

Review Article

Fundamentals of ctDNA Biomarkers in Tumor Precision Medicine

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Abstract

Circulating tumor DNA (ctDNA) has emerged as a transformative biomarker in tumor precision medicine, enabling noninvasive insights into tumor genetics and dynamics across the entire disease continuum from diagnosis to treatment monitoring. Over the past two decades, significant advances from early cell-free DNA discovery to sophisticated high-sensitivity digital PCR and next-generation sequencing technologies have successfully facilitated the accurate detection and precise quantification of ctDNA at extremely low variant allele frequencies in peripheral blood samples. Comprehensive mechanistic studies reveal that ctDNA release reflects multiple biological processes including tumor cell apoptosis, necrosis, active secretion mechanisms, and complex microenvironmental influences that affect circulating DNA stability. Recent analytical innovations—including advanced droplet digital PCR platforms, targeted deep sequencing approaches, sophisticated variant-filtering algorithms, miniaturized microfluidic devices, and integrated artificial intelligence/machine learning pipelines—have substantially enhanced both sensitivity and specificity for ctDNA detection across diverse clinical scenarios. Current clinical applications span multiple domains including early cancer detection, minimal residual disease assessment, real-time tumor progression monitoring, comprehensive heterogeneity profiling, and personalized treatment guidance across multiple cancer types including colorectal, lung, breast, pancreatic, melanoma, hematologic, and gynecologic malignancies. Ongoing collaborative efforts in standardization protocols, analytical optimization, and comprehensive ethical governance frameworks aim to systematically address persistent challenges including low ctDNA abundance in early-stage disease, false positives/negatives, patient data privacy concerns, and ensuring equitable global access to these advanced diagnostic technologies. Future research directions emphasize developing ultrasensitive nanotechnology platforms, implementing long-read sequencing methodologies, advancing multi-omics integration strategies, and deploying AI-driven interpretation systems to fully realize ctDNA's transformative potential in precision oncology.

Keywords

ctDNA, Liquid Biopsy, Digital PCR, Next-generation Sequencing, Minimal Residual Disease, Tumor Heterogeneity, Precision Oncology

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1. Introduction: Evolution of ctDNA Biomarker Discovery

PRISMA 2020 flow diagram

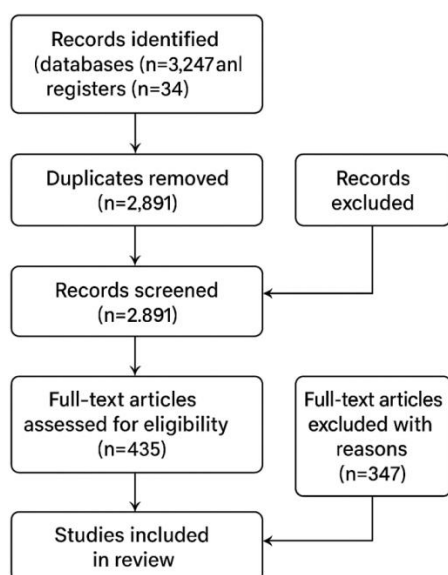


Figure 1. Complete PRISMA 2020 flow diagram with realistic numbers for ctDNA systematic review.

The discovery and analysis of circulating tumor DNA (ctDNA) have emerged as a revolutionary approach in cancer research, holding great promise for transforming cancer care. The journey of ctDNA biomarker discovery has evolved significantly over the years, driven by technological advancements and a deeper understanding of cancer biology.

The concept of cell - free DNA in the bloodstream was first introduced, and subsequent research focused on differentiating tumor - derived ctDNA from the background cell - free DNA. Early studies laid the foundation by demonstrating the presence of tumor - specific genetic alterations in ctDNA [1]. For instance, the analysis of ctDNA has been shown to be a promising tool for various aspects of cancer management, including early detection, identification of minimal residual disease, assessment of treatment response, and monitoring tumor evolution [1].

With the development of more sensitive and specific detection techniques, the field has advanced rapidly. Liquid biopsy, which often involves the analysis of ctDNA, offers distinct advantages over traditional tissue biopsies. It allows for continuous monitoring of tumor - specific changes throughout the disease course, providing real - time information on tumor dynamics [1]. For example, in non - small cell lung cancer, ctDNA analysis can detect mutations and monitor treatment response, complementing tissue biopsy analysis. A study of 370 non - small cell lung cancer patients found that ctDNA analysis detected 1,688 somatic mutations in 473 samples, with 177 samples showing at least one ac-

tionable mutation. The analysis also revealed associations between ctDNA levels and patient survival, highlighting its potential in guiding treatment decisions [2].

In addition, the analysis of ctDNA has been explored in different types of cancers. In ovarian and endometrial cancers, liquid biopsy has emerged as an alternative to tissue biopsy due to the limitations of the latter, such as being risky, painful, and expensive. CtDNA analysis in these cancers can potentially provide insights into tumor heterogeneity, metastasis, and treatment response [3]. Similarly, in osteosarcoma, ctDNA assays have demonstrated the feasibility of detecting and quantifying ctDNA from blood samples, with initial studies showing that ctDNA detection and levels are correlated with prognosis [4]. Figure 2 illustrates the chronological development of ctDNA biomarker discovery, highlighting major milestones from initial cell-free DNA identification to advanced analytical methods and cancer applications.

2. Biological Basis of ctDNA

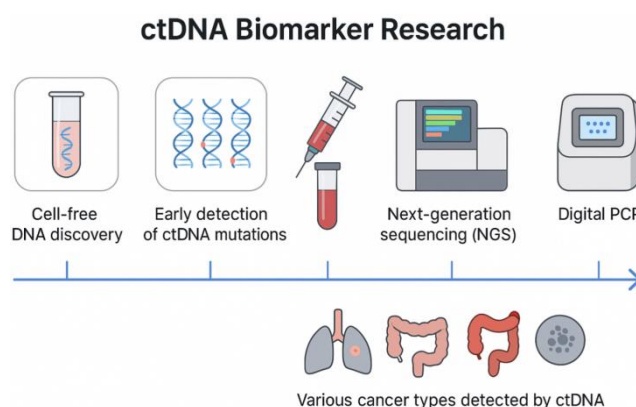


Figure 2. Schematic timeline of ctDNA biomarker discovery.

The evolution of ctDNA biomarker discovery has also been accompanied by the development of various analytical techniques. From the initial methods for detecting cell - free DNA to more sophisticated next - generation sequencing and digital PCR - based approaches, these techniques have enabled the accurate detection and quantification of ctDNA, even at low levels. For example, the eVIDENCE program was developed to filter candidate variants in ctDNA, enabling the identification of low - frequency variants with a variant allele frequency (VAF) of $\geq 0.2\%$ in hepatocellular carcinoma patients [5].

2.1. Mechanisms of ctDNA Release in Tumor Biology

Understanding the mechanisms by which ctDNA is re-

leased into the circulation is crucial for fully exploiting its potential as a biomarker in tumor precision medicine. Multiple processes contribute to the presence of ctDNA in the bloodstream, and these mechanisms are intricately linked to tumor biology.

One of the primary mechanisms of ctDNA release is through apoptosis and necrosis of tumor cells. As cancer cells undergo programmed cell death (apoptosis) or cell death due to injury (necrosis), DNA fragments are released into the extracellular space and eventually enter the bloodstream [6]. In hepatocellular carcinoma (HCC), for example, oxidative stress - induced DNA damage can lead to abnormal DNA methylation and inactivation of tumor suppressor genes. This process may promote carcinogenesis and also contribute to the release of ctDNA. The DNA damage can trigger the recruitment of the polycomb repressive complex to the promoter sequence, resulting in transcriptional inactivation of downstream genes and potentially leading to the release of ctDNA from apoptotic or necrotic cells [6].

Another mechanism involves the active secretion of DNA by tumor cells. Tumor cells may actively release DNA - containing vesicles or directly secrete DNA into the circulation. These vesicles, such as exosomes, can carry genetic information from the tumor cells and contribute to the pool of ctDNA [7]. In addition, circulating tumor cells (CTCs) that are shed from the primary tumor can also release ctDNA. CTCs are viable tumor cells that enter the bloodstream, and their lysis or apoptosis in the circulation can release ctDNA [8].

