



Review Article

Liquid Biopsy: A Breakthrough Technology in Early Cancer Screening



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Abstract

Cancer is the leading cause of death globally, with nearly 20 million new cases and 9.7 million deaths in 2022. Due to its vague initial symptoms, cancer is often difficult to detect in its early stages. Liquid biopsy, a revolutionary approach in oncology, provides a minimally invasive, real-time method for cancer detection, monitoring, and characterization by examining circulating tumor components in body fluids. This review presents current technologies and clinical applications of liquid biopsy, focusing particularly on its value for early cancer diagnosis. Liquid biopsy enables molecular profiling of cancer for precision oncology by isolating circulating extracellular nucleic acids (cell-free DNA), circulating tumor DNA, and circulating tumor cells from blood and other body fluids. Cell-free DNA, which circulates freely in the blood, may or may not be tumor-derived, while circulating tumor DNA is specifically of tumor origin. Additionally, circulating tumor cells can be isolated from blood; these cells, shed from tumors into the bloodstream, typically survive only 1–2.5 h before immune clearance, though a small fraction can persist and metastasize to distant sites. Exosomes, small membrane-bound vesicles secreted by tumor cells, also carry molecular information about the tumor and have become a valuable source of biomarkers in liquid biopsy. Advances in detection technologies for these analytes have expanded the utility of liquid biopsy, facilitating the identification of somatic mutations and actionable genomic alterations in tumors. Finally, this review discusses the opportunities and challenges facing liquid biopsy and offers insights into its future development.

Introduction

Cancer is a leading cause of death worldwide. According to the latest malignancy statistics report published by the International Agency for Research on Cancer, a subsidiary of the World Health Organization, in February 2024, there were approximately 19.976 million new cancer cases and 9.7 million cancer deaths globally in 2022. The age-standardized incidence and mortality rates were 196.9 and 91.7 per 100,000, respectively.¹ Global cancer incidence is projected to rise from 20.0 million cases in 2022 to 32.6 million cases in 2045, indicating a significant increase in the global cancer burden. According to the latest cancer incidence and mortality estimates for the United States in 2024, published in *CA: A Cancer Journal for Clinicians* in April 2024, approximately 2,001,140 new cancer cases and 611,720 cancer deaths are expected to oc-

cur.² Given the high incidence and mortality rates, cancer imposes a substantial public health burden, ranking as the first or second leading cause of death in most countries. Early detection plays a crucial role in reducing cancer-related morbidity and mortality. However, due to the subtle early symptoms of malignant tumors and limited public awareness of early screening, many cancers are diagnosed at advanced stages. This delayed diagnosis often results in missed opportunities for timely intervention, leading to poorer prognoses. Effective early screening strategies enable the identification of suspicious lesions and precancerous conditions, significantly improving patient outcomes.³ For instance, lung cancer screening with low-dose computed tomography has been shown to reduce lung cancer mortality by 20% compared to conventional chest X-rays.^{4–6} Despite the benefits, current screening modalities vary in sensitivity, specificity, accessibility, and cost-effectiveness, underscoring the need for innovative approaches to enhance early cancer detection.⁷

Liquid biopsy is a transformative, non-invasive diagnostic approach that examines circulating tumor components in bodily fluids. Initially introduced for detecting circulating tumor cells (CTCs), the concept has expanded to include circulating tumor DNA (ctDNA) and other tumor-associated biomarkers.⁸ Compared to traditional tissue biopsy, which is invasive and may fail

Keywords: Liquid biopsy; Cancer diagnosis; Circulating tumor cells; Cell-free DNA; Precision oncology; Minimally invasive techniques; Early cancer detection.

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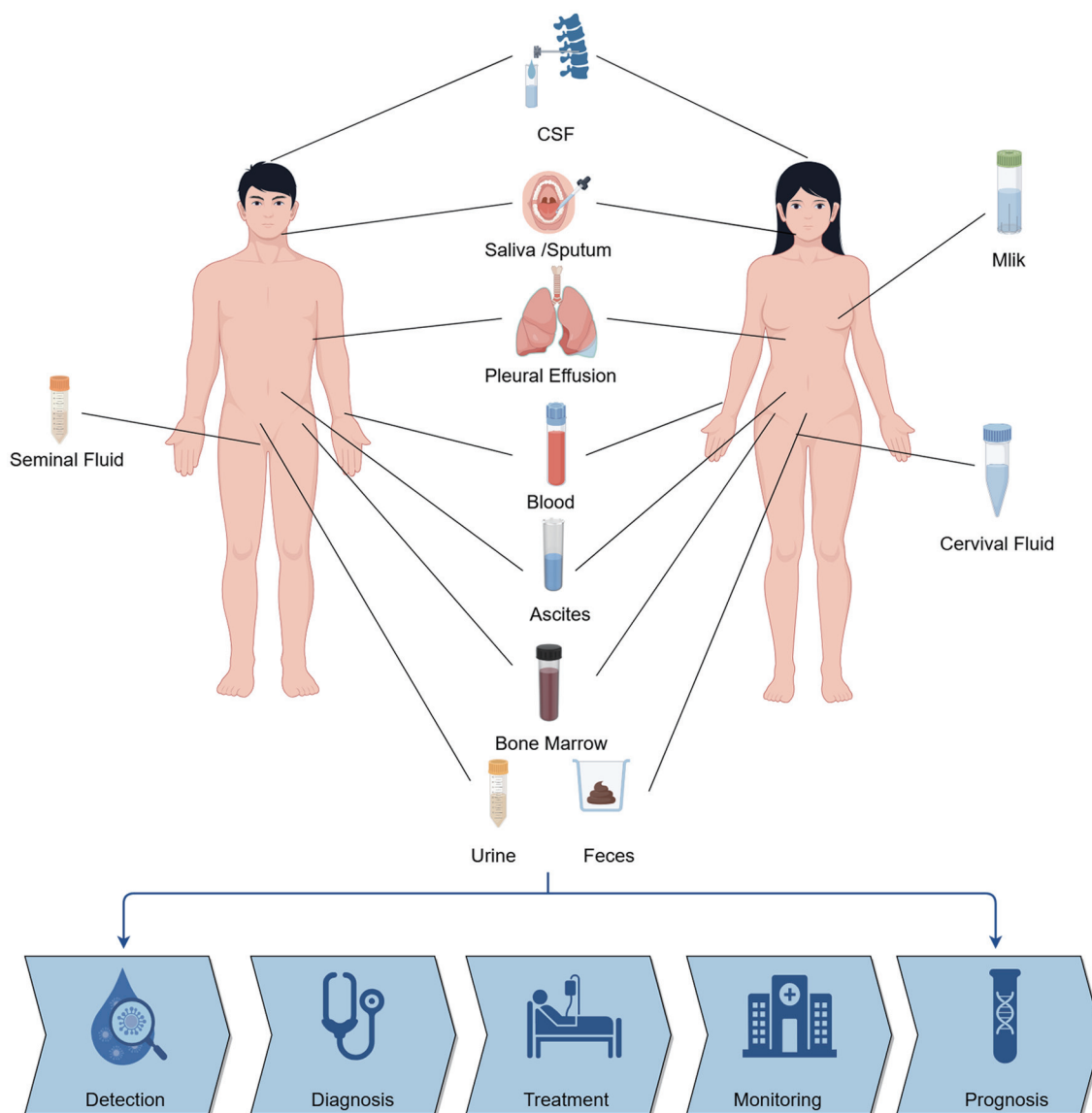


