World Journal of Biology Pharmacy and Health Sciences
eISSN: 2582-5542
Cross Ref DOI: 10.30574/wjbphs
Journal homepage: https://wjbphs.com/

(REVIEW ARTICLE)

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Breakthroughs and challenges in liquid biopsy technologies for cancer diagnosis and treatment monitoring

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World Journal of Biology Pharmacy and Health Sciences, 2025, 22(01), 112-138

Publication history: Received on 26 February 2025; revised on 05 April 2025; accepted on 07 April 2025

Article DOI: https://doi.org/10.30574/wjbphs.2025.22.1.0327

Abstract

Cancer remains a leading global health challenge, with early detection and precise monitoring playing a crucial role in improving patient outcomes. Traditional tissue biopsies, while essential for diagnosis, are invasive, limited in scope, and often fail to capture tumor heterogeneity or track disease progression dynamically. Liquid biopsy technologies have emerged as a transformative alternative, offering a minimally invasive approach to cancer detection and management by analyzing circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), extracellular vesicles, and tumor-derived exosomes in bodily fluids. This review explores the technological advancements that have enhanced the sensitivity and specificity of liquid biopsies, including next-generation sequencing (NGS), droplet digital PCR (ddPCR), and machine learning-driven bioinformatics. The clinical applications of liquid biopsies are vast, encompassing early cancer detection, monitoring of therapeutic responses, identification of minimal residual disease (MRD), and real-time tracking of resistance mutations. These capabilities support the paradigm shift toward precision oncology, allowing clinicians to tailor treatments based on a dynamic understanding of tumor evolution. Despite their promise, liquid biopsies face challenges such as low biomarker abundance, standardization issues, cost barriers, and regulatory complexities, which hinder widespread clinical implementation. However, emerging innovations, including single-cell liquid biopsies, point-of-care diagnostic devices, and AI-assisted biomarker analysis, are set to overcome these limitations. As research advances, liquid biopsies are poised to revolutionize cancer diagnostics, providing a non-invasive, comprehensive, and personalized approach to cancer management that could significantly enhance survival rates and treatment efficacy.

Keywords: Liquid biopsy; Circulating tumor DNA; Cancer diagnostics; Treatment monitoring; Precision oncology; Minimal residual disease; Next-generation sequencing; Biomarker detection

1. Introduction

Cancer remains a formidable challenge in global health, and according to the World Health Organization, it was responsible for around 10 million mortalities in 2020 alone [1]. The relentless pursuit of improved diagnostic and therapeutic strategies has been at the forefront of medical research. Traditional cancer diagnostics have long relied on tissue biopsies, which, while informative, present several inherent limitations [2]. Recent years have seen the emergence of liquid biopsies as a viable, minimally invasive substitute that may improve cancer surveillance and early

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diagnosis. Examining the advances and difficulties of liquid biopsy technology in cancer diagnosis and therapy monitoring is the goal of this study [1,2].

1.1. Background on Cancer Diagnostics and the Limitations of Traditional Biopsies

Conventional cancer diagnosis often necessitates the extraction of tissue samples through surgical or needle biopsies. These procedures provide valuable histopathological information, enabling pathologists to determine tumor type, grade, and molecular characteristics essential for guiding treatment decisions [3]. However, the invasive nature of these procedures poses significant challenges. Patients may experience discomfort, pain, and potential complications such as bleeding or infection. Moreover, certain anatomical locations of tumors, such as those in the brain or pancreas, render biopsies particularly risky or, in some cases, unfeasible [3].

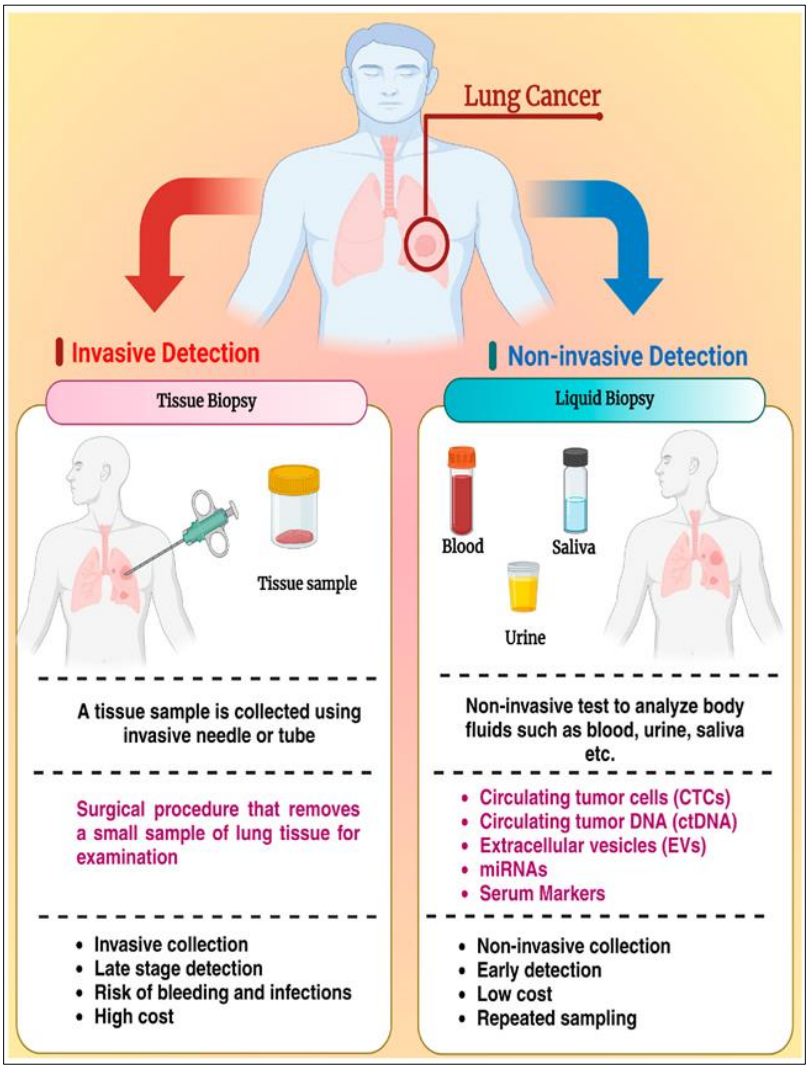

Figure 1 Lung cancer diagnosis and the two contrasting approaches: conventional tissue biopsy vs. liquid biopsy. Reproduced with permisiion from Ref. [3]

Beyond the immediate procedural risks, tissue biopsies offer a static snapshot of the tumor's genetic landscape. Tumors are inherently heterogeneous, comprising subpopulations of cells with distinct genetic mutations and phenotypic traits. A single biopsy may not capture this intratumoral heterogeneity, leading to an incomplete understanding of the disease. This limitation becomes pronounced when considering tumor evolution over time, especially under therapeutic pressure. As tumors adapt and develop resistance to treatments, their molecular profiles shift, necessitating updated information to guide subsequent therapeutic interventions. However, repeated tissue biopsies for monitoring purposes are impractical due to their invasiveness and associated complications [4,5]. To appreciate the advancements in cancer diagnostics, it is essential to compare traditional tissue biopsies with emerging liquid biopsy techniques (see Figure 1).

Table 1 outlines the key differences between these two approaches, highlighting aspects such as invasiveness, sampling frequency, and ability to capture tumor heterogeneity.

Table 1 Comparison of Traditional Biopsy and Liquid Biopsy

Aspect	Traditional Biopsy	Liquid Biopsy
Invasiveness	Involves surgical or needle procedures to extract tissue samples, which can be painful and carry risks such as infection.	Minimally invasive; requires a simple blood draw, reducing discomfort and associated risks.
Sampling Frequency	Limited due to invasiveness; repeated sampling is often impractical.	Allows for frequent sampling, enabling real-time monitoring of tumor dynamics.
Tumor Heterogeneity Assessment	May not capture the full genetic diversity of tumors, especially if sampling is from a single site.	Provides a comprehensive view by detecting circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) from multiple tumor sites, offering insights into tumor heterogeneity.
Cost	Generally higher due to surgical procedures and hospitalization requirements.	Potentially lower costs associated with outpatient blood draws and reduced need for surgical interventions.
Clinical Applicability	Standard method for initial diagnosis and histopathological analysis; essential for determining tumor architecture and microenvironment.	Useful for early detection, monitoring treatment response, and detecting minimal residual disease; however, it may not replace the need for tissue biopsies entirely, especially when detailed histological information is required [6].

