, then validated in an independent cohort of 207 specimens from CNSL and non-CNSL patients, demonstrating sensitivity for PCNSL detection of 21% for plasma and 57% for CSF at 100% specificity (21). Patients with detectable ctDNA during induction chemoimmunotherapy experienced decreased PFS/OS (21). Clearance of ctDNA has also been associated with response to ibrutinib-based combination therapy, suggesting a general role for molecular response assessment in CNS lymphoma (129).

Screening for secondary central nervous system involvement. Secondary CNS involvement of aggressive systemic lymphomas is a feared complication evaluated at staging via CSF cytology and flow cytometry with limited sensitivity. ctDNA detection may help stratify patients at highest risk of CNS relapse. An exploratory study utilizing IgHTS detected tumor-derived clonotypes in the CSF of 42% (8/19) of a cohort of high-risk patients without known CNS involvement at diagnosis (130). At a median follow-up of 11 months, 2 ctDNA-positive patients experienced CNS relapse despite negative staging imaging and conventional CSF studies, while no ctDNA-negative patients developed CNS involvement (130). Given emerging evidence for the limited efficacy of traditional methotrexate-based CNS prophylaxis in DLBCL, such an approach could help identify the highest-risk patients for CNS-directed interventions.

Virus-Associated Lymphomas

In most healthy individuals, EBV-specific cytotoxic T lymphocytes prevent viral reactivation by recognizing EBV antigens on expanding infected B cells. Systemic reactivation of EBV, particularly in immunocompromised states associated with HIV or solid organ transplantation, has been associated with several lymphoma subtypes, including Burkitt lymphoma (BL), HL, ENKTL, and posttransplant lymphoproliferative disorder (PTLD) (51). For many EBV-associated malignancies, monitoring of viral loads in peripheral blood can play an important role in diagnosis and management (52).

Technical approach and choice of analyte. The standard method for clinical EBV load measurement is RT-PCR, a rapid, sensitive, and inexpensive technique (52). While RT-PCR has historically been hindered by lack of standardization across institutions, the 2011 World Health Organization international standard for EBV improved harmonization across institutions, facilitating management guidelines for EBV-associated lymphomas (131).

The circulating burden of EBV-infected tumor cells varies across lymphoma subtypes, impacting the relative utility of measuring EBV in plasma versus whole blood or PBMCs. Most EBV-infected cHL and ENKTL tumor cells are thought to remain localized, shedding cell-free EBV DNA, while PTLD may involve circulating EBV+ cells (52, 132). However, several studies have concluded that plasma specimens are overall superior to cellular specimens in sensitivity and specificity across a range of EBV+ diseases (133, 134).

Surveillance of Epstein-Barr virus–positive lymphomas. Despite these limitations, EBV monitoring has demonstrated clinical utility in certain contexts including PTLD (135). While routine EBV surveillance is not recommended in unselected adult solid organ transplant recipients, guidelines suggest prospective monitoring of viral loads in high-risk patients, coupled with interventions including reduction in immunosuppression to reconstitute the EBV-directed immune response or rituximab targeting $CD20^+$ B cells (136, 137). Despite a lack of randomized controlled trials, some evidence suggests that these preemptive strategies have reduced the incidence of PTLD compared with historical cohorts (135).

In cHL, plasma EBV cfDNA is highly correlated to tumor EBV status inferred by EBV-encoded small RNA in situ hybridization (138). In addition, plasma EBV levels have been shown to have prognostic significance both pretreatment and at 6-month follow-up in advanced HL, with elevated plasma EBV load being associated with worse failure-free survival (139). Sequencing techniques evaluating lymphoma ctDNA have also been utilized for simultaneous EBV detection in cHL patients with strong concordance with tumor EBV status (88).

ENKTL is a rare subtype of non-Hodgkin lymphoma that commonly involves the nasal cavity and has high prevalence in East Asia and South America. Like endemic BL, ENKTL has near-universal association with EBV infection, and circulating viral loads are useful in making a diagnosis (140). Several studies have shown that the EBV-DNA load correlates with clinical stage and can be used to monitor disease progression and predict prognosis (141, 142).

Novel sequencing-based approaches. Sequencing-based techniques may offer additional prognostic benefit. In nasopharyngeal carcinoma (NPC), an epithelial neoplasm with 97% EBV positivity, a prospective study of 20,000 high-risk participants demonstrated that determination of plasma EBV load by RT-PCR is useful in screening for early disease (143). Furthermore, the addition of NGS-based EBV cfDNA fragment size and methylation metrics improved the positive predictive value of early NPC detection to 35.1%, compared with 11% using RT-PCR alone (144, 145). Another advantage of EBV cfDNA sequencing is the noninvasive detection of high-risk viral variants, which may increase lymphoma risk (146).

CONCLUSIONS AND DISCUSSION

Liquid biopsies are promising tools in many aspects of clinical lymphoma care. Studies to date have been limited by heterogenous methodologies, relatively small cohort sizes, and the ongoing rapid development of novel analytic techniques. An overview of selected published studies, as discussed above by subtype, is summarized in **Table 3**.

Table 3 Selected contemporary studies in the molecular monitoring of lymphoma

Study	Year	Subtype	Molecular technique	Total patient count	Monitoring time points	Intervention	DOI
Weng et al. (121)	2013	CTCL	IgHTS	10	Pretreatment, posttreatment	Allogeneic HSCT	10.1126/scitranslmed.3007420
Kurtz et al. (3)	2015	DLBCL	IgHTS	75	Pretreatment; 39 patients On-treatment; 123 time points Surveillance; 102 time points Progression; 47 patients	R-CHOP-like	10.1182/blood-2015-03-635169
Roschewski et al. (84)	2015	DLBCL	IgHTS	126	Pretreatment; 126 patients On-treatment; 108 time points Posttreatment; 107 patients	EPOCH ± R	10.1016/s1470-2045(15)70106-3
Vandenberghe et al. (93)	2015	cHL	LP-WGS	10	Pretreatment, posttreatment	ABVD	10.1016/S2352-3026(14)00039-8
Camus et al. (80)	2016	DLBCL	ddPCR	14	Pretreatment; 14 patients Posttreatment; 1 patient	GA101-ACVBP	10.3109/10428194.2016.1139703
Kanakry et al. (133)	2016	PTLD	qPCR	2,146	EBV+ disease; 105 patients Pre/posttreatment	Multiple	10.1182/blood-2015-09-672030
Herrera et al. (155)	2016	Multiple	IgHTS	68	Pretreatment, posttreatment, surveillance	Allogeneic HSCT	10.1111/bjh.14311
Rossi et al. (79)	2017	DLBCL	Targeted capture	50	Pretreatment; 50 patients Posttreatment; 30 patients	R-CHOP	10.1182/blood-2016-05-719641

(*Continued*)

Table 3 (*Continued*)

Study	Year	Subtype	Molecular technique	Total patient count	Monitoring time points	Intervention	DOI
Sakata-Yanagimoto et al. (115)	2017	AITL	Amplicon	14	Pretreatment	NA	10.1007/s00277-017-3038-2
Fontanilles et al. (124)	2017	PCNSL	Amplicon	24	Pretreatment	NA	10.18632/oncotarget.18325
Sarkozy et al. (110)	2017	FL	IgHTS	34	Pretreatment	R-CHOP	10.18632/oncotarget.14448
Bohers et al. (82)	2018	DLBCL	Targeted capture	30	Pretreatment; 30 patients Posttreatment; 20 patients	R-CHOP-like	10.1038/s41408-018-0111-6
Suehara et al. (20)	2018	Intravascular LBCL	Amplicon	9	Pretreatment	NA	10.3324/haematol.2017.178830
Kurtz et al. (24)	2018	DLBCL	Targeted capture	217	Pretreatment, on-treatment	R-CHOP-like	10.1200/JCO.2018.78.5246
Spina et al. (23)	2018	cHL	Targeted capture	112	Pretreatment; 80 patients Posttreatment; 24 patients Refractory; 32 patients	Multiple	10.1182/blood-2017-11-812073
Hattori et al. (125)	2018	PCNSL	ddPCR, amplicon	14	Pretreatment; 14 patients Posttreatment; 5 patients	EORTC, MPV	10.1111/cas.13450
Delfau-Larue et al. (25)	2018	FL	ddPCR	133	Pretreatment plasma; 61 patients PB; 68 patients	NA	10.1182/bloodadvances.2017015164
Hiemcke-Jiwa et al. (126)	2019	PCNSL	ddPCR	38	Pretreatment; 26 patients On-treatment; 12 time points	Multiple	10.1111/bjh.15674
Rimelen et al. (127)	2019	PCNSL	ddPCR	11	Pretreatment; 9 patients Relapse; 5 patients	NA	10.1186/s40478-019-0692-8

