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Research Article

Liquid Biopsy and Circulating Tumor DNA (ctDNA) as a Molecular Tools for Early Detection and Monitoring of Cancer: A review

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Abstract

Background: Liquid biopsy of circulating tumor DNA (ctDNA) is a groundbreaking technology that facilitates cancer screening and surveillance on a much less invasive basis than conventional tissue biopsies. Aims: The literature presented in this review integrates the clinical applications of ctDNA for early diagnosis, treatment response, minimal residual disease (MRD) followed up longitudinally, and resistance mutation characterization.

Methodology: A literature review was conducted in global biomedical databases across peer-reviewed studies of polymerase chain reaction (PCR) and next-generation sequencing (NGS) methods in liquid biopsy processes. Literature Review: Based on all literature up to October 2023, the current state of literature related to polymerase chain reaction (PCR) in liquid biopsy workflows for this topic is extensive. The literature realizes that PCR and NGS techniques have equally improved oncology, but common application suffers from formidable limitations. This includes gaps in genome sequence amplification and biological interference through clonal hematopoiesis of indeterminate potential (CHIP) and overpriced methods.

Results: The data suggest that new technologies especially artificial intelligence, advanced bioinformatics, and multi-analytic biomarkers (methylation patterns, microRNAs, and proteins) are addressing some of the technological challenges. Moreover a recent study emphasizes the applicability of liquid biopsy principles to neurological and cardiologic fields.

Conclusion: Liquid biopsy in precision medicine will inevitably depend on extensive laboratory assay standardization, cost-control, and sound clinical validation. Over time, as these techniques mature, liquid biopsy will transform from being a classical cancer management tool to a more personalized therapeutic method for people of differing disease profiles, transforming to a state of clinical workflows oriented toward the specific client.

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1.Introduction

Liquid biopsy has established notable attention as a robust, noninvasive method for cancer detection and surveillance which exhibits advantages in comparison with traditional tissue-based methods. Advances in next-generation sequencing (NGS) and digital polymerase chain reaction (dPCR) are also substantially increasing the sensitivity and specificity of circulating tumor DNA (ctDNA) (1,2). In addition, liquid biopsies are crucial in the clinic for clonal monitoring and assessment for resistance mutations in order to develop adaptive therapeutic approaches (3). Nevertheless, despite the great potential of this method, low ctDNA concentrations in early-stage malignancies and urgent assays that can be integrated into standardized formats represent two of the major challenges in the clinic (4). At the heart of the assay is the analysis of the circulating tumor DNA (ctDNA) liberated by apoptotic (or necrotic) tumor cells into the blood stream which allows real-time molecular characterization of tumor heterogeneity, the evaluation of therapeutic response, and the identification of minimal residual disease (MRD) in tumors (5,6).

Nevertheless, they remain far from compatible with the conventional clinical paradigm, as a major gap exists between the potential for liquid biopsy to be analyzed and their potential clinical utility to be uniform. Although there is abundant evidence of the potential for utilizing ctDNA in the existing literature, the pre-analytical uniformity of analysis, best timing for MRD monitoring and methods to circumvent low tumor fraction shedding in early stage tumors are missing in the existing literature (7, 8). Moreover, although the prognostic impact of ctDNA on recurrence is well-known, whether this approach is better-suited to provide clinicians with real-time ctDNA dynamic feedback to steer them in guiding dynamic or de-escalated therapeutic intervention rather than with a passive recall approach is largely unarticulated within a variety of solid tumors (9, 10). Most previous studies have narrowly focused on individual sequencing modalities or single cancer types, which leaves a broad literature gap for understanding how new multi-omic integration and AI technologies can be collectively scaled into deployable, clinician-friendly practice workflows (11,12).

To address these critical limitations, this review examines where liquid biopsies from ctDNA are now. More precisely, its usage is expected to help in the early detection of cancer, continuous disease monitoring, and a prospective role in precision oncology paradigms is explored.

2. Methodology

A systematic search helped to guide the strict, transparent, and equitable synthesis of the current literature strategy was established and executed. Although called narrative review, the literature selection process was clearly defined to avoid selection bias and provide high-impact, peer-reviewed evidence in clinical practice.

2.1 Search Strategy and Available Data:

Electronic database searches were carried out systematically in three major biomedical and scientific repositories: PubMed/MEDLINE, Scopus, and Google Scholar. We focused on published articles within the period January 2016 to May 2026, with particular emphasis on more recent developments in technology and computational oncology paradigms. Boolean operators were used together with Medical Subject Headings terms and free-text keywords in the search. An exact search string structure was defined as: ("liquid biopsy" OR "circulating tumor DNA") AND ("minimal residual disease" OR "cancer diagnosis" OR "disease surveillance") AND ("next-generation sequencing" OR "digital polymerase chain reaction" OR "artificial intelligence")

2.2. Criteria for inclusion and exclusion:

Articles were screened using a dual screening process with title/abstract screening and full-text review from the initial search lists, according to strict eligibility standards.

