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REVIEW ARTICLE

Circulating tumor DNA: clinical roles in diffuse large B cell lymphoma

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Abstract

Diffuse large B cell lymphoma (DLBCL), the most common non-Hodgkin lymphoma (NHL), is a clinically and molecularly heterogeneous malignant lymphoproliferative disease. In the era of personalized medicine, genetic information is critical to early diagnosis, aiding risk stratification, directing therapeutic option, and monitoring disease relapse. However, lacking a circulating disease with most DLBCL cases hampers the acquisition of tumor genomic landscapes and disease dynamics. Circulating tumor DNA (ctDNA) is a novel noninvasive, real-time, and tumor-specific biomarker, reliably reflecting the comprehensive tumor genetic profiles, thus holds great promise in individualized medicine, including precise diagnosis and prognosis, response monitoring, and relapse detection of DLBCL. Here, we reviewed the recent advances of ctDNA in DLBCL and discussed its clinical values at different time points during the disease courses by comparing with the current routine methods in clinical practice. Collectively, we anticipated that ctDNA will ultimately be integrated into the management of DLBCL to facilitate precision medicine.

Keywords Diffuse large B cell lymphoma · Circulating tumor DNA · Cell-free DNA · Plasma DNA · Liquid biopsy

Introduction

Diffuse large B cell lymphoma (DLBCL), the most prevalent subtype of non-Hodgkin lymphoma (NHL) accounting for up to 30–40% of newly diagnosed cases [1], exhibits marked clinical and biological heterogeneity [2]. As an aggressive lymphoma, the goal of therapy for DLBCL is to cure. However, about one third of patients do not respond to the first-line treatment or will ultimately relapse, leading to dismal outcomes [3]. Current strategies for initial risk stratification, interim/end-of-treatment response assessment, and surveillance after therapy mainly rely on radiographic methods and clinical parameters, but are less than optimum (Table 1) [7, 15, 16].

With the rapid development of novel targeted regimens, the treatment for DLBCL is undergoing a shift from conventional chemotherapy to combination with molecular targeted agents [17–19]. In the era of targeted therapy, biomarkers reflecting tumor genetic characteristics hold great promise, owing to their potential in identifying the specific population who can actually benefit from a particular molecular inhibitor and in studying mutation-driven resistant mechanisms of targeted agents [17, 19, 20]. In parallel, gene expression profiling (GEP) and next-generation sequencing (NGS) have illustrated the molecular heterogeneity of DLBCLs, delineating cell of origin (COO) subtypes and genetic alterations of clinical importance [21–23].

However, due to lacking a leukemic involvement in most DLBCL cases, to acquire these information needs tissue biopsy, which is hampered by several problems in clinical practice (Table 1). The molecular stratification and dynamic genomic monitoring of DLBCL therefore remain unmet medical needs.

Circulating cell-free DNA (cfDNA) is shed into peripheral bloodstream by normal or tumor cells undergoing apoptosis and necrosis [24]. Accessing tumor-specific DNA simply through plasma, serum, or other body fluid, without the necessity of an invasive tumor biopsy, led to the concept of "liquid biopsy" (Fig. 1), which recently aroused considerable interest in DLBCL [25]. As a promising noninvasive approach reflecting the entire tumor genetics, circulating tumor DNA

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Table 1 Comparison between the clinical applications of ctDNA, tissue biopsy and PET/CT in DLBCL

	Advantages	Limitations	Clinical utilities								
			Pretreatment					During treatment			After treatment
			Diagnosis	Staging	Prognosis	Genotyping	Directing targeted therapy	Interim assessment	Tracking genetic alterations	End-of-treatment evaluation	Surveillance and relapse detecting
ctDNA	• Noninvasive; • Painless; • Low-risk; • No radiation; • Real-time; • Tumor specific (i.e., targeting lymphoma-related genes); • Easily to obtain; • Serially sampling; • Reflecting tumor heterogeneity; • Detecting subclinical disease	• Confounded by sampling noise, some mutations with low AFs might be missed in ctDNA, especially in low-burden diseases [4–6]; • Histological features and immunophenotype cannot be acquired; • Lack of anatomical information; • Genetic changes originating from other tumors or benign lesions (e.g., clonal hematopoiesis) carried by the same patient can confound results and reduce specificity.	✓ (Assisted, valuable when tumor tissues are inaccessible)	✗	✓	✓	✓	✓	✓	✓ (Potential to determine CMR)	✓ (Early detection of relapse)
Tissue biopsy	• Gold standard for diagnosis; • Providing morphologic information; • Available for immunohistochemical staining; • More likely to detect mutations with low-AFs than ctDNA; • Already be routinely used	• Invasive; • Limitation in obtaining enough materials; • Difficult to implement when tumors are inaccessible, such as PCNSL; • Subjected to selection bias arising from tumor heterogeneity; • Unrepeatable	★	✗	✓	✓	✓	NA	NA	NA	NA
PET/CT	• Improved sensitivity compared to CT; • Gold standard for staging and end-of-treatment assessment; • Providing anatomical information; • Already available in many hospitals	• High false positive rate; • Costly; • Radiation exposure; • Confounded by other circumstances with elevated glycolysis, like inflammation; • Tumor genetic information cannot be provided	✓ (Assisted)	★	✓	✗	✗	Controversial [7–11]	✗	★	PET/CT: not recommended CT: controversial [12–14]

✓, yes; ✗, no; ★, current gold standard; *AFs*, allele frequencies; *CMR*, complete molecular remission; *ctDNA*, circulating tumor DNA; *NA*, not applicable; *PCNSL*, primary central nervous lymphoma

