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REVIEW ARTICLE

Beyond blood: Advancing the frontiers of liquid biopsy in oncology and personalized medicine

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Abstract

Liquid biopsy is emerging as a pivotal tool in precision oncology, offering a noninvasive and comprehensive approach to cancer diagnostics and management. By harnessing biofluids such as blood, urine, saliva, cerebrospinal fluid, and pleural effusions, this technique profiles key biomarkers including circulating tumor DNA, circulating tumor cells, microRNAs, and extracellular vesicles. This review discusses the extended scope of liquid biopsy, highlighting its indispensable role in enhancing patient outcomes through early detection, continuous monitoring, and tailored therapy. While the advantages are notable, we also address the challenges, emphasizing the necessity for precision, cost-effectiveness, and standardized methodologies in its broader application. The future trajectory of liquid biopsy is set to expand its reach in personalized medicine, fueled by technological advancements and collaborative research.

KEYWORDS
circulating tumor DNA (ctDNA), liquid biopsy, noninvasive cancer detection, personalized medicine, precision oncology

1 | INTRODUCTION

Oncology has experienced a revolution driven by diagnostic innovations that have fundamentally altered cancer management. Traditional tumor biopsies, once relatively straightforward procedures, have evolved to incorporate complex technologies, enabling precise exploration of genetic mutations within tumors and the personalization of cancer therapies.[1] However, these traditional biopsy methods, offering direct access to tumor tissue, come with inherent invasiveness and associated risks, including patient discomfort and challenges in representing tumor heterogeneity.[2]

In contrast, liquid biopsy has emerged as a groundbreaking alternative, expanding the diagnostic toolkit to include bodily fluids like blood, urine, saliva, cerebrospinal fluid, and pleural effusions. This technique assesses cancer biomarkers such as CTCs, ctDNA, circulating miRNAs, tumor-derived EVs, and specific proteins, offering a less invasive yet comprehensive genomic profile of both primary and metastatic cancer sites.[3] Liquid biopsy's potential for continuous monitoring of tumor dynamics and detection of resistance or recurrence has garnered widespread attention.[4]

Advancements in liquid biopsy techniques, including ultrasensitive methods like digital PCR and NGS, have significantly improved the accuracy of ctDNA analysis. These tools are pivotal for early cancer detection, monitoring minute changes in tumor DNA, tracking minimal residual disease, and observing the real-time evolution of cancer.[5] The integration of AI into liquid biopsy data analysis

Abbreviations: AI, artificial intelligence; cfDNA, cell-free DNA; CGP, comprehensive genomic profiling; CSF, cerebrospinal fluid; CTC, circulating tumor cell; ctDNA, circulating tumor DNA; EGFR, epidermal growth factor receptor; EV, extracellular vesicle; miR, microRNA; miRNA, microRNA; MRD, minimal residual disease; NGS, next-generation sequencing; NSCLC, non-small-cell lung cancer; ScfDNA, salivary cell-free DNA.

promises to enhance the precision of clinical decision-making by revealing complex biomarker patterns.[6]

While liquid biopsy is frequently celebrated as an innovative approach in cancer management, often referred to as oncology's "liquid gold," it demands a thoughtful and critical evaluation. Addressing this question is essential for the clinical community and the broader scientific discourse.[7]

2 | ISOLATION TECHNIQUES FOR LIQUID BIOPSY BIOMARKERS

The extraction of biomarkers such as cfDNA and CTCs is a critical step in the liquid biopsy process, essential for delineating the intricate dynamics of cancer dynamics.[8] This section expands on the various isolation techniques used, highlighting their efficiencies, specificities, and the trade-offs inherent in each method.

2.1 | Immunomagnetic-based methods

Immunomagnetic-based methods, like the CellSearch System (Menarini Silicon Biosystems, Huntingdon Valley, PA, USA) provide high specificity by capturing CTC expressing epithelial cell adhesion molecule (EpCAM) and other targeted antigens.[9] Their strength lies in the precise selection of cells, providing a high degree of purity in the isolated samples. Nonetheless, their effectiveness is contingent on the expression of the selected antigens, potentially missing CTCs that do not express these markers or cfDNA fragments lacking them. This limitation necessitates a fine-tuned approach to balance the specificity of target antigens with a broader catchment sensitivity.[10]

2.2 | Microfluidic systems

Microfluidic systems, exemplified by ClearCell FX (Biologics Limited, Singapore) and IsoFlux (Fluxion Biosciences, Oakland, CA, USA), separate cfDNA and CTCs based on physical properties, such as size or specific interactions. These systems offer a label-free, high-throughput alternative that mitigates the need for antigen-based selection, thereby circumventing some of the limitations of immunomagnetic methods. The primary advantage of microfluidics lies in its ability to sort cells and DNA with minimal processing, albeit sometimes at the expense of specificity when compared to antigen-based methods.[11]

2.3 | Filter-based techniques

Filter-based techniques, including ScreenCell (ScreenCell, Paris, France) and ISET (Isolation by Size of Epithelial Tumor Cells, Rarecells Diagnosis, Paris, France), are designed for rapidity and ease, capturing larger DNA fragments and CTCs through size-based filtration. Although they excel in processing speed and simplicity, they might not offer the same level of specificity as other techniques and can be limited by their inability to capture smaller or nonconforming tumor cells and DNA fragments.[12]

2.4 | Advanced imaging and dielectrophoresis methods

Techniques such as dielectrophoresis array (DEPArray, Menarini Silicon Biosystem, Bologna, Italy) and the RareCyte Platform (RareCyte Inc., Seattle, WA, USA) incorporate advanced imaging and dielectrophoresis to increase the specificity and precision of isolating cfDNA and CTCs. These methods allow for the visualization and individual selection of target cells, providing an unparalleled level of detail in the analysis. However, they often involve more complex workflows and higher operational costs, which can be a barrier to routine clinical use.[13]

