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LIQUID BIOPSIES IN HEMATOLOGICAL MALIGNANCIES: EXPLORING ANALYSIS FOR ENHANCED PATIENT MANAGEMENT

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ABSTRACT

Evaluating the malignant profile of mutations is essential for effective patient classification, treatment planning, as well as management. Currently, genomic profiling of hematological cancers along with solid masses, i.e., lymphomas, is primarily conducted on the tissue biopsies. However, tumors may contain distinct genetic alterations in different anatomical regions. The circulating tumor DNA (ctDNA) exploration from liquid biopsies is a developing technique, allowing for genotyping, disease monitoring while treatment and patient follow-up. The ctDNA evaluation from liquid biopsies of Hodgkin lymphoma (HL) and diffuse large B-cell lymphoma (DLBCL) can mirror the mutation profile of biopsies in tissues and detect mutations not found in tissue samples. Additionally, variations in ctDNA levels following chemotherapy cycles can expressively predict the outcome of the patients. Promising results are also seen with ctDNA analysis in myeloid neoplasms. Beyond mutational analysis, liquid biopsies hold potential for future applications, such as examination of ctDNA epigenetic patterns and fragments examination. As a result, numerous clinical trials are currently investigating the integration of ctDNA analysis for personalized treatment in hematological malignancies. This review explores various methods for ctDNA analyses and use of liquid biopsies in hematological cancers.

Keywords: Hematological malignancies, lymphoma, leukemia, circulating tumor DNA, genomic profiling.

Introduction

Evaluating the mutational profile of cancer is essential for the patient stratification, management, as well as making treatment assessments. In hematological cancers along with solid masses, i.e., lymphomas, the genome profiling of tumor is typically executed on the tissue biopsies. These molecular and genomic investigations strengthen the histological diagnoses by detecting molecular biomarkers having therapeutic and prognostic significance[1]. Nevertheless, the need for invasive procedures limits the feasibility of sequential sampling for real-time observation. Furthermore, tissue biopsies typically focus on a single site of tumor, restricting widespread tumor genomic description, which can differ across diverse structural locations[2,3].

Liquid biopsy is a developing method that characterizes malignancies by isolating and analyzing the components derived from tumors among various bodily fluids, e.g., cerebrospinal fluid, urine, and blood[4]. These components can include circulating tumor cells (CTCs), tumor-educated platelets (TEPs), cell-free nucleic acids, or exosomes. In hematological cancers, the liquid biopsy offers a negligibly invasive procedure for real-time that can help the tissue biopsy limitations, including procedural risks and the inability to reason for the spatial intra-tumor heterogeneity[4]. Various synchronous tumor DNA sources provide complementary information on mutational driver genes with possible value for prognosis or prediction. The exploration of circulating tumor DNA (ctDNA) from plasma is one of the most common liquid biopsy applications in hematological malignancies, used for monitoring and genotyping of disease and its response during chemotherapy[5]. This review will converse the present applications of the liquid biopsy in myeloid and lymphoid tumors and explore prospective applications in future that can enable a more personalized therapeutic management approach for each patient.

1. Analysis and Detection of ctDNA

The circulating cell-free DNA (cfDNA) comprises short fragments of DNA, typically 180-200 base pairs in length that enter the bloodstream from apoptotic or necrotic cells[6]. In healthy individuals, cfDNA levels in the plasma range from 1-16.8 ng/mL and have a brief half-life of about two hours. These levels can rise due to factors i.e., infection, exercise, stroke, or trauma. In tumor patients, the cfDNA concentration is notably increased than in normal individuals because the necrotic and apoptotic debris in tumors is not efficiently removed by phagocytes. This inefficiency results in the collection of cell debris, such as cfDNA, which consequently enters the bloodstream. For these patients, cfDNA includes DNA from both healthy and cancerous cells[7]. The portion of cfDNA that comes from tumor cells, released through apoptosis, secretion, and necrosis, is ctDNA. In patients with lymphoma, the levels of ctDNA fluctuate widely among various subtypes and correlates with the type of tumor, tumor burden, and disease stage. On average, ctDNA concentration in patients with lymphoma is approximately 30 ng/mL[8].