Fig. 1. Sources of human biofluids and their clinical applications: The diagram illustrates the collection sites and types of various biofluids (e.g., blood, urine, saliva, pleural effusion, cerebrospinal fluid, etc.) from different parts of the human body. These samples provide critical insights for precision medicine and personalized therapy. By Figdraw. CSF, cerebrospinal fluid.

to capture tumor heterogeneity, liquid biopsy offers a safer, repeatable, and convenient alternative. It enables dynamic monitoring of tumor evolution, assessment of treatment response, and early identification of resistance mechanisms. Biomarkers detected in biological fluids such as blood, urine, saliva, and cerebrospinal fluid provide valuable insights into tumor biology, addressing some limitations of conventional diagnostic approaches (Fig. 1).^{9,10}

This review systematically examines the progress of liquid biopsy in early cancer diagnosis, highlighting its applications, advantages, and limitations. We further discuss the key challenges that must be addressed for clinical translation and explore future directions to optimize its diagnostic potential. By integrating recent advancements and emerging trends, we aimed to provide a comprehensive perspective on the role of liquid biopsy in transforming early cancer screening and improving patient outcomes.

Liquid biopsy components and analytical techniques

Components of liquid biopsy

Circulating cell-free DNA (cfDNA) and ctDNA

Circulating cfDNA refers to short DNA fragments present in bodily fluids, such as blood, primarily released from cells through biological processes such as apoptosis, necrosis, or active secretion.^{11,12} These fragments exist freely in plasma or serum, unencapsulated within cells or membranous vesicles. In healthy individuals, cfDNA is present at very low concentrations in the bloodstream (<10 ng/mL), with a modal fragment size of approximately 167 bp.¹³ In contrast, in cancer patients, the concentration of cfDNA is highly variable and often includes ctDNA, a subset of cfDNA derived from tumor cells. Notably, ctDNA fragments demonstrate

Table 1. Comparison of cfDNA and ctDNA

Characteristic	cfDNA	ctDNA
Source	Derived from normal cells (blood cells, healthy tissues, etc.) and tumor cells	Specifically derived from tumor cells
Proportion	Accounts for the majority of cfDNA in the bloodstream	Represents a small fraction of cfDNA but increases in cancer patients
Length	Fragment length is mainly around 167 bp	Fragment length is shorter, around 143 bp
Specificity	Non-specific with no tumor-specific mutations or methylation patterns	Contains tumor-specific mutations or epigenetic changes
Clinical significance	Widely used for monitoring various diseases	Focused on tumor diagnosis, classification, and treatment response monitoring

cfDNA, cell-free DNA; ctDNA, circulating tumor DNA.

distinct biophysical profiles, averaging 143 bp in length, significantly shorter than their non-malignant counterparts, and remain virtually undetectable in healthy populations.^{11,14}

cfDNA serves as an ideal biomarker for early cancer detection, as it can be obtained non-invasively through blood, urine, or other bodily fluids and provides genetic (e.g., mutations) and epigenetic (e.g., methylation) information. These characteristics enable cfDNA to reveal tumor burden, tissue of origin, and dynamic changes in tumor biology. The short half-life of ctDNA in circulation allows for real-time monitoring of tumor dynamics, making it the biomarker of choice for various clinical applications.¹² Table 1 compares cfDNA and ctDNA in key characteristics and clinical significance.

CTCs

CTCs are tumor cells that detach from the primary tumor and enter the bloodstream, serving as key mediators in the metastatic cascade.¹⁵ Experimental evidence indicates that tumor cells may disseminate as early as the initial stages of tumor development.¹⁶ While the majority of CTCs survive in circulation for only 1–2.5 h before being eliminated by the immune system, a small subset evades immune surveillance and seeds secondary metastatic sites.¹⁷

Traditional high-throughput sequencing of tumor tissues, despite providing insights into overall genomic characteristics, is constrained by its reliance on bulk sample analysis. This limitation obscures the heterogeneity of tumor cells and dilutes the genomic information of low-abundance but biologically critical cell populations, such as CTCs and cancer stem cells, thereby limiting the resolution of genetic analyses. In contrast, CTCs exhibit pronounced heterogeneity, encompassing epithelial-type cells and mesenchymal-type cells that have undergone epithelial-to-mesenchymal transition. These cells demonstrate invasive capacities, stem-cell-like properties, and a dynamic ability to transition between epithelial-to-mesenchymal transition and mesenchymal-to-epithelial transition.¹⁵ Molecular characterization of CTCs is typically achieved through markers such as epithelial markers (e.g., EpCAM), mesenchymal markers (e.g., N-cadherin), and proliferation markers (e.g., Ki-67). Single-cell sequencing technologies have emerged as powerful tools for delineating the genomic, transcriptomic, and epigenomic variations in CTCs across peripheral blood, primary tumors, metastatic lesions, and metastatic lymph nodes. By mitigating the confounding effects of tumor heterogeneity, these approaches provide unprecedented insights into the mechanisms underlying tumor initiation, progression, and metastasis.^{18–20}

Exosomes

Exosomes are lipid bilayer vesicles with a diameter ranging from 40 to 160 nm, actively released by most cells and stably circulating in body fluids. Increasing evidence indicates that various bioactive molecules, including nucleic acids, proteins, and lipids, are enriched in exosomes and can be transferred from donor cells to recipient cells, resulting in intracellular signal transmission.^{21–23} The bioactive substances in exosomes can be absorbed by recipient cells, promoting tumor initiation and progression. Compared to cfDNA and CTCs, exosomes offer several advantages in liquid biopsy applications. Firstly, the abundant presence of exosomes in bodily fluids (about 10⁹ particles/mL) facilitates the relatively easy isolation of vesicles, whereas only a few CTCs are found in 1 mL of blood.²⁴ Secondly, exosomes are secreted by living cells and carry rich biological information from the parent cells. Thus, exosomes are more representative than ctDNA, although they only partially reflect the information from tumor cell apoptosis or death.²⁵ Moreover, exosomes exhibit inherent stability due to their lipid bilayer, allowing them to circulate stably under physiological conditions, even in the harsh tumor microenvironment.²⁵ This high biological stability enables long-term storage of specimens, which is essential for exosome isolation and detection.

However, the application of exosomes in liquid biopsy faces several challenges. Efficient isolation with high purity remains a primary issue due to their nanoscale size and inherent heterogeneity.^{26,27} Furthermore, since cancer-associated exosomes represent only a small fraction of all exosomes in body fluids, ultrasensitive and specific detection is a prerequisite for developing exosome-based cancer diagnostics.²⁸ Several methods have been developed for exosome isolation and the detection of exosomal proteins and nucleic acids. Despite significant progress, limitations in sensitivity and specificity, low purity, and low throughput continue to be major challenges for both academic research and practical applications.

