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Circulating tumor DNA in lymphoma: technologies and applications

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Abstract

Lymphoma, a malignant tumor derived from lymphocytes and lymphoid tissues, presents with complex and heterogeneous clinical manifestations, requiring accurate patient classification for appropriate treatment. While invasive pathological examination of lymph nodes or lymphoid tissue remains the gold standard for lymphoma diagnosis, its utility is limited in cases of deep-seated tumors such as intraperitoneal and central nervous system lymphomas. In addition, biopsy procedures carry an inherent risk of complications. Computed tomography (CT) and positron emission tomography/computed tomography (PET/CT) imaging are essential for treatment assessment and monitoring, but lack the ability to detect early clonal evolution and minimal residual disease (MRD). Liquid biopsy-based analysis of circulating tumor DNA (ctDNA) offers a non-invasive alternative that allows for repeated sampling and overcomes the limitations of spatial heterogeneity and invasive biopsies. ctDNA provides genetic and epigenetic insights into lymphoma and serves as a dynamic, quantifiable biomarker for diagnosis, risk stratification, and treatment response. This review comprehensively summarizes common genetic variations in lymphoma and systematically evaluates ctDNA detection technologies, including PCR-based assays and next-generation sequencing (NGS). Applications of ctDNA detection in noninvasive genotyping, risk stratification, therapeutic response monitoring, and MRD detection are discussed across various lymphoma subtypes, including diffuse large B-cell lymphoma, Hodgkin lymphoma, follicular lymphoma, and T-cell lymphoma. By integrating recent research findings, the review highlights the role of ctDNA profiling in advancing precision medicine, enabling personalized therapeutic strategies, and improving clinical outcomes in lymphoma.

Keywords Lymphoma, Circulating tumor DNA, Next-generation sequencing, Liquid biopsy, Minimal residue disease

Background

Lymphoma, a malignant neoplasm that originates from lymphocytes and lymphoid tissues, has experienced a significant increase in incidence over recent years [1]. The complexity of its pathological types necessitates a comprehensive classification system. The latest World Health Organization (WHO) classification, authored by a diverse group of experts in hematopathology, hematology, oncology, genetics, and molecular biology, categorizes lymphomas based on cell lineage, such as B-, T-, or natural killer (NK) cells, and further stratifies subtypes within each lineage according to morphology, immunophenotype, genetic features, and clinical presentation [2].

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The clinical manifestations of different lymphoma subtypes exhibit marked heterogeneity, resulting in different treatments [3–5].

Recent advances in biomedical technologies, such as next-generation sequencing (NGS) and high-throughput drug screening, have enhanced our understanding of lymphoma and facilitated the development of targeted therapies and immunotherapy, thereby improving treatment outcomes and survival rates [5–7]. Although aggressive B-cell lymphomas are often curable with combination chemotherapy and immunotherapy, indolent lymphomas typically achieve durable remissions but require lifelong monitoring due to their incurable nature. Among aggressive subtypes, diffuse large B-cell lymphoma (DLBCL) poses a significant challenge, as resistance or relapse after first-line therapy is common for a subset of patients [8]. Therefore, accurately identifying patients at risk of refractory disease or relapse, along with early prognostic predictions at diagnosis, has become increasingly important. Such prognostic assessments are crucial for guiding personalized treatment strategies [5, 9, 10].

Circulating cell-free DNA (cfDNA) refers to small fragments of DNA, typically ~ 70–200 base pairs in length, that are released into the bloodstream or other body fluids as a result of cellular apoptosis (programmed cell death) or necrosis (cell death due to injury or disease) [11–14]. Originating from various organs and tissues, cfDNA can be detected in blood, urine, saliva, and additional body fluids [15]. Notably, as a dynamic biomarker, its concentration fluctuates under different physiological and pathological conditions, such as pregnancy, organ transplantation, and cancer. In recent years, cfDNA has garnered attention in clinical and research settings due to its non-invasive nature and utility as a biomarker for conditions such as cancer, prenatal testing, organ transplant monitoring, and infectious diseases [16–20]. Analysis of cfDNA can reveal genetic and epigenetic changes, offering insights into underlying physiological or pathological processes [21–23].

Circulating tumor DNA (ctDNA), a subset of cfDNA that originates from tumor cells, carries distinct genetic and epigenetic signatures specific to cancer (Fig. 1). ctDNA constitutes a variable fraction of cfDNA and has been extensively studied across a range of cancers, including lung, breast, colorectal, kidney, and hematological malignancies [23]. As ctDNA reflects the molecular characteristics of the tumor, it is increasingly being used for cancer prognosis, diagnosis, and monitoring of therapeutic responses. Recent research highlights the potential of ctDNA in lymphoma for risk stratification, therapeutic response assessment, and disease progression monitoring [24–27].

In this review, we provide a comprehensive overview of common genetic alterations in lymphoma and evaluate the current ctDNA detection technologies. We also examine recent advancements in the application of ctDNA in malignant lymphoma, emphasizing its pivotal role in advancing personalized medicine through non-invasive approaches.

Molecular genetics of lymphoma

Lymphomas are broadly classified into two main types: non-Hodgkin lymphoma (NHL), accounting for about 85–90% of cases, and Hodgkin lymphoma (HL), comprising the remaining 10–15% [3]. NHL itself represents a diverse group of malignancies with distinct genetic profiles [28, 29]. Approximately 85–90% of NHL cases originate from B cells, including DLBCL, follicular lymphoma (FL), marginal zone lymphoma (MZL), and mantle cell lymphoma (MCL), while the remainder derive from T cells or natural killer (NK) cells. Recent advances in NGS have provided comprehensive insights into the genomic landscape of these lymphoma subtypes, as illustrated in Fig. 2.

Diffuse large B-cell lymphoma

DLBCL, accounting for 30–40% of B-cell NHL, exhibits significant genetic heterogeneity that complicates a complete definition of its landscape (Fig. 2) [30, 31]. Integrative analyses of large biopsy cohorts have clarified its genetic variation, while ctDNA analysis detects alterations missed in tissue biopsies [32–34]. The pathogenic alterations affect key processes: B-cell differentiation (*BCL-6* translocations disrupt germinal center responses and *PRDM1* alterations enhance NF-κB activation and impair plasma cell differentiation); B-cell receptor signaling (mutations in *CD79B* and *CARD11*, *BCL10* amplifications, the $MYD88^{L265P}$ mutation in Toll-like receptor signaling, *TNFAIP3* mutations, increased *REL* expression, *PTEN* deletions, and *PIK3CA* amplifications or activating mutations in the PI3K–AKT–mTOR pathway); apoptosis (alterations in *BCL2* and *FAS*); epigenetic regulation (mutations in *KMT2D, CREBBP*, *EZH2,* and *EP300*); and immune evasion (*PD-L1* overexpression) [30, 35].

Molecular subtypes based on these features enable risk stratification through RNA-based cell-of-origin classifications (germinal center B-cell-like and activated B-cell-like) and genomic aberration-based systems defining five clusters (C1- C5) and seven subtypes (EZB MYC+, EZB MYC-, ST2, BN2, A53, N1, and MCD) (Fig. 2) [36–40], with plasma ctDNA genotyping further complementing RNA-based classification [41, 42]. Collectively, these genetic insights underscore the complexity of DLBCL and suggest avenues for targeted therapies and prognostic markers.

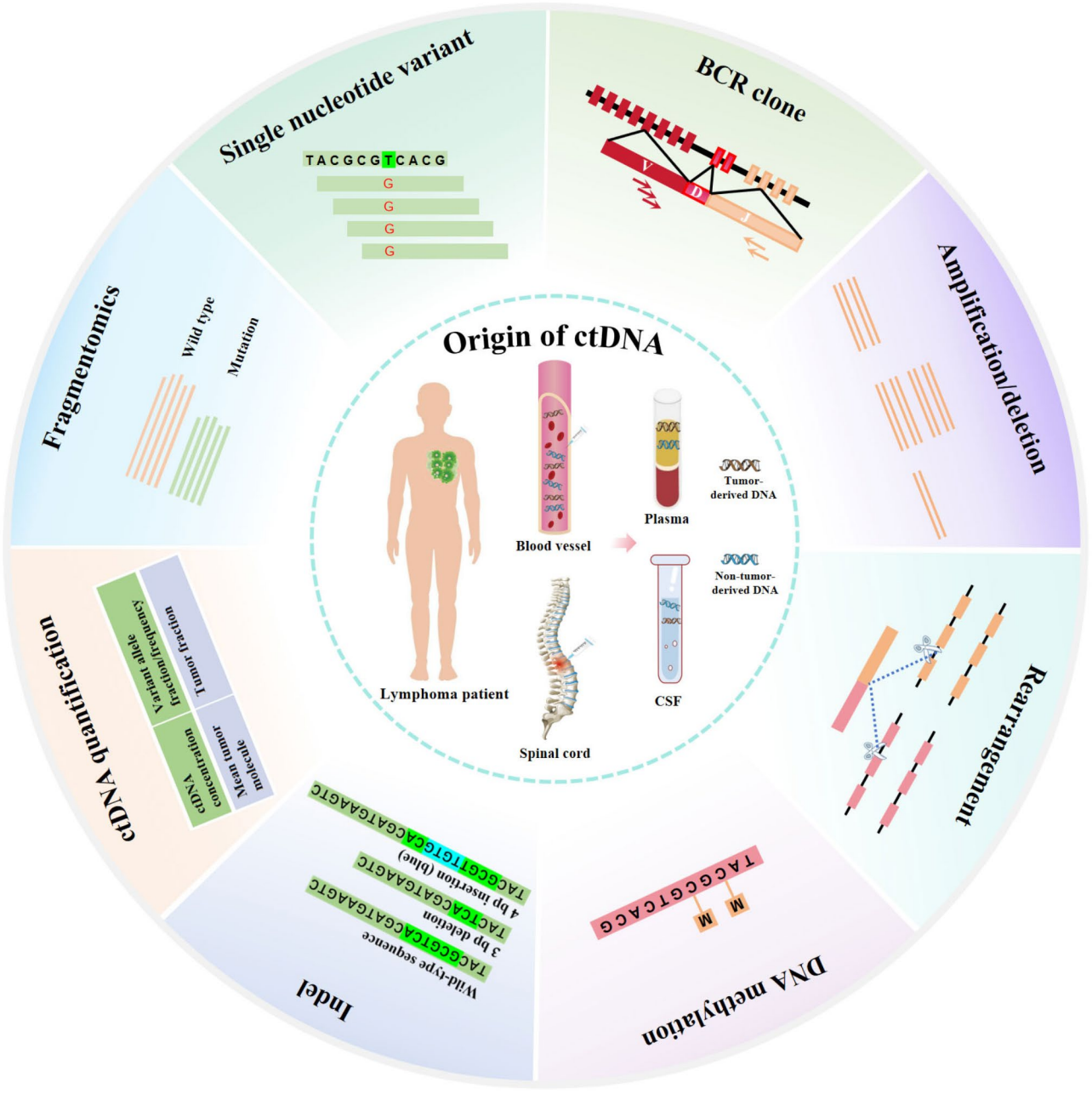

Fig. 1 Genetic features of ctDNA in lymphoma. Quantitative and qualitative analysis of ctDNA provide insights into cellular turnover, genetic and epigenetic alterations, immunoglobulin gene rearrangements, and fragmentomics, revealing underlying physiological and pathological processes in lymphoma