As discussed previously, the isolation of ctDNA can be done from various bodily fluids, but peripheral blood is the most commonly used source due to its easy accessibility. Serum and plasma are typically used for ctDNA analysis, with plasma being preferred. This preference is because plasma has lower levels of cfDNA from normal leukocytes, which helps to reduce dilution of the ctDNA. Blood should be collected in K2EDTA tubes followed by processing for separation of plasma within 6 hours to prevent leukocyte genomic DNA contamination[9]. Alternatively, specialized tubes like Streck BCT can preserve and stabilize ctDNA for 14 days, permitting safe transportation of samples. In order to preserve the cfDNA integrity and diminish lysis of leukocyte, peripheral blood should be processed through two sequential centrifugations before storage or extraction of cfDNA[10]. Investigations have shown that the purified plasma can be frozen at cold temperature and stored in the single-use aliquots without compromising its quality[11,12].

1.1. Methods to Analyze ctDNA

In hematological cancers, ctDNA analysis is used to detect gene mutations and examine distinctive immunoglobulin heavy chain (IGHV) rearrangements in B-cell malignancies. This allows to identify somatic mutations in cancerous genes without needing prior tumor biopsy analysis, making it a tissue biopsy-free approach. This method can reveal mutations from different tumor sites, offering a comprehensive view of tumor heterogeneity[13]. Advanced next-generation sequencing (NGS) techniques enables the analogous sequencing of many DNA components and molecules, identifying various genetic changes, including deletions/insertions, copy number alterations, rearrangements, and point mutations[14]. Cancer Personalized Profiling by deep sequencing (CAPP-seq) is one of the targeted method of NGS that detects tumor-specific mutations in the ctDNA within diverse malignancies. CAPP-seq uses a disease-related selector that includes intronic and exonic targets wrapper regions with recognized recurrent mutations in particular tumor types. These target regions are then amplified and sequenced, allowing for the quantification of ctDNA by identifying cancer-related mutations. Nearly all B-cell malignancies have district rearrangement of IGHV, detectable in ctDNA with use of PCR or NGS-dependent technologies. If the IGHV rearrangement is unproductive, the IGL or IGL light chain sequence can serve as an alternative markers. To confirm that the IGHV rearrangement detected in a liquid biopsy is of tumor origin, it should first be identified in a tissue biopsy.(15) The NGS-dependent assays e.g., clonoSEQ, which use universal primers to target immunoglobulin light/heavy chains, are FDA-approved for detecting minimal residual disease (MRD) among chronic lymphocytic leukemia (CLL), B-cell acute lymphoblastic leukemia (B-ALL), and multiple myeloma (MM) patients[16–19]. These assays are widely utilized to monitor MRD in patients with lymphoma. However, these immunoglobulin analysis methods focus on a one molecular markers and may not be able to identify clonal V (D) J rearrangement in about 20% of lymphoma cases because of increased somatic hypermutations (SHM) rate[16,19,20].

2. Applications of ctDNA Hematological Malignancies

2.1. Diffuse Large B-cell Lymphoma

The diffuse large B-cell lymphoma (DLBCL) exhibits substantial molecular and clinical diversity. Within a single patient, different anatomical regions may possess unique genetic mutations (Table 1)[21]. Liquid biopsy has been shown to effectively identify mutations that might be missed in a single tissue biopsy. Research indicates that genetic alterations associated with lymphoma can be identified from the ctDNA with use of CAPP-seq, even in the absence of an initial tumor tissue biopsy[8,22]. Several independent studies have assessed the specificity and sensitivity of the target gene mutations exploration in ctDNA compared to tissue biopsy in patients with DLBCL[23,24]. The true-positive rate for mutations found in both range of ctDNA and tissue biopsy varies from 95% to 99%. Furthermore, the liquid biopsy uncover about 15% to 20% of mutations not recognized in the lymph node biopsies, while merely about 1% to 5% of low-abundance mutations are detected solely in lymph node biopsies and not in ctDNA[23,24].