Analytical techniques

As shown in Figure 2, liquid biopsy primarily relies on samples such as cfDNA, ctDNA, CTCs, and exosomes, processed through steps of detection, enrichment, identification, and analysis. With technological advancements, enrichment methods have been continuously optimized, offering greater potential for liquid biopsy in non-invasive cancer screening.

Analytical techniques for cfDNA/ctDNA

The analysis of ctDNA has historically relied on polymerase chain

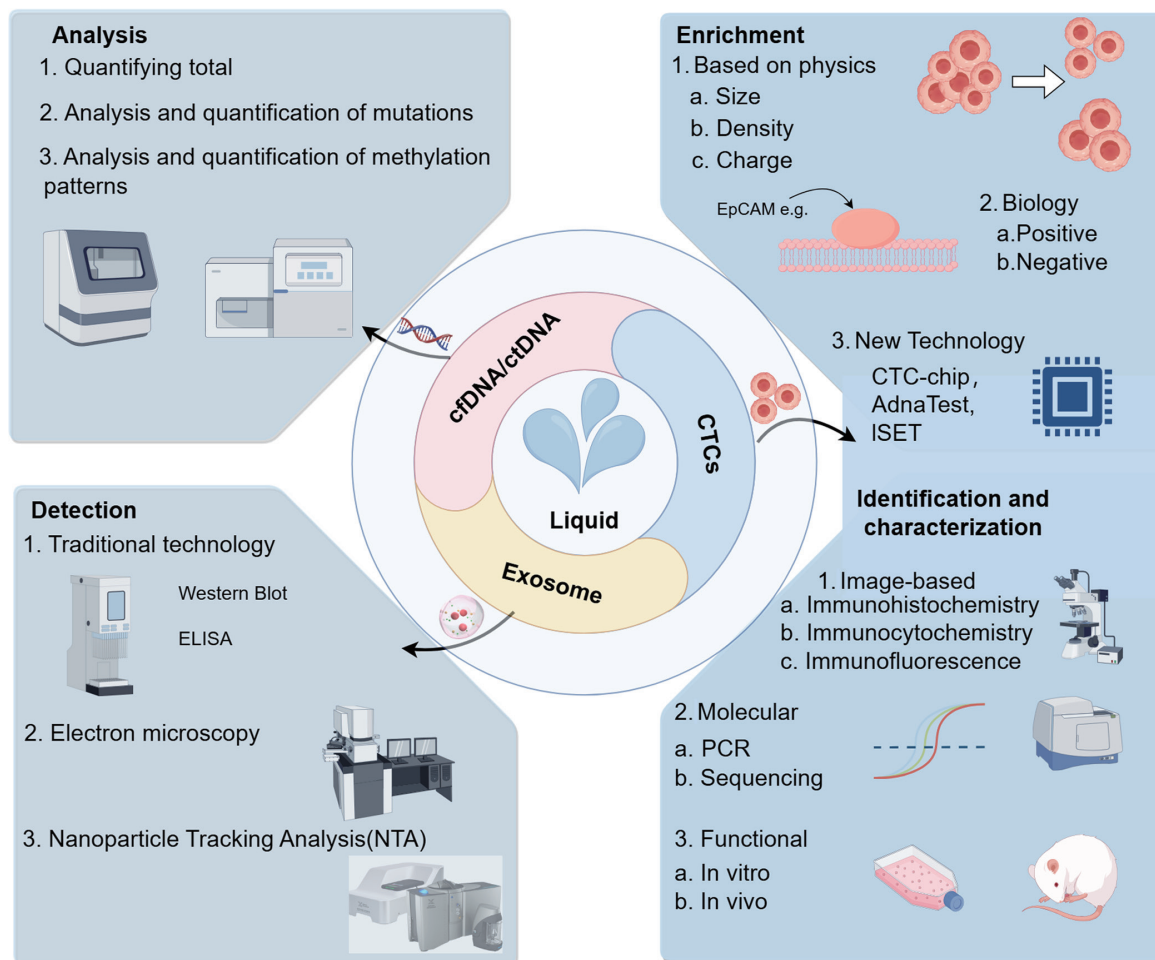


Fig. 2. Overview of liquid biopsy techniques and applications. By Figdraw. cfDNA, cell-free DNA; CTC, circulating tumor cell; ctDNA, circulating tumor DNA; ELISA, enzyme-linked immunosorbent assay; ISET, isolation by size of epithelial tumor cells; PCR, polymerase chain reaction.

reaction (PCR)-based assays or next-generation sequencing (NGS) combined with specialized bioinformatics tools. Digital PCR, initially proposed by Kinzler and Vogelstein, introduced the concept of single-molecule amplification, enabling the evaluation of rare mutant alleles amidst a background of abundant wild-type alleles.²⁹ The first high-throughput digital PCR method, known as BEAMing (i.e., beads, emulsions, amplification, and magnetics), integrated PCR with flow cytometry, facilitating highly sensitive detection of known mutations.³⁰ This method demonstrated strong concordance with tissue biopsy results and successfully validated the utility of digital PCR in detecting ctDNA from metastatic diseases and monitoring therapeutic responses. Numerous studies have established the analytical and clinical validity of droplet digital PCR.³¹ However, droplet digital PCR is currently limited to analyzing one potential mutation per reaction, necessitating large quantities of cfDNA for the analysis of multiple mutations.

Nevertheless, compared to contemporary NGS methods, a key advantage of digital PCR is that its results do not require bioinformatic analysis. The data output is similar to flow cytometry, significantly reducing turnaround times, which is clinically advantageous.³²

The maturation of NGS technologies has introduced orders-of-magnitude higher multiplexing capabilities for cfDNA analy-

sis. With the advent of molecular barcoding for individual DNA molecules and novel bioinformatics pipelines, NGS has evolved to overcome limitations associated with PCR amplification or NGS-induced errors, achieving limits of detection comparable to or even exceeding those of digital PCR. NGS provides a deeper and more comprehensive analytical approach to identifying genetic alterations.³³ Unlike digital PCR, which uses molecular probes to query known mutations, NGS enables the relatively unbiased discovery of genetic perturbations, as sequencing inherently identifies all base pairs in a given DNA molecule. However, the inherent nature of strand synthesis and PCR amplification makes NGS prone to sequencing errors. Despite these limitations, the ability of NGS to detect a wide range of genetic changes with high multiplexing potential underscores its value in cfDNA-based diagnostics.

Additionally, methylation alterations are closely associated with tumorigenesis. These changes often persist in a stable manner and can occur earlier than genomic mutations.³⁴ cfDNA also reflects tissue-specific methylation signatures, providing a means to identify the tissue of origin.^{12,35} Numerous studies have focused on developing high-performance multi-cancer early detection (MCED) tests utilizing cfDNA methylation patterns.

