

Review

Liquid Biopsy in Precision Oncology: Clinical Applications and Emerging Roles of Circulating Tumor DNA, Cell-Free DNA, and Extracellular Vesicles

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Abstract

Liquid biopsy has emerged as a transformative approach in modern oncology, offering minimally invasive access to tumor-derived biomarkers through the analysis of circulating tumor DNA, cell-free DNA, and extracellular vesicles such as exosomes. Unlike conventional tissue biopsies, liquid biopsy enables real-time monitoring of tumor dynamics, molecular heterogeneity, treatment response, and the development of therapeutic resistance. Recent advances in ultra-sensitive molecular technologies, including digital droplet polymerase chain reaction, next-generation sequencing, methylation profiling, and fragmentomic analysis, have substantially improved the sensitivity and specificity of circulating nucleic acid detection, facilitating their integration into precision cancer medicine. ctDNA analysis has demonstrated significant clinical utility across multiple malignancies, including lung, breast, colorectal, pancreatic, and prostate cancers, particularly in the identification of actionable genomic alterations, minimal residual disease, and mechanisms of acquired resistance. In parallel, cell-free DNA provides broader insights into tumor biology and systemic genomic alterations, while exosomes contribute additional layers of molecular information through the transport of nucleic acids, proteins, and signaling molecules involved in intercellular communication and tumor microenvironment modulation. The integration of artificial intelligence and machine learning approaches further enhances the interpretative power of liquid biopsy-derived datasets and supports the development of personalized therapeutic strategies. Despite these advances, important challenges remain, including low tumor fraction in early-stage disease, biological and technical variability, clonal hematopoiesis-associated false positives, assay standardization, and cost-effectiveness considerations. Nevertheless, the expanding clinical applicability of liquid biopsy technologies positions them as essential components of contemporary precision oncology. This review summarizes the biological foundations, analytical methodologies, current clinical applications, technological innovations, and future perspectives of circulating tumor DNA, cell-free DNA, and exosome-based liquid biopsies in cancer diagnosis, monitoring, and personalized treatment strategies.

Keywords: liquid biopsy; circulating tumor DNA; cell-free DNA; exosomes



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1. Introduction

The emergence of liquid biopsy technologies has significantly transformed the landscape of contemporary oncology by enabling minimally invasive molecular characterization of malignant diseases through the analysis of tumor-derived biomarkers present in body fluids. Unlike conventional tissue biopsies, which are invasive, spatially limited, and often difficult to repeat during disease progression, liquid biopsy approaches provide dynamic and real-time insights into tumor evolution, molecular heterogeneity, therapeutic response, and mechanisms of resistance [1–3]. Among the most extensively investigated analytes are circulating tumor DNA (ctDNA), cell-free DNA (cfDNA), and extracellular vesicles, particularly exosomes, which collectively represent promising tools for precision cancer diagnostics and personalized therapeutic management.

Traditional tissue-based diagnostics remain the gold standard for histopathological classification and molecular profiling of tumors. However, tissue biopsies are associated with several limitations, including procedural risks, limited accessibility in anatomically challenging lesions, sampling bias resulting from intratumoral heterogeneity, and the inability to adequately capture temporal genomic changes during treatment [4]. These limitations have accelerated the development of liquid biopsy methodologies capable of providing a more comprehensive overview of tumor biology through repeated, non-invasive sampling.

ctDNA consists of short fragments of tumor-derived nucleic acids released into the bloodstream predominantly through apoptosis, necrosis, and active cellular secretion [5–7]. Because ctDNA harbors tumor-specific genomic and epigenomic alterations, including point mutations, copy number variations, rearrangements, and methylation signatures, its analysis offers valuable information regarding tumor burden, clonal evolution, minimal residual disease (MRD), and therapeutic resistance mechanisms [8,9]. In parallel, cfDNA encompasses a broader pool of extracellular nucleic acids originating from both malignant and non-malignant cells, reflecting systemic physiological and pathological processes [10]. The increasing refinement of molecular technologies has enabled the detection of extremely low-frequency tumor-derived alterations within the complex background of circulating nucleic acids.

Recent technological advances, particularly in digital droplet polymerase chain reaction (ddPCR), next-generation sequencing (NGS), methylation profiling, and fragmentomic analyses, have markedly improved the analytical sensitivity and specificity of liquid biopsy assays [11–13]. Furthermore, the integration of artificial intelligence (AI) and machine learning algorithms into genomic data interpretation has facilitated the identification of complex molecular patterns and supported the development of increasingly personalized oncological strategies [14]. These advances have expanded the clinical applicability of liquid biopsy approaches across multiple tumor types, including lung, colorectal, breast, pancreatic, and prostate cancers [3,15–17].

Beyond ctDNA and cfDNA, exosomes have emerged as an additional source of clinically relevant molecular information. These nanoscale extracellular vesicles participate in intercellular communication and transport a diverse molecular cargo consisting of nucleic acids, proteins, lipids, and signaling molecules reflective of their cellular origin [18–21]. Their potential role in cancer diagnosis, prognosis, immune modulation, and treatment monitoring has attracted substantial scientific interest in recent years.

Despite the considerable progress achieved in the field, several biological, technical, and clinical challenges continue to limit the widespread implementation of liquid biopsy technologies. These include low tumor fraction in early-stage disease, preanalytical variability, assay standardization difficulties, interference from clonal hematopoiesis, limited concordance between tissue and plasma findings in certain contexts, and issues related to

cost-effectiveness and clinical validation [18]. Addressing these limitations will be essential for the broader integration of liquid biopsy into routine oncological practice.

This review aims to provide a comprehensive overview of the biological foundations, analytical methodologies, current clinical applications, emerging technological innovations, and future perspectives of ctDNA-, cfDNA-, and exosome-based liquid biopsy approaches in precision oncology. Particular emphasis is placed on their evolving role in early cancer detection, treatment monitoring, MRD assessment, resistance profiling, and personalized therapeutic decision-making (Figure 1).

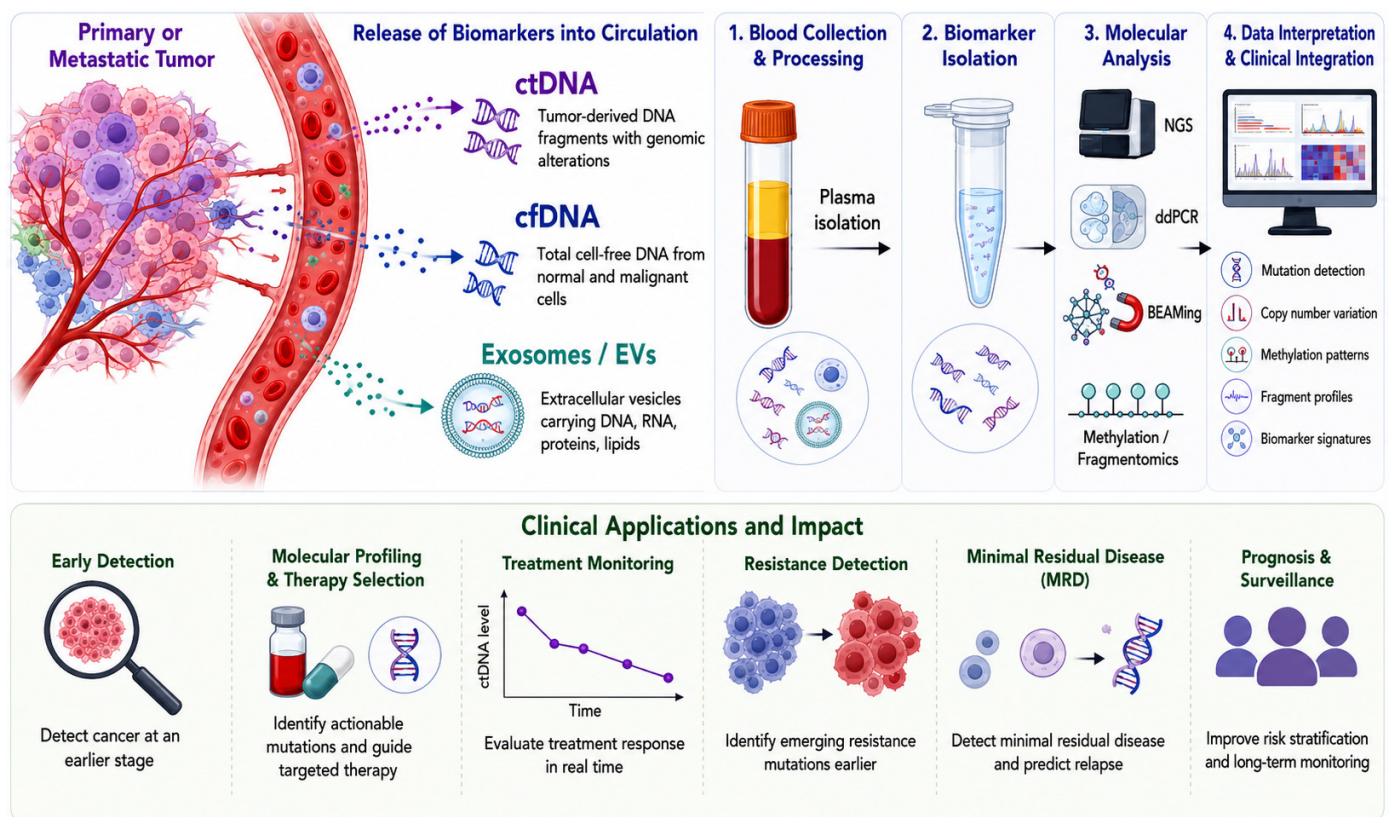


Figure 1. Overview of liquid biopsy in precision oncology. Tumor-derived biomarkers, including ctDNA, cfDNA, exosomes, and CTCs are released into the bloodstream and isolated from plasma samples. Molecular analyses, including digital PCR, next-generation sequencing, methylation profiling, and fragmentomic analysis, provide clinically relevant information for early cancer detection, molecular profiling, treatment monitoring, minimal residual disease assessment, resistance detection, and longitudinal patient surveillance.

2. Biological Basis of Liquid Biopsy

2.1. Cell-Free DNA and Circulating Tumor DNA Biology

The biological foundation of liquid biopsy relies on the detection and characterization of extracellular nucleic acids released into body fluids during physiological and pathological cellular processes. Among these analytes, cfDNA and ctDNA have emerged as the most clinically relevant biomarkers in precision oncology due to their ability to reflect the molecular landscape of malignant tumors in a minimally invasive manner [3,22].

cfDNA consists of short extracellular DNA fragments that circulate freely in the bloodstream and originate from multiple cellular sources, including hematopoietic cells, normal tissues, inflammatory cells, and neoplastic lesions [23–25]. In healthy individuals, cfDNA is typically present at low concentrations and is primarily generated through physiological apoptosis. These DNA fragments generally exhibit a modal fragment size of approximately

160–180 base pairs, corresponding to nucleosomal DNA fragmentation patterns produced during programmed cell death [26]. Additional mechanisms contributing to cfDNA release include necrosis, active cellular secretion, neutrophil extracellular trap formation, and cellular turnover associated with inflammation or tissue injury [26].

In patients with malignancies, a proportion of cfDNA originates specifically from tumor cells and is referred to as ctDNA. ctDNA carries tumor-associated molecular alterations and therefore represents a highly informative biomarker for cancer detection and molecular profiling [27]. The fraction of ctDNA within total cfDNA is highly variable and depends on several factors, including tumor type, stage, vascularization, proliferative activity, metastatic burden, and therapeutic response [1,28–30]. In advanced metastatic disease, ctDNA may constitute a substantial proportion of total cfDNA, whereas in early-stage tumors or minimal residual disease settings, ctDNA levels can be exceedingly low, often below 0.1% of the total circulating DNA pool [1].

The release of ctDNA into the circulation occurs predominantly through apoptosis and necrosis of tumor cells, although active secretion mechanisms have also been proposed [26]. Apoptotic fragmentation typically produces shorter and more uniform DNA fragments, while necrotic cell death results in more heterogeneous and longer DNA fragments. Recent evidence additionally suggests that viable tumor cells may actively release nucleic acids through extracellular vesicles and exosomal pathways, contributing further to the circulating tumor-derived nucleic acid pool [31].

