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Advances in Liquid Biopsy for Early Cancer Detection: A Comprehensive Review of Circulating Tumor DNA and Other Biomarkers

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ABSTRACT

Liquid biopsy is a non-invasive diagnostic tool that detects cancer biomarkers in bodily fluids, revolutionising early cancer detection and monitoring. This approach analyses circulating tumour DNA (ctDNA), circulating tumour cells (CTCs), and extracellular vehicles (EVs) to provide insights into tumour biology and progression. Liquid biopsy has emerged as a promising alternative to traditional tissue biopsies, offering a more accurate and less invasive approach. The technology has gained significant attention in recent years due to its potential in detecting cancer at an early stage. This review seeks to explore the advances in liquid biopsy for early cancer detection with emphasis on circulating tumour DNA and other biomarkers. Studies show liquid biopsy effectively detects cancer, particularly when combining ctDNA and CTC analysis. ctDNA analysis identifies genetic mutations, while CTCs provide information on tumour heterogeneity. Liquid biopsy also monitors treatment response and detects minimal residual disease. Advances in technologies like next-generation sequencing (NGS) and polymerase chain reaction (PCR) enhance sensitivity and specificity. Liquid biopsy applications include early detection, prognosis, and personalised treatment strategies. The technology has been explored in various cancer types, including lung, breast, and colorectal cancer. Researchers have also investigated its potential in detecting cancer recurrence and metastasis. Liquid biopsy is a promising tool for early cancer detection, offering a minimally invasive alternative to traditional tissue biopsies. Its applications in precision oncology are vast, enabling personalised treatment and improved patient outcomes. The technology has the potential to revolutionise cancer diagnosis and treatment. Further research is needed to validate its clinical utility.

***Keywords**: Liquid biopsy, Circulating tumor DNA, Circulating tumor cells, Cancer detection, Biomarkers.*

Introduction

Cancer remains a leading cause of morbidity and mortality worldwide, with projections indicating a substantial escalation in burden over the coming decades [1]. In 2023, approximately 18.5 million new cases and 10.4 million deaths occurred globally, excluding non-melanoma skin cancers, with forecasts suggesting rises to around 30.5 million new cases and 18.6 million deaths by 2050, driven largely by population growth, aging, and persistent disparities in low- and middle-income regions where access to early intervention is limited [2-5].

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Early detection profoundly influences outcomes, as many cancers are curable when identified at localised stages, potentially averting a significant portion of deaths through timely prevention and treatment strategies that target modifiable risk factors and improve survival [6].

Conventional tissue biopsy, while providing definitive histopathological confirmation, faces inherent drawbacks that hinder its utility in routine screening and longitudinal monitoring. The procedure's invasiveness often entails risks such as pain, infection, bleeding, and anaesthesia-related complications, rendering it unsuitable for repeated sampling or patients in poor condition [7]. Moreover, tissue biopsies capture only a limited spatial snapshot of the tumour, exacerbating challenges from intratumoral heterogeneity where genetically diverse subclones coexist and spatial sampling bias, which may miss critical mutations or fail to reflect the full evolutionary landscape of the disease [8]. These constraints limit the ability to monitor dynamic changes, detect minimal residual disease, or guide precision therapies effectively in heterogeneous or metastatic settings.

In response, liquid biopsy has emerged as a transformative, minimally invasive alternative that analyses tumour-derived components shed into bodily fluids, primarily blood, enabling real-time insights into tumour biology with greater accessibility and repeatability [9, 10]. This approach circumvents many pitfalls of tissue sampling by providing a systemic view that better captures heterogeneity and temporal evolution,

facilitating applications in early detection, prognosis, treatment selection, and monitoring of response or resistance across diverse cancers [11].

Key circulating biomarkers underpinning liquid biopsy include circulating tumor DNA (ctDNA), which consists of fragmented tumor-derived DNA bearing somatic mutations, methylation patterns, and fragmentomic features for sensitive detection even in early stages; circulating tumor cells (CTCs), intact viable cells offering insights into metastatic potential and heterogeneity through molecular profiling; cell-free RNA (cfRNA), encompassing miRNAs and other non-coding RNAs that reflect tumor gene expression and regulatory alterations; exosomes and other extracellular vehicles (EVs), which carry multifaceted cargo (proteins, lipids, nucleic acids) for multi-omic analysis and early signaling; and additional entities such as proteins and metabolites that complement genetic markers [12, 13]. In ovarian cancer, for instance, emerging biomarkers like ctDNA, CTCs, exosomes, and cfRNA show promise for overcoming diagnostic delays common in this malignancy, where traditional markers (e.g., CA-125) lack specificity, highlighting liquid biopsy's potential to enhance early detection and prognostic stratification [14, 15].

This review aimed at investigating recent advances in liquid biopsy for early cancer detection, with a primary emphasis on ctDNA alongside complementary biomarkers such as CTCs, cfRNA, exosomes, and others. The scope encompasses biological mechanisms, detection technologies, performance evidence across cancer types (including ovarian), clinical applications, challenges including standardisation and low tumour shedding in early stages, and future directions toward multi-analyte integration and clinical translation. The structure proceeds from foundational biology and technologies to empirical performance, specific applications, limitations, and perspectives to guide ongoing research and implementation.