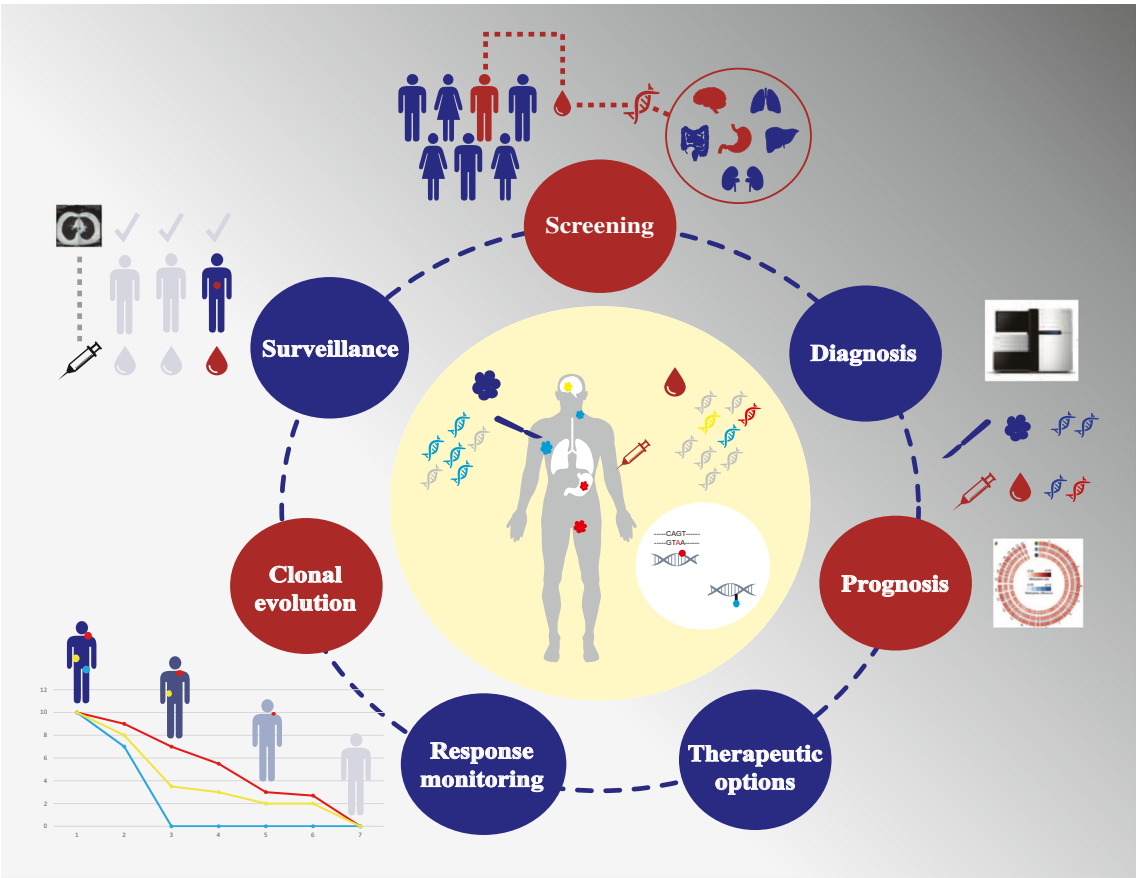

Fig.1 A paradigm of clinical use of liquid biopsy in tumor management. Fig. 1 illustrated the clinical use of circulating tumor DNA (ctDNA) in the management of patients with malignant diseases. The ctDNA from peripheral blood reflects comprehensive molecular profiles of neoplasms, including genetic and epigenetic alterations from all malignant sites, revealing tumor heterogeneity. Screening for ctDNA could detect asymptomatic tumor individuals at a population level. Before initial treatment, demonstrating tumor genomic features by next-generation sequencing (NGS) using both tissue and plasma samples facilitates accurate diagnosis, risk stratification, and therapeutic options. Dynamic monitoring of ctDNA during treatment can delineate real-time response to drugs and track clonal evolution of tumor. In surveillance, liquid biopsy could sensitively identify subclinical relapse under the detection threshold of image scans and therefore enables prompt therapeutic intervention

(ctDNA) has clear sampling superiority which provides the possibility of serial monitoring tumor molecular profile, clonal evolution, and disease burden. The advantages and limitations of ctDNA were summarized in Table 1.

In the present review, we mainly highlighted and explored recent advances of liquid biopsy in DLBCL and summarized the clinical roles of ctDNA in different time points during disease courses (Fig. 1). Additionally, we provided a brief overview of the biological, historical (Fig. 2), and technical (Table 2) aspects of ctDNA and discussed the problems and prospects of ctDNA in lymphoma.

Overview of ctDNA

During apoptosis and necrosis, tumor cells as well as normal cells release nucleic acids (DNA, mRNA, and microRNA) into the peripheral blood and other body fluids, such as cerebrospinal fluid (CSF), pleural fluid, and urine [49–52]. cfDNA is comprised of double-stranded DNA fragments, which are overwhelmingly short (< 200 base pairs [bp]) [24, 53]. Previous studies showed cfDNA had a typical length pattern with a dominant peak at 166–167 bp, and ctDNA was even shorter than normal cfDNA [24, 53, 54]. The proportion of ctDNA can vary largely from only 3 to 93% of total cfDNA [55]. Fast clearance by the liver and kidney makes ctDNA a real-time snapshot of tumor genome, with a half-life ranging from 15 min to several hours [56]. The average concentration of cfDNA was measured to be 180 ng/ml with tumor patients and 30 ng/ml with healthy subjects [56, 57]. Furthermore, cfDNA might also increase in other conditions, such as infection, trauma, exercise, transplantation, and autoimmune disorders [4, 58].