Inclusion Criteria: Articles were included if they met the following criteria:

1. Primary research articles, prospective or retrospective clinical trials, and meta-analyses examining circulating tumor DNA kinetics in human malignancies.
2. Analyses that examined analytical sensitivity, specificity, or the clinical usefulness of next-generation sequencing and digital polymerase chain reaction platforms.
3. Studies that describe the use of machine learning or artificial intelligence pipelines for interpreting low-abundance cell-free DNA fragments.
4. Paper studies in peer-reviewed journals and published in the English language.

Exclusion Criteria: materials were excluded if they met one of these criteria:

1. Conference abstracts, case reports, editorials, and/or correspondence letters that did not present complete datasets and/or elaborate technical approaches.
2. Non-peer-reviewed pre-prints or grey literature.
3. Studies focusing exclusively on in vitro cell lines or animal models without direct translational validation using human clinical samples.

2.3 Data Synthesis and Quality Assessment

Data were synthesized according to the criteria for the inclusion criteria. After duplicate records were excluded and selection filters were applied, meaningful scientific literature was prioritized for thematic synthesis. After reviewing eligible articles based on their sample size, detection limits, clinical endpoints, and structural robustness, some were treated critically. Results were then qualitatively synthesized and integrated into thematic sections around technological platforms, early screening, and minimal residual disease tracking.

3. Origin of Circulating Tumor DNA (ctDNA)

Circulating tumor DNA (ctDNA) consists of small-size fragments of cell-free DNA (cfDNA) secreted into the blood by malignant cells through apoptosis, necrosis, and active leakage (Figure 1; 13,14). These fragments often span 140 to 170 bp, indicative of nucleosomal shielding in apoptosis and contain site-specific genetic and epigenetic modifications such as somatic alterations, copy number variations (CNVs) and aberrant methylation patterns (15). Release of ctDNA depends on various factors -Tumor burden, vascularization and cell turnover rate and higher levels are usually found for late-stage and metastatic disease (16). There is a requirement for highly sensitive assays for use in clinic for ctDNA release that in some cases is even detectable during early-stage cancers and it is released, but reduced in the sample concentration. Besides passive release mechanism, growing evidence supports the involvement of extracellular vesicles (EVs) most obviously exosomes in the transport of ctDNA, resulting in its resistance to degradation and improvement of its capacity as a biomarker (17). Due to its dynamic nature, ctDNA is suited to monitoring tumor progression, therapeutic response and emergence of a resistance mechanism in real time and is thus poised; ctDNA becomes an essential tool in precision oncology (18). Although there is great potential for its use, numerous biological factors that contribute to its low levels of abundance in early-stage disease and heterogeneity of shedding patterns need to be resolved for optimal clinical and prognostic value (19).