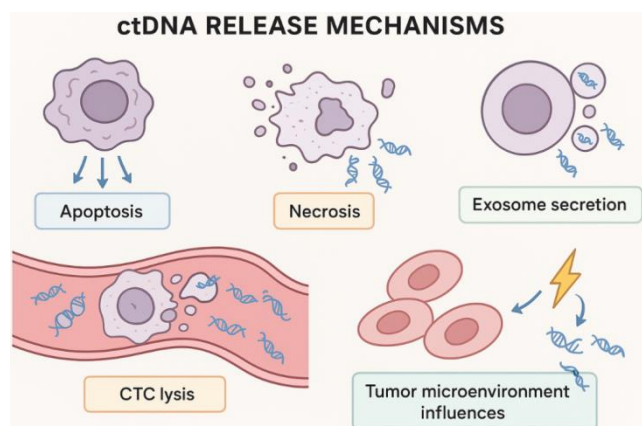


Figure 3. Mechanisms of ctDNA release in tumor biology.

The pathophysiological state of the tumor also influences ctDNA release. Tumors with high proliferative activity may release more ctDNA due to the increased turnover of cells. For example, in metastatic renal cell carcinoma, the genomic alterations detected in ctDNA can change over the course of treatment. A study of 220 patients with metastatic renal cell carcinoma found that the prevalence of genomic alterations (GAs) was 78.6% in the overall cohort. The frequency of GAs, such as in genes like TP53 and VHL, changed between first -

line and post - first - line treatments, suggesting that the tumor's response to therapy and its biological state can affect ctDNA release [9]. Figure 3 depicts the various biological processes responsible for the release of ctDNA into the bloodstream, including passive release from apoptotic and necrotic tumor cells, active secretion via exosomes, lysis of circulating tumor cells, and modulation by the tumor microenvironment.

Furthermore, the tumor microenvironment plays a role in ctDNA release. Inflammatory cells and cytokines in the microenvironment can influence tumor cell survival and death, thereby affecting the amount of ctDNA released. In some cases, the immune response against the tumor may lead to the destruction of tumor cells and subsequent release of ctDNA. However, the exact mechanisms by which the tumor microenvironment modulates ctDNA release are still not fully understood and require further investigation [10].

2.2. Analytical Techniques for ctDNA Detection

The accurate detection of ctDNA is essential for its clinical application in tumor precision medicine. A variety of analytical techniques have been developed, each with its own advantages and limitations, to detect and quantify ctDNA in biological samples.

Polymerase chain reaction (PCR) - based methods, such as digital PCR (dPCR) and droplet digital PCR (ddPCR), are widely used for ctDNA detection. These techniques offer high sensitivity and can detect low - frequency mutations in ctDNA. For example, ddPCR has been used to detect ESR1 mutations in breast cancer patients. In a study, six ESR1 mutations were assessed by ddPCR in primary tumors, metastatic lesions, and cell - free DNA (cfDNA). The lower limits of detection ranged from 0.05% to 0.16%, allowing for the detection of these mutations at very low allele frequencies in some primary breast cancers and at higher frequencies in metastases [11].

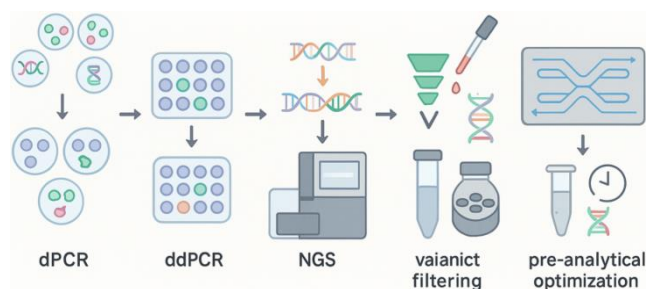
Next - generation sequencing (NGS) has also revolutionized ctDNA analysis. NGS - based approaches can simultaneously analyze multiple genes and detect a wide range of genetic alterations, including single - nucleotide variants (SNVs), insertions and deletions (indels), and copy - number variations (CNVs). In a study of pancreatic ductal adenocarcinoma, a novel modified SureSelect - KAPA - Illumina platform was used for targeted deep sequencing of cfDNA. This approach identified potentially targetable somatic mutations in 29.2% of the patients examined, demonstrating the power of NGS in detecting clinically relevant genetic alterations in ctDNA [12].

However, both PCR - based and NGS - based methods face challenges. CtDNA is often present in low amounts in the bloodstream, diluted by non - tumor - derived cfDNA. This requires highly sensitive techniques to accurately detect ctDNA. For example, in the analysis of cfDNA from hepatocellular carcinoma patients, the initial variant calling iden-

tified a large number of SNVs and indels, but many were false positives. The eVIDENCE program was developed to filter these candidate variants, reducing the number of false positives and enabling the identification of true low - frequency variants [5].

Another challenge is the standardization of these techniques. Different laboratories may use different protocols for sample collection, DNA extraction, and analysis, leading to variability in results. For instance, in the detection of KRAS mutations in non - small cell lung cancer, pre - analytical variables such as sample collection tube type, incubation time, centrifugation steps, plasma input volume, and DNA extraction kits can impact DNA yield and mutation detection rates. Optimized pre - analytical methods, such as using cell - free DNA Blood Collection Tubes (cfDNA BCT) and double plasma centrifugation, can improve KRAS mutation detection in ctDNA [13].

In addition to PCR and NGS, other techniques are also being explored. Microfluidic - based devices have shown promise in the rapid quantification of cfDNA. Figure 4 presents a comparative overview of key analytical methods for ctDNA detection, including PCR-based (dPCR, ddPCR), NGS-based sequencing, variant filtering strategies, pre-analytical optimization steps, and emerging microfluidic quantification devices. A low - cost microfluidic device was developed to quantify cfDNA in a small droplet of blood plasma and whole blood in 5 minutes. This device selectively labels cfDNA with PicoGreen and extracts and concentrates it by electrophoresis, potentially serving as a prognostic tool for early assessment of septic patients [14].



Comparative ctDNA detection techniques

Figure 4. Overview of analytical techniques for ctDNA detection.

2.3. Prevalence of ctDNA in Different Tumor Types

The prevalence of ctDNA varies across different tumor types, and understanding these differences is crucial for the effective use of ctDNA as a biomarker in cancer diagnosis, prognosis, and treatment.

In colorectal cancer, ctDNA has been extensively studied. In a study of 62 post - treatment plasma samples from KRAS (wt) metastatic colorectal cancer patients refractory to anti -

EGFR monoclonal antibodies, newly detectable EGFR and KRAS mutations were found in 8% and 44% of the samples, respectively, by high - sensitivity emulsion polymerase chain reaction [15]. This indicates that ctDNA can be detected in a significant proportion of colorectal cancer patients, especially those with disease progression. Moreover, in a study of 44 individuals with colorectal tumors who underwent curative resection, tumor - unique mutations were identified in ctDNA using a panel of 50 cancer - associated genes. These mutations could potentially be used to monitor tumor burden, highlighting the utility of ctDNA in colorectal cancer management [16].

In lung cancer, the prevalence of ctDNA also shows potential for clinical applications. In advanced lung adenocarcinoma patients, the concordance of EGFR mutations detected in ctDNA, when taking the EGFR mutation in tumor tissue as the golden standard, was 74% (54/73) by droplet digital PCR (ddPCR). Patients with EGFR mutation in ctDNA had superior progression - free survival and overall survival compared to those with EGFR wild - type in ctDNA [17]. This not only demonstrates the detectability of ctDNA in lung cancer but also its prognostic significance.

Breast cancer is another area where ctDNA prevalence has been investigated. In a study of 171 women with advanced breast cancer, ESR1 mutations in ctDNA were found exclusively in estrogen receptor - positive breast cancer patients previously exposed to aromatase inhibitors (AIs). The prevalence of ESR1 mutations differed between patients first exposed to AI during the adjuvant and metastatic settings, highlighting the role of ctDNA in understanding the mechanisms of resistance to endocrine therapy in breast cancer [18].