Importantly, ctDNA reflects the genomic and epigenomic architecture of the underlying malignancy. Molecular alterations detectable in ctDNA include single-nucleotide variants, insertions and deletions, copy number alterations, structural rearrangements, microsatellite instability, methylation changes, and fragmentation signatures [6]. This molecular diversity enables comprehensive characterization of tumor biology through liquid biopsy analyses and provides insights into clonal evolution and spatial heterogeneity that may not be fully captured by conventional tissue biopsies [32].

One of the most clinically relevant characteristics of ctDNA is its short half-life, estimated to range from approximately 16 min to several hours [1,29,30]. This rapid turnover enables real-time monitoring of tumor dynamics and therapeutic response. Changes in ctDNA levels frequently correlate with tumor burden and may precede radiological progression or clinical relapse by several weeks or months [10]. Consequently, ctDNA analysis has become increasingly important for longitudinal disease monitoring, treatment stratification, and minimal residual disease assessment.

However, several biological complexities complicate ctDNA interpretation. One major challenge is clonal hematopoiesis of indeterminate potential (CHIP), a phenomenon characterized by age-related somatic mutations arising in hematopoietic stem cells rather than tumor cells [3]. CHIP-associated mutations, particularly involving genes such as DNMT3A, TET2, and ASXL1, may generate false-positive findings during ctDNA analysis and therefore require careful interpretation, especially in low-frequency variant detection settings.

In addition to genomic alterations, recent studies have highlighted the diagnostic significance of cfDNA fragmentation patterns, methylation signatures, and nucleosomal positioning profiles [24,33–35]. Fragmentomic analyses evaluate fragment size distributions and fragmentation motifs, while methylation profiling enables tissue-of-origin identification and early cancer detection with increasing sensitivity. These emerging approaches expand the biological and clinical relevance of liquid biopsy beyond conventional mutation analysis.

Collectively, the biological properties of cfDNA and ctDNA establish them as dynamic and highly informative biomarkers in oncology. Their capacity to provide real-time

molecular information regarding tumor burden, heterogeneity, clonal evolution, and treatment response underpins the rapidly expanding role of liquid biopsy in precision cancer medicine (Figure 2).

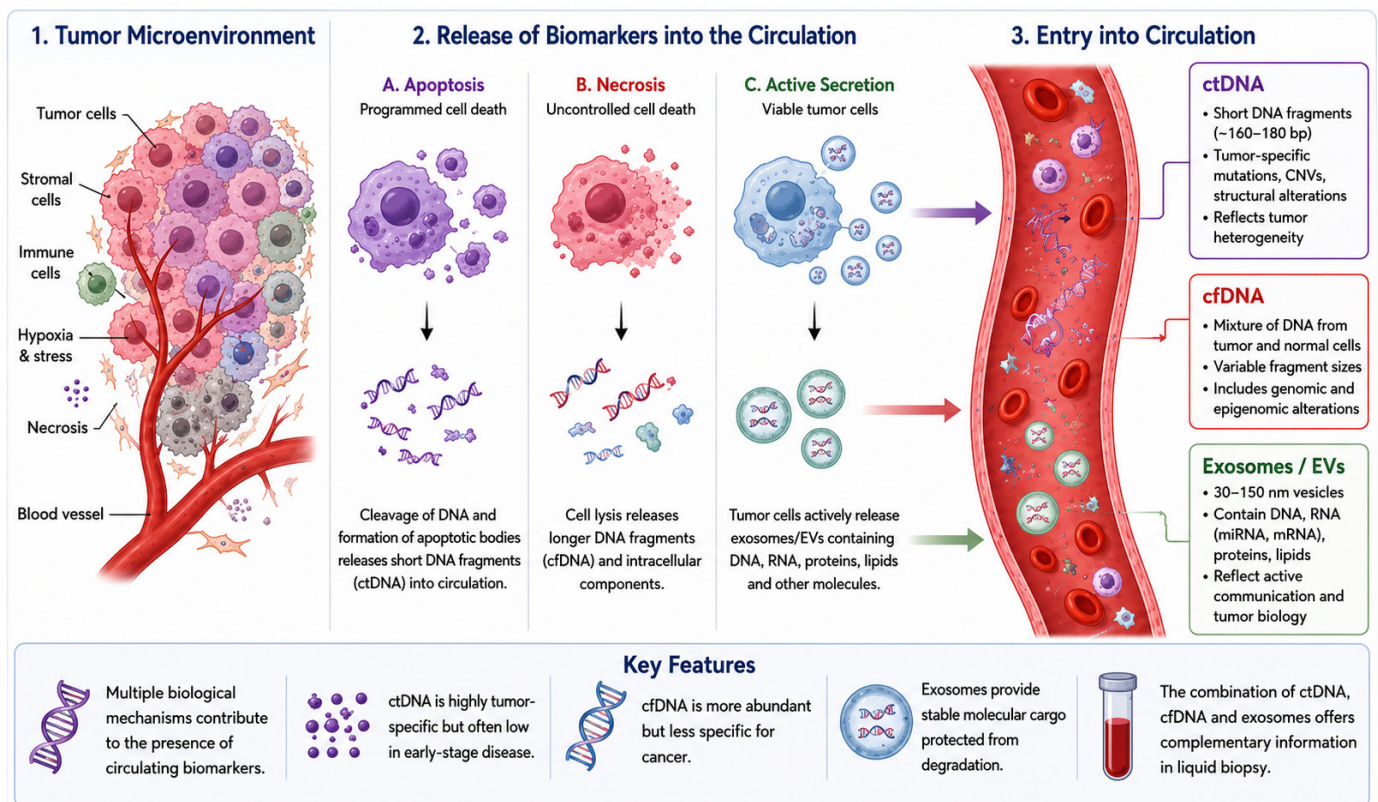


Figure 2. Biological origin and release mechanisms of circulating biomarkers used in liquid biopsy. Tumor-derived biomarkers enter the circulation through several biological processes, including apoptosis, necrosis, active cellular secretion, and extracellular vesicle release. cfDNA originates from both normal and malignant cells, whereas ctDNA represents the tumor-specific fraction carrying somatic mutations, copy number alterations, structural rearrangements, methylation changes, and other cancer-associated molecular alterations. Exosomes and other extracellular vesicles transport DNA, RNA, proteins, lipids, and signaling molecules protected by a lipid bilayer, preserving molecular integrity during circulation. Together, these complementary analytes provide information regarding tumor burden, clonal evolution, tumor heterogeneity, and tumor–microenvironment interactions, forming the biological basis of liquid biopsy.

2.2. Technologies for ctDNA and cfDNA Detection

The clinical implementation of liquid biopsy has been driven by substantial technological advances that have improved the sensitivity, specificity, and reproducibility of circulating nucleic acid detection. Because ctDNA frequently represents only a minor fraction of the total circulating cfDNA pool, particularly in early-stage disease or minimal residual disease settings, highly sensitive analytical methodologies are required to accurately identify tumor-associated molecular alterations [17,24,27,33,36,37]. Contemporary liquid biopsy platforms combine advanced molecular biology techniques with sophisticated bioinformatics pipelines to enable comprehensive genomic characterization from limited quantities of circulating DNA (Table 1).

Table 1. Comparison of the principal molecular technologies used for liquid biopsy analysis in precision oncology.

Technology	Analytical Principle	Main Analyte	Typical Sensitivity *	Clinical Maturity	Representative Applications	Key References
ddPCR	Partitioning DNA into thousands of droplets followed by endpoint PCR amplification	ctDNA	Very high (<0.1% VAF)	Routine clinical use	Detection of known mutations, MRD monitoring, treatment response	O’Leary et al., 2019 [13]
BEAMing	Emulsion PCR on magnetic beads combined with flow cytometry	ctDNA	Very high (<0.1% VAF)	Clinical use	Monitoring resistance mutations (EGFR, KRAS), quantitative mutation analysis	O’Leary et al., 2019 [13]
Targeted NGS	Deep sequencing of selected cancer-associated genes	ctDNA/cfDNA	High (~0.1–1% VAF)	Routine clinical use	Comprehensive genomic profiling, therapy selection	Forsheew et al., 2012 [38]
CAPP-Seq	Hybrid-capture targeted NGS with deep sequencing and error suppression	ctDNA	Very high (~0.02–0.1% VAF)	Advanced clinical/research	MRD detection, longitudinal monitoring, early relapse detection	Newman et al., 2014 [36]
Whole-exome sequencing (WES)	Sequencing of all protein-coding regions	ctDNA	Moderate	Mainly research	Discovery of novel mutations, clonal evolution	Chen M et al., 2019 [39]
Whole-genome sequencing (WGS)	Sequencing of the complete genome	ctDNA	Moderate	Research	Structural variants, copy-number alterations, genome-wide profiling	Chen M et al., 2019 [39]
DNA methylation assays	Detection of tumor-specific methylation patterns	cfDNA/ctDNA	High	Emerging clinical implementation	Early cancer detection, tissue-of-origin prediction	Lissa D et al., 2016 [40]
Fragmentomics	Analysis of cfDNA fragment size, end motifs and nucleosome positioning	cfDNA	High	Emerging	Early cancer detection, cancer classification	Avgeris M et al., 2021 [41]
CellSearch®	EpCAM-based immunomagnetic enrichment and cytokeratin staining	CTCs	Cell-based	FDA-cleared	Prognostic assessment in metastatic breast, prostate and colorectal cancers	Cristofanilli et al., 2009 [42]
Exosome isolation	Ultracentrifugation, immunoaffinity, size-exclusion chromatography or microfluidics	Exosomes	—	Mainly research	miRNA, protein and extracellular vesicle biomarker analysis	Yu et al., 2022 [18]
Multi-analyte liquid biopsy	Integration of ctDNA, CTCs, exosomes, proteins and AI-assisted analysis	Multiple analytes	Variable	Emerging	Early detection, comprehensive molecular profiling, precision oncology	Hofmann et al., 2020 [43]

* VAF = Variant Allele Frequency.

2.3. Preanalytical Considerations

The accuracy of ctDNA and cfDNA analysis is strongly influenced by preanalytical variables, making sample collection and processing critical components of liquid biopsy workflows [30,34]. Plasma is generally preferred over serum because serum samples are more susceptible to contamination by genomic DNA released from lysed leukocytes during coagulation [2]. Blood is commonly collected in EDTA or specialized cfDNA stabilization

tubes that minimize cellular degradation and preserve circulating nucleic acid integrity during transport and storage [25,30,34].

Following blood collection, rapid plasma separation through sequential centrifugation is essential to reduce contamination with cellular DNA. Delays in processing, repeated freeze–thaw cycles, and inappropriate storage conditions may significantly alter cfDNA quantity and fragment integrity, potentially compromising downstream analyses. Standardization of preanalytical procedures therefore remains a major prerequisite for reliable clinical implementation.

2.4. Digital Droplet Polymerase Chain Reaction (ddPCR)

Digital droplet polymerase chain reaction [12,13] represents one of the most sensitive methodologies currently available for targeted ctDNA detection. This technique partitions DNA samples into thousands of nanoliter-sized droplets, each functioning as an independent PCR microreaction. Following amplification, droplets are analyzed individually to determine the presence or absence of target mutations, enabling highly precise quantification of mutant allele frequencies.

ddPCR demonstrates excellent analytical sensitivity and can detect rare mutant alleles present at frequencies below 0.1% within a high background of wild-type DNA [19]. This high sensitivity makes ddPCR particularly suitable for monitoring known driver mutations, treatment resistance alterations, and minimal residual disease. In clinical oncology, ddPCR is widely used for detecting EGFR mutations in non-small-cell lung cancer, KRAS mutations in colorectal cancer, and ESR1 mutations in breast cancer [7,8,10,12,13,44].

Despite its advantages, ddPCR is primarily limited to the analysis of predefined mutations and lacks the broad genomic coverage offered by sequencing-based approaches. Consequently, its utility is greatest in targeted clinical applications where specific genomic alterations are already known.