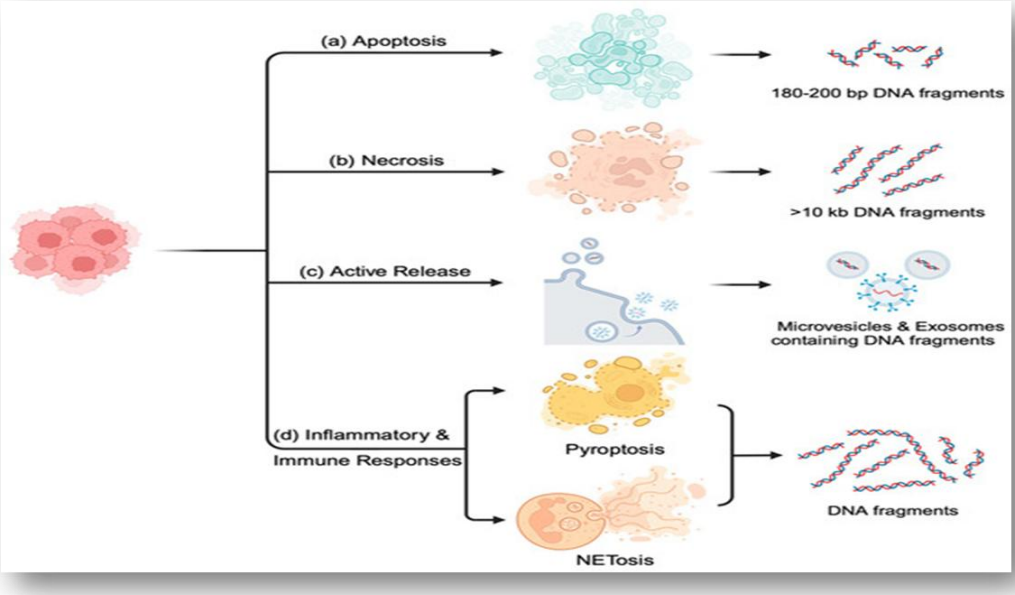

Figure 1:Circulating cell-free DNA (cfDNA) release into circulatory circulation mechanisms. Cell-free DNA, including ctDNA in the form of circulating tumor DNA (ctDNA), can be released into the blood stream via apoptosis (release short fragments of DNA) followed by necrosis and necrosis to longer undigested fragments, and active release to the blood stream (e.g., microvesicles, exosomes, pyroptosis and NETosis and an inflammatory immune response (13)

4.Technologies Used to Detect ctDNA

Detection of circulating tumor DNA is based on highly sensitive molecular techniques with low frequency tumor – derived modifications in the presence of a background of mainly wild type DNA. These methodologies fluctuate in sensitivity, throughput, cost, and clinical adoption, each potentially benefiting from other approaches for the specific application of cancer diagnosis and disease monitoring.

4.1. PCR-based techniques

PCR based methods such as qPCR and dPCR(which can be used both at small scales and are thus highly specific) are widely used in the field of ctDNA detection. They are based on the idea that proteins make up a part of the genome, and using these different DNA molecules provides a way to quantify their presence such as ctDNA. While quantitative PCR provides an economical means of identifying established hotspot mutations, its potential limit is that it cannot significantly quantify variants with allele frequencies lower than ~1%. On the other hand, dPCR separates DNA into large quantities of individual reactions. Thus allowing for absolute quantification of mutations, with sensitivities between 0.01% and 0.1%. As such, dPCR is especially suited to monitoring minimal residual disease (MRD) and low-abundance ctDNA (20). However, dPCR will only detect known mutations and will not have the capabilities to multiplex with next-generation sequencing (NGS)

4.2. Next-Generation Sequencing (NGS)

Second generation sequencing is widely applied in ctDNA analysis, where multi-feature and molecular identification (multiple alterations) in broad genomic regions, like single nucleotide variants (SNVs), insertion and deletion (indels), and copy number alterations can be detected at the same time (21). New targeted NGS techniques, like CAPP-Seq, improved sensitivity of analysis (allele frequency detection as low

as 0.1% and minimizing sequencing-generated noise). However, challenges remain with regards to cost and complexity of bioinformatics requirements as well as the requirement of deep sequencing for truly unique mutations to be reliably differentiated from technical artifacts (22). Despite these limitations, NGS is still the gold standard for full ctDNA profiling in precision oncology

4.3. Emerging Technologies

Longstanding issues in detection of ctDNA have recently been being tackled by new approaches. CRISPR technology and, in particular, CRISPR-Dx allow targeting of circulating tumor DNA to enhance sensitivity without extensive sequencing (23). BEAMing (Beads, Emulsion, Amplification and Magnetics) uses combined PCR with flow technologies to detect rare mutations with single molecule precision (24), and epigenetic profiling of ctDNA, especially via methylation-based assays, has been lauded for its ability to identify cancer specific biomarkers (25). These new technologies provide more accurate, scalable, and clinically applicable solutions in the booming field of liquid biopsy

5. Clinical Applications of Liquid Biopsy

Liquid biopsy, primarily by studying circulating tumor DNA, changed everything about oncology by enabling a non-invasive and live diagnostic experience of the disease process. Clinical applications range from early diagnosis and therapeutic response monitoring to minimal residual disease (MRD) surveillance and resistance mechanisms characterization that substantially support precision medicine.

5.1. Early Cancer Detection

As patient survival rate rises greatly with early diagnosis of cancer, traditional screening is generally performed in the target location of the cancer, only covering a limited set of malignancies such as gastric, colorectal, lung, breast and cervical cancers for example. Consequently, a large fraction of cancers ~ 45.5% of cancers are poorly screened, impeding early detection. On the other hand, multi-cancer early detection (MCED) analyses are available to detect multiple cancer varieties through a single liquid biopsy and help to identify molecular alterations in a patient before presentation to the clinic. Along with confirming cancer, these tests provide insight into molecular alterations in DNA, altered methylation patterns and fragments and other markers linked to tumors, and predict the tissue's origin. Further, MCED tests can detect various malignancies simultaneously, including malignancies for which no screening guidelines exist today, and could transform cancer screening and therapy overall. Large 0-scale studies have shown good performance for both sensitivity ranging from 50% to 95% and specificity between 89% and 99% for all types of cancer. However, the widespread clinical adoption requires resolution of several challenges including improving diagnosis accuracy, ethical issues (e.g., effect of the data on the psyche), and appropriate incorporation of MCED assays in integrated healthcare systems. Moreover, future multi-institutional follow-up is needed to determine clinical applicability in heterogeneous populations (26). Although there is ongoing cancer support for MCED assessment to markedly reduce late-stage cancer diagnosis, such a finding is limited to high-risk patients (27).