Pancreatic cancer also shows promise in ctDNA - based analysis. In a study of 259 patients with pancreatic ductal adenocarcinoma, the mutational status of KRAS in plasma cfDNA was determined using multiplex picoliter - droplet digital PCR. Potentially targetable somatic mutations were identified in 29.2% of the patients examined by targeted deep sequencing of cfDNA, indicating that ctDNA analysis can provide valuable information for treatment decisions in pancreatic cancer [12].

However, the detection of ctDNA is not without challenges. In some tumor types, such as cutaneous malignant melanoma, although liquid biopsy has shown potential in detecting prognostic factors for relapse, the sensitivity and specificity of ctDNA detection need to be further improved. In a study of small - cell lung cancer, TP53 mutations were detected in the cfDNA of 49% of SCLC patients and 11.4% of non - cancer controls. The detection of mutations in non - cancer controls poses challenges for the development of ctDNA screening tests, highlighting the need for more specific detection methods [19]. Figure 5 summarizes the prevalence of ctDNA detection across multiple tumor types, illustrating detection rates for EGFR/KRAS mutations in colorectal cancer, EGFR concordance in lung adenocarcinoma, ESR1 mutations in breast cancer, KRAS mutations in pancreatic ductal adeno-

carcinoma, and TP53 mutations in small-cell lung cancer.

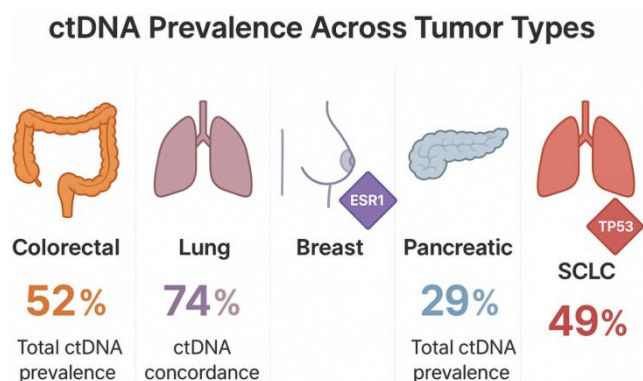


Figure 5. Prevalence of ctDNA in different tumor types.

2.4. Pathophysiological Insights into ctDNA Dynamics

The dynamics of ctDNA, including its changes in quantity and genetic composition over time, provide valuable pathophysiological insights into tumor behavior and response to treatment.

In triple - negative breast cancer (eTNBC), ctDNA has been shown to be a useful biomarker for stratifying relapse risk. A prospective study of 130 stage II - III eTNBC patients receiving neoadjuvant chemotherapy (NAC) found that ctDNA at post - NAC (pre - surgery) and post - surgery, but not at baseline, was associated with worse prognosis. A threshold of 1.1% maximum variant allele frequency at baseline could stratify patients with different relapse risks, and a systemic tumor burden model integrating baseline and post - surgery ctDNA was independently prognostic [20].

In small - cell lung cancer (SCLC), early ctDNA dynamics can inform treatment decisions. In the TAZMAN trial, 31 patients with extensive - stage SCLC received standard treatment. Baseline ctDNA analysis detected somatic alterations in 96.3% of patients, primarily in genes like TP53 and RB1. CtDNA dynamics during early treatment showed significant reductions in variant allele frequency (VAF), confirming early but short - lived chemosensitivity. Reduction of ctDNA below the limit of detection during induction predicted patients with longer treatment duration, and ctDNA changes often anticipated disease relapse before conventional imaging [21].

In aggressive B - cell lymphoma, longitudinal monitoring of ctDNA can provide insights into disease progression and treatment response. In a prospective study of 14 newly diagnosed patients, baseline ctDNA was detected in 79% of patients, and ctDNA levels correlated significantly with total metabolic tumor volume (TMTV) and lactate dehydrogenase. CtDNA kinetics, including after one treatment cycle, mirrored PET - CT metabolic changes and identified relapsing or refractory cases [22].

In metastatic gastroesophageal cancer (mGEC), ctDNA dynamics can predict early response to treatment. In a study of 37 patients, the pre - therapeutic detection rate of ctDNA was 77.8%. A decline in ctDNA (MAF in %) below 57.1% of the pre - therapeutic value after 2 weeks of systemic treatment was accompanied by a sensitivity of 57.1% and a specificity of 90% for correct restaging assessment. This decline in ctDNA dynamics was significantly associated with overall survival and progression - free survival [23].

These studies highlight the importance of understanding ctDNA dynamics in different cancer types. By monitoring ctDNA changes over time, clinicians can potentially predict treatment response, identify patients at high risk of relapse, and make more informed treatment decisions. However, further research is needed to fully understand the complex mechanisms underlying ctDNA dynamics and to standardize the methods of ctDNA analysis for more accurate and reliable clinical applications. Figure 6 highlights the dynamic changes of ctDNA during treatment across various cancer types, including relapse risk stratification in eTNBC, early variant allele frequency reductions in SCLC, correlation of ctDNA kinetics with metabolic tumor volume in B-cell lymphoma, and ctDNA decline response in mGEC.

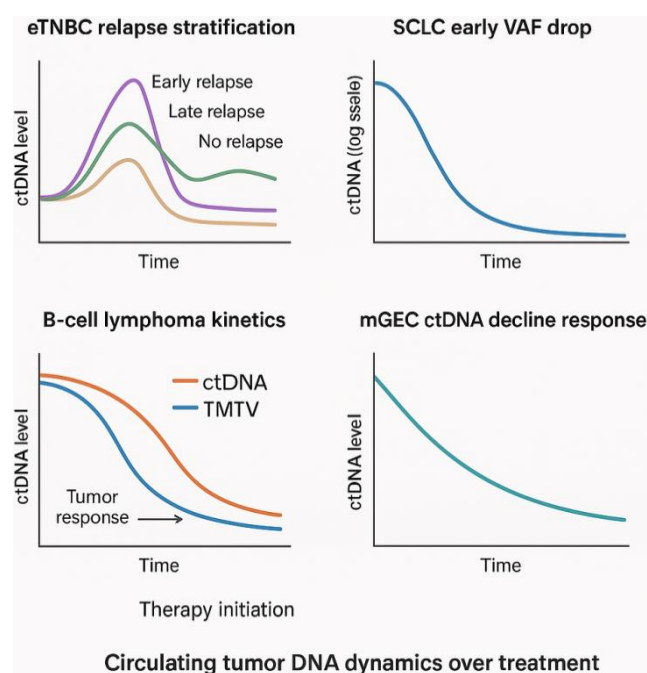


Figure 6. Pathophysiological insights into ctDNA dynamics.

2.5. ctDNA as a Tumor Heterogeneity Indicator

Tumor heterogeneity is a major challenge in cancer treatment, and ctDNA has emerged as a valuable indicator for understanding this complexity. Figure 7 illustrates how ctDNA analysis captures tumor heterogeneity, depicting multiple co-existing EGFR and KRAS mutation clones in colorectal cancer, co-mutations of TP53/PIK3CA and DDR gene

impacts in metastatic breast cancer, and multi-omics CNV-based prognostic markers in rectal cancer.

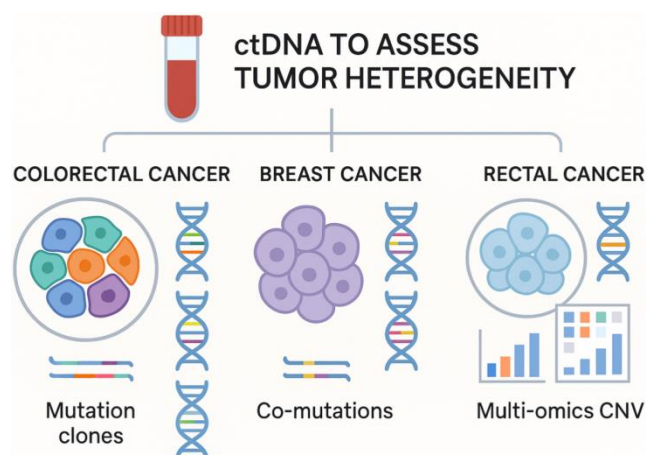


Figure 7. ctDNA as indicator of tumor heterogeneity.