2.5. BEAMing Technology

BEAMing (Beads, Emulsion, Amplification, and Magnetics) combines principles of digital PCR and flow cytometry to achieve highly sensitive mutation detection [13]. In this methodology, DNA fragments are amplified on magnetic beads within water-in-oil emulsions, followed by fluorescent labeling and flow cytometric analysis.

BEAMing enables precise quantification of low-frequency variants and has demonstrated strong analytical performance in the detection of mutations associated with therapeutic resistance and disease progression [13]. Similar to ddPCR, however, BEAMing is primarily optimized for targeted mutation analysis rather than comprehensive genomic profiling.

2.6. Next-Generation Sequencing

Next-generation sequencing (NGS) has revolutionized liquid biopsy diagnostics by enabling simultaneous analysis of multiple genomic alterations across large genomic regions or entire exomes and genomes [12,39]. NGS-based liquid biopsy approaches include targeted sequencing panels, whole-exome sequencing (WES), and whole-genome sequencing (WGS), each offering varying degrees of coverage and analytical depth.

Targeted NGS panels are currently the most widely implemented clinical approach due to their balance between sensitivity, cost-efficiency, and clinically actionable information [6]. These panels enable the detection of point mutations, insertions/deletions, copy number alterations, gene fusions, and structural rearrangements across selected cancer-associated genes.

Advanced error-correction strategies, including unique molecular identifiers (UMIs) and deep sequencing algorithms, have significantly improved the analytical sensitivity of NGS platforms, allowing reliable detection of variants at very low allele frequencies [6,12,39].

Furthermore, ultra-deep sequencing approaches facilitate longitudinal monitoring of clonal evolution and resistance mechanisms during therapy.

NGS additionally enables broader molecular analyses beyond mutation detection, including:

- methylation profiling,
- fragmentomic analysis,
- mutational signature analysis,
- and microsatellite instability assessment.

These emerging applications are increasingly important in early cancer detection and tissue-of-origin prediction [31,45].

Nevertheless, NGS-based liquid biopsy approaches remain associated with higher costs, longer turnaround times, increased bioinformatic complexity, and the need for specialized infrastructure and expertise [39].

2.7. Methylation-Based Assays

DNA methylation alterations represent some of the earliest molecular events in carcinogenesis and therefore constitute attractive biomarkers for liquid biopsy applications [46]. Methylation-based assays evaluate tumor-specific epigenetic signatures within cfDNA and have demonstrated promising sensitivity for early cancer detection.

Unlike mutation-based approaches, methylation profiling may provide information regarding tissue of origin because methylation patterns are highly tissue-specific [40]. Multi-cancer early detection assays based on cfDNA methylation analysis have recently demonstrated encouraging performance across multiple tumor types, highlighting the expanding role of epigenomic liquid biopsy technologies in precision oncology.

2.8. Fragmentomics and Emerging Approaches

Fragmentomic analysis represents an emerging field focused on evaluating the physical characteristics of circulating DNA fragments, including fragment length distributions, nucleosomal positioning, end motifs, and fragmentation patterns [26,41]. Tumor-derived cfDNA fragments often display distinct fragmentation profiles compared with non-tumor-derived cfDNA, providing additional diagnostic information independent of mutation status.

Combined fragmentomic and methylation-based approaches have shown potential to improve sensitivity for early-stage cancer detection, particularly in tumors characterized by low ctDNA abundance [41]. Additional emerging methodologies include:

- single-molecule sequencing,
- multi-omics liquid biopsy platforms,
- proteogenomic integration,
- and artificial intelligence-assisted molecular classification systems.

2.9. Bioinformatic Analysis and Data Interpretation

The increasing complexity of liquid biopsy data necessitates advanced computational and bioinformatic approaches for accurate interpretation [47,48]. Bioinformatic pipelines are used to:

- align sequencing reads,
- identify variants,
- remove technical artifacts,
- distinguish true mutations from sequencing noise,
- and annotate clinically relevant alterations.

Artificial intelligence and machine learning algorithms are increasingly integrated into liquid biopsy workflows to identify complex molecular patterns and improve diagnostic accuracy [49]. These approaches may facilitate earlier cancer detection, treatment response prediction, and prognostic stratification through multidimensional analysis of genomic, epigenomic, and fragmentomic datasets.

Despite major technological progress, several analytical challenges remain unresolved, including inter-platform variability, limited standardization, false-positive results associated with clonal hematopoiesis, and difficulties in detecting extremely low-frequency variants. Continued technological refinement and harmonization of analytical methodologies will therefore be essential for the broader clinical implementation of liquid biopsy technologies in precision oncology (Figure 3).

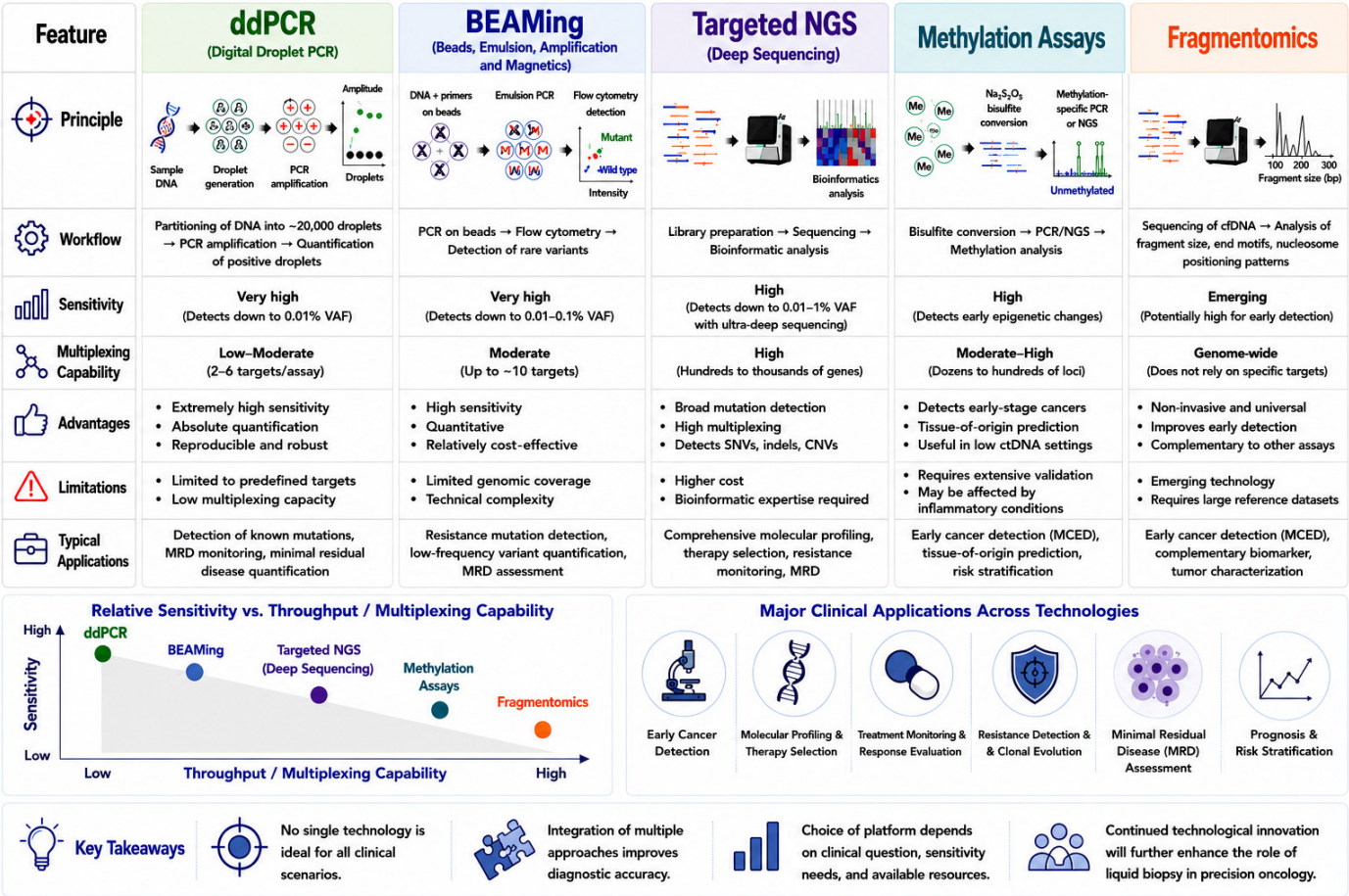


Figure 3. Principal analytical technologies used for ctDNA and cfDNA analysis. The figure summarizes the most commonly employed molecular techniques for liquid biopsy, including ddPCR, BEAMing, NGS, methylation assays, and fragmentomic analysis. Each platform differs with respect to analytical sensitivity, genomic coverage, multiplexing capacity, turnaround time, and clinical applications. Highly targeted approaches such as ddPCR and BEAMing provide exceptional sensitivity for predefined mutations, whereas NGS-based methods enable broader genomic profiling, including mutation detection, copy number analysis, structural variants, microsatellite instability, and longitudinal assessment of clonal evolution. Emerging fragmentomic and methylation-based assays further improve early cancer detection and tissue-of-origin prediction. The selection of a particular analytical platform depends on the clinical question, required sensitivity, available sample quantity, and intended application.

2.10. Clinical Applications of Liquid Biopsy in Oncology

The rapid evolution of liquid biopsy technologies has significantly expanded their clinical applicability across multiple stages of cancer management. By enabling minimally invasive and longitudinal assessment of tumor-derived molecular alterations, ctDNA and cfDNA analyses have emerged as valuable tools for early cancer detection, therapeutic stratification, treatment monitoring, minimal residual disease assessment, and identification of resistance mechanisms [3,27,50]. The integration of liquid biopsy into clinical oncology increasingly supports precision medicine approaches tailored to the molecular dynamics of individual tumors.

2.11. Early Cancer Detection

One of the most promising applications of liquid biopsy involves the early detection of malignant disease. Conventional screening modalities often exhibit limited sensitivity for early-stage tumors, particularly in cancers lacking effective population-based screening strategies. Because ctDNA can harbor tumor-specific molecular alterations before clinical or radiological manifestation, liquid biopsy offers the potential for earlier diagnosis and intervention [5,37,39].

Early detection strategies based on ctDNA analysis include mutation profiling, methylation-based assays, fragmentomic analyses, and multi-analyte approaches integrating genomic and proteomic biomarkers [41]. Methylation profiling has demonstrated particular promise because epigenetic alterations frequently occur early during carcinogenesis and may provide tissue-specific information regarding tumor origin [40,46].

Several multi-cancer early detection platforms have recently demonstrated encouraging sensitivity and specificity across diverse malignancies, including colorectal, lung, pancreatic, ovarian, and hepatocellular carcinomas. However, significant challenges remain, particularly in detecting very low ctDNA concentrations in early-stage disease and minimizing false-positive findings associated with benign conditions or clonal hematopoiesis.

2.12. Molecular Profiling and Therapeutic Stratification

Liquid biopsy has become increasingly important for molecular profiling and therapeutic decision-making, particularly when tissue biopsy is unavailable, insufficient, or difficult to obtain. ctDNA analysis enables the identification of actionable genomic alterations that guide targeted therapy selection across multiple tumor types.

In non-small-cell lung cancer (NSCLC), plasma-based detection of EGFR mutations has become an established clinical application of ctDNA analysis [51]. Liquid biopsy facilitates both initial identification of sensitizing EGFR mutations and subsequent detection of resistance-associated alterations such as T790M during tyrosine kinase inhibitor therapy [52]. Similar applications have been demonstrated for ALK rearrangements, KRAS mutations, and MET amplification.

In colorectal cancer, ctDNA testing is widely utilized for detecting KRAS, NRAS, and BRAF mutations relevant to anti-EGFR therapeutic selection [53,54]. Likewise, in breast cancer, ctDNA analysis may identify ESR1, PIK3CA, and HER2-associated alterations associated with endocrine resistance and targeted therapeutic eligibility [55–58].