2.5 | Density gradient centrifugation

Density gradient centrifugation methods, exemplified by OncoQuick (Greiner Bio-One GmbH, Frickenhausen, Germany), exploit differences in buoyant density to isolate cfDNA and CTCs effectively. Density gradient centrifugation methods, exemplified by OncoQuick, exploit differences in buoyant density to isolate cfDNA and CTCs effectively. They offer a balance between yield and purity but may require more extended processing times. This method's advantage is its ability to process larger sample volumes, increasing the likelihood of capturing rare CTCs, albeit with a potential trade-off in terms of throughput efficiency.[14]

2.6 | Advanced nucleic acid analysis techniques

Liquid biopsy harnesses advanced techniques like digital droplet PCR for sensitive detection of mutations, essential for MRD monitoring, and NGS for a comprehensive genomic profile. Digital droplet PCR's precision in quantifying nucleic acids posttreatment and next-generation-targeted amplicon sequencing's rapid profiling of multiple genes in cfDNA samples are crucial for the ongoing monitoring of metastatic breast cancer.[15]

High-throughput platforms such as Illumina's MiSeq and Thermo Fisher Scientific's Ion Torrent, alongside bioinformatics tools GATK and VarScan, enhance the specificity of targeted mutation panels in both CGP and MRD applications, making them critical for precise cancer gene analysis.

The inclusion of miRNAs and EVs broadens the scope of cancer biomarkers, providing additional insights for disease characterization and therapy planning.[16] The strategic selection of these techniques is tailored to fulfill specific clinical or research needs, from early detection to treatment response evaluation.[3,8]

2.7 | Start material considerations for biomarker isolation

Biofluid choice is crucial for biomarker isolation in liquid biopsies. Blood, rich in ctDNA, CTCs, and miRNAs, is commonly used despite challenges such as high nontumor DNA background.[3] Centrifugation and magnetic separation are among the methods enhancing biomarker recovery.

Urine's noninvasiveness makes it ideal for detecting urological cancer biomarkers. However, challenges like dilution and enzymatic degradation necessitate concentration and preservation techniques for accurate analysis.[17]

Saliva is becoming important for head and neck cancer detection, with innovative microfluidics and PCR-based methods to differentiate tumor-derived biomarkers from microbial content.[18]

Cerebrospinal fluid is vital for brain cancer diagnostics, offering sensitive ctDNA and CTC detection. The low biomarker concentration requires highly sensitive methods for isolation.[19]

Pleural effusions, rich in nucleic acids and proteins, are essential for diagnosing thoracic malignancies. Distinguishing malignant from benign effusions often involves density gradient centrifugation and immunoaffinity methods.[20]

Overall, biomarker recovery optimization involves preanalytical processes like centrifugation for debris removal and the use of stabilizers. Bioinformatics and digital quantification enhance the sensitivity and specificity of detection across biofluids.

2.8 | Comparative summary

Each isolation technique possesses unique attributes that make it suitable for different scenarios in liquid biopsy applications. Immunomagnetic methods are highly specific but could miss atypical CTCs, while microfluidic and filter-based techniques offer a broader range of capture with varying degrees of specificity.[21] Advanced imaging and dielectrophoresis provide the highest precision but at increased complexity and cost.[3] Density gradient centrifugation is a good middle ground, offering a decent balance of yield and purity.[20] The choice of technique will largely depend on the specific requirements of the clinical or research objectives, factoring in the trade-offs between speed, specificity, cost, and the need for comprehensive capture of tumor-derived materials.

2.9 | Clinical relevance of isolating different biological molecules in liquid biopsy

Liquid biopsy biomarkers like ctDNA, CTCs, miRNAs, and EVs offer crucial insights into the genetic and molecular landscape of tumors, facilitating precision oncology through noninvasive means.

Circulating tumor DNA reflects the genetic profile of tumors, aiding in the detection of actionable mutations and the monitoring of tumor dynamics. It is particularly useful for guiding targeted therapies, such as EGFR inhibitors in lung cancer, and for tracking treatment response.[22] Circulating tumor cells provide a snapshot of the tumor cells themselves, offering data on tumor heterogeneity and potential metastatic activity.[23] Their analysis can assist in establishing a prognosis and in the evaluation of therapeutic effectiveness. MicroRNAs, as regulators of gene expression, act as biomarkers for the diagnosis and progression of cancer, as well as for predicting responses to treatment. For example, elevated levels of miR-21 have been frequently associated with negative outcomes in various cancers.[24] Extracellular vesicles carry nucleic acids and proteins from their cells of origin, thus reflecting the state of the tumor microenvironment. They are promising for early cancer detection and for understanding resistance to therapies such as immune checkpoint inhibitors.[25]

Combining the analysis of these biomarkers allows for a multifaceted approach to cancer management. It enables initial mutation screening, assessment of tumor heterogeneity, prognosis, and monitoring of drug resistance, providing a dynamic and comprehensive molecular picture for individualized therapy.

As we delve into the specificities of liquid biopsy biomarkers and their isolation techniques, Figure 1 offers a visual encapsulation of the journey of biofluids from collection to their pivotal roles in clinical applications. This diagram serves as a concise guide through the nuanced landscape of liquid biopsy, underscoring the integration of ctDNA, CTCs, EVs, and miRNAs into a cohesive framework for advancing personalized oncology.

3 | BENEFITS OF LIQUID BIOPSY ACROSS FLUIDS

Liquid biopsy offers numerous benefits across various bodily fluids, revolutionizing oncological diagnostics and cancer management.[26] This section highlights these advantages, emphasizing the transformative role of liquid biopsy in cancer diagnosis and patient care.