To capture these methylation patterns, various methods based on distinct technologies have been developed to selectively enrich

genome-wide CpG regions, followed by high-throughput NGS. For example, cell-free methylated DNA immunoprecipitation and sequencing enables the detection of methylation patterns from small amounts of cfDNA.³⁶ This approach employs immunoprecipitation with antibodies specifically recognizing methylated cytosine residues to enrich methylated DNA fragments. Cell-free methylated DNA immunoprecipitation and sequencing has been effectively used to differentiate plasma samples from pancreatic cancer, lung cancer, and acute myeloid leukemia from those of other cancer types.

In addition to genome-wide methylation sequencing, targeted methylation sequencing has emerged as a powerful and more commonly used approach for MCED tests, showing remarkable results. These methods typically use a set of DNA probes or primers to capture methylation patterns of target genomic regions via NGS. The target regions are often selected based on comparisons between non-cancer samples and samples from each type of target cancer. Consequently, the panel size can be highly flexible, allowing for cost-effective deep sequencing depending on the number of target regions.¹² Ample research has demonstrated the accuracy and sensitivity of targeted methylation sequencing. Klein *et al.*³⁷ showcased a highly targeted methylation detection method capable of detecting over 50 types of cancer and pinpointing their tissue of origin. Overall, targeted methylation sequencing represents a promising approach for MCED, offering high sensitivity and specificity while maintaining cost-effectiveness and flexibility.

CTC isolation and analysis

The extremely low proportion of CTCs in the blood presents a significant challenge for accurately isolating these cells from the vast number of blood cells. CTCs can be enriched and captured based on either physical or biological properties. Physical methods leverage differences between CTCs and blood cells in size, density, deformability, and electrical properties. For example, the Oncoquick system utilizes density gradients to separate red and white blood cells, while the Apostream system employs dielectrophoresis in microfluidic chambers to isolate CTCs.^{38,39} These approaches are cost-effective and preserve cell viability but are generally limited by low efficiency, poor purity, and lack of specificity. Furthermore, they may fail to capture CTCs that share similar physical characteristics with white blood cells. In contrast, biological property-based methods rely on antibody-antigen interactions. Technologies like the CellSearch system use epithelial (EpCAM) and mesenchymal (vimentin) markers to positively enrich CTCs, or CD45 to deplete unwanted white blood cells.^{40,41} However, the high heterogeneity of CTC surface antigens can result in under-detection of EpCAM-low CTCs, leading to inaccuracies. Physical property-based methods, by comparison, are unaffected by such antigen variability.

Microfluidics and nanotechnology have further enhanced CTC sorting capabilities. For example, CTC-chip technology isolates viable CTCs from whole blood without pre-labeling or extensive sample processing, improving cell viability and purity.^{15,42} Advances such as the NP-HB CTC-Chip, which combines herringbone microfluidics with gold nanoparticles for chemical ligand exchange reactions, allow for efficient isolation and safe release of viable CTCs for further analysis.⁴³ Despite their high capture efficiency and cell viability, these microfluidic platforms face barriers to clinical implementation due to high initial costs, lengthy setup times, bulky instrumentation, and limited capacity for single-cell molecular analysis.

CTC detection methods include fluorescence microscopy, fluorescence spectrophotometry, flow cytometry, surface-enhanced

Raman scattering, and electrical impedance. Morphological analysis using immunocytochemistry (hereinafter referred to as ICC) with antibodies against cytokeratins is a widely applied approach for qualitative and quantitative CTC analysis.⁴⁴ To address the labor-intensive nature of conventional immunofluorescence, automated technologies such as laser scanning cytometers have been developed to screen highly enriched CTCs more efficiently. The Ariol automated image analysis system is another widely used method for imaging CTCs, meeting high-resolution diagnostic pathology standards. This system has been validated across multiple cancer types, including lung, colorectal, and prostate cancers, and eliminates the need to collect proteins released by CTCs.⁴⁵⁻⁴⁷

PCR-based methods offer higher sensitivity and specificity for CTC detection. RT-qPCR, for instance, can specifically target tumor-related genes while excluding non-cancerous blood cells. This method is capable of detecting extremely low concentrations of CTCs, such as one CTC among more than 10^6 leukocytes. By extracting total RNA from CTCs, RT-qPCR amplifies tumor-specific gene sequences, such as cancer-associated markers (EpCAM, mucin1, ERBB2), EMT-related transcription factors (Twist1, Snail, PI3Ka, Akt-2), and stem cell markers (CD34, CD133, ALDH1).⁴⁸

The isolated and enriched CTCs are valuable for downstream analyses, including genomics, transcriptomics, proteomics, and cell culture. These approaches enable in-depth investigations into the molecular characteristics and biological functions of CTCs, contributing to advances in precision medicine, cancer progression studies, and therapeutic strategies.

Application of liquid biopsies in early cancer diagnosis

Most cancers are classified according to disease staging, which measures the extent of spread in the primary and other organs: Stage 0 (in situ), I, II, III, and IV. Compared to late-stage cancers (III/IV), early-stage cancers (I/II) are associated with lower treatment costs and better prognosis.⁴⁹ The majority of patients diagnosed with stage I cancer (approximately 70%) undergo surgery as part of their treatment. Surgery has been proven to be the most effective curative treatment, offering fewer side effects and a greater chance of eradication compared to radiotherapy and chemotherapy.¹⁰ Therefore, early cancer detection significantly reduces treatment costs and improves patients' expected survival and quality of life. However, due to the subtle nature of early cancer symptoms and the difficulty of detecting them with conventional diagnostic methods, the rate of early cancer diagnosis remains low. Current clinical trials also lack sufficient sensitivity and specificity for early cancer detection.⁵⁰ As illustrated in Figure 3, various types of cancer release biomarkers such as RNAs, DNA, CTCs, and exosomes into the bloodstream, which can be utilized for liquid biopsy.

In recent years, many countries have implemented cancer screening programs for asymptomatic high-risk populations, based on risk factors such as age, gender, family history, and epidemiological data, with the aim of reducing mortality and incidence. For example, breast ultrasound is used for large-scale screening of breast diseases, and mammography is the gold standard for clinical practice in breast cancer screening.⁵¹ For lung cancer, low-dose computed tomography screening has significantly reduced mortality rates.⁵² The diagnosis of gastric cancer is primarily based on imaging and pathological biopsy after endoscopy.⁵³ For high-risk patients with liver cancer, early diagnosis is conducted using ultrasound or alpha-fetoprotein testing.⁵⁴ However, single-cancer screening methods are costly and have limitations, such as high false-positive rates and the need for radiation exposure or invasive