The ability of liquid biopsy to capture tumor heterogeneity represents a major advantage over single-site tissue biopsy. Because ctDNA may originate from multiple primary and metastatic lesions simultaneously, liquid biopsy can provide a more comprehensive overview of the genomic landscape and clonal architecture of advanced malignancies.

2.13. Treatment Response Monitoring

The short half-life of ctDNA enables dynamic real-time monitoring of tumor burden and therapeutic response [59]. Quantitative changes in ctDNA levels often correlate closely with treatment efficacy and may precede radiological progression by several weeks or months [60].

Serial ctDNA measurements have demonstrated clinical utility in multiple malignancies, including:

- lung cancer,
- colorectal cancer,
- breast cancer,
- pancreatic cancer,
- melanoma,
- and hematologic malignancies.

Rapid decreases in ctDNA levels during treatment are generally associated with favorable therapeutic response, whereas persistent or rising ctDNA levels may indicate residual disease or emerging resistance [61].

In metastatic breast cancer, ctDNA dynamics have been shown to predict progression earlier than conventional imaging modalities [55,56]. Similarly, in pancreatic cancer, longitudinal cfDNA and ctDNA profiling during chemotherapy may provide valuable information regarding treatment response and disease evolution [59].

Liquid biopsy-based monitoring may additionally reduce dependence on repeated imaging studies and facilitate earlier therapeutic modifications in cases of treatment failure.

2.14. Minimal Residual Disease Detection

Minimal residual disease assessment represents one of the most clinically impactful emerging applications of ctDNA analysis [7–10]. Following curative-intent surgery or systemic therapy, residual microscopic disease may persist despite the absence of radiologically detectable tumors. ctDNA detection in the post-treatment setting can identify molecular evidence of residual malignancy with remarkable sensitivity.

Numerous studies have demonstrated that postoperative ctDNA positivity strongly correlates with increased recurrence risk across several tumor types, particularly colorectal cancer, lung cancer, and breast cancer [15,51,58]. In colorectal cancer, postoperative ctDNA detection has emerged as a powerful prognostic marker capable of identifying patients at high risk of relapse who may benefit from adjuvant therapy escalation.

Importantly, ctDNA-based MRD detection frequently identifies molecular relapse months before conventional imaging methods, creating opportunities for earlier therapeutic intervention [10]. As a result, MRD-guided treatment strategies are increasingly being investigated in prospective clinical trials.

Despite its considerable promise, MRD analysis remains technically challenging because ctDNA concentrations are often extremely low in this setting. Ultra-sensitive sequencing approaches and optimized bioinformatic algorithms are therefore essential for reliable MRD detection.

2.15. Detection of Resistance Mechanisms and Clonal Evolution

Acquired therapeutic resistance represents a major obstacle in oncology, particularly in patients receiving targeted therapies and immunotherapies. Liquid biopsy enables non-invasive characterization of evolving resistance mechanisms through serial genomic monitoring [62].

During treatment, selective therapeutic pressure may drive the emergence of resistant subclones harboring secondary genomic alterations. ctDNA analysis enables early identi-

cation of these alterations, facilitating adaptive therapeutic strategies before overt clinical progression occurs.

Examples include:

- EGFR T790M mutations in NSCLC,
- ESR1 mutations in hormone receptor-positive breast cancer,
- KRAS-mediated resistance in colorectal cancer,
- and AR gene alterations in prostate cancer [44,55,58,61,63].

Longitudinal ctDNA profiling additionally provides insights into clonal evolution and intratumoral heterogeneity during disease progression. This dynamic molecular surveillance capability represents one of the most important advantages of liquid biopsy over conventional tissue-based diagnostics.

2.16. Immunotherapy Applications

Liquid biopsy is increasingly being explored in the context of immunotherapy response prediction and monitoring [15,53,64]. ctDNA dynamics may correlate with response to immune checkpoint inhibitors, and reductions in ctDNA burden during therapy are often associated with improved clinical outcomes.

Emerging applications include:

- assessment of tumor mutational burden (TMB),
- microsatellite instability (MSI) detection,
- immune-related genomic signatures,
- and monitoring of hyperprogression or pseudoprogression.

Although these applications remain under active investigation, liquid biopsy may eventually contribute significantly to immunotherapy personalization and response stratification (Figure 4).

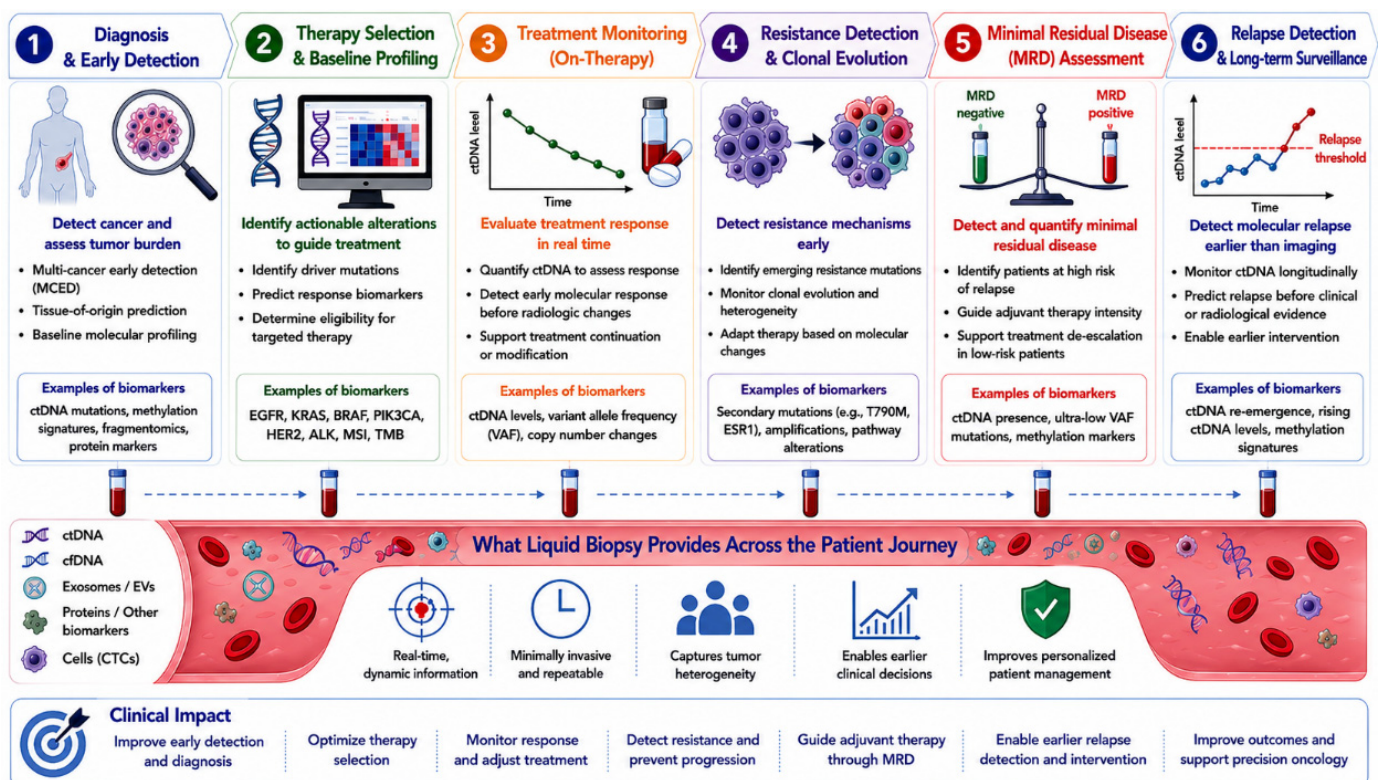


Figure 4. Clinical applications of liquid biopsy throughout cancer management. Liquid biopsy enables longitudinal molecular assessment through the analysis of circulating tumor DNA, cell-free

DNA, circulating tumor cells, and extracellular vesicles. These biomarkers support multiple stages of cancer management, including early cancer detection, molecular profiling for treatment selection, therapeutic response monitoring, identification of acquired resistance mechanisms, minimal residual disease assessment, and detection of molecular relapse before clinical or radiological progression. Integration of liquid biopsy with advanced analytical technologies, such as digital droplet PCR, next-generation sequencing, methylation profiling, fragmentomic analysis, and artificial intelligence-assisted data interpretation, provides complementary insights into tumor heterogeneity and disease evolution. Although further standardization and prospective clinical validation are required, liquid biopsy is becoming an increasingly important component of precision oncology and personalized patient management.

2.17. Current Clinical Limitations

Despite substantial progress, several limitations continue to restrict the universal clinical implementation of liquid biopsy approaches. These include:

- low ctDNA abundance in early-stage disease,
- biological variability between tumor types,
- preanalytical and analytical standardization challenges,
- clonal hematopoiesis-associated false positives,
- incomplete concordance with tissue biopsies,
- and economic considerations [1].

Furthermore, interpretation of low-frequency variants requires careful integration with clinical, radiological, and pathological findings to avoid overdiagnosis or inappropriate therapeutic decisions.

Nevertheless, the expanding evidence base supporting ctDNA and cfDNA applications continues to position liquid biopsy as a central component of contemporary precision oncology and personalized cancer management.

2.18. Cancer-Specific Applications of ctDNA and cfDNA Analysis

The clinical value of liquid biopsy has been investigated across a wide range of malignancies, with particularly relevant applications in lung, colorectal, breast, pancreatic, and prostate cancers. Although the principles of ctDNA and cfDNA analysis are broadly applicable, the clinical utility of liquid biopsy varies according to tumor biology, disease stage, metastatic burden, and the availability of actionable molecular alterations.

2.19. Lung Cancer

Non-small-cell lung cancer (NSCLC) represents one of the most established clinical settings for liquid biopsy implementation. Molecular profiling is essential for therapeutic decision-making in NSCLC, particularly for identifying actionable alterations involving EGFR, ALK, ROS1, BRAF, MET, RET, NTRK, and KRAS. In patients in whom tissue biopsy is unavailable, insufficient, or clinically difficult to obtain, plasma ctDNA analysis provides an important alternative for molecular characterization [5,9,51,65–68].

One of the best validated applications of ctDNA testing in NSCLC is the detection of EGFR mutations. Plasma-based assays can identify sensitizing EGFR alterations that support the use of tyrosine kinase inhibitors and can also detect resistance-associated mutations such as T790M during disease progression [9,69]. This enables clinicians to adapt treatment strategies according to the evolving molecular profile of the tumor.

Beyond therapy selection, ctDNA analysis in lung cancer is increasingly used for treatment response monitoring and minimal residual disease assessment. Longitudinal ctDNA dynamics may provide earlier evidence of relapse or progression than conventional imaging, particularly after curative-intent surgery or radiotherapy [51]. However, the

sensitivity of ctDNA detection remains lower in early-stage disease, and negative plasma results should be interpreted cautiously, especially when tumor shedding is limited.

2.20. Colorectal Cancer

Colorectal cancer is another major area in which liquid biopsy has demonstrated significant clinical relevance. ctDNA analysis is particularly useful for detecting mutations in KRAS, NRAS, and BRAF, which are essential biomarkers for therapeutic stratification, especially in the context of anti-EGFR therapy [70]. The detection of RAS mutations in plasma can guide treatment selection and may identify emerging resistance during targeted therapy.

One of the most promising applications of ctDNA in CRC is postoperative minimal residual disease detection. Following surgical resection, ctDNA positivity is strongly associated with an increased risk of recurrence and may identify patients who could benefit from intensified adjuvant treatment [70–72]. Conversely, ctDNA-negative patients may represent a lower-risk group in whom treatment de-escalation strategies could be considered in future prospective trials.

Liquid biopsy also allows longitudinal monitoring of clonal evolution in metastatic CRC. During anti-EGFR therapy, resistant subclones harboring RAS pathway alterations may emerge and can be detected through serial ctDNA testing. This dynamic monitoring may support adaptive treatment strategies and provide insight into mechanisms of acquired resistance.