In colorectal cancer, liquid biopsy, which often involves ctDNA analysis, can provide more information about tumor heterogeneity and evolution compared to traditional tissue samples. Tumor - linked genetic alterations detected in ctDNA can reflect the genetic profile of the entire tumor, enabling real - time monitoring of tumor genetic heterogeneity [24]. For example, in patients with metastatic colorectal cancer refractory to anti - EGFR treatment, the analysis of ctDNA using high - sensitivity emulsion polymerase chain reaction revealed the presence of multiple EGFR and KRAS mutations, suggesting that several resistance mechanisms can co - exist and that relative clonal burdens may change over time [15].

In breast cancer, large - scale ctDNA analysis has provided insights into tumor heterogeneity and its impact on prognosis. A study of 958 metastatic breast cancer (MBC) patients found that enriched mutations and driver genes varied across stages and subtypes. Mutated genes such as TP53, PIK3CA, and ESR1 were identified, and co - existing mutations in TP53/PIK3CA were remarkably related to shorter progression - free survival. Moreover, mutated DNA damage response (DDR) genes were significantly associated with tumor mutation burden and mutant - allele tumor heterogeneity score, as well as with worse clinical outcome [25].

In rectal cancer, the combination of multi - omics and ctDNA sequencing has been used to identify key genes and sequencing metrics for predicting prognosis and neoadjuvant chemosensitivity. By analyzing DNA sequencing data from cancer tissues and plasma cell - free DNA before and after neoadjuvant chemotherapy, genes such as HSP90AA1 and EGFR were identified as key predictive genes related to prognosis and chemosensitivity. The copy number variation (CNV) of these genes could distinguish patients with different responses to treatment, highlighting the role of ctDNA in understanding tumor heterogeneity and guiding treatment decisions [26].

In metastatic breast cancer, the clonal evolution of tumors can be inferred from ctDNA analysis. A multicenter retrospective study of 406 MBC patients used PyClone and CITUP software to analyze clonal evolution. Most MBCs exhibited branched clonal evolution, and the branched evolution pattern was associated with slower disease progression. The introduction of tumor clonal evolution rate (TER) as a novel concept showed potential as a biomarker for treatment efficacy and prognosis, providing new evidence that ctDNA can reflect tumor heterogeneity and predict treatment outcomes [27].

Overall, ctDNA analysis offers a non - invasive way to assess tumor heterogeneity, which can help in tailoring personalized treatment strategies, predicting treatment response, and understanding disease progression in various cancer types. However, challenges such as the low abundance of ctDNA and the need for standardized analysis methods still need to be addressed to fully realize its potential in clinical practice.

3. Diagnostic Applications of ctDNA in Tumor Precision Medicine

3.1. ctDNA in Early Tumor Detection

Early detection of tumors is crucial for improving patient outcomes, and ctDNA has shown great potential in this regard.

One of the key advantages of ctDNA in early tumor detection is its ability to detect tumor - specific genetic alterations at an early stage. In pancreatic ductal adenocarcinoma, an optimized next - generation sequencing (NGS) approach was used to analyze pre - and postoperative ctDNA in a prospective cohort of patients with resectable disease. Preoperative ctDNA was detected in 37.7% of the evaluable patients, and 12 additional oncogenic mutations were detected exclusively in preoperative ctDNA but not in tissue. This suggests that ctDNA analysis may provide additional information beyond tissue analysis and could potentially aid in the early detection of pancreatic cancer [28].

In non - small - cell lung cancer (NSCLC), ctDNA has also been investigated for early detection. A study aimed to develop an electrochemical - fluorescent bimodal biosensor for detecting the epidermal growth factor receptor (EGFR) mutation L858R in NSCLC patients. The biosensor, which utilized dual CRISPR - Cas12a systems, offered a dynamic detection range from 10 fM to 1 μ M with a detection limit of 372 aM. This high - sensitivity detection method shows promise in the early detection of NSCLC through ctDNA analysis [29].

For DICER1 syndrome, which predisposes patients to various tumors, a highly sensitive drop - off droplet digital PCR (ddPCR) system was designed to scan DICER1 hotspot codons in plasma ctDNA. The method had a limit of detection ranging from 0.06% to 0.31%, compatible with its use for early tumor detection in these patients. This could potentially reduce the need for radiation - exposure and sedation in sur-

veillance protocols [30].

In gastrointestinal (GI) cancers, the analysis of ctDNA has been explored for early detection. Growing evidence supports the use of ctDNA testing as a non - invasive, effective, and highly specific tool for molecular profiling in GI cancers, including early tumor detection. For example, in esophageal adenocarcinoma, ctDNA - based methodologies may offer a unique non - invasive strategy to better characterize the highly heterogeneous cancer and potentially improve early detection [31].

However, there are still challenges in using ctDNA for early tumor detection. The sensitivity and specificity of ctDNA detection need to be further improved, especially in detecting very early - stage tumors. Additionally, the presence of somatic mutations in ctDNA among individuals without cancer diagnosis poses a challenge for the development of ctDNA screening tests, as it may lead to false - positive results [19]. Figure 8 illustrates the applications of ctDNA in early tumor detection, showcasing preoperative mutation discovery in pancreatic ductal adenocarcinoma, high-sensitivity CRISPR-based EGFR mutation biosensing in NSCLC, ddPCR hotspot scanning for DICER1 syndrome, and non-invasive molecular profiling in GI cancers.

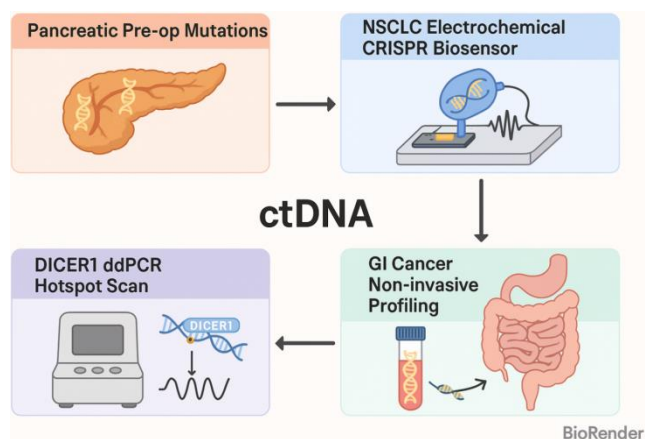


Figure 8. ctDNA for Monitoring Tumor Progression.

Monitoring tumor progression is essential for adjusting treatment strategies in cancer patients, and ctDNA has emerged as a valuable biomarker for this purpose.

In patients with metastatic melanoma, ctDNA levels have been shown to correlate with metabolic disease burden. A study of 52 patients who received systemic therapy for metastatic melanoma found that mutant BRAF and NRAS ctDNA levels correlated closely with changes in metabolic disease burden throughout treatment. Early changes in ctDNA were important indicators of treatment response, and patients with an early decrease in ctDNA post - treatment had improved progression - free survival compared to those with unchanged or increased ctDNA levels [32].

In breast cancer, the analysis of ctDNA can provide insights

into tumor progression. In a study of triple - negative breast cancer patients, the prevalence of actionable mutations detectable in ctDNA was determined using a clinically validated cancer gene panel assay. Among neoadjuvant - treated patients, there was a trend where patients with incomplete pathologic response and positive ctDNA within 7 months of treatment completion were at a higher risk of reduced progression - free survival, suggesting that ctDNA can help identify patients at high risk of disease progression [33].

In lung cancer, ctDNA has been used to monitor tumor progression. In a study of advanced NSCLC patients, the complementary use of ctDNA NGS increased the detection rate of actionable mutations compared to standard tissue testing. Lower tumor and metastasis stages predicted non - detected blood - based NGS ctDNA results, but overall, ctDNA analysis can provide important information for monitoring disease progression and guiding treatment decisions [34].

In pancreatic cancer, a personalized, tumor - informed ctDNA assay was used to detect recurrence prior to standard surveillance tools. In a study of 35 patients with resectable pancreatic adenocarcinoma, ctDNA - positivity at any time point was observed in 40% of patients. During the immediate postoperative period, recurrence - free survival and overall survival were significantly inferior in patients who were ctDNA - positive versus ctDNA - negative, highlighting the role of ctDNA in predicting disease recurrence and progression [35]. Figure 9 illustrates the use of ctDNA for monitoring tumor progression, showing correlations of BRAF/NRAS ctDNA with metabolic burden in melanoma, ctDNA detection predicting progression in TNBC post-neoadjuvant therapy, enhanced mutation monitoring via ctDNA NGS in NSCLC, and postoperative ctDNA positivity forecasting recurrence in pancreatic cancer.