Mantle cell lymphoma and Burkitt lymphoma(BL)

MCL and BL have distinctive genetic hallmarks (Fig. 2). MCL is defined by the t(11;14)(p13;q32) translocation leading to *CCND1* overexpression and recurrent variations in DNA damage repair (*ATM*, *TP53*), epigenetic regulation (*NSD2*, *KMT2D*, *MEF2B*, *KMT2C*, *SMARCA4*), and cellular homeostasis and growth (*CCND1*, *CDKN2A*, *BIRC3*, *CARD11*, *TRAF2*, *RB1*, *POT1*, *NOTCH1/2*) [43–46]. BL is characterized by the t(8;14) translocation that activates MYC, along with mutations in *TCF3*, *ID3*, *CCND3*, *TP53*, and *CDKN2A* [47–51]. Both MCL and BL can be divided into outcome-related subtypes based on comprehensive genomic and transcriptomic profiles [50, 52].

Follicular lymphoma and marginal zone lymphoma

FL and MZL are common indolent B-NHLs, with approximately 15% of cases transforming to aggressive B-cell lymphomas. FL is characterized by the t(14;18)(q32;q21) translocation, which is necessary but not sufficient for

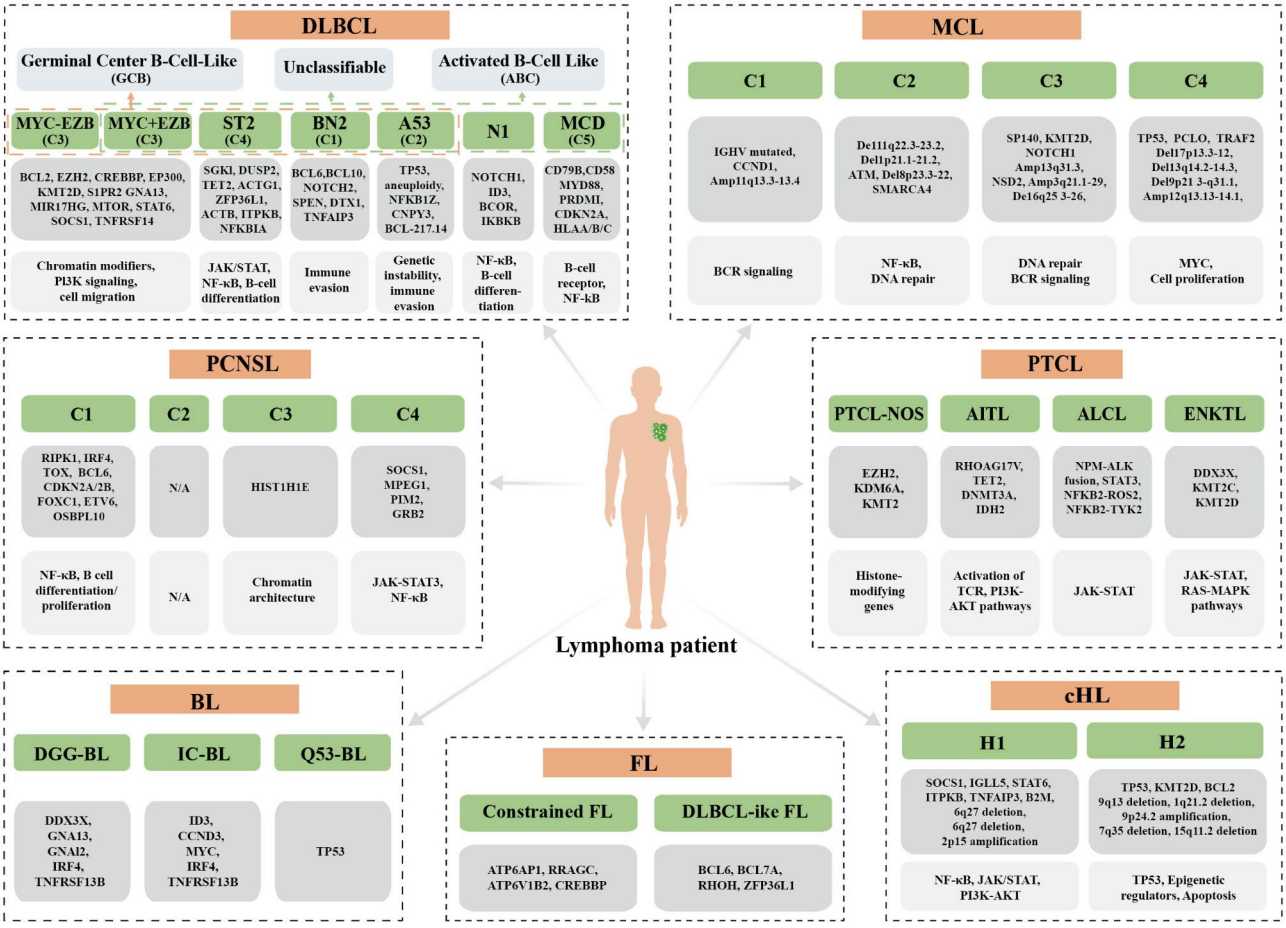

Fig. 2 Overview of lymphoma types, subtypes, genetic changes, and impacted signaling pathways in this review. Orange: lymphoma subtypes; light blue: the cell-of-origin classification of DLBCL; green: genetic subtypes of lymphoma; dark gray: genetic hallmarks of lymphoma; light gray: deregulated biological pathways. Abbreviations: DLBCL, diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; PCNSL, primary central nervous system lymphoma; BL, Burkitt lymphoma; PTCL, peripheral T-cell lymphoma; FL, follicular lymphoma; cHL, classical Hodgkin lymphoma. N/A, not available

its development [53, 54], and frequently shows alterations in epigenetic regulators such as *CREBBP*, *KMT2D*, *EZH2*, and *EP300* [55, 56]. MZL comprises three subtypes: extranodal, splenic, and nodal. Extranodal MZL is frequently associated with the t(11;18)(q21;q21) translocation that produces the *API2-MALT1* fusion and is less often linked to the t(14;18)(q32;q21) translocation (*IGH-MALT1*) or the t(1;14)(p22;q32) translocation (*BCL10-IGH*) [57, 58]. Both splenic and nodal MZL share genomic alterations including mutations in *KMT2D*, *NOTCH2*, *PTPRD*, *TNFAIP3*, and *KLF2* [59]. Predictive models based on these genetic features have been developed to assess the risk of histological transformation of FL and MZL to aggressive lymphoma [60–62].

Central nervous system lymphoma (CNSL)

CNSL is a rare, aggressive NHL subtype comprising primary CNS lymphoma (PCNSL) and secondary CNS lymphoma (SCNSL), the latter indicating lymphoma metastasized to the CNS from systemic disease. PCNSL, confined to the CNS at diagnosis, is marked by recurrent somatic mutations in key genes (*PIM1*, *MYD88*, *CD79B*, *KMT2D*, and *BTG2*) that affect critical signaling pathways (JAK-STAT, NF-κB, and B-cell receptor) and drive lymphomagenesis and progression [63, 64]. Additional alterations include recurrent amplifications and deletions at 18q21.23 and 6p21, along with a notable loss of MHC class I expression that may aid immune evasion. An integrative analysis of 240 PCNSL cases identified four distinct molecular clusters (C1, C2, C3, and C4) (Fig. 2), each with unique genetic and epigenetic profiles and prognostic outcomes [65]. These classifications offer valuable insights into PCNSL heterogeneity and have significant implications for personalized therapeutic strategies and prognostication.

Peripheral T-cell lymphoma (PTCL)

PTCL refers to an uncommon and diverse collection of aggressive NHL originating from mature T cells and NK cells [66]. Its major subtypes include PTCL-NOS,

angioimmunoblastic T-cell lymphoma (AITL), ALK-positive/negative anaplastic large cell lymphoma (ALCL), and extranodal NK/T-cell lymphoma (ENKTL) (Fig. 2). PTCL-NOS often harbors mutations in histone-modifying genes (*EZH2*, *KDM6A*, and *KMT2*) [67], while AITL is marked by recurrent inactivating $RHOA^{G17V}$ mutations and alterations in epigenetic regulators (*TET2*, *DNMT3A*, and *IDH2*), alongside activation of TCR and PI3K-AKT pathways [68, 69]. ENKTL exhibits mutations in RNA helicase genes (e.g., *DDX3X*), aberrations in the JAK-STAT and RAS-MAPK pathways, and alterations in epigenetic modulators (*KMT2C*, *KMT2D*) [70]. ALK-positive ALCL is defined by the NPM-ALK fusion that activates STAT3, whereas ALK-negative ALCL involves *NFKB2-ROS2* and *NFKB2-TYK2* fusions driving STAT3 activation [71]. Comprehensive genomic profiling has delineated four distinct molecular and microenvironmental PTCL subtypes with unique features [72].