One of the primary advantages of liquid biopsy is its noninvasive nature. Unlike traditional tumor biopsies that involve surgical procedures, liquid biopsy only requires a blood sample, eliminating the need for surgery, anesthesia, and associated risks and recovery time.[27,28] This noninvasiveness enables frequent and timely monitoring, providing continuous insights into tumor dynamics for early detection of disease progression and therapy response.[29]

Liquid biopsy extends its utility beyond blood to include various bodily fluids such as urine, saliva, and CSF,[30] each offering unique diagnostic insights into different types of cancers. For example, urinary biomarkers show promise in urological cancers like bladder and prostate cancer, serving as noninvasive indicators of tumor burden and progression.[31]

The scope of liquid biopsy also captures the evolving genomic landscape of tumors. It allows for continuous sampling, allowing clinicians to obtain a comprehensive view of cancer cell genetics across different fluidic environments. This ongoing monitoring is crucial for assessing treatment effectiveness, identifying resistance, and making prompt treatment adjustments.[32]

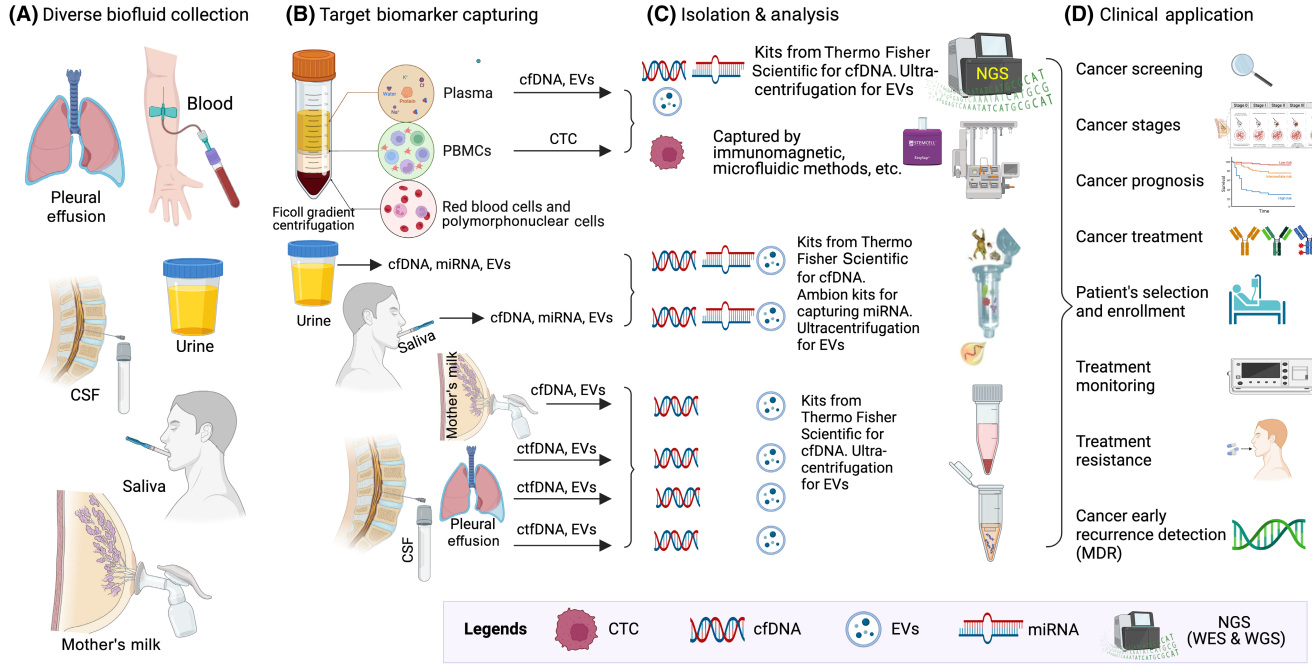

FIGURE 1 Comprehensive workflow of liquid biopsy from biofluid collection to clinical application. (A) Diverse biofluid collection. The liquid biopsy workflow begins with the collection of various biofluids capable of harboring tumor-derived components. This panel illustrates the procurement of primary liquid biopsy sources, including blood, cerebrospinal fluid (CSF), urine, saliva, and breast milk. (B) Target biomarker capturing. After collection, specific biomarkers such as circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), extracellular vesicles (EVs), and microRNAs (miRNAs) are targeted and captured from the biofluids. Techniques used include Ficoll gradient centrifugation for plasma and PBMCs, alongside filtration methods for cfDNA and miRNAs. (C) Isolation and analysis. The isolated biomarkers are analyzed using a CTC capture platform or kits provided by companies such as Thermo Fisher Scientific and Qiagen. Further analysis may involve next-generation sequencing (NGS). Techniques cover immunomagnetic and microfluidic methods for CTCs and ultracentrifugation for EVs. This crucial step prepares biomarkers for detailed molecular analysis. (D) Clinical application. The final panel links the isolated and analyzed biomarkers to their respective clinical applications. It underscores the contribution of ctDNA, CTCs, miRNAs, and EVs to diverse aspects of patient care, including cancer screening, staging, prognostication, treatment selection, therapeutic response monitoring, and early detection of treatment resistance or cancer recurrence. This integrative approach aims to personalize patient care by providing real-time molecular insights into cancer. MRD, minimal residual disease; WES, whole exome sequencing; WGS, whole genome sequencing.