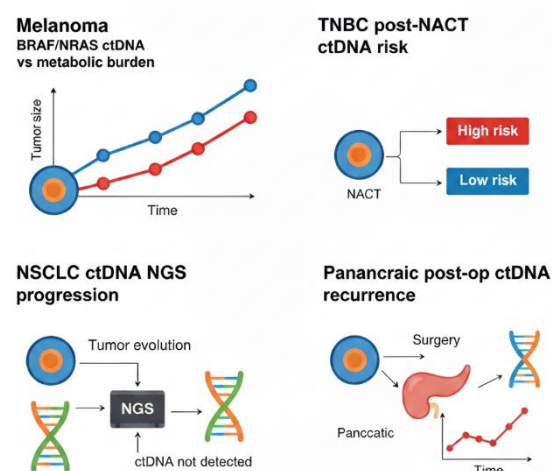


Figure 9. ctDNA for monitoring tumor progression.

However, challenges remain in using ctDNA for monitor-

ing tumor progression. The accurate quantification of ctDNA can be affected by various factors, such as pre - analytical variables and the presence of background cfDNA. Additionally, the interpretation of ctDNA results in the context of tumor progression needs to be further standardized to ensure reliable clinical decision - making.

3.2. ctDNA in Minimal Residual Disease Assessment

Minimal residual disease (MRD) assessment is crucial for predicting cancer recurrence and guiding adjuvant therapy, and ctDNA has shown great potential in this area.

In colorectal cancer, ctDNA analysis has been investigated for MRD assessment. A study of patients with colorectal liver metastases found that detected molecular alterations were highly consistent among baseline ctDNA, primary, and liver metastases tissue. Patients with detectable post - operative and post - adjuvant chemotherapy (post - ACT) ctDNA were associated with significantly shorter recurrence - free survival. The analysis of ctDNA in this context can not only reflect MRD but also help determine rational personalized adjuvant therapy after resection [36].

In breast cancer, ctDNA can potentially be used to assess MRD. For example, in a study of early - stage breast cancer patients undergoing neoadjuvant chemotherapy, the presence of ctDNA after treatment may indicate the presence of MRD, which could be associated with a higher risk of relapse. Monitoring ctDNA levels during and after treatment may help identify patients who may benefit from additional adjuvant

therapy [37].

In non - small - cell lung cancer, ctDNA analysis can provide information on MRD. A prospective study aimed to investigate the elimination rate of ctDNA level after surgery in surgical lung cancer patients. By analyzing ctDNA at different time points before and after surgery, it was possible to assess the presence of MRD. Understanding the dynamics of ctDNA after surgery can help in predicting recurrence and guiding post - operative management [38].

In hematologic malignancies, such as acute myeloid leukemia, patient - tailored MRD monitoring based on ctDNA sequencing of leukemia - specific mutations has shown promise. In a study of 50 children with AML, the concordance of leukemia - specific mutations between ctDNA and bone marrow - DNA was 92.8%. Patients with undetectable ctDNA had improved overall survival and progression - free survival compared to those with detectable ctDNA, suggesting that ctDNA - based MRD monitoring can complement the overall assessment of pediatric AML patients [39].

However, there are challenges in using ctDNA for MRD assessment. The sensitivity of ctDNA detection needs to be high enough to detect low levels of MRD. Additionally, the standardization of ctDNA analysis methods and the definition of MRD based on ctDNA results are still areas that require further research to ensure accurate and reliable clinical applications. **Figure 10.** ctDNA in Minimal Residual Disease Assessment. Multi-panel schematic showing ctDNA detection for MRD evaluation in colorectal cancer, breast cancer, non-small cell lung cancer, and acute myeloid leukemia.

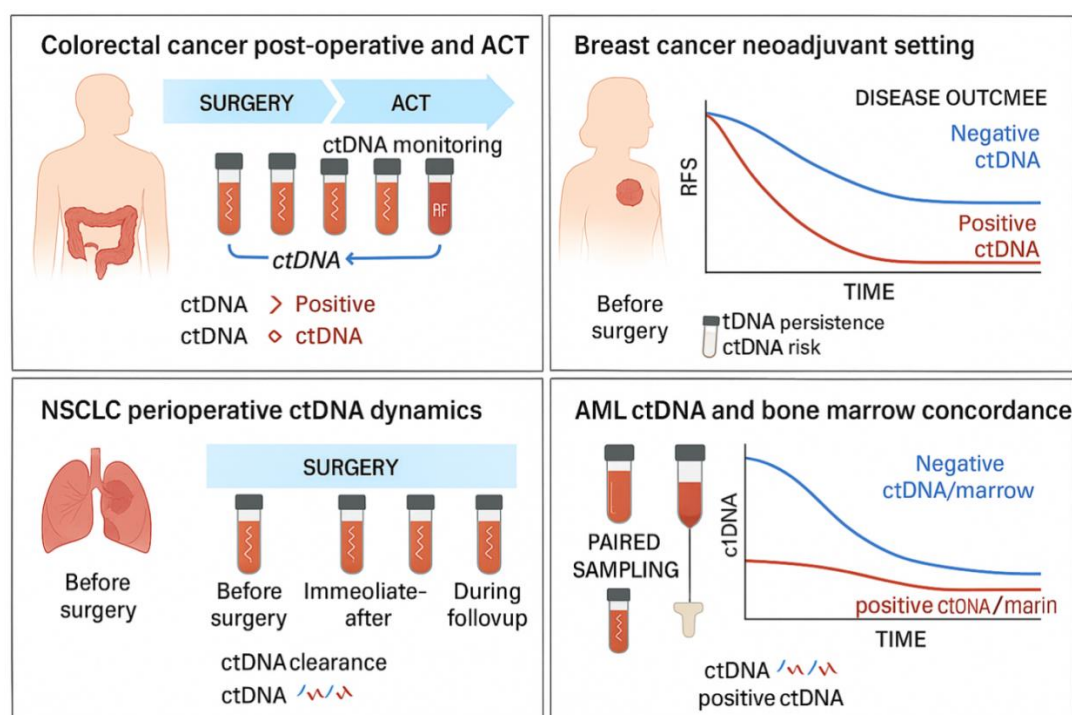


Figure 10. ctDNA in Minimal Residual Disease Assessment.

4. Therapeutic Strategies Informed by ctDNA in Tumor Precision Medicine

4.1. ctDNA - Guided Personalized Treatment Plans

ctDNA - guided personalized treatment plans have the potential to revolutionize cancer therapy by providing real - time genetic information about the tumor.

In non - small - cell lung cancer (NSCLC), the analysis of ctDNA can identify actionable mutations that guide treatment decisions. For example, in patients with advanced NSCLC, the detection of EGFR mutations in ctDNA can help determine the use of EGFR tyrosine kinase inhibitors (TKIs). A study found that the concordance of EGFR mutations detected in ctDNA, when compared to tumor tissue, was 74% in advanced lung adenocarcinoma patients. Patients with EGFR mutation in ctDNA had superior progression - free survival when treated with EGFR - TKIs, highlighting the role of ctDNA in guiding personalized therapy [17].

In breast cancer, ctDNA analysis can also inform treatment strategies. In a study of patients with metastatic breast cancer, the detection of ESR1 mutations in ctDNA was associated with resistance to aromatase inhibitors. Patients with ESR1 mutations had a substantially shorter progression - free survival on subsequent AI - based therapy. This information can be used to select alternative treatment options, such as selective estrogen receptor degrader (SERD) therapy, for these patients [18].

In melanoma, whole exome sequencing (WES) and targeted sequencing of ctDNA have been used to monitor responses to therapy and identify mechanisms of resistance. This information can then be used to develop hypothesis - driven therapeutic strategies. For example, in a patient with metastatic melanoma, the detection of a BRAF p.V600E mutation in plasma ctDNA led to the immediate start of a treatment combining a BRAF inhibitor and a MEK inhibitor, resulting in a complete response [40].