1.2. Definition and Significance of Liquid Biopsy in Oncology

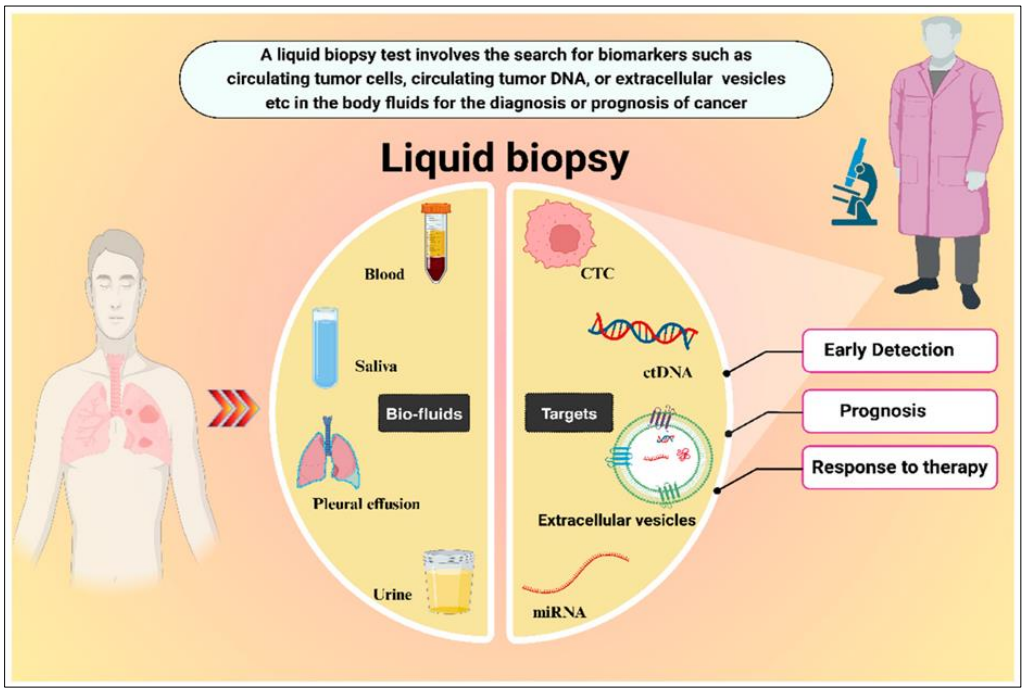

Figure 2 Mechanism of Liquid Biopsy in Cancer Detection. Reproduced with permission from Ref. [3]

In order to find biomarkers linked to cancer, non-solid biological tissues—most often blood—are sampled and analysed using a technique known as liquid biopsy. These biomarkers consist of extracellular vesicles, tumor-derived exosomes, circulating tumour cells (CTCs), and circulating tumour DNA (ctDNA) (see figure 2) [7]. Unlike traditional biopsies, liquid biopsies are minimally invasive, involving simple blood draws, and can be performed repeatedly, enabling real-time monitoring of tumor dynamics. The significance of liquid biopsies in oncology is multifaceted. Firstly, they facilitate

early detection of genetic mutations and alterations associated with cancer, potentially identifying malignancies before they become clinically apparent. This early detection is crucial, as it increases the likelihood of successful treatment outcomes. Secondly, liquid biopsies allow for the assessment of treatment efficacy by monitoring changes in ctDNA levels, providing insights into tumor response or progression. Thirdly, they enable the timely identification of resistance mechanisms, such as the emergence of new mutations that confer resistance to targeted therapies, allowing clinicians to adjust treatment strategies accordingly [8,9].

For instance, the National Health Service (NHS) in England has implemented liquid biopsies to detect specific mutations in breast cancer patients, allowing for more individualized and successful approaches of therapy. Liquid biopsies being included into clinical practice represents a significant advancement in precision oncology, offering the potential for improved patient outcomes and more tailored therapeutic interventions [10].

1.3. Objectives and Scope of the Review

This review attempts to offer a thorough analysis of the current state of liquid biopsy technologies in oncology. It will delve into the biological components detectable through liquid biopsies, such as ctDNA and CTCs, and discuss the technological advancements that have enhanced their detection and analysis. The review will also explore the therapeutic uses of liquid biopsies, including early cancer identification, therapy monitoring, and detection of minimum residual illness. Furthermore, it will address the current limitations and challenges hindering widespread adoption, such as concerns of standardization, sensitivity, and specificity. Finally, the review will highlight future directions and emerging innovations in the field, offering insights into how liquid biopsies may continue to revolutionize cancer diagnostics and treatment monitoring. By examining these aspects, this review seeks to elucidate the revolutionary possibilities of liquid biopsy technologies in oncology, while also acknowledging the obstacles that need to be eliminated in order to properly utilize them in clinical settings.

2. Biological Components of Liquid Biopsies

The use of liquid biopsy technologies is revolutionizing the detection and tracking of cancer by analyzing tumor-derived materials found in bodily fluids. These biological components provide crucial molecular insights into tumor behavior, allowing clinicians to track disease progression and treatment responses in real time. Among these components, circulating tumor DNA (ctDNA) carries genetic mutations, epigenetic modifications, and methylation patterns that reflect the evolving nature of cancer [11]. Circulating tumour cells (CTCs), which are released into the circulation from primary or metastatic tumours, are essential for metastasis and can provide important information about the phenotypic features of tumours. Extracellular vesicles and exosomes, once thought to be mere cellular debris, have recently been identified as essential mediators in communication between cells, carrying biomarkers that can aid in cancer detection. Additionally, microRNAs (miRNAs) and tumor-derived proteins are emerging as powerful tools for cancer classification and diagnosis (See Figure 3) [11,12]. This section explores the biological significance of these components, their role in cancer pathology, and their potential to enhance precision oncology. Liquid biopsies analyze various biomarkers to detect and monitor cancer. Table 2 summarizes the primary biomarkers, their sources, clinical significance, and current applications.

Table 2 Key Biomarkers Detected in Liquid Biopsy

Biomarker Type	Source	Clinical Significance	Current Applications
Circulating Tumor DNA (ctDNA)	Blood	Reflects the genetic alterations of tumors; used to identify mutations, monitor treatment response, and detect resistance mechanisms.	Detection of specific mutations (e.g., EGFR in non-small cell lung cancer) to guide targeted therapy decisions [13].
Circulating Tumor Cells (CTCs)	Blood	Presence indicates tumor shedding into the bloodstream; associated with prognosis and metastatic potential.	Prognostic indicator in cancers such as breast, prostate, and colorectal cancers; monitoring disease progression [6].
Exosomes	Blood, Urine	Nano-sized vesicles containing proteins, RNA, and DNA; involved in cell communication and may reflect tumor status.	Investigational use in cancer diagnostics and monitoring; potential for early detection and understanding tumor behavior.

MicroRNAs (miRNAs)	Blood, Urine	Small non-coding RNAs that regulate gene expression; aberrant expression patterns are linked to cancer development and progression.	Research-stage biomarker for cancer diagnosis, prognosis, and therapeutic targets; not yet widely implemented in clinical practice.

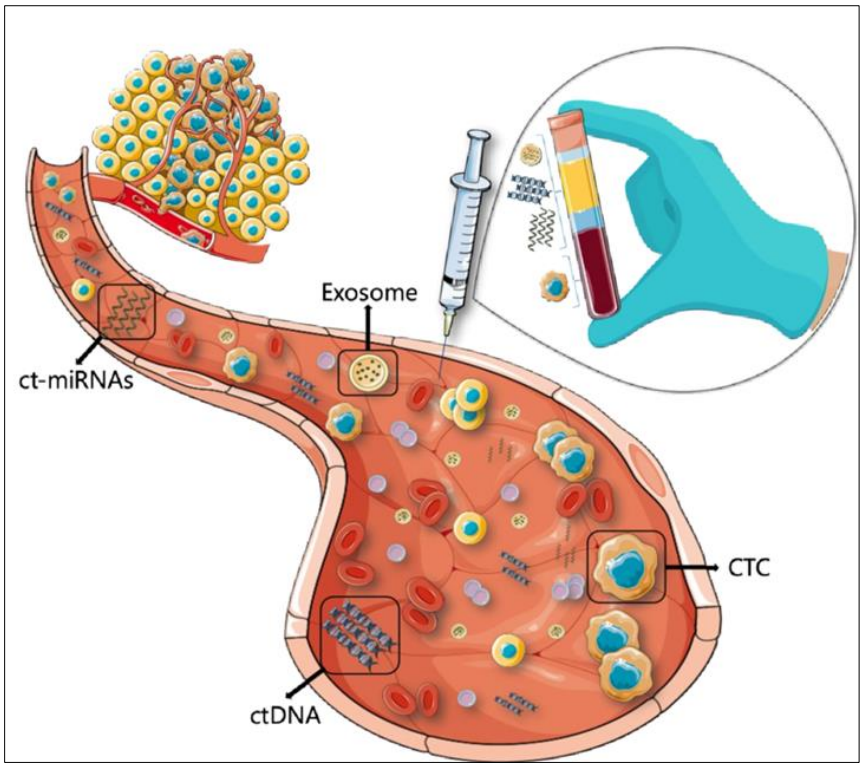

Figure 3 A schematic view of liquid biopsy. Blood collected from cancer patients contains circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), exosomes, and circulating tumor microRNA (ct-miRNA). Reproduced with perssion from Ref. [12]

2.1. Circulating Tumor DNA (ctDNA): Mutation Profiling, Epigenetic Alterations, Methylation Patterns

The term "circulating tumour DNA" (ctDNA) describes tiny DNA fragments released into the circulation by tumour cells. These fragments, typically less than 200 base pairs in length, carry genetic and epigenetic information that mirrors the tumor's molecular landscape, offering a non-invasive window into cancer biology [14]. The analysis of ctDNA encompasses various aspects, including mutation profiling, epigenetic alterations, and methylation patterns, each providing unique insights into tumor behavior and potential therapeutic strategies.

2.1.1. Mutation Profiling

Mutation profiling of ctDNA involves identifying genetic changes like point mutations, copy number variations, insertions, and deletions that drive cancer development and progression. Although useful, traditional tissue biopsies are invasive and could miss the variety of metastatic tumours. In contrast, ctDNA analysis enables the detection of mutations across all tumor sites through a simple blood draw, facilitating real-time monitoring of tumor dynamics [15].

The therapeutic usefulness of ctDNA mutation profiling has been shown in several research. For instance, Leary et al. [16] employed whole-genome sequencing of ctDNA to detect chromosomal alterations in patients with colorectal and breast cancers. Their findings revealed that ctDNA could identify structural variations and copy number changes concordant with those found in primary tumors, underscoring its potential for non-invasive genomic profiling.