5.2. Assessment of Treatment Efficacy

Serial ctDNA analysis allows for a dynamic evaluation of therapeutic response and provides quicker and more sensitive feedback than conventional imaging or tissue biopsy. During chemotherapy or immunotherapy, the rapidly decreasing ctDNA level correlates with treatment efficacy; meanwhile stable or increasing concentration of ctDNA may suggest resistant response (28). This approach is doubly useful in aggressive cancer like lung or colorectal cancers, in which treatment can actually affect the course of a given patient's illness (29).

5.3. Detection of Minimal Residual Disease (MRD)

Post-treatment detection by ctDNA analysis of MRD follows treatment and helps in prediction of relapse months before clinical or radiographic sign of recurrence may be noticed. Of all breast and colorectal cancers, ctDNA presence after surgery or during adjuvant treatment is highly correlated with the prognostication and follow-on metastatic advancement (30). We are in the process of evaluating the clinical trial IMvigor11 to confirm a ctDNA-guided therapy intensification pathway for the eradication of MRD by improving the cure rate (31).

5.4. Determination of Resistance Mutations

Real-time application of liquid biopsy facilitates the detection of acquired resistance mutations like EGFR T790M and KRAS G12C that allow tumors to evade targeted therapy (32). This enables clinicians to proactively adjust treatment regimens by monitoring clonal evolution. For example, a shift in EGFR inhibitor status from 1st line to Osimertinib with the tumor progression of the T790M mutation (33).

6. Comparison between Liquid Biopsy and Traditional Biopsy

Liquid biopsy and traditional tissue biopsy are two very different methods for cancer diagnosis and surveillance which have their own distinctive strengths and drawbacks. Table 1 compares these approaches with notable differences and clinical instances for which liquid biopsy could be performed with preferred options (34-40).

Table 1: Compression Between Liquid Biopsy and Traditional Tissue Biopsy

Feature	Liquid biopsy	Traditional tissue biopsy
Sample type	Blood (plasma / serum), urine, other body fluids	Solid tumor tissue such as core needle and surgical biopsy.
Invasiveness	Minimally invasive (blood draw)	Invasive (risk of bleeding , infection, complications)
Reversal time	Rapid (hours to days)	Slower (days to weeks due to processing)
Cost	Moderate (varies by technology , NGS is expensive)	High (surgical procedure, pathology and sequencing)
Sensitivity	Lower for early stage tumors (60-90)%	High (gold standard for mutation detection)
Specificity	High for known mutations but risk for false positives	Very high (direct tumor analysis)
Spatial heterogeneity capture	Limited (averaged signal from all tumor sites)	Captured regional tumor heterogeneity
Temporal resolution	Enable real-time monitoring (serial testing)	Single snapshot in time (unless repeated)
Clinical use cases	Early detection, MRD, therapy monitoring, resistance tracking	Initial diagnosis , histopathology, biomarker confirmation

This distinction emphasizes the complementary nature of these processes: liquid biopsy is more active and more effective for monitoring tumor evolution while the traditional tissue biopsy should still be conducted for histopathological diagnosis as well as characterization of genomic details. These are examples in clinical practice in which liquid biopsy is indicated:

1. Initial diagnosis and screening for cancer.

Liquid biopsy can be non-invasive, advantageous clinically, particularly among high-risk populations such as familial cancer syndromes (e.g., BRCA mutation carriers) and Lynch syndrome patients who may benefit from serial monitoring of cancers, in order to reveal it earlier and/or more successfully, without the added danger and time-consuming and repetitive routine tissue biopsy. At this point, multi-cancer early detection (MCED) assays, such as Galleri TM, integrate sophisticated genomic and epigenomic analyses of circulating tumor DNA to identify a large range of tumors at the same time. It can also screen for cancers that aren't detected in routine screening modalities: pancreatic, ovarian and hepatocellular carcinomas. These tests identify tumor-derived molecular changes that often occur before clinical symptoms and could inform further diagnostic work-up by predicting the tissue of origin expected (35).