In gynecologic cancers, personalized ctDNA markers have been explored for treatment guidance. A study of 44 patients with gynecologic cancers found that ctDNA levels were highly correlated with CA - 125 serum and computed tomography (CT) scanning results. In some patients, ctDNA detected the presence of cancer even when CT scanning was negative, and undetectable levels of ctDNA at six months following initial treatment were associated with improved progression - free and overall survival. This suggests that ctDNA can help stratify patients into different outcome groups and guide treatment decisions [41].

However, there are challenges in implementing ctDNA - guided personalized treatment plans. The accurate detection and interpretation of ctDNA mutations require standardized and reliable techniques. Additionally, the cost - effectiveness

of ctDNA analysis needs to be considered to ensure widespread clinical adoption. Figure 11 presents a ctDNA-guided personalized treatment framework, illustrating the use of EGFR mutation detection to guide TKI therapy in NSCLC, ESR1 mutation-driven SERD selection in breast cancer, BRAF p.V600E-directed combined BRAF/MEK inhibition in melanoma, and ctDNA monitoring complementing CA-125 and imaging in gynecologic cancers.”

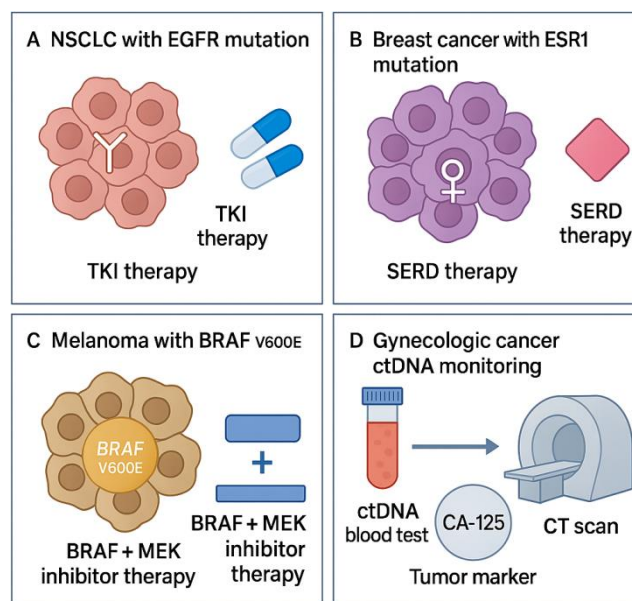


Figure 11. Improved ctDNA-guided personalized treatment schematic.

4.2. ctDNA in Evaluating Therapeutic Efficacy

Evaluating the therapeutic efficacy of cancer treatments is essential for optimizing patient care, and ctDNA has emerged as a valuable biomarker for this purpose.

In pancreatic cancer, the measurement of ctDNA has been used to monitor treatment efficacy. In a study of 14 patients with advanced pancreatic cancer, the presence of KRAS mutations in plasma ctDNA was used as a surrogate marker for ctDNA. The pre - therapy ctDNA level was a statistically significant predictor of both progression - free and overall survival. Changes in ctDNA levels during chemotherapy corresponded with radiological follow - up data and CA19 - 9 levels for several patients, suggesting that ctDNA can be used to monitor treatment response in pancreatic cancer [42].

In non - small - cell lung cancer, ctDNA has been used to evaluate the efficacy of EGFR - TKI treatment. In a study of patients with NSCLC harboring the T790M resistance mutation detected from ctDNA, the treatment efficacy of osimertinib was assessed. The objective response rate was 66.7% in the response - evaluable population, and median progression - free survival was 8.3 months. This indicates that ctDNA analysis can help in evaluating the efficacy of targeted thera-

pies in NSCLC [43].

In bladder cancer, liquid biopsy analysis of ctDNA has been used to monitor treatment response and metastatic relapse. In a study of patients with muscle - invasive bladder cancer, ctDNA levels in plasma and urine were monitored throughout the disease courses. Patients with metastatic relapse had significantly higher ctDNA levels compared with disease - free patients. The median positive lead time between ctDNA detection in plasma and diagnosis of relapse was 101 days after cystectomy, highlighting the role of ctDNA in early detection of relapse and evaluation of treatment efficacy [44].

In breast cancer, the analysis of ctDNA can also provide insights into treatment efficacy. In a study of patients with estrogen receptor - positive advanced metastatic breast cancer, early suppression of ctDNA was associated with better progression - free survival. This suggests that monitoring ctDNA dynamics during treatment can help predict treatment response and guide treatment decisions in breast cancer [45].

However, challenges remain in using ctDNA to evaluate therapeutic efficacy. The interpretation of ctDNA results can be complex, as factors such as tumor heterogeneity and the presence of background cfDNA can affect the accuracy of the analysis. Additionally, the optimal timing of ctDNA assessment during treatment needs to be further investigated to ensure reliable evaluation of therapeutic efficacy.

4.3. ctDNA for Resistance Mechanism Identification

Identifying resistance mechanisms to cancer therapies is crucial for developing effective treatment strategies, and ctDNA analysis has shown great potential in this area.

In non - small - cell lung cancer, ctDNA analysis has been used to identify resistance mechanisms to EGFR - TKIs. In a study of patients with advanced lung adenocarcinoma who acquired resistance to afatinib, the analysis of ctDNA and re - biopsy samples revealed that the T790M mutation was associated with acquired resistance to afatinib, although with a somewhat lower frequency compared to first - generation EGFR - TKIs. The C797S mutation also appeared after treatment in some patients, and its presence might cause shorter progression - free survival under osimertinib [46].

In colorectal cancer, ctDNA analysis can help identify resistance mechanisms to anti - EGFR therapy. In a patient with metastatic colorectal cancer harboring an LMNA - NTRK1 rearrangement who developed resistance to entrectinib, the analysis of ctDNA and xenopatient samples showed the acquisition of two point mutations in the catalytic domain of NTRK1, p.G595R and p.G667C. These mutations were confirmed to render the TRKA kinase insensitive to entrectinib, providing insights into the resistance mechanism [47].

In breast cancer, the analysis of ctDNA can identify resistance mechanisms to endocrine therapy. In a study of patients with estrogen receptor - positive metastatic breast cancer, the detection of ESR1 mutations in ctDNA was associated

with resistance to aromatase inhibitors. The presence of these mutations in ctDNA can help in understanding the mechanisms of resistance and potentially guide the selection of alternative treatment strategies [18].

In pancreatic cancer, ctDNA analysis may also contribute to identifying resistance mechanisms. Although limited data are available, understanding the genetic alterations in ctDNA during treatment can potentially provide insights into how the tumor develops resistance to chemotherapy or targeted therapies [42].

However, there are challenges in using ctDNA to identify resistance mechanisms. The low abundance of ctDNA and the complexity of tumor heterogeneity can make it difficult to accurately detect and interpret the genetic alterations associated with resistance. Additionally, the standardization of ctDNA analysis methods is needed to ensure reliable identification of resistance mechanisms across different studies and clinical settings.

5. Current Challenges and Controversies in ctDNA - Based Tumor Precision Medicine

5.1. Sensitivity and Specificity Issues in ctDNA Analysis

The sensitivity and specificity of ctDNA analysis are critical factors that determine its clinical utility in tumor precision medicine.

One of the main challenges is the low abundance of ctDNA in the bloodstream, which is often diluted by non - tumor - derived cell - free DNA (cfDNA). This makes it difficult to accurately detect ctDNA, especially in early - stage cancers or in patients with low tumor burden. For example, in a study of early - stage non - small - cell lung cancer, the sensitivity of deep sequencing of plasma DNA for detecting EGFR mutations was relatively low in early - stage NSCLC (22.2% for stages IA - IIIA), although the specificity was high (98.0% for exon 19 deletions and 94.1% for L858R mutation) [48].

Another issue is the presence of false - positive and false - negative results. False - positive results can occur due to contamination during sample collection, processing, or sequencing, or due to the presence of somatic mutations in normal cells, such as clonal hematopoiesis of indeterminate potential (CHIP). In a study of patients with various cancers, somatic mutations in cfDNA were detected in some healthy individuals, which may pose challenges for the development of ctDNA screening tests [19]. False - negative results can be caused by limitations in the detection techniques, such as insufficient sensitivity to detect low - frequency mutations.