2.21. Breast Cancer

In breast cancer, ctDNA and cfDNA analyses have gained increasing attention for disease monitoring, therapeutic stratification, and resistance detection. Liquid biopsy is particularly relevant in metastatic breast cancer, where repeated tissue sampling is often impractical and tumor biology may evolve under therapeutic pressure [55–57].

ctDNA levels can reflect tumor burden and may correlate with clinical response or disease progression. Serial monitoring of ctDNA has shown potential for detecting relapse earlier than imaging-based methods and for evaluating treatment efficacy in real time [58]. In hormone receptor-positive breast cancer, ctDNA analysis can identify ESR1 mutations associated with endocrine therapy resistance, while PIK3CA mutations may guide eligibility for targeted PI3K pathway inhibition. In HER2-positive disease, ctDNA-based assessment may provide additional information regarding HER2 amplification dynamics and treatment response [57].

Despite these promising applications, liquid biopsy in breast cancer is still influenced by disease burden, metastatic site, and assay sensitivity. Therefore, ctDNA findings should be integrated with histopathological, radiological, and clinical information rather than interpreted in isolation.

2.22. Pancreatic Cancer

Pancreatic cancer remains one of the most challenging malignancies for early diagnosis and clinical management. Because tissue acquisition can be technically difficult and the disease is often diagnosed at an advanced stage, liquid biopsy offers an attractive non-invasive tool for molecular assessment and monitoring [73,74].

ctDNA analysis in pancreatic cancer may detect tumor-associated mutations, most commonly involving KRAS, and may provide information regarding tumor burden, prognosis, and treatment response. Serial cfDNA or ctDNA profiling during systemic therapy may help assess response to chemotherapy and identify early disease progression [74]. However, the sensitivity of ctDNA detection in pancreatic cancer can be variable, particularly in localized disease or in patients with low tumor shedding.

At present, liquid biopsy is not yet a replacement for tissue diagnosis in pancreatic cancer, but it may serve as a complementary tool for molecular profiling, monitoring, and future early detection strategies.

2.23. Prostate Cancer

In prostate cancer, ctDNA analysis has particular relevance in advanced and castration-resistant disease. Liquid biopsy can detect genomic alterations associated with resistance to androgen receptor pathway inhibitors, including androgen receptor amplification, point mutations, and structural alterations [61].

The identification of AR-related alterations may support treatment adaptation in patients receiving androgen deprivation therapy or second-generation hormonal agents. In addition, ctDNA analysis may detect DNA damage repair gene alterations, including BRCA1, BRCA2, and ATM, which may have therapeutic implications for PARP inhibitor eligibility and platinum-based treatment strategies [75].

As in other malignancies, ctDNA detection in prostate cancer is more informative in advanced metastatic disease than in localized tumors. The interpretation of plasma-based findings requires attention to tumor fraction, prior treatments, and concordance with tissue-based molecular results.

2.24. Comparative Clinical Relevance Across Tumor Types

The clinical performance of liquid biopsy varies substantially across cancer types. Tumors with high proliferative activity, extensive metastatic burden, and rich vascularization tend to release higher amounts of ctDNA, improving detection sensitivity. By contrast, tumors with low shedding capacity, limited disease burden, or confined anatomical localization may produce low ctDNA levels, increasing the risk of false-negative results.

Therefore, liquid biopsy should be understood as a complementary approach rather than a universal replacement for tissue biopsy. Its greatest current value lies in contexts where repeated molecular monitoring is clinically useful, tissue biopsy is difficult, or rapid detection of actionable or resistance-associated alterations can directly influence treatment decisions.

Overall, cancer-specific applications of ctDNA and cfDNA analysis demonstrate the expanding clinical relevance of liquid biopsy in precision oncology. Continued prospective validation will be essential to define the optimal use of these technologies across tumor types, disease stages, and therapeutic contexts (Table 2).

2.25. Circulating Tumor Cells: Biological Significance and Clinical Applications

Circulating tumor cells represent another major component of the liquid biopsy landscape and provide unique biological information that complements circulating tumor DNA, cell-free DNA, and extracellular vesicle analyses. Unlike ctDNA, which consists of fragmented nucleic acids released predominantly during apoptosis or necrosis, CTCs are intact viable or apoptotic malignant cells that detach from either the primary tumor or metastatic deposits and enter the bloodstream. Although their presence was first described more than a century ago, technological advances over the past two decades have enabled increasingly reliable isolation and molecular characterization, leading to renewed interest in their clinical application [56].

Table 2. Clinical applications of liquid biopsy in the major cancer types included in this review.

Cancer Type	Principal Liquid Biopsy Analyte(s)	Clinically Relevant Biomarkers	Current Clinical Applications	Clinical Implementation	Representative References
Non-small-cell lung cancer	ctDNA, cfDNA, CTCs	<i>EGFR, ALK, ROS1, KRAS, MET, RET, BRAF, NTRK</i>	Molecular profiling, targeted therapy selection, detection of acquired resistance (e.g., EGFR T790M), treatment monitoring, MRD assessment	Routine clinical use	Tartarone et al., 2017, Paez et al., 2004, Farago et al., 2018 [51,66,76]
Colorectal cancer	ctDNA, cfDNA, CTCs	<i>KRAS, NRAS, BRAF, MSI</i>	Selection of anti-EGFR therapy, postoperative MRD detection, recurrence prediction, monitoring treatment response	Routine clinical use for selected indications	Xu et al., 2024, Zhou et al., 2022 [53,77]
Breast cancer	ctDNA, CTCs, exosomes	<i>PIK3CA, ESR1, HER2</i>	Monitoring endocrine resistance, treatment response, prognostic assessment, molecular profiling	Increasing clinical implementation	Schwarzenbach et al., 2015, Cristofanilli et al., 2004 [55,56]
Pancreatic cancer	ctDNA, exosomes	<i>KRAS, TP53, SMAD4</i>	Disease monitoring, prognostic assessment, investigational early detection	Investigational	Jamal et al., 2025, Wei et al., 2019, Tanaka et al., 2025 [59,73,74]
Prostate cancer	ctDNA, CTCs	Androgen receptor (AR), <i>BRCA1, BRCA2, ATM</i>	Monitoring resistance to androgen receptor-targeted therapies, therapy stratification, PARP inhibitor selection	Routine use in advanced disease	Miyamoto et al., 2015, Hofmann et al., 2020 [43,78]
Melanoma	ctDNA	<i>BRAF, NRAS</i>	Monitoring targeted therapy and immunotherapy response, detection of relapse, MRD assessment	Increasing clinical implementation	Heidrich et al., 2025 [79]
Hepatocellular carcinoma	cfDNA, ctDNA, methylation markers	<i>TP53, TERT</i> promoter mutations, methylation signatures	Early detection, surveillance of high-risk patients, prognostic assessment	Emerging clinical application	Yang et al., 2024 [80]

The biological significance of CTCs extends beyond their value as biomarkers. They are considered active participants in the metastatic cascade, representing tumor cells that have successfully acquired the ability to invade surrounding tissue, intravasate into the

circulation, survive hemodynamic stress and immune surveillance, extravasate into distant organs, and eventually establish metastatic lesions. Only a very small proportion of circulating cells complete this process successfully; nevertheless, the presence of CTCs reflects the metastatic potential of the tumor and has consistently been associated with adverse clinical outcomes across multiple malignancies [81].

CTCs exhibit remarkable phenotypic and molecular heterogeneity. Within the circulation, tumor cells may retain epithelial characteristics, undergo partial or complete epithelial-to-mesenchymal transition (EMT), or display hybrid epithelial–mesenchymal phenotypes. This phenotypic plasticity contributes to metastatic competence but also presents important analytical challenges, as many enrichment strategies rely on epithelial markers such as epithelial cell adhesion molecule (EpCAM). Cells that have undergone EMT frequently downregulate epithelial surface antigens, reducing capture efficiency and potentially leading to underestimation of CTC burden. Furthermore, CTCs may circulate as single cells or as multicellular clusters. Although clusters are detected less frequently, accumulating evidence indicates that they possess substantially greater metastatic capacity than individual circulating tumor cells, likely owing to enhanced resistance to apoptosis, preservation of intercellular signaling, and improved survival within the bloodstream [81].

2.26. Technologies for CTC Isolation and Characterization

The rarity of CTCs represents the principal technical challenge for their clinical implementation. In many patients with advanced solid tumors, only one to several dozen tumor cells may be present within billions of circulating blood cells per milliliter of whole blood. Consequently, highly efficient enrichment and detection methods are required before downstream molecular analyses can be performed.

Current isolation strategies can be broadly divided into biological and physical approaches. Biological enrichment methods exploit differences in surface antigen expression through immunoaffinity-based capture. The most widely established platform is the CellSearch® system, which employs magnetic nanoparticles coated with antibodies against EpCAM to isolate epithelial tumor cells, followed by cytokeratin staining, nuclear labeling, and exclusion of CD45-positive leukocytes. CellSearch remains the only United States Food and Drug Administration (FDA)-cleared platform for CTC enumeration in metastatic breast, prostate, and colorectal cancers, where baseline CTC counts have demonstrated independent prognostic value [81].

Alternative technologies have sought to overcome the limitations associated with EpCAM-dependent enrichment. Microfluidic devices integrate sophisticated channel geometries, antibody-coated surfaces, or inertial flow dynamics to increase capture efficiency while preserving cellular integrity. Size-based filtration systems exploit the generally larger diameter and reduced deformability of tumor cells relative to hematopoietic cells, whereas density-gradient centrifugation and dielectrophoretic separation provide label-free enrichment based on physical properties. More recently, integrated microfluidic platforms combining immunocapture with physical separation have improved both recovery rates and sample purity, facilitating downstream molecular analyses [82].

Following enrichment, CTCs may undergo extensive phenotypic and molecular characterization. Immunocytochemistry enables assessment of protein expression patterns relevant to diagnosis or therapeutic targeting, whereas fluorescence in situ hybridization (FISH) can identify clinically actionable chromosomal alterations. Advances in single-cell technologies have further expanded the analytical potential of CTCs, allowing whole-genome sequencing, targeted next-generation sequencing, transcriptomic profiling, epigenetic analysis, and even functional assays using viable isolated cells. Such approaches provide

insights into intratumoral heterogeneity, clonal evolution, and mechanisms of therapeutic resistance that cannot be obtained through cell-free nucleic acid analysis alone [30,41,48].

2.27. Clinical Applications of CTCs

The strongest clinical evidence supporting CTC analysis currently lies in prognostic assessment. Numerous studies have demonstrated that elevated CTC counts are independently associated with shorter progression-free and overall survival in metastatic breast, prostate, colorectal, and lung cancers. Importantly, serial CTC enumeration during systemic treatment may reflect therapeutic efficacy, with declining CTC numbers generally indicating favorable treatment response, whereas persistent or increasing counts frequently precede radiological evidence of disease progression [61,83].

Beyond simple enumeration, molecular characterization of CTCs has expanded their clinical utility. Expression of therapeutic targets, including androgen receptor splice variants in metastatic castration-resistant prostate cancer, HER2 expression in breast cancer, or programmed death-ligand 1 (PD-L1) in several solid tumors, may provide additional information for treatment selection and resistance monitoring. Likewise, genomic analyses performed at the single-cell level permit longitudinal assessment of tumor evolution and facilitate identification of emerging resistant subclones during targeted therapy [61].

Despite these promising applications, routine clinical implementation remains more limited than for ctDNA. The low abundance of CTCs, biological heterogeneity, variability among enrichment platforms, and incomplete analytical standardization continue to restrict widespread clinical adoption. In addition, different isolation technologies frequently recover distinct CTC subpopulations, complicating inter-study comparisons and establishment of universally applicable diagnostic thresholds.

2.28. Complementarity of CTCs and ctDNA

Rather than representing competing biomarkers, CTCs and ctDNA provide complementary information regarding tumor biology. ctDNA offers highly sensitive detection of tumor-derived genomic alterations from fragmented DNA released by dying tumor cells and is particularly well suited for mutation profiling, minimal residual disease detection, and longitudinal monitoring. Conversely, CTCs preserve the entire cellular architecture, enabling simultaneous evaluation of DNA, RNA, protein expression, cellular morphology, and functional characteristics within individual tumor cells.