Tumour Microenvironment and Biomarker Release Mechanisms

The tumour microenvironment (TME) forms an intricate, heterogeneous network encompassing malignant epithelial cells, supportive stromal cells (including carcinoma-associated fibroblasts [CAFs]), diverse immune populations (such as tumour-associated macrophages [TAMs], lymphocytes, and mast cells), pericytes, endothelial cells, and a remodelled extracellular matrix (ECM). This ecosystem not only sustains tumour proliferation, invasion, and immune evasion but also drives the continuous shedding of biomarkers into circulation, making it foundational for liquid biopsy utility [16, 17].

Tumour angiogenesis, triggered by hypoxia and pro-angiogenic factors including vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF), generates aberrant, tortuous blood vessels with structural defects: enlarged endothelial gaps, fenestrations, discontinuous basement membranes, and reduced pericyte ensheathment [18, 19]. These abnormalities create "leaky vasculature," characterised by increased permeability that enhances nutrient diffusion but also enables passive extravasation of tumour-derived entities into the bloodstream [20]. Leaky vessels facilitate the dissemination of fragmented circulating tumour DNA (ctDNA) from dying cells, intact circulating tumour cells (CTCs) through active intravasation, and exosomes/extracellular vehicles (EVs) via enhanced permeation and retention-like effects in the TME [21].

Hypoxia, prevalent in rapidly growing tumours due to mismatched perfusion, activates hypoxia-inducible factor-1α (HIF-1α), which upregulates angiogenesis-related genes, glycolytic pathways, and exosome biogenesis/secretion. Hypoxic stress promotes cell death (apoptosis, yielding nucleosome-protected short ctDNA fragments; necrosis, producing larger, irregular ones) while viable hypoxic cells release enriched exosomes carrying hypoxia-responsive cargo (e.g., pro-angiogenic miRNAs, proteins, and metabolites) that reprogram distant sites and amplify biomarker availability in circulation [22, 23].

Elevated cellular turnover in malignant tissues, driven by unchecked proliferation offset by apoptosis, necrosis, and active secretion, constitutes the core mechanism for biomarker generation. Stromal and immune interactions intensify this: CAFs remodel ECM via protease secretion (e.g., matrix metalloproteinases) to aid invasion and vascular access; TAMs and mast cells secrete cytokines/growth factors perpetuating angiogenesis and inflammation; pericytes and endothelial cells contribute to vessel instability and leakiness. These collaborative processes create an environment permissive for efficient biomarker entry into systemic circulation via the described leaky vasculature [24, 25].

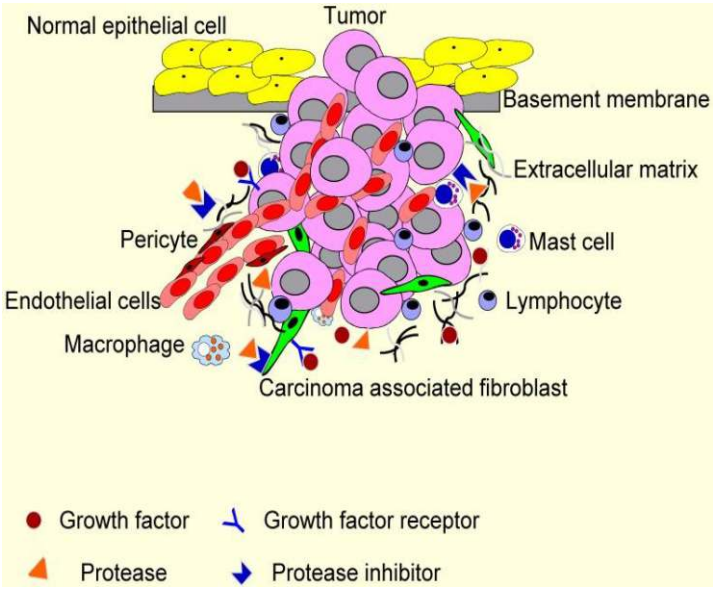

Figure 1. Tumour microenvironment illustrating cancer cells, stromal cells, immune cells, Leaky tumour vasculature and routes of biomarker entry into circulation.
***Source:** [19]*

Figure 1 above illustrates the tumour microenvironment and its role in biomarker release. At the top, normal epithelial cells line a structured basement membrane, contrasting with the disorganized tumour mass below (pink/purple carcinoma cells clustered amid ECM). The tumour features leaky, irregular vasculature (red endothelial tubes with gaps/fenestrations, reduced pericyte coverage shown as sparse wrapping), allowing biomarker shedding into the bloodstream flow. Key stromal and immune elements include elongated carcinoma-associated fibroblasts remodelling ECM (egret fibrous network), pericytes (red wrapping), macrophages (irregular blue/purple), lymphocytes (small round), and mast cells (granulated). Signaling is depicted with red dots (growth factors), blue triangles (proteases), and arrows indicating interactions and release pathways: ctDNA fragments from apoptotic/necrotic tumour cells drifting passively, CTCs actively migrating/intravasating through vessel gaps, and exosomes budding from viable cells into circulation.

This visual emphasises how TME components, hypoxia-driven angiogenesis, ECM degradation, and cellular interactions promote leaky vasculature and routes for ctDNA, CTCs, exosomes, and other biomarkers to enter blood, underpinning liquid biopsy detection (adapted from common TME schematics in liquid biopsy reviews [26, 27].

