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CIRCULATING TUMOR DNA LIQUID BIOPSY IN COLORECTAL CANCER: ADVANCES IN EARLY DETECTION, MINIMAL RESIDUAL DISEASE, AND PRECISION ONCOLOGY

Muhammad Shoaib Rafiq[1], Sehar Fatima[2], Nadia Sarwar[3], Ayesha Qayyum[4], Ibtisham Elahi[5], Muhammad Aden[6], Umair Qaisar[7], Aqeel Murtaza[8], Amna Maqsood[9], Muhammad Yasir Malik[*10]

[1,6]Department of Pathology, The Islamia University of Bahawalpur, Bahawalpur, Punjab, Pakistan
[2,3,4,6,9, 10]Virology lab, Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture Faisalabad Punjab Pakistan
[5]Federal State Autonomous Educational Institution of Higher Education I.M. Sechenov First Moscow State Medical University of the Ministry of Health of the Russian Federation (Sechenov University)
[7]Department of Biochemistry, Government College University Faisalabad Pakistan
[8]Institute of Microbiology, University of Agriculture Faisalabad Punjab Pakistan

[*10]malikyasir6500@gmail.com

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Muhammad Yasir Malik

Abstract

Colorectal cancer (CRC) remains a major global health challenge, with disease outcomes largely dependent on early detection, accurate molecular characterization and effective therapeutic management. Conventional diagnostic approaches, including colonoscopy, tissue biopsy, and imaging, are associated with limitations such as invasiveness, sampling bias, and inability to provide real-time information on tumor evolution. Circulating tumor DNA (ctDNA)-based liquid biopsy has emerged as a promising minimally invasive biotechnology approach for detecting and monitoring tumor-derived molecular alterations in blood. This review summarizes recent advances in ctDNA analysis for CRC, focusing on molecular mechanisms, analytical platforms, and clinical applications. The roles of polymerase chain reaction-based assays, digital droplet PCR, next-generation sequencing, DNA methylation profiling, and multi-omics approaches in ctDNA characterization are discussed. Furthermore, the applications of ctDNA in early cancer detection, prognosis prediction, minimal residual disease monitoring, treatment response assessment, and precision oncology are highlighted. Despite significant progress, challenges including low ctDNA abundance, tumor heterogeneity, technical variability, assay standardization, and clinical validation remain barriers to widespread implementation. Future integration of artificial intelligence, advanced sequencing technologies, and personalized

molecular surveillance strategies may enhance the clinical utility of ctDNA. Overall, ctDNA-based liquid biopsy represents a promising molecular diagnostic approach with potential to transform CRC detection and personalized patient management.

1. INTRODUCTION

Colorectal cancer (CRC) is one of the biggest health problems in the world, being one of the main causes of cancer incidence and cancer death in the world (1). CRC is estimated to represent about 10% of all new cases of cancer worldwide, and is projected to be the second most common cause of cancer-related death, largely because of population aging, lifestyle changes, obesity, dietary patterns and decreased physical activity (2). Despite the improved prognosis for CRC patients due to improvements in screening programs, surgical techniques, chemotherapy, targeted therapies and immunotherapy, the prognosis remains strongly dependent on the stage of diagnosis. CRC patients with localized disease have better survival rates, while those with advanced and metastatic disease have significantly lower survival rates (3). Thus, developing sensitive, minimally invasive and molecularly informative diagnostic strategies is a key challenge in modern oncology.

The current methods of CRC screening and diagnosis are mostly based on colonoscopy, fecal molecular tests, imaging, and histological analysis of biopsies (4). Colonoscopy is the most valuable diagnostic tool as it gives the physician direct access to the colorectal lesions as well as the opportunity to remove any precancerous polyps simultaneously. Its invasiveness, bowel preparation, and cost, coupled with poor patient compliance, limit its use, though (5). Non-invasive stool-based tests, like fecal immunochemical testing (FIT) and multitarget stool DNA testing have increased the accessibility of screening, but both tests may have varying sensitivities, especially for early-stage cancers and advanced adenomas (6). While tissue biopsy can be useful for molecular characterization and therapeutic decision making, there is a limited tissue volume that is analyzed and biopsy may not accurately reflect the large molecular heterogeneity found in primary or metastatic tumor (7). These restrictions underscore the need to find alternative methods that will yield real-time rich, minimally invasive molecular information.

Liquid biopsy has become a revolutionary biotechnology platform for cancer detection and monitoring by analyzing components from the tumor in biological fluids such as blood plasma (8).

As opposed to tissue biopsies, liquid biopsy gives a snapshot of a tumor's biology and can be repeated during disease progression and treatment. Circulating tumor cells (CTCs), extracellular vesicles, circulating RNA molecules and circulating tumor DNA (ctDNA) are several circulating biomarkers that have been studied. Of these biomarkers, ctDNA has garnered much attention due to the fact that it harbors tumor-specific genetic and epigenetic changes that are reflective of molecular alterations of the malignant cells (9, 10). ctDNA is released into the circulation primarily by apoptosis, necrosis and active secretion from tumor cells, and is composed of fragments of DNA. The short half-life of ctDNA makes it an accurate real-time marker of tumor burden, response to therapy and disease progression (11, 12).

CRC has been one of the most promising applications for ctDNA-based liquid biopsy, showing significant promise in enhancing early detection, risk stratification, minimal residual disease (MRD) monitoring, and personalized treatment approaches (13). Specific molecular changes that are commonly found in CRC, including mutations in APC, KRAS, TP53, BRAF and DNA mismatch repair genes, can be identified by using advanced ctDNA platforms like digital droplet PCR (ddPCR), next-generation sequencing (NGS), and methylation-based assays (14, 15). Specifically, postoperative ctDNA detection has become an important marker for prognosis as patients with postoperative-detectable ctDNA have been found to have a much higher risk of recurrence than those with negative postoperative ctDNA (16, 17). In addition, ctDNA-based treatment strategies are being explored to expand the patient population that might benefit from the use of adjuvant chemotherapy, to monitor therapeutic resistance and to aid in precision-based treatment decisions (18).