Hodgkin lymphoma (HL)

Over 90% of HL cases are classified as classical HL (cHL), while nodular lymphocyte-predominant HL (NLPHL) accounts for about 5–10%. Tissue-based genomic profiling of cHL is limited by the low abundance of Hodgkin and Reed-Sternberg (HRS) cells, which represent only 0.1–10% of tumor cellularity [73, 74]. In contrast, noninvasive cfDNA profiling has demonstrated superior performance [34, 75–77]. cfDNA and flow-sorted HRS cell sequencing have revealed recurrent mutations in *SOCS1*, *TNFAIP3*, *B2M*, *STAT6*, *CSF2RB*, *GNA13*, *PTPN1*, *ARID1A*, *ZNF217*, *IL4R*, *NFKBIA*, *ACTB*, *PCBP1*, *CISH*, *NFKB2*, and linker histone *H1-5*, along with recurrent copy number variants (CNVs), including 2p15 (*REL*), 9p24.1-9p24.2 (*PDL1*), 5p15.33 (*TERT*), 17q21.31 (*MAP3K14*), 6q27 (*TNFAIP3*), 17p13.1 (*TP53*), 9p21.3 (*CDKN2A/B*), 11q22.3 (*BIRC3*) and 6p21-22 (*H1-5*, *HLA-A* and *HLA-C*) [75–82]. Targeted ctDNA sequencing of 366 patients has defined two cHL subtypes: cluster H1 (68% of cases), characterized by mutations in the NF-κB, JAK/STAT, and PI3K-AKT pathways, and cluster H2 (32%), which exhibits broader structural abnormalities and harbors mutations in *TP53* and *KMT2D* [34].

NLPHL displays a distinct genetic profile. It is defined by the presence of lymphocyte-predominant cells and lacks many of the mutations common in cHL [2, 73] In NLPHL, targeted sequencing reveals mutations in *SGK1*, *DUSP2*, and *JUNB*, as well as frequent *BCL6* translocations - a finding that is rare in cHL. Moreover, mutations in S*TAT6*, *JAK2*, *TNFAIP3*, and *NFKBIA*, which are prevalent in cHL, are uncommon in NLPHL [83, 84].

Methods for ctDNA detection and analysis

The clinical application of conventional genomic profiling in lymphoma management faces challenges due to spatial heterogeneity and the limited tumor material typically obtained from fine-needle aspirations and core needle biopsies. ctDNA profiling presents a noninvasive approach to capture comprehensive molecular characteristics without these sampling limitations, showing potential for genotyping, response assessment, and MRD monitoring in lymphomas.

Liquid biopsy technologies, particularly through analysis of ctDNA in body fluids like peripheral blood, have emerged as valuable tools for lymphoma detection and monitoring. Optimal ctDNA collection and processing depend on key preanalytical factors, including blood volume, timing of plasma isolation, and use of cell-stabilizing tubes to prevent cellular DNA contamination [25, 85–88]. Among ctDNA profiling technologies, PCR-based methods and NGS-based approaches are most common and have been extensively studied in recent years (Table 1; Fig. 3).

PCR-based methods

PCR assays, including BEAMing (beads, emulsion, amplification, and magnetics) [89], allele-specific oligonucleotide PCR (ASO-PCR) [90] and digital droplet PCR (ddPCR) [91], are cost-effective and provide rapid turnaround times, making them well-suited for ctDNA-based assays in lymphomas (Fig. 3). BEAMing leverages magnetic bead-based PCR amplification within microemulsions and flow cytometry, employing streptavidin-coated beads and biotinylated oligonucleotides for the highly sensitive detection and quantification of nucleotide variations through fluorescent labeling and analysis of PCR products (Fig. 3a) [89]. With a sensitivity as low as 0.01%, this technique reliably detects genetic alterations in ctDNA and shows strong concordance with alterations identified in patient tissue samples (Table 1) [92].

ASO-PCR, or amplification refractory mutation system (ARMS), utilizes uniquely designed primers to amplify DNA when there is a perfect match at single-nucleotide variant (SNV) or wild-type sequences, enabling precise SNV detection through specific PCR product patterns (Fig. 3b) [90]. For example, Jimenez et al. used ASO-PCR to reliably detect the $MYD88^{L265P}$ mutation in lymphoproliferative disorders, highlighting its utility as a sensitive, cost-effective diagnostic tool [87].

ddPCR is considered the gold standard for quantifying DNA mutations due to its ability to partition DNA molecules into thousands of droplets for individual PCR amplification, enabling high sensitivity and absolute quantification without needing standard curves (Fig. 3c) [93]. The method is effective in identifying genetic alternations in lymphomas, such as detecting t(14;18)

Table 1 Comparison of ctDNA detection technologies for lymphoma analysis

	Methods	Sensitivity	Advantages	Limitations
PCR-based	BEAMing [186]	~0.01%	High concordance with tissue-based assays	Detection of known mutations only
	ASO-PCR [90]	~1%	Simple workflow and clear results	Detection of known mutations only
	ddPCR [96]	~0.01%	Simple workflow and clear results	Detection of known mutations only
NGS-based	WGS/WES [98]	~1%	Broad variant coverage Oncogene and suppressor gene detection	Costly and time consuming Low sensitivity Difficult data interpretation
	CAPP-seq [99]	~0.002%	Simultaneous mutation identification Capture patient-specific variations Detect SNVs, insertions, rearrangements, CNVs	Complex, multi-step workflow Requires bioinformatics support Difficult data interpretation Unable to detect gene fusions
	PhasED-seq [97]	~0.00005%	High sensitivity Detects any type of somatic change, including fusions	Complex workflow Requires bioinformatics support Difficult data interpretation
	IgHTS [100, 187]	~0.005%	Simple workflow and high accuracy	Identification of a single marker; Need to identify tissue specific dominant clonality
	TBS [23]	-	High accuracy	Target regions need to be defined

Abbreviations: PCR, polymerase chain reaction; BEAMing, beads, emulsion, amplification and magnetics; ASO-PCR, allele specific oligonucleotide polymerase chain reaction; CNVs, copy number variants; ddPCR, droplet digital polymerase chain reaction; NGS, next generation sequencing; IgHTS, immunoglobulin high-throughput sequencing; VDJ, variable, diversity, joining; WGS, whole genome sequencing; WES, whole exome sequencing; CAPP-seq, cancer personalized profiling by deep sequencing; SNVs, single nucleotide variants; PhasED-seq, phased variant enrichment and detection sequencing; TBS, Targeted bisulfite sequencing

translocation in FL [54], t(11;14) translocation in MCL [94], and $MYD88^{L265P}$ in PCNSL [95].

Despite their advantages, PCR-based methods are typically limited to detecting a single or few known mutations, with a sensitivity threshold of approximately 0.01% allele frequency (AF) (Table 1) [96].

NGS-based methods

NGS-based technologies allow massive parallel sequencing of DNA molecules, enabling comprehensive assessment of mutational landscapes, including SNVs, insertions and deletions and CNVs [97–100]. Targeted amplicon-based and hybrid-capture NGS approaches offer advantages over single-gene assays by identifying a broad range of genetic alterations in lymphoma without the need for patient-specific optimization [101]. For example, Dubois et al. developed Lymphopanel-a 34-gene panel applied to samples from 215 patients-that revealed the molecular heterogeneity among DLBCL subtypes and identified mutations with potential therapeutic and prognostic significance [102].

Immunoglobulin high-throughput sequencing (IgHTS), marketed as clonoSEQ by Adaptive Biotechnology, is FDA approved for the detection of MRD in chronic lymphocytic leukemia (CLL), multiple myeloma (MM), and B-cell acute lymphoblastic leukemia (B-ALL) [85]. This technique uses universal primers for PCR amplification of immunoglobulin genomic regions, followed by sequencing to identify the amplified IgV(D)J clonotypes (Fig. 3d) [103]. IgHTS achieves a sensitivity of ~0.005%, although its effectiveness may be limited by somatic hypermutation and the amount of cfDNA analyzed (Table 1) [104]. Because it doesn't require patient-specific primers, it is widely applicable, although there are some limitations for highly mutated lymphoma subtypes [105].

Cancer personalized profiling by deep sequencing (CAPP-seq) [99], initially developed for non-small cell lung cancer with approximately 125 kb coverage, combines unique barcoding strategies with bioinformatics algorithms to improve sensitivity and enable ctDNA detection down to allele frequencies of ~0.002% (Fig. 3e; Table 1) [106]. This ultra-sensitive assay is used in diverse oncology research areas, including early detection, non-invasive genotyping, resistance mutation identification, and disease burden quantification [41, 43, 107].

Phased variant enrichment and detection sequencing (PhasED-Seq) is a hybrid capture method that uses phased variants, detects mutations within 150 base pairs on the same DNA strand for higher ctDNA sensitivity (Fig. 3f) [97]. Phased mutations are commonly found in specific genomic regions of B-cell lymphoma due to both normal and abnormal somatic hypermutation. Analytical sensitivity, tested by diluting lymphoma ctDNA in healthy cfDNA, achieved a detection threshold of 0.00005% (Table 1) [108].