The integration of both analytes has the potential to improve the accuracy of liquid biopsy by combining the high analytical sensitivity of ctDNA with the biological richness of intact tumor cells. Multi-analyte approaches incorporating ctDNA, CTCs, extracellular vesicles, circulating proteins, and clinical variables are increasingly being investigated to improve early cancer detection, therapeutic stratification, and prediction of treatment response. As single-cell sequencing technologies, microfluidic enrichment platforms, and computational analytical methods continue to evolve, combined liquid biopsy strategies are expected to provide increasingly comprehensive characterization of tumor heterogeneity and disease evolution, supporting the continued development of precision oncology [78].

2.29. Exosomes and Extracellular Vesicles in Liquid Biopsy

In addition to ctDNA and cfDNA, extracellular vesicles (EVs), particularly exosomes, have emerged as important components of liquid biopsy with considerable diagnostic and therapeutic potential in oncology. Exosomes are nanosized membrane-bound vesicles, typically ranging from 30 to 150 nm in diameter, that are actively secreted by virtually all cell types into the extracellular environment [19–21,49,84]. Their molecular cargo reflects the biological state of the originating cells, making them attractive biomarkers for cancer detection, disease monitoring, and therapeutic response assessment.

2.30. Biology and Biogenesis of Exosomes

Exosomes originate through the endosomal pathway. Their formation begins with inward budding of the plasma membrane, leading to the generation of early endosomes that subsequently mature into multivesicular bodies (MVBs). Intraluminal vesicles formed within MVBs are eventually released into the extracellular space following fusion of MVBs with the plasma membrane, at which point they are referred to as exosomes [85].

The molecular composition of exosomes includes:

- DNA
- messenger RNA (mRNA),
- microRNA (miRNA),
- long non-coding RNA,
- proteins,
- lipids,
- cytokines,
- and signaling molecules.

Because this cargo reflects the molecular and functional characteristics of the parent cells, exosomes provide valuable insight into tumor biology and intercellular communication [85,86].

Tumor-derived exosomes participate in multiple processes associated with cancer progression, including:

- tumor growth,
- angiogenesis,
- immune modulation,
- epithelial-mesenchymal transition,
- metastatic dissemination,
- and therapeutic resistance.

Their ability to circulate in various body fluids, including blood, urine, saliva, cerebrospinal fluid, and pleural effusions, further supports their utility as minimally invasive biomarkers.

2.31. Exosomal Nucleic Acids and Molecular Profiling

One of the most clinically relevant features of exosomes is their ability to transport stable nucleic acids protected from enzymatic degradation by the surrounding lipid bilayer [18,84]. Exosomal DNA and RNA may therefore provide highly informative molecular material for liquid biopsy analyses.

Exosomal microRNAs have attracted substantial attention as potential cancer biomarkers because their expression patterns frequently correlate with tumor type, stage, progression, and treatment response. Numerous studies have identified cancer-associated exosomal miRNA signatures in:

- lung cancer,
- colorectal cancer,
- breast cancer,
- pancreatic cancer,
- glioblastoma,
- and hepatocellular carcinoma [87].

Similarly, exosomal DNA may contain tumor-specific mutations that mirror the genomic profile of the primary tumor. In certain contexts, exosomal DNA may provide improved stability and sensitivity compared with freely circulating ctDNA, particularly in tumors characterized by low ctDNA shedding.

The combined analysis of exosomal cargo alongside ctDNA and cfDNA may therefore improve overall liquid biopsy sensitivity and provide complementary biological information regarding tumor activity and microenvironmental interactions.

2.32. Role in Tumor Microenvironment and Metastasis

Exosomes play a major role in mediating communication between tumor cells and the surrounding microenvironment [88]. Through the transfer of signaling molecules and regulatory nucleic acids, tumor-derived exosomes influence stromal cells, immune cells, endothelial cells, and distant metastatic niches.

Several mechanisms by which exosomes contribute to cancer progression have been described, including:

- suppression of anti-tumor immune responses,
- promotion of angiogenesis,
- extracellular matrix remodeling,
- induction of pre-metastatic niche formation,
- and enhancement of invasive potential.

Exosomal signaling has additionally been implicated in resistance to chemotherapy, targeted therapy, and immunotherapy. The ability of exosomes to transfer resistance-associated molecules between tumor cells may contribute to intratumoral heterogeneity and therapeutic failure.

Because exosomes actively participate in tumor biology rather than merely reflecting it passively, they represent both biomarkers and potential therapeutic targets.

2.33. Diagnostic and Prognostic Applications

Exosome-based liquid biopsy approaches are increasingly being investigated for:

- early cancer detection,
- prognostic stratification,
- treatment monitoring,
- and recurrence surveillance.

Compared with ctDNA, exosomes may offer several theoretical advantages. Their lipid membrane provides protection from nuclease degradation, resulting in improved molecular stability. Furthermore, exosomes are actively secreted by viable cells rather than released primarily during cell death, potentially enabling earlier detection of biologically active disease.

Exosomal biomarkers have demonstrated promising diagnostic accuracy in several malignancies. For example:

- exosomal glypican-1 has been investigated in pancreatic cancer,
- exosomal EGFR-related signatures in lung cancer,
- and exosomal HER2-associated molecules in breast cancer [67,73,89,90].

Nevertheless, many proposed biomarkers still require large-scale prospective validation before routine clinical implementation.

2.34. Technical Challenges and Standardization Issues

Despite growing scientific interest, the clinical application of exosome-based diagnostics remains limited by important technical challenges [46–50]. One of the primary obstacles is the lack of standardized methodologies for exosome isolation, purification, quantification, and characterization.

Common isolation techniques include:

- ultracentrifugation,

- size-exclusion chromatography,
- precipitation-based methods,
- immunoaffinity capture,
- and microfluidic platforms.

However, these methods differ substantially in yield, purity, reproducibility, and scalability. Contamination with other extracellular vesicles, proteins, and lipoproteins may additionally compromise analytical accuracy [91].

Another challenge involves the heterogeneity of extracellular vesicle populations. Exosomes coexist with other vesicles such as microvesicles and apoptotic bodies, complicating precise characterization and interpretation. International efforts toward methodological standardization are therefore essential for future clinical translation.

2.35. Future Perspectives of Exosome-Based Liquid Biopsy

Recent technological advances in high-throughput sequencing, proteomics, nanotechnology, and microfluidics are accelerating the development of exosome-based diagnostics. Emerging multi-omics approaches integrating exosomal nucleic acids, proteins, lipids, and metabolomic signatures may substantially improve diagnostic sensitivity and biological insight.

Artificial intelligence-assisted analysis of exosomal molecular patterns may further enhance biomarker discovery and predictive modeling [91]. In parallel, exosomes are increasingly being explored as therapeutic vehicles because of their natural biocompatibility and ability to transport molecular cargo across biological barriers.

Although exosome-based liquid biopsy remains less clinically established than ctDNA analysis, its rapidly expanding scientific foundation suggests considerable future potential in precision oncology. The integration of exosomal biomarkers with ctDNA, cfDNA, imaging, and clinical data may ultimately contribute to more comprehensive and dynamic cancer characterization (Table 3).

Table 3. Comparative overview of the principal liquid biopsy analytes used in precision oncology.

Characteristic	ctDNA	cfDNA	Circulating Tumor Cells (CTCs)	Exosomes/Extracellular Vesicles
Definition	Tumor-derived fraction of circulating cell-free DNA	Total extracellular DNA circulating in body fluids	Intact malignant cells released from primary or metastatic tumors into the bloodstream	Membrane-bound extracellular vesicles actively secreted by cells
Primary origin	Apoptotic, necrotic, or actively secreting tumor cells	Normal and malignant cells	Primary tumor and metastatic lesions	Viable tumor cells, stromal cells, immune cells and other normal tissues
Biological material	DNA	DNA	Whole viable or apoptotic cells	DNA, mRNA, miRNA, lncRNA, proteins, lipids and metabolites
Tumor specificity	High	Moderate	Very high	Moderate to high (depending on isolation strategy)

Table 3. Cont.

Characteristic	ctDNA	cfDNA	Circulating Tumor Cells (CTCs)	Exosomes/Extracellular Vesicles
Information obtained	Somatic mutations, CNVs, structural variants, methylation, fragmentomics	Global circulating DNA profile	Morphology, DNA, RNA, protein expression, chromosomal alterations, functional analyses	Gene expression, protein cargo, intercellular communication, signaling pathways
Major clinical applications	Molecular profiling, MRD detection, treatment monitoring, resistance detection	Early detection, methylation analysis, tissue-of-origin identification	Prognostic assessment, treatment monitoring, metastatic potential, single-cell molecular profiling	Biomarker discovery, disease monitoring, tumor microenvironment characterization
Major advantages	High analytical sensitivity; suitable for serial monitoring; reflects tumor evolution	Abundant and easily accessible	Intact cells allow multi-omic and functional analyses; preserves cellular morphology	Molecular cargo protected by lipid membrane; reflects active biological processes
Major limitations	Low abundance in early-stage disease; CHIP-associated false positives	Lower tumor specificity; background DNA from normal tissues	Extremely rare cells; technical complexity; lack of assay standardization	Isolation methods remain poorly standardized; heterogeneous vesicle populations
Typical analytical methods	ddPCR, BEAMing, targeted NGS, CAPP-Seq, methylation assays	qPCR, NGS, methylation assays, fragmentomics	CellSearch [®] , immunomagnetic enrichment, microfluidics, size-based filtration, single-cell sequencing	Ultracentrifugation, size-exclusion chromatography, immunoaffinity capture, microfluidic isolation
Clinical implementation	Routine clinical practice for selected indications	Routine component of liquid biopsy workflows	FDA-cleared for prognostic assessment in selected cancers; broader applications under investigation	Predominantly investigational

2.36. Challenges and Limitations of Liquid Biopsy

Despite the rapid progress of liquid biopsy technologies, several biological, analytical, and clinical limitations continue to restrict their universal implementation in oncology. Although ctDNA, cfDNA, and exosome-based assays offer major advantages over conventional tissue biopsy, their interpretation requires careful consideration of tumor biology, assay performance, preanalytical variability, and clinical context [18–21,84] (Table 4).

Table 4. Emerging technologies and future perspectives of liquid biopsy in precision oncology.

Advantages	Limitations
Minimally invasive	Low ctDNA fraction in early-stage disease
Allows serial real-time monitoring	Risk of false-negative results
Captures tumor heterogeneity	Clonal hematopoiesis-associated false positives
Enables early detection of resistance mechanisms	Lack of assay standardization
Facilitates MRD detection	Variable sensitivity across tumor types
Useful when tissue biopsy is unavailable	Bioinformatic complexity
Potential for earlier relapse detection	High cost of advanced platforms
Supports personalized therapy selection	Limited prospective clinical validation in some settings

2.37. Low Tumor Fraction and False-Negative Results

One of the most important limitations of ctDNA analysis is the frequently low proportion of tumor-derived DNA within the total cfDNA pool. The ctDNA fraction varies widely according to tumor type, stage, anatomical location, vascularization, metastatic burden, and treatment status. In advanced metastatic disease, ctDNA levels are often sufficient for reliable molecular profiling, whereas in early-stage tumors, minimal residual disease, or tumors with low shedding capacity, ctDNA may be present at extremely low levels.

This biological limitation increases the risk of false-negative results. A negative liquid biopsy result does not necessarily exclude the presence of malignancy or a clinically relevant genomic alteration, particularly when tumor burden is low or when the tumor releases limited amounts of DNA into the circulation [92]. Therefore, liquid biopsy should not be interpreted as a complete replacement for tissue biopsy in all clinical scenarios.

2.38. Preanalytical Variability

Preanalytical factors have a major impact on the quality and reliability of cfDNA and ctDNA analyses [34]. Variables such as blood collection tube type, time from venipuncture to plasma separation, centrifugation protocol, storage temperature, freeze–thaw cycles, and DNA extraction methodology can significantly influence cfDNA concentration, fragment integrity, and background genomic DNA contamination.