Moreover, ctDNA has been used to identify minimum residual diseases and track therapy results. In a study by Olsson et al. [17], ctDNA serial measures in individuals with breast cancer that has spread correlated with changes in tumor burden and provided early indications of treatment efficacy, often preceding radiographic assessments. These observations highlight the role of ctDNA as a dynamic biomarker for personalized cancer management.

2.1.2. Epigenetic Alterations and Methylation Patterns

ctDNA contains epigenetic changes that affect gene expression without changing the DNA sequence, in addition to genetic mutations [18]. One important epigenetic process controlling gene activity is DNA methylation, which involves the addition of methyl groups to cytosine residues. Cancer is characterized by aberrant methylation patterns, which can act as biomarkers for diagnosis and prognosis [18,19]. Examples of these patterns include hypermethylation of tumour suppressor genes and hypomethylation of oncogenes. The stability of methylation marks in ctDNA makes them attractive targets for liquid biopsy assays. Sun et al. [20] investigated 5-hydroxymethylcytosine (5hmC) signatures in ctDNA across various cancer types. Their study demonstrated that 5hmC profiles could distinguish between tumor and normal tissues and differentiate among cancer types, suggesting their potential for early detection and tumor classification.

Advancements in sequencing technologies have enabled comprehensive methylation analysis of ctDNA. Techniques such as whole-genome bisulfite sequencing allow for the detection of methylation changes across the genome, providing insights into the epigenetic landscape of tumors. These approaches have been applied to identify cancer-specific methylation patterns, offering opportunities for developing non-invasive screening tests [19].

2.1.3. Technological Advances and Clinical Applications

The sensitivity and specificity of ctDNA analysis have been enhanced by technological innovations. Next-generation sequencing (NGS) technologies and digital PCR enable the detection of low-frequency mutations and subtle methylation changes in ctDNA, even when present at low concentrations [21]. These advancements have paved the way for integrating ctDNA assays into clinical practice. One notable application is the identification of resistance mutations in metastatic cancer. In non-small cell lung cancer (NSCLC), for example, the emergence of the EGFR T790M mutation confers resistance to first-line tyrosine kinase inhibitors [22]. Monitoring ctDNA allows for the timely identification of such mutations, guiding the selection of subsequent therapies. Desai et al. [23] demonstrated that ctDNA analysis could detect EGFR mutations with high concordance to tissue biopsies, facilitating rapid and non-invasive assessment of resistance mechanisms.

Furthermore, ctDNA methylation patterns have been explored for early cancer detection. A study by Wang et al. [24] developed a blood-based assay targeting methylation markers across multiple cancer types. The assay achieved high sensitivity and specificity in detecting cancers at various stages, highlighting the promise of ctDNA methylation analysis as a screening tool.

2.1.4. Challenges and Future Directions

Despite the promising applications, several challenges hinder the widespread adoption of ctDNA analysis. The low abundance of ctDNA, especially in early-stage cancers, necessitates highly sensitive detection methods. Pre-analytical variables, such as blood collection, processing, and storage conditions, can impact ctDNA yield and integrity [25]. Standardization of protocols is necessary to guarantee dependability and repeatability across labs. Additionally, distinguishing ctDNA from normal cell-free DNA requires precise analytical techniques. Bioinformatics tools capable of differentiating tumor-specific alterations from benign variations are crucial for accurate interpretation. Integrating ctDNA analysis with other biomarkers, such as circulating tumor cells and protein markers, may enhance diagnostic performance and provide a more comprehensive view of tumor biology [25,26].

To determine the therapeutic value of ctDNA biomarkers, future studies should concentrate on confirming them in sizable, prospective clinical trials. Exploring the combination of ctDNA analysis with imaging modalities and other diagnostic tools could lead to more robust cancer detection and monitoring strategies [25]. As technologies evolve and our understanding of ctDNA biology deepens, liquid biopsies have the potential to transform cancer therapy by allowing individualized and flexible therapeutic strategies.

2.2. Circulating Tumor Cells (CTCs): Role in Metastasis and Phenotypic Analysis

Cancer cells that separate from the main or metastasized tumours and enter the bloodstream are known as circulating tumour cells, or CTCs. Their presence is a critical factor in the metastatic cascade, leading to the spread of cancer to distant organs. Understanding the role of CTCs in metastasis and their phenotypic characteristics offers valuable insights into cancer progression and potential therapeutic interventions [27].

2.2.1. Role in Metastasis

The phases involved in the metastatic process include local invasion, bloodstream intravasation, extravasation into distant tissues, survival in circulation, and colonization to produce new tumours [28]. CTCs are essential to this procedure, particularly during intravasation and dissemination.

Recent studies have challenged the traditional view that CTCs travel as single cells [27,29]. The "cancer exodus hypothesis" posits that CTC clusters—aggregates of two or more tumor cells—maintain their multicellular structure throughout metastasis. These clusters intravasate, circulate, and extravasate as cohesive units, significantly enhancing their metastatic potential compared to single CTCs [30,31]. This multicellularity provides advantages such as increased survival, proliferation, and resistance to apoptosis. For instance, research has shown that patients with prostate cancer exhibiting CTC clusters have a shorter mean survival rate compared to those with only single CTCs, underscoring the aggressive nature of clustered CTCs [30]. Moreover, because of their distinct gene expression patterns, CTC clusters are more resistant than individual tumour cells and can avoid several cancer treatments. This resistance further complicates treatment strategies and highlights the need for targeted therapies addressing the specific characteristics of CTC clusters [32].

2.2.2. Phenotypic Analysis

Phenotypic characterization of CTCs involves assessing their morphological and molecular attributes, which can provide insights into their origin, metastatic potential, and resistance mechanisms. CTCs can be categorized based on the expression of epithelial markers, size, and apoptotic status:

- ***Traditional CTCs****:* These cells exhibit an intact, viable nucleus; express epithelial markers such as EpCAM and cytokeratins; lack hematopoietic markers like CD45; and are typically larger with irregular shapes [33].
- ***Cytokeratin-negative CTCs****:* These lack epithelial markers, possibly indicating an undifferentiated state or a mesenchymal phenotype due to epithelial-mesenchymal transition (EMT). Such cells may be more resistant to therapies and possess higher metastatic potential [34].
- ***Apoptotic CTCs****:* These are CTCs undergoing programmed cell death, identifiable by nuclear fragmentation or cytoplasmic blebbing. Monitoring the ratio of viable to apoptotic CTCs can provide clues to treatment efficacy [35].
- ***Small CTCs****:* These are cytokeratin-positive and CD45-negative but similar in size and shape to white blood cells. They have been implicated in aggressive disease progression and may differentiate into small cell carcinomas, requiring distinct therapeutic approaches [36].

CTC clusters can be homotypic, consisting solely of tumor cells, or heterotypic, incorporating other cell types such as white blood cells, fibroblasts, endothelial cells, and platelets. Heterotypic clusters, also known as microemboli, might enhance metastatic potential by facilitating immune evasion and promoting survival in circulation [36,37].

2.2.3. Clinical Implications

Identifying and evaluating CTCs, especially clusters, using liquid biopsies provides useful prognostic data. The presence of CTC clusters is associated with increased metastatic potential and poorer prognosis. For example, research has indicated that individuals with prostate cancer

who have only single CTCs exhibit an eight-fold longer mean survival rate compared to those with CTC clusters [38]. Understanding the phenotypic diversity of CTCs can inform treatment strategies. Identifying EMT markers or stem cell-like properties in CTCs may indicate resistance to conventional therapies, suggesting the need for alternative or combination treatments. Additionally, monitoring apoptotic CTCs can serve as a real-time indicator of therapeutic efficacy, allowing for timely adjustments to treatment plans [39].

2.3. Extracellular Vesicles & Exosomes: Biomarker Potential and Intercellular Communication

Cells release particles into the extracellular environment called extracellular vesicles (EVs), which include exosomes and microvesicles and are encased in a lipid bilayer. These vesicles carry bioactive substances including nucleic acids, proteins, and lipids between cells, facilitating intercellular communication. In oncology, EVs have garnered interest due to their potential as biomarkers and their role in mediating tumor progression [40].

2.3.1. Biogenesis and Composition of Exosomes

Exosomes, a subset of EVs, originate from the endosomal pathway. Their formation starts with the endosomal membranes budding inward, which produces multivesicular bodies (MVBs) containing intraluminal vesicles. These intraluminal vesicles are discharged as exosomes into the extracellular area when MVBs fuse with the plasma membrane. Exosomes usually have a diameter of 30 to 150 nanometres and contain molecular cargo that is representative of the cell from which they originated. This load includes various proteins, lipids, mRNAs, and microRNAs, enabling exosomes to influence recipient cell behavior [41].

Biomarker Potential of Exosomes

The molecular composition of exosomes provides a snapshot of the parent cells' physiological or pathological condition, making them valuable as non-invasive biomarkers for the detection and tracking of cancer. Tumor-derived exosomes have been found to contain specific proteins and nucleic acids associated with malignancy. For instance, exosomes from glioblastoma multiforme (GBM) patients have been shown to carry amplified oncogene sequences and retrotransposon elements, which can be detected in the circulation, offering a potential diagnostic avenue.

Moreover, exosomal integrins have been implicated in organ-specific metastasis. Hoshino et al. [42] demonstrated that tumor exosome integrins determine organotropic metastasis, suggesting that the integrin profiles of circulating exosomes could predict metastatic sites, thereby aiding in prognosis and personalized treatment strategies.