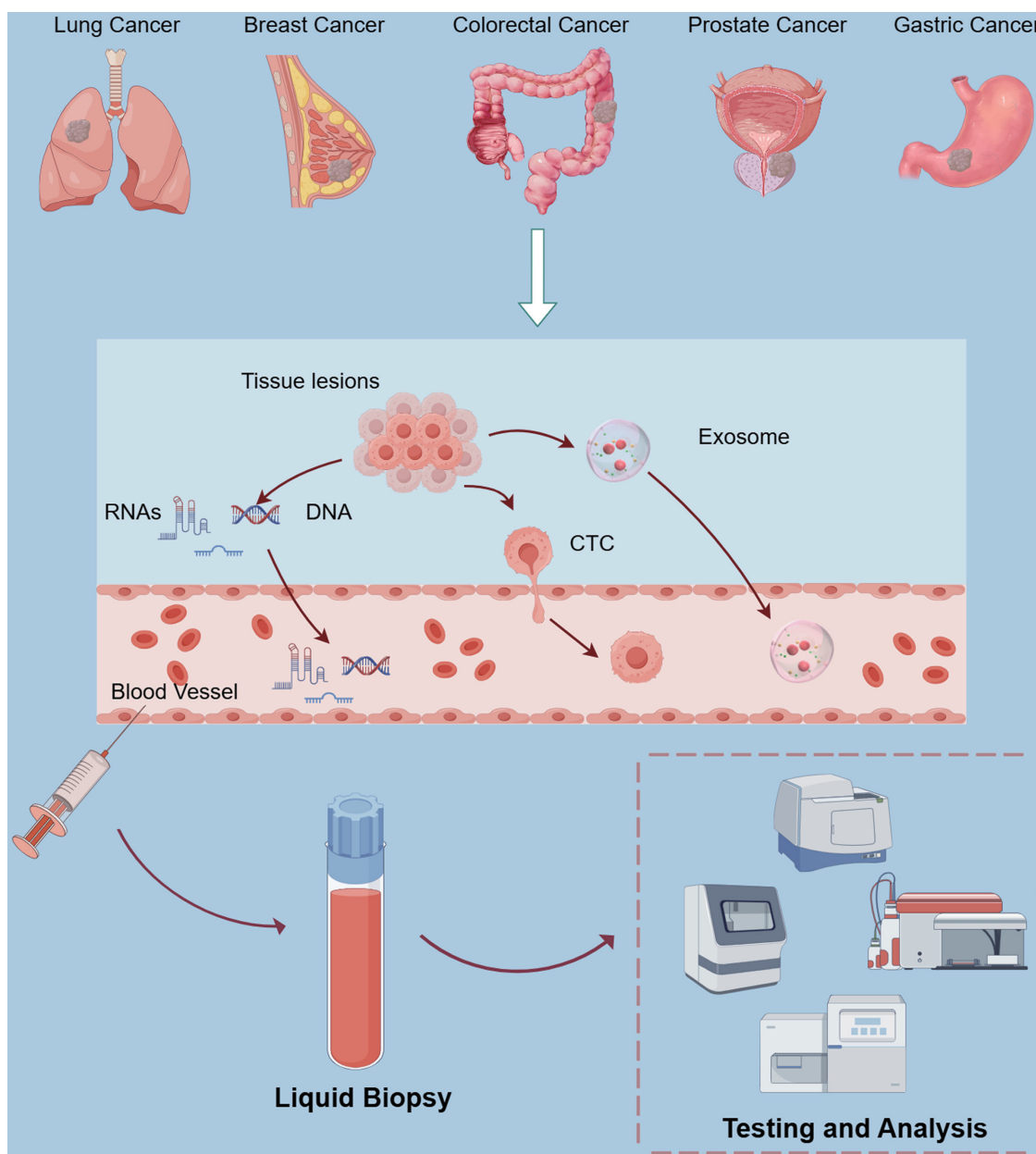


Fig. 3. Liquid biopsy workflow in cancer detection and analysis. The image illustrates the process of liquid biopsy for cancers such as lung, breast, colorectal, prostate, and gastric cancers. Tumor-derived biomarkers, including RNAs, DNA, exosomes, and circulating tumor cells, are released from tissue lesions into the bloodstream. These biomarkers are collected through blood sampling, followed by advanced testing and analysis to aid in cancer diagnosis, monitoring, and personalized treatment. By Figdraw. CTC, circulating tumor cell.

procedures. In the future, multi-cancer testing may prove more effective, allowing a single test to detect multiple cancers. This approach is also valuable for patients exhibiting non-specific symptoms but with a low likelihood of cancer.^{35,55} Moreover, low-cost blood tests could serve as a triage tool, helping doctors to consider potential disease directions in patients with non-specific symptoms and low suspicion of cancer, facilitating early investigation.

Tissue biopsy is considered the “gold standard” for tumor profiling in cancer diagnosis, and in most cases, tissue biopsy is required to determine the specific type of cancer.⁵⁶ The biopsy methods can be divided into excisional biopsy, which involves removing the

entire abnormal lesion, and incisional biopsy, which removes only part of the lesion. Open surgical biopsy offers precise excision but carries increased risks of infection and bleeding. Moreover, tissue sections cannot capture the spatial and temporal heterogeneity of tumors or their clonal evolution, thus limiting their utility for tumor monitoring.

Liquid biopsy involves analyzing tumor-related biomarkers circulating in body fluids, such as blood. Early tumors are small in size, meaning the level of tumor-related biomarkers released into circulation is very low. As a result, biomarkers used for detection at this stage may not necessarily be directly related to can-

Table 2. Summary of reported liquid biopsy-based analytical techniques in various cancers

Cancer	Biofluid	Analyte	Potential analytic techniques
Lung	Plasma; Pleural fluid	EVs; CTCs; ctDNA; miRNA	PCR; qRT-PCR; ARMS-PCR; CAPP-seq; EFIRM; NGS; Methylation-specific RT-PCR; ISET; CellSearch; nano-quantum dots microarray
Breast	Plasma; Saliva	CtDNA; CTCs; cfmiRNA	BEAMing ddPCR; qRT-PCR; TEC-seq; Personalized and ultra deep sequencing; Large NGS panels; Nanotube CTC chip; CTC ichipmicroarray
Colon	Plasma; Serum; Saliva	ctDNA; CTCs; EV RNA; EV proteins; TEPs	CellMax biomimetic platform (cMx); CellSearch; Safe-SeqS; NGS; Digital PCR (BEAMing) ddPCR; qPCR; ALU-qPCR; Electrochemical sensing
Prostate	Plasma; Serum	ctDNA; CTCs; EVs; ctRNA; ucrRNA	qPCR; Sodium bisulfite-PCR; qRT-PCR; ELISA
Gastric	Plasma; Gastric juice; Saliva; Urine	ctDNA; CTCs; EVs; ctRNA; miRNA; TEPs	ddPCR; NGS; MSP; cfMeDIP-seq; cfMeDIP-seq; RNA-seq

ALU-qPCR, ALU quantitative PCR; ARMS-PCR, amplification refractory mutation system PCR; BEAMing, beads, emulsion, amplification, and magnetics; CAPP-seq, cancer personalized profiling by deep sequencing; cfMeDIP-seq, cell-free methylated DNA immunoprecipitation sequencing; cfmiRNA, cell-free microRNA; CTCs, circulating tumor cells; ctDNA, circulating tumor DNA; ddPCR, droplet digital PCR; EFIRM, electric field-induced release and measurement; ELISA, enzyme-linked immunosorbent assay; EVs, extracellular vesicles; ISET, isolation by size of tumor cells; miRNA, microRNA; MSP, methylation-specific PCR; NGS, next-generation sequencing; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RNA-seq, RNA sequencing; RT-PCR, reverse transcription polymerase chain reaction; TEC-seq, tumor-educated circulating DNA sequencing; TEPs, tumor-educated platelets; ucrRNA, urinary circular RNA.