The history of cfDNA can be traced back to 1948 when it was first described by French scientists Mandel and Métais [26]. But it was not until recently when rapid molecular biology technical advances, especially digital PCR (dPCR) and NGS, gave tremendous boost to liquid biopsy that ctDNA

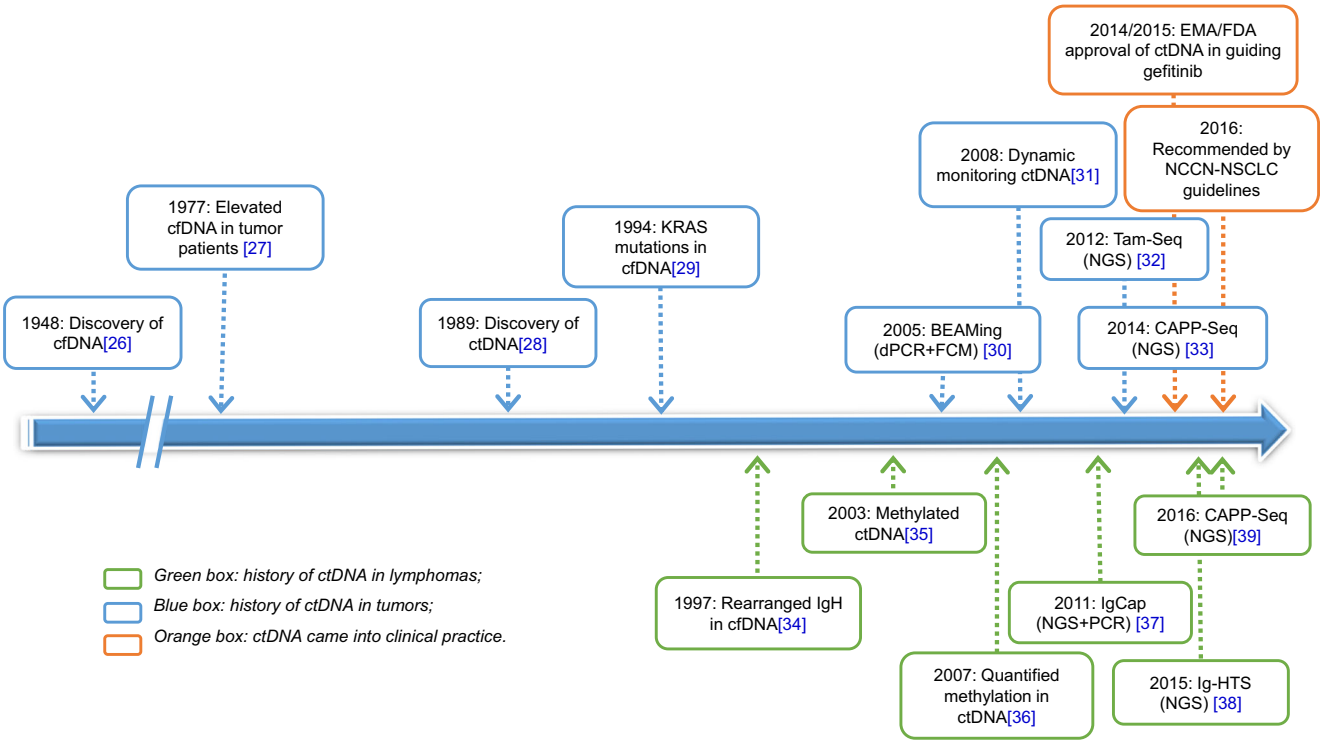

Fig. 2 A brief developmental history of ctDNA. Fig. 2 overviewed the developmental history of ctDNA in lymphomas and other tumors from 1948 to now, including the major discoveries, technical breakthroughs, and clinical milestones of ctDNA. BEAMing, beads, emulsion, amplification and magnetics; CAPP-Seq, cancer personalized profiling by deep sequencing; cfDNA, cell-free DNA; ctDNA, circulating tumor DNA; EMA, the European Medicines Agency; FCM, flow cytometry; FDA, Food and Drug Administration; IgCap, capturing and sequencing the IgH genomic regions; IgH, immunoglobulin heavy chain; Ig-HTS, Ig high-throughput sequencing; NCCN, the National Comprehensive Cancer Network; NGS, next-generation sequencing; NSCLC, non-small cell lung cancer; Tam-Seq, tagged-amplicon deep sequencing

finally gained great research interests [27–33] (see more details in Fig. 2).

Lacking single typical tumor-specific mutation distinguished the ctDNA detection strategies of most lymphocytic malignant diseases from those of other solid malignancies, while the clonally rearranged immunoglobulin (Ig) genes of mature B cell lymphomas provided a novel tactics. Rearranged Ig heavy chain (IgH) was first detected in plasma DNA from patients with NHL in 1997 [34]. In 2003, the presence of methylated plasma DNA in lymphoproliferative diseases was reported by Deligezer et al., and later in 2006, Shi and colleagues observed DLC-1 methylation in matched tumor tissue and plasma in NHL [35, 36]. By capturing and sequencing the IgH gene (IgCap), He et al. first explored the feasibility of using NGS combined with PCR to detect ctDNA in lymphoma [37]. During the last decade, the studies exploiting ctDNA as a tumor-specific biomarker in lymphoid malignancies were intermittently until the recent 2 years when high-throughput sequencing methods were adopted by several large-scale researches to monitor ctDNA in DLBCL [38–40, 59, 60] (Fig. 2).