Intercellular Communication Mediated by Exosomes

By delivering their chemical payload to recipient cells, exosomes play a crucial part in intercellular communication and influence a number of physiological and pathological processes. This transfer can influence immune responses, angiogenesis, and tumor progression. For example, bone marrow progenitor cells can be taught to adopt a pro-metastatic phenotype by tumor-derived exosomes via the MET receptor tyrosine kinase pathway, facilitating the establishment of pre-metastatic niches [43]. Additionally, exosomes have been shown to mediate the horizontal movement of genetic material across cells, including microRNAs and mRNAs. This mechanism allows for the modulation of gene expression in recipient cells, contributing to tumorigenesis and the tumor microenvironment's dynamic nature. For instance, exosomes derived from melanoma cells can transfer microRNAs to recipient cells, promoting tumor growth and metastasis [43,44].

2.3.2. Clinical Applications and Future Directions

The unique properties of exosomes have spurred interest in their clinical applications. Their stability in bodily fluids and capacity to mirror their parent cells' molecular traits make them attractive candidates for liquid biopsy approaches in cancer diagnostics. Standardized techniques for isolating and characterizing exosomes are being developed to harness their full potential as biomarkers. Furthermore, exosomes are being explored as therapeutic vehicles due to their natural biocompatibility and ability to deliver cargo to specific cells. Modifying exosomes to transport antimicrobial agents like short interfering RNAs or chemotherapeutic drugs, offers a promising strategy for targeted cancer therapy [45].

2.4. MicroRNAs (miRNAs) and Proteins: Emerging Roles in Cancer Detection and Classification

With respect to liquid biopsies, microRNAs (miRNAs) and proteins have emerged as pivotal biomarkers, offering profound insights into cancer detection and classification. These molecular entities, detectable in various body fluids, provide a non-invasive window into the oncogenic processes, facilitating early diagnosis and personalized treatment strategies [46].

2.4.1. MicroRNAs (miRNAs) in Cancer Detection

Small, non-coding RNA molecules called miRNAs control post-transcriptional expression of genes. Their dysregulation has been linked to the development and spread of a number of cancers. Notably, miRNAs exhibit remarkable stability in body fluids, making them ideal candidates for liquid biopsy-based diagnostics. New developments have resulted in the development of miRNA biosensors, which enable the detection of specific miRNA signatures associated with different cancer types. These biosensors have been integrated into point-of-care (POC) testing devices, allowing for sensitive and quick miRNA analysis in clinical settings. For instance, urine samples containing miR-21 can be detected using electrochemical biosensors, achieving detection limits as low as 2 nanomolar within a two-hour timeframe. Such innovations underscore the potential of miRNA-based diagnostics in facilitating early cancer detection and monitoring disease progression [47].

2.4.2. Proteins as Biomarkers in Liquid Biopsies

Proteins, being the functional executors of cellular processes, reflect the physiological and pathological states of an organism. In the context of cancer, aberrant protein expression, post-translational modifications, and the presence of tumor-specific isoforms serve as valuable biomarkers for disease detection and classification [48]. A notable advancement in this arena is the development of a urine-based test for early lung cancer detection. Researchers have identified proteins released by senescent cells—often referred to as "zombie" cells—that can reprogram their environment to support cancer cell emergence. By utilizing an injectable sensor that interacts with these proteins, a detectable compound is released into the urine, signaling potential early-stage lung cancer. This innovative approach, currently progressing towards human trials, exemplifies the utility of protein biomarkers in non-invasive cancer diagnostics [48,49].

2.4.3. Integration of miRNA and Protein Biomarkers

The convergence of miRNA and protein biomarker analysis holds promise for enhancing the liquid biopsies' sensitivity and specificity. Combining these molecular signatures can give a thorough rundown of tumour biology, enabling more accurate cancer detection and classification [46,50]. As research advances, the integration of multi-omic approaches in liquid biopsy platforms is anticipated to revolutionize personalized oncology, leading to improved patient outcomes through tailored therapeutic interventions.

3. Technological Advancements in Liquid Biopsy

The evolution of liquid biopsy technology has transformed how cancer is detected, monitored, and managed. With continuous improvements in sensitivity, specificity, and throughput, novel platforms now enable the identification of minute traces of tumor-derived material circulating in body fluids. These advancements (see figure 4) are redefining early diagnosis, allowing clinicians to capture critical molecular changes in real time without relying on invasive tissue biopsies. From next-generation sequencing (NGS) to digital PCR and microfluidic-based enrichment techniques, the growing arsenal of cutting-edge tools is enhancing the precision of cancer diagnostics and treatment monitoring [51]. This section delves into the key breakthroughs that are influencing liquid biopsy's future, highlighting the technologies that are improving the identification of extracellular vesicles, circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), and other critical biomarkers. Various technologies have been developed to detect circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) in liquid biopsies. Table 3 compares major detection techniques, focusing on sensitivity, specificity, cost, and their respective advantages and limitations.

Table 3 Comparison of Detection Techniques for ctDNA and CTCs

Technology	Target	Sensitivity	Specificity	Cost	Major Advantages	Limitations
Next-Generation Sequencing (NGS)	ctDNA	High	High	High	Comprehensive genomic profiling; detects multiple mutations simultaneously.	Requires complex data analysis; higher cost; longer turnaround time.
Droplet Digital PCR (ddPCR)	ctDNA	Very High	Very High	Moderate	High sensitivity and specificity; quantifies rare mutations; faster results.	Limited to known mutations; not suitable for broad mutation discovery.
BEAMing (Beads, Emulsion, Amplification, Magnetics)	ctDNA	Very High	Very High	High	Combines digital PCR and flow cytometry; highly sensitive; allows for rare mutation detection.	Complex and labor-intensive; higher cost; limited availability.
CellSearch System	CTCs	Moderate	High	High	FDA-approved for certain cancers; standardized method; provides prognostic information.	Limited sensitivity; may miss CTC subpopulations; expensive equipment.

Microfluidic Devices	CTCs	High	High	Variable	Captures CTCs based on size and deformability; potential for high purity and viability of captured cells.	Technology still under development; variability in performance; not yet widely available clinically.

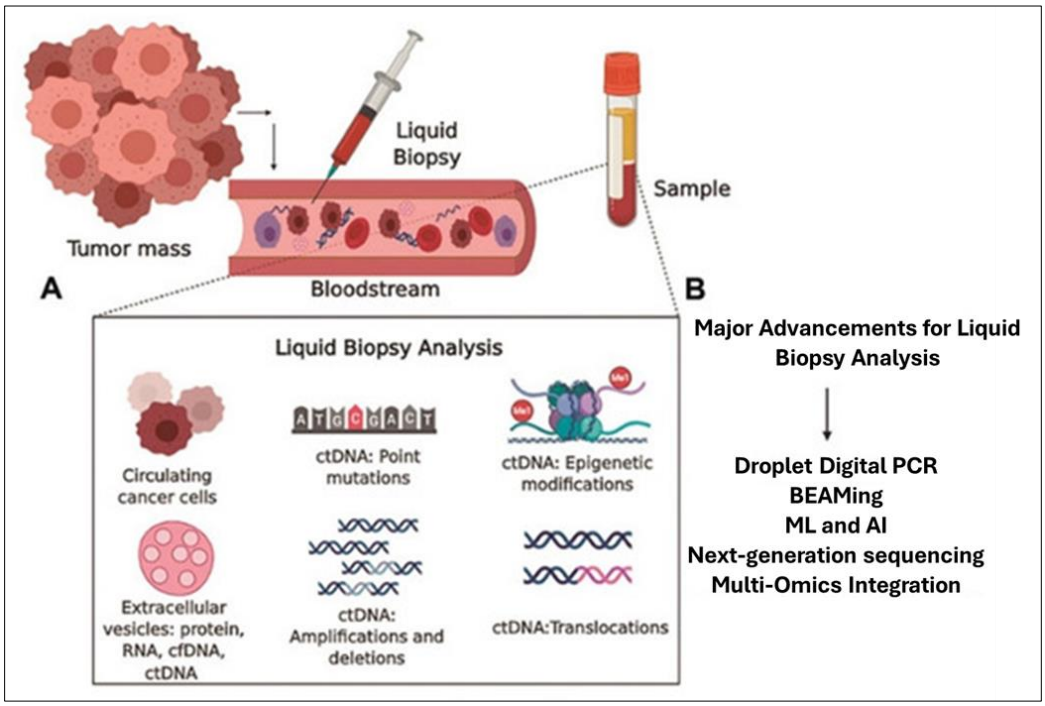

Figure 4 Schematic representation of Liquid Biopsy Analysis and Major Technological Advancements in Liquid Biopsy. Modified from Ref. [48], with permission

3.1. Next-Generation Sequencing (NGS) and Its Role in ctDNA Analysis

The examination of circulating tumour DNA (ctDNA) has been transformed by next-generation sequencing (NGS), offering unprecedented insights into the genetic landscape of cancers through minimally invasive means [21]. By enabling comprehensive genomic profiling from peripheral blood samples, NGS facilitates early detection, real-time monitoring, and personalized treatment strategies for cancer patients. One notable advancement in this field occurred when researchers at Stanford University developed the technique known as Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq) in 2014. CAPP-Seq enhances the sensitivity of ctDNA detection, ability to recognize one mutated DNA molecule out of 10,000 normal ones. Because of its great sensitivity, low-frequency mutations may be detected, which is essential for monitoring minimal residual illness and early cancer diagnosis. The technique has been effectively used to treat a number of cancer types, including pancreatic cancer, diffuse large B-cell lymphoma (DLBCL), and non-small-cell lung cancer (NSCLC), among others [52].