Despite these progressions, there are a number of hurdles to overcome before liquid biopsy can be used routinely in clinical practice, such as the low amount of biomarkers in early-stage disease, variability between detection platforms, a lack of standardization of methodologies, high analytical cost, and a

need for prospective clinical validation. Hence, this review aims to give a comprehensive insight into molecular mechanism, technology platforms, and clinical applications of liquid biopsy based on ctDNA in colorectal cancer. This review aims to critically review the state of the art in detection technologies, to summarize the evidence for clinical use, to discuss current limitations and to point towards future directions, such as using artificial intelligence, multi-omics integration and precision medicine approaches. This review is useful as it connects molecular pathology and biotechnology and offers a fresh look at the potential for the implementation of ctDNA-based liquid biopsy in the diagnosis, monitoring, and personalized therapeutic management of CRC.

2. Molecular Pathogenesis of Colorectal Cancer and Biological Basis of ctDNA Release

Colorectal cancer is a genetically and epigenetically diverse disease that is a step-wise accumulation of molecular changes in pathways involved in cell proliferation, differentiation, DNA repair, apoptosis, and genomic stability (Table 1).

To develop circulating tumor DNA (ctDNA) based liquid biopsy approaches (Figure 1), it is crucial to understand the molecular pathogenesis of CRC, as the detection of genetic and epigenetic signatures of the tumor in blood is the basis of the ctDNA approach. There are several different molecular pathways of CRC, such as chromosomal instability (CIN), microsatellite instability (MSI) and CpG island methylator phenotype (CIMP), all of which play a role in tumor initiation, progression, metastasis and response to treatment (19, 20).

2.1 Molecular Pathways Involved in Colorectal Cancer Development

Sporadic CRC cases occur in about 70-85% of cases and follow the chromosomal instability pathway. Mutations in critical tumor suppressor genes and oncogenes, along with progressive chromosomal abnormalities and loss of heterozygosity are key features of this pathway. The classical adenoma–carcinoma sequence (21) suggests that CRC development is a multistep process involving the activation of oncogenic signaling pathways (such as KRAS) and the loss of tumor suppressor function via alterations in TP53. This alteration in the molecular structure helps to produce excessive cell growth, resistance to apoptosis, and transformation into a cancer cell.

The microsatellite instability pathway is another important pathway resulting from defects in DNA mismatch repair (MMR) systems.

Mismatch repair genes (such as MLH1, MSH2, MSH6, and PMS2) normally are responsible for correcting errors in the replication of repetitive DNA sequences, or microsatellites, that are prone to errors. MSI-high tumors account for about 15% of CRC cases, are often linked to immune activation and higher TMB and show better response to immune checkpoint inhibitors (ICIs) (22, 23). Moreover, the CpG island methylator phenotype (CIMP) pathway is characterized by extensive DNA hypermethylation of promoters, resulting in silencing of the expression of tumor suppressor genes and is involved in the initiation of CRC, especially in serrated pathway tumors (24). A better understanding of these molecular pathways can lead to distinct alterations in the DNA that can be identified through ctDNA analysis, which is vital for an accurate diagnosis and treatment of colorectal cancer, as well as molecular classification

2.2 Genetic Alterations Associated With Colorectal Cancer Progression

APC Mutations

APC is one of the genes that is mutated in the earliest and most common colorectal tumorigenesis. About 70-80% of colorectal cancers have mutations in this gene (Table 1 Figure 1). APC is a negative regulator of the Wnt/β-catenin pathway, a pathway that regulates intestinal epithelial cell proliferation and differentiation. Mutations that result in loss of APC function cause abnormal nuclear levels of β-catenin, activation of oncogenic transcription programs, and unchecked growth of epithelial cells (25). The presence of APC mutations in ctDNA could be useful for early molecular diagnosis and cancer surveillance, as they are located in early stages of CRC development.

Table 1 Major Molecular Biomarkers Detected in Colorectal Cancer ctDNA

Biomarker	Molecular Function/Pathway	Clinical Significance
APC mutations	Wnt/β-catenin pathway; tumor suppressor loss	Early CRC development marker and tumor evolution monitoring
KRAS mutations	RAS–RAF–MEK–ERK signaling activation	Predicts resistance to anti-EGFR therapy and guides treatment selection
NRAS mutations	RAS pathway activation	Associated with anti-EGFR therapy resistance
TP53 alterations	DNA repair, apoptosis, and cell-cycle regulation	Marker of genomic instability and poor prognosis
BRAF V600E mutation	MAPK pathway activation	Prognostic marker and therapeutic stratification
PIK3CA mutations	PI3K/AKT/mTOR signaling pathway	Associated with tumor progression and treatment response
MMR gene defects (MLH1, MSH2, MSH6, PMS2)	DNA mismatch repair pathway	Identifies dMMR tumors and predicts immunotherapy response
Microsatellite instability (MSI)	Genome instability due to MMR deficiency	Biomarker for immune checkpoint inhibitor therapy
SEPT9 methylation	Epigenetic promoter methylation	Blood-based biomarker for CRC screening
CIMP-associated methylation	CpG island promoter hypermethylation	Defines molecular CRC subtype
Copy number alterations	Chromosomal gains/losses	Indicates tumor progression and genomic changes
Fragmentomic signatures	Altered ctDNA fragment patterns	Emerging biomarker for early detection
Gene fusions/structural alterations	Genomic rearrangements	Identification of rare therapeutic targets

KRAS and NRAS Mutations

Mutations in genes of the RAS oncogene family (KRAS and NRAS) are important events in CRC progression and therapeutic resistance. The mutations cause down-stream signaling pathways such as the RAS–RAF–MEK–ERK pathway to be continually stimulated, favoring the development and survival of the tumor. KRAS mutations are present in about 40–50% of CRC patients, and are especially significant in the prediction of resistance to anti-epidermal growth factor receptor (anti-EGFR) drugs like cetuximab and panitumumab (26). Non-invasive detection of RAS mutations by ctDNA analysis can also be used for continuous monitoring of the emergence of resistant clones during targeted therapy.