Cellular Processes Generating Circulating Biomarkers

Circulating biomarkers central to liquid biopsy, including circulating tumour DNA (ctDNA), circulating tumour cells (CTCs), and components within extracellular vehicles (EVs), originate from specific cellular death and secretion pathways in tumour tissues, providing insights into tumour burden, heterogeneity, and dynamics for non-invasive cancer detection and monitoring [28]. Apoptosis, a regulated programmed cell death process, predominantly generates short ctDNA fragments through caspase-mediated chromatin fragmentation and apoptotic body formation, yielding nucleosome-protected DNA typically 145–180 bp in length with characteristic laddering and periodicity that reflects nucleosomal structure and enhances tumour-specific signal enrichment even at low fractions [29]. This mechanism predominates in early-stage or well-vascularized tumours, contributing to ctDNA's utility in sensitive early detection via fragmentomic patterns.

Necrosis, an uncontrolled lytic process triggered by severe stress or hypoxia, releases larger, irregular high-molecular-weight DNA fragments often >1 kb or exceeding 10 kb due to membrane rupture, organelle breakdown, and inflammatory content leakage, resulting in less protected cfDNA with reduced specificity but elevated total levels in advanced, necrotic tumours [30, 31]. These differences in fragment size, short/protected from apoptosis versus long/unprotected from necrosis, enable bioinformatic enrichment strategies to improve ctDNA detection sensitivity by prioritising shorter fragments characteristic of tumour origin.

Active secretion provides a non-lytic pathway where viable tumour cells release protected nucleic acids and other molecules via extracellular vesicles, such as exosomes (30–150 nm) or microvesicles, shielding ctDNA, cfRNA, miRNAs, proteins, and lipids from degradation while facilitating intercellular signaling, metastasis promotion, and immune evasion [32, 33]. This continuous mechanism sustains biomarker presence in circulation during low tumour burden or therapy, supporting multi-omic profiling for prognosis and resistance monitoring.

Shedding of intact CTCs involves active detachment from primary or metastatic sites, often via epithelial-mesenchymal transition, intravasation into vasculature, and survival in blood, offering viable cells for genomic, transcriptomic, and functional analysis of metastatic potential and heterogeneity [34, 35].

Panel A in figure 2 depicts the divergence from a normal cell to apoptosis (ordered shrinkage, chromatin condensation, nuclear collapse, membrane budding, apoptotic body formation, and phagocytosis) versus necrosis (swelling, organelle breakdown, content leakage, and inflammation), directly linking to biomarker release: apoptosis produces short ctDNA fragments (~150 bp, nucleosome-protected) while necrosis yields larger, irregular high-molecular-weight DNA with inflammatory spillover.

The panel B outlines the extrinsic pathway (ligands TNF-α/FASL binding death receptors, DISC assembly with FADD/TRAF2/TRADD/cIAPs/RIP, activation of Casp8/10, leading to effector caspases Casp3/7 via Casp9/apoptosome and PARP cleavage) and intrinsic pathway (stimuli like oncogenes/DNA damage/hypoxia/growth factor deprivation activating BCL-2 family/BH3-only proteins, BAK/BAX mitochondrial oligomerization, cytochrome C release, apoptosome formation, Casp9 activation, and downstream effector caspases), emphasizing apoptosis's caspase cascade and cleaved PARP as hallmarks of orderly DNA fragmentation into short ctDNA pieces.

Active secretion (EVs budding from viable cells, protecting cargo like ctDNA/RNA) and CTC shedding (intact tumor cells entering circulation) complement these death pathways, with the figure illustrating apoptosis (short ctDNA, programmed execution), necrosis (large DNA, chaotic/inflammatory), active secretion (EV-protected multi-omic cargo from living cells), and CTCs (whole-cell dissemination), explaining variations in biomarker abundance, size, protection, and clinical informativeness essential for liquid biopsy optimization [35-37].