Addressing the challenge of tumor heterogeneity, liquid biopsy provides a holistic view of cancer's genetic diversity. Unlike traditional biopsies that might capture only a limited area of the tumor, liquid biopsy can detect a broader spectrum of genetic material shed into various body fluids. This CGP is essential for precision medicine, enabling the customization of treatment plans targeting both predominant and less abundant but clinically relevant cancer cell populations.[33]

MicroRNAs and EVs enhance the utility of liquid biopsy by offering additional layers of molecular information.[34] MicroRNAs, stable across various body fluids, can regulate gene expression and reflect the biological state of the tumor. Extracellular vesicles, including exosomes, carry nucleic acids and proteins from their cells of origin, providing a rich source of tumor-derived biomarkers.[35] The analysis of miRNAs and EVs complements other liquid biopsy components, especially when cellular components like CTCs and ctDNA are scarce or challenging to isolate. For example, miRNAs in saliva have been investigated for their potential in oral cancer detection, while EVs in CSF have shown promise in brain tumor diagnostics.[36]

Furthermore, urinary EVs are gaining attention for their role in noninvasive cancer diagnostics.[37] They can encapsulate tumor-specific proteins and genetic material, offering a wealth of information about the tumor's state and environment. Studies have reported the potential of urinary EVs in providing early diagnostic cues and insights into the efficacy of therapeutic interventions in bladder and prostate cancers, among others.[38]

Incorporating these fluidic mediums and their respective biomarkers, liquid biopsy enables a holistic approach to cancer management. This multifaceted strategy enhances diagnostic precision and therapeutic efficacy, ultimately leading to improved patient outcomes and personalized care in oncology.

4 | CHALLENGES AND LIMITATIONS IN DIVERSE BIOPSY FLUIDS

Liquid biopsies offer transformative potential for cancer diagnostics and personalized treatments, but they come with significant

challenges and limitations across various biofluids that require careful consideration.[39] These challenges range from technical issues to broader concerns about the clinical implementation of liquid biopsy techniques.[39,40]

A critical challenge is striking the right balance between sensitivity and specificity. High sensitivity is essential to detect all instances of a disease or mutation, while high specificity ensures that those without the condition are not falsely identified.[41] Achieving this balance is complex due to the low abundance of ctDNA and the presence of other genetic materials,[42] which can lead to false negatives or false positives that could impact treatment decisions.[39,43] Rigorous quality control is crucial to minimize such diagnostic errors.

Tumor heterogeneity also poses a significant challenge.[44] A tumor's diverse genetic profile can be difficult to fully capture through liquid biopsy, potentially leading to incomplete genetic portraits that could overlook critical therapeutic targets or misrepresent the cancer's progression.[45,46]

From a technical perspective, the low concentration of ctDNA and CTCs in various biofluids,[47] such as blood, urine, saliva, CSF, and pleural effusions, complicates their isolation and analysis. This is further exacerbated by the technical intricacies of differentiating these from the background of normal DNA and other cellular components.[32,48]

The isolation and standardization of miRNAs and EVs pose additional specific challenges.[34] These molecules and vesicles offer valuable insights into the tumor's genetic and molecular landscape but require sensitive and standardized methods for detection and quantification. The small size and variability of miRNAs and the complex biogenesis of EVs demand precise analytical techniques and reference standards, which are still under development.[49] Addressing these challenges is crucial for ensuring the reliable use of miRNAs and EVs as biomarkers in liquid biopsy.[50]

Other challenges include the risk of contamination with nontumor DNA, the dynamic nature of tumors that require timely capture for accurate diagnosis, and the economic and accessibility issues associated with cost and availability.[39] Regulatory, ethical, and privacy concerns also play a significant role in the adoption and success of liquid biopsies.[51] The integrity and storage of samples, as well as the interpretation of complex genetic data, are other areas that need to be carefully managed.[52]

As the field progresses, it is imperative to address these challenges with diligence and innovation to harness the full potential of liquid biopsy technology across all relevant biofluids.[53] As we navigate the complexities of liquid biopsy, Figure 2 provides a visual synopsis of the multifaceted challenges and limitations encountered across different biofluids. It succinctly illustrates the technical and clinical hurdles that must be overcome to fully realize the potential of liquid biopsy in personalized cancer care.

5 | DEBUNKING LIQUID BIOPSY MYTHS IN A BROADER CONTEXT

The advent of liquid biopsies has been marked by both optimism and skepticism, necessitating a clear understanding of what these innovative diagnostics can and cannot do. Myths surrounding liquid biopsies could give rise to unrealistic expectations or unwarranted dismissals of their potential.

One common myth is the alleged inability of liquid biopsies to detect a comprehensive set of genetic markers compared to traditional