TP53 Alterations

The TP53 tumor suppressor gene has been shown to be mutated in about 50-60% of colorectal cancers. TP53 is the gene that codes for the p53 protein, a regulator of DNA damage response, cell-cycle arrest and apoptosis. When TP53 is lost, it enables the survival of cells with additional genetic changes, which can facilitate tumor progression and metastasis. TP53 mutations are important molecular markers that can be identified utilizing ctDNA sequencing methods as a result of their prevalence in advanced CRC (27).

BRAF Mutations

In about 8-12% of colorectal cancers there are mutations in the BRAF gene, including mutations called the BRAF V600E mutation. Mutations of BRAF lead to constant activation of the MAPK signaling pathway and are often found in right-sided tumors, in patients with poor prognosis and in those who

are resistant to standard treatments. The detection of mutations in BRAF using ctDNA can be useful for prognosis and therapeutic decisions, especially for the use of BRAF inhibitors (28).

DNA Mismatch Repair Defects and Microsatellite Instability

Two particular molecular characteristics of CRC are defective DNA mismatch repair (MMR) and microsatellite instability (Figure 1). Lack of MMR leads to a genome-wide accumulation of insertion and deletion mutations, creating tumor-specific neoantigens, which affect immune recognition. MSI status can be clinically significant as MSI-high CRCs have better prognosis and are more responsive to immune checkpoint inhibitors like pembrolizumab. Non-invasive detection of MSI using ctDNA-based methods is being explored as an alternative to tissue testing (29).

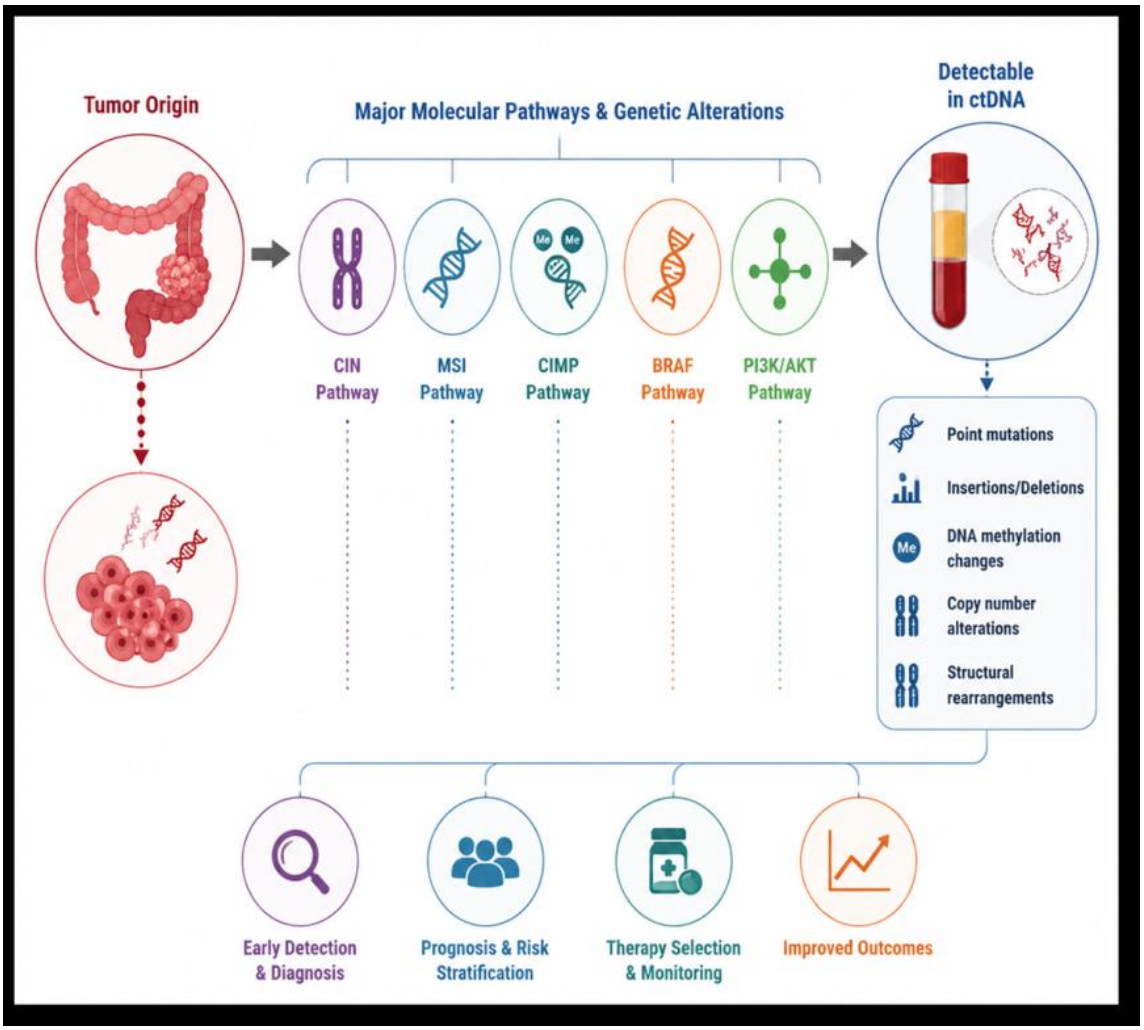


Figure 1 Molecular pathways and genetic alterations involved in colorectal cancer detectable through ctDNA

2.3 Mechanisms of ctDNA Release Into Circulation

The ctDNA is fragments of DNA that circulate in the blood due to several biological processes of tumor cells. Apoptosis, necrosis, and active secretion of cells are the main sources of ctDNA. In apoptosis, programmed cell death, the DNA is broken into fragments of about 150-200 base pairs that may be released into the bloodstream when the tumor cells disintegrate. The rapid growth, the limited supply of oxygen and the metabolic stress of the necrotic tumor areas release larger DNA fragments in the extra-cellular environment (11). In addition, recent studies indicate that exosomal release of extracellular DNA may be an active process of living tumor cells, and constitute a source of circulating tumor derived genetic material. Multiple factors affect the plasma level of ctDNA such as tumor size,

disease stage, vascularization, metastatic burden, and individual tumor biology. In general, advanced and metastatic cancers shed more ctDNA than localized cancers, but low levels of ctDNA can only be detected using sensitive techniques.