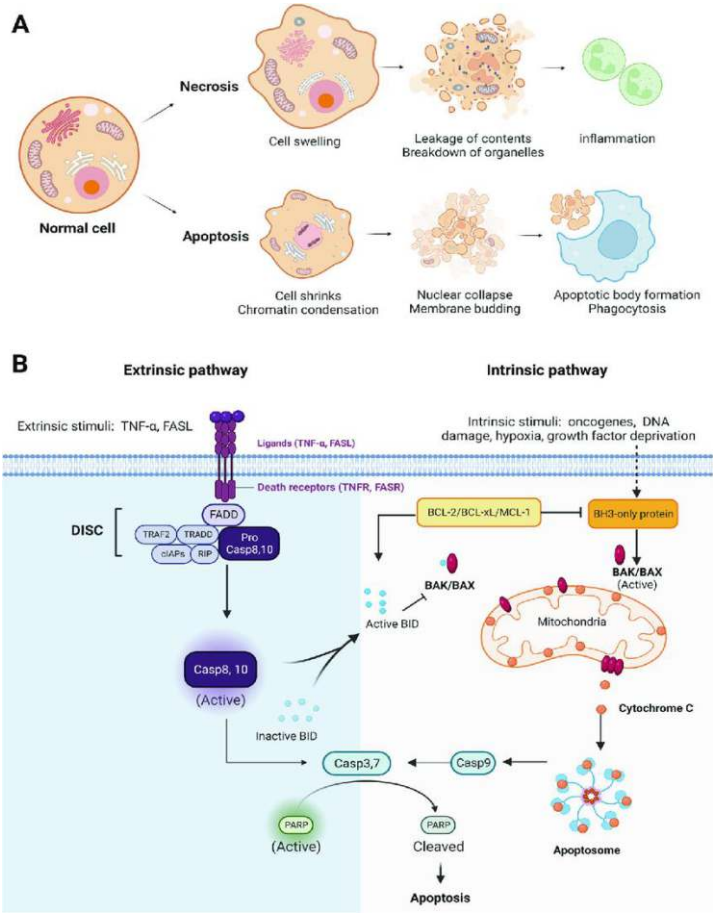

Figure 2. Necroptosis, pyroptosis and apoptosis: an intricate game of cell death
***Source:** [36]*

Release Mechanisms of Circulating Biomarkers

Circulating tumour DNA (ctDNA) displays unique biophysical and kinetic properties that underpin its value as a sensitive, real-time biomarker in liquid biopsy. ctDNA fragments are characteristically shorter than those of non-tumour cell-free DNA (cfDNA), with predominant enrichment in the 90–150 bp range (often peaking near 145 bp, aligning with mononucleosomal protection minus linker DNA), in contrast to the ~166–167 bp mononucleosomal peak typical of physiological cfDNA from apoptotic normal cells [38, 39]. This size bias stems from tumour-specific chromatin accessibility and nuclease activity, enabling fragment omics approaches to amplify tumour signal by prioritising shorter fragments, frequently achieving substantial sensitivity gains [40, 41].

The plasma half-life of ctDNA is brief, typically spanning 16 minutes to 2.5 hours (commonly cited as ~0.5–2 hours), attributable to rapid nuclease degradation, hepatic/renal clearance, and phagocytic uptake, which confers near-real-time tracking of tumour burden fluctuations, therapeutic responses, and minimal residual disease [42, 43]. Tumour fraction (TF), the proportion of ctDNA within total cfDNA, ranges from <0.1% in early-stage or low-shedding cancers to >10% in advanced/metastatic disease, directly correlating with tumour volume, stage, and shedding efficiency; low TF demands ultrasensitive assays, while higher TF (>1%–5%) bolsters variant detection reliability and reduces false negatives [44].

Complementing ctDNA, other biomarkers expand the liquid biopsy's multi-omic scope. Circulating tumour cells (CTCs) are rare intact malignant cells (often 1 per million of leukocytes) released via active intravasation following epithelial-mesenchymal transition (EMT), exhibiting short half-life (~1–2 hours) due to shear stress, anoikis, and immune clearance; they offer direct cellular heterogeneity, surface marker expression (e.g., EpCAM), and functional metastatic insights [45, 46]. Exosomes and extracellular vehicles (EV**s**, 30–150 nm) are actively secreted by viable tumour/stromal cells, encapsulating stable cargo (miRNAs, cfRNA, proteins, lipids, metabolites) that resists degradation and mediates paracrine signaling, angiogenesis, and pre-metastatic niche formation; their abundance and content enable prognostic multi-omic profiling even in low-burden settings [47, 48]. Cell-free RNA (cfRNA), including miRNAs, mirrors tumour transcriptional dysregulation and is often EV-protected for enhanced stability, serving as diagnostic/prognostic markers in diverse cancers [49]. Circulating proteins (e.g., antigens) and metabolites provide functional/epigenetic complements, though with lower specificity, and synergise in integrated panels for improved accuracy [50].

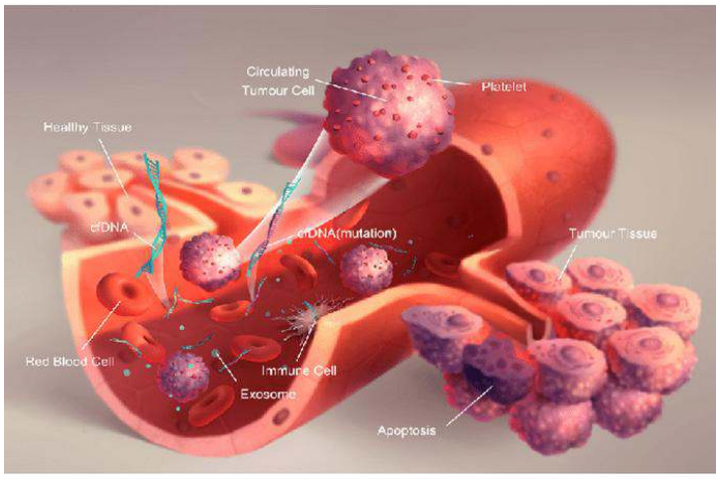

Figure 3. *Release mechanisms of ctDNA, CTCs, exosomes, and other biomarkers from tumor cells into the bloodstream (e.g., arrows showing apoptosis/necrosis/secretion pathways and biomarker types in circulation).*
Sources: *[51]*

Figure 3 presents a simple schematic of biomarker release from tumour cells (pink clustered masses) into the bloodstream (red flow). Arrows illustrate key processes: cell death pathways (apoptosis/necrosis), releasing fragmented ctDNA (short wavy lines) into circulation; active secretion/budding of exosomes/EVs (small vesicle icons) carrying cfRNA/miRNA, proteins, and metabolites from viable tumour cells; and active migration/intravasation of intact CTCs (whole cell figures) through leaky vasculature.