Current strategies for detecting ctDNA in lymphoma entities mainly include the following: (1) Ig gene sequencing, e.g., Ig high-throughput sequencing (Ig-HTS) and Ig VDJ gene sequencing; (2) targeted sequencing of lymphoma-related genes, e.g., cancer personalized profiling by deep sequencing (CAPP-Seq) and Lymphopanel; (3) dPCR of recurrent mutations in lymphoma; (4) aberrant methylation detecting, e.g., pyrosequencing and genome-wide methylation sequencing (see more details in Table 2).

Recent advances of ctDNA in DLBCL

Table 3 summarized the main studies of ctDNA in DLBCL in the recent 5 years.

Pretreatment

Prognosis

The elevated level of cfDNA indicating more aggressive disease in lymphomas was first described in 2009 using a quantitative PCR for the beta-globin gene, which is not tumor specific however [62]. Technical developments in molecular biology enabled quantitative identification and measurement of malignant cell-free nucleic acids. In 2015, a group from Stanford University evaluated the prognostic value of ctDNA by Ig-HTS in 75 DLBCL patients [40]. Among 34

Table 2 Current techniques for ctDNA detection in lymphocytic malignancies

Detecting methods			Information provided	Advantages	Limitations	Application examples
Genetic aberration	NGS	Ig gene sequencing Targeted sequencing of lymphoma-related genes (e.g., CAPP-Seq, Lymphopanel)	• Ig gene rearrangement • Mutations • Translocations • Ig VDJ recombination • (Copy number variations*)	• High-throughput; • Comprehensive genetic profiles; • Massive genetic information; • Almost universal	• Costly; • Technically demanding; • Time consuming; • Larger panels are subjected to lower sequencing depth; • Less sensitive than dPCR	• DLBCL [6, 38–40] • CLL [41] • FL [42]
	dPCR		• A few mutations	• More sensitive, especially *suitable for rare aberrations*; • Relatively low cost	• Less universal; • Less information	• DLBCL [43] • HL [44] • CLL [41] • FL [45]
Epigenetic aberration	Pyrosequencing		• A few methylation alterations	• Economical; • Technically simple	• Less universal; • Less information	• DLBCL [46]
	Genome-wide bisulfite sequencing		• Genome-wide methylation alterations • Tissue mapping	• Comprehensive methylation profiles; • Tracing the tissue of origin of ctDNA	• Costly; • Technically demanding; • Time consuming	• FL [47]

*Although not been used for detecting copy number variants in lymphoma yet, CAPP-Seq has been successfully adopted for this purpose in non-small cell lung cancer [48]

CAPP-Seq, cancer personalized profiling by deep sequencing; *CLL*, chronic lymphocytic leukemia; *DLBCL*, diffuse large B cell lymphoma; *dPCR*, digital PCR; *FL*, follicular lymphoma; *HL*, Hodgkin lymphoma; *Ig*, immunoglobulin; *NGS*, next-generation sequencing

Table 3 Recent studies of ctDNA in DLBCL (2013–2017)

First author/year of publication	Technical platform	Detection approach	Patients enrolled year (follow-up)	Main study aims	Patients (*n*)	Disease (stage)	Plasma/ serum samples (*n*)	Positive rate[§], % (*n*/total)	Concordance with tumor biopsy, % (*n*/total)	Sensitivity[*], % (n/total)	Specificity[*], % (n/total)
Armand 2013 [60]	NGS	Ig-HTS	/	To see if ctDNA is detectable in DLBCL	17	DLBCL, MLBCL	/	11/16	/	/	/
Roschewski2015 [38]	NGS	Ig-VDJ rearrangement	1993–2013 (~11 years)	Interim assessment and surveillance	126	DLBCL (II~IV and bulky stage I mediastinal)	1558 serum	37%, 32/86	/	Interim: 46.9%, surveillance: 88.2%	Interim: 88.2%, surveillance: 97.8%
Bohers 2015 [6]	NGS	Lymphopanel (34 genes relevant to lymphomagenesis)	2014	To validate ctDNA reliably reflects somatic alterations of tumor	12	DLBCL	12 plasma	/	85%	/	/
Kurtz 2015 [40]	NGS	Ig-HTS	2010–2014	Prognosis and response monitoring and surveillance	75	DLBCL	311 plasma	82%, 18/22	/	Surveillance: 31%	Surveillance: 100%
Camus 2016 [43]	dPCR	XPO1 E571K, EZH2 Y641N, and MYD88 L265P mutations	/	To develop a platform for directing treatment options	14	DLBCL, PMBL	/	13/82	13/14	XPO1 E571K: 100%, EZH2 Y641N: 100%, MYD88 L265P: 80%	100%
Scherer 2016 [39]	NGS	CAPP-Seq	/	Tumor genotyping and tracking mutations and identifying tFL and early detection of relapse	92	DLBCL, tFL	166 plasma	87%, 39/45	Mutant AFs: 82%, 37/45; COO classify: 88%, 36/41	100%	99.8%
Herrera 2016 [61]	NGS	IgH or TCR	2009–2012 (33 m)	Surveillance after HSCT	68	B/T-NHL, HL, CLL	393 plasma	55% (prior to HSCT)	/	73% (3 m after HSCT)	55% (3 m after HSCT)
Assouline 2016 [20]	NGS + dPCR	CAPP-Seq	2010–2013	Directing panobinostat treatment and monitoring response	26	DLBCL	52 plasma	96.15%, 25/26	/	71.4% (at day 15 of treatment)	100% (at day 15 of treatment)
	Pyrosequencing				74	DLBCL			/	/	/

Table 3 (continued)