Other techniques, including pyrosequencing [109], whole exome sequencing (WES) [110] and whole genome sequencing (WGS) [82], have been widely used for ctDNA analysis in lymphoma patients (Fig. 3g). Additionally, methods assessing epigenetic modifications as biomarkers for lymphoma diagnosis and monitoring are being explored (Fig. 3h) [111]. Using pyrosequencing, Kristensen and colleagues demonstrated the feasibility of detecting aberrant promoter DNA methylation in cfDNA from the plasma of DLBCL patients. They identified aberrant *DAPK1* methylation as an independent

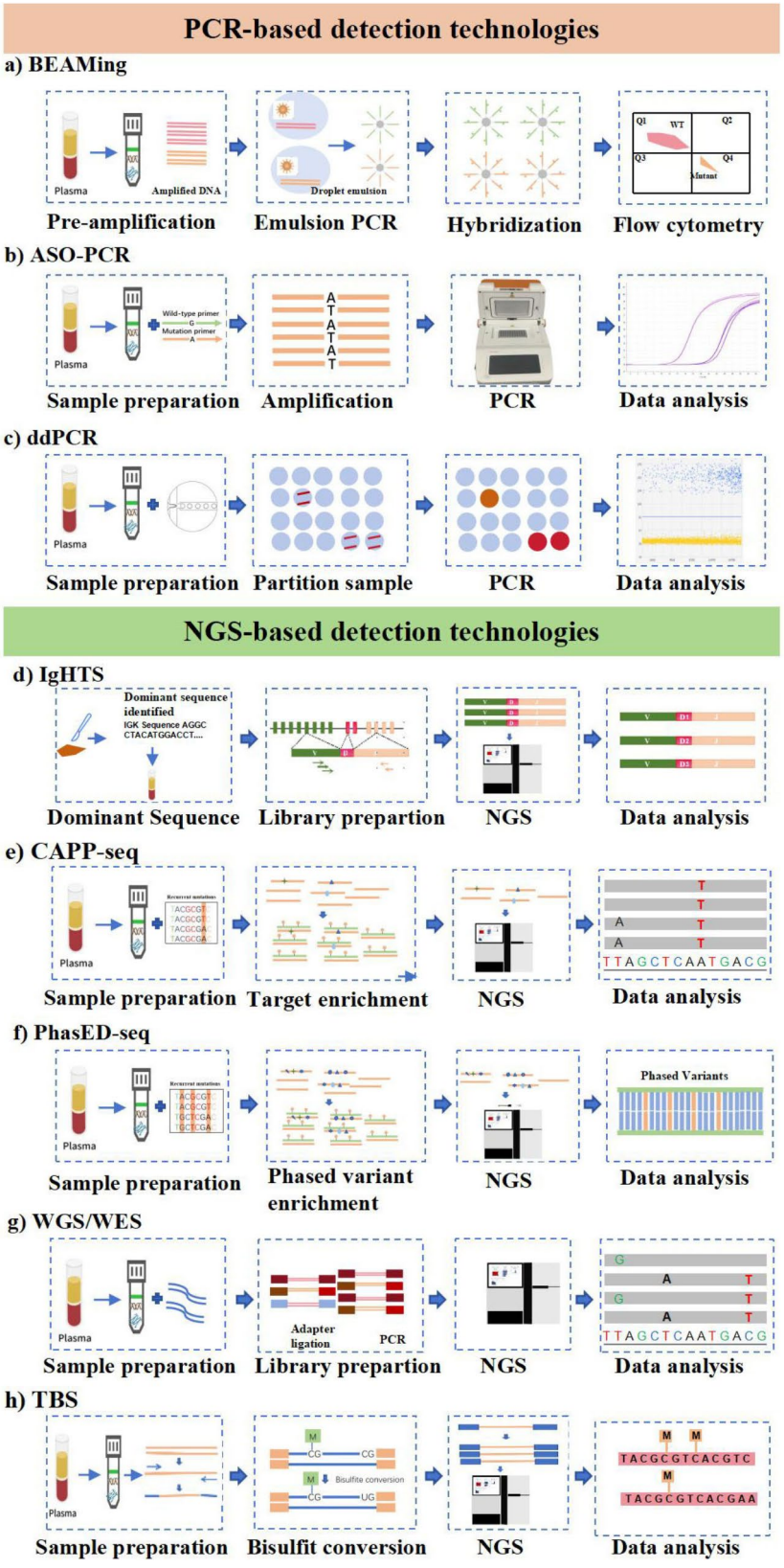

Fig. 3 (See legend on next page.)

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Fig. 3 Summary of key ctDNA detection technologies in lymphoma. Abbreviations: PCR, polymerase chain reaction; BEAMing, beads, emulsion, amplification and magnetics; ASO-PCR, allele specific oligonucleotide polymerase chain reaction; CNVs, copy number variants; ddPCR, droplet digital polymerase chain reaction; NGS, next generation sequencing; IgHTS, immunoglobulin high-throughput sequencing; VDJ, variable, diversity, joining; WGS, whole genome sequencing; WES, whole exome sequencing; CAPP-seq, cancer personalized profiling by deep sequencing; SNVs, single nucleotide variants; PhasED-seq, phased variant enrichment and detection sequencing; TBS, Targeted bisulfite sequencing

prognostic marker associated with treatment response and patient survival [112]. Furthermore, targeted bisulfite sequencing (TBS) of over 100,000 genomic regions using ctDNA has enabled the detection of more than 50 cancer types, including lymphoma, across all stages, achieving an average sensitivity of 54.9% at a specificity exceeding 99% and a single false positive rate below 1% [23].

Although numerous techniques have been reported, significant challenges remain in the analysis of ctDNA in lymphoma, including the detection of low absolute and fractional amounts (often less than 0.5%) of ctDNA, as well as the diversity of mutations present in lymphoma (Table 1) [85, 86, 105].

Application of ctDNA detection in lymphoma

Studies on the application of ctDNA in lymphoma have predominantly focused on DLBCL, with several investigations extending to other lymphoma subtypes (Tables 2 and 3). This section summarizes the clinical applications of ctDNA for noninvasive genotyping, treatment response monitoring, and MRD assessment across various lymphoma subtypes (Fig. 4).

Clinical significance of ctDNA testing in diffuse large B-cell lymphoma

ctDNA as a non-invasive genotyping biomarker

Genetic profiling of lymphoma tissue obtained via biopsy or surgery is essential for diagnosis and subtype classification. However, such tissue analysis can be challenging due to spatial heterogeneity and limited material from fine needle aspirations or core needle biopsies. To overcome these limitations, ctDNA has been investigated as a noninvasive biomarker for capturing the complete molecular landscape of DLBCL (Table 2). Studies have demonstrated a concordance rate greater than 70% between ctDNA and tissue-based genotyping in DLBCL [32, 113–115]. This high concordance highlights the potential for ctDNA genotyping in individualized therapy selection for DLBCL. For instance, patients with the ABC-DLBCL subtype harboring B-cell receptor mutations, particularly those with *MYD88* mutations, have shown high response rates to ibrutinib (80%) [116]. Furthermore, ctDNA-based cell of origin (COO) classification tools have shown strong concordance with tumor biopsies and have been used effectively for individualized risk stratification [41, 42]. Additionally, ctDNA analysis often detects novel mutations not found in tissue biopsies, which could be due to tumor spatial heterogeneity, clonal evolution in recurrence, or selective pressures from targeted therapy [32, 41, 113, 117–119]. These findings underscore that ctDNA genotyping is a clinically feasible, noninvasive tool for DLBCL patients.

ctDNA quantification in evaluating tumor burden and treatment efficacy

Beyond genotyping, pretreatment ctDNA levels can provide a reliable assessment of disease burden and predict outcomes in DLBCL. Baseline ctDNA levels correlate with total metabolic tumor volume (TMTV), international prognostic index (IPI), lactate dehydrogenase (LDH) levels, and Ann Arbor stage, with high predictive value for clinical outcomes in patients receiving standard immunochemotherapy [41, 107, 113, 120–123]. A study by Alig et al. demonstrated that pretreatment ctDNA levels also predicted a short diagnosis-to-treatment interval and served as an independent prognostic marker for event-free survival (EFS) in 267 DLBCL patients [124].

Traditional risk stratification tools like IPI and TMTV are often used only once before treatment and have not achieved the desired precision for personalized treatment [125–129]. Inspired by molecular response models in chronic myelogenous leukemia and CLL, recent studies have investigated the prognostic utility of ctDNA molecular responses in DLBCL treated with anthracycline-based regimens [130, 131]. In an early study, DLBCL patients with undetectable interim ctDNA by lgHTS after two cycles of dose-adjusted EPOCH-R treatment demonstrated favorable 5-year progression-free survival (PFS) [120]. Based on these findings, Kurtz and colleagues proposed thresholds for early molecular response (EMR) using CAPP-seq after a single cycle of front-line R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone) therapy. In their framework, a 2-log ctDNA reduction defined EMR, and a 2.5-log reduction after two cycles was classified as major molecular response (MMR). Both EMR and MMR were significantly correlated with improved EFS at 24 months for patients receiving either front-line or salvage therapy, with multivariate analyses indicating that EMR and MMR independently predicted EFS and overall survival (OS) [107]. It is also demonstrated that the molecular response (EMR or MMR, hazard ratios for EMR and MMR were 6.5–10 and 11–26) showed more strongly prognostic of outcomes than pretreatment ctDNA (hazard ratio of 2.4–2.6). Additionally, ctDNA clearance after

Table 2 Clinical application of ctDNA detection in diffuse large B cell lymphoma

Reference	Origin of ctDNA	Technologies	Patient counts (Lymphoma subtypes)	Clinical applications
Daigle et al. [114]	Plasma	62-gene panel NGS	185 (DLBCL, FL)	Treatment response
Rivas-Delgado et al. [113]	Plasma	112-gene panel NGS	100 (DLBCL)	Treatment response; Prognosis
Rossi et al. [32]	Plasma	59-gene panel CAPP-seq	50 (DLBCL)	Treatment response; MRD monitoring
Sworder et al. [117]	Plasma	Custom 608 kb oligonucleotide panel CAPP-seq	138 (R/R LBCL)	Treatment response; Prognosis
Bruscaggin et al. [118]	Plasma	Custom 608 kb oligonucleotide panel CAPP-seq	67 (DLBCL, FL, RS)	Treatment response; Prognosis
Meriranta et al. [122]	Plasma	126-gene panel NGS	101 (DLBCL, PMBCL, FL3b, THRLBCL)	Treatment response; Prognosis
Esfahani et al. [42]	Plasma	EPIC-seq targeting on 236 TSS regions	114 (DLBCL)	Prognosis
Olszewski et al. [170]	Cerebrospinal fluid	IgHTS	37 (Aggressive lymphoma)	Diagnostic
Alig et al. 2021 [124].	Plasma	Custom 608 kb oligonucleotide panel CAPP-seq	267 (DLBCL, TRHRLBCL)	Prognosis
Roschewski et al. [120]	Plasma	NGS targeting on IgH and IgK locus	126 (DLBCL)	MRD monitoring; Prognosis
Kurtz et al. [107]	Plasma	466-gene panel CAPP-seq	217 (DLBCL, PMBL)	Prognosis
Zou et al. [33]	Plasma	188-gene panel NGS	23 (DLBCL, HGBL, tFL)	Prognosis; MRD monitoring
Frank et al. [121]	Plasma	IgHTS	72 (DLBCL, TFL, PMBL)	Prognosis
Herrera et al. [135]	Plasma	320 kb panel NGS	33 (R/R DLBCL)	Prognosis; MRD monitoring
Kurtz et al. [104]	Plasma	IgHTS	75 (DLBCL, PTLD)	MRD monitoring
Scherer et al. [41]	Plasma	Custom 608 kb oligonucleotide panel CAPP-seq	116 (DLBCL)	MRD monitoring; Prognosis
Kurtz et al. [97]	Plasma	PhasED-Seq; Custom 608 kb oligonucleotide panel CAPP-seq	213 (B-cell lymphomas)	MRD monitoring