2. Longitudinal Therapy Monitoring

Liquid biopsy would be able to constantly, non-invasively assess treatment efficacy in metastatic neoplasms such as non-small cell lung cancer (NSCLC) and colorectal malignancy by examining the active changes of ctDNA level during therapy. Such an approach allows for temporal real-time monitoring of tumor response or disease advancement, where rapid decreasing concentrations of ctDNA correlate with favorable therapeutic outcomes, while stable or increasing concentration, may denote therapeutic resistance or disease progression (36). In addition, ctDNA-based monitoring possesses a considerable temporal advantage compared to traditional imaging modalities, as molecular markers of progression might facilitate early treatment intervention and possibly enhance patient outcomes from timely adjustment of therapies (37).

3. Inaccessible Tumors

To patients with tumors that occupy important sites which may be too important to be missed surgically (such as in the brain where small amounts of intervention can impair normal neurologic function); the lung, where biopsy can be a source of pneumothorax or a haemorrhage; or the pancreas, where an extended, deeply-placed, and vascularized pancreas with supporting organs have serious clinical risks, a tissue sample by routine biopsy may be dangerous. In addition, even if biopsy is technically possible, the retrieved tissue sample may be inadequate (i.e., insufficient in volume, quality or cellularity), restricting or precluding a diagnostic histopathology. This joint obstacle of anatomical inaccessibility and possibility of nondiagnostic sampling demonstrates one the main limitations of traditional biopsy methods for specific tumor types (38).

4. Detection of Resistance Mutations

In patients on targeted therapy, dynamic identification of acquired resistance mutations for instance, EGFR T790M gene mutations in non-small cell lung cancer, KRAS G12C mutations in other solid tumors or ESR1 mutations are responsible for determining which therapy to pursue in hormone receptor-positive breast cancer. By monitoring gene changes detected in circulating tumor DNA or tissue biopsies as part of targeted treatment, coupled with ongoing feedback and monitoring of results, physicians can identify new resistant clones before symptoms, and immediately shift the patient to the next-line therapy (e.g., osimertinib for T790M-positive patients or KRAS G12C inhibitors such as

sotorasib or adagrasib, or novel endocrine drugs against ESR1-mutant disease). Such dynamic method not only reduces the disadvantage of single point tumor genotyping, but also provides the basis for a dynamic strategy in which therapy could be adapted dynamically to evolving tumor biology and in order to have better outcomes of the patient whether as a result of deferring the failure of therapy or not (39).

5. Minimal Residual Disease (MRD) Assessment

MRD (minimal residual disease) is defined as a noninvasive, non-destructive investigation in which ctDNA or occult tumor cells will be detected at minimal levels after extensive surgical resection of the breast or colorectal tumours, usually long before radiotherapy or clinical examination. This post-operative surveillance strategy allows oncologists to identify patients who have residual disease after complete gross resection, thus giving them a powerful prognostic tool and early opportunity to choose adjuvant therapy strategies. For example, in the case of early-stage breast cancer, MRD positivity after surgery can prompt up-scaling to aggressive chemotherapy or even targeted agents, while in colorectal cancer, a positive MRD signal could inform adjuvant treatment using fluoro-pyrimidine-based chemotherapy and surveillance for early management strategy. Conversely, patients with prolonged negative MRD in patients diagnosed with surgery will generally not be exposed to the toxicities and costs associated with unnecessary adjuvant therapy. By allowing for this risk-stratified, personalized approach to post-operative management, MRD assessment embodies a paradigm shift from traditional clinicpathological factors of breast/ colorectal cancers of a single patient to real-time, molecularly guided decision-making in clinical practice (40).

7. Limitations and Challenges of Liquid Biopsy

Despite the revolutionary potential available in cancer, liquid biopsy faces various technical and clinical challenges regarding the broad application. Sensitivity limitations, biological interference, and economic issues are still to be solved to allow for improvements of the application such as sensitivity that needs to be resolved to make it more effective.

7.1. Detection Sensitivity (Low concentration of ctDNA)

Liquid biopsies are especially challenging to use with both minimal residual disease and for early detection given the very low ctDNA concentration, but the problem is how to get detection sensitivity. The problem of low-contents, ctDNA levels of ctDNA in early-stage malignancy or low shedding tumors such as gliomas and renal cell carcinoma can be an even greater concern because levels of ctDNA levels range from minute 0.01% allele frequency which is close to many detection thresholds in sequencing technologies (41). There are serious clinical implications that arise from this limitation, as such a false-negative diagnosis may delay diagnosis and cause an underestimate or even not a full estimation of burden in tumors with ultimately delayed treatment, thereby limiting treatment or accurate control of treatment response. To overcome these sensitivity limitations, many sophisticated methodological choices have been proposed. One method, ultra-deep sequencing, with coverage >50,000x, significantly increases the likelihood to detect infrequent ctDNA isolates in a backdrop of relatively abundant cell-free DNA (42). Another method, currently used more frequently, uses methylation-based assays that exploit tumor-specific epigenetic patterns, e.g., the presence of differentially methylated CpG islands which tend to be far more common and more stable than somatic mutations, and thus increase the sensitivity of the detection tool to detect ctDNA at very low ctDNA levels (43).