(*Continued*)

Table 3 (*Continued*)

Study	Year	Subtype	Molecular technique	Total patient count	Monitoring time points	Intervention	DOI
Raman et al. (85)	2020	DLBCL/HL	LP-WGS	123	Pretreatment; 123 patients On-treatment; 31 time points	Multiple	10.3324/haematol.2020.268813
Li et al. (116)	2020	ENKTL	Targeted capture	65	Pretreatment; 65 patients On-treatment; 10 time points	CHOP-like	10.1186/s40364-020-00205-4
Qi et al. (156)	2021	ENKTL	Targeted capture	47	Pretreatment	NA	10.1182/bloodadvances.2020001637
Alig et al. (22)	2021	DLBCL	Targeted capture	267	Pretreatment	R-CHOP-like	10.1200/JCO.20.02573
Meriranta et al. (81)	2021	DLBCL	Targeted capture, WGS	101	Pretreatment; 100 patients On-treatment; 58 time points Posttreatment; 71 patients	R-CHOP14 with CNS prophylaxis	10.1182/blood.2021012852
Rivas-Delgado et al. (83)	2021	DLBCL	Targeted capture	79	Pretreatment	NA	10.1158/1078-0432.Ccr-20-2558
Chiu et al. (32)	2021	DLBCL/FL	Methylation	73	Pretreatment	NA	10.1038/s41525-021-00179-8
Frank et al. (60)	2021	DLBCL	IgHTS	72	Pretreatment, on-treatment, surveillance	CAR-T/axi-cel	10.1200/jco.21.00377
Miljkovic et al. (122)	2021	PTCL	IgHTS	45	Pretreatment; 45 patients On-treatment; 24 time points Posttreatment; 24 patients	DA-EPOCH	10.1182/bloodadvances.2020003679
Bobillo et al. (128)	2021	PCNSL	ddPCR	19	Pretreatment, on-treatment	IT methotrexate, R-CHOP, BAM-R	10.3324/haematol.2019.241208
Buedts et al. (94)	2021	cHL	LP-WGS	177	Pretreatment; 177 patients On-treatment; 136 time points	ABVD, BV-AVD	10.1182/bloodadvances.2020003039

(*Continued*)

Table 3 (*Continued*)

Study	Year	Subtype	Molecular technique	Total patient count	Monitoring time points	Intervention	DOI
Sobesky et al. (88)	2021	cHL	Targeted capture	121	Pretreatment; 121 patients Posttreatment; 77 patients	BEACOPP, BRECADD, nivolumab/AVD	10.1016/j.medj.2021.09.002
Camus et al. (91)	2021	cHL	Amplicon	60	Pretreatment; 60 patients Interim (post-C2); 54 patients	ABVD, BEACOPP	10.3324/haematol.2019.237719
Chen et al. (26)	2021	DLBCL	Methylation	86	Pretreatment; 86 patients	R-CHOP	10.1186/s13148-020-00973-8
Mutter et al. (21)	2021	CNSL	Targeted capture	92	Pretreatment, on-treatment	Multiple	10.1182/blood-2021-149644
Bruscaggin et al. (86)	2021	DLBCL, FL, RS	Targeted capture	67	Pretreatment, on-treatment	Ibrutinib, nivolumab	10.1182/bloodadvances.2021004528
Lakhotia et al. (102)	2022	MCL	IgHTS	53	Pretreatment; 45 patients On-treatment; 33 time points Surveillance; 16 time points	DA-EPOCH-R with bortezomib	10.1182/bloodadvances.2021006397
Herrera et al. (87)	2022	DLBCL	Targeted capture	33	Pretreatment, posttreatment	Polatuzumab/BR versus BR	10.1182/bloodadvances.2021006415

Abbreviations: ACVBP, doxorubicin, cyclophosphamide, vindesine, bleomycin, and prednisone; axi-cel, axicabtagene ciloleucel; AITL, angioimmunoblastic T cell lymphoma; ABVD, doxorubicin, bleomycin, vinblastine, and dacarbazine; BEACOPP, bleomycin, etoposide, doxorubicin hydrochloride (Adriamycin), cyclophosphamide, vincristine (Oncovin), procarbazine, and prednisone; BAM-R, carmustine, cytarabine, methotrexate, and rituximab; BR, bendamustine-rituximab; BRECADD, brentuximab-vedotin, etoposide, cyclophosphamide, doxorubicin, dacarbazine, and dexamethasone; BV-AVD, brentuximab-vedotin and adriamycin, vinblastine, and dacarbazine; C2, second chemotherapy cycle; CAR-T, chimeric antigen receptor T cell; cHL, classic Hodgkin lymphoma; CHOP, cyclophosphamide, hydroxydaunorubicin, vincristine (Oncovin), and prednisone; CNSL, central nervous system lymphoma; CTCL, cutaneous T cell lymphoma; DA, dose-adjusted; ddPCR, droplet digital polymerase chain reaction; DLBCL, diffuse large B cell lymphoma; EBV+, Epstein-Barr virus–positive; ENKTL, extranodal natural killer/T cell lymphoma; EORTC, European Organisation for Research and Treatment of Cancer; EPOCH, etoposide, prednisone, vincristine (Oncovin), cyclophosphamide, and hydroxydaunorubicin; FL, follicular lymphoma; HL, Hodgkin lymphoma; HSCT, hematopoietic stem cell transplantation; IgHTS, immunoglobulin high-throughput sequencing; IT, intrathecal; LBCL, large B cell lymphoma; LP-WGS, low-pass whole-genome sequencing; MCL, mantle cell lymphoma; MPV, methotrexate, procarbazine, and vincristine; NA, not applicable; PB, peripheral blood; PCNSL, primary central nervous system lymphoma; PTCL, peripheral T cell lymphoma; PTLD, posttransplant lymphoproliferative disorder; qPCR, quantitative polymerase chain reaction; R, rituximab, R-CHOP, rituximab plus CHOP; RS, Richter's syndrome; WGS, whole-genome sequencing.

Considerations for Prospective Studies

Seizing the opportunity for clinical progress will require several important next steps. When using decentralized testing in laboratories across the world, two major issues in establishing cfDNA as a replicable biomarker across studies are the standardization of preanalytic workflows and harmonization of clinical response metrics. A working statement regarding these clinical priorities was formulated at the 15-ICML workshop on ctDNA, the first international consensus meeting of lymphoma ctDNA experts (70).

Logistical and analytical considerations. Steps in preanalytic standardization include prompt plasma processing of blood specimens and fluorometric quality control of extracted DNA to detect high-molecular-weight DNA contamination and ensure adequate cfDNA input of >5,000 hGE (70). Several metrics have been proposed for ctDNA quantification, but their harmonization could improve broader utility and adoption for lymphoma monitoring. The fraction of tumor-derived to total cfDNA is frequently estimated by the mean AF of tumor-confirmed variants. However, response assessment via relative measures can be deceptive, as total cfDNA levels demonstrate large shifts in response to exercise, granulocyte colony-stimulating factor growth factors, or other systemic stressors. Calculating the absolute plasma concentration of tumor-derived molecules (in hGE or other mass-based metrics per milliliter of plasma) can yield a more consistent quantification. Prespecified minimum variant counts and sequencing depths may also help standardize tumor-informed prospective investigations.