First author/year of publication	Technical platform	Detection approach	Patients enrolled year (follow-up)	Main study aims	Patients (*n*)	Disease (stage)	Plasma/ serum samples (*n*)	Positive rate[§], % (*n*/total)	Concordance with tumor biopsy, % (*n*/total)	Sensitivity[*], % (n/total)	Specificity[*], % (n/total)
Kristensen 2016 [5]		DAPK1, DBC1, MIR34A, and MIR34B/C methylations	2003–2007 (> 5 years)	Prognosis and monitoring response			158 plasma	DAPK1: 19%, DBC1: 16%, MIR34A: 8%, MIR34B/C: 10%			
Alcaide 2016 [45]	dPCR	EZH2, STAT6, MYD88, CCND3 mutations	/	To develop ctDNA detecting techniques	21	DLBCL, FL, LPL	/	/	/	mutant DNA > 0.01%	/
Rossi 2017 [59]	NGS	CAPP-Seq	2013–2016	Tumor genotyping and tracking clonal evolution, and monitoring response	50	DLBCL	127 plasma	66.6%, 20/30	96%	82.8%, 87/105; (for mutations with AF > 20% in tumor biopsy: 97.1%, 68/70)	99.99%
Wedge 2017 [46]	Pyrosequencing	Global hypomethylation: LINE1 methylation	/	Prognosis	74	DLBCL	74	6/74	0/2	/	/

AF, allele frequency; *B/T-NHL*, B cell/T cell non-Hodgkin; *CAPP-Seq*, cancer personalized profiling by deep sequencing; *COO*, cell of origin; *DLBCL*, diffuse large B cell lymphoma; *dPCR*, digital PCR; *Ig*, immunoglobulin; *Ig-HTS*, immunoglobulin high-throughput sequencing; *m*, month(s); *NGS*, next-generation sequencing; *PMBL*, primary mediastinal B cell lymphoma; *tFL*, transformed follicular lymphoma; *TCR*, T cell receptor; *y*, year(s)

§Positive rate = positive patients (*n*)/total available patients (*n*)

*Sensitivity and specificity: if no special instruction, refer to the rates before treatment

patients with active disease measured by PET/CT, plasma Ig-HTS had a higher sensitivity (88%, 30/34) than serum lactate dehydrogenase (LDH) (59%, 20/34) ($P = 0.01$). In addition, ctDNA was significantly correlated with metabolic tumor volume (MTV) measured from PET/CT ($P = 0.002$). More recently, the same group published their new results from more sensitive deep targeted sequencing, indicating that ctDNA is a strong predictive index in DLBCL at initial diagnosis [39]. It was demonstrated that patients with higher pretreatment ctDNA levels were linked with inferior progression-free survival (PFS) and more risk of subsequent disease progression. Herrera and colleagues tested plasma DNA prior to allogeneic stem cell transplantation (HSCT) in different lymphomas including DLBCL; however, basal ctDNA status did not significantly associate with survival in their study [61].

Targeting aberrant DNA methylations of ctDNA balances cost and scope against accuracy [63, 64]. Kristensen and colleagues adopted pyrosequencing to analyze the DNA methylation in the promoter regions of DAPK1, DBC1, MIR34A, and MIR34B/C in plasma from DLBCL cases. Aberrant DAPK1 methylation was significantly correlated with lower 5-year overall survival (OS) rates [5]. Recently, another study assessed the feasibility of global hypomethylation in both tissue and plasma samples [46]. Among 74 DLBCL patients, global hypomethylation in plasma was linked with inferior OS. Interestingly, their results additionally showed that global hypomethylation coexisted with DAPK1 promoter hypermethylation.

Tumor genotyping

Latest improvements in the sensitivity and accuracy of molecular analysis have allowed tumor genotyping by liquid biopsy in a group of solid cancers [65–67]. In 2015, Bohers et al. first validated that in DLBCL, this is also possible [6]. Applying NGS targeting 34 lymphomagenesis-associated genes in 12 DLBCL cases, they found that in most cases, mutations detected in blood samples were similar or partially the same as those in tumor tissue, with a high median concordance rate (85%). A groundbreaking study in 2016 used CAPP-Seq, a targeted ultra-deep sequencing for ctDNA mutation detecting already verified in non-small-cell lung cancer (NSCLC) in 2014, to explore the possibility and capacity of biopsy-free genotyping in DLBCL [33, 39]. The target panel covers recurrently mutated regions, recurrent breakpoint regions, and Ig VDJ recombination sites. In 45 pretreatment plasma samples, noninvasive approach successfully identified mutations and translocations confirmed by matched tumor tissues in most cases. Rossi et al. prospectively assessed the capacity of ctDNA for tumor genotyping and illustrated mutations with ≥ 20% AF (allele frequency) in tumor biopsy samples could be discovered by ultra-deep targeted sequencing of plasma DNA with high sensitivity (> 90%) and specificity (~ 100%) [59].

Of note, tumor genotyping by plasma cfDNA not simply mirrors the genomic information within the tumor biopsy, but instead complements to it. Tissue biopsy is restricted to clones spatially distant from the biopsy site, while ctDNA genotyping allows discovering somatic alterations that might not be detected by tissue biopsy, capturing tumor genomic heterogeneity. In DLBCL, as well as other cancers, disease-associated mutations only observed in plasma samples but not in initial tumor biopsy tissues have been reported and subsequently been confirmed not false-positive calls [39, 59, 68].

Accessory diagnosis

According to the Revised European American Classification (REAL)/the World Health Organization (WHO) classification, current diagnosis of lymphoma is mainly based on morphology and immunophenotyping, which liquid biopsy cannot provide. But analyses of ctDNA from plasma or other body fluids can be helpful as an alternative tool when tissue biopsy is difficult to implement, such as for primary central nervous system lymphoma (PCNSL) [49, 50, 69].