Abbreviations: NGS, next-generation sequencing; IgHTS, immunoglobulin high-throughput sequencing; Ig-NGS, immunoglobulin next generation sequencing; CAPP-seq, cancer personalized profiling by deep sequencing; PhasED-seq, phased variant enrichment and detection sequencing; EPIC-seq, epigenetic expression inference from cell-free DNA sequencing; DLBCL, diffuse large B-cell lymphoma; PMBCL, primary mediastinal B-cell lymphoma; FL3b, grade 3b follicular lymphoma; THRLBCL, T-cell/histiocyte-rich large B-cell lymphoma; PMBL, primary mediastinal B-cell lymphoma; RS, Richter's syndrome; FL, follicular lymphoma; LBCL, large B-cell lymphoma; CNS, central nervous system; HGBL: high grade B cell lymphoma; tFL, transformed follicular lymphoma; PTLD, posttransplant lymphoproliferative disorders; MRD, molecular residual disease; R/R, relapsed/refractory

four treatment cycles has been identified as an independent prognostic biomarker in multivariate analyses [132]. To address limitations associated with fixed-point ctDNA assessments, the Kurtz group developed the continuous individualized risk index (CIRI), a dynamic model incorporating IPI, pretreatment ctDNA, COO, EMR, MMR, and interim PET/CT to provide individualized risk profiles. The CIRI model outperformed traditional methods in predicting outcomes [133]. Furthermore, combining MMR with interim PET/CT improved PFS stratification, yielding 2-year PFS rates of 84%, 17%, and 0% across different risk groups ($p < 0.001$) [134].

ctDNA for risk stratification and treatment response in relapsed/refractory (R/R) diffuse large B cell lymphoma

The clinical utility of ctDNA levels in R/R DLBCL has shown promise across various therapies, notably chimeric antigen receptor (CAR) T-cell therapy, where risk stratification and response assessment remain challenging. In a study of 72 R/R DLBCL patients treated with axicabtagene ciloleucel, undetectable ctDNA one-week post-infusion was observed in 70% of patients with durable responses compared to only 13% of those with disease progression ($p < 0.0001$), while detectable ctDNA at day 28 predicted poorer PFS and OS [121]. An additional study using CAPP-seq in two independent CAR T-cell cohorts revealed that higher ctDNA levels at weeks 1 and 4 were significantly associated with progression ($p < 0.05$), with pretreatment ctDNA ($p = 0.003$) and minimal molecular residual at week 4 ($p = 0.028$) predicting EFS [117].

Baseline ctDNA levels have also been evaluated by CAPP-seq in R/R DLBCL patients receiving polatuzumab vedotin in combination with bendamustine and rituximab (BR) or BR alone, with results showing a correlation between baseline ctDNA and both PFS and OS. ctDNA levels decreased significantly in patients who achieved complete response (CR) compared to non-CR patients [135]. In 40 patients with R/R DLBCL (27 de novo and 13 transformed) treated with panobinostat, with or

Table 3 Clinical application of ctDNA detection in other non-Hodgkin lymphoma and Hodgkin lymphoma

Reference	Origin of ctDNA	Technologies	Patient counts (Lymphoma subtypes)	Clinical applications
Lakhotia, R et al. [139]	Plasma	NGS targeting on IgH genomic regions	53 (MCL)	MRD monitoring; Prognosis
Agarwal et al. [140]	Plasma	42-gene panel NGS	24 (R/R MCL)	Treatment response
Martínez-Laperche et al. [141]	Plasma	RT-PCR	154 (FL)	Treatment response; Prognosis
Nagy et al. [142]	Plasma	ddPCR	123 (FL)	Treatment response
Hatipoğlu et al. [145]	Plasma	110-gene panel NGS	20 (FL)	Diagnosis; Prognosis
Zhao et al. [146]	Plasma	59-gene panel NGS	28 (FL)	Prognosis; MRD monitoring
Yoon et al. [147]	Plasma	61-gene penel NGS	40 (FL)	Prognosis; MRD monitoring
Delfau-Larue et al. [148]	Plasma	ddPCR	133 (FL)	Prognosis; MRD monitoring
Fernández-Miranda et al.2023 [150].	Plasma	78-gene panel NGS	36 (FL)	Treatment response; MRD monitoring
Jiménez-Ubieto et al. [151]	Plasma	56-gene Panel NGS	11 (FL)	MRD monitoring
Schroers-Martin et al. [153]	Plasma	188-gene panel CAPP-seq	48 (FL)	Diagnosis
Tatarczuch et al. [154]	Plasma	48-gene panel NGS	18 (MZL)	Treatment response
Yoon et al. [155]	Plasma	54-gene panel NGS	42 (PCNSL)	Diagnostic
He et al. [156]	Plasma	NGS targeting on IgH genomic regions	5 (PCNSL)	Diagnosis
Bobillo et al. [157]	CSF; plasma	ddPCR	7 (PCNSL)	Diagnosis
Mutter et al. [159]	CSF; plasma	214-gene panel CAPP-seq	136 (CNSL)	Prognosis; MRD monitoring
Hiemcke-Jiwa et al. [162]	Aqueous humor; vitreous fluid	ddPCR	63 (PCNSL)	Diagnosis
Wang et al. [160]	Aqueous humor; vitreous fluid	400-gene panel NGS	15 (PCNSL)	Diagnosis
Downs et al. [163]	Plasma	MSP	26 (PCNSL)	Diagnosis
Heger et al. [164]	Plasma, CSF	Multiple gene panel NGS	67 (PCNSL, SCNSL)	Prognosis; MRD monitoring
Hiemcke-Jiwa et al. [168]	CSF, plasma	ddPCR	29 (PCNSL)	Diagnosis
Liang et al. [169]	CSF	475-gene panel NGS	150 (DLBCL, PCNSL)	Prognosis; MRD monitoring; Treatment response
Hayashida et al. [171]	Plasma	ASO-PCR	20 (AITL)	Diagnosis
Qi et al. [173]	Plasma	112-gene panel NGS	24 (ENKTL)	Prognosis
Ottolini et al. [174]	Plasma	81-gene panel CAPP-seq; 12-gene panel NGS; ddPCR	25 (PTCL-NOS, AITL, ALCL)	Treatment response; MRD monitoring
Kim et al. [175]	Plasma	171-gene panel NGS	45 (ENKTL)	Treatment response; MRD monitoring
Li et al. [176]	Plasma	41-gene panel NGS	65 (ENKTL)	MRD monitoring
Kim et al. [177]	Plasma	66-gene panel NGS	94 (TFHL, PTCL, ALCL, CTCL)	Prognosis
Miljkovic et al. [178]	Plasma	TCR sequences	45 (PTCL)	Prognosis
Herrera et al. [188]	Plasma	LgHTS; TCR sequencing	68 (B-NHL, T-NHL, HL, CLL)	MRD monitoring; Prognosis
Gao et al. [180]	Plasma	475-gene panel NGS	38 (R/R-ENKTL)	Prognosis
Jin-Hua et al. [179]	Plasma	475-gene panel NGS	64 (PTCL)	MRD monitoring; Prognosis
Tian et al. [181]	Plasma	WGBS; TBS	480 (ENKTL)	Diagnosis
Alig et al. [34]	Plasma	Custom 608 kb oligonucleotide panel CAPP-seq and PhasED-Seq	366 (cHL)	MRD monitoring; Prognosis

Table 3 (continued)

Reference	Origin of ctDNA	Technologies	Patient counts (Lymphoma subtypes)	Clinical applications
Spina et al. [76]	Plasma	Custom 608 kb oligonucleotide panel CAPP-seq	80 (cHL)	MRD monitoring; Treatment response
Desch et al. [77]	Plasma	106-gene and 121-gene panel NGS	96 (PHL)	MRD monitoring; Treatment response
Camus et al. [81]	Plasma	9-gene panel NGS	60 (cHL)	MRD monitoring
Camus et al. [182]	Plasma	ddPCR	94 (cHL)	Prognosis

Abbreviations: CSF, cerebrospinal fluid; ddPCR, droplet digital polymerase chain reaction; NGS, next generation sequencing; IgHTS, immunoglobulin high-throughput sequencing; WGBS, whole genome bisulfite sequencing; TBS, targeted bisulfite sequencing; RT-PCR, real time polymerase chain reaction; CAPP-seq, cancer personalized profiling by deep sequencing; Ig-NGS, immunoglobulin next generation sequencing; ASO-PCR, allele specific oligonucleotide polymerase chain reaction; TCR, T-cell receptor; MSP, Methylation-specific PCR; PHL, pediatric Hodgkin lymphoma; cHL, classical Hodgkin lymphoma; FL, follicular lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; PCNSL, primary central nervous system lymphoma; CNSL, central nervous system lymphoma; CNS, central nervous system; SCNSL, secondary central nervous system Lymphoma; DLBCL, diffuse large B-cell lymphoma; AITL, Angioimmunoblastic T-cell lymphoma; ENKTL, Extranodal natural killer/T-cell lymphoma; NOS, not otherwise specified; TFHL, T follicular helper lymphoma; PTCL, peripheral T-cell lymphoma; ALCL, anaplastic large cell lymphoma; CTCL, cutaneous T-cell lymphoma; B-NHL: B-cell non-Hodgkin lymphoma, T-NHL: T-cell non-Hodgkin lymphoma; CLL: chronic lymphocytic leukemia; HL, Hodgkin lymphoma; MRD, minimal residual disease; R/R, relapsed/refractory