Response-directed therapy and trial design considerations. In contrast to hematologic malignancies including chronic myeloid leukemia, molecular response and MRD levels are not yet routinely assayed biomarkers in lymphomas. cfDNA levels typically change rapidly during treatment, and in patients receiving treatment for aggressive B cell lymphomas, a 2-log reduction after one cycle of chemoimmunotherapy (EMR) or a 2.5-log reduction after two cycles (MMR) was shown to be predictive of both EFS and OS (24). This threshold has subsequently been validated in cHL and should be evaluated in future studies of other lymphoma subtypes (23). Importantly, additional validation in prospective cohorts will be required to demonstrate either on-treatment kinetics or posttreatment MRD status as a robust surrogate for survival, as well as to utilize these measures as clinical trial end points. Potential trial design strategies are summarized graphically in **Figure 3**. While optimal time points for molecular monitoring are not conclusively established, current recommendations suggest evaluation pretreatment, after two cycles of therapy, at the end of treatment, and every 3–6 months during surveillance or maintenance (70).

In considering potential interventional strategies based on molecular data, the analytic lead time required for sample processing and analysis becomes an important consideration. Typical processing times currently range from 1–3 weeks, which may be acceptable for posttreatment interventions (e.g., maintenance therapy for MRD-positive patients) but potentially challenging for pretreatment evaluation of patients with active disease. One trial design that addresses this concern is a prephase or window approach in which patients receive a first intervention while molecular data is processed.

Opportunities for Technical and Clinical Development

In addition to the mutation- and translocation-based analyses detailed above, several emerging techniques target tumor-derived epigenetic signals including cfDNA methylation and fragmentation profiles (16, 50). Alternative nucleic acid sources including RNA are also under development for cancer detection—however, preliminary studies evaluating the tissue of origin of circulating

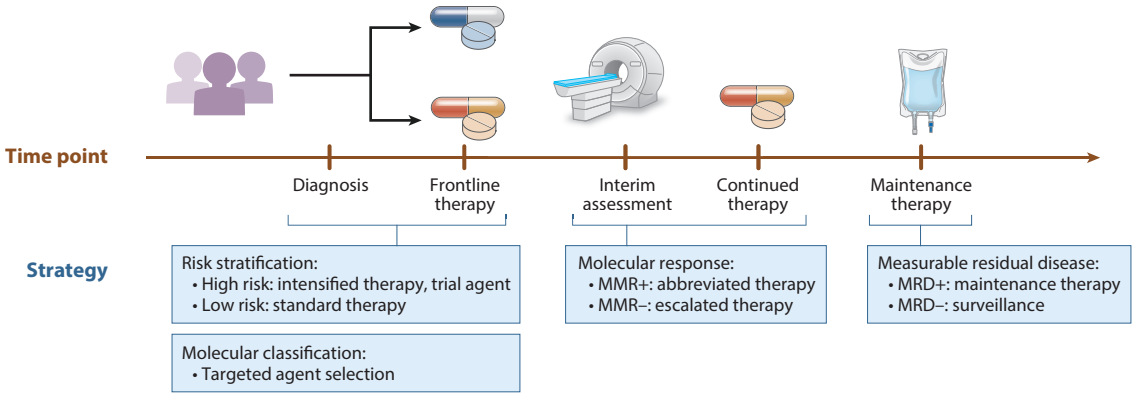

Figure 3

A schematic overview of potential molecular strategies for lymphoma clinical trial design, grouped by time point of ctDNA assessment. Abbreviations: ctDNA, circulating tumor DNA; MMR, major molecular response; MRD, measurable residual disease.

RNA molecules have estimated that the majority are of hematopoietic origin, and the ability to detect blood cancer signals against this background is incompletely characterized (56). An ultimate limiting factor in the sensitivity of cfDNA is the total number of tumor-derived molecules in an individual plasma specimen. As collection of larger blood volumes is unlikely to be feasible in routine clinical practice, several approaches have been developed to enrich tumor-derived nucleic acids. These include magnetic bead sorting or microfluidic approaches leveraging the shorter fragment length of tumor-derived molecules.

To date, most lymphoma patients assessed via molecular monitoring have undergone chemoimmunotherapy for aggressive disease. Indolent subtypes such as FL, marginal zone lymphoma, and small lymphocytic lymphoma remain understudied, including the critical clinical issues of early progression and histologic transformation. Likewise, response assessment to novel targeted therapeutics, immunotherapies, and cellular therapies will require additional validation. Emerging approaches to noninvasively assess the immune microenvironment alongside tumor-derived molecules may prove of particular interest in this context.

Conclusions

Molecular monitoring of lymphomas promises to augment clinical and radiographic response assessment and disease surveillance, with potential applications in risk stratification and targeted therapeutics. While fundamental principles are now established, several important technical and logistical hurdles remain for the broader clinical application of these techniques. These include the standardization of preanalytical and response assessment workflows when using decentralized ctDNA testing. Separately, additional validation of ctDNA measurements as surrogate biomarkers for survival in prospective clinical cohorts is needed, allowing utilization as a clinical trial end point. One opportunity for near-term development is the evaluation of molecular surveillance techniques in studies including R/R disease or high-risk subtypes such as double-hit lymphomas or ABC-DLBCL. Here, higher expected event rates may facilitate the more rapid refinement of technical sensitivity and clinical response criteria while serving patients with critical unmet clinical needs. Early detection of inadequate response or relapse in high-risk populations can serve as an important first step in translating the broader promise of molecular monitoring to improve outcomes for lymphoma patients.

SUMMARY POINTS

1. Cell-free DNA (cfDNA) and its malignant fraction, circulating tumor DNA (ctDNA), have important roles for the genotyping and monitoring of diverse cancers.
2. Lymphoma is well suited for molecular monitoring due to high ctDNA concentrations especially in aggressive disease, high somatic mutational burden, and the existence of stereotyped variants enabling focused interrogation of recurrently altered regions targeted by somatic hypermutation.
3. Emerging clinical applications for cfDNA in lymphoma include pretreatment genotyping and risk stratification, response assessment during therapy, and evaluation for measurable residual disease.
4. Potential future applications include molecular subtyping and classification, targeted therapy selection, detection of resistance mechanisms, and the surveillance of cellular therapies.

FUTURE ISSUES

1. Clinical circulating tumor DNA (ctDNA) studies in lymphoma to date have employed heterogenous molecular biology and bioinformatics workflows. Where centralized testing is unavailable, standardization of sample processing and harmonization of analysis and response criteria will allow improved comparison across clinical cohorts.
2. While treatment-related changes in ctDNA levels are strongly associated with clinical response and outcomes, additional prospective validations will more firmly establish ctDNA as a surrogate biomarker for progression-free and overall survival.
3. Given widespread epigenetic dysregulation in lymphomas, emerging epigenetic markers including ctDNA methylation and fragmentation profiles may provide additional information for subtype characterization, risk stratification, and response assessment.