The performance of different detection techniques also varies. For instance, in the evaluation of the Idylla ctEGFR mutation assay for detecting EGFR mutations in plasma from NSCLC patients, the overall agreement, sensitivity, and

specificity were 92.1%, 86.7%, and 95.7% for one analytical condition (C1) and 94.7%, 86.7%, and 100% for another condition (C2). The assay was able to detect the exon 19 deletion from 6 copies/mL and up to 91 copies/mL for the G719S mutation, but careful interpretation is still needed [49].

To improve sensitivity and specificity, various strategies are being explored. Some studies have focused on optimizing pre - analytical procedures, such as sample collection and DNA extraction, to increase the yield and purity of ctDNA.

Others are developing more sensitive and specific detection methods, such as using molecular barcoding and digital PCR techniques to reduce sequencing artifacts and improve the detection of low - frequency mutations [5]. Figure 12 presents key challenges and standardization efforts in ctDNA analysis, including low ctDNA abundance and ctDNA dilution impacting sensitivity, sources of false positives and negatives, comparative performance metrics of the Idylla ctEGFR assay, and proposed standardized testing workflows.

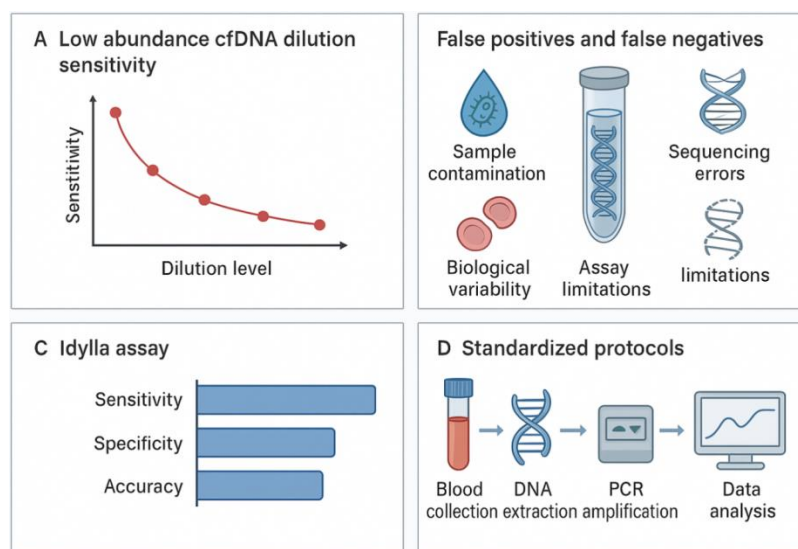


Figure 12. Sensitivity, Specificity, and Standardization in ctDNA Analysis.

5.2. Standardization of ctDNA Testing Protocols

The lack of standardization in ctDNA testing protocols is a significant hurdle that needs to be overcome for the widespread clinical implementation of ctDNA - based tumor precision medicine.

Pre - analytical variables, such as blood collection tubes, plasma processing, and DNA extraction methods, can significantly affect the quantity and quality of ctDNA available for analysis. For example, different blood collection tubes may have varying effects on cell lysis and DNA stability. A study comparing two cell - stabilizing reagents, the cell - free DNA BCT tube and the PAXgene tube, found that blood stored for 7 days in BCT tubes did not show evidence of cell lysis, while PAXgene tubes showed a significant increase in genome equivalents, indicative of cellular lysis [50].

Analytical considerations also play a crucial role. The choice of assay, such as PCR - based methods or next - generation sequencing (NGS), and the analytical input parameters can vary widely between laboratories. This can lead to inconsistent results and difficulties in comparing data from different studies. For instance, in the analysis of mRNA splicing assays across multiple laboratories, differences in PCR primer design strategies, PCR conditions, and product

detection methods were found to be key factors affecting the accuracy of the results [51].

To address these issues, efforts are being made to standardize ctDNA testing protocols. The Blood Profiling Atlas Consortium (BloodPAC) has attempted to define a set of generic analytical validation protocols tailored for ctDNA - based NGS assays. These protocols aim to address the unique challenges of ctDNA analysis, such as the need for high sensitivity and specificity, the potential for false negatives, and the reliance on contrived samples for validation [52].

In addition, inter - laboratory studies are being conducted to evaluate the performance, concordance, and sensitivity of different ctDNA testing methods. For example, an inter - laboratory massively parallel sequencing (MPS) study in the framework of the SeqForSTRs project evaluated forensically relevant parameters using a standardized sequencing library. The study found that despite some variation in performance between sequencing runs, all laboratories obtained quality metrics within the manufacturer's recommended ranges, and inter - laboratory concordance was high for most markers [53].

5.3. Ethical Considerations in ctDNA Utilization

The utilization of ctDNA in tumor precision medicine raises several important ethical considerations.

One ethical concern is the potential for incidental findings. As ctDNA analysis becomes more comprehensive, there is a risk of detecting genetic mutations or alterations that are not directly related to the cancer being treated but may have implications for the patient's future health or the health of their family members. For example, the detection of germline mutations in genes associated with other diseases may lead to complex ethical decisions regarding disclosure and follow-up [54].

Another issue is the privacy and confidentiality of patient data. CtDNA analysis involves the collection and analysis of highly personal genetic information. Ensuring the secure storage, handling, and use of this data is crucial to protect patient privacy. In clinical trials and research studies, strict data protection and privacy regulations need to be observed to prevent unauthorized access or misuse of patient genetic data [55].

The cost - effectiveness of ctDNA - based tests also has ethical implications. If these tests are too expensive, it may limit access to potentially beneficial personalized treatments, leading to disparities in healthcare. For example, in the context of prenatal screening, the high cost of cell - free DNA (cfDNA) testing for fetal trisomies may prevent some women from accessing this advanced screening method [56].

Furthermore, the interpretation of ctDNA results can be complex, and there is a risk of over - or under - interpretation. This may lead to unnecessary treatments or missed opportunities for treatment, both of which can have significant ethical consequences for patients. Clinicians need to be well - informed about the limitations and uncertainties of ctDNA analysis to provide accurate and appropriate counseling to patients [57].

Ethical Considerations in ctDNA Utilization

Incidental Findings



Data Privacy



Cost-Effectiveness



Interpretation Training



Figure 13. Ethical considerations in ctDNA utilization.

To address these ethical considerations, clear guidelines and regulations are needed. **Figure 13.** Ethical Considerations in ctDNA Utilization. An infographic highlighting potential incidental findings, patient genetic data privacy, cost-effectiveness implications for equitable access, and clinician education for accurate result interpretation. These should cover aspects such as the management of incidental findings, data privacy, cost - effectiveness evaluation, and the training of healthcare providers in the interpretation of ctDNA results.

6. Future Perspectives in ctDNA Biomarkers for Tumor Precision Medicine

6.1. Innovations in ctDNA Detection Technologies

The field of ctDNA detection technologies is rapidly evolving, with several innovative approaches on the horizon that have the potential to revolutionize cancer diagnosis and treatment.

One area of innovation is the development of more sensitive and specific detection methods. **Figure 14.** Innovations in ctDNA Detection Technologies. An infographic depicting novel microfluidic bead-based biosensors, combined digital PCR and NGS workflows, advanced long-read sequencing platforms, and AI/ML-based data analytics.

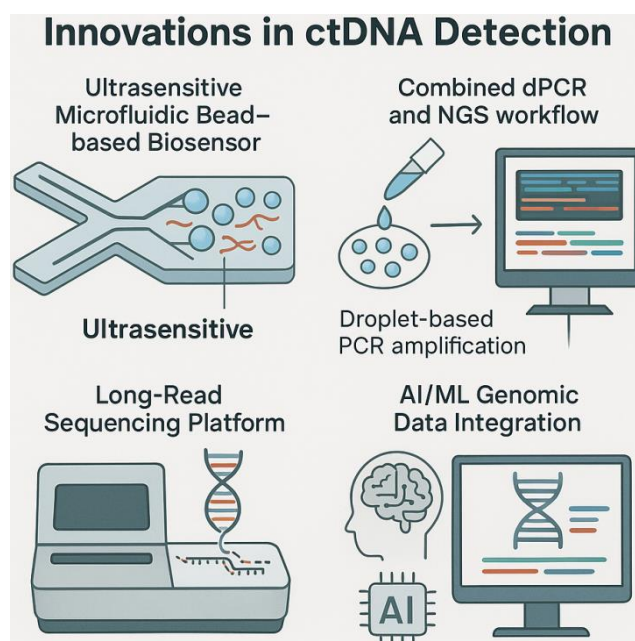


Figure 14. Innovations in ctDNA detection technologies.