The diagram emphasises directional release of passive from dying cells for ctDNA, active secretion for EVs/cfRNA/proteins/metabolites, and cellular entry for CTCs, highlighting how these mechanisms generate detectable circulating biomarkers with distinct properties (e.g., short ctDNA fragments, protected EV cargo, viable CTCs), facilitating non-invasive liquid biopsy applications [14-17].

Detection Technologies and Methodological Advances

Detection of circulating biomarkers in liquid biopsy has evolved rapidly, with technologies tailored to the low abundance and heterogeneity of analytes like ctDNA, CTCs, and EVs. PCR-based methods remain foundational for targeted ctDNA analysis, particularly droplet digital PCR (ddPCR) and BEAMing. ddPCR partitions samples into thousands of droplets for absolute quantification of known mutations, achieving high analytical specificity (limits of detection ~0.01%–0.1% variant allele frequency) through Poisson statistics and binary readout, making it ideal for monitoring hotspot variants, minimal residual disease, and therapeutic response [18, 19]. BEAMing combines emulsion PCR on beads with flow cytometry for clonal amplification and mutation-specific probing, offering comparable or slightly superior sensitivity (~0.02%–0.04% LOD) for predefined targets, though with more complex workflows and instrumentation [18].

Next-generation sequencing (NGS)-based approaches provide broader genomic coverage, transitioning from targeted panels (e.g., hybrid capture or amplicon-based like CAPP-Seq, TAm-Seq) to whole-exome or whole-genome sequencing, enabling detection of mutations, copy number variations, and structural alterations at LODs ~0.1% or lower with error-correction via unique molecular identifiers [19]. Fragmentomics enhances NGS by analysing ctDNA size distributions (enriching shorter ~90–150 bp fragments), nucleosome positioning, and end motifs for improved early-stage sensitivity without requiring prior mutation knowledge [20].

Emerging techniques focus on epigenetic and integrative strategies: methylation profiling (e.g., whole-genome bisulfite sequencing or targeted methylation sequencing) captures tumour-specific patterns for multi-cancer early detection (MCED), often outperforming mutation-based methods in specificity and tissue-of-origin prediction [19]. Multi-omics integration combines ctDNA mutations, methylation, fragmentomics, cfRNA, proteins, and EVs for complementary signals, boosting overall sensitivity (e.g., multimodal classifiers achieving ~75% sensitivity at 98% specificity) [21]. Machine learning-enhanced analysis, including deep learning on fragmentation patterns, methylation arrays, or multi-omic datasets, refines signal detection, reduces background noise (e.g., clonal hematopoiesis), and improves predictive accuracy in MCED and resistance monitoring [22].

For other biomarkers, CTC technologies like the FDA-cleared CellSearch system use EpCAM-based immunomagnetic enrichment and cytokeratin/CD45 fluorescent labelling for enumeration in metastatic settings (e.g., breast, prostate, colorectal cancers), providing prognostic value despite limitations in EpCAM-negative subpopulations [23]. Exosome/EV isolation employs nanoparticle-based methods (e.g., ultracentrifugation alternatives like size-exclusion chromatography, acoustic nanofiltration, or immunoaffinity magnetic beads), enabling cargo analysis (miRNAs, proteins) with high purity and yield for downstream multi-omic profiling [24].

Table 1. Comparison of major liquid biopsy detection technologies

Technology Category	Sensitivity (LOD)	Specificity	Cost	Throughput	Turnaround	Applications	Key Limitations
ddPCR / BEAMing (PCR-based)	High (~0.01–0.1% VAF)	Very high	Low–moderate	Low–moderate	Hours–1 day	Hotspot variant monitoring, MRD	Requires predefined mutations; limited multiplexing
NGS-targeted panels (e.g., CAPP-Seq, Guardant360)	Good–high (~0.1% VAF; ~0.01% with UMIs)	High	Moderate–high	High	Days–1 week	Genomic profiling, resistance mechanisms	Higher cost; longer turnaround
Fragmentomics / Methylation assays	Enhanced (early/low-shedding)	Very high	Moderate–high	Moderate–high	Days	Multi-cancer detection, epigenetics	Data complexity; standardization needed
CTC enrichment (e.g., CellSearch)	Moderate	High	High	Low	Days	Prognostic use in metastatic disease	Misses EMT/EpCAM-negative cells; low early-stage yield
EV / Exosome isolation	Variable (cargo-dependent)	Moderate–high	Moderate	Moderate	Days	Biomarker discovery, exploratory diagnostics	Assay variability; evolving standards

Sources: *[20-24].*

In table 1, droplet digital polymerase chain reaction (ddPCR/BEAMing (PCR-based): High sensitivity (~0.01%–0.1% LOD), excellent specificity for known hotspots, moderate throughput (limited multiplexing), lower cost per target, fast turnaround (~hours–1 day), but requires predefined mutations and is less suited for broad profiling. NGS-targeted panels (e.g., CAPP-Seq, Guardant360/FoundationOne): Good sensitivity (~0.1% LOD), high throughput (hundreds of genes), broad coverage including novel variants, higher cost, longer turnaround (~days–week), moderate-high specificity with error correction.