DISCLOSURE STATEMENT

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LITERATURE CITED

1. Hughes T, Deininger M, Hochhaus A, Branford S, Radich J, et al. 2006. Monitoring CML patients responding to treatment with tyrosine kinase inhibitors: review and recommendations for harmonizing current methodology for detecting BCR-ABL transcripts and kinase domain mutations and for expressing results. *Blood* 108:28–37
2. Landgren O. 2018. MRD testing in multiple myeloma: from a surrogate marker of clinical outcomes to an every-day clinical tool. *Semin. Hematol.* 55:1–3
3. Kurtz DM, Green MR, Bratman SV, Scherer F, Liu CL, et al. 2015. Noninvasive monitoring of diffuse large B-cell lymphoma by immunoglobulin high-throughput sequencing. *Blood* 125:3679–87

4. Mandel P, Metais P. 1948. Nuclear acids in human blood plasma. *C. R. Des Seances Soc. Biol. Ses Filiales* 142:241–43
5. Leon SA, Shapiro B, Sklaroff DM, Yaros MJ. 1977. Free DNA in the serum of cancer patients and the effect of therapy. *Cancer Res.* 37:646–50
6. Hohaus S, Giachelia M, Massini G, Mansueto G, Vannata B, et al. 2009. Cell-free circulating DNA in Hodgkin's and non-Hodgkin's lymphomas. *Ann. Oncol.* 20:1408–13
7. Newman AM, Lovejoy AF, Klass DM, Kurtz DM, Chabon JJ, et al. 2016. Integrated digital error suppression for improved detection of circulating tumor DNA. *Nat. Biotechnol.* 34:547–55
8. Heitzer E, Haque IS, Roberts CES, Speicher MR. 2019. Current and future perspectives of liquid biopsies in genomics-driven oncology. *Nat. Rev. Genet.* 20:71–88
9. van Dessel LF, Beije N, Helmijr JCA, Vitale SR, Kraan J, et al. 2017. Application of circulating tumor DNA in prospective clinical oncology trials – standardization of preanalytical conditions. *Mol. Oncol.* 11:295–304
10. Pasqualucci L, Trifonov V, Fabbri G, Ma J, Rossi D, et al. 2011. Analysis of the coding genome of diffuse large B-cell lymphoma. *Nat. Genet.* 43:830–37
11. Schmitz R, Wright GW, Huang DW, Johnson CA, Phelan JD, et al. 2018. Genetics and pathogenesis of diffuse large B-cell lymphoma. *N. Engl. J. Med.* 378:1396–407
12. Chapuy B, Stewart C, Dunford AJ, Kim J, Kamburov A, et al. 2018. Molecular subtypes of diffuse large B cell lymphoma are associated with distinct pathogenic mechanisms and outcomes. *Nat. Med.* 24:679–90
13. Xu-Monette ZY, Wu L, Visco C, Tai YC, Tzankov A, et al. 2012. Mutational profile and prognostic significance of TP53 in diffuse large B-cell lymphoma patients treated with R-CHOP: report from an International DLBCL Rituximab-CHOP Consortium Program Study. *Blood* 120:3986–96
14. Scholze H, Stephenson RE, Reynolds R, Shah S, Puri R, et al. 2020. Combined EZH2 and Bcl-2 inhibitors as precision therapy for genetically defined DLBCL subtypes. *Blood Adv.* 4:5226–31
15. Scherer F, Kurtz DM, Newman AM, Stehr H, Craig AF, et al. 2016. Distinct biological subtypes and patterns of genome evolution in lymphoma revealed by circulating tumor DNA. *Sci. Transl. Med.* 8:364ra155
16. Esfahani MS, Hamilton EG, Mehrmohamadi M, Nabet BY, Alig SK, et al. 2022. Inferring gene expression from cell-free DNA fragmentation profiles. *Nat. Biotechnol.* 40(4):585–97
17. Araf S, Wang J, Korfi K, Pangault C, Kotsiou E, et al. 2018. Genomic profiling reveals spatial intra-tumor heterogeneity in follicular lymphoma. *Leukemia* 32:1258
18. Magnes T, Wagner S, Thorner AR, Neureiter D, Klieser E, et al. 2021. Spatial heterogeneity in large resected diffuse large B-cell lymphoma bulks analysed by massively parallel sequencing of multiple synchronous biopsies. *Cancers* 13:650
19. Green MR, Kihira S, Liu CL, Nair RV, Salari R, et al. 2015. Mutations in early follicular lymphoma progenitors are associated with suppressed antigen presentation. *PNAS* 112:E1116–25
20. Suehara Y, Sakata-Yanagimoto M, Hattori K, Nanmoku T, Itoh T, et al. 2018. Liquid biopsy for the identification of intravascular large B-cell lymphoma. *Haematologica* 103:e241–44
21. Mutter JA, Alig S, Lauer EM, Esfahani MS, Mitschke J, et al. 2021. Profiling of circulating tumor DNA for noninvasive disease detection, risk stratification, and MRD monitoring in patients with CNS lymphoma. *Blood* 138(Suppl. 1):6
22. Alig S, Macaulay CW, Kurtz DM, Duhrsen U, Huttmann A, et al. 2021. Short diagnosis-to-treatment interval is associated with higher circulating tumor DNA levels in diffuse large B-cell lymphoma. *J. Clin. Oncol.* 39:2605–16
23. Spina V, Bruscaggin A, Cuccaro A, Martini M, Di Trani M, et al. 2018. Circulating tumor DNA reveals genetics, clonal evolution, and residual disease in classical Hodgkin lymphoma. *Blood* 131:2413–25
24. Kurtz DM, Scherer F, Jin MC, Soo J, Craig AFM, et al. 2018. Circulating tumor DNA measurements as early outcome predictors in diffuse large B-cell lymphoma. *J. Clin. Oncol.* 36:2845–53
25. Delfau-Larue M-H, Van Der Gucht A, Dupuis J, Jais J-P, Nel I, et al. 2018. Total metabolic tumor volume, circulating tumor cells, cell-free DNA: distinct prognostic value in follicular lymphoma. *Blood Adv.* 2:807–16
26. Chen H-Y, Zhang W-L, Zhang L, Yang P, Li F, et al. 2021. 5-Hydroxymethylcytosine profiles of cfDNA are highly predictive of R-CHOP treatment response in diffuse large B cell lymphoma patients. *Clin. Epigenet.* 13:33