In addition to CAPP-Seq, companies like Guardant Health have developed NGS-based liquid biopsy tests such as Guardant360. Launched in 2014, Guardant360 profiles multiple ctDNA genes simultaneously from blood samples, aiding oncologists in guiding personalized treatment plans for late-stage cancer patients and matching them with appropriate clinical trials. The test's ability to provide comprehensive genomic information without the need for invasive tissue biopsies represents a significant advancement in cancer care [53,54].

The integration of NGS into ctDNA analysis has also facilitated the detection of various genomic changes, such as insertions and deletions (indels), point mutations, structural variations, and copy number variations. This comprehensive detection capability is essential for understanding tumor heterogeneity and developing targeted therapies. Moreover, the cost-effectiveness of targeted NGS approaches, compared to whole-genome or whole-exome sequencing, makes them more accessible for routine clinical use [52].

Despite these advancements, challenges remain in ctDNA analysis using NGS. Factors such as low ctDNA concentrations, potential sample contamination, and sequencing errors can affect the accuracy of detection. Ongoing research aims to address these limitations by improving error suppression techniques and enhancing the sensitivity of NGS platforms.

3.2. Droplet Digital PCR (ddPCR) and BEAMing for High-Sensitivity Detection

Advancements in molecular diagnostics have significantly enhanced the sensitivity and precision of liquid biopsies, particularly through techniques like BEAMing (Beads, Emulsion, Amplification, Magnetics) and Droplet Digital PCR (ddPCR). These methodologies enable the detection and quantification of rare genetic mutations, even amidst a high background of wild-type DNA, thereby facilitating early cancer detection and monitoring [55,56].

3.2.1. Droplet Digital PCR (ddPCR)

As an improvement on conventional PCR, ddPCR divides a DNA sample into thousands of nanoliter-sized droplets, each of which functions as a separate PCR reaction vessel. This partitioning allows for absolute quantification of target DNA molecules without the need for standard curves [57]. By isolating DNA molecules into separate droplets, ddPCR minimizes competition between rare mutant sequences and abundant wild-type sequences, thereby enhancing the detection sensitivity of rare mutations. This high sensitivity is particularly beneficial in liquid biopsy applications, where the concentration of circulating tumor DNA (ctDNA) can be exceedingly low. Studies have demonstrated that Even when there is a 200,000-fold excess of wild-type DNA present, ddPCR can capture mutant DNA, offering a sensitivity that is 2,000 times greater than conventional quantitative PCR methods [58].

3.2.2. BEAMing (Beads, Emulsion, Amplification, Magnetics)

BEAMing integrates principles from digital PCR and flow cytometry to identify and quantify specific somatic mutations with superior sensitivity. The inception of the procedure is marked by DNA extraction from a patient's blood or plasma sample, followed by a pre-amplification step targeting regions of interest. The amplified DNA is then mixed with magnetic beads and emulsified into millions of microdroplets, each containing a single DNA molecule and a bead. Within these droplets, PCR amplification occurs, coating each bead with thousands of copies of the DNA fragment. Subsequently, the beads are recovered, hybridized with fluorescent probes specific to either wild-type or mutant sequences, and analyzed via flow cytometry. This approach allows for the precise quantification of mutant alleles, even when they are present at very low frequencies. BEAMing has demonstrated a sensitivity threshold of 0.01%, making it a powerful tool for detecting rare tumor DNA molecules in a high background of normal DNA [59-61].

3.2.3. Clinical Applications and Impact

The high sensitivity and specificity of ddPCR and BEAMing have made them invaluable in clinical oncology, particularly for non-invasive cancer diagnostics and monitoring. These techniques quantify the concentrations of ctDNA in bodily fluids, thereby allowing for the discovery of minimum residual disease, evaluation of therapy response, and early relapse detection. For instance, in colorectal cancer patients, BEAMing has been utilized to detect mutations in plasma, supplying up-to-date information on tumour dynamics and supporting individualised treatment plans [61]. Similarly, ddPCR has been employed to monitor tumor load and treatment efficacy in various cancers by measuring rare mutations in ctDNA from patients' blood samples [62].

3.3. Machine Learning and Artificial Intelligence in Liquid Biopsy Data Interpretation

The incorporation of machine learning (ML) and artificial intelligence (AI) into liquid biopsy data interpretation marks a transformative leap in oncology diagnostics. These technologies improve the capacity to identify and track cancers through non-invasive means, offering unprecedented accuracy and efficiency.

3.3.1. Enhancing Early Cancer Detection

The analysis of complex genomic data derived from liquid biopsies has benefited greatly from the use of AI and ML techniques. A notable example is the Cancer Likelihood in Plasma (CLiP) method, which integrates various genomic features to detect early-stage lung cancer [63]. Developed by Chabon et al. [63] at Stanford, CLiP employs ensemble learning techniques to differentiate tumor-derived mutations from those arising due to clonal hematopoiesis. By considering factors such as fragment length and mutational signatures, CLiP has demonstrated the capability to differentiate risk-matched controls from early-stage lung cancers in several cohorts [63,64].

3.3.2. Advancements in Spectroscopic Analysis

Beyond genomic data, AI has been applied to spectroscopic analysis of biofluids. Dxcover Limited, a Scottish company, has developed a platform that combines infrared spectroscopy with AI algorithms to detect early-stage cancers. Their 'Drop, Dry, Detect' technology analyzes blood samples to identify spectral patterns indicative of malignancies. This approach offers rapid results and has the potential to screen for multiple cancer types simultaneously, thereby improving early detection rates [65].

3.3.3. Fragmentomics and Tissue-of-Origin Analysis

AI and ML have also been pivotal in fragmentomics, the study of cfDNA fragmentation patterns. Techniques such as EPIC-Seq utilize AI to analyze cfDNA fragment sizes and end motifs, providing insights into the tissue of origin and aiding in cancer detection. By employing supervised machine learning methods like Random Forest and Logistic Regression on shallow whole-genome sequencing data, these approaches can classify cancerous and healthy patients, offering a non-invasive diagnostic tool [66].

3.3.4. Integration with Imaging Modalities

The application of AI extends to integrating liquid biopsy data with imaging modalities. In partnership with United International University and Australian Catholic University, researchers at Charles Darwin University have created an AI model that can accurately diagnose respiratory conditions including pneumonia and COVID-19 from lung ultrasound videos with a 96.57% accuracy rate [67]. This model examines video frames for lung features and patterns, classifying the ultrasound into diagnostic categories. Such integration exemplifies the potential of AI in enhancing diagnostic accuracy by combining molecular and imaging data [67].

3.4. Multi-Omics Integration: Combining Genomics, Proteomics, and Metabolomics for Enhanced Accuracy

The advent of multi-omics integration—melding genomics, proteomics, and metabolomics— has brought about a new age in liquid biopsy diagnostics by providing a more thorough understanding of cancer biology and improving diagnostic precision. This holistic approach (see figure 5) makes it possible to analyze several molecular levels at once, making it easier to find complex biomarker signatures and enhancing the accuracy of cancer detection and classification [68].

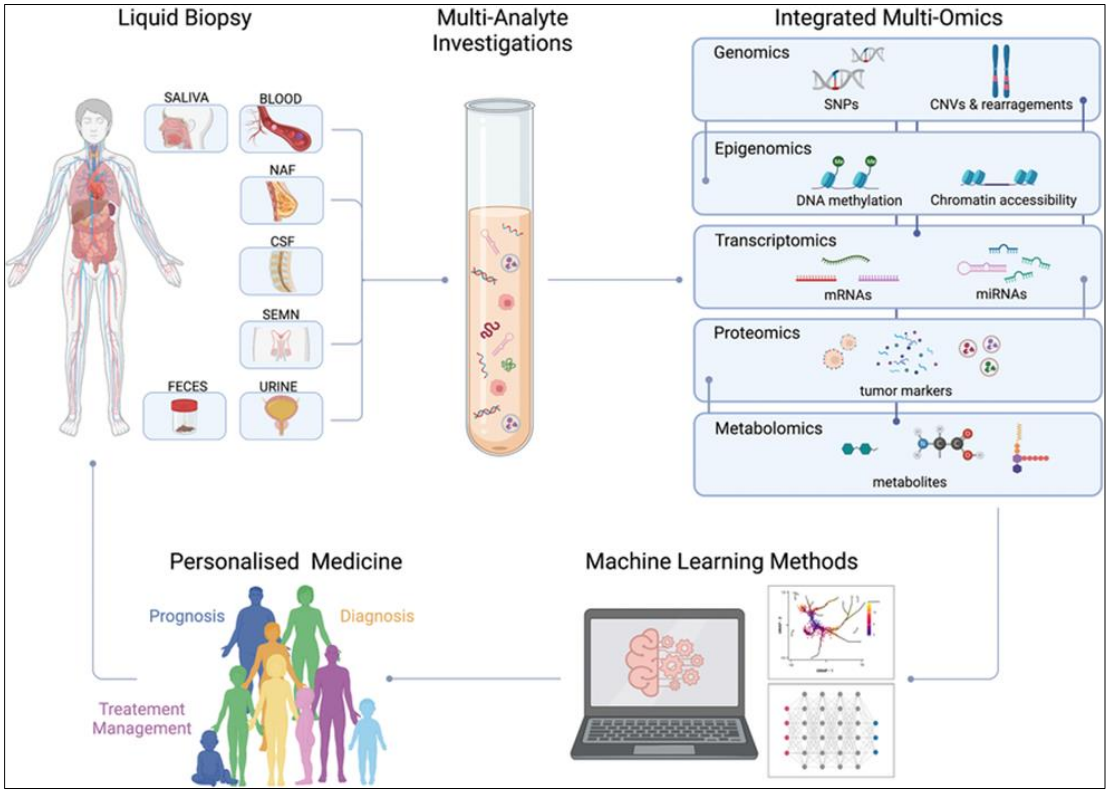

Figure 5 Schematic diagram showing the Multi-Omics Integration (ie, the integration of genomics, proteomics, and metabolomics, etc) in liquid biopsy. Reproduced with permission from Ref. [68]

3.4.1. Genomics in Liquid Biopsy

Genomic analysis in liquid biopsies primarily focuses on detecting circulating tumor DNA (ctDNA), which harbors tumor-specific genetic alterations. Next-generation sequencing (NGS) and other advanced sequencing technologies allow for the identification of insertions, deletions, copy number changes, and single nucleotide mutations in ctDNA [69]. These genomic aberrations serve as critical biomarkers for detecting cancer early, tracking treatment results, and determining minimal residual disease. For instance, clinicians can establish precise diagnoses and adjust targeted therapy by using NGS-based molecular diagnostics, which offer thorough genomic information regarding tumor-related variants and structural alterations [70].