Another potential utility of ctDNA generating enthusiasm is tumor screening and early diagnosis [70–72]. Sun et al. developed a novel strategy enabling the quantitative measurement of the relative contributions of different tissues to the plasma DNA pool, by comparing the genome-wide bisulfite sequencing results of ctDNA to methylation profiles of each tissue [47]. In this study, they observed copy number aberrations in the plasma of a pregnant woman, and further analysis identified the origin as B cells, which showed an extremely increased contribution to the whole circulating DNA pool, indicating B cell malignant disease. She was diagnosed with follicular lymphoma (FL) during pregnancy later. More recently, Guo et al. using methylation haplotypes illustrated tissue-of-origin mapping by ctDNA in cancers [73]. The capacity of plasma DNA to inform the likely origin tissues of malignancies would be of grossly clinical significance for tumor screening and early detection.

Directing treatment options

At the era of individualized precision medicine, tumor genetic profiling by plasma DNA holds great promise for directing targeted therapy [74]. In a phase 2 study of panobinostat (a histone deacetylase inhibitor) for relapse DLBCL, MEF2B mutations were tested in both tumor and plasma samples and showed prognostic value for predicting early response of this novel agent [20]. Researches demonstrated that DLBCL patients with the myeloid differentiation primary response gene (MYD88) mutations frequently responded to ibrutinib, the exportin-1 (XPO1) alterations potentially affect sensitivity to selective inhibitors of nuclear exports (SINEs such as selinexor), and activating EZH2 mutations is targeted by EZH2 inhibitors [17, 75, 76]. Camus et al. devised dPCR assays

for detecting XPO1, MYD88, and EZH2 mutations using plasma DNA in DLBCL patients, with a sensitivity of 0.05%, aiming to select the subset most likely to benefit from particular targeted drug [43]. Although so far, no evidence from prospective researches has shown that ctDNA can guide pharmaceutical options in patients with lymphoma, these studies highlight the potential of liquid biopsy in predicting response to therapies with molecularly targeted agents in lymphoma.

During treatment

Monitoring response

The latest study from Rossi et al. demonstrated the dynamic patterns of somatic aberrations in cfDNA during therapy in DLBCL patients [59]. Among responding patients, basal disease-associated mutations showed a rapid clearance upon treatment, while genetic variants did not disappear from circulating DNA among those resistant to therapy. Moreover, new emerging mutations were observed in the plasma DNA of resistant patients, indicating resistant clones were selected through the clonal evolution. In targeted therapy with panobinostat, a remarkable elevated ctDNA level at day 15 compared with baseline was significantly correlated to lack of response, with sensitivity of 71.4% and specificity of 100% [20]. If further studies could confirm that a very early change in plasma DNA abundance is a reliable prediction of later clinical benefit, it will help clinicians to identify the patients not likely to benefit from the initial treatment and so that they can be earlier switched to another therapy. In the National Cancer Institute (NCI) study for ctDNA in DLBCL, Roschewski et al. analyzed the kinetic patterns of ctDNA during initial therapy of 25 patients who subsequently progressed within 6 months, out of 126 DLBCL cases [38]. Results showed patients who never cleared tumor-specific DNA in plasma are suffering from both the shortest time to progression and the poorest median survival after tumor recurrence.

In addition to researches based on high-throughput sequencing monitoring the mean AFs of a massive number of single nucleotide variants (SNVs) in ctDNA, dPCR targeting a few recurrent point mutations (such as EZH2 and STAT6 mutations) or sequentially quantitative measurement of aberrant methylations of genes like DAPK1 also exhibited potential for disease monitoring during treatment in DLBCL [5, 45]. However, the limitation of these strategies is obvious, as only a small group of patients harboring a specific mutation or abnormal methylation, such methods may not be as universal as those using NGS techniques.

Interim assessment

The exploratory utility of interim PET has generated enthusiasm in DLBCL and other FDG-avid lymphomas, such as Hodgkin lymphoma (HL). However, unlike HL, in which preliminary data forcefully support the clinical use of interim PET, interim PET in DLBCL cases is controversial and not recommended, resulting from frequently false-positive results [7–11]. Thus, for interim assessment, alternative methods with more accuracy are needed. The NCI study of ctDNA retrospectively analyzed the clinical impact of interim ctDNA at the end of two treatment cycles among 108 patients with DLBCL [38]. Patients with detectable interim ctDNA had shorter time to progression and worse OS compared to those without detectable ctDNA after two cycles of therapy. Moreover, this research showed detectable interim ctDNA had relatively favorable performance with a positive predictive value (PPV) of 62.5% and a negative predictive value (NPV) of 79.8%, suggesting it might provide a surrogate or complement tool for interim PET scan.

Tracking clonal evolution

Along with the rapid development of targeted treatment for DLBCL patients, related resistant mutations are increasingly emerging [17]. Dynamic monitoring cfDNA may have particular utility for adaptive treatment, if acquisition of targeted drug-resistance is prospectively detected and therapy can be adapted in real time [4]. This has been well established in the anti-EGFR therapies of colorectal and lung cancers [77–79]. Using biopsy-free detection of mutations emerging during BTK inhibitor treatment, Scherer et al. identified resistant clones in two of three DLBCL patients undergoing ibrutinib therapy [39]. Interestingly, they observed two independent resistant subclones in one patient, indicating convergent evolution, which can be further studied to reveal relevant evolutional mechanisms. Besides, serial analysis of plasma DNA from a transformed DLBCL patient treated with idelalisib (the PI3K δ inhibitor) captured the acquisition of an emerging mutation in EZH2, a potential drug target [39].