27. Han HS, Escalón MP, Hsiao B, Serafini A, Lossos IS. 2009. High incidence of false-positive PET scans in patients with aggressive non-Hodgkin's lymphoma treated with rituximab-containing regimens. *Ann. Oncol.* 20:309–18
28. Spina V, Bruscaggin A, Cuccaro A, Martini M, Di Trani M, et al. 2018. Circulating tumor DNA reveals genetics, clonal evolution, and residual disease in classical Hodgkin lymphoma. *Blood* 131:2413–25
29. Woyach JA, Furman RR, Liu T-M, Ozer HG, Zapatka M, et al. 2014. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. *N. Engl. J. Med.* 370:2286–94
30. Sworder B, Kurtz D, Alig S, Frank M, Macauley C, et al. 2021. Determinants of resistance to engineered T-cell therapies targeting CD19 in lymphoma. *Hematol. Oncol.* 39(S2) (Abstr.). **https://doi.org/10.1002/hon.6_2879**
31. Pasqualucci L, Khiabanian H, Fangazio M, Vasishtha M, Messina M, et al. 2014. Genetics of follicular lymphoma transformation. *Cell Rep*. 6:130–40
32. Chiu BCH, Chen C, You Q, Chiu R, Venkataraman G, et al. 2021. Alterations of 5-hydroxymethylation in circulating cell-free DNA reflect molecular distinctions of subtypes of non-Hodgkin lymphoma. *NPJ Genom. Med.* 6:11
33. Roulland S, Kelly RS, Morgado E, Sungalee S, Solal-Celigny P, et al. 2014. t(14;18) translocation: a predictive blood biomarker for follicular lymphoma. *J. Clin. Oncol.* 32:1347–55
34. Kurtz DM, Soo J, Co Ting Keh L, Alig S, Chabon JJ, et al. 2021. Enhanced detection of minimal residual disease by targeted sequencing of phased variants in circulating tumor DNA. *Nat. Biotechnol.* 39:1537–47
35. Schmitt MW, Fox EJ, Prindle MJ, Reid-Bayliss KS, True LD, et al. 2015. Sequencing small genomic targets with high efficiency and extreme accuracy. *Nat. Methods* 12:423
36. Suehara Y, Sakata-Yanagimoto M, Hattori K, Kusakabe M, Nanmoku T, et al. 2019. Mutations found in cell-free DNAs of patients with malignant lymphoma at remission can derive from clonal hematopoiesis. *Cancer Sci*. 110:3375–81
37. Nadeu F, Martin-Garcia D, Clot G, Díaz-Navarro A, Duran-Ferrer M, et al. 2020. Genomic and epigenomic insights into the origin, pathogenesis, and clinical behavior of mantle cell lymphoma subtypes. *Blood* 136:1419–32
38. Pott C, Hoster E, Delfau-Larue M-H, Beldjord K, Böttcher S, et al. 2010. Molecular remission is an independent predictor of clinical outcome in patients with mantle cell lymphoma after combined immunochemotherapy: a European MCL intergroup study. *Blood* 115:3215–23
39. Rambaldi A, Carlotti E, Oldani E, Della Starza I, Baccarani M, et al. 2005. Quantitative PCR of bone marrow BCL2/IgH$^+$ cells at diagnosis predicts treatment response and long-term outcome in follicular non-Hodgkin lymphoma. *Blood* 105:3428–33
40. Weinberg OK, Ai WZ, Mariappan MR, Shum C, Levy R, Arber DA. 2007. "Minor" *BCL2* breakpoints in follicular lymphoma. *J. Mol. Diagnost.* 9:530–37
41. Greisman HA, Greiner TC, Yi HS, Hoffman NG. 2008. High-throughput cloning of t(11;14) breakpoints outside the major translocation cluster in mantle cell lymphoma. *Blood* 112:3752
42. Voena C, Ladetto M, Astolfi M, Provan D, Gribben J, et al. 1997. A novel nested-PCR strategy for the detection of rearranged immunoglobulin heavy-chain genes in B cell tumors. *Leukemia* 11:1793–98
43. Pasqualucci L, Neumeister P, Goossens T, Nanjangud G, Chaganti RSK, et al. 2001. Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. *Nature* 412:341–46
44. Green MR, Monti S, Rodig SJ, Juszczynski P, Currie T, et al. 2010. Integrative analysis reveals selective 9p24.1 amplification, increased PD-1 ligand expression, and further induction via JAK2 in nodular sclerosing Hodgkin lymphoma and primary mediastinal large B-cell lymphoma. *Blood* 116:3268–77
45. Bea S, Zettl A, Wright G, Salaverria I, Jehn P, et al. 2005. Diffuse large B-cell lymphoma subgroups have distinct genetic profiles that influence tumor biology and improve gene-expression-based survival prediction. *Blood* 106:3183–90
46. Zviran A, Schulman RC, Shah M, Hill STK, Deochand S, et al. 2020. Genome-wide cell-free DNA mutational integration enables ultra-sensitive cancer monitoring. *Nat. Med.* 26:1114–24
47. Alexandrov LB, Kim J, Haradhvala NJ, Huang MN, Tian Ng AW, et al. 2020. The repertoire of mutational signatures in human cancer. *Nature* 578:94–101

48. Pinyol M, Cobo F, Bea S, Jares P, Nayach I, et al. 1998. p16^{INK4a} gene inactivation by deletions, mutations, and hypermethylation is associated with transformed and aggressive variants of non-Hodgkin's lymphomas. *Blood* 91:2977–84
49. Sermer D, Pasqualucci L, Wendel H-G, Melnick A, Younes A. 2019. Emerging epigenetic-modulating therapies in lymphoma. *Nat. Rev. Clin. Oncol.* 16:494–507
50. Liu M, Oxnard G, Klein E, Swanton C, Seiden M, et al. 2020. Sensitive and specific multi-cancer detection and localization using methylation signatures in cell-free DNA. *Ann. Oncol.* 31:745–59
51. Engels EA. 2007. Infectious agents as causes of non-Hodgkin lymphoma. *Cancer Epidemiol. Biomarkers Prev.* 16:401–4
52. Kanakry J, Ambinder R. 2015. The biology and clinical utility of EBV monitoring in blood. *Curr. Top. Microbiol. Immunol.* 391:475–99
53. Chan KCA, Yeung S-W, Lui W-B, Rainer TH, Lo YMD. 2005. Effects of preanalytical factors on the molecular size of cell-free DNA in blood. *Clin. Chem.* 51:781–84
54. Lee T-H, Montalvo L, Chrebtow V, Busch MP. 2001. Quantitation of genomic DNA in plasma and serum samples: higher concentrations of genomic DNA found in serum than in plasma. *Transfusion* 41:276–82
55. De Mattos-Arruda L, Mayor R, Ng CKY, Weigelt B, Martínez-Ricarte F, et al. 2015. Cerebrospinal fluid-derived circulating tumour DNA better represents the genomic alterations of brain tumours than plasma. *Nat. Commun.* 6:8839
56. Ibarra A, Zhuang J, Zhao Y, Salathia NS, Huang V, et al. 2020. Non-invasive characterization of human bone marrow stimulation and reconstitution by cell-free messenger RNA sequencing. *Nat. Commun.* 11:400
57. Metzenmacher M, Váraljai R, Hegedüs B, Cima I, Forster J, et al. 2020. Plasma next generation sequencing and droplet digital-qPCR-based quantification of circulating cell-free RNA for noninvasive early detection of cancer. *Cancers* 12:353
58. Garcia V, Garcia JM, Silva J, Martin P, Peña C, et al. 2009. Extracellular tumor-related mRNA in plasma of lymphoma patients and survival implications. *PLOS ONE* 4:e8173
59. Alidousty C, Brandes D, Heydt C, Wagener S, Wittersheim M, et al. 2017. Comparison of blood collection tubes from three different manufacturers for the collection of cell-free DNA for liquid biopsy mutation testing. *J. Mol. Diagnost.* 19:801–4
60. Frank MJ, Hossain NM, Bukhari A, Dean E, Spiegel JY, et al. 2021. Monitoring of circulating tumor DNA improves early relapse detection after axicabtagene ciloleucel infusion in large B-cell lymphoma: results of a prospective multi-institutional trial. *J. Clin. Oncol.* 39:3034–43
61. Treon SP, Xu L, Yang G, Zhou Y, Liu X, et al. 2012. MYD88 L265P somatic mutation in Waldenström's macroglobulinemia. *N. Engl. J. Med.* 367:826–33
62. Nakamura T, Tateishi K, Niwa T, Matsushita Y, Tamura K, et al. 2016. Recurrent mutations of *CD79B* and *MYD88* are the hallmark of primary central nervous system lymphomas. *Neuropathol. Appl. Neurobiol.* 42:279–90
63. Jardin F, Pujals A, Pelletier L, Bohers E, Camus V, et al. 2016. Recurrent mutations of the exportin 1 gene (XPO1) and their impact on selective inhibitor of nuclear export compounds sensitivity in primary mediastinal B-cell lymphoma. *Am. J. Hematol.* 91:923–30
64. Ching T, Duncan ME, Newman-Eerkes T, McWhorter MME, Tracy JM, et al. 2020. Analytical evaluation of the clonoSEQ assay for establishing measurable (minimal) residual disease in acute lymphoblastic leukemia, chronic lymphocytic leukemia, and multiple myeloma. *BMC Cancer* 20:612
65. Borowitz MJ, Wood BL, Devidas M, Loh ML, Raetz EA, et al. 2015. Prognostic significance of minimal residual disease in high risk B-ALL: a report from Children's Oncology Group study AALL0232. *Blood* 126:964–71
66. Mateos M-V, Dimopoulos MA, Cavo M, Suzuki K, Jakubowiak A, et al. 2017. Daratumumab plus bortezomib, melphalan, and prednisone for untreated myeloma. *N. Engl. J. Med.* 378:518–28
67. Thompson PA, Srivastava J, Peterson C, Strati P, Jorgensen JL, et al. 2019. Minimal residual disease undetectable by next-generation sequencing predicts improved outcome in CLL after chemoimmunotherapy. *Blood* 134:1951–59