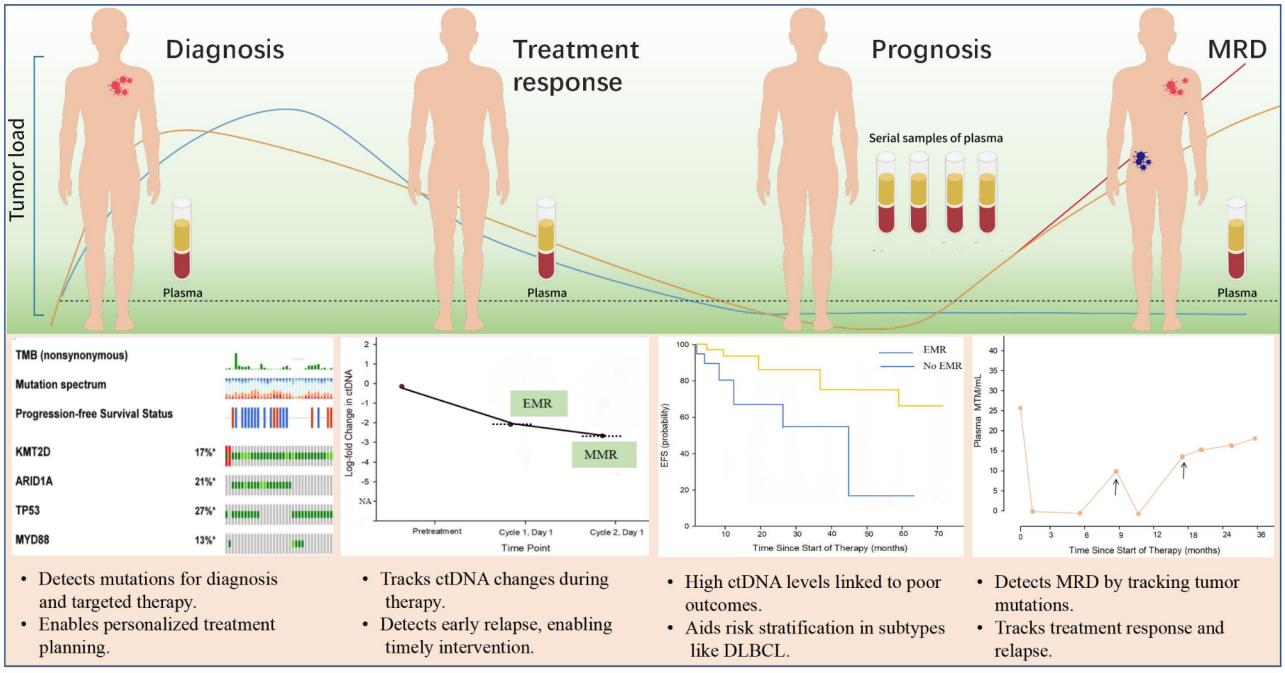

Fig. 4 Clinical applications of ctDNA testing in lymphoma. ctDNA profiling provides a noninvasive liquid biopsy method to capture comprehensive molecular characteristics, overcoming the limitations of traditional tissue sampling. Applications include diagnosis, genotyping, outcome prediction, response assessment, and MRD monitoring in lymphoma

without rituximab, ctDNA changes at day 15 showed 71.4% sensitivity and 100% specificity in predicting treatment response, compared to baseline ctDNA levels [136]. Dynamic ctDNA monitoring in an Asian cohort of 23 R/R DLBCL patients indicated that undetectable ctDNA at day 28 post-CAR19 T-cell therapy was linked to longer PFS ($p = 0.004$) and OS ($p = 0.004$). In addition, shorter ctDNA fragments (< 170 bp) were associated with poorer PFS ($P = 0.002$) and OS ($p = 0.008$) [33]. These findings suggest that baseline ctDNA levels, early ctDNA dynamics, and fragment size are promising predictors of treatment response and survival outcomes in R/R DLBCL.

ctDNA for minimal residual disease monitoring

Relapse remains a concern in DLBCL, even among patients achieving remission following first-line anthracycline-based therapy. Conventional surveillance techniques, including clinical examinations and imaging, often lack the sensitivity and specificity to detect early relapse [137, 138]. ctDNA monitoring offers a noninvasive and radiation-free alternative, allowing for personalized MRD detection through tumor-informed or disease-specific assays. Multiple studies have highlighted the utility of ctDNA over imaging for MRD monitoring in DLBCL. Two independent studies demonstrate

that IgHTS analysis of plasma ctDNA provided superior specificity and a high negative predictive value, detecting relapse three to 3.5 months before radiographic evidence [104, 120]. Scherer and co-workers applied CAPP-seq to monitor plasma ctDNA in DLBCL patients with CR or recurrence, finding that MRD detection anticipated relapse by an average of 6 months, outperforming IgHTS and imaging techniques [41]. PhasED-Seq further improved sensitivity, detecting MRD in all samples that were undetectable by CAPP-seq prior to biopsy-confirmed recurrence [97]. Moreover, combining ctDNA fragment analysis with mutation-based MRD monitoring may enhance detection accuracy in patients who test negative with mutation-based assays or show positive PET results [122].

Clinical significance of ctDNA testing in other lymphoma types

The application of ctDNA has been also investigated in other NHL types, including MCL, FL, MZL, CNSL, Peripheral T-cell lymphoma and HL as outlined in Table 3.

Mantle cell lymphoma

In untreated MCL, higher pretreatment ctDNA levels have been associated with several prognostic indicators, including the IPI ($P = 0.0004$) and TMTV ($r = 0.73$; $p = 0.0001$). However, no statistically significant survival difference was observed between patients with high pretreatment ctDNA (above the median) and those with low pretreatment ctDNA (below the median) [139]. Noninvasive ctDNA genotyping may also provide insights into treatment resistance mechanisms. For instance, in MCL patients undergoing ibrutinib-venetoclax therapy, ctDNA analysis revealed chromosome 9p21.1–p24.3 loss and specific mutations associated with resistance in all five study participants. Although these findings are promising, further research is needed to determine if such genotypic data can guide personalized treatments in MCL [140].

In a longitudinal study of 53 untreated MCL patients with a median follow-up of 12.7 years, patients achieving undetectable ctDNA after two cycles of induction therapy had significantly longer PFS and OS than those with detectable ctDNA (median PFS: 2.7 vs. 1.8 years, $p = 0.005$; median OS: 13.8 vs. 7.4 years, $p = 0.03$). Molecular relapse was detectable before clinical progression in seven patients, suggesting that MRD-guided treatment strategies could improve outcomes [139]. Nevertheless, further investigation is needed to assess MRD-guided treatment by comparing preemptive treatment with conventional therapy for MCL.

Follicular lymphoma and marginal zone lymphoma

EZH2 is an important biomarker for guiding frontline treatment in FL, with tazemetostat being approved for patients harboring *EZH2* mutations [141]. In a cohort of 123 FL patients, multiplex ddPCR identified an *EZH2* mutation frequency of 41.5% using paired biopsy tissue and ctDNA, which is higher than previous estimates of 20–27% [142–144]. This highlights the potential need for routine *EZH2* mutation screening in ctDNA sample of FL to refine patient selection for targeted therapies.

In addition to genetic mutation screening, liquid biopsy analyses have provided further insights into the prognostic landscape of FL. High levels of plasma ctDNA mutations in genes such as *BCL2*, *KMT2D*, *EP300*, *STAT6*, *CREBBP*, and *TP53* have been linked to poor survival outcomes in FL [145–147]. The distinct prognostic value of ctDNA, CTCs, and TMTV has been clearly demonstrated. FL patients with elevated TMTV (> 510 cm^3, $p = 0.0004$), high CTC counts (> 0.0018 PB cells, $p = 0.03$), or increased cfDNA levels (> 2,550 equivalent genomes/ml, $p = 0.04$) showed a lower 4-year PFS. Both cfDNA and TMTV remained independently predictive of outcomes in multivariate Cox analysis, underscoring their prognostic relevance [148].

Pretreatment ctDNA levels are particularly prognostic in FL, with high levels emerging as the only independent factor associated with PFS in multivariate analysis (HR 4, 95% CI: 1.1–37, $p = 0.039$). Patients with elevated ctDNA before treatment had a notably poorer prognosis, with higher levels observed in those who failed to achieve CR or who experienced disease progression within 24 months (POD24), compared to patients who achieved CR or were POD24-negative ($p = 0.02$ and $p < 0.001$, respectively) [149, 150]. In a longitudinal study of 13 FL patients who achieved CR after frontline chemotherapy, persistent or re-emergent ctDNA mutations were closely linked to disease relapse, emphasizing the utility of ctDNA in monitoring for early signs of recurrence [147].

ctDNA levels have also shown promise in assessing treatment responses across different FL therapies. Among FL patients treated with anti-CD19 CAR T-cell therapy, two of four patients who were PET/CT-positive post-treatment were found to be MRD-negative by ctDNA analysis and experienced no relapse after a median follow-up of 34 months [151]. This suggests that ctDNA monitoring may complement PET/CT in confirming treatment success and assessing MRD status in FL.

Transformation from indolent FL to aggressive DLBCL often indicates a worsening prognosis [152]. Scherer et al. developed a noninvasive ctDNA-based prediction model with 83% sensitivity and 89% specificity to identify FL transformation, allowing for earlier detection. In one case, the ctDNA model captured both indolent and

aggressive clones prior to clinical transformation, highlighting the ability of ctDNA to reflect tumor heterogeneity [41]. Similarly, a study by Schroers-Martin et al. using CAPP-seq on pre-diagnostic samples found that *CREBBP* mutations in blood could help identify patients at risk of developing FL, suggesting a potential role for ctDNA in early detection and risk stratification [153].