7.2. Distinguishing Tumor from Benign Genetic Contributions

Another important challenge in ctDNA

analysis is differentiating between tumour-derived mutations and benign genetic mutations; because somatic mutations detected in plasma may not originate from the cancer itself, but rather from clonal hematopoiesis of indeterminate potential (CHIP), such as chronic inflammation or age-disproportionate hematopoietic clonal expansion; and as a result, they may look similar to true ctDNA signals closely (44). For instance, pathogenic mutations in genes such as DNMT3A or TP53 that are synonymous with solid tumors might occur from clonally expanded haematopoietic cells, in the absence of neoplasm, and lead to clouded interpretations of the liquid biopsy. The clinically significant impact of this ambiguity is considerable, since positive identification could produce false-positive, non-tumor-associated findings, which could result in unnecessary diagnostic and treatment, patient concern, and costs to treatment. To minimize such damage with this risk several integrative methodologies are proposed. The most common method is paired WBC sequencing, where genomic DNA from peripheral blood leukocytes is sequenced with plasma ctDNA and a CHIP variant was found that was not isolated from solid tumor cells and compared with plasma ctDNA (45). Moreover, fragmentomics characterized by cell-free DNA fragmentation patterns provides a robust orthogonal approach since cancer/tumor-derived ctDNA fragments typically show systematically reduced fragment lengths compared to non-cancer cell-free DNA, allowing computational discrimination between true neoplastic and benign background mutations (46).

7.3 Restricted Commercial Accessibility and Elevated Expenses

One of the practical barriers to a widespread use of liquid biopsy technology is the limited commercial uptake and high cost of a next-generation sequencing (NGS)-based assay, that many healthcare facilities have and have not adopted (at between $500 - $5,000 a test, depending platform and breadth of analysis) (47). The high cost is exacerbated by the lack of consistency among competing technologies, such as variations in gene panels, sequence depth, bioinformatic algorithms, error correction protocols for the use of different assays such as CAPP-Seq (Cancer Personalized Profiling by deep Sequencing) and Guardant360, which limits clinical application by generating uncertainty regarding cross-platform comparability, reimbursement, and regulatory compliance. The financial and standardization challenges remain limited to low-hanging fruit within the context of financial and commercial liquid biopsy, ultimately keeping this service limited to well-to-do healthcare establishments and academic medical centers with some exceptions, and the potential use of these services is limited for patients in the resource-poor context or even in un-funded health systems. Solutions to solve these issues are on the horizon. The availability of FDA-approved assays, notably Foundation One Liquid CDx, is a key component of expanding access by establishing a confirmed standard of performance and streamlining insurance reimbursements for a screening tool, however, ongoing reductions in per-test cost may also be required for routine use in community oncology settings (48). Crucially, public health efforts to incorporate liquid biopsy into population-based cancer screening programs (adjunct to mammography, low-dose CT lung screening, or colonoscopy triage) may leverage economies of scale, reduce costs and increase access for individuals at higher risk of cancer such that this powerful molecular diagnostic tool would be moved out to clinical practice from research labs (48).

8. Pre-Analytical Variables and Assay Relevance

The availability of quality of clinical utility and analytical sensitivity of circulating tumor DNA (ctDNA) experiments relies very heavily on careful handling of pre-analytical conditions. ctDNA, on average, only makes up a small fraction of total cell-free DNA (cfDNA) in the

circulation and therefore may be easily damaged in abnormal way by incorrect handling. This results in the release of an overabundance of high-molecular-weight genomic DNA (gDNA), which weakens tumor-derived signals and can cause false-negative readings.

8.1 Blood collection tubes and separation time for plasma

The blood collection tube set the timing that was necessary for the processing of the sample downstream. Standard Ethylene-diamine-tetra-acetic-acid (EDTA) tubes do not provide any cell-stabilizing agents and consequently, rapid plasma isolation is required to prevent leukocyte lysis. Previous studies indicate that plasma must be separated 2 to 4 hours following venipuncture with EDTA tubes to maintain the integrity of cfDNA (49). To maximize the time to processing time, specialized cell-stabilizing tubes (such as Streck Cell-Free DNA BCT®, Roche Cell-Free DNA Collection Tubes) contain proprietary fixatives that hold nucleated blood cells into stability. At ambient temperature, these stabilization tubes maintain sample integrity for 7 to 14 days, thereby preventing sample transport contamination by background gDNA (50).