68. Newman AM, Bratman SV, To J, Wynne JF, Eclov NC, et al. 2014. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat. Med.* 20:548–54
69. Cohen JD, Douville C, Dudley JC, Mog BJ, Popoli M, et al. 2021. Detection of low-frequency DNA variants by targeted sequencing of the Watson and Crick strands. *Nat. Biotechnol.* 39:1220–27
70. Rossi D, Kurtz DM, Roschewski M, Cavalli F, Zucca E, Wilson WH. 2020. The development of liquid biopsy for research and clinical practice in lymphomas: report of the 15-ICML workshop on ctDNA. *Hematol. Oncol.* 38:34–37
71. Adalsteinsson VA, Ha G, Freeman SS, Choudhury AD, Stover DG, et al. 2017. Scalable whole-exome sequencing of cell-free DNA reveals high concordance with metastatic tumors. *Nat. Commun.* 8:1324
72. Shen SY, Singhania R, Fehringer G, Chakravarthy A, Roehrl MHA, et al. 2018. Sensitive tumour detection and classification using plasma cell-free DNA methylomes. *Nature* 563:579–83
73. Ivanov M, Baranova A, Butler T, Spellman P, Mileyko V. 2015. Non-random fragmentation patterns in circulating cell-free DNA reflect epigenetic regulation. *BMC Genom.* 16(Suppl. 13):S1
74. Snyder MW, Kircher M, Hill AJ, Daza RM, Shendure J. 2016. Cell-free DNA comprises an in vivo nucleosome footprint that informs its tissues-of-origin. *Cell* 164:57–68
75. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, et al. 2000. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 403:503–11
76. Nowakowski GS, Chiappella A, Gascoyne RD, Scott DW, Zhang Q, et al. 2021. ROBUST: a phase III study of lenalidomide plus R-CHOP versus placebo plus R-CHOP in previously untreated patients with ABC-type diffuse large B-cell lymphoma. *J. Clin. Oncol.* 39:1317–28
77. Younes A, Sehn LH, Johnson P, Zinzani PL, Hong X, et al. 2019. Randomized phase III trial of ibrutinib and rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone in non-germinal center B-cell diffuse large B-cell lymphoma. *J. Clin. Oncol.* 37:1285–95
78. Wilson WH, Wright GW, Huang DW, Hodkinson B, Balasubramanian S, et al. 2021. Effect of ibrutinib with R-CHOP chemotherapy in genetic subtypes of DLBCL. *Cancer Cell* 39:1643–53.e3
79. Rossi D, Diop F, Spaccarotella E, Monti S, Zanni M, et al. 2017. Diffuse large B-cell lymphoma genotyping on the liquid biopsy. *Blood* 129:1947–57
80. Camus V, Sarafan-Vasseur N, Bohers E, Dubois S, Mareschal S, et al. 2016. Digital PCR for quantification of recurrent and potentially actionable somatic mutations in circulating free DNA from patients with diffuse large B-cell lymphoma. *Leuk. Lymphoma* 57:2171–79
81. Meriranta L, Alkodsi A, Pasanen A, Lepistö M, Mapar P, et al. 2021. Molecular features encoded in the ctDNA reveal heterogeneity and predict outcome in high-risk aggressive B-cell lymphoma. *Blood* 139(12):1863–77
82. Bohers E, Viailly P-J, Becker S, Marchand V, Ruminy P, et al. 2018. Non-invasive monitoring of diffuse large B-cell lymphoma by cell-free DNA high-throughput targeted sequencing: analysis of a prospective cohort. *Blood Cancer J.* 8:74
83. Rivas-Delgado A, Nadeu F, Enjuanes A, Casanueva-Eliceiry S, Mozas P, et al. 2021. Mutational landscape and tumor burden assessed by cell-free DNA in diffuse large B-cell lymphoma in a population-based study. *Clin. Cancer Res.* 27:513–21
84. Roschewski M, Dunleavy K, Pittaluga S, Moorhead M, Pepin F, et al. 2015. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. *Lancet Oncol.* 16:541–49
85. Raman L, Van der Linden M, De Vriendt C, Van den Broeck B, Muylle K, et al. 2020. Shallow-depth sequencing of cell-free DNA for Hodgkin and diffuse large B-cell lymphoma (differential) diagnosis: a standardized approach with underappreciated potential. *Haematologica* 107:211–20
86. Bruscaggin A, Di Bergamo LT, Spina V, Hodkinson B, Forestieri G, et al. 2021. Circulating tumor DNA for comprehensive noninvasive monitoring of lymphoma treated with ibrutinib plus nivolumab. *Blood Adv.* 5:4674–85
87. Herrera AF, Tracy S, Croft B, Opat S, Ray J, et al. 2022. Risk profiling of patients with relapsed/refractory diffuse large B-cell lymphoma by measuring circulating tumor DNA. *Blood Adv.* 6:1651–60
88. Sobesky S, Mammadova L, Cirillo M, Drees EEE, Mattlener J, et al. 2021. In-depth cell-free DNA sequencing reveals genomic landscape of Hodgkin's lymphoma and facilitates ultrasensitive residual disease detection. *Med* 2:1171–93.e11

89. Desch A-K, Hartung K, Botzen A, Brobeil A, Rummel M, et al. 2020. Genotyping circulating tumor DNA of pediatric Hodgkin lymphoma. *Leukemia* 34:151–66
90. Johnson P, Federico M, Kirkwood A, Fosså A, Berkahn L, et al. 2016. Adapted treatment guided by interim PET-CT scan in advanced Hodgkin's lymphoma. *N. Engl. J. Med.* 374:2419–29
91. Camus V, Viennot M, Lequesne J, Viailly P-J, Bohers E, et al. 2021. Targeted genotyping of circulating tumor DNA for classical Hodgkin lymphoma monitoring: a prospective study. *Haematologica* 106:154–62
92. Roemer MGM, Redd RA, Cader FZ, Pak CJ, Abdelrahman S, et al. 2018. Major histocompatibility complex class II and programmed death ligand 1 expression predict outcome after programmed death 1 blockade in classic Hodgkin lymphoma. *J. Clin. Oncol.* 36:942–50
93. Vandenberghe P, Wlodarska I, Tousseyn T, Dehaspe L, Dierickx D, et al. 2015. Non-invasive detection of genomic imbalances in Hodgkin/Reed-Sternberg cells in early and advanced stage Hodgkin's lymphoma by sequencing of circulating cell-free DNA: a technical proof-of-principle study. *Lancet Haematol.* 2:e55–65
94. Buedts L, Wlodarska I, Finalet-Ferreiro J, Gheysens O, Dehaspe L, et al. 2021. The landscape of copy number variations in classical Hodgkin lymphoma: a joint KU Leuven and LYSA study on cell-free DNA. *Blood Adv.* 5:1991–2002
95. Liu H, Johnson JL, Koval G, Malnassy G, Sher D, et al. 2012. Detection of minimal residual disease following induction immunochemotherapy predicts progression free survival in mantle cell lymphoma: final results of CALGB 59909. *Haematologica* 97:579–85
96. Hermine O, Hoster E, Walewski J, Bosly A, Stilgenbauer S, et al. 2016. Addition of high-dose cytarabine to immunochemotherapy before autologous stem-cell transplantation in patients aged 65 years or younger with mantle cell lymphoma (MCL Younger): a randomised, open-label, phase 3 trial of the European Mantle Cell Lymphoma Network. *Lancet* 388:565–75
97. Andersen NS, Pedersen LB, Laurell A, Elonen E, Kolstad A, et al. 2009. Pre-emptive treatment with rituximab of molecular relapse after autologous stem cell transplantation in mantle cell lymphoma. *J. Clin. Oncol.* 27:4365–70
98. Simonsen AT, Schou M, Sørensen CD, Bentzen HHN, Nyvold CG. 2015. SOX11, CCND1, BCL1/IgH and IgH-VDJ: a battle of minimal residual disease markers in mantle cell lymphoma? *Leuk. Lymphoma* 56:2724–27
99. Pott C. 2011. Minimal residual disease detection in mantle cell lymphoma: technical aspects and clinical relevance. *Semin. Hematol.* 48:172–84
100. Smith M, Jegede O, Parekh S, Hanson CA, Martin P, et al. 2019. Minimal residual disease (MRD) assessment in the ECOG1411 randomized phase 2 trial of front-line bendamustine-rituximab (BR)-based induction followed by rituximab (R) ± lenalidomide (L) consolidation for mantle cell lymphoma (MCL). *Blood* 134:751
101. Ladetto M, Brüggemann M, Monitillo L, Ferrero S, Pepin F, et al. 2014. Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. *Leukemia* 28:1299–307
102. Lakhotia R, Melani C, Dunleavy K, Pittaluga S, Saba NS, et al. 2022. Circulating tumor DNA predicts therapeutic outcome in mantle cell lymphoma. *Blood Adv.* 6:2667–80
103. Agarwal R, Chan Y-C, Tam CS, Hunter T, Vassiliadis D, et al. 2019. Dynamic molecular monitoring reveals that SWI–SNF mutations mediate resistance to ibrutinib plus venetoclax in mantle cell lymphoma. *Nat. Med.* 25:119–29
104. Martin P, Chadburn A, Christos P, Weil K, Furman RR, et al. 2009. Outcome of deferred initial therapy in mantle-cell lymphoma. *J. Clin. Oncol.* 27:1209–13
105. Clot G, Jares P, Giné E, Navarro A, Royo C, et al. 2018. A gene signature that distinguishes conventional and leukemic nonnodal mantle cell lymphoma helps predict outcome. *Blood* 132:413–22
106. Schroers-Martin JG, Kurtz DM, Soo J, Jin M, Scherer F, et al. 2017. Determinants of circulating tumor DNA levels across lymphoma histologic subtypes. *Blood* 130:4018
107. Hatipoğlu T, Esmeray E, Hu XH, Yuan HL, Erşen Danyeli A, et al. 2020. Ultra-deep targeted sequencing reveals cancer-associated, prognostically significant mutations in circulating cell-free DNA in treatment-naive patients with follicular lymphoma. *Blood* 136(Suppl. 1):20–21