cer cells. In contrast, systemic, non-tumor-derived markers may be more prevalent.^{57,58} Liquid biopsies can be performed rapidly and provide genomic, proteomic, and metabolomic information. Using pan-omics approaches that combine both tumor- and non-tumor-derived information may improve the success of early cancer detection.^{59,60} Additionally, liquid biopsies are less expensive than tissue biopsies, easier to repeat, and more reliable, making them more suitable for use in low- and middle-income countries.⁴⁹ Furthermore, unlike tissue biopsies, which are typically preserved through processes such as fixation, embedding, and freezing for immunohistochemistry, liquid biopsies are not affected by contamination from preservatives.⁶¹ Below, we will elaborate on the current status of the application of liquid biopsies in the early diagnosis of various types of cancer. Table 2 summarizes liquid biopsy-based analytical techniques applied to various cancers and associated biofluids.

Lung cancer

Lung cancer had the highest incidence (12.4%) and mortality (18.7%) rates among all types of cancer in 2022, according to the International Agency for Research on Cancer report published in February 2024.¹

Liquid biopsy has shown significant progress in the clinical application and technological research of CTCs in lung cancer. Since the concept of CTCs was first proposed by an Australian physician in 1869,⁶² studies on CTCs in patients with early-stage lung adenocarcinoma have demonstrated a significant increase in CTC counts in all patients with tumor progression.⁶³ NGS analysis of CTCs from lung cancer patients revealed that over 50% of them harbor four common mutated genes (Notch1, IGF2, EGFR, and PTCH1).⁶⁴ Additionally, liquid chromatography-mass spectrometry analysis identified 100 differential metabolites, among which 10 were determined to have potential clinical value for the diagnosis of CTC-positive early-stage lung cancer.⁶⁵ This suggests that NGS and metabolomics of CTCs may offer new tumor markers for the early diagnosis of lung cancer. Furthermore, CTCs have been isolated from a subset of high-risk lung cancer patients, specifically smokers with chronic obstructive pulmonary disease and without pulmonary nodules on chest computed tomography (CT) scans.⁶³ In a study that followed this group with annual CT

scans, lung adenocarcinoma-related nodules were observed in patients with CTCs. Notably, the predictive value of CTC detection for identifying a second primary lung cancer in these patients approached 100%.⁶⁵

Regarding plasma ctDNA, studies have demonstrated that ctDNA levels in non-small cell lung cancer (NSCLC) patients were significantly higher compared to patients with chronic respiratory inflammation and healthy controls.^{65,66} In a recent study, the detection rate of ctDNA in plasma samples from patients with stage II-IV NSCLC was 100%, and in stage I NSCLC patients, it was 50%.⁶⁷ It appears that while ctDNA demonstrates significant potential as a non-invasive biomarker for advanced NSCLC detection, its utility in early-stage diagnosis (particularly stage I) remains constrained by current technological sensitivity limits. This necessitates the integration of multi-omics approaches or ultrasensitive detection platforms to overcome biological and technical barriers. However, it has also been suggested that ctDNA levels are highly correlated with tumor volume, capable of distinguishing residual lesions and treatment-related radiological changes, indicating that ctDNA level detection may assess therapeutic efficacy earlier than imaging methods.⁶⁷

Beyond CTCs and ctDNA, exosome-derived microRNAs (miRNAs) can be analyzed to differentiate between lung adenocarcinoma and pulmonary granuloma patients. miRNA analysis can also be used to distinguish lung cancer patients from healthy individuals. Further research has confirmed that plasma exosomes containing miR-30e-3p, miR-30a-3p, miR-181-5p, and miR-361-5p are specific diagnostic biomarkers for adenocarcinoma, while those containing miR-15b-5p, miR-10b-5p, and miR-320b can serve as specific diagnostic biomarkers for squamous cell carcinoma.⁶⁸

Despite these advancements, challenges remain, including the standardization of detection methods, low sensitivity for early-stage lung cancer, and the need for further validation in large-scale clinical trials. Several clinical trials have demonstrated the effectiveness of ctDNA analysis in lung cancer diagnosis and treatment monitoring. For instance, the NILE trial (NCT03615443) compared ctDNA-based EGFR mutation detection with traditional tissue biopsy, showing comparable accuracy and faster turnaround times. Therefore, liquid biopsy holds great potential for lung cancer screening and may complement existing imaging techniques.

Breast cancer (BC)

BC is the most common cancer among women worldwide. The incidence (11.5%) of breast cancer is notably higher among females, but its mortality rate (6.8%) is significantly lower than that of lung cancer, reflecting advancements in early detection and treatment.^{1,2}

To date, biomarkers based on cfDNA, ctDNA, CTCs, and miRNA have been described in numerous studies. Some studies have identified cfDNA as an early detection biomarker for BC based on the analysis of DNA damage and DNA methylation changes. Li *et al.*⁶⁹ were the first to assess the methylation status of the EGFR and PPM1E promoters in plasma using next-generation bisulfite sequencing. Their study found significantly higher methylation levels in BC patients compared to healthy controls, highlighting the potential of cfDNA methylation as an early detection biomarker. Consistent with known promoter hypermethylation in cancer, they observed significantly higher methylation levels in BC patients compared to healthy control subjects. ctDNA can also serve as a potential biomarker in liquid biopsy samples to identify specific mutations in BC.⁷⁰

The detection of CTCs as a non-invasive biomarker for the early diagnosis of BC has yielded promising results. Kruspe *et al.*⁷¹ developed a rapid, highly sensitive diagnostic method for detecting CTCs based on nuclease-activated probe technology, which can distinguish between BC patients and healthy controls through plasma analysis.

The profiling of miRNA molecules offers an opportunity to identify minimally invasive biomarkers for the early diagnosis of BC. Shimomura *et al.*⁷⁰ evaluated the expression profiles of miRNAs in the serum of BC patients and healthy women. A combination of five miRNAs (miR-1246, miR-1307-3p, miR-4634, miR-6861-5p, and miR-6875-5p) was found to be helpful in detecting BC (with a sensitivity of 97.3%, specificity of 82.9%, and accuracy of 89.7%) and individuals with early-stage BC (with a sensitivity of 98.0% for in situ carcinoma).

While liquid biopsy technologies for BC are promising, challenges remain in terms of sensitivity and specificity. For example, the detection of CTCs in early-stage BC patients remains difficult, and more research is needed to enhance the sensitivity of these methods.⁷² Current research suggests that the use of liquid biopsy, in conjunction with positron emission tomography/computed tomography, is not an alternative but rather a complementary analytical approach for diagnosing various types of malignant tumors, locations, and stages of disease. However, further studies are needed to assess the clinical utility, risks, and cost-effectiveness of these tests.⁷³

Colorectal cancer (CRC)

CRC is the second leading cause of cancer incidence (9.6%) and cancer-related mortality (9.3%) worldwide.¹ Early detection of CRC is crucial, as the survival rate for patients with early-stage cancer exceeds 90%.