3.4.2. Proteomics in Liquid Biopsy

Proteomic profiling in liquid biopsies consists of the extensive investigation of proteins, including their expression levels, post-translational modifications, and interactions. Mass spectrometry-based techniques have been instrumental in identifying protein indicators found in bodily fluids including urine and blood, which reflect the physiological and pathological states of an individual. These protein biomarkers can indicate tumor presence, subtype, and progression, thereby aiding in cancer diagnosis and prognosis. For example, the identification of particular protein signatures in urine samples has been investigated as a potential early lung cancer screening method, highlighting the potential of proteomics in non-invasive cancer diagnostics [71,72].

3.4.3. Metabolomics in Liquid Biopsy

Metabolomics is the thorough examination of metabolites (tiny compounds involved in metabolism) within biological samples. Cancer cells exhibit distinct metabolic profiles due to altered metabolic pathways, leading to the production of unique metabolite signatures. By analyzing these metabolomic patterns in body fluids, researchers can identify biomarkers indicative of cancer presence and progression. Metabolites are detected and quantified using techniques like mass spectrometry and nuclear magnetic resonance (NMR) spectroscopy, offering insights into tumor metabolism and potential therapeutic targets [73,74].

3.4.4. Integration of Multi-Omics Data

The convergence of proteomics, metabolomics, and genomics data through integrative computational approaches enables a more comprehensive understanding of tumor biology. This multi-omics integration allows for the development of innovative biomarkers and elucidates the complex molecular mechanisms underlying cancer development and progression. By combining diverse datasets, researchers can construct detailed molecular profiles of tumors, leading to improved diagnostic accuracy and personalized treatment strategies. For instance, in order to provide integrated multi-omics methods for precision diagnostics, the Human Personal Omics Profiling project was founded, underscoring the potential of this strategy in enhancing patient care [75].

3.5. Challenges and Future Directions

Despite its promise, multi-omics integration in liquid biopsy faces challenges, including data complexity, standardization of analytical methods, and the need for sophisticated bioinformatics tools to handle large datasets. Ongoing research aims to address these issues by developing robust computational frameworks and validating multi-omics biomarkers in clinical settings [76]. As these challenges are overcome, multi-omics integration is poised to revolutionize liquid biopsy diagnostics, offering more accurate, non-invasive, and personalized approaches to cancer detection and management.

4. Clinical Applications of Liquid Biopsy

In clinical practice, liquid biopsy is revolutionizing cancer diagnosis, monitoring, and treatment. By capturing tumor-derived materials such as extracellular vesicles from bodily fluids, circulating tumor cells (CTCs), and circulating tumor DNA (ctDNA), this approach provides valuable molecular insights instead of requiring the sampling of invasive tissue. The ability to detect minimal residual disease, assess treatment response, and track tumor evolution in real time has positioned liquid biopsy as a powerful tool in precision oncology [77]. This section explores how this technology is being integrated into routine cancer care, highlighting its role in early detection, therapeutic decision-making, and patient monitoring. Liquid biopsy offers multiple clinical applications, ranging from early detection to real-time monitoring and treatment adaptation. Table 4 below provides a comparative overview of these applications and their respective biomarkers.

Table 4 Comparison of Different Liquid Biopsy Applications in Cancer Management

Application	Biomarkers Analyzed	Key Benefits	Clinical Challenges
Early Cancer Detection	ctDNA, miRNAs	Enables non-invasive screening for early-stage cancers.	Sensitivity issues in early-stage disease.
Treatment Monitoring	ctDNA, CTCs	Tracks treatment response and resistance mutations.	Requires frequent testing for dynamic assessment.
Minimal Residual Disease Detection	ctDNA, Exosomes	Identifies residual cancer post-treatment.	Low ctDNA levels can affect detection accuracy.
Predicting Drug Resistance	ctDNA, CTCs, Proteins	Detects emerging mutations that confer resistance to therapy.	Requires rapid turnaround for clinical decisions.

4.1. Early Cancer Detection: Current FDA-Approved Tests and Emerging Methodologies

The landscape of early cancer detection has been significantly transformed by the advent of liquid biopsy technologies, which enable the identification of malignancies through non-invasive blood tests [78]. These advancements have resulted in the creation and acceptance of numerous tests that are now integral to clinical practice. Over the past decade, the U.S. Food and Drug Administration (FDA) has approved several liquid biopsy tests that have transformed cancer diagnostics and treatment strategies [10,79]. These tests enable non-invasive detection and monitoring of various cancers by analyzing biomarkers such as circulating tumor DNA (ctDNA) and circulating tumor cells (CTCs) in blood samples. Table 5 provides an overview of FDA-approved liquid biopsy tests, their target cancers, detected biomarkers, approval years, and clinical applications.

Table 5 FDA-Approved Liquid Biopsy Tests and Their Applications

Test Name	Company	Cancer Type	Biomarkers Targeted	Approval Year	Clinical Applications
Guardant360 CDx	Guardant Health	Non-Small Cell Lung Cancer (NSCLC)	EGFR mutations	2020	Detection of EGFR mutations to guide treatment with osimertinib in NSCLC patients [80].
FoundationOne Liquid CDx	Foundation Medicine	Multiple cancers, including NSCLC and prostate cancer	BRCA1/2, EGFR mutations, ALK rearrangements, PIK3CA mutations	2020	Comprehensive genomic profiling to guide targeted therapies across various cancer types; approved as a companion diagnostic for multiple therapies [80].
CellSearch CTC Test	Menarini Silicon Biosystems	Metastatic breast, prostate, and colorectal cancers	Circulating Tumor Cells (CTCs)	2004	Enumeration of CTCs to monitor disease progression and prognosis in metastatic cancer patients [81].
Epi proColon	Epigenomics AG	Colorectal Cancer	SEPT9 gene methylation	2016	Screening test for colorectal cancer by detecting methylated SEPT9 DNA in plasma samples. [82]
Cobas EGFR Mutation Test v2	Roche Diagnostics	Non-Small Cell Lung Cancer (NSCLC)	EGFR mutations	2016	Detection of specific EGFR mutations to identify NSCLC patients eligible for targeted therapy with erlotinib [83].

Guardant360 CDx (Expanded Use)	Guardant Health	Multiple solid tumors	Comprehensive genomic profiling (over 60 genes)	2020	Broad genomic profiling to guide treatment decisions across various solid tumors; approved as a companion diagnostic for multiple therapies [84].
FoundationOne Liquid CDx (Expanded Use)	Foundation Medicine	Multiple solid tumors	Comprehensive genomic profiling (over 300 genes)	2020	Extensive genomic profiling to inform targeted therapy decisions across various solid tumors; approved as a companion diagnostic for multiple therapies [85].
Shield™	Guardant Health	Colorectal Cancer	ctDNA mutations specific to colorectal cancer	2024	Non-invasive screening test for colorectal cancer by detecting tumor-specific DNA mutations in blood samples [86].

Note: The approval years indicate the initial FDA approval for each test. Some tests have received expanded approvals for additional applications in subsequent years.

4.1.1. FDA-Approved Liquid Biopsy Tests

One notable example is Guardant Health's Shield test, designed for colorectal cancer screening. Shield was approved by the United States Food and Drug Administration in July 2024, marking a significant milestone in non-invasive cancer diagnostics. This blood-based test offers an alternative to traditional screening methods, aiming to improve patient compliance and early detection rates [87,88].

4.1.2. Emerging Methodologies in Early Cancer Detection

Beyond FDA-approved tests, several innovative methodologies are under investigation, aiming to improve the specificity and sensitivity of early cancer detection.

- ***Nanorobotics****:* Researchers are exploring the use of nanorobots—tiny devices smaller than blood cells—that can deliver drugs directly to tumor sites, minimizing harm to healthy cells. British biotech firm Nanovery is developing nanorobots for early cancer detection, highlighting their potential in liquid biopsies by identifying cancer DNA in blood samples [89,90].
- ***Infrared Spectroscopy****:* Dxcover Limited has developed a technology that detects signs of cancer using artificial intelligence trained models. This 'Drop, Dry, Detect' technology works in minutes to detect signs of cancer, offering a rapid and non-invasive diagnostic alternative [65].
- ***Protease Activity Mapping****:* Researchers from Oregon Health & Science University have developed an inexpensive blood test, called PAC-MANN, that detects pancreatic cancer even in its early stages. The test measures changes in protease enzyme activity, a hallmark of cancer progression. When combined with the existing CA 19-9 test, it achieves 85% accuracy in diagnosing early-stage pancreatic cancer. The PAC-MANN test uses only a small blood sample, takes 45 minutes to run, and costs less than a penny per sample, making it accessible for rural and underserved areas [91].