108. Pastore A, Jurinovic V, Kridel R, Hoster E, Staiger AM, et al. 2015. Integration of gene mutations in risk prognostication for patients receiving first-line immunochemotherapy for follicular lymphoma: a retrospective analysis of a prospective clinical trial and validation in a population-based registry. *Lancet Oncol*. 16:1111–22
109. Jurinovic V, Passerini V, Oestergaard MZ, Knapp A, Mundt K, et al. 2019. Evaluation of the m7-FLIPI in patients with follicular lymphoma treated within the GALLIUM trial: *EZH2* mutation status may be a predictive marker for differential efficacy of chemotherapy. *Blood* 134(Suppl. 1):122
110. Sarkozy C, Huet S, Carlton VEH, Fabiani B, Delmer A, et al. 2017. The prognostic value of clonal heterogeneity and quantitative assessment of plasma circulating clonal IG-VDJ sequences at diagnosis in patients with follicular lymphoma. *Oncotarget* 8:8765–74
111. Morschhauser F, Tilly H, Chaidos A, McKay P, Phillips T, et al. 2020. Tazemetostat for patients with relapsed or refractory follicular lymphoma: an open-label, single-arm, multicentre, phase 2 trial. *Lancet Oncol*. 21:1433–42
112. Delfau-Larue M-H, Boulland M-L, Beldi-Ferchiou A, Feugier P, Maisonneuve H, et al. 2020. Lenalidomide/rituximab induces high molecular response in untreated follicular lymphoma: LYSA ancillary RELEVANCE study. *Blood Adv*. 4:3217–23
113. Luminari S, Manni M, Galimberti S, Versari A, Tucci A, et al. 2021. Response-adapted postinduction strategy in patients with advanced-stage follicular lymphoma: the FOLL12 study. *J. Clin. Oncol.* 40:729–39
114. Jiminez Ubieto AI, Heredia Y, de la Rosa JM, Rodriguez Izquierdo A, Rufian L, et al. 2020. Minimal residual disease monitoring from liquid biopsy by next generation sequencing in follicular lymphoma patients. *Blood* 136(Suppl. 1):31–33
115. Sakata-Yanagimoto M, Nakamoto-Matsubara R, Komori D, Nguyen TB, Hattori K, et al. 2017. Detection of the circulating tumor DNAs in angioimmunoblastic T-cell lymphoma. *Ann. Hematol.* 96:1471–75
116. Li Q, Zhang W, Li J, Xiong J, Liu J, et al. 2020. Plasma circulating tumor DNA assessment reveals KMT2D as a potential poor prognostic factor in extranodal NK/T-cell lymphoma. *Biomarker Res.* 8:27
117. Damm-Welk C, Busch K, Burkhardt B, Schieferstein J, Viehmann S, et al. 2007. Prognostic significance of circulating tumor cells in bone marrow or peripheral blood as detected by qualitative and quantitative PCR in pediatric NPM-ALK–positive anaplastic large-cell lymphoma. *Blood* 110:670–77
118. Kalinova M, Krskova L, Brizova H, Kabickova E, Kepak T, Kodet R. 2008. Quantitative PCR detection of NPM/ALK fusion gene and CD30 gene expression in patients with anaplastic large cell lymphoma—residual disease monitoring and a correlation with the disease status. *Leuk. Res.* 32:25–32
119. Damm-Welk C, Kutscher N, Zimmermann M, Attarbaschi A, Schieferstein J, et al. 2020. Quantification of minimal disseminated disease by quantitative polymerase chain reaction and digital polymerase chain reaction for NPM-ALK as a prognostic factor in children with anaplastic large cell lymphoma. *Haematologica* 105:2141–49
120. Quelen C, Grand D, Sarot E, Brugières L, Sibon D, et al. 2021. Minimal residual disease monitoring using a 3′ALK universal probe assay in ALK-positive anaplastic large-cell lymphoma. *J. Mol. Diagnost.* 23:131–39
121. Weng W-K, Armstrong R, Arai S, Desmarais C, Hoppe R, Kim YH. 2013. Minimal residual disease monitoring with high-throughput sequencing of T cell receptors in cutaneous T cell lymphoma. *Sci. Transl. Med.* 5:214ra171
122. Miljkovic MD, Melani C, Pittaluga S, Lakhotia R, Lucas AN, et al. 2021. Next-generation sequencing-based monitoring of circulating tumor DNA reveals clonotypic heterogeneity in untreated PTCL. *Blood Adv*. 5:4198–210
123. Mehta-Shah N, Jacobsen ED, Fehniger TA, Kahl BS, Bartlett NL, et al. 2021. End of treatment peripheral blood TCR evaluation for minimal residual disease evaluation in peripheral T-cell lymphomas. *Blood* 138:3506
124. Fontanilles M, Marguet F, Bohers É, Viailly P-J, Dubois S, et al. 2017. Non-invasive detection of somatic mutations using next-generation sequencing in primary central nervous system lymphoma. *Oncotarget* 8:48157–68