8.2 Storage Temperature & Centrifugation Protocols

Once collected, it is important to preserve a temperature profile. The liquid in EDTA tubes contains whole blood and should never be frozen, as ice crystals lead to cell lysis. The plasma should be stored at -80 °C or in liquid nitrogen to prevent enzymatic degradation by circulating nucleases (51). Additionally, the exact centrifugation regimen determines assay reliability. In standard practice, it is necessary to initiate centrifugation with a low-speed spin (e.g., 1,600 *g) to separate plasma from cells, followed by a high-speed micro-centrifugation spin (e.g., 16,000 *g) to pellet residual cellular debris and apoptotic bodies (52). Falling short of the secondary high-speed spin allows the remaining genomic contaminants to remain intact, compromising library preparation for next-generation sequencing (NGS).

8.3 Methods of DNA Extraction

The last pre-analytical factor influencing the accuracy of the assay will be the efficient extraction of. cfDNA fragments are highly stereotyped, generally with a peak at about 166 bp corresponding to chromatosome sizes, and extraction kits need to be optimized to extract short, fragmented nucleic acids (53). Silicon membrane column-based systems (e.g., QIAamp Circulating Nucleic Acid Kit) and automated magnetic bead-based protocols are high-efficiency benchmarks for achieving maximum yield with low-volume inputs (e.g., 1–5 mL plasma), which directly affects the LoD of the following analytical method (54).

9. Prospective Trends and Future Directions in Liquid

Biopsy Liquid biochemical techniques are developed with high-throughput technologies and advances in cancer biology. The shift is on the horizon, and the original oncological paradigm is changing. Promising developments in the future will greatly promote the sensitivity, specificity, and clinical relevance of cell-free nucleic acid analysis in general biomedical applications for the generalizing of these tools to the clinical diagnostic environment as well as further application across different therapeutic lines.

1.Artificial Intelligence and Machine Learning are revolutionizing analytics in circulating tumor DNA (ctDNA), replacing traditional bioinformatics algorithms for advanced deep learning models trained on large genomic datasets, significantly boosting the accuracy of information collected. Most notably, they are able to distinguish real cancer-specific somatic mutations from background noise, polymerase misprints and genome sequencing artifacts, and achieve a sensitivity that enables discovery of ultra-low variant allele frequencies (<0.1). A huge

challenge in this domain is how to bring the bioinformatic sensitivity needed for identifying low-abundance tumor signatures from huge wild-type genomic backgrounds at the correct degree. But when properly optimized, these AI techniques can identify not only rare malignancies, but also accurately predict their tissue-of-origin (55). AI also excels at observing patients over the long haul, dynamically. Combining systematic time-series ctDNA data with multimodal clinical cues, such as longitudinal radiographic imaging, serial biomarker measures, and electronic health records (EHRs), ML models can predict recurrence of cancer or emergent therapeutic resistance several months before there is a distinct clinical or radiological deterioration (56). This transition provides clinicians the capacity to migrate from the classical nonspecific and reactive pathology of oncology into a proactive approach to predictive treatment intervention.

2. Integrating multi-analyte biomarkers to be able to address the innate limitations of ctDNA; both in light of the minimal presence of ctDNA in disease early stages, variable shedding, and its inability to elucidate non-genomic malignancy determinants as well as the use of multi-analyte liquid biopsies is the next phase in the future of cancer detection and therapy (57). Multisystematic biopsy with bio molecules is a big advance, and could provide comprehensive anti-cancer diagnostic treatment and treatment for cancers of the early stages and cancers in non-cancerous cancers. For proteomic and exosomal synergy, ctDNA in combination with known protein markers, such as CA-125 in ovarian cancer or PSA in prostate cancer, or exosome cargos (e.g., PD-L1 protein), can provide essential biological context which can account for low ctDNA shedding rates (58). Transcriptomic Amplification, combining tumor-specific microRNA (miRNA) or cell-free RNA signatures into liquid biopsy panels, broadens applicability to low-shedding tumors. As an example, in central nervous system tumors such as gliomas, where blood-based ctDNA is often not detectable owing to the blood-brain barrier, tumor-derived RNA species can often be collected and quantified (59). Epigenetic Layering, epigenetic biomarkers, which consist of profiles of DNA methylation as well as patterns of chromatin conformation, provide an additional level of diagnostic refinement. For comparison, ctDNA mutations combined with methylated SEPT9 promoter sequences (a clinically approved biomarker for colorectal cancers), signal the presence of malignancy, which would also help identify the tissue of origin, facilitating precise clinical imaging of cancer of unknown primary origin (60). By applying established multi-analytic techniques, isolated liquid biopsy assays have become powerful diagnostic ecosystems.