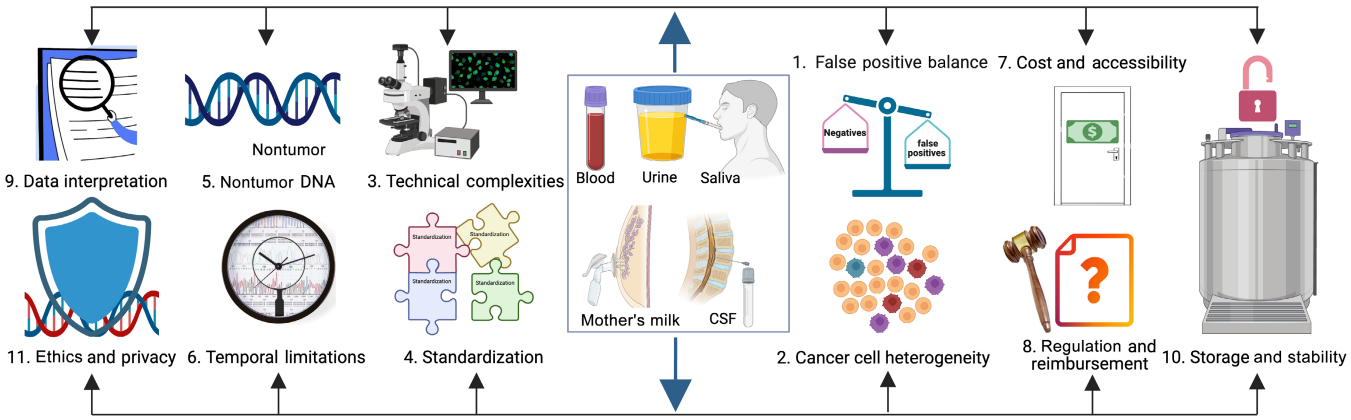

FIGURE 2 Multifaceted challenges in liquid biopsy implementation. This figure outlines key challenges encountered in liquid biopsy from sample procurement to clinical utility. (1) Diagnostic accuracy. Balancing the detection of genuine cancer markers while reducing false positives. (2) Tumor diversity. Capturing the genetic heterogeneity of tumors to form a comprehensive molecular profile. (3) Technical hurdles. Overcoming the complexities in isolating and analyzing delicate biomarkers from biofluids. (4) Standardizing protocols. Establishing uniform procedures for liquid biopsy to guarantee reproducibility and reliability. (5) Contamination risk. Reducing the contamination of analyses by nontumor DNA. (6) Timing of tests. Optimizing sample collection timing for an accurate reflection of the tumor's state. (7) Economics and access. Making liquid biopsy cost-effective and accessible for widespread use. (8) Regulatory obstacles. Overcoming regulatory and insurance challenges in liquid biopsy implementation. (9) Data analysis. Using advanced analytics to interpret complex genetic data accurately. (10) Sample integrity. Preserving biofluid sample stability and integrity for reliable results. (11) Ethics and privacy. Safeguarding patient data and addressing the ethical aspects in genetic testing.

tumor biopsies. However, liquid biopsies have proven their efficacy in capturing a broad genetic profile through the continuous shedding of DNA by tumors into various biofluids, not just blood fields.[27,54] They provide a dynamic view of tumor evolution, offering insights that might not be accessible through a one-time traditional biopsy. Furthermore, it is a misconception that miRNAs and EVs are inferior biomarkers compared to direct tumor sampling. Contrary to this belief, numerous studies have established the robust diagnostic and prognostic capabilities of miRNAs and EVs.[55] These molecules and vesicles can reflect the tumor's genetic and molecular landscape, providing valuable information for cancer prognosis, monitoring therapeutic responses, and detecting disease recurrence.[56] Their role in liquid biopsy complements ctDNA and CTC analysis, enhancing the diagnostic spectrum and offering a noninvasive avenue for comprehensive tumor evaluation.[29,57]

While the value of traditional biopsies is unequivocal, the noninvasive nature of liquid biopsies allows for a complementary approach. They facilitate ongoing monitoring of genetic changes, enabling clinicians to adapt treatment strategies in real time. It is essential to recognize that liquid biopsies are not meant to supplant traditional methods but rather to supplement them, enriching the overall understanding of a patient's cancer journey.[39,58]

However, it is a misconception to regard liquid biopsies as a panacea for all types of cancer diagnostics. Their efficacy can vary depending on the type of cancer and its biology. For instance, tumors that shed limited DNA into biofluids or are in areas with low shedding rates might not be ideal candidates for liquid biopsy detection. The utility of ctDNA in assessing minimal residual disease is still under investigation, with ongoing research needed to validate its clinical impact.[59] Tumor stage and location are also crucial factors, as early-stage cancers might not release detectable levels of tumor-specific genetic markers into the bloodstream or other biofluids.[60]

Hence, clinicians and patients must approach liquid biopsies with a nuanced understanding. Although they offer substantial benefits in many cases, their application should be carefully considered within the context of each unique cancer diagnosis, avoiding a one-size-fits-all mentality. Acknowledging the strengths and addressing the limitations of liquid biopsies will ensure their optimal use in precision oncology.

6 | FUTURE DIRECTION IN MULTIFLUID BIOPSIES

The horizon of liquid biopsy is expanding, with innovative technological breakthroughs, redefining the realms of oncology and diagnostic medicine. The integration of NGS enhances genetic precision, enabling the detection of subtle genetic variations that eluded earlier methods.[61] Concurrently, advances in machine learning and AI promise to refine mutation and resistance pattern analyses.[6] Microfluidic advancements are anticipated to expedite sample processing, collectively broadening the scope of liquid biopsies beyond oncology to early cancer detection and personalized treatment strategies.

Emerging multicancer detection tests mark a revolution in early screening, allowing multiple cancer types to be identified from a single blood sample.[62] These tests, based on sophisticated biomarker analysis, pave the way for routine, noninvasive cancer diagnosis akin to standard blood tests. Liquid biopsy's utility is also extending into nononcological fields, with potential markers for neurodegenerative and cardiovascular diseases under investigation. This shift from concept to clinical application heralds a new era in health-care, with detection and monitoring becoming integral to patient management strategies.

The potential of miRNAs and EVs within the realm of liquid biopsies is particularly promising.[63] These biomarkers offer a wealth of information about the tumor's genetic and molecular landscape and could play a crucial role in the future of personalized medicine. Technological advancements are expected to overcome current limitations in their isolation and analysis, allowing for their integration into routine clinical practice. With their capacity for indicating disease presence, progression, and response to therapy, miRNAs and EVs are poised to be at the forefront of the next wave of personalized medical diagnostics.[41]

In infectious diseases, liquid biopsy techniques could hasten pathogen detection and genomic tracing, leading to quicker therapeutic responses.[64] The potential for transformative discoveries in rheumatology, cardiology, psychiatry, and other disciplines is on the horizon as liquid biopsy uncovers molecular nuances indicative of disease emergence or progression.

The ongoing refinement of liquid biopsy methods points to a future where diagnostics are revolutionized across various medical fields,[65] upholding the highest standards of precision and innovation.

The transformation of liquid biopsy from an innovative concept to a pivotal diagnostic tool exemplifies the rapid progress in medical science. Its ascent to a recognized position in clinical practice is a tribute to relentless research and innovation, offering clinicians a noninvasive window into the molecular landscape of cancer.