2.4 Biological Characteristics and Clinical Significance of ctDNA

ctDNA has several properties which make it a useful biomarker in the management of colorectal cancer. In contrast to the traditional serum biomarkers like carcinoembryonic antigen (CEA), ctDNA offers direct molecular data on tumor-specific mutations, copy number alteration, methylation and genomic evolution. The short half-life of ctDNA, lasting several hours, makes it a good indicator of changes in tumor burden and response to treatment (12). In a clinical context, ctDNA has shown promise for the detection of early diagnosis of cancers, prediction of prognosis, monitoring MRD, and therapeutic decision making. Postoperative ctDNA positivity has been shown to have a clear link to the likelihood of recurrence, which can enable patients to be targeted for enhanced surveillance or potentially adjuvant treatment. Moreover, serial ctDNA detection can be used as a tool to identify emerging resistance mutations before radiological progression, thus helping to adopt a strategy of adaptive treatment (30).

Hence, the molecular features of CRC and the biological properties of ctDNA are the backbone of liquid biopsy technology, which has the potential to revolutionize CRC diagnosis and precision management. As sequencing technology, bioinformatics analysis and assay standardization continue to advance, the clinical potential of ctDNA-based methods will further be improved.

3. Biotechnology Platforms for Detection and Characterization of ctDNA

The clinical utility of circulating tumor DNA (ctDNA) based liquid biopsy to CRC is highly dependent on the availability of highly sensitive, specific and reproducible biotechnology platforms to detect very low levels of tumor derived DNA fragments in the blood stream (Table 2). However, because the amount of ctDNA is a small fraction of the total circulating cell-free DNA (cfDNA), high-tech molecular methods are needed to correctly identify tumor-specific genetic and epigenetic changes, especially in early disease (31). These ten years have seen substantial advances with methods such as PCR-based assays, digital PCR technologies, next-generation sequencing (NGS), methylation-based assays, and multi-omics assays. Such technologies have given rise to much more than just the detection of mutations: the comprehensive tumor profiling, the minimal residual disease (MRD) monitoring and the selection of precision treatment.

Table 2 Comparison of ctDNA Detection Technologies

Technology	Principle	Advantages	Limitations	Major Applications
qPCR	Target-specific DNA amplification and quantification	Rapid, simple, low cost	Limited sensitivity; requires known mutations	Mutation screening
Digital droplet PCR (ddPCR)	Partitioning DNA into thousands of droplets for absolute quantification	Very high sensitivity and accuracy	Limited to targeted alterations	MRD detection, mutation monitoring
Next-generation sequencing (NGS)	Parallel sequencing of multiple genomic regions	Comprehensive mutation profiling; detects multiple alterations	Expensive; complex data analysis	Tumor profiling, precision oncology
Whole-exome/genome sequencing	Large-scale analysis of coding regions or entire genome	Identifies novel mutations and genomic changes	High cost and computational requirements	Tumor evolution studies

(WES/WGS)				
Methylation-based assays	Detection of tumor-specific DNA methylation patterns	Early detection potential; stable biomarkers	Requires assay standardization	CRC screening and diagnosis
Fragmentomic analysis	Analysis of ctDNA fragment size and genomic distribution patterns	Improves early cancer detection accuracy	Requires advanced sequencing and bioinformatics	Early detection and risk assessment

3.1 Polymerase Chain Reaction (PCR)-Based Detection Approaches

One of the earliest and most popular detection methods for ctDNA is the use of polymerase chain reaction (PCR)-based methods, which are simple, fast, and economically viable. Conventional PCR and quantitative PCR (qPCR) both are capable to amplify specific areas of the DNA, where tumor-related mutations are known, thus detect genetic mutations in circulating DNA. PCR-based assays have been mainly used for the detection of clinically relevant mutations in genes like KRAS, NRAS, BRAF and TP53 that affect prognosis and therapeutic decisions in colorectal cancer (12).

qPCR techniques involve real-time monitoring of DNA amplification by measuring a fluorescent signal, allowing the determination of the abundance of mutations in a sample. These strategies have proven of value in the identification of common "hotspot" mutations for CRC progression and treatment resistance. When using a PCR-based approach to analyze ctDNA, for instance, the presence of activating KRAS mutations can inform treatment personalization, particularly when targeting anti-EGFR treatments, which are known to be ineffective in patients with these mutations (32). Standard PCR based methods, however, are not ideal as they are only sensitive for detecting mutations that have been previously identified and are usually very sensitive when mutant DNA is present at a very low level compared to normal DNA.

New techniques, like allele-specific PCR and amplification-refractory mutation system (ARMS)-PCR, have been developed that increase the sensitivity of mutation detection by selectively amplifying mutant DNA sequences. However, they are limited in their ability to fully capture the tumor heterogeneity due to the fact that they only test for pre-selected genetic changes and do not assess the full molecular spectrum.

3.2 Digital Droplet PCR (ddPCR) for Sensitive Mutation Detection

Digital droplet PCR (ddPCR) is a great improvement on standard PCR as it allows for absolute and extremely sensitive quantification of the number of ctDNA molecules (33). ddPCR involves breaking up DNA samples into thousands of microdroplets for each of which it acts as a separate PCR reaction. This partitioning allows the accurate detection of rare mutant DNA molecules even if they occur at very low frequencies.

The high analytical sensitivity of ddPCR makes it a highly valuable tool for precise quantification, e.g. MRD detection following surgical tumor resection or follow-up for treatment efficacy. The use of ddPCR has been successful for detecting mutations in the genes KRAS, APC, and TP53 to distinguish the evolution of colorectal cancer and the risk of recurrence (17). ddPCR offers greater sensitivity, reproducibility, and quantitative accuracy over conventional PCR, and does not require extensive bioinformatics analysis.

There are, however, some limitations to ddPCR. Typically affects a specific area of the genome, meaning it will not identify unknown mutations or complete genome information. Furthermore, the approach of creating very specific assays for a number of mutations is technically difficult, especially for genetically diverse tumors. As a result, ddPCR is frequently applied in addition to the other sequencing-related technologies.