In MZL, ctDNA profiling through targeted NGS assays showed increases in primary ctDNA levels at disease progression, with ctDNA mutation burden decreasing in patients achieving partial remission, indicating the potential for ctDNA to serve as a marker for treatment response [154].

Central nervous system lymphoma

Minimally invasive ctDNA analysis of cerebrospinal fluid (CSF) or plasma offers significant potential for enhancing the diagnosis, surveillance, and prognosis of CNSL. Studies employing targeted NGS of ctDNA in PCNSL patients have demonstrated a 45% sensitivity for detecting mutations found in primary tumor tissue [155]. However, assessment of *IgH* gene rearrangements in plasma for residual disease tracking has shown limited effectiveness, with only one tracking clone detected out of four patients, highlighting the challenges of ctDNA monitoring in PCNSL [156]. Interestingly, the genetic profiles of CSF ctDNA show greater concordance with tissue findings compared to plasma ctDNA, suggesting CSF as a more reliable medium for analysis in CNSL [157, 158].

Building upon these findings, ultrasensitive ctDNA profiling detected ctDNA in 78% of plasma and all CSF samples from CNSL patients before treatment. Patients with detectable plasma ctDNA prior to treatment had significantly shorter PFS ($P < 0.0001$) and OS ($P = 0.0001$), with plasma ctDNA-based MRD monitoring effectively identifying high-risk patients (PFS, $P = 0.0002$; OS, $P = 0.004$) [159]. Additionally, a biopsy-free CNSL identification model based on ctDNA mutation patterns and burden demonstrated sensitivities of 59% in CSF and 25% in plasma, maintaining 100% specificity and positive predictive value. Moreover, for vitreoretinal lymphomas, ctDNA sequencing from aqueous humor has shown high concordance with vitreous fluid, suggesting that aqueous humor ctDNA may be a viable, noninvasive alternative to vitreous fluid for diagnosis and monitoring therapeutic response [160–162].

In parallel to these genetic analyses, epigenetic biomarkers in ctDNA, established for various solid tumors, are also being evaluated for CNSL. In a pilot study, two methylated markers (cg054 and SCG3) showed a sensitivity of 20% for distinguishing PCNSL from other CNS tumors [163]. Moreover, a peripheral residual disease biomarker has demonstrated high predictive value for relapse, and when integrated with clinical risk factors and radiographic response into a molecular prognostic index, it provided strong predictive power for CNSL outcomes [164].

Beyond primary CNSL, predicting CNS relapse in DLBCL remained challenging. Mutations in $MYD88^{L265P}$ and $CD79B^{Y196}$ were detectable in CSF ctDNA approximately one month before clinical diagnosis, suggesting their utility in early detection of CNS relapse in lymphoma patients [165–168]. However, as 15-20% of CNSL cases lack these mutations, negative results should be interpreted with caution. In a cohort study of 126 newly diagnosed DLBCL and 24 PCNSL cases, pretreatment CSF ctDNA demonstrated 100% sensitivity and 77.3% specificity for predicting CNS relapse when analyzed with a panel of 475 leukemia- and lymphoma-related genes [169]. Furthermore, clonotypic DNA was identified in all CSF ctDNA samples from patients with parenchymal CNS involvement and in 36% of aggressive lymphomas, indicating a 29% risk of CNS recurrence [170].

Peripheral T-cell lymphoma

ctDNA analysis has emerged as a noninvasive diagnostic and genetic profiling tool with broad clinical applications in PTCL. Detection of mutations in genes such as *TET2*, *RHOA*, *DNMT3A*, and *IDH2* in plasma ctDNA has provided a noninvasive method for diagnosing AITL (a subtype of PTCL) [171, 172]. In ENKTL patients, plasma ctDNA testing demonstrated a sensitivity of 72.4% for detecting tumor biopsy variants [173]. Furthermore, CAPP-seq analysis of cfDNA from PTCL patients identified novel *RHOA* mutations, including c.73 A > G (p.Phe25Leu) and c.48 A > T (p.Cys16*), which were validated in additional tissue cohorts. This finding suggests that ctDNA sequencing can identify somatic mutations not detected in tumor genomic DNA, overcoming tumor spatial heterogeneity and providing comprehensive genotypic information [174]. The diagnostic capabilities, combined with the ability to identify novel mutations, highlight the potential of ctDNA analysis not only for initial diagnosis but also for guiding subsequent treatment strategies.

Despite chemotherapy being the standard first-line treatment for most PTCL subtypes, treatment resistance limits its efficacy. Noninvasive ctDNA monitoring offers dynamic assessment of molecular burden, treatment response, prognostic risk, and MRD. Mutations in *DDX3X* and *KMT2D* detected in ctDNA from ENKTL patients have been associated with poor PFS [175, 176]. In cases where Epstein-Barr virus (EBV) DNA was undetectable in whole blood, ctDNA mutations were identified in 7 of 14 patients, suggesting that ctDNA profiling can complement EBV DNA quantification in ENKTL monitoring [175]. Analyzing plasma ctDNA mutation profiles in 94 PTCL patients using targeted NGS

revealed a significant association between post-treatment ctDNA levels and survival outcomes [177]. Moreover, tumor-specific clones were identified in 76% of patients using NGS-based TCR sequencing of ctDNA; detectable ctDNA after treatment predicted worse survival, although the prognostic significance throughout treatment was not statistically significant [178]. Another study involving 64 Chinese PTCL patients found that high pretreatment ctDNA levels were significantly associated with adverse clinical markers, and MRD negativity correlated with higher remission rates [179]. Collectively, these data indicate that ctDNA may have potential for noninvasive monitoring of treatment response and predicting outcomes in PTCL patients, with emerging evidence highlighting its role in high-risk subgroups.

In high-risk ENKTL patients, targeted NGS of tumor tissue and longitudinal plasma ctDNA showed that low pretreatment cfDNA concentrations were associated with favorable survival outcomes (1-year PFS: 90.0% vs. 36.4%; $p = 0.012$). Patients with rapid clearance of ctDNA mutations achieved significantly higher complete remission rates (80.0% vs. 0%; $p = 0.004$) and more favorable PFS (79.0% vs. 20.0%; $p = 0.002$) compared to those with persistent detectable mutations [173]. A phase 1b/2 study assessing ctDNA biomarkers in 38 R/R ENKTL patients treated with anti-PD-1 antibodies found that integrating plasma ctDNA with EBV DNA provided better prognostic value than either biomarker alone; notably, the presence of *STAT3* mutations predicted an inferior prognosis [180]. These findings reinforce the clinical utility of ctDNA dynamics in risk stratification and treatment response assessment. However, due to the limited sample size, larger cohorts are needed to validate the predictive value of ctDNA monitoring for treatment outcomes.

Apart from tumor-specific genetic variations, epigenetic alterations in ctDNA have been investigated for diagnostic and prognostic purposes in ENKTL. A diagnostic prediction model incorporating seven ctDNA methylation markers achieved an area under the curve (AUC) of 0.988 in an independent validation cohort. By combining the seven-marker ctDNA methylation prognostic score with the prognostic index of natural killer (PINK) risk system, the PINK-C risk stratification model was developed, achieving an AUC of 0.773 in predicting prognosis [181]. The PINK-C model demonstrated distinct prognostic stratification levels. However, as these models are based on retrospective data, their specificity and sensitivity require validation in future prospective studies.

Hodgkin's lymphoma

For cHL, ctDNA profiling with CAPP-seq has shown great promise. Specifically, ctDNA analysis identified approximately 87.5% of tumor variants present in biopsy samples from 80 newly diagnosed and 32 refractory patients, supporting ctDNA as a potential noninvasive profiling tool [76]. Notably, plasma ctDNA exhibited a higher median variant allele fraction than biopsy samples, likely reflecting the low tumor cell content typically present in cHL biopsies. This observation further underscores the utility of ctDNA in molecular profiling [34]. Certain mutations detected by ctDNA, including $XPO1^{E571K}$, *STAT6* and *SOCS1*, help distinguish cHL from other lymphoma types such as DLBCL, PMBL, ALCL and MGZL [76, 81, 182–184]. In addition to diagnostic insights, ctDNA profiling offers valuable information about the clonal structure and evolution in cHL. Some mutations in oncogenes and tumor suppressors, such as *GNA13*, *XPO1*, *NFKBIE*, *IKBKB*, *CSF2RB*, and *B2M*, are clonal, present across most cells, while others, like *PRBM1*, *NOTCH2*, *CHD2*, and *BCR*, appear as subclonal mutations [75]. Longitudinal ctDNA monitoring (41 samples from 13 patients) revealed that chemotherapy partially reshapes subclonal diversity, while salvage therapy with nivolumab suppresses dominant clones and promotes the emergence of new ones, thereby highlighting the utility of ctDNA in tracking clonal shifts over the course of treatment [76].

ctDNA profiling in cHL also carries prognostic significance. For example, detecting the $XPO1^{E571K}$ mutation via ddPCR is associated with shorter PFS, with a 2-year PFS of 57.1% in mutation-positive patients compared to 90.5% in mutation-negative patients [182]. Similarly, *TP53* mutations in ctDNA correlate with inferior PFS ($p = 0.0038$) [75]. Baseline ctDNA levels before treatment initiation have been linked to clinical features such as elevated TMTV, higher Hasenclever international prognostic scores (≥ 3), increased LDH levels, and advanced disease stages, suggesting that baseline ctDNA may serve as a valuable supplement to traditional prognostic markers [77, 81, 185].

Moreover, the role of ctDNA in monitoring treatment response and predicting relapse has also been demonstrated in HL. Longitudinal ctDNA monitoring combined with PET/CT imaging identified disease progression in 38% of patients, with a negative predictive value of 99% when both ctDNA and PET/CT results were negative, suggesting that ctDNA may improve the predictive accuracy of PET/CT in clinical management [76]. Furthermore, in patients with advanced cHL, a 2-log reduction in ctDNA levels after two cycles of ABVD (doxorubicin, bleomycin, vinblastine, dacarbazine) chemotherapy was predictive of CR, supporting a threshold previously validated in DLBCL [76].