Liquid biopsy has indeed initiated a revolution in diagnostics, altering the approach to early cancer detection and influencing therapeutic strategies. As we advance, it is crucial to balance optimism with critical evaluation. While the progress has been significant, continuous refinement and validation remain essential. It is through this sustained commitment to research and development that liquid biopsy will realize its full potential, enhancing patient care and ushering in an era of tailored and proactive medical interventions.

7 | CLINICAL APPLICATIONS AND CASE STUDIES IN DIVERSE FLUIDS

Liquid biopsy represents a paradigm shift in oncology, serving as a noninvasive adjunct to traditional tissue biopsies and transforming patient care.[32] This innovative approach is particularly vital when conventional biopsies are either infeasible or pose undue risk to patients.[66] The sections herein synthesize key clinical applications and research findings, reflecting the significant impact and

integration of liquid biopsies across various medical domains.[27,54] They underscore the broad spectrum of case studies and clinical trials that emphasize the profound influence of liquid biopsies, highlighting their role in offering real-time insights into disease progression and therapeutic response, and ultimately enhancing patient outcomes.[67]

7.1 | Blood-based liquid biopsies

Blood serves as a cornerstone for liquid biopsy analysis due to its ready accessibility and the diverse range of biomarkers it contains, such as ctDNA, CTCs, and miRNAs. These biomarkers are invaluable in various clinical scenarios, including NSCLC, where the detection of ctDNA is crucial for real-time insights into tumor mutations, directly impacting the selection and administration of targeted therapies. The clinical utility of blood-based liquid biopsies extends to conditions where traditional tissue biopsies are infeasible, as reported by studies from the West Japan Oncology Group, which highlight their importance in guiding therapy for NSCLC.[68] Additionally, blood-based liquid biopsy plays a pivotal role in metastatic breast cancer by monitoring disease progression and assisting in therapeutic decision-making, as shown in the Translational Breast Cancer Research Consortium (TBCRC) 005 trial.[69] Furthermore, the realm of prenatal diagnostics has been transformed by noninvasive prenatal sequencing (NIPS), which leverages ctDNA for the early detection of genetic disorders, demonstrating the wide-ranging applicability of blood-based biomarkers in both oncological and non-oncological contexts.[70]

7.2 | Urinary liquid biopsies in urologic oncology

Urinary liquid biopsies harness urine's unique molecular composition to noninvasively detect and monitor urological cancers. Cell-free DNA, miRNAs, proteins, and peptides in urine provide insights into tumor dynamics, aiding in the management of prostate and bladder cancers. Chen et al. underscore the clinical value of these biomarkers for tracking disease progression and evaluating therapeutic responses, with implications for understanding tumor burden and resistance.[71]

Innovative techniques like attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy are improving biomarker detection in urine, offering less invasive monitoring of cancers such as bladder cancer with high sensitivity and specificity.[72] These advances, along with necessary precautions like addressing the dilution effect and ensuring sample preservation, are enhancing the clinical application of urinary liquid biopsies.

The comprehensive evaluation of urinary biomarkers illustrates their increasing role in precision medicine, providing a window into the disease state and influencing treatment strategies in urologic oncology.

7.3 | Salivary liquid biopsies

Salivary liquid biopsies are carving out a significant niche in the liquid biopsy landscape, offering a convenient and noninvasive means to detect and monitor various cancers. The utilization of ScfDNA as a diagnostic biomarker is gaining traction, particularly in the detection of gastric cancer. Swarup et al. have contributed significantly to this field with their application of broad-range cell-free DNA sequencing (BRcfDNA-Seq) for ScfDNA analysis. This technique leverages the fragmentomics and end-motif profiles unique to ScfDNA to differentiate between cancerous and non-cancerous states, providing a potential tool for early, noninvasive detection of gastric cancer.[73]

This pioneering work indicates that pathophysiological changes in cancer could alter the molecular profile of ScfDNA, influencing factors such as nuclear cleavage and the composition of microbial cfDNA. The team's comprehensive analysis, which examines fragmentomic patterns, genomic element distribution, end-motif sequences, and unique oral microbiome signatures, has successfully differentiated gastric cancer patients from controls. This highlights the potential of salivary liquid biopsy in clinical settings for early cancer detection and the real-time monitoring of patients.[73]

The diagnostic potential of saliva extends beyond gastric cancer. Research has indicated that salivary biomarkers can be used to detect noncoding RNAs associated with oral cancer, providing a rapid, localized diagnostic tool for this malignancy.[74] Saliva, rich in molecular information, presents a unique opportunity for the development of targeted biosensors. For instance, the use of molecularly imprinted polymers for the detection of transforming growth factor-beta-1 (TGF-β1) is an example of how advances in biosensor technology are tapping into salivary diagnostics, paving the way for noninvasive, point-of-care cancer screening and monitoring.[75]

7.4 | Cerebrospinal fluid in liquid biopsy for brain cancer

Cerebrospinal fluid is increasingly recognized as a valuable source for liquid biopsies in the context of central nervous system malignancies. Its proximity to brain and spinal cord tumors allows for the detection of tumor-derived DNA, often with greater sensitivity than blood-based biopsies, especially for tumors shielded by the blood–brain barrier.

Recent advancements in molecular diagnostics have enabled the detection of tumor-specific mutations in CSF, thereby offering a powerful tool for the noninvasive diagnosis and monitoring of brain tumors. For example, studies have reported the successful identification of key mutations in CSF from glioma patients, providing actionable insights into tumor genomics. These findings are particularly crucial for patients with tumors in locations inaccessible to surgical biopsy or those who cannot undergo such procedures due to medical comorbidities.[76]

However, the application of CSF-based liquid biopsies in clinical practice is at a nascent stage, with several challenges that need addressing. The invasiveness of CSF collection, typically through lumbar puncture, poses risks and could limit the frequency of sampling. Moreover, the variability in the concentration of tumor DNA in CSF necessitates highly sensitive detection techniques, and the interpretation of results can be complex.