Tsai *et al.*⁵⁵ were the first to demonstrate the utility of CTCs for early cancer detection. In a prospective study, CTC detection based on the Cellmax platform showed a sensitivity of 86.9% and a specificity of 97.3% in CRC patients, with an area under the curve of 0.88.^{55,74} The study also found a correlation between CTC counts and CRC disease staging, with the lowest sensitivity at 89.2% for stage I CRC and the highest at 99.9% for stage IV CRC. Different detection methods may yield varying detection rates; however, standardized approaches and new technologies will enhance the detection of CTCs in early-stage malignant tumors.

cfDNA is an emerging potential biomarker for guiding early

CRC screening. In a multicenter cohort study, 88.5% of patients with stages I-III CRC tested positive for ctDNA. The methylation profile of cfDNA is beneficial for the early diagnosis of CRC.⁷⁵ Wu *et al.*⁷⁶ identified a novel cfDNA methylation model based on 11 methylation biomarkers to improve the detection rate of early CRC patients. Additionally, methylation markers like EYA4, GRIA4, and ITGA4 in metastatic CRC patients have shown promising results for monitoring tumor burden and treatment efficacy.⁷⁷ Moreover, promoter hypermethylation of septin 9 in cfDNA has been confirmed as an effective biomarker for CRC, and the Epi proColon 2.0 kit for cell-free circulating methylated septin 9 detection has been approved by the FDA as the first blood-based CRC screening test.⁷⁸⁻⁸⁰

Due to the wide availability and high specificity of exosomal miRNAs for CRC, they have been proposed as prospective target biomarkers for the diagnosis of both early and advanced CRC. Overexpression of exosomal miR-17-92a or miR-19a in CRC patients is closely related to tumorigenesis and recurrence, especially in the early stages of the disease.⁸¹ Furthermore, in a study by Wang *et al.*,⁸² miR-125a-3p and miR-320c were highly upregulated in plasma exosomes of patients with early-stage colon cancer.

Despite the promising potential of liquid biopsy in CRC, challenges such as variability in detection sensitivity, particularly for early-stage cancer, remain. Standardization of methods and larger clinical validation studies are essential to improve reliability and accuracy. Combining liquid biopsy with other diagnostic techniques, like colonoscopy, may offer a more comprehensive approach for early detection, with future advancements focusing on enhancing sensitivity and minimizing false negatives, especially in high-risk populations.

Prostate cancer (PCa)

PCa is a prevalent malignant tumor and the second leading cause of cancer-related death among men, with an incidence rate of 7.3% and a mortality rate of 4.1%.¹

DNA methylation-based liquid biopsy has demonstrated significant diagnostic potential in early-stage prostate cancer detection, offering a non-invasive approach for identifying epigenetic alterations associated with tumorigenesis. Studies have indicated that hypermethylation at specific CpG sites of RARB2 and GSTP1 can be utilized for the diagnosis of PCa.⁸³ Additionally, the analysis of miRNA expression profiles has increasingly been employed for the early diagnosis of PCa. Mitchell *et al.*⁸⁴ were the first to confirm the presence of miRNAs in the plasma of PCa patients. In 2018, Liu *et al.*⁸⁵ conducted an RT-PCR analysis on plasma samples from 229 PCa patients under active surveillance and identified three miRNAs (miR-24, miR-223, and miR-375) that were significantly expressed in tumor patients. The authors concluded that a 3-miR score, combined with prostate-specific antigen (PSA), may serve as a non-invasive tool with high negative predictive value for identifying asymptomatic PCa patients under active surveillance.⁸⁶ As for CTCs, the ISET-CTC-ICC method has been found to have a positive predictive value of 99% and a negative predictive value of 97% in studies. However, the rarity of CTCs in the bloodstream limits their use for diagnosis.⁸⁷

In addition to blood, urine is also considered a suitable source for liquid biopsies in the early diagnosis of prostate cancer.⁸⁸ From urine samples, various analytes can be isolated and detected, among which cfDNA/RNA, CTCs, and extracellular vesicles contribute to the clinical diagnosis and treatment of patients with urogenital system malignancies. A retrospective study by Casadio *et al.*⁸⁹ demonstrated that the integrity of urine DNA could

distinguish between PCa patients and healthy individuals with an accuracy of approximately 80%. However, a study by Salvi *et al.*⁹⁰ showed that ctDNA had lower clinical predictive values in terms of sensitivity (0.58 vs. 0.95) and specificity (0.44 vs. 0.69) compared to PSA.

Despite the potential of liquid biopsy in prostate cancer detection, several obstacles remain, including the limited sensitivity of ctDNA and the low abundance of CTCs, which complicate early diagnosis. Urinary biomarkers, while promising, are also still under investigation and need further validation for clinical use. However, combining liquid biopsy with established methods like PSA testing and imaging could enhance early detection and ongoing monitoring of PCa. Future research should aim at improving sensitivity, particularly for detecting early-stage tumors and assessing treatment response.

Gastric cancer

Gastric cancer is one of the most common types of cancer, with over a million patients diagnosed worldwide each year, an incidence rate of 4.9%, and a mortality rate of 6.8%. Early detection plays a crucial role in improving prognosis, as late-stage gastric cancer often has a poor survival rate.¹

CTCs have shown potential in the diagnosis of gastric cancer, but their sensitivity needs improvement.⁹¹ A subset of gastric cancer patients has a low number of CTCs in their blood, leading to a high rate of false negatives in diagnosis. Yoon-Kyung Cho stated that they collected 7.5 mL of blood from 115 gastric cancer patients and 31 healthy controls and isolated CTCs using a centrifugal microfluidic system. Among the subjects with more than two CTCs, 97.1% were gastric cancer patients, with a specificity of 90.3%. However, 38% of gastric cancer patients had fewer than two CTCs per 7.5 mL, highlighting the issue of insufficient sensitivity.⁹² A recent study indicated that the key membrane receptor protein tyrosine kinase 7, discovered for the first time in colon cancer cell lines, in combination with epithelial cell adhesion molecule (EpCAM), improved the sensitivity of CTC detection in gastric cancer cell line samples. However, further research is required to test the effectiveness of protein tyrosine kinase 7 and EpCAM in blood samples.⁹³

When it comes to cfDNA, Zhong *et al.* found, through receiver operating characteristic analysis, that cfDNA has a higher diagnostic value than traditional biomarkers such as CA199, CA125, and alpha-fetoprotein.^{94,95} Methylated cfDNA and ctDNA are significant research topics. A genome-wide methylation analysis based on 1,781 gastrointestinal tumor and adjacent normal tissue methylation profiles was conducted, followed by validation with 300 cfDNA samples. The results suggested that gastrointestinal cancers can be distinguished by differentially methylated regions obtained from blood samples.⁹⁶ Additionally, research has found that the methylation of tumor suppressor genes in cfDNA, including PCDH10, RASSF1A, RUNX3, and RPRML, particularly in the blood samples of gastric cancer patients, has produced satisfactory sensitivity and specificity, indicating that the methylation of these genes could potentially serve as diagnostic biomarkers.^{97,98}

While liquid biopsy shows promise for gastric cancer detection, challenges such as low CTC sensitivity, especially in early-stage disease, and the need for further validation of cfDNA and exosomal miRNAs remain. Additionally, cfDNA methylation studies require more extensive clinical validation before becoming reliable biomarkers for routine screening. Combining liquid biopsy with imaging techniques like endoscopy or CT scans could improve early detection. Future research should focus on enhancing sen-

sitivity, particularly for early-stage gastric cancer, and confirming the clinical utility of cfDNA and miRNA-based biomarkers.