3. Non-Cancer Applications:

The primary method for liquid biopsy, collection and profiling of cell-free nucleic acids (cfDNA) removed from damaged or dying cells into bio fluids, is in great accelerating motion, being applied in the treatment of non-cancer diseases and allowing for versatile diagnostic platforms in every medical field: Neurodegenerative & Cerebrovascular disorders: In addition to measurement of standard biomarkers such as amyloid-beta or hyper-phosphorylated tau in cerebrospinal fluid, the characterization of cell-free DNA (blood-derived cfDNA) is being described as minimally invasive tool used to detect central nervous system pathology (61). In acute ischemic stroke, distinct cfDNA methylation patterns can be employed to predict the real-time trajectory of cerebral injury, and to quickly assess neuronal damage in a non-invasive way to develop emergent therapy (62). Cardiovascular Disease: cell-free mitochondrial DNA (cf-mtDNA) released by cellular necrosis or hypoxia is a powerful marker for myocardial infarction and heart failure. This association between high cf-mtDNA concentration and myocardial tissue injury is strong and often higher (63), and often more accurate than usual cardiac enzymes. Prenatal Screening and

Transplantation: Liquid biopsy has already changed the standard of care for obstetricians and transplant providers globally. The isolation of fetal cfDNA from maternal circulation supports NIPT by preventing miscarriage without the adverse effects of amniocentesis. In solid-organ transplantation, monitoring donor-derived Strengths of the review can be summarized as,

1. Holistic/futuristic vision on liquid biopsy: this paper goes not just under current oncology application, but future clinical applications as well into things like multi-omics plus AI analytics in liquid biopsy.
2. Multidisciplinary Approach: correctly articulates the critical importance of multidisciplinary collaboration between clinicians, scientists, and policymakers that ensures translation of laboratory research into routine clinical practice.
3. This should demonstrate pragmatic, real-world barriers to the clinical adoption and clinical use of this technology, particularly in terms of cost, regulatory frameworks, and equitable access to the technology in the clinical setting, not just the technical mechanics of the technology.

This review is thorough but has multiple limitations that may need to be addressed:

1. Dependence on new technologies as the future of liquid biopsy is highly dependent on technology with fast evolution like artificial intelligence and multi-omics, so the future use cases mentioned above can be fairly speculative and change due to rapid technological evolution.
2. Standardization Needed: This stresses the significance of standardization for analytic procedures but the global scarcity of standardized protocols limits consensus formation with which to establish any one standard that can be enacted in practice instantly upon their request.
3. The wide scope of this review could lead to the article not being able to capture the extremely narrow clinical trial data/technical parameters for any given cancer class, if it covers a broad and heterogeneous set of literature (which includes non-oncology settings).

cell-free DNA (dd-cfDNA) levels in the recipient's blood provides a highly sensitive, ultra-early indicator of graft rejection, permitting rapid titration of immunosuppressive therapy before the body gradually contracts irreversible organ damage (64).

Conclusions:

Liquid biopsy is now a groundbreaking innovation in precision oncology; the clinical world today is in great difficulty of harmonizing its technical capabilities with practical scalability. This paper demonstrates the high analytic sensitivity that was previously achievable with deep learning models and multi-omic integration is now unachievable for such methods; however, a systematic literature review found that there is a wide gap between a controlled research environment and a large discrepancy between the performance of the assay in real-life research and what we observe in the clinic. The flagship attribute of modern liquid biopsy is its multi-analyze evolution of ctDNA with proteomic, transcriptomic and epigenetic markers. This approach solves the biologic bottlenecks of low tumor shedding in early-stage malignancies in a new way. But such success in analysis creates a large clinical barrier: exponential increases in assay complexity and sequencing expense both directly threaten equitable patient access. Moreover, although AI-based bioinformatics has the ability to extract background genomic noise to ultra-low allele frequencies (<0.1), the industry suffers from fragmentation from a lack of cross-platform standardization. Conflicting results among major trials can result not from biological variability, but rather from different pre-analytical extraction techniques, different plasma-separation timelines, and a lack of shared regulatory compliance toolkits. Finally, the rate-limiting step for universal liquid

biopsy adoption no longer resides in the technology, but in the validation framework. Translating this platform from a powerful research asset to everyday, non-oncological and oncological medical practice requires a shift in response from reactive testing to proactive and integrated clinical protocols. Collaboration across practitioners, titlists and policymakers is increasingly required to establish globally accepted reference standards. Addressing these methodological disparities and economic discrepancies is undoubtedly also the single key issue driving liquid biopsy: how to evolve from an expensive, high-end specialized lab diagnostic resource to a low-cost, patient-oriented preventive tool.

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