While many patients of DLBCL are successfully treated with first-line chemoimmunotherapy regimen R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone), new treatment modalities have recently been permitted[25,26]. Despite these advancements, about 40% of DLBCL patients experience relapse or are refractory to therapy[27,28]. Identifying molecular and clinical biomarkers to enhanced pinpoint patients at higher risk, could progress outcomes and rate of cure. Currently, criteria for response to lymphoma treatment do not consists markers of MRD at the completion of treatment. Monitoring ctDNA levels after and during treatment presents a prospective method for assessing MRD in patients with DLBCL. Multiple methods are employed to assess MRD in DLBDL utilizing genetic mutation as well as IGHV rearrangement through CAPP-seq. High-throughput quantitative methods for analyzing IGHV rearrangements in ctDNA have shown the ability to identify the progression of disease months prior to conventional methods of imaging[29]. Another strategy involves using CAPP-seq to measure ctDNA levels at baseline and throughout R-CHOP treatment. A 2-log decrease in ctDNA from the baseline after one treatment cycle, and a 2.5-log reduction after two cycles have been recognized as optimal thresholds for predicting patient outcomes[8,18,30,31]. Furthermore, ctDNA-based molecular response remains an independent factor of prognosis for event-free survival (EFS), and overall survival (OS), even when considering the international prognostic index (IPI) score, interim CT/PET scan results, and cell of origin[32].

Advancements in molecular biology techniques and *in silico* methods, which reduce technical inaccuracies have significantly increased sensitivity of the targeted approaches based on NGS. Techniques to suppress errors have lowered the rate of background, achieving a sensitivity of approximately 2.5:100,000[22,33]. For instance, combining CAPP-seq to distinctive

barcoding and downstream bioinformatics mainly eradicates errors of sequencing and background noise, enabling the detection of ctDNA at 0.002% allele frequency (AF)[8,24]. To further enhance diagnostic sensitivity and minimize rate of background errors, an innovative technique named phased variant enrichment and detection sequencing (PhasED-seq) has been established. This technique monitors two or further variants ("phased variants") on the similar DNA strand, reducing biological as well as technical background signals while maintaining enhanced genomic recovery[34]. PhasED-seq enables monitoring of ctDNA at an detection minimum limit of about 0.000005%[8,22]. This method is predominantly beneficial in B-cell tumor, where multiple-phased variants happen in stereotypes regions of malignant genome because of on-target and unusual somatic hypemutations (SHM) regulated by activation-induced deaminsae (AID)[35]. Although PhasED-seq shows great potential in improving sensitivity to detect ctDNA in patients with lymphoma, further research is essential to ratify its advantage over CAPP-seq in early relapse detection and to determine the paramount clinical applications for this technique[22].

2.2. Central Nervous System Lymphoma

The diffuse large B-cell lymphomas (DLBCLs) which involve central nervous system (CNS), terms CNS lymphomas (CNSLs), are divided into primary and secondary types. Primary CNS lymphoma (PCNSL) is a rare subclass of DLBCL that affects the brain, spinal cord, eyes, or leptomeninges without spreading outside the CNS. Secondary CNS lymphoma (SCNSL) describes either a DLBCL relapse confined to CNS or simultaneous involvement of the CNS and other body systems[36]. Diagnosing CNSL often requires invasive neurosurgical procedures, which can be risky or delayed due to concurrent treatments. Consequently, clinical outcomes for CNSL patients vary widely, with many facing early mortality or recurrence after initial treatment[37]. This highlights the need for innovative diagnostic methods and biomarkers that can identify CNSL non-invasively, better stratify the patients by risk, and calculate responses to therapy.

Primarily, in PCNSL, the liquid biopsy focused on cerebrospinal fluid (CSF) due to decreased plasmas ctDNA levels, which made recognition difficult[38,39]. However, advancements in liquid biopsy methods, including the integration of PhasED-seq and CAPP-seq, have significantly improved the sensitivity of ctDNA detection, achieving high concordance rates in both CSF and plasma of CNSL patients[40,41]. Plasma ctDNA analysis has successfully recognized recurrent mutations in the genes related to the B-cell receptor signaling pathway, such as CD79b, MYD88, and PIM1[42,43]. Expanding on the capability of ctDNA analysis to genotype CNSL, researchers have explored its prognostic value before and after treatment. Patients having detectable ctDNA in plasma before treatment, exhibited considerably shorter progression-free survival (PFS) and overall survival (OS), even when accustomed for known radiological and clinical risk factors. Furthermore, like DLBCL, the recognition of MRD through plasma ctDNA while

therapy identified the patients having bad prognosis after immunochemotherapy regimen[40].