These emerging methodologies, alongside FDA-approved tests, represent a significant shift toward more accessible and accurate early cancer detection strategies. As research progresses, these innovations hold the promise of further improve patient outcomes by intervening and diagnosing problems early.

4.1.3. Real Case Studies with The Galleri Test from GRAIL

The Galleri test from GRAIL is a multi-cancer early detection (MCED) blood test that can detect more than 50 cancer types in asymptomatic people 50 years of age and older. The test identifies aberrant methylation patterns linked to cancer by examining bloodstream cell-free DNA (cfDNA), and when a cancer signal is found, it predicts the tissue of origin [92,93].

In a clinical validation study involving 2,823 participants with cancer and 1,254 without, the Galleri test demonstrated a specificity of 99.5%, demonstrating a low false-positive rate. The sensitivity increased with the degree of the

malignancy, reaching 51.5% overall: First degree - 16.8%; second degree - 40.4%; third degree - 77.0%; and fourth degree - 90.1%. For 12 pre-specified cancer categories that cause almost two-thirds of all cancer-related fatalities in the United States each year, the sensitivity from first degree to third degree was 67.6%. Additionally, in 88.7% of true-positive instances, the test correctly identified the target tissue for the malignancy [93].

The Galleri test is intended to complement existing cancer screenings, such as mammography and colonoscopy, and is recommended for adults at a higher risk of developing cancer, particularly those aged 50 or older. It requires a prescription from a licensed healthcare provider [93].

The NHS in England is conducting a randomized controlled trial, known as NHS-Galleri, to evaluate how well the test works to lower the incidence of late-stage cancer. Over 140,000 individuals aged 50 to 77 without a cancer diagnosis have been enrolled, with results expected in the summer of 2026 [94].

While the Galleri test shows promise in detecting multiple cancer types early, it is essential to consider its limitations, such as varying sensitivity across different cancer stages and types. Ongoing studies aim to further validate its clinical efficacy and cost-effectiveness before widespread implementation.

4.2. Real-Time Treatment Monitoring: Tracking Tumor Evolution and Therapeutic Response

The dynamic nature of cancer necessitates continuous monitoring to effectively assess therapeutic efficacy and adapt treatment strategies accordingly. Traditional imaging modalities and tissue biopsies, while informative, often fall short in recording the tumours' temporal variability due to their invasive nature and limited sampling frequency. In this regard, liquid biopsy has become a game-changing technique that allows for the study of circulating biomarkers including circulating tumour DNA (ctDNA) and circulating tumour cells (CTCs), allowing for real-time monitoring of tumour progression and therapy response [95].

4.2.1. Monitoring Therapeutic Response

The quantification of ctDNA levels in plasma serves as a non-invasive biomarker for evaluating tumor burden and therapeutic response. A decline in ctDNA concentrations post-treatment initiation often correlates with a favorable response, whereas stable or rising levels could be a sign of disease progression or resistance. For instance, the Guardant360 test, a comprehensive liquid biopsy assay, has been utilized to monitor ctDNA dynamics in patients undergoing targeted therapies, facilitating timely adjustments to treatment regimens [96,97].

4.2.2. Detecting Minimal Residual Disease (MRD)

Assays for liquid biopsies have been useful in identifying minimal residual disease (MRD), which is the existence of cancer cells that may recur. The Guardant Reveal test, for example, is a blood-only liquid biopsy designed to detect recurring and persistent disease in colorectal cancer by identifying ctDNA. This enables oncologists to detect recurrence earlier than using conventional approaches and to identify individuals with residual disease who might benefit from extra therapy [97].

4.2.3. Assessing Treatment Resistance

In oncology, the formation of clones that are resistant to treatment presents serious difficulties. The identification of such mutations linked to resistance is made possible via liquid biopsy, allowing for the timely modification of therapeutic strategies. In breast cancer management within the NHS, liquid biopsies have been implemented to identify mutations such as ESR1, which can develop after hormone treatment and promote cancer growth. Patients testing positive for the ESR1 mutation can now access elacestrant, a targeted therapy, thereby personalizing treatment and potentially improving outcomes [8].

4.2.4. Advantages Over Traditional Monitoring

Compared to conventional tissue biopsies, liquid biopsies offer several advantages in monitoring tumor evolution and therapeutic response. One of the primary benefits is their non-invasiveness, as they require only a simple blood draw, minimizing patient discomfort and the risks associated with surgical or needle biopsies. Additionally, liquid biopsies enable real-time monitoring of tumor dynamics through serial sampling, allowing clinicians to track disease progression and detect relapse at an earlier stage. This continuous assessment is particularly valuable in guiding timely therapeutic adjustments [8]. Furthermore, liquid biopsies provide a more comprehensive tumor profile by capturing circulating tumor DNA (ctDNA) from multiple tumor sites, thereby reflecting the heterogeneity of the disease more accurately than

a single-site tissue biopsy. This holistic approach enhances precision medicine strategies, improving patient outcomes by tailoring treatments based on a more complete understanding of tumor evolution [69].

4.2.5. Real Case Studies with Guardant360

Guardant360 is a liquid biopsy test that looks for genetic changes in cancer patients by analysing circulating tumour DNA (ctDNA) in blood samples. This non-invasive method offers a comprehensive genomic profile, aiding in the selection of targeted therapies, particularly for metastatic non-small cell lung cancer (NSCLC) patients.

Clinical Validation and Impact on Survival Rates

A prospective study involving 193 advanced cancer patients, including those with NSCLC, demonstrated the clinical utility of the Guardant360 assay. In the NSCLC cohort, patients matched to targeted therapy based on Guardant360 results exhibited an objective response rate of 87% and a disease control rate of 100%. Notably, median overall survival more than doubled for these patients (31.8 months) compared to those receiving non-targeted cytotoxic therapy (12.7 months) [98]. Additionally, a study published in JCO Precision Oncology assessed the use of Guardant360 to monitor molecular response in metastatic NSCLC patients undergoing pembrolizumab-based therapy. Individuals who showed a molecular response—which is characterised by a reduction of at least 50% in the mean variant allele fraction—had better median progression-free survival (14.1 months vs. 4.4 months) and overall survival (22.1 months vs. 12.0 months) than those who did not [99].

Advantages Over Traditional Tissue Biopsy

A head-to-head study comparing Guardant360 liquid biopsy to standard tissue biopsy in advanced NSCLC patients revealed that Guardant360 detected 23.6% more actionable mutations when used as a first-line test. This suggests that liquid biopsy can uncover additional therapeutic targets, potentially leading to improved patient outcomes [100].

4.3. Minimal Residual Disease (MRD) Detection

The term "minimum residual disease" (MRD) describes the little quantity of cancer cells that might still be present in a patient's body following therapy, which may cause recurrence. Traditional imaging methods often lack the sensitivity to detect these residual cells, making early intervention challenging. Liquid biopsies have emerged as a transformative approach in this context, enabling the detection of circulating tumor DNA (ctDNA) in blood samples to monitor MRD with high precision [101,102]. Guardant Health's Guardant Reveal test exemplifies this advancement. This blood-only liquid biopsy test is intended to identify ctDNA after surgery, which helps identify individuals who may benefit from further treatment. It is used to detect residual and recurrent disease in colorectal cancer (CRC). Notably, Medicare's recent decision to cover Guardant Reveal for colon cancer patients underscores its clinical utility and potential to enhance patient outcomes [103]. By facilitating early detection of MRD, liquid biopsies empower clinicians to make informed decisions regarding adjuvant therapies, thereby reducing recurrence risk and improving survival rates.

4.3.1. Real Case Studies with Signatera for MRD Test

Signatera, developed by Natera, is a personalized molecular residual disease (MRD) test designed to detect circulating tumor DNA (ctDNA) in the bloodstream of colorectal cancer patients. By identifying ctDNA, Signatera can detect minimal residual disease that may not be visible through conventional imaging, thereby predicting cancer recurrence earlier and informing treatment decisions [104,105].

Clinical Evidence Supporting Signatera's Efficacy

A European study involving 265 patients with stage I-III colorectal cancer utilized the Signatera test shortly after surgery and periodically over several months. The findings revealed that among the 20 patients with detectable ctDNA post-surgery, 75% experienced relapse, compared to only 13.6% of those who tested negative. Moreover, serial ctDNA analysis predicted recurrence before imaging scans by a median of eight months, demonstrating greater accuracy than the carcinoembryonic antigen (CEA) blood test [106]. According to a different study, with a mean lead time of 8.7 months, serial ctDNA analyses could predict disease recurrence up to 16.5 months before radiologic imaging [105].

4.4. Predicting Drug Resistance: Identifying Resistance Mutations for Adaptive Therapy Strategies

One major obstacle to cancer treatment is the establishment of medication resistance, which frequently results in therapeutic failure. Real-time, non-invasive detection of resistance mutations by liquid biopsies enables prompt modification of treatment approaches. In breast cancer management, the integration of liquid biopsies into clinical practice has marked a significant advancement. The NHS in England, for instance, has adopted ultra-sensitive blood

tests to detect tumor DNA, enabling the identification and monitoring of mutations such as ESR1. This mutation can develop after hormone treatment and promote cancer progression. Patients testing positive for the ESR1 mutation can now access elacestrant, a targeted therapy that offers a more personalized treatment approach [107-109]. Furthermore, the work of researchers like Alberto Bardelli has shed light on the molecular mechanisms underlying resistance to targeted therapies. By analyzing liquid biopsies, Bardelli's team has uncovered how drug-resistant clones emerge and evolve, providing insights that inform the development of adaptive therapy strategies [110,111]. By making it possible to identify resistance mutations early, liquid biopsies facilitate the implementation of adaptive therapy strategies. This proactive approach allows clinicians to modify treatment plans in response to evolving tumor profiles, thereby enhancing the effectiveness of cancer therapies and improving patient outcomes.