Fragmentomics/methylation assays (integrated NGS): Enhanced early detection sensitivity (via size/methylation enrichment), high specificity for tumour discrimination, moderate-high throughput, variable cost (improving with scale), supports MCED/TOO prediction. CTC (CellSearch): Moderate sensitivity/specificity for EpCAM+ cells, low throughput (enumeration-focused), high cost/instrumentation, clinical validation in metastatic disease. EV/exosome isolation (nanoparticle/microfluidic): High purity/yield for cargo, variable sensitivity (cargo-dependent), emerging throughput, moderate cost, enables multi-omics but is less standardized.

Performance of ctDNA in Early Cancer Detection

The performance of circulating tumour DNA (ctDNA) in early cancer detection has been extensively studied in the context of multi-cancer early detection (MCED) assays, where sensitivity and specificity are the critical benchmarks for clinical utility. Across large prospective trials, ctDNA-based assays consistently demonstrate very high specificity, often exceeding 99%, which is essential to reduce false positives in population screening. Sensitivity, however, is more variable and strongly dependent on cancer type and stage. For example, in stage I cancers, detection rates remain modest, with sensitivities ranging from 30–40%, while stage II cancers show improved detection rates of 60–70% [24]. These differences highlight the challenge of identifying minimal residual disease in early-stage tumours, where ctDNA fragments are scarce.

Evidence from landmark studies underscores the promise of ctDNA in MCED. In colorectal and lung cancers, detection rates in stage II disease are consistently higher than in stage I, reflecting the correlation between tumour burden and ctDNA release [25]. Breast cancer studies have shown that fragmentomic and methylation signatures can enhance sensitivity, allowing detection even in early-stage disease [26]. Importantly, tissue-of-origin prediction has advanced significantly: methylation-based classifiers can correctly localize the cancer signal in over 90% of positive cases, a crucial step for guiding diagnostic follow-up [27]. Trials published in *BMC Medicine* and *The Lancet Oncology* further validate these findings, demonstrating that ctDNA assays can detect multiple cancers simultaneously in asymptomatic populations, with specificity maintained above 99% [28, 29].

The molecular features of ctDNA provide the foundation for its utility in early detection. Somatic mutations offer direct evidence of tumour-derived DNA, while aberrant methylation patterns provide tissue-specific signatures that enhance both sensitivity and localisation [29, 30]. Fragment omics differences in fragment size, end motifs, and nucleosome positioning between tumour-derived and normal cfDNA add another layer of discriminatory power [31]. Multimodal approaches that integrate mutations, methylation, and fragment omics consistently outperform single-feature assays, with forest plot analyses showing improved sensitivity across lung, colorectal, and breast cancers when these features are combined [32, 33].

Other Biomarkers and Multi-Biomarker/Multi-Analyte Approaches

While ctDNA remains the most extensively validated biomarker for early cancer detection, other circulating analytes have gained traction as complementary tools. Circulating tumour cells (CTCs) provide direct evidence of malignant cells in the bloodstream, but their rarity in early-stage disease limits sensitivity. Clinical studies have shown that while CTCs are robust in metastatic cancers, their detection rates in stage I/II cancers often remain below 30%, underscoring their limited utility as standalone early detection markers [33, 34].

Exosomes and extracellular vehicles (EVs) represent another promising class of biomarkers. These vesicles carry proteins, DNA, and RNAs, including microRNAs that reflect tumour biology. Their cargo has been shown to contain early signals of malignancy, with exosomal miRNA panels distinguishing cancer patients from healthy controls with sensitivities approaching 70% in pancreatic and breast cancers [35]. Because exosomes are abundant and stable in circulation, they offer practical advantages for longitudinal monitoring.

Epigenetic markers, particularly cfDNA methylation, have emerged as highly tissue-specific signals. Aberrant methylation patterns can precede detectable mutations, making them valuable for early detection and tissue-of-origin prediction. Several studies have demonstrated that cfDNA methylation assays achieve specificity above 95% while improving sensitivity in stage I/II cancers compared to mutation-only approaches [36]. Similarly, circulating free RNA (cfRNA) and tumour-educated platelets (TEPs) provide transcriptomic insights.

TEPs undergo RNA splicing changes in response to tumour-derived signals, and their RNA profiles have been shown to distinguish cancer patients from controls with sensitivities exceeding 80% in certain tumour types [37].

Figure 4 ideally illustrates these comparisons using a Venn diagram, showing ctDNA's strength in mutation detection and specificity, exosomes' advantage in transcriptomic coverage, CTCs' cellular context but limited abundance, and TEPs' unique RNA splicing signatures. Integration strategies would appear at the intersection, combining strengths across modalities to maximise detection accuracy.