Plasma is generally preferred over serum because serum is more prone to leukocyte-derived genomic DNA contamination during clotting [34]. However, even plasma-based workflows require strict standardization to ensure reproducibility. The absence of harmonized preanalytical protocols across laboratories remains a significant obstacle to broad clinical adoption.

2.39. Analytical Sensitivity and Specificity

Liquid biopsy assays require very high analytical sensitivity to detect low-frequency tumor-derived variants within a large background of wild-type cfDNA. Technologies such as ddPCR, BEAMing, and ultra-deep NGS have improved detection limits, but each method has specific advantages and limitations [13].

Targeted assays such as ddPCR and BEAMing provide excellent sensitivity for predefined mutations but cannot detect unexpected or novel alterations. Conversely, NGS-based approaches offer broader genomic coverage but are more expensive, technically complex, and dependent on robust bioinformatic error correction [12,13].

False-positive results may arise from sequencing artifacts, polymerase errors, contamination, or biological confounders. Therefore, variant allele frequency thresholds,

assay validation standards, and orthogonal confirmation strategies are essential for reliable interpretation.

2.40. Clonal Hematopoiesis

Clonal hematopoiesis represents one of the most clinically relevant biological confounders in liquid biopsy interpretation. Age-related somatic mutations arising in hematopoietic cells may be released into plasma and mistakenly interpreted as tumor-derived alterations. This is particularly problematic when analyzing low-frequency variants or genes frequently mutated in hematopoietic clones, such as DNMT3A, TET2, ASXL1, TP53, and JAK2.

Without matched white blood cell sequencing, it may be difficult to distinguish true ctDNA variants from clonal hematopoiesis-associated mutations. This limitation is especially important in early cancer detection, MRD assessment, and screening contexts, where the consequences of false-positive results may be clinically significant.

2.41. Tissue–Plasma Discordance

Although ctDNA can capture tumor heterogeneity across multiple disease sites, discordance between tissue and plasma molecular findings may occur. Tissue biopsy provides spatially localized information from a specific tumor region, whereas liquid biopsy reflects DNA released from multiple tumor sites and non-tumor sources. Differences between tissue and plasma results may therefore reflect true biological heterogeneity, temporal evolution, treatment pressure, or technical limitations [18,19].

In some cases, plasma may detect resistance mutations not identified in archival tissue. In other cases, tissue biopsy may reveal actionable alterations absent from plasma due to low ctDNA shedding. For this reason, liquid biopsy and tissue biopsy should often be regarded as complementary rather than competing diagnostic approaches.

2.42. Standardization and Clinical Validation

The lack of universal standardization remains a major barrier to routine implementation. Assays differ in sample input requirements, target regions, sequencing depth, bioinformatic pipelines, reporting thresholds, and clinical interpretation frameworks [93]. This heterogeneity makes comparison between platforms difficult and may affect reproducibility across institutions.

Clinical validation is particularly important for applications such as early detection, MRD assessment, and treatment de-escalation. While many retrospective studies support the prognostic value of ctDNA, prospective interventional trials are still required to demonstrate that liquid biopsy-guided clinical decisions improve patient outcomes.

2.43. Cost, Accessibility, and Implementation

Liquid biopsy technologies, particularly NGS-based platforms and multi-omics assays, may be associated with substantial costs. Implementation requires specialized laboratory infrastructure, validated workflows, trained personnel, and bioinformatic expertise [94]. These requirements may limit accessibility, especially in resource-constrained healthcare systems.

Cost-effectiveness also depends on the clinical scenario. Liquid biopsy may be highly valuable when it avoids invasive procedures, accelerates therapeutic decision-making, or enables early detection of resistance. However, routine use in contexts with uncertain clinical benefit may increase healthcare costs without improving outcomes.

2.44. Exosome-Specific Limitations

Although exosomes represent a promising liquid biopsy analyte, their clinical translation is less advanced than ctDNA-based testing. Major limitations include the lack of

standardized isolation methods, contamination with other extracellular vesicles or plasma proteins, variability in yield and purity, and difficulty in distinguishing tumor-derived exosomes from vesicles released by normal cells [85].

Furthermore, many proposed exosomal biomarkers have been identified in small discovery cohorts and require validation in larger, prospective, clinically annotated studies. Until these methodological and validation issues are resolved, exosome-based liquid biopsy will remain largely investigational.

2.45. Ethical and Clinical Interpretation Challenges

The increasing sensitivity of liquid biopsy assays raises additional ethical and clinical questions. Detection of low-level molecular abnormalities may not always correspond to clinically meaningful disease. This is particularly relevant in early cancer detection, MRD surveillance, and screening of asymptomatic individuals.

False-positive findings may lead to anxiety, unnecessary investigations, or overtreatment, whereas false-negative results may provide inappropriate reassurance. Therefore, liquid biopsy results should always be interpreted in the context of clinical presentation, imaging findings, histopathology, and multidisciplinary decision-making.

2.46. Artificial Intelligence and Emerging Directions in Liquid Biopsy

The rapid expansion of liquid biopsy technologies has generated increasingly large and complex molecular datasets that require sophisticated computational approaches for accurate interpretation. Artificial intelligence (AI), machine learning (ML), and advanced bioinformatic methods are therefore becoming integral components of contemporary liquid biopsy analysis [95]. These technologies have the potential to enhance diagnostic sensitivity, improve risk stratification, identify clinically relevant molecular patterns, and facilitate personalized therapeutic decision-making in precision oncology.

2.47. Artificial Intelligence in Molecular Data Interpretation

Liquid biopsy analyses frequently generate multidimensional datasets that include:

- genomic alterations,
- methylation profiles,
- fragmentomic signatures,
- transcriptomic data,
- proteomic patterns,
- and longitudinal molecular dynamics [96–99].

The complexity of these datasets often exceeds the interpretative capacity of conventional statistical methods. Machine learning algorithms can identify subtle molecular relationships and predictive patterns that may not be apparent through traditional analytical approaches [95].

AI-assisted models have demonstrated promising applications in:

- early cancer detection,
- tissue-of-origin prediction,
- treatment response assessment,
- prognostic classification,
- and resistance mechanism identification.

By integrating large-scale molecular and clinical datasets, machine learning systems may improve diagnostic accuracy and facilitate individualized patient management strategies.

2.48. Multi-Cancer Early Detection (MCED)

One of the most rapidly developing areas of liquid biopsy research involves multi-cancer early detection approaches. These platforms aim to identify molecular signals associated with multiple cancer types simultaneously from a single blood sample [100].

AI-driven algorithms play a central role in MCED systems by integrating and analyzing complex combinations of:

- methylation signatures,
- cfDNA fragmentation patterns,
- mutational profiles,
- protein biomarkers,
- and clinical variables.

Methylation-based machine learning models have shown particular promise because methylation signatures are highly tissue-specific and may enable simultaneous cancer detection and tissue-of-origin classification [100]. Fragmentomic approaches additionally exploit characteristic fragmentation profiles of tumor-derived cfDNA to improve sensitivity for early-stage disease.

Although MCED technologies remain under active investigation, they may eventually transform population-based cancer screening strategies, particularly for malignancies lacking effective conventional screening methods.

2.49. Fragmentomics and Epigenomic Profiling

Emerging liquid biopsy approaches increasingly extend beyond mutation detection alone. Fragmentomics analyzes physical properties of cfDNA fragments, including:

- fragment length distributions,
- nucleosome positioning,
- end motif frequencies,
- and fragmentation patterns [26,41].

Tumor-derived cfDNA exhibits distinct fragmentation characteristics compared with non-malignant cfDNA, and machine learning models can use these differences for cancer classification and detection.

Similarly, epigenomic liquid biopsy approaches evaluate:

- DNA methylation patterns,
- chromatin accessibility,
- histone modifications,
- and nucleosomal organization.

Because epigenetic alterations often occur early in carcinogenesis, these methods may improve sensitivity in early-stage tumors where mutation burden is limited [24].

The integration of fragmentomic and methylation-based analyses with AI-driven classification systems represents one of the most promising directions for next-generation liquid biopsy development.

2.50. Multi-Omics Liquid Biopsy Approaches

Future liquid biopsy strategies are increasingly focused on multi-omics integration. Rather than relying exclusively on ctDNA mutation analysis, contemporary platforms aim to combine information derived from:

- ctDNA,
- cfDNA,
- exosomal nucleic acids,
- circulating tumor cells,

- proteomics,
- metabolomics,
- and immune biomarkers.

Multi-omics approaches may provide a more comprehensive representation of tumor biology and host-tumor interactions. Artificial intelligence systems are particularly well suited for integrating these highly complex datasets and identifying clinically actionable molecular signatures.

Such integrative strategies may ultimately improve:

- diagnostic precision,
- prognostic stratification,
- therapeutic response prediction,
- and longitudinal disease monitoring.

2.51. Minimal Residual Disease and Dynamic Monitoring

AI-assisted liquid biopsy analysis is also being explored in minimal residual disease detection and longitudinal surveillance [101]. In MRD settings, ctDNA concentrations are often extremely low, making signal discrimination technically challenging.

Machine learning-based error correction algorithms can improve sensitivity by distinguishing true low-frequency variants from sequencing artifacts or background noise. Furthermore, dynamic modeling of serial ctDNA measurements may enhance prediction of relapse risk and treatment response over time.

Future MRD platforms may integrate:

- genomic evolution,
- temporal ctDNA kinetics,
- immune response markers,
- and radiological data to generate individualized relapse prediction models [101].

2.52. Exosome-Based Innovations

Advances in nanotechnology, microfluidics, and high-throughput sequencing are accelerating the development of exosome-based diagnostics [100–102]. Emerging technologies aim to improve:

- exosome isolation,
- single-vesicle analysis,
- cargo characterization,
- and real-time molecular profiling.

AI-assisted analysis of exosomal protein and RNA signatures may further enhance biomarker discovery and disease classification. In parallel, exosomes are being investigated as potential therapeutic delivery vehicles because of their natural biocompatibility and ability to transport molecular cargo across biological barriers.

Although these applications remain largely experimental, they illustrate the rapidly expanding scope of extracellular vesicle research in oncology.

2.53. Integration into Precision Oncology

The future of liquid biopsy is closely linked to the broader evolution of precision oncology. Increasingly, clinical management strategies are expected to rely on dynamic molecular characterization rather than static baseline tissue profiling alone.

Liquid biopsy may contribute to:

- adaptive treatment selection,
- earlier detection of resistance,
- personalized monitoring schedules,
- real-time therapeutic adjustment,
- and individualized prognostic assessment [103,104].

Combined with AI-driven predictive modeling, liquid biopsy could facilitate a transition toward continuously evolving, patient-specific oncological management.

2.54. Current Challenges of AI Integration

Despite considerable enthusiasm, the integration of artificial intelligence into liquid biopsy workflows also presents important challenges. These include:

- limited availability of high-quality annotated datasets,
- variability between sequencing platforms,
- lack of algorithm transparency,
- overfitting risks,
- and difficulties in external validation.

Furthermore, regulatory, ethical, and medico-legal considerations surrounding AI-assisted diagnostics remain incompletely defined. Clinical implementation will therefore require rigorous validation, reproducibility assessment, and multidisciplinary oversight [105,106].

2.55. Future Perspectives

The future development of liquid biopsy will likely involve increasing convergence between:

- ultra-sensitive molecular technologies,
- multi-omics profiling,
- artificial intelligence,
- and real-time clinical analytics.

As technological sensitivity improves and standardization advances, liquid biopsy may progressively transition from a complementary diagnostic tool to a central component of precision oncology workflows. Prospective clinical trials and large-scale validation studies will be essential to establish the clinical utility, cost-effectiveness, and long-term impact of these emerging technologies on patient outcomes.

Overall, the integration of AI and next-generation molecular profiling approaches has the potential to fundamentally redefine cancer diagnostics, disease monitoring, and personalized therapeutic strategies in modern oncology.

Liquid biopsy technologies are expected to play an increasingly central role in the future of precision oncology. Continuous advances in molecular biology, sequencing technologies, computational analysis, and biomarker discovery are rapidly expanding the clinical capabilities of ctDNA-, cfDNA-, and exosome-based diagnostics. Although current applications remain concentrated primarily in advanced disease monitoring and targeted therapy selection, future developments may substantially broaden the role of liquid biopsy across the entire continuum of cancer care (Figure 5).