2.3. Hodgkin Lymphoma

The main obstacle in fully understanding Hodgkin lymphoma (HL) lies in the scant presence of malignant Hodgkin/Reed-Sternberg (HRS) cells among biopsy specimens, typically ranging from 0.1% to 3%[44]. To overcome this challenge in tissue-based profiling, plasmas ctDNA emerged as a promising alternative for characterization HL mutations[45,46]. Notably, HL patients exhibit approximately double the levels of cfDNA compared to healthy individuals, with a median ctDNA levels of around 200 hGE/mL[47]. Despite HL's smaller tumor cell volume relative to other aggressive lymphomas, there is a notable association between levels of ctDNA and volume of radiologic malignancy, suggesting substantial release of ctDNA in HL, possibly attributable to its high apoptotic HRS cell rate[45]. As a result, initial attention has been directed towards HL genotyping via liquid biopsy. Leveraging a panel of CAPP-seq comprising recurrently mutated genes in HLA, ctDNA efficaciously identified 87% of genetics. Furthermore, longitudinal ctDNA profiling unveiled therapy-based arrangements of clonal progression in relapsed individuals post-treatment and those upholding partial remission in immunotherapy[48]. Given immune system evasion's pivotal role in HL, investigations into genetic alterations of the immune checkpoint genes in ctDNA were prompted. cfDNA genomic profiling accurately identified and characterized approximately 80% of copy number aberrations of chromosome 9p24.1, associated with overexpression of PD-1 ligand and favorable outcomes in classic HL (cHL)[49,50].

Computed tomography/positron emission tomography (CT/PET) stands as the gold standard for the staging and evaluating response in cHL[51]. Total metabolite tumor volume (TMTV) at diagnosis strongly predicts outcomes in cHL. Notable, plasma ctDNA levels correlate with TMTV, suggesting that combining ctDNA quantification with TMTV assessment could improve outcome prediction at diagnosis[52–54]. Interim CT/PET following two ABVD cycles in advanced cHL characterizes an essential therapy juncture. Patients with negative interim CT/PET continue the ABVD, while those with positive results switch to intensified regimens. Nevertheless, meta-analyses reveal some inaccuracies in this approach, prompting CT/PET alongside ctDNA analysis. A >2-log decrease in the ctDNA following two chemotherapy cycles signals complete response and potential care, whereas a <2-log reduction indicates progression and shorter survival. Hence, ctDNA quantification supplements interim CT/PET in evaluating residual disease in cHL, enabling differentiation between cured and relapsing patients despite discrepancies in interim CT/PET interpretations[55].

Table 1: Clinical Application of ctDNA in Lymphoma

Application	Type of lymphoma	Target	Method	Disease stage	Findings	Ref.
Concordance	HL NHL	β-globin gene	qPCR	Diagnosis	Average levels of cfDNA in lymphoma patients were increased	[56]
	PCNSL	APP gene	RT-qPCR	Diagnosis	MYD88 was identified in cfDNA of CSF and plasma samples	[57]
Prognosis	HL NHL	β-globin gene	qPCR	Diagnosis	Increased cfDNA levels were linked to bad prognosis in HL; not significant in DLBCL	[56]
	NHL	APP gene	RT-qPCR	Diagnosis	Higher cfDNA concentrations were linked to bad 2-year PFS, while lower concentration was correlated with significantly higher 2-years PFS rates	[58]
	FL	IgH gene rearrangement	Multiplex PCR ddPCR	Diagnosis	Patients with higher cfDNA levels had a reduced 4-year PFS	[59]
		V(D)J gene	NGS	Diagnosis	Elevated cfDNA levels were linked to bad progression-free survival; also detected subclones	[19]
	DLBCL	MIR34B/C MIR34A DBC1 APK1	PCR	Diagnosis	Methylation of DAPK1 and DBC1 was correlation with poor 5-year OS	[60]
		LINE-1	PCR	Diagnosis	Hypomethylation of LINE-1 emerged as an independent risk factor for poor survival	[61]
Staging	DLBCL	IgH gene rearrangement	NGS PCR	Diagnosis/ post-treatment	Patients with detectable cfDNA had higher hazard ratio for clinical disease progression	[18]
Response assessment	DLBCL	Somatic mutations	CAPP-Seq Fluorometry	Diagnosis/ Relapse	Patients with EMR demonstrated EFS at 24 months	[31]

APP, amyloid beta precursor protein; BCL2, B-cell lymphoma 2; CAPP-Seq, cancer personalized profiling by deep sequencing; CCND1, cyclin D1; cfDNA, cell-free DNA; CLL, chronic lymphocytic leukemia; ddPCR, digital droplet PCR; DLBCL, diffuse large B-cell lymphoma; EFS, event free survival; EMR, early molecular response; FL, follicular lymphoma; HL, Hodgkin lymphoma; IgH, immunoglobulin heavy chain; LINE-1, long interspersed element-1; MYD88, myeloid differentiation primary response 88; NGS, next generation sequencing; NHL, non-Hodgkin lymphoma; OS, overall survival; PCR, polymerase chain reaction; PFS, progression free survival; PCNSL, primary central nervous system lymphoma; qPCR, quantitative PCR; RT-qPCR, reverse transcriptase quantitative PCR; TCR, T-cell receptor