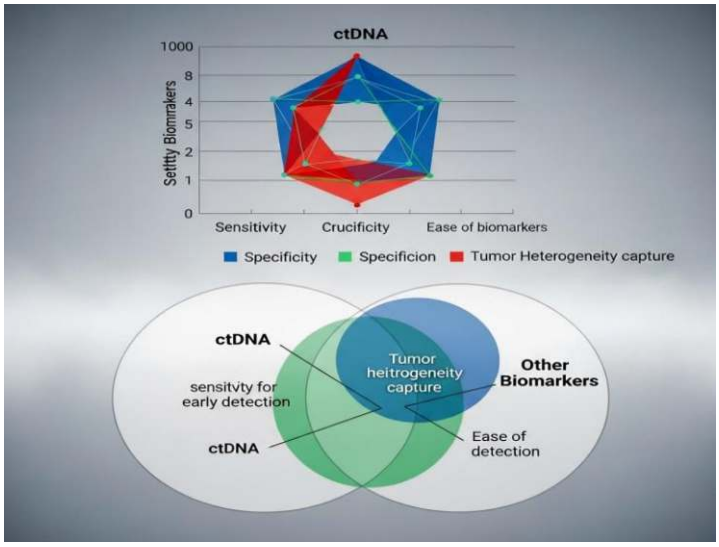

Figure 4. Venn diagram comparing strengths/weaknesses of ctDNA vs. other biomarkers
***Source:** [38].*

Clinical Applications and Evidence in Specific Cancer Types

Liquid biopsy has demonstrated strong clinical utility in high-burden cancers where early detection significantly improves survival. In lung cancer, circulating tumour DNA (ctDNA) profiling has been shown to detect molecular residual disease months before radiographic recurrence, offering a lead-time advantage that enables earlier therapeutic intervention [39]. In colorectal cancer, ctDNA positivity after curative surgery predicts relapse with a median lead time of 8–10 months compared to imaging, highlighting its role in surveillance and guiding adjuvant therapy decisions [40]. Pancreatic cancer, which is often diagnosed at advanced stages, has benefited from multi-analyte panels integrating ctDNA, exosomal RNA, and protein biomarkers, achieving sensitivities above 70% in stage II disease, an improvement over imaging alone [41]. Similarly, ovarian cancer studies have demonstrated that cfDNA methylation signatures can detect disease earlier than conventional CA-125 assays, with specificity exceeding 95% [42].

Multi-cancer detection panels are now being validated in large-scale trials. The Circulating Cell-free Genome Atlas (CCGA) study showed that cfDNA methylation-based MCED assays could detect over 50 cancer types with high specificity, while tissue-of-origin prediction was accurate in more than 90% of cases [41, 42]. The PATHFINDER trial further demonstrated that liquid biopsy could identify cancers missed by standard screening modalities, supporting its integration into clinical workflows [43]. The lead-time advantage of liquid biopsy over imaging is one of its most compelling features. In early-stage lung and colorectal cancers, ctDNA detection has preceded radiographic recurrence by several months, allowing clinicians to initiate treatment earlier and potentially improve survival outcomes [44, 45].

Figure 5 tracks the concentration of circulating tumour DNA as a dynamic biomarker across the continuum of cancer care. The process begins during the early detection and diagnosis phases, where ctDNA levels rise as the primary tumour develops, allowing for initial molecular profiling to identify actionable mutations. Following neoadjuvant therapy, a significant intervention such as surgery causes a rapid decline in ctDNA levels, marking the transition into the adjuvant setting. At this critical juncture, the detection of minimal residual disease or MRD serves as a molecular indicator of whether microscopic cancer cells remain despite the removal of the visible tumour.

As the timeline moves into surveillance, the levels ideally remain low; however, a subsequent rise in the curve signals a molecular recurrence, often providing a lead time of several months before clinical symptoms or radiological evidence appear. During the stage of metastatic relapse, the diagram depicts a fluctuating pattern where therapy 1, therapy 2, and therapy 3 are administered. Each peak in this phase represents the emergence of resistance mutations that allow the cancer to evade the current treatment, while the subsequent dips reflect the treatment response monitoring as new interventions are introduced to suppress the evolving tumour burden.

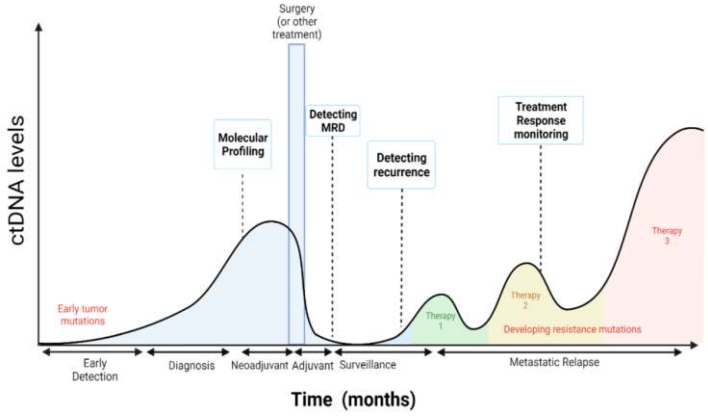

Figure 5. Timeline or waterfall plot illustrating lead time of liquid biopsy (ctDNA/other) detection vs. clinical/imaging recurrence in early-stage or screening cohorts.
***Source:** [43]*

Challenges, Limitations, and Standardisation

Liquid biopsy technologies face several critical challenges that must be addressed before they can be fully integrated into routine early cancer detection. One of the most significant limitations is low tumour shedding in early stages, which reduces sensitivity and leads to false negatives. Early-stage tumours often release minimal ctDNA or other analytes into circulation, making detection difficult even with advanced sequencing methods [44]. Another major issue is false positives caused by clonal hematopoiesis of indeterminate potential (CHIP), where age-related mutations in hematopoietic stem cells mimic tumour-derived signals. This biological noise complicates specificity and requires advanced bioinformatic filtering to distinguish benign from malignant variants (**Table 2)** [45, 46].