3.3 Next-Generation Sequencing (NGS)-Based ctDNA Profiling

Next generation sequencing (NGS) has enabled a new way of analyzing ctDNA, allowing multiple genomic changes to be analyzed in several genes simultaneously. NGS platforms offer a complete molecular profile of mutations, copy number variations, structural alterations and mutational signatures, without the need for predefined targets, which is not possible with PCR-based methods (34).

In light of the high-depth sequencing provided by targeted NGS panels, high analytical sensitivity and the ability to sequence cancer-associated genes, targeted NGS panels are becoming more common in CRC (35). The panels detect clinically significant changes in genes coding for APC, KRAS, NRAS, BRAF, PIK3CA, TP53 and DNA mismatch repair genes Furthermore, whole-exome sequencing (WES) and whole genome sequencing (WGS) can be used to obtain more comprehensive information on the genome, which can be used to analyze tumor evolution, clonal diversity, and therapy resistance mechanisms (36).

The use of NGS-based ctDNA detection has shown great promise in the field of MRD detection after therapy. There are highly sensitive approaches to sequencing tumors in patients, known as tumor-informed sequencing, in which the mutations from primary tumor tissue are identified and then monitored in plasma, which has been shown to predict recurrence after CRC surgery (37). Nevertheless, the use of NGS methods is hindered by a number of limitations such as the high cost, complex bioinformatics needs, and error in sequencing and standardization issues.

3.4 DNA Methylation-Based ctDNA Detection Strategies

DNA methylation changes are potential biomarkers for the detection of CRC through ctDNA. Methylation changes do not have to be genetic mutations and are often early events in colorectal cancer and can yield very specific tumor signatures (38). Promoter methylation of genes, including SEPT9, BCAT1, IKZF1 and VIM, have been examined as potential markers for non-invasive CRC screening and monitoring.

mSEPT9 has been widely studied and has been integrated into CRC screening programs in a few countries based on blood based markers (39). One of the examples that has made it into clinical practice is the detection of methylated SEPT9 DNA in plasma which is a moderate sensitive and specific biomarker to detect CRC (40).

Methylation-based assays have multiple benefits, such as improved stability of the epigenetic signal and the ability to detect cancer in its early stages (41). However, there are challenges to assay performance, tumor heterogeneity, and population differences in methylation patterns that exist, but must be addressed before validation.

3.5 Multi-Omics Approaches for Advanced ctDNA Characterization

The utilization of ctDNA with single markers has evolved to multi-omics approaches in recent years. Multi-omics approach integrates the genomic, epigenomic, transcriptomic and proteomic data to provide a holistic picture of tumor biology (42). The combination of ctDNA sequencing, DNA methylation, fragmentomics, and machine learning analysis has significantly boosted cancer detection accuracy, enabling the detection of complex molecular patterns instead of relying on individual biomarkers. The pattern of cfDNA fragmentation, the nucleosome positioning, and the genome-wide methylation signatures are becoming important sources of information for the early detection of cancer (43).

Multi-omics techniques could be a solution to the challenges of low levels of ctDNA and tumor heterogeneity in the context of CRC. These approaches hold great promise for the creation of next generation EDPs to detect cancer in its earliest stages before clinical symptoms are seen.

3.6 Current Advances in Assay Sensitivity and Standardization

Although there have been significant advances in technology, analytical sensitivity and developing a standard protocol for implementation of ctDNA in clinical practice are still big challenges. Blood collection, plasma processing time, DNA extraction procedure, depth of sequence, bio-informatic pipelines and reporting criteria influence the performance of assays.

Progress in recent years such as error-corrected sequencing, unique molecular identifiers (UMIs), and the development of more sophisticated bioinformatics algorithms has greatly improved the ability to detect low frequency tumor mutations (44). These methods minimize the occurrence of sequencing errors and facilitate the detection of any rare molecules of ctDNA when present at very low levels.

There is currently international work in progress to create a consensus guideline for ctDNA analysis that includes recommendations for sample handling, analytical validation, quality control and clinical interpretation. To successfully incorporate the liquid biopsy of ctDNA into clinical practice, future advances in technology standardization, automation, and cost will be crucial (45).

In general, the platforms that use biotechnology to detect ctDNA have developed from targeted mutation detection to more complete molecular profiling platforms. The integration of highly sensitive detection technologies, multi-omics approaches and advanced sequencing techniques lays the foundation to make ctDNA liquid biopsy a clinically valuable tool for early detection, disease monitoring and precision treatment of colorectal cancer.

4. Clinical Applications of ctDNA-Based Liquid Biopsy in Colorectal Cancer

Circulating tumor DNA (ctDNA) liquid biopsy is an important breakthrough in the molecular field of oncology (Figure 2), which offers a minimally invasive method to monitor the tumor biology in real-time in CRC management. In contrast to traditional diagnostic approaches which involve taking samples at a single time, ctDNA can be used to track tumor-derived genetic and epigenetic changes over time and during treatment (13). The clinical uses of ctDNA have grown from experimental cancer research biomarker studies to the use of early detection, prognosis prediction, minimal residual disease (MRD) monitoring, therapeutic monitoring, and precision oncology applications. The molecular profile that ctDNA represents offers important information for personalized management of patients and could be able to overcome some of the limitations of conventional imaging and tissue-based methods.