Current research is focused on refining CSF-based liquid biopsy methods, with studies exploring the use of NGS and other high-sensitivity platforms to enhance detection capabilities. Although the potential of CSF liquid biopsies is evident, further validation through larger clinical trials is essential to establish standardized protocols and determine the clinical significance of various biomarkers found in CSF.

7.5 | Breast milk as a liquid biopsy medium in postpartum breast cancer

Breast milk has recently been recognized as a valuable medium for liquid biopsy, especially pertinent to postpartum breast cancer screening. The close anatomical relationship between breast milk and mammary tissues, which are frequently the origin of breast cancers, underscores its potential for early detection through the analysis of ctDNA.[77,78]

Studies have established that ctDNA, reflective of the tumor's genetic profile, is present in breast milk and can serve as a biomarker for breast cancer. This is particularly advantageous in the postpartum period when traditional imaging methods like mammography are less effective due to increased breast density. The detection of ctDNA in breast milk could offer a noninvasive, patient-friendly alternative for early diagnosis, leveraging the physiological changes during lactation to facilitate cancer detection.[77,78]

Further research into the constituents of breast milk could expand its utility as a liquid biopsy medium. For instance, exploring the presence and significance of other biomarkers, such as miRNAs and EVs, could provide a broader understanding of the molecular changes associated with postpartum breast cancer. This could lead to the development of comprehensive panels that not only detect cancer presence but also monitor tumor dynamics and response to treatment during the postpartum period.[77,78]

7.6 | Advancements in pleural effusion analysis

The diagnostic landscape of oncology is being reshaped by the advancements in pleural effusion analysis through liquid biopsy techniques. The ability to differentiate between malignant and benign pleural effusions is paramount in cancer diagnostics and patient management, especially in the context of lung diseases.[79,80]

Pleural effusions can be a direct consequence of malignancy or other diseases such as tuberculosis. Zhang et al.'s research points to exosomal miRNAs in pleural effusions as reliable biomarkers for distinguishing lung adenocarcinoma from other conditions, offering a noninvasive diagnostic approach that can significantly impact clinical decisions.[79]

Furthermore, studies like those conducted by Vukovic et al. have shown that pleural effusions can be a more sensitive medium than blood plasma for detecting mutations like EGFR T790M, known for its association with treatment resistance in NSCLC. This enhanced sensitivity could lead to better treatment strategies and monitoring of resistance, particularly crucial in settings where advanced diagnostic facilities could be limited.[80]

The utility of pleural effusions in liquid biopsy extends beyond mutation detection. The molecular analysis of pleural fluid can reveal a comprehensive profile of the tumor environment, offering insights into tumor biology, prognosis, and response to therapy. With the integration of NGS and other sophisticated platforms, pleural effusion analysis is becoming an increasingly valuable tool for oncologists.

Table 1 underscores this point by listing current clinical trials that assess the efficacy of pleural effusion analysis in various cancer types. These trials are pivotal in validating pleural effusions as a routine part of cancer diagnostics and could pave the way for new standards in oncological care.

The research detailed in Table 1 reflects a growing trend toward the use of less invasive, more dynamic diagnostic methods. From the profiling of miRNAs in pleural effusions to uncovering biomarkers in other biofluids like ascites and urine, the scope of liquid biopsy is rapidly expanding. These advancements not only enhance the precision of cancer detection and monitoring but also play a crucial role in customizing treatment plans to improve patient outcomes.

Clinical trials serve as a foundational pillar for the verification of liquid biopsy's effectiveness across a spectrum of cancer conditions and stages. Each trial mentioned in Table 1 adds to the robust evidence base advocating for the integration of liquid biopsy into everyday clinical practice and contributes to the evolution of personalized oncology care.

In the context of clinical management, miRNAs and EVs are proving to be invaluable tools. Specific miRNAs found in biofluids are now associated with disease prognosis and therapeutic outcomes in malignancies such as lung and breast cancers. Extracellular vesicles offer a window into the molecular intricacies of tumors, especially where CTCs may be elusive. The comprehensive nature and profound implications of liquid biopsy applications showcased in these clinical trials are forging the path for precision medicine. This advancement empowers healthcare providers with the necessary capabilities for the early detection of treatment resistance, fine-tuning therapeutic approaches, and meticulous tracking of disease evolution.

Through these detailed case studies, liquid biopsy reveals its transformative power, paving the way for concluding insights on its potential to redefine personalized medicine and proactive healthcare strategies.

Liquid biopsy marks a significant milestone in medical diagnostics, reflecting the culmination of extensive research and innovation. It provides a less invasive option for probing cancer's molecular landscape and has become an essential part of modern clinical practice.

TABLE 1 Clinical trials exploring the diagnostic and therapeutic utility of liquid biopsies in oncology (http://www.clinicaltrials.gov)