Discussion

Liquid biopsy has emerged as a promising tool in early cancer diagnosis, offering a minimally invasive alternative to traditional diagnostic methods. By analyzing cfDNA, ctDNA, CTCs, and miRNAs, liquid biopsy provides significant advantages, including real-time monitoring and the potential to overcome tumor heterogeneity. These features offer exciting opportunities for early cancer detection, which could lead to better patient outcomes through timely interventions. Encouragingly, a growing number of ongoing clinical trials are actively evaluating the potential of liquid biopsy in early cancer detection, as cataloged in Table 3.

However, the journey from a promising diagnostic tool to standard clinical practice is fraught with challenges. The sensitivity and specificity of liquid biopsy assays vary across different cancer types and stages, necessitating further optimization to ensure reliability. For instance, the rarity of CTCs in the bloodstream, as observed in gastric cancer, and the heterogeneity of ctDNA present significant hurdles that require innovative solutions. In our view, these challenges are not insurmountable but will require significant innovation in both technology and understanding of tumor biology. For example, advancements in amplification technologies and improved methods for profiling ctDNA could potentially overcome some of these limitations. Still, addressing the rarity of CTCs may require a more tailored approach, such as focusing on specific subtypes or utilizing multi-modal approaches to increase detection rates.

The field is also in dire need of standardized protocols for sample collection, processing, and analysis to ensure the reproducibility and comparability of results across different studies and clinical settings. This is a critical issue that could hinder the broader adoption of liquid biopsy in clinical practice. From our perspective, establishing standard operating procedures for liquid biopsy assays is essential for ensuring consistency and reliability. Furthermore, collaborations between academic researchers, clinicians, and industry partners will be crucial in developing these standards. The cost-effectiveness of liquid biopsy in the context of early cancer detection also warrants thorough evaluation, particularly in light of the potential for widespread screening programs.

It is important to highlight that liquid biopsy demonstrates substantial potential in precision oncology, not only serving as a tool for early cancer diagnosis but also providing critical value in prognostic prediction, personalized therapeutic guidance, and recurrence monitoring. Continuous advancements in detection technologies have significantly enhanced the analytical accuracy of biomarkers such as cfDNA and CTCs, thereby improving the clinical reliability of liquid biopsy. Further research is warranted to address existing challenges and optimize methodologies, which will ultimately advance the development of precision medicine.

Looking ahead, the integration of liquid biopsy with existing diagnostic modalities, such as imaging and tissue biopsy, could enhance the accuracy and comprehensiveness of cancer diagnosis. Future research should focus on the discovery of novel biomarkers, the development of more sensitive and specific assays, and the conduct of large-scale clinical trials to validate the clinical utility of liquid biopsy.

Conclusions

Liquid biopsy has demonstrated substantial promise as a minimally invasive tool for early cancer detection, providing significant

Table 3. Summary of selected clinical trials evaluating liquid biopsy approaches for early cancer detection

Trial name	Cancer type	Sample	Study type	Analyte	Trial identifier
Fluid biopsy for the diagnosis of lung cancer	Lung cancer	Blood	Observational	CTC	NCT04162678
Liquid biopsy in lung cancer	Lung cancer	Blood	Observational	CTC; ctDNA	NCT03479099
A study to compare tissue and liquid Biopsies in people with different types of cancer	NSCLC; CRC; PDAC	Blood	Interventional	ctDNA	NCT05708599
Liquid biopsy for early non-small lung cancer detection	NSCLC	Blood	Observational	ctDNA	NCT05462795
Assessment of early-detection based on liquid biopsy in lung cancer	Lung cancer	Blood	Observational	ctDNA; cfDNA; CTC	NCT04817046
Early breast cancer detection based on liquid biopsies and micrornas	Breast cancer	Plasma	Observational	MicroRNA	NCT06439940
Exploring a breast cancer early screening model Based on cfDNA	Breast cancer	Plasma	Observational	cfDNA	NCT06016790
Evaluation of circulating tumor cells (CTC) Relevance in breast cancer follow-up using the ScreenCell device (PROBE-CTC)	Breast cancer	Blood	Interventional	CTC	NCT06807502
Early detection of five common cancers using the ctDNA analysing test	Breast cancer; Liver cancer; Gastric cancer; Colorectal cancer; Lung cancer	Blood	Observational	ctDNA	NCT05227261
Serial circulating tumor DNA (ctDNA) monitoring during adjuvant capecitabine in early triple-negative breast cancer	Breast cancer	Blood	Interventional	ctDNA	NCT04768426
Early onset colorectal cancer detection	Colorectal cancer	Blood	Observational	MicroRNA	NCT06342401
Exploratory study of a novel based rbcDNA liquid biopsy technique for colorectal cancer early detection	Colorectal cancer	Blood; Stool	Observational	rbcDNA	NCT05875584
Assessment of early-detection based on liquid biopsy in hepatobiliary cancer malignancies	Hepatobiliary cancer	Serum	Observational	ctDNA; RNA	NCT04835675
Assessment of early-detection based on liquid biopsy in gastric cancer	Gastric cancer	Serum	Observational	ctDNA; cfDNA	NCT05224596
Stomach cancer exosome-based detection	Gastric cancer	Serum	Observational	miRNA; cfDNA	NCT06342427
Early-stage detection of liver, biliary tract and pancreatic cancers	Liver Cancer; Biliary Tract Cancer; Pancreatic Cancer	Serum	Observational	cfDNA; microRNA	NCT06139042

cfDNA, cell-free DNA; CRC, colorectal carcinoma; CTC, circulating tumor cells; ctDNA, circulating tumor DNA; NSCLC, non-small cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; rbcDNA, red blood cell-derived circulating DNA.

advantages in terms of real-time monitoring, overcoming tumor heterogeneity, and enabling early interventions. Despite its potential, challenges related to sensitivity, specificity, and standardization remain, requiring continued innovation and validation in the field. Moving forward, integrating liquid biopsy with traditional diagnostic methods and advancing research into novel biomarkers will be essential to overcoming these obstacles and maximizing its clinical utility.

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Conflict of interest

The authors declare no conflicts of interest related to this manuscript.

Author contributions

Conceptualized the review topic, defined research questions, designed the framework, wrote the main body of the manuscript (XL), assisted in literature search and screening, performed data extraction, supplemented critical references, prepared the figures and tables (QT, JW), and critically revised and edited the final version (YW). All authors approved the final manuscript.

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