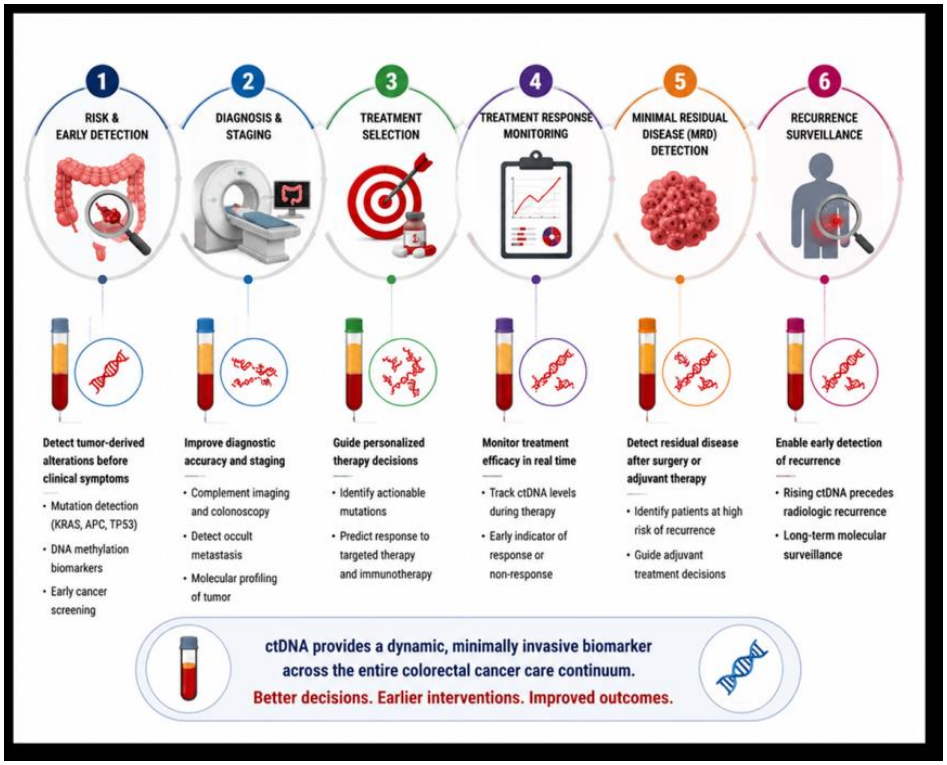


Figure 2 Clinical applications of ctDNA-based liquid biopsy throughout the colorectal cancer patient journey, from early detection and diagnosis to treatment monitoring, MRD assessment, recurrence surveillance, and precision oncology

4.1 Role of ctDNA in Early Detection and Population Screening

Early detection is one of the most critical tools to reduce colorectal cancer deaths; outcomes of colorectal cancer strongly depend on the stage at diagnosis. Current screening methods, such as colonoscopy and fecal screening, have made a huge improvement in detecting CRC; however there are some limitations with these tests, such as invasiveness, patient acceptability, sensitivity for early detection and access to healthcare, which have led to the need for alternative, non-invasive, molecular-based screening. The possibility of detecting tumor-specific DNA alterations in blood before clinical symptoms of disease occurs has led to the development of a technology termed ctDNA-based liquid biopsy, which has shown great promise (46).

Initial CRC is a very difficult time for detection of ctDNA, as tumor-derived DNA only accounts for a proportion of the total amount of circulating cell-free DNA (cfDNA). However, sequencing depth, error-correction and methylation assays have improved the detectability of ctDNA. Alterations in tumor genes such as APC, KRAS, TP53 and BRAF or DNA methylation patterns have been studied as possible biomarkers for early diagnosis of CRC (47).

The methylation-based ctDNA assays have been particularly promising for population screening because early changes in the methylation of genes are frequently seen during colorectal tumorigenesis. The presence of methylated SEPT9 (mSEPT9) in plasma is one of the most advanced CRC screening blood markers in clinical trials. Its sensitivity is still lower than colonoscopy, but it does show the potential for molecular screening techniques using blood (40). Moreover, there is a move towards using a multi-analyte approach, integrating multiple ctDNA mutations, methylation signatures and protein biomarkers, to enhance the diagnostic sensitivity (38).

A large-scale study testing multi-cancer early detection platforms has shown the ability to detect cancer-associated molecular patterns using multi-cancer circulating DNA with greater accuracy when adding genomic and epigenomic information. This is because the use of ctDNA as a screening method needs to be validated before it can be used in place of the current screening methods, in order to assess its cost-effectiveness, its applicability in the population and its detection of precancerous lesions (48).

4.2 Prognostic Significance of ctDNA in Colorectal Cancer Patients

In addition to diagnosis, ctDNA also has proven to have significant prognostic potential in providing molecular data linked to tumor burden, recurrence risk and survival (Table 3). The main pathological factors used in traditional assessment of prognosis in CRC are tumor invasion depth, lymph node involvement, and the presence of metastasis. Patients with identical clinical stages may have different results, though, because of the molecular heterogeneity of the tumors. ctDNA analysis also offers more biological information which could be used to better stratify the risk (49).

Multiple studies have shown that detectable ctDNA following diagnosis or treatment is correlated with worse clinical outcomes. The higher the basal level of ctDNA, the more likely the disease is to be in an advanced stage, burden of disease is high, and the disease is metastatic. Patients who have undetectable ctDNA following treatment, however, tend to have better disease-free survival than patients who have detectable ctDNA. In stage II and III CRC, the prognostic role of ctDNA is especially relevant, where the decision of adjuvant chemotherapy timings remains a difficult one. Patients with a high risk of recurrence may not be correctly identified with conventional clinical parameters. Low levels of ctDNA after the surgery to remove the primary tumor may suggest the presence of molecular residual disease, and help distinguish patients who may need continued systemic treatment. Additionally, serial ctDNA testing can yield dynamic information about tumor evolution. Higher levels of ctDNA at follow-up could signal early molecular recurrence, which may enable earlier intervention and better clinical outcomes, before radiological recurrence is seen (50).

4.3 Detection of Minimal Residual Disease (MRD) After Surgery

One of the most important and clinically relevant uses of ctDNA in CRC is the detection of minimal residual disease (MRD). After localized CRC surgery, some recurrences occur when cancer cells remain undetectable even after using routine imaging techniques. It is important to diagnose them at an early

molecular stage in order to provide the best treatment options in combination with an adjuvant treatment strategy (15).

MRD detection using ctDNA is based on the detection of genetic changes that are present following surgery that are characteristic of the tumor. Tumor informed approaches generally start with sequencing the primary tumor to find patient-specific mutations and then monitor the disease very sensitively through plasma samples, looking for the patient's specific mutations. Recurrent ctDNA positivity following surgery has been found to have consistently been associated with significantly higher risk of recurrence (51).