NCT number	Study title	Study type	Conditions	Interventions	Phase	Enrollment	Start date	Completion date
NCT05885009	Feasibility and impact of liquid biopsy genomic profiling on treatment patients with metastatic prostate cancer in Spain	Observational	Metastatic prostate cancer	Genetic: Liquid biopsy; Genetic: Archival tumor DNA sequencing	–	240	3/28/2023	3/28/2028
NCT02485691	Cabazitaxel versus the switch to an alternative AR-targeted Agent (enzalutamide or abiraterone) in metastatic castration-resistant prostate cancer (mCRPC) patients previously treated with docetaxel and who rapidly failed a prior AR-targeted Agent	Interventional	Prostate cancer metastatic	Drug: Cabazitaxel XRP6258; Drug: Enzalutamide; Drug: Abiraterone acetate; Drug: Prednisone	IV	255	11/09/2015	03/15/2021
NCT05475366	Personalized first-line chemotherapy choice in advanced pancreatic adenocarcinoma using transcriptomic signatures	Interventional	Carcinoma, pancreatic ductal, prognosis	Other: Clinical value of five transcriptomic signatures to personalize the therapeutic decision for L1 in PDAC; Other: Biomarkers of tumor signatures	NA	62	12/12/2022	03/30/2025
NCT04566614	Preventing viral pandemic associated risk of cancer death using less invasive diagnostic tests – liquid biopsies	Observational	Neoplasm, colorectal, neoplasm lung, neoplasm, bladder, neoplasms pancreatic, biliary tract neoplasms, gastrointestinal stromal tumor	Other: ctDNA blood sampling	–	294	06/18/2020	12/31/2025
NCT03142516	FOLFIRI + panitumumab first-line treatment in elderly patients with unresectable metastatic colorectal cancer, RAS/BRAF wild-type and good performance status	Interventional	Colorectal neoplasms, colorectal carcinoma, colorectal cancer metastatic, neoplasm metastasis	Drug: Panitumumab; Drug: Irinotecan; Drug: Folinic acid; Drug: 5-FU	II	20	10/31/2017	01/21/2021
NCT04776655	Study in mCRC patients RAS/BRAF with tissue and RAS mutated liquid biopsy to compare FOLFIRI plus cetuximab or bevacizumab	Interventional	Colorectal cancer, metastatic colorectal cancer, RAS mutation	Drug: Bevacizumab; Drug: Cetuximab; Drug: 5-FU; Drug: Irinotecan; Drug: Calcium levofolinate	III	280	04/30/2021	04/29/2024
NCT033832158	International PPB/DICER1 Registry	Observational	Pleuropulmonary blastoma, Sertoli-Leydig cell tumor, DICER1 syndrome, cystic nephroma, Wilms tumor, pineoblastoma, renal sarcoma, nodular hyperplasia of thyroid, nasal chondromesenchymal	–	–	3400	12/06/2016	12/06/2028

TABLE 1 (Continued)

NCT number	Study title	Study type	Conditions	Interventions	Phase	Enrollment	Start date	Completion date
NCT02530658	Next-generation sequencing of normal tissues prospectively in pediatric oncology patients	Observational	Solid, Liquid, Central Nervous System Tumors	Other: Study introduction visit; Other: Informed consent visit; Other: Informed consent follow-up visit; Other: Return of results conversation; Other: Return of results follow-up visits; Procedure: Blood sample; Procedure: Skin biopsy	–	5000	08/28/2015	07/01/2025
NCT02934529	Metastatic colorectal cancer (RAS-wild type) after response to first-line treatment with FOLFIRI plus cetuximab	Interventional	Metastatic colorectal cancer	Drug: Irinotecan; Drug: Folinic acid; Drug: 5-FU; Drug: Cetuximab; Drug: Bevacizumab; Drug: Capecitabine; Drug: Regorafenib; Drug: Irinotecan 125 mg; Drug: Cetuximab weekly	III	550	03/2015	12/2024
NCT04526587	Biomarkers and clinical features of metastatic breast cancer in patients treated with CDK4/6 inhibitors	Observational	Anatomic stage IV breast cancer AJCC version 8, metastatic breast carcinoma, prognostic stage IV breast cancer AJCC version 8	Other: Cytology specimen collection procedure; Other: Diagnostic laboratory biomarker analysis; Other, medical chart review	–	400	07/03/2020	07/03/2025
NCT05790460	Telehealth based synchronous navigation to improve molecularly informed care for patients with lung cancer	Interventional	Non-small-cell lung cancer	Other: Telehealth	NA	138	05/08/2023	09/20/2025
NCT04484636	PLATON—Platform for analyzing targetable tumor mutations (pilot study)	Interventional	Hepatocellular cancer, cholangiocarcinoma, gallbladder cancer, pancreatic cancer, esophageal cancer, stomach cancer	Diagnostic test: FoundationOne CDx and FoundationOne Liquid	NA	400	10/28/2020	06/30/2024

Abbreviations: 5-FU, 5-fluorouracil; AJCC, American Joint Committee on Cancer; AR, androgen receptor; CDK, cyclin-dependent kinase; ctDNA, circulating tumor DNA; FOLFIRI, folinic acid, fluorouracil, and irinotecan; mCRPC, metastatic castration-resistant prostate cancer; mCRC, metastatic colorectal cancer; NA, not applicable; PDAC, pancreatic ductal adenocarcinoma; RAS, rat sarcoma.

This technique is transforming oncology, enabling early cancer detection, real-time disease monitoring, and the personalization of treatment. The incorporation of miRNAs and EVs into liquid biopsies has especially enhanced our insight into the tumor environment and patient-specific therapeutic needs.

As the field progresses, careful evaluation of liquid biopsy's capabilities and ongoing research are imperative to maximize its potential. The strategic use of miRNAs and EVs, combined with technological advances, is poised to shift clinical practices significantly, offering prospects for improved patient care. Sustained innovation will ensure that liquid biopsy remains at the forefront of precision medicine and proactive healthcare.

AUTHOR CONTRIBUTIONS

Ying Bao: Conceptualization; data curation; methodology; project administration; writing – original draft; writing – review and editing. **Dejing Zhang:** Conceptualization; data curation; methodology; resources; visualization; writing – original draft. **Huihui Guo:** Data curation; methodology; resources; writing – original draft. **Wenxue Ma:** Conceptualization; data curation; formal analysis; methodology; project administration; resources; software; supervision; validation; writing – original draft; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

ETHICS STATEMENT

Approval of the research protocol by an institutional review board: N/A.

Informed consent: N/A.

Registry and the registration no. of the study/trial: N/A.

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