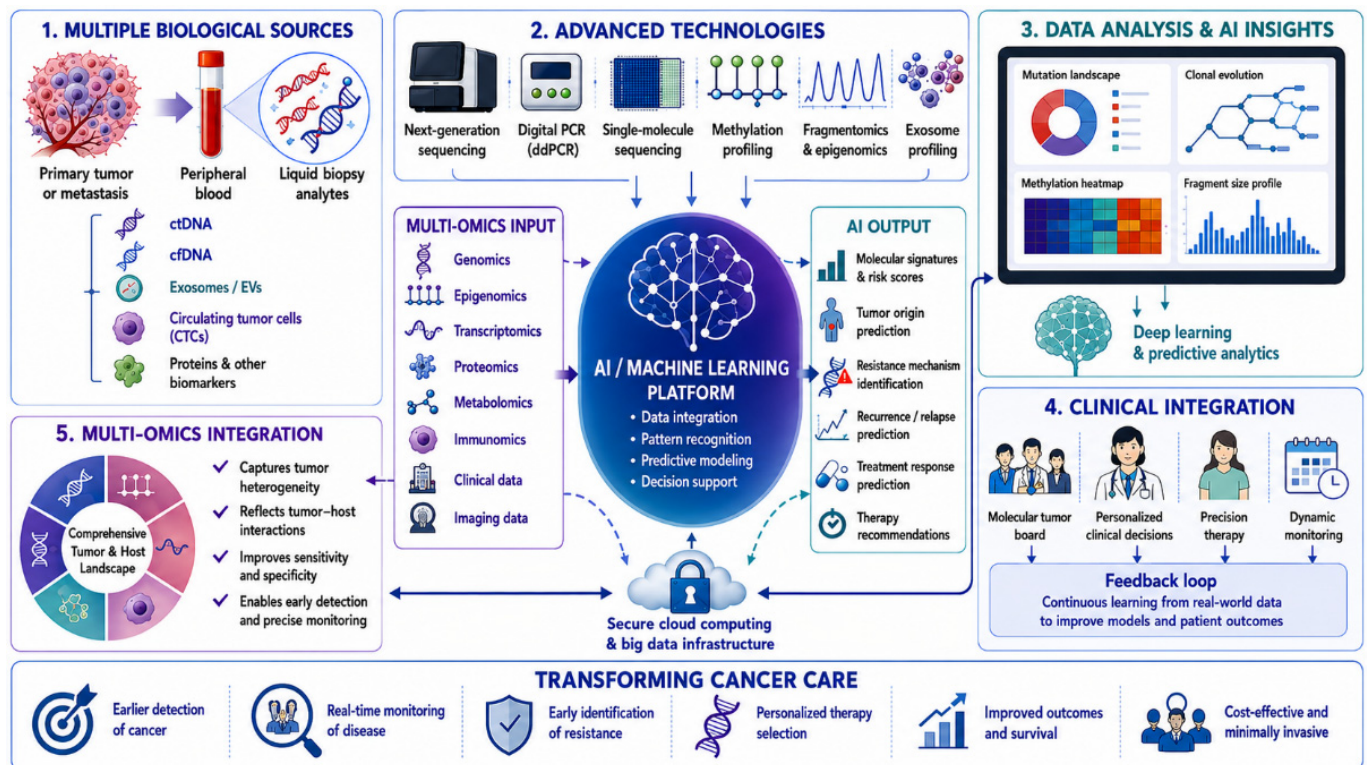


Figure 5. Future perspectives of liquid biopsy in precision oncology. Emerging developments in liquid biopsy include the integration of ctDNA, cfDNA, CTCs, EVs, methylation profiling, fragmentomics, and other circulating biomarkers into multi-analyte diagnostic platforms. Advances in next-generation sequencing, ultra-sensitive molecular assays, and AI-assisted data analysis are expected to improve early cancer detection, tissue-of-origin prediction, MRD assessment, treatment monitoring, and personalized therapeutic decision-making. Continued technological innovation, assay standardization, and prospective clinical validation will be essential for the broader implementation of liquid biopsy in routine precision oncology.

2.56. Transition Toward Earlier Cancer Detection

One of the most important future directions involves improving the sensitivity of liquid biopsy for early-stage cancer detection. Current ctDNA assays often face limitations in localized tumors because of low tumor fraction and limited DNA shedding. However, ongoing refinement of ultra-sensitive sequencing methodologies, methylation-based assays, fragmentomic profiling, and multi-analyte detection platforms may significantly improve early detection performance [37,39,102].

The development of reliable multi-cancer early detection assays has the potential to transform population-based cancer screening strategies. Blood-based screening platforms capable of simultaneously detecting multiple malignancies and predicting tissue of origin may eventually complement or partially replace certain conventional screening modalities. Such approaches could be particularly valuable for cancers that currently lack effective screening programs, including pancreatic, ovarian, and hepatobiliary malignancies [107].

Nevertheless, the implementation of population-level liquid biopsy screening will require careful evaluation of:

- diagnostic accuracy,
- false-positive rates,
- overdiagnosis risk,
- cost-effectiveness,
- and clinical outcome benefits.

Large prospective validation studies will therefore be essential before routine implementation can be recommended.

2.57. Increasing Role in Minimal Residual Disease-Guided Therapy

Minimal residual disease detection is likely to become one of the most clinically impactful future applications of ctDNA analysis [108]. As assay sensitivity improves, ctDNA-guided treatment strategies may allow increasingly personalized approaches to adjuvant and surveillance therapy.

Future oncology management may incorporate ctDNA-based MRD assessment to:

- identify patients requiring treatment escalation,
- guide treatment de-escalation in low-risk individuals,
- optimize surveillance intervals,
- and enable earlier therapeutic intervention at molecular relapse.

Such approaches may reduce overtreatment while improving relapse prevention and long-term outcomes. Several ongoing clinical trials are currently evaluating ctDNA-guided therapeutic decision-making in colorectal, breast, lung, and other solid tumors.

2.58. Dynamic Real-Time Precision Oncology

Liquid biopsy supports a transition from static tumor characterization toward continuously adaptive molecular oncology. Traditional tissue biopsies provide only a limited snapshot of tumor biology at a single time point, whereas serial ctDNA analyses allow dynamic monitoring of clonal evolution and therapeutic resistance [109].

Future treatment paradigms may increasingly rely on longitudinal molecular surveillance to:

- detect resistance mechanisms before radiological progression,
- monitor therapeutic efficacy in real time,
- adjust treatment according to evolving tumor profiles,
- and optimize personalized treatment sequencing.

This dynamic approach may become particularly important in metastatic disease and in patients receiving targeted therapies or immunotherapies.

2.59. Expansion of Multi-Omics Approaches

The future of liquid biopsy will likely involve increasing integration of multi-omics data rather than isolated mutation analysis alone. Emerging platforms are beginning to combine:

- genomic profiling,
- epigenomic signatures,
- transcriptomics,
- proteomics,
- metabolomics,
- immune biomarkers,
- and extracellular vesicle analysis.

Such integrative approaches may provide a more comprehensive representation of tumor biology and host-tumor interactions. Artificial intelligence and machine learning systems will be essential for interpreting these highly complex datasets and identifying clinically relevant molecular signatures [104].

The integration of multiple biomarker classes may additionally improve sensitivity in low-shedding tumors and early-stage disease.

2.60. Exosome-Based Diagnostics and Therapeutics

Exosome research continues to evolve rapidly, and future developments may substantially expand their clinical relevance [110]. Improved isolation technologies, single-vesicle analyses, and advanced molecular profiling techniques may facilitate more reliable exosome-based diagnostics.

In addition to their diagnostic role, exosomes are increasingly being investigated as potential therapeutic tools. Their natural biocompatibility, low immunogenicity, and ability to transport molecular cargo across biological barriers make them attractive candidates for:

- targeted drug delivery,
- RNA-based therapeutics,
- immunomodulation,
- and gene therapy applications.

Although many of these applications remain experimental, exosome-based therapeutics may eventually emerge as an important area of translational oncology research.

2.61. Artificial Intelligence-Driven Clinical Integration

Artificial intelligence is expected to become progressively integrated into routine liquid biopsy workflows. AI-assisted systems may facilitate:

- automated molecular interpretation,
- risk stratification,
- relapse prediction,
- therapeutic response modeling,
- and clinical decision support.

Machine learning algorithms may additionally improve assay sensitivity through advanced signal discrimination and artifact correction. As datasets become larger and more standardized, AI-driven predictive oncology models may significantly improve personalized cancer management.

However, successful implementation will require:

- robust validation,
- transparency of algorithms,
- regulatory oversight,
- and careful integration into multidisciplinary clinical practice [103,106].

2.62. Standardization and Regulatory Development

Future progress in liquid biopsy will depend heavily on international standardization efforts. Harmonized protocols for:

- sample collection,
- processing,
- sequencing,
- bioinformatic analysis,
- and result interpretation will be necessary to ensure reproducibility and clinical reliability.

Regulatory frameworks will also need to evolve alongside technological advances. Clear guidelines regarding assay validation, clinical utility, quality assurance, and reporting standards will be essential for widespread clinical adoption.

Professional organizations and regulatory agencies are increasingly working toward standardized implementation pathways, but substantial heterogeneity remains between platforms and institutions [95].

2.63. Accessibility and Global Implementation

Although liquid biopsy technologies continue to advance rapidly, future implementation must also address issues of accessibility and healthcare equity. High costs, specialized infrastructure requirements, and limited access to advanced molecular diagnostics may restrict availability in lower-resource settings.

Technological simplification, automation, and cost reduction will therefore be essential for ensuring broader global access to precision oncology approaches. Point-of-care liquid biopsy platforms and simplified molecular workflows may eventually facilitate implementation beyond highly specialized academic centers.

3. Conclusions

Liquid biopsy has emerged as one of the most promising developments in precision oncology, providing minimally invasive access to dynamic tumor-derived biomarkers that complement conventional tissue-based diagnostics. The rapid evolution of analytical technologies has substantially improved the detection and characterization of circulating tumor DNA (ctDNA), cell-free DNA (cfDNA), circulating tumor cells (CTCs), and extracellular vesicles, enabling increasingly comprehensive molecular profiling of solid malignancies. Each of these analytes offers distinct yet complementary biological information, and their combined application has the potential to provide a more complete understanding of tumor heterogeneity, disease evolution, therapeutic response, and mechanisms of treatment resistance.

Despite these remarkable advances, several challenges continue to limit the widespread implementation of liquid biopsy in routine clinical practice. Biological variability among tumor types, low biomarker abundance in early-stage disease, pre-analytical and analytical variability, lack of universal assay standardization, and the need for further prospective clinical validation remain important considerations. Consequently, liquid biopsy should currently be regarded as a complementary approach that augments, rather than replaces, conventional tissue-based molecular diagnostics in many clinical settings.

Continued technological innovation, including improvements in ultra-sensitive sequencing methods, methylation profiling, fragmentomic analyses, single-cell characterization of CTCs, extracellular vesicle profiling, and multi-analyte liquid biopsy platforms, is expected to further enhance the analytical performance and clinical utility of these approaches. In parallel, advances in artificial intelligence and machine learning are increasingly facilitating the integration and interpretation of complex molecular datasets, supporting biomarker discovery, predictive modelling, and more informed clinical decision-making.

Looking ahead, the progressive integration of multiple circulating biomarkers with imaging, clinicopathological data, and computational analytical tools is expected to further advance personalized oncology. As ongoing prospective studies continue to refine the clinical indications for liquid biopsy and international efforts toward methodological harmonization mature, these technologies are likely to assume an increasingly important role in early cancer detection, molecular stratification, treatment monitoring, minimal residual disease assessment, and longitudinal patient management. Together, these developments are expected to strengthen the role of liquid biopsy as an integral component of precision cancer medicine while highlighting the need for continued multidisciplinary collaboration to facilitate its safe, standardized, and evidence-based implementation.

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