Stage II colon cancer patients with detectable postoperative ctDNA had a higher risk of disease recurrence than those who were ctDNA-negative. Similarly, serial postoperative ctDNA analysis demonstrated a strong predictive value for recurrence in patients with stage I–III CRC (16).

MRD detection becomes clinically relevant because it can help to inform the choices of adjuvant therapy. The presence of ctDNA could signal the need for more aggressive treatment; otherwise, patients without the presence of ctDNA may not need to undergo the toxicity of unnecessary chemotherapy. Multiple exciting clinical trials are currently underway to assess the efficacy of making therapeutic decisions based on ctDNA status on patient outcomes, including randomized ctDNA-based treatment trials.

Table 3 Clinical Studies Evaluating ctDNA-Guided Colorectal Cancer Management

Clinical Setting	ctDNA Application	Major Findings	Study (Year)
Stage II colon cancer after surgery	Minimal residual disease (MRD) detection	Postoperative ctDNA positivity predicted a significantly higher risk of recurrence	(16)
Stage I–III colorectal cancer	Recurrence monitoring	Serial ctDNA analysis detected molecular recurrence and predicted relapse risk	(17)
Stage II colon cancer	ctDNA-guided adjuvant therapy	ctDNA-guided treatment reduced unnecessary chemotherapy without affecting recurrence outcomes	(14)
Stage II–IV colorectal cancer	MRD detection and prognosis assessment	ctDNA status was strongly associated with recurrence risk and survival outcomes	(14)
Resected colorectal cancer	Personalized ctDNA monitoring	Tumor-informed ctDNA assays demonstrated clinical utility for recurrence surveillance	(15)
Stage II–III colorectal cancer	Treatment escalation/de-escalation guidance	Evaluated ctDNA-guided strategies for optimizing adjuvant therapy decisions	(52)
Stage II colon cancer	Adjuvant treatment selection	Investigated the role of ctDNA-guided chemotherapy decision-making	(53)

4.4 Monitoring Chemotherapy and Targeted Therapy Response

One advantage of ctDNA is that it's a live indicator of treatment response, as the degree of the change in circulating tumor DNA may appear earlier than imaging changes. Lowering of ctDNA levels is associated with a reduction in the burden of disease and therapeutic response, and a non-reduction or rising of ctDNA levels often point to resistance or disease progression during chemotherapy (54).

In metastatic CRC, ctDNA has been shown to be useful to monitor the response to targeted treatments, such as anti-EGFR agents. It is possible to monitor changes in the genomic composition of the tumor over time using serial ctDNA assessment, thereby enabling the clinician to track treatment response and adjust treatment as needed (55). Patients taking EGFR inhibitors can benefit initially and eventually stop responding to the drug because of secondary mutations to other genes such as KRAS, NRAS, BRAF or others in the same pathway. ctDNA analysis can allow the detection of these changes without

the need for repeated tissue biopsies, and is a minimally invasive way to monitor the molecular evolution of these changes. Also, the use of ctDNA monitoring could aid in the evaluation of immunotherapies, especially in the case of mismatch repair deficient or microsatellite unstable tumors. The alterations in ctDNA throughout immune checkpoint inhibitor treatment could offer early signs of therapeutic response or resistance (56).

4.5 Detection of Molecular Resistance Mechanisms

Genetic evolution plays a major role in cancer progression; "the more a cancer is treated, the more selection pressure is applied, the more resistant cancer cells survive and grow." Current tissue biopsy is inadequate to give any information about tumor heterogeneity since it samples one anatomical area at one time. Unlike ctDNA, which is able to capture DNA released from several tumor sites throughout the body (57).

Acquired genetic changes in signaling pathways are common in CRC that are resistant to targeted therapies. Acquired mutations in KRAS or NRAS, amplification of MET and activation of alternative signaling pathways, for instance, are examples of mechanisms by which resistance to anti-EGFR therapy may develop. ctDNA testing can identify these resistance mechanisms early, to help the doctor change treatment before there is serious disease progression (58).

Beyond this, ctDNA has helped to support the idea of molecular disease monitoring, whereby decisions on treatment are made based on changes in the genetics of the tumor, not just the radiological progress. This is a new method towards adaptive oncology, whereupon therapies may be adjusted given to the changing characteristics of the tumor (59).

4.6 ctDNA-Guided Personalized and Precision Oncology Approaches

The goal of precision oncology is to tailor the treatment of each patient's cancer to the molecular makeup of their tumor. Rapid, repeated and minimally invasive genome profiling, which is provided by the ctDNA-based liquid biopsy, lends strong support to this approach (60).

In CRC, ctDNA can be used to detect clinically relevant mutations that impact therapeutic selection. RAS mutations enable the diagnosis of eligibility for EGFR-targeted therapy; in addition, BRAF mutations and MSI status as well as other molecular changes aid the selection of targeted and/or immune-based therapy (61).

In addition, precision medicine using ctDNA enables real-time tumor monitoring as the disease progresses and evolves treatment strategies accordingly. Unlike classic methods using initial tumor characterization, ctDNA offers a molecular snapshot of the disease at a given time.

Combined with AI, machine learning algorithms, and multi-omics analysis, the future integration of ctDNA could enhance the ability to predict treatment and personalize cancer management. Such strategies might allow the discovery of molecular signatures linked to predictive treatment outcomes, recurrence risk, and metastatic potential (62).

In conclusion, ctDNA liquid biopsy has revolutionized the management of CRC by offering valuable molecular information throughout the disease spectrum, from early diagnosis to advanced precision treatment. While sensitivity, standardization and clinical validation of the technology remain an issue, future technological advancements are anticipated to make ctDNA a key element of future colorectal cancer diagnostics and personalized oncology.

5. Comparison of ctDNA With Other Liquid Biopsy Biomarkers

Liquid biopsy is a new biotechnology strategy that allows the non-invasive diagnosis and monitoring of cancer based on the analysis of tumor-derived components found in the biological fluids (Figure 3) (8). In addition to the circulating tumor DNA (ctDNA) which has proven to be one of the most promising liquid biopsy biomarkers in colorectal cancer (CRC), other circulating components are also showing promise as diagnostic and prognostic markers for CRC, such as circulating tumor cells (CTCs), extracellular vesicles (EVs), exosomes, and circulating RNA molecules. Different types of biomarkers offer different types of biological information about the nature of the tumor, but abundance, stability, analytical complexity and clinical applicability differ among them and affect their suitability for routine

clinical use. The comparative advantages and limitations of these biomarkers are crucial for precision oncology integrated liquid biopsy strategies (63).