For example, new techniques are being designed to overcome the challenges of detecting low - abundance ctDNA in

the presence of high levels of non - tumor - derived ctDNA. Some researchers are exploring the use of novel nanoprobe and microfluidic devices to enhance the capture and detection of ctDNA. A microfluidic bead - based biosensor, for instance, was developed for ultrasensitive ctDNA detection. This biosensor, which combined duplex - functional split - DNAzyme and dendritic enzyme - free signal amplification, achieved an excellent detection limit of 0.36 fM and a wide linear range, showing great promise for early cancer detection [58].

Another innovation is the integration of multiple detection techniques. Combining different methods, such as PCR - based assays with next - generation sequencing (NGS), may provide a more comprehensive and accurate analysis of ctDNA. This approach could potentially increase the sensitivity for detecting low - frequency mutations while also allowing for the simultaneous detection of a wide range of genetic alterations. For example, in some studies, digital PCR is used for the initial screening of known mutations, and NGS is then employed for a more in - depth analysis of the ctDNA genome [59].

Advancements in sequencing technologies are also driving innovation in ctDNA detection. Newer sequencing platforms offer higher throughput, lower costs, and improved accuracy. For instance, the development of long - read sequencing technologies may enable the detection of more complex genetic rearrangements and structural variations in ctDNA, which are often associated with cancer progression and treatment resistance [60].

In addition, the use of artificial intelligence (AI) and machine learning (ML) algorithms is emerging as a powerful tool in ctDNA analysis. These algorithms can analyze large - scale genomic data from ctDNA, helping to identify patterns and biomarkers that may be difficult to detect using traditional methods. AI - based approaches can also improve the interpretation of ctDNA results by integrating multiple data sources, such as clinical information, imaging data, and other omics data, to provide more accurate predictions of treatment response and patient prognosis [61].

6.2. Integration of ctDNA with Multi - Omics Approaches

The integration of ctDNA analysis with multi - omics approaches holds great promise for a more comprehensive understanding of cancer biology and the development of personalized treatment strategies.

One aspect of this integration is the combination of ctDNA with genomics. By analyzing the genomic alterations in ctDNA along with the genomic profiles of tumor tissues, a more complete picture of the tumor's genetic landscape can be obtained. This can help in identifying driver mutations, understanding tumor heterogeneity, and predicting treatment response. For example, in pancreatic cancer, the analysis of ctDNA in combination with genomic data from tumor tissue can provide insights into the genetic alterations that drive the

disease and potentially guide the selection of targeted therapies [62].

Transcriptomics can also be integrated with ctDNA analysis. The study of gene expression patterns in ctDNA or in combination with the transcriptomic profiles of tumor cells can help in understanding the functional implications of genetic mutations. In lung cancer, for instance, integrating ctDNA analysis with transcriptomic data may reveal how certain mutations affect gene expression and signaling pathways, providing valuable information for developing more effective treatment strategies [63].

Proteomics and metabolomics are other omics approaches that can be integrated with ctDNA analysis. Proteomic analysis can identify proteins that are associated with ctDNA - detected mutations or that are involved in cancer progression. Metabolomic analysis can provide insights into the metabolic changes occurring in the tumor, which may be related to the presence of ctDNA - detected genetic alterations. In breast cancer, the integration of ctDNA analysis with proteomic and metabolomic data may help in understanding the complex biological processes underlying the disease and in developing more personalized treatment plans [64].

Furthermore, the integration of ctDNA with multi - omics approaches can be enhanced by the use of artificial intelligence and machine learning. These technologies can analyze the large and complex datasets generated from multi - omics analysis to identify patterns, biomarkers, and potential therapeutic targets. For example, in the MOREOVER project, multi - omics data including radiomics, metagenomics, metabolomics, metatranscriptomics, human genomics, and ctDNA are being integrated and analyzed using AI and ML techniques to improve the prediction of pathological complete response in locally advanced rectal cancer patients [65].

6.3. Prospective Clinical Trials and ctDNA Applications

Prospective clinical trials are essential for validating the clinical utility of ctDNA and translating its potential into routine clinical practice.

In cancer screening, prospective trials are needed to determine the effectiveness of ctDNA - based screening methods. For example, studies are underway to evaluate whether ctDNA analysis can be used to detect early - stage cancers in asymptomatic populations. These trials aim to assess the sensitivity, specificity, and positive predictive value of ctDNA - based screening tests. A large - scale prospective study could help determine if ctDNA screening can reduce cancer - related mortality by enabling early detection and treatment [57].

In the context of treatment monitoring, prospective clinical trials can evaluate the role of ctDNA in guiding treatment decisions. For instance, trials are exploring whether changes in ctDNA levels during treatment can be used to predict treatment response and adjust therapy in real - time. In a study of metastatic colorectal cancer patients, early changes in

ctDNA during first - line chemotherapy were found to predict the later radiologic response, but further prospective trials are needed to confirm these findings and establish the optimal use of ctDNA for treatment monitoring [66].

For minimal residual disease (MRD) assessment, prospective trials are crucial for validating the use of ctDNA as a biomarker. These trials can determine the accuracy of ctDNA in detecting MRD and its ability to predict cancer recurrence. In colorectal cancer, for example, ongoing trials are investigating whether ctDNA - based MRD assessment can help identify patients who may benefit from adjuvant chemotherapy, potentially reducing unnecessary treatment and improving patient outcomes [36].

In addition, prospective clinical trials can also evaluate the cost - effectiveness of ctDNA - based applications. As ctDNA analysis technologies become more widespread, understanding the cost - effectiveness of using ctDNA in different clinical scenarios is important for healthcare decision - making. Trials can assess the economic impact of ctDNA - guided treatment strategies compared to traditional treatment approaches, taking into account factors such as the cost of testing, treatment, and potential improvements in patient survival and quality of life [56].

Overall, well - designed prospective clinical trials are necessary to fully realize the potential of ctDNA in tumor precision medicine, from early detection to treatment monitoring and MRD assessment.

Abbreviations

cfDNA	Cell-Free DNA
ctDNA	Circulating Tumor DNA
PCR	Polymerase Chain Reaction
dPCR	Digital PCR
ddPCR	Droplet Digital PCR
NGS	Next-Generation Sequencing
VAF	Variant Allele Frequency
AI	Artificial Intelligence
ML	Machine Learning
CRC	Colorectal Cancer
NSCLC	Non-Small Cell Lung Cancer
SCLC	Small-Cell Lung Cancer
HCC	Hepatocellular Carcinoma
CTCs	Circulating Tumor Cells
CNV	Copy-Number Variation
SNV	Single-Nucleotide Variant
indel	Insertion/Deletion
CHIP	Clonal Hematopoiesis of Indeterminate Potential
EGFR	Epidermal Growth Factor Receptor
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog
ESR1	Estrogen Receptor 1
BRAF	B-Raf Proto-Oncogene
TP53	Tumor Protein p53

RB1	Retinoblastoma 1
DDR	DNA Damage Response
TMTV	Total Metabolic Tumor Volume
TKIs	Tyrosine Kinase Inhibitors
OS	Overall Survival
PFS	Progression-Free Survival
MRD	Minimal Residual Disease
WES	Whole Exome Sequencing
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
AI/ML	Artificial Intelligence/Machine Learning
SERD	Selective Estrogen Receptor Degradator
CA-125	Cancer Antigen 125
PET-CT	Positron Emission Tomography–Computed Tomography
TKIs	Tyrosine Kinase Inhibitors
TER	Tumor Clonal Evolution Rate
HSP90AA1	Heat Shock Protein 90 Alpha Family A Member 1
POTS	Postural Orthostatic Tachycardia Syndrome
SES	Socioeconomic Status
VR	Virtual Reality

Author Contributions

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Minghang Li: Conceptualization, Formal Analysis, Project administration

Yue Huang: Conceptualization, Investigation, Methodology, Validation

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Conflicts of Interest

The authors declare no conflicts of interest.

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