125. Hattori K, Sakata-Yanagimoto M, Suehara Y, Yokoyama Y, Kato T, et al. 2018. Clinical significance of disease-specific *MYD88* mutations in circulating DNA in primary central nervous system lymphoma. *Cancer Sci*. 109:225–30
126. Hiemcke-Jiwa LS, Leguit RJ, Snijders TJ, Bromberg JEC, Nierkens S, et al. 2019. *MYD88* p.(L265P) detection on cell-free DNA in liquid biopsies of patients with primary central nervous system lymphoma. *Br. J. Haematol.* 185:974–77
127. Rimelen V, Ahle G, Pencreach E, Zinniger N, Debliquis A, et al. 2019. Tumor cell-free DNA detection in CSF for primary CNS lymphoma diagnosis. *Acta Neuropathol. Commun.* 7:43
128. Bobillo S, Crespo M, Escudero L, Mayor R, Raheja P, et al. 2021. Cell free circulating tumor DNA in cerebrospinal fluid detects and monitors central nervous system involvement of B-cell lymphomas. *Haematologica* 106:513–21
129. Grommes C, Tang SS, Wolfe J, Kaley TJ, Daras M, et al. 2019. Phase 1b trial of an ibrutinib-based combination therapy in recurrent/refractory CNS lymphoma. *Blood* 133:436–45
130. Olszewski AJ, Chorzalska AD, Petersen M, Ollila TA, Zayac A, et al. 2020. Cerebrospinal fluid (CSF) analysis of tumor-specific cell-free DNA (cfDNA) as a diagnostic and prognostic tool for central nervous system (CNS) invasion in lymphoma. *Blood* 136(Suppl. 1):21–22
131. Fryer JF, Heath AB, Wilkinson DE, Minor PD, Kessler H, et al. 2011. *Collaborative study to evaluate the proposed 1st WHO international standards for Epstein-Barr virus (EBV) for nucleic acid amplification technology (NAT)-based assays*. WHO Rep. WHO/BS/2011.2172, World Health Organ., Geneva
132. Riddler SA, Breinig MC, McKnight JL. 1994. Increased levels of circulating Epstein-Barr virus (EBV)-infected lymphocytes and decreased EBV nuclear antigen antibody responses are associated with the development of posttransplant lymphoproliferative disease in solid-organ transplant recipients. *Blood* 84:972–84
133. Kanakry JA, Hegde AM, Durand CM, Massie AB, Greer AE, et al. 2016. The clinical significance of EBV DNA in the plasma and peripheral blood mononuclear cells of patients with or without EBV diseases. *Blood* 127:2007–17
134. Ruf S, Behnke-Hall K, Gruhn B, Bauer J, Horn M, et al. 2012. Comparison of six different specimen types for Epstein-Barr viral load quantification in peripheral blood of pediatric patients after heart transplantation or after allogeneic hematopoietic stem cell transplantation. *J. Clin. Virol.* 53:186–94
135. Allen UD, Preiksaitis JK, AST Infect. Dis. Community Practice. 2013. Epstein-Barr virus and posttransplant lymphoproliferative disorder in solid organ transplantation. *Am. J. Transplant.* 13(Suppl. 4):107–20
136. Choquet S, Varnous S, Deback C, Golmard JL, Leblond V. 2014. Adapted treatment of Epstein-Barr virus infection to prevent posttransplant lymphoproliferative disorder after heart transplantation. *Am. J. Transplant.* 14:857–66
137. Bakker NA, Verschuuren EA, Erasmus ME, Hepkema BG, Veeger NJ, et al. 2007. Epstein-Barr virus–DNA load monitoring late after lung transplantation: a surrogate marker of the degree of immunosuppression and a safe guide to reduce immunosuppression. *Transplantation* 83:433–38
138. Gandhi MK, Lambley E, Burrows J, Dua U, Elliott S, et al. 2006. Plasma Epstein-Barr virus (EBV) DNA is a biomarker for EBV-positive Hodgkin's lymphoma. *Clin. Cancer Res.* 12:460–64
139. Kanakry JA, Li H, Gellert LL, Lemas MV, Hsieh WS, et al. 2013. Plasma Epstein-Barr virus DNA predicts outcome in advanced Hodgkin lymphoma: correlative analysis from a large North American cooperative group trial. *Blood* 121:3547–53
140. Haverkos BM, Coleman C, Gru AA, Pan Z, Brammer J, et al. 2017. Emerging insights on the pathogenesis and treatment of extranodal NK/T cell lymphomas (ENKTL). *Discov. Med.* 23:189–99
141. Suzuki R, Yamaguchi M, Izutsu K, Yamamoto G, Takada K, et al. 2011. Prospective measurement of Epstein-Barr virus–DNA in plasma and peripheral blood mononuclear cells of extranodal NK/T-cell lymphoma, nasal type. *Blood* 118:6018–22
142. Wang ZY, Liu QF, Wang H, Jin J, Wang WH, et al. 2012. Clinical implications of plasma Epstein-Barr virus DNA in early-stage extranodal nasal-type NK/T-cell lymphoma patients receiving primary radiotherapy. *Blood* 120:2003–10
143. Chan KCA, Woo JKS, King A, Zee BCY, Lam WKJ, et al. 2017. Analysis of plasma Epstein-Barr virus DNA to screen for nasopharyngeal cancer. *N. Engl. J. Med.* 377:513–22

144. Lam WKJ, Jiang P, Chan KCA, Cheng SH, Zhang H, et al. 2018. Sequencing-based counting and size profiling of plasma Epstein-Barr virus DNA enhance population screening of nasopharyngeal carcinoma. *PNAS* 115:E5115–24
145. Lam WKJ, Jiang P, Chan KCA, Peng W, Shang H, et al. 2019. Methylation analysis of plasma DNA informs etiologies of Epstein-Barr virus-associated diseases. *Nat. Commun.* 10:3256
146. Hatton O, Smith MM, Alexander M, Mandell M, Sherman C, et al. 2019. Epstein-Barr virus latent membrane protein 1 regulates host B cell microRNA-155 and its target FOXO3a via PI3K p110α activation. *Front. Microbiol.* 10:2692
147. Rambaldi A, Lazzari M, Manzoni C, Carlotti E, Arcaini L, et al. 2002. Monitoring of minimal residual disease after CHOP and rituximab in previously untreated patients with follicular lymphoma. *Blood* 99:856–62
148. Hirt C, Schüler F, Kiefer T, Schwenke C, Haas A, et al. 2008. Rapid and sustained clearance of circulating lymphoma cells after chemotherapy plus rituximab: clinical significance of quantitative t(14; 18) PCR monitoring in advanced stage follicular lymphoma patients. *Br. J. Haematol.* 141:631–40
149. Goff L, Summers K, Iqbal S, Kuhlmann J, Kunz M, et al. 2009. Quantitative PCR analysis for Bcl-2/IgH in a phase III study of yttrium-90 ibritumomab tiuxetan as consolidation of first remission in patients with follicular lymphoma. *J. Clin. Oncol.* 27:6094–100
150. Morschhauser F, Recher C, Milpied N, Gressin R, Salles G, et al. 2012. A 4-weekly course of rituximab is safe and improves tumor control for patients with minimal residual disease persisting 3 months after autologous hematopoietic stem-cell transplantation: results of a prospective multicenter phase II study in patients with follicular lymphoma. *Ann. Oncol.* 23:2687–95
151. Ladetto M, Lobetti-Bodoni C, Mantoan B, Ceccarelli M, Boccomini C, et al. 2013. Persistence of minimal residual disease in bone marrow predicts outcome in follicular lymphomas treated with a rituximab-intensive program. *Blood* 122:3759–66
152. Pott C, Schrader C, Gesk S, Harder L, Tiemann M, et al. 2006. Quantitative assessment of molecular remission after high-dose therapy with autologous stem cell transplantation predicts long-term remission in mantle cell lymphoma. *Blood* 107:2271–78
153. Geisler CH, Kolstad A, Laurell A, Andersen NS, Pedersen LB, et al. 2008. Long-term progression-free survival of mantle cell lymphoma after intensive front-line immunochemotherapy with in vivo–purged stem cell rescue. *Blood* 112:2687–93
154. Cheminant M, Derrieux C, Touzart A, Schmit S, Grenier A, et al. 2016. Minimal residual disease monitoring by 8-color flow cytometry in mantle cell lymphoma: an EU-MCL and LYSA study. *Haematologica* 101:336
155. Herrera AF, Kim HT, Kong KA, Faham M, Sun H, et al. 2016. Next-generation sequencing-based detection of circulating tumour DNA after allogeneic stem cell transplantation for lymphoma. *Br. J. Haematol.* 175:841–50
156. Qi F, Cao Z, Chen B, Chai Y, Lin J, et al. 2021. Liquid biopsy in extranodal NK/T-cell lymphoma: a prospective analysis of cell-free DNA genotyping and monitoring. *Blood Adv.* 5:2505–14