5.1 Circulating Tumor Cells (CTCs)

During tumor progression, circulating tumor cells (CTCs) are intact malignant cells which break off from the primary tumor, or metastatic lesions, into the bloodstream. The advantage of CTCs is that they are actually tumor cells and are able to offer direct information regarding cellular characteristics, such as morphology, protein expression, genomic alterations and metastatic potential. Detection of CTC has been explored as a biomarker of prognosis in colorectal cancer due to the observation that the higher the number of CTC, the more advanced the disease, the more likely the presence of metastases, and the poorer the survival outcomes (64).

Isolated tumor cells can be further examined using genomic, transcriptomic and functional studies – one major advantage of CTC analysis. This allows scientists to understand how metastasis, drug resistance and tumor evolution occur. In such cases, molecular analysis of CTCs may allow for the detection of expression of therapeutic targets and alterations associated with resistance, which can be used to direct therapy (65).

There are several drawbacks in the use of the CTC-based approaches, however. The isolation of CTCs is technically challenging, because they are very rare in peripheral blood, especially in early-stage cancers (66). In addition, during metastasis, tumor cells change into an epithelial-to-mesenchymal transition (EMT), thereby altering their surface markers, which can decrease the detection efficiency. The existing CTC isolation tools, such as immunomagnetic separation and microfluidic technology, need to be optimized and standardized for broad clinical applications. The amount of DNA in the blood stream is higher with ctDNA than with CTCs since DNA fragments are released continuously as cells undergo apoptosis and necrosis in the tumor. Thus, ctDNA can more frequently show higher sensitivity for the detection of molecular changes, especially in monitoring the metastatic disease or in the surveillance of postoperative disease (67).

5.2 Extracellular Vesicles and Exosome-Based Biomarkers

Extracellular vesicles (EVs), such as exosomes, are nanoparticles of membrane and released into circulation by normal and tumor cells. The vesicles harbor a variety of biological molecules with different molecular properties such as DNA, mRNA, microRNA (miRNA), proteins, lipids, etc., that are typical of the cells giving rise to the vesicles. Tumor-derived exosomes have been shown to play a role in tumor progression through immune regulation, angiogenesis, metastasis and communication among tumor cells and the microenvironment (68).

One area of colorectal cancer that has garnered substantial interest is the use of exosome based biomarkers due to their enzymatic stability and protection of nucleic acids. The involvement of exosomal miRNAs in tumor proliferation, invasion and resistance to therapy has been examined and led to the identification of potential diagnosis and prognosis markers like miR-21, miR-92a and miR-17-5p (69).

One of the big benefits of the exosome analysis is that it offers information about the active biological communication between cells in the tumor and other cells. Moreover, the lipid membrane composition of exosomes ensures the stability of their molecular content, which may help stabilize the biomarkers. The clinical application has been limited so far, however, because the isolation of pure tumor-derived exosomes is difficult, and there are no standardized methods for their isolation, as well as variations in the analytical procedures applied (70).

In comparison to exosome-based biomarkers, the advantages of ctDNA analysis include well-developed detection approaches and their clinical interpretation, especially in the context of actionable genomic alterations.

5.3 Circulating RNA Biomarkers (miRNA, lncRNA, mRNA)

Another important type of liquid biopsy markers are circulating RNA molecules (Figure 3). These include microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and messenger RNAs (mRNAs) that

help control the expression of genes and are known to be important in the progression of colorectal cancer (71).

MicroRNAs are small non-coding RNA molecules regulating post transcriptional gene expression and modulating processes like proliferation, apoptosis, invasion, and immune response. A few circulating miRNAs, such as miR-21, miR-29a and miR-92a have shown potential for CRC diagnosis and prognosis (72). LncRNAs also play a role in the regulation of tumor biology, and circulating lncRNAs have been suggested as markers for diagnosis and monitoring of treatment.

The key benefit of circulating RNA biomarkers is that they can reflect functional changes to gene regulation as well as just genetic changes. RNA profiles can offer clues about the activity of a tumor and its biological pathways and how well a treatment is working. But RNAs are less stable than DNAs, as they are more easily degraded by RNases (73). Furthermore, they are not standardized in clinical use due to the different processing methods, the different normalization methods, and the different detection platforms that are used. In addition to being more stable molecular information, ctDNA can also be directly identified tumor-specific mutations and epigenetic changes, compared with circulating RNA biomarkers.

5.4 Advantages and Limitations of ctDNA Compared With Other Biomarkers

The liquid biopsy biomarker is currently one of the most clinically advanced due to its ability to directly reflect tumor-specific genetic alterations and its ability to be analyzed with highly sensitive technologies, such as digital droplet PCR and next-generation sequencing (Figure 3). The benefits of ctDNA include the ability to be collected in a minimally invasive manner, longitudinally monitored in real time, molecular resistance mechanisms can be detected, and the molecular genetic material can be analyzed repeatedly over time (74).

But there are also drawbacks to ctDNA. In some cases, the amount of ctDNA may be very low, especially in early stage CRC, and special highly sensitive detection methods are required. Additionally, the heterogeneity of the tumor may contribute to the differences in ctDNA representation, as the molecular signature released from different regions of the tumor could be different. Additionally, there is no universal standardization, there is analytic variability, and the cost of sequencing is still high, which is why it is not adopted routinely in the clinical environment (13).

Overall, none of the liquid biopsies biomarkers is able to give a comprehensive picture of tumor biology. The major difference with ctDNA is the superior genomic information that it gives, with CTCs giving you cellular characteristics and exosomes giving you intercellular communication and circulating RNAs giving you functional gene regulation. Integrated multi-omics-based platforms could generate more comprehensive and accurate diagnostic, monitoring and personalized treatment approaches for CRC in the future.