Assay variability and lack of standardisation across platforms also hinder reproducibility. Differences in pre-analytical handling, sequencing depth, and computational pipelines can yield inconsistent results, limiting cross-study comparisons and clinical confidence [47]. As shown in Table 2, cost and accessibility remain barriers as well, with ultrasensitive assays and multimodal platforms being resource-intensive and often restricted to specialized centres. Health economic analyses highlight the need for scalable, cost-effective technologies to ensure equitable access [48].

It is worthy to note that regulatory and validation gaps persist. While several assays have shown promise in clinical trials, few have achieved full regulatory approval for population-level screening. Standardisation initiatives such as BLOODPAC are working to establish analytical and clinical validation frameworks, but large-scale prospective trials are still needed to demonstrate utility in diverse populations [49].

Table 2. Key challenges and mitigation strategies in liquid biopsy for early cancer detection

Challenge	Impact on Early Detection	Current/Future Strategies
Low tumor shedding in early stages	Reduced sensitivity, false negatives in stage I cancers	Ultrasensitive assays, multimodal biomarker integration (ctDNA + CTCs + exosomes)
False positives from clonal hematopoiesis (CHIP)	Specificity issues, misclassification of benign mutations	Bioinformatic filtering, CHIP-aware algorithms, AI-driven signal discrimination
Assay variability and lack of standardization	Inconsistent results across labs, poor reproducibility	Harmonization of protocols, international standards, proficiency testing
High cost and limited accessibility	Restricted use to specialized centers, inequitable access	Cost reduction via scalable sequencing, point-of-care technologies
Regulatory and validation gaps	Limited approval for screening, uncertain clinical utility	Large-scale prospective trials, evolving regulatory frameworks, real-world evidence integration

Source: *[14-17]*

Future Directions and Perspectives

The trajectory of liquid biopsy research points toward increasingly sophisticated and clinically integrated applications. One of the most promising directions is the integration of artificial intelligence (AI) and machine learning (ML) to enhance signal detection and interpretation. AI-driven algorithms can distinguish tumour-derived signals from background noise such as clonal hematopoiesis, improving both sensitivity and specificity. Recent work has demonstrated that ML models trained on multimodal data, including ctDNA mutations, methylation, fragment omics, and exosomal cargo, can outperform traditional single-feature assays in early-stage cancer detection [50].

Prospective large-scale trials are another critical step. Studies such as the PATHFINDER and CCGA trials have already validated multi-cancer early detection (MCED) panels in diverse populations, but ongoing and future trials will be essential to establish real-world utility, cost-effectiveness, and integration into screening programs [51]. These trials are expected to provide the evidence base needed for regulatory approval and eventual adoption in population-level cancer screening.

Personalised and hybrid approaches are also emerging, where liquid biopsy is combined with imaging modalities to maximise detection accuracy. For example, integrating ctDNA analysis with low-dose CT scans in lung cancer screening has been shown to reduce false positives while improving early detection rates [52]. Similarly, hybrid strategies in colorectal cancer surveillance combine ctDNA monitoring with colonoscopy, offering both molecular and anatomical insights [53].

Finally, liquid biopsy holds great potential for risk-stratified screening. By integrating genetic predisposition, lifestyle factors, and biomarker profiles, screening programs could be tailored to individuals at the highest risk, thereby optimising resource allocation and minimizing unnecessary interventions. This approach aligns with precision medicine goals and could transform cancer screening from a one-size-fits-all model to a dynamic, personalised system [54].

Conclusion

Liquid biopsy has emerged as a transformative tool in oncology, offering the potential to shift cancer detection from late-stage diagnosis to proactive early intervention. By capturing tumour-derived signals such as ctDNA, cfRNA, exosomes, and CTCs, these assays provide a window into tumour biology that can precede clinical or imaging recurrence. This lead-time advantage is particularly impactful in high-burden cancers, where earlier detection directly translates into improved survival outcomes.

At the same time, challenges remain. Low tumour shedding in early stages, false positives from clonal hematopoiesis, assay variability, and regulatory gaps highlight the need for rigorous validation and standardisation. Addressing these limitations through ultrasensitive technologies, AI-driven signal enhancement, harmonized protocols, and large-scale prospective trials will be essential to ensure reproducibility and clinical confidence. Looking forward, integration of liquid biopsy into risk-stratified screening and hybrid approaches with imaging promises to personalise cancer detection strategies. With continued innovation and validation, liquid biopsy is poised to become a cornerstone of precision oncology, transforming early detection, guiding treatment, and ultimately improving patient outcomes.

Authors' Contributions

The authors of this research have significantly contributed to the study's conception, data collection, and manuscript development. All authors were involved in writing the manuscript or critically reviewing it for its intellectual value. They have reviewed and approved the final version for submission and publication and accepted full responsibility for the content and integrity of the work.

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Conflict of Interest

The authors declared that there are no conflicts of interest.

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