The timing of ctDNA assessment during treatment has been shown to impact prognostic accuracy. Patients with high pretreatment ctDNA levels and detectable ctDNA throughout treatment (e.g., at C1D15, C3D1, and post-four cycles) experienced significantly worse PFS ($p < 0.05$)

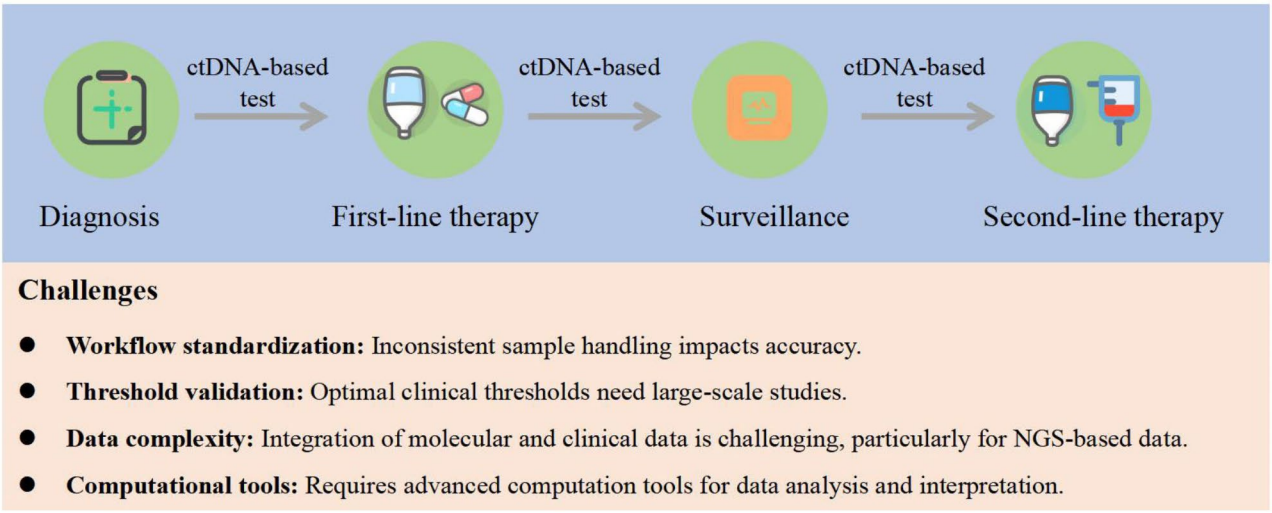

Fig. 5 Key challenges in implementing ctDNA testing in clinical practice

[34]. Molecular remission rates improved at sequential time points (C1D15, C2D1, and C3D1), with MRD negativity rates reaching 38%, 85% and 90%, respectively. Additionally, ctDNA monitoring as early as one week into treatment correlated with PET response and predicted PFS [75]. In pediatric Hodgkin lymphoma, targeted NGS showed that ctDNA was undetectable in patients who achieved an early PET response (qPET < 3), suggesting favorable outcomes [77]. Although ctDNA has shown considerable potential for clinical applications in HL, further prospective clinical trials are necessary to determine if MRD status, whether detectable or undetectable, can reliably inform decisions on treatment intensification or de-escalation.

Conclusions and perspectives

The emergence of ctDNA as a biomarker represents a major advance in lymphoma management, with applications spanning diagnosis, risk stratification, treatment monitoring, and MRD assessment. By providing comprehensive molecular insights through non-invasive sampling, ctDNA enables real-time tracking of disease burden, response to therapy, and clonal evolution, paving the way for personalized approaches to lymphoma care. Recent innovations in ctDNA analysis, including ctDNA fragmentation and methylation profiling, further expand its diagnostic and prognostic capabilities, potentially improving precision medicine in lymphoma.

The opportunities presented by ctDNA are substantial. It facilitates non-invasive genotyping, overcoming the limitations of tissue biopsies, such as spatial heterogeneity and insufficient sample material. ctDNA quantification provides a dynamic assessment of tumor burden, correlating with established prognostic factors like the IPI and TMTV. Moreover, its role in MRD detection surpasses traditional imaging techniques in sensitivity and specificity, allowing for earlier intervention upon relapse. When combined with emerging technologies such as NGS and epigenetic profiling, ctDNA can refine prognostic models and identify therapeutic targets. However, several challenges must be overcome before ctDNA can be fully integrated into routine clinical practice (Fig. 5). Standardizing preanalytical workflows remains critical, as variability in sample handling and processing can impact test accuracy. Furthermore, while ctDNA provides valuable insights, establishing optimal thresholds for clinical decision making, such as MRD or treatment response, requires further validation in large, prospective studies. In addition, the complexity of ctDNA data requires robust computational methods to integrate multiple molecular and clinical parameters. Advances in machine learning may help overcome these challenges and ultimately advance the clinical application of ctDNA as a reliable tool in lymphoma management.

Despite challenges in clinical implementation, emerging evidence supports ctDNA as a dynamic biomarker to guide lymphoma therapy [25–27]. Integrating ctDNA detection into clinical trial designs may improve therapeutic precision by enabling early and accurate monitoring of treatment response, MRD, and clonal evolution - potentially surpassing conventional imaging and biopsy methods. For instance, future trials in aggressive lymphomas such as DLBCL could incorporate early ctDNA monitoring to stratify patients based on their molecular response to frontline chemoimmunotherapy (e.g., R-CHOP). Patients who demonstrate rapid ctDNA clearance after one or two cycles of treatment could be candidates for de-escalated therapy, thereby reducing exposure to potentially toxic regimens without compromising efficacy. Conversely, patients with persistent

ctDNA positivity could be assigned to intensified or alternative therapeutic arms incorporating novel agents (e.g., targeted therapies or immunomodulators) to overcome early resistance. This adaptive approach leverages ctDNA dynamics to inform real-time, personalized treatment decisions that may ultimately improve progression-free and overall survival.

In indolent lymphomas such as FL, which exhibit a slow yet variable course and significant clonal heterogeneity, serial ctDNA-based testing could help track clonal evolution and emerging resistance mutations during targeted therapies (e.g., PI3K inhibitors, immunomodulators) [147]. A future trial might regularly assess ctDNA profiles throughout treatment and follow-up, enabling early identification of resistance-associated genetic alterations. This, in turn, could prompt a timely therapeutic switch or the addition of combination strategies aimed at suppressing resistant clones before overt relapse. Such a personalized approach not only refines treatment decisions but also provides deeper insights into the molecular mechanisms driving disease progression and resistance.

Abbreviations

ABC	Activated B-cell-like
AITL	Angioimmunoblastic T-cell lymphoma
ALCL	Anaplastic large cell lymphoma
ASO-PCR	Allele specific oligonucleotide polymerase chain reaction
B-NHL	B-cell non-Hodgkin lymphoma
BEAMing	Beads, emulsion, amplification and magnetics
Bp	Base pairs
CAPP-seq	Cancer personalized profiling by deep sequencing
cfDNA	Circulating cell-free DNA
cHL	Classical Hodgkin lymphoma
CHOP	Rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone
CIRI	Continuous individualized risk index
CLL	Chronic lymphocytic leukaemia
CNS	Central nervous system
CNS	Central nervous system
CNSL	Central nervous system lymphoma
CNVs	Copy number variants
COO	Cell of origin
CR	Complete response
CSF	Cerebrospinal fluid
CT	Computed tomography
CTCL	Cutaneous T-cell lymphoma
ctDNA	Circulating tumor DNA
ddPCR	Droplet digital polymerase chain reaction
DLBCL	Diffuse large B-cell lymphoma
EFS	Event-free survival
EMR	Early molecular response
ENKTL	Extranodal natural killer/T-cell lymphoma
EPIC-seq	Epigenetic expression inference from cell-free DNA sequencing
FL3b	Grade 3b follicular lymphoma
FL	Follicular lymphoma
GCB	Germinal center B-cell-like
HGBL	High grade B cell lymphoma
HL	Hodgkin Lymphoma
HR	Hazard ratio
Ig-NGS	Immunoglobulin next generation sequencing
IgHTS	Immunoglobulin high-throughput sequencing
IPI	International prognostic index
LBCL	Large B-cell lymphoma
LDH	Lactate dehydrogenase
MCL	Mantle cell lymphoma
MMR	Major molecular response
MRD	Molecular residual disease
MZL	Marginal zone lymphoma
NGS	Next generation sequencing
NK	Natural killer
OS	Overall survival
PCNSL	Primary central nervous system lymphoma
PCR	Polymerase chain reaction
PET/CT	Positron emission tomography/computed tomography
PFS	Progression-free survival
PhasED-seq	Phased variant enrichment and detection sequencing
PHL	Pediatric Hodgkin lymphoma
PINK	Prognostic index of natural killer
PMBCL	Primary mediastinal B-cell lymphoma
PMBL	Primary mediastinal B-cell lymphoma
POD24	Progression within 24 months.
PTCL-NOS	PTCL not otherwise specified
PTCL	Peripheral T-cell lymphoma
PTLD	Posttransplant lymphoproliferative disorders
R/R	Relapsed/refractory
RS	Richter's syndrome
RT-PCR	Real time polymerase chain reaction
SNVs	Single nucleotide variants
T-NHL	T-cell non-Hodgkin lymphoma
TCR	T-cell receptor
TFHL	T follicular helper lymphoma
tFL	Transformed follicular lymphoma
THRLBCL	T-cell/histiocyte-rich large B-cell lymphoma
TMTV	Total metabolic tumor volume
VDJ	Variable, diversity, joining
WES	Whole exome sequencing
WGBS	Whole genome bisulfite sequencing
WGS	Whole genome sequencing
WHO	World health organization

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LF, XRZ, and XYZ conducted the literature search and drafted the manuscript. XL and FZ edited the figures and proofread the manuscript. HG and XW critically reviewed and edited the manuscript. All authors have read and approved the final version of the manuscript for publication.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

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The authors declare no competing interests.

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