In addition to tracking emerging mutations during targeted therapies, liquid biopsy may hold great promise in illustrating the molecular mechanism of histological transformation from indolent lymphoma to DLBCL [80]. In order to picture the patterns of genomic evolution among three subsets of patients, i.e., transforming FL (tFL), FL undergoing progression but without transformation (ntFL), and rrDLBCL, tumor and plasma samples from 26 patients were collected at multiple time points [39]. Results showed tFL had a higher fraction of emergent alterations distinguishing it from other histologic subtypes. Based on the key genetic divergences between the three subtypes, researchers were able to noninvasively discriminate tFL from ntFL (sensitivity, 83%; specificity, 89%) and successfully identified tFL ahead of clinical diagnosis on an average of 66 days. The early suspicion, accurate diagnosis, and prompt therapy of Richter's syndrome (RS) are often challenging in current clinical setting. Yeh et al. recently assessed

the capacity of ctDNA for tracking the clonal evolution of CLL undergoing RS transformation [41]. Performing NGS in matched plasma and tumor tissues at baseline and RS in three patients, they illustrated that the genetic aberrations in tumor samples were closely reflected by ctDNA. Moreover, serial analyses of ctDNA were able to detect new emerging mutations 2 months ahead of the clinical diagnosis of RS in one patient.

Altogether, these results demonstrated that longitudinal liquid biopsy may facilitate real-time adaption of molecular inhibitor treatment in DLBCL and may achieve early detection of histologic transformation, while further parallel in vitro or in vivo experiments would give new insights into tumor biology and resistant mechanisms of novel agents.

After treatment

End-of-treatment evaluation

Currently, PET-CT is the international standard care for end-of-treatment response assessment for FDG-avid lymphomas, including DLBCL [81]. However, a meta-analysis reviewing totally 737 DLBCL cases found that a non-negligible proportion of patients experience disease recurrence during follow-up [82]. One of the possible interpretations for this might be the present typical spatial resolution (6–7 mm) of PET techniques in clinical practice is unable to detect microscopic residual lesions, which are likely to be the origin of relapse [82, 83]. The concept of "minimal residual disease" (MRD) is an excellent surrogate for therapy outcome at the end-of-treatment in many B cell lymphomas, i.e., mantle cell lymphoma (MCL), chronic lymphocytic leukemia (CLL), and FL [84–86]. However, at variance with these lymphoma entities, DLBCL patients typically lack leukemic involvement; therefore, MRD cannot be determined by conventional methods in DLBCL. Against this background, ctDNA holds great potential in detecting MRD below the detection threshold of PET-CT. To date, few studies have been carried out to assess the clinical value of liquid biopsy at end-of-treatment in DLBCL. Kurtz et al. tested plasma DNA in seven DLBCL patients who achieved CR by PET/CT after four cycles of treatment, and all seven cases had undetectable ctDNA, consistent with PET/CT [40]. In the NCI study, researchers analyzed ctDNA from 17 patients who progressed after achieving CR at the end of initial treatment and found positive ctDNA in 15 patients before progression [38]. But the clinical capacity of ctDNA at the end-of-therapy was not further assessed in this research. Referring to solid cancers also treated with curative intent, such as breast cancer and colorectal cancer, detectable ctDNA is regarded as a signal of MRD, and evaluation of ctDNA at the first follow-up visit after surgery was proved to be a strong predictor of disease recurrence [87, 88]. Therefore, presumably, end-of-treatment liquid biopsy in DLBCL may be able to further divide CR (determined by PET/CT) patients into subgroups to achieve more accurate risk stratification of relapse. Furthermore, additional therapeutic intervention may be taken for patients without CMR to induce deeper remission and may partially overcome the inferior outcomes of some rrDLBCL cases. Nevertheless, these hypotheses still need further researches for validation.

Surveillance and early detection of relapse

Due to the improved cure rates in patients with DLBCL, early and accurate detection of relapse allows implement of a prompt salvage therapy and would potentially prolong survival. However, current clinical follow-ups mainly including physical exams, routine labs, and imaging scans are less than satisfying. Recently, Cohen et al. systematically reviewed the appropriateness of routine surveillance imaging in DLBCL from 8 relevant studies and recommended that patients with DLBCL who achieve CR should not have routine surveillance imaging [89]. Some studies reported the likelihood of detecting recurrence by routine imaging in an asymptomatic patient after CR was low (1/40–1/50), and there was also no superiority for survival in relapse patients who were identified by current standard follow-up compared to those were found outside of regular visits, perhaps implicating limited sensitivity and specificity of present methods for relapse detection [90, 91]. In addition, CT and PET/CT scans pose a threat of radiological hazard, which may lead to high risk of developing second malignancies [12].

Peripheral blood monitoring of tumor-derived nucleic acids does not expose patients to radiation and can detect radiographically occult recurrence. The NCI study represented a milestone, in which surveillance monitoring of serum DNA from 107 DLBCL patients who achieved CR with the EPOCH regimen (comprising etoposide, prednisone, vincristine cyclophosphamide, and doxorubicin) was done by NGS targeting the VDJ gene segments of the rearranged Ig receptor genes [38]. With a median of 11 years follow-up, 17 patients ultimately relapsed after complete remission, and 15 (88%) of them developed detectable ctDNA prior to clinical progression, at a median lead time of 3.5 months, especially for those with late recurrence (median, 7.4 months). According to their data, detectable tumor DNA in surveillance serum samples was a strong predictor for disease progression (HR, 228), with a PPV of 88.2% and a NPV of 97.8%. Recently, researchers from Stanford University also corroborated the compelling ability of liquid biopsy for early detecting relapse in a separate group of DLBCL patients who finally experienced disease progression after remission (totally 11) [39]. Using ultrasensitive NSG technique (CAPP-Seq), they were able to identify recurrence before the emergence of clinical evidence for disease progression

with a mean lead time of 188 days in 8 (73%) patients. Furthermore, they compared this method with Ig-HTS, which they had previously adopted for surveillance relapse detection in DLBCL (median lead time, 88 days), and found CAPP-Seq was more superior [39, 40]. Herrera et al. and Rossi et al. also assessed ctDNA in follow-up surveillance [59, 61]. Herrera and co-workers evaluated plasma DNA monitoring after HSCT in lymphoma entities, including DLBCL, and illustrated that 16 of 19 (84%) patients progressed after HSCT developed detectable ctDNA ahead of progression with a median lead time of 3.7 months [61]. Collectively, these researches demonstrated the tremendous potential of liquid biopsy for early relapse detection, while further prospective studies to ascertain its value and impact on clinical outcome are definitely required.

Future perspectives

Collectively, circulating tumor DNA can reliably provide real-time monitoring of disease burden and tumor genomic information, thus may represent a paradigm switch in the upcoming implementation of personalized precision medicine in patients with DLBCL and other hematologic malignant diseases. There is no doubt that these noninvasive, tumor-specific methods will be grossly valuable to patients and aid clinical decisions of clinicians. Nevertheless, we should realize that liquid biopsy is not a proxy of tumor biopsy or imaging scans, but instead offers complementary information, and in turn, routine tissue biopsy and CT or PET/CT will not be replaced, at least in the near future, due to the intrinsic limitations of ctDNA (Table 1). Definitely, before integrating it into the management of lymphoma, large-scale prospective cohorts are needed to draw precise conclusions and determine whether additional therapies driven by liquid biopsy really improve survival [92]. Besides, several issues of ctDNA remain to be solved: (1) although some recommendations were published, there are no widely accepted standard protocols for sample collecting, processing, and storing to date resulting in possible pre-analytical bias and disparate results [93–96]; (2) the optimal timing and duration of ctDNA monitoring remain unclear; (3) different groups set inconsistent cutoff levels for detection, leading to lack of comparability between some results; (4) commercially available ctDNA detecting platforms are limited and technical obstacles have to be addressed; (5) well-designed prospective studies for verification should be carried out; and (6) further understanding of the biological features of ctDNA would facilitate the optimization of use.

At present, a large number of clinical trials incorporating ctDNA analyses are undergoing, including those assessing the ability of liquid biopsy in lymphoma (NCT02339805, NCT02620527). The future of monitoring ctDNA in lymphocytic malignancies can be envisioned with great momentum. Taking NSCLC (the "pioneer" of ctDNA application) as an example, from the initial approval by the European Medicines Agency (EMA) in 2014 for using ctDNA to aid the selection of patients with mutant EGFR for gefitinib to the current recommendation in the National Comprehensive Cancer Network (NCCN) NSCLC guidelines of considering plasma biopsy when repeat biopsy is unfeasible, these noninvasive methods of tumor molecular analysis have gradually transformed into clinical tools.

Besides in DLBCL, the compelling results of monitoring ctDNA in CLL from Yeh et al. for the first time demonstrated that liquid biopsy played an important role even in lymphocytic neoplasms with a circulating disease component [41]. According to their study, dynamic ctDNA analysis reflected the genomic information not only within leukemic cells but also from various tumor compartments, heralding the possibility of early detecting RS. Researches exploiting the clinical applications of plasma DNA in HL and FL were also reported by several groups [42, 44, 97].

Conclusions

In conclusion, liquid biopsy holds tremendous promise and is poised to be an indispensable tool for tumor comprehensive genetic profiling and tumor real-time dynamic monitoring in DLBCL and other hematologic malignancies in the near future.

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Compliance with ethical standards

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

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Abbreviations AF, allele frequency; CAPP-Seq, cancer personalized profiling by deep sequencing; cfDNA, cell-free DNA; CLL, chronic lymphocytic leukemia; CMR, complete molecular remission; COO, cell of origin; CR, complete response; CSF, cerebrospinal fluid; ctDNA, circulating tumor DNA; DLBCL, diffuse large B cell lymphoma; dPCR, digital polymerase chain reaction; EMA, European Medicines Agency; GEP, gene expression profiling; FL, follicular lymphoma; HL, Hodgkin lymphoma; HSCT, allogeneic stem cell transplantation; HTS, high-throughput sequencing; Ig, immunoglobulin; IgCap, capturing and sequencing the IgH gene; IgH, immunoglobulin heavy chain; IPI, International Prognostic Index; LDH, serum lactate dehydrogenase; MCL, mantle cell lymphoma; MRD, minimal residual disease; MTV, metabolic tumor volume; MYD88, myeloid differentiation primary response gene; NCI, National Cancer Institute; NCCN, National Comprehensive Cancer Network; NGS, next-generation sequencing; NHL, non-Hodgkin lymphoma; NPV, negative predictive value; NSCLC, non-small-cell lung cancer; OS, overall survival; PCNSL, primary central nerve system lymphoma; PCR, polymerase chain reaction; PET/CT, 18-fluorodeoxyglucose positron emission tomography combined with CT; PFS, progression-free survival; PPV, positive predictive value; REAL, the Revised European American Classification; rrDLBCL, refractory/relapse DLBCL; RS, Richter's syndrome; SINE, selective inhibitors of nuclear export; SUV, single nucleotide variant; tFL, transforming follicular lymphoma; WES, whole exome sequencing; WHO, the World Health Organization; XPO1, exportin-1

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