2.4. Myeloid Leukemia and Neoplasm

The analysis of many myeloid cancers depends upon a comprehensive evaluation involving morphological, molecular, and immunophenotypic analyses of the bone marrow[62]. While monitoring the MRD for these malignancies typically relies on sequential bone marrow assessments, recent research has shed light on the ability of circulating cfDNA as a valuable diagnostic modality. Initial investigations have shown increased cfDNA levels in patients having myeloid neoplasms in contrast to healthy individuals, making it a reliable indicator for identifying disease-specific genomic abnormalities (Table 2)[63,64]. The significance of cfDNA in myeloid neoplasms was initially recognized in acute myeloid leukemia (AML)[65]. The targeted ctDNA NGS has further advanced our understanding by uncovering clinically relevant mutations that may be missed by traditional bone marrow analysis, thereby complementing AML patient evaluation and monitoring. In patients with AML undergoing allogeneic hematopoietic stem cell transplantation (alloSCT), monitoring MRD based on ctDNA has shown promising results. Using NGS, researchers identified driver mutations among 51 patients, therefore personalized digital PCR assays were developed to assess MRD. Analysis of multiple time points post-alloSCT revealed the perseverance of ctDNA mutations was linked with poorer outcomes[66,67]. Analogous strategies have been applied in the MDS, where serial monitoring of ctDNA with use of digital PCR enables the recognition and monitoring of the driver mutations and karyotyping aberrations while treatment, predicting treatment failure[68,69].

A recent research investigated the cytogenetic and molecular profiles of the MDS through NGS analysis of ctDNA, comparing findings with DNA samples of paired bone marrow. The mutation profile identified in ctDNA displayed a high level of agreement (92.1%) with bone marrow analysis, and the variant allele frequency correlated well between ctDNA and bone marrow samples. Notably, NGS analysis of ctDNA and microarrays demonstrated high concordance in identifying chromosomal aberrations, with all cytogenetic abnormalities identified in bone marrow DNA also discovered in ctDNA. The findings highlights that ctDNA determination holds promise for the molecular representation and monitoring of MDS[70]. While there is limited data on liquid biopsy among patients with Philadelphia-negative myeloproliferative neoplasms (MPNs), an investigation reported elevated concentration of ctDNA in these disorders compared to healthy individuals. Furthermore, patients with primary myelofibrosis exhibited higher ctDNA levels than those with polycythemia vera or essential thrombocythemia. Like other hematological malignancies, ctDNA reveals the mutation profile recognized in the genomic DNA from granulocytes in peripheral blood or bone marrow samples[71].

Table 2: Application of ctDNA in Leukemia

Application	Type of leukemia	Target	Method	Disease stage	Findings	Ref.
Concordance	ALL	TCR/IgH rege rearrangement	RQ-PCR	Diagnosis	In T-ALL, there was a robust correlation between MRD levels in matched bone marrow and peripheral blood cfDNA; no correlation in B-ALL	[72]
	AML	Somatic mutations	NGS	Diagnosis	cfDNA revealed a median of 3 novel mutations in patients that were not detected in bone marrow at diagnosis; also detected additional and identical mutations	[73]
	AML ALL MDS	FLT3-ITD	PCR	Diagnosis	Detection rate was same for both cfDNA and bone marrow in AML and MDS patients, but not in ALL patients	[74]
Early relapse detection	AML ALL	Somatic mutations	NGS	Diagnosis/ Relapse	Mutations detected in both cfDNA and bone marrow; IDH1 and ASXL1 mutations in cfDNA found months before relapse	[75]
MRD response assessment	ALL	TCR/IgH gene rearrangement	RQ-PCR/ Flow cytometry	Diagnosis/ Post-treatment	Weak correlation observed between two methods when assessing the level of MRD	[76]
Prognosis	ALL	TCR/IgH gene rearrangement	RQ-PCR/ Flow cytometry	Diagnosis/ Post-treatment	Positive MRD by flow cytometry on day 15 of treatment significantly increased the risk of relapse by 20-35 fold	[76]
Response assessment	ALL	TCR/IgH gene rearrangement	RQ-PCR	Diagnosis/ Post-treatment	In precursor B-ALL, MRD levels were notably elevated in bone marrow samples	[72]

ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; ASXL, additional sex combs-like; cfDNA, cell-free DNA; FLT3-ITD, FMS-like tyrosine kinase internal tandem repeat; IDH1, isocitrate dehydrogenase 1; IgH, immunoglobulin heavy chain; MDS, myelodysplastic syndrome; MRD, minimal residual disease; NGS, next generation sequencing; PCR, polymerase chain reaction; RQ-PCR, real-time quantitative PCR; TCR, T-cell receptor

3. Future Applications

The investigation into patterns of cfDNA fragmentation among liquid biopsy, called as "fragmentomics," and its relationship with therapeutic outcomes has gained significant attention in recent era[77]. The thought of cfDNA fragmentomics was initially presented in 2015, leading to the establishment of various experimental and computational methods to assess the patterns of fragmentation for cfDNA in the plasma[78,79]. Typically, cfDNA demonstrates peaks around 166 base pairs or their multiples, indicating apoptosis as the primary cfDNA release mechanism. Cancer patients often exhibit molecules of ctDNA having smaller size distributions compared to the hematopoietic cells-derived background cfDNA. Recent studies suggest that the lengths of ctDNA fragments in lymphoma patients may vary individually and correlate with stage of disease[80–83]. Furthermore, these patterns of fragmentation have shown predictive capability in DLBCL, underscoring fragmentomics as a promising prognostic marker for this condition[28].

Tumor-specific changes in methylation of DNA play essential part in gene expression regulation and appear promptly in neoplastic progression. Such variations can potentially be identified in the plasma even earlier than clinical diagnosis of cancer is established[84]. Epigenetic sequencing of cfDNA encompasses the complete cfDNA pool, enabling the detection of methylation alterations beyond somatically mutated cfDNA alone. This method holds promise, given the widespread distribution of methylation sites through human genome[85]. Among hematological cancers, patterns of aberrant methylation identified in the cfDNA have been related with unfavorable DLBCL outcomes[60,61]. Additionally, abnormal alterations among 5-hydroxymethylcytosine (5hmC), a distinctive epigenetic characteristic observed in numerous tumors, have been detected in cfDNA, emerging as a more specific marker for diagnosing and prognosing AML[86]. Furthermore, specific patterns of DNA methylation can precisely differentiate between cancer types in cfDNA samples, facilitating non-invasive cancer classification[87]. The incorporation of mutation and epigenetic investigations of ctDNA molecules offers a favorable avenue for comprehensive tumor characterization.

4. Conclusion

The exploration of ctDNA from the liquid biopsy is gaining momentum in hematological cancers as a trustworthy method for cancer genotyping, treatment monitoring, and prediction of outcome. Recent technological advancements have facilitated the combination of conventional molecular profiling with the liquid biopsy to identify and analyze biomarkers. The adoption of techniques like PhasED-seq and CAPP-seq in HL and DLBCL has emerged as a well-established method, enabling baseline outcome prediction and evaluation of minimal residual disease post-chemoimmunotherapy, thereby aiding in the early detection of relapses. Numerous clinical trials are

currently investigating ctDNA examination for personalized therapy strategies. Nevertheless, the challenge lies in ensuring the reproducibility of these high-throughput technologies across different laboratories, necessitating standardization and validation before clinical use. The predominant focus of the liquid biopsy endeavors in hematological cancers has been on gene mutation analysis using PhasED-seq and CAPP-seq techniques. However, liquid biopsy holds potential for exploring other disease indicators, including ctDNA epigenetic patterns, copy number abnormalities, and fragmentomics. The integration of these factors with innovative statistical techniques and machine learning approaches has the potential to enhance the molecular categorization of blood disorders. The ctDNA examination from liquid biopsy characterizes a significant step onward in precision medicine for patients having hematological cancers, particulatly those with cHL and DLBCL. The integration of ctDNA dynamics with the CT/PEAT scans at provisional stages shows promise for enhancing outcome prediction and tailoring personalized treatment strategies during therapy. Patients without residual disease may benefit from treatment reduction, while those with persistent conditions may require intensified therapy. Ongoing clinical trials are poised to provide crucial insights into these questions in the near future.

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