4.4.1. Clinical Examples

The introduction of the T790M mutation frequently results in resistance to first- and second-generation tyrosine kinase inhibitors (TKIs) in the treatment of non-small cell lung cancer (NSCLC) with epidermal growth factor receptor (EGFR) mutations [112]. Finding this mutation is essential for directing further treatment.

Role of Liquid Biopsy in Detecting T790M Mutation

To find genetic changes like the T790M mutation, circulating tumour DNA (ctDNA) in the bloodstream is analysed using liquid biopsy, which is a non-invasive technique. When tumour tissue is inaccessible, this method can be very helpful since it provides a less intrusive substitute for conventional tissue biopsies. Research has demonstrated that liquid biopsies can successfully identify the T790M mutation, enabling timely therapeutic interventions [113,114].

Osimertinib: Targeted Therapy for T790M-Positive NSCLC

Osimertinib is a third-generation EGFR-TKI that is specifically intended to target the T790M resistant mutation as well as EGFR-sensitizing mutations. Clinical trials have demonstrated its efficacy in patients with T790M-positive NSCLC, leading to its approval for this indication [115,116].

Implementing liquid biopsy to detect the T790M mutation allows for the early identification of resistance, facilitating a timely switch to osimertinib. This strategy has been associated with improved progression-free survival and overall outcomes in patients with EGFR-mutant NSCLC [115].

5. Current Limitations and Challenges

Despite the ability of liquid biopsies to transform in oncology, several obstacles continue to hinder its widespread clinical adoption. Sensitivity and specificity remain critical concerns, as distinguishing tumor-derived signals from normal cell-free DNA can be challenging, particularly in early-stage cancers. Standardization of protocols across different platforms and laboratories is another pressing issue, with variations in sample collection, processing, and data interpretation affecting reproducibility. Moreover, the high cost of some advanced techniques limits accessibility, making integration into routine clinical practice difficult in resource-limited settings. This section delves into these challenges, examining the technological, biological, and regulatory barriers that must be addressed to fully realize the promise of liquid biopsy in precision oncology [117].

5.1. Sensitivity and Specificity Issues

Liquid biopsies hold promise for non-invasive cancer diagnostics, yet challenges persist in ensuring high sensitivity and specificity. Detecting low levels of circulating tumor DNA (ctDNA) amidst abundant normal cell-free DNA can lead to false negatives, particularly in early-stage cancers [78]. Conversely, benign mutations or clonal hematopoiesis may result in false positives. Advanced techniques like Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq) have enhanced detection capabilities, achieving sensitivity to detect one mutant DNA molecule among 10,000 healthy ones [118]. However, issues such as sample cross-contamination, allelic bias, and PCR or sequencing errors can still affect accuracy. Moreover, the lack of standardized protocols across laboratories contributes to variability in results, underscoring the need for consistent methodologies to ensure reliable clinical applications [78].

5.2. Cost and Accessibility: Economic Barriers to Widespread Clinical Adoption

The implementation of liquid biopsy technologies is often hindered by economic factors. The development and execution of sophisticated assays require substantial financial investment, leading to high costs that can limit accessibility, especially in resource-constrained settings. This economic barrier poses challenges to the widespread

clinical adoption of liquid biopsies, potentially exacerbating healthcare disparities. Efforts to streamline technologies and reduce costs are essential to make these advanced diagnostics more affordable and universally accessible [79,119].

5.3. Regulatory Hurdles: FDA Approvals and Global Implementation Challenges

Navigating the regulatory landscape presents significant challenges for the integration of liquid biopsy technologies into clinical practice. In the United States, obtaining FDA approval necessitates rigorous validation to demonstrate safety and efficacy, a process that can be time-consuming and costly [120]. Globally, disparate regulatory standards and approval processes further complicate the implementation of these technologies. Harmonizing regulatory frameworks and establishing clear guidelines are crucial steps toward facilitating the global adoption of liquid biopsies in standard clinical care [25].

5.4. Ethical Considerations

The utilization of liquid biopsies raises important ethical considerations, particularly concerning data privacy and the implications of genetic risk information. Safeguarding patient genetic data is paramount to prevent misuse or discrimination. Additionally, the interpretation of genetic risk requires careful consideration to avoid unnecessary anxiety or interventions. Establishing robust ethical guidelines and engaging in transparent communication with patients are essential to address these challenges responsibly [121,122].

6. Future Directions and Emerging Innovations

The rapid evolution of liquid biopsy technologies is paving the way for breakthroughs that could redefine cancer diagnostics and treatment monitoring. Researchers are refining analytical methods to improve sensitivity, enhance multi-analyte detection, and integrate artificial intelligence for more precise data interpretation. Innovations in microfluidics and single-molecule sequencing are pushing the boundaries of early cancer detection, while novel biomarkers, such as tumor-educated platelets and circulating mitochondrial DNA, are expanding the scope of liquid biopsy applications [123]. Table 6 highlights key emerging technologies and their potential impact on the field. As these advancements progress, the focus is shifting toward making liquid biopsy not only a complementary tool but a primary diagnostic strategy in precision oncology. This section explores the promising developments shaping the future of this field and the challenges that must be addressed to bring these innovations into routine clinical practice.

Table 6 Emerging Technologies and Their Potential Impact on Liquid Biopsy

Technology	Development Stage	Potential Advantages	Current Challenges
Single-Cell Liquid Biopsy	Experimental	Allows analysis of individual tumor cells, offering insights into heterogeneity and resistance mechanisms.	Expensive and complex; requires advanced microfluidics.
Wearable Biosensors for Liquid Biopsy	Early research	Real-time monitoring; non-invasive detection of biomarkers from sweat, saliva, or interstitial fluids.	Limited biomarker range; requires validation for accuracy.
AI-Integrated Liquid Biopsy Analysis	Pilot trials	Improves accuracy by analyzing large datasets; enhances early cancer detection.	Data privacy concerns; regulatory approval challenges.
Microfluidics-Based ctDNA Isolation	Experimental	High efficiency in capturing rare ctDNA fragments; minimal sample requirement.	Requires standardization for clinical use.

6.1. Single-Cell Liquid Biopsies: Advancing Precision in CTC Analysis

Single-cell liquid biopsies are enhancing the precision of circulating tumor cell (CTC) analysis by allowing for the examination of individual tumor cells isolated from blood samples. This approach provides detailed insights into tumor heterogeneity, metastatic potential, and treatment resistance mechanisms. Technologies such as the NanoVelcro Chip have been developed to capture and analyze single CTCs, facilitating personalized treatment strategies and real-time monitoring of tumor dynamics [124-126].

6.2. Wearable and Point-of-Care Liquid Biopsy Devices

The development of wearable and point-of-care liquid biopsy devices is revolutionizing real-time patient monitoring. These innovations enable continuous tracking of biomarkers, allowing for early detection of cancer recurrence and timely therapeutic interventions. Integrating microfluidic technologies into portable devices facilitates rapid, on-site analysis of bodily fluids, enhancing patient convenience and enabling more responsive healthcare delivery [127].

6.3. Combination with AI and Blockchain for Secure Data Management

Integrating artificial intelligence (AI) and blockchain technology with liquid biopsy data management systems enhances diagnostic accuracy and ensures secure handling of sensitive patient information. AI algorithms can analyze complex datasets to identify patterns indicative of cancer, while blockchain provides a decentralized, immutable ledger for secure data storage and sharing, addressing concerns related to data privacy and integrity. However, regulatory scrutiny is essential to validate the efficacy and safety of these AI-driven diagnostic tools, as highlighted by recent evaluations of health tech firms' claims [128].

6.4. Potential for Universal Cancer Screening

The potential of liquid biopsies for universal cancer screening lies in their ability to detect multiple cancer types through a simple blood test, facilitating large-scale, population-based applications. Implementing such screening programs could lead to early detection and improved survival rates across diverse populations. However, challenges such as ensuring test accuracy, managing healthcare infrastructure, and addressing ethical considerations related to widespread genetic testing must be carefully navigated to realize this potential fully [129,130].

7. Conclusion

Liquid biopsies have transformed cancer diagnostics and monitoring by enabling the detection of circulating tumor DNA (ctDNA) in the bloodstream, providing a non-invasive alternative to traditional tissue biopsies.This advancement allows for real-time insights into tumor dynamics, facilitating early detection of mutations and personalized treatment strategies. The integration of artificial intelligence (AI) and genetic sequencing has further enhanced the precision of these diagnostics, offering tailored therapies based on individual cancer profiles.

Despite these advancements, challenges persist. Economic factors, such as the high costs associated with advanced diagnostics, limit accessibility, particularly in resource-constrained settings. Additionally, disparities in research funding and pharmaceutical interest have slowed progress in treating less common but lethal cancers, highlighting the need for equitable investment across all cancer types.

Looking ahead, the continued evolution of liquid biopsy technologies, coupled with AI integration, holds the potential to revolutionize precision oncology. These innovations could lead to earlier detection, more accurate monitoring, and personalized treatment plans, ultimately improving patient outcomes and survival rates. Addressing current challenges through collaborative efforts and equitable resource allocation will be crucial in realizing the full potential of these advancements in cancer care.

Compliance with ethical standards

Acknowledgments

The authors wish to acknowledge the collaborative effort of all contributing scholars and colleagues who jointly authored and edited this review paper. This work was conducted entirely through the intellectual and academic contributions of the authoring team, without external funding or assistance from any individual, institution, or organization.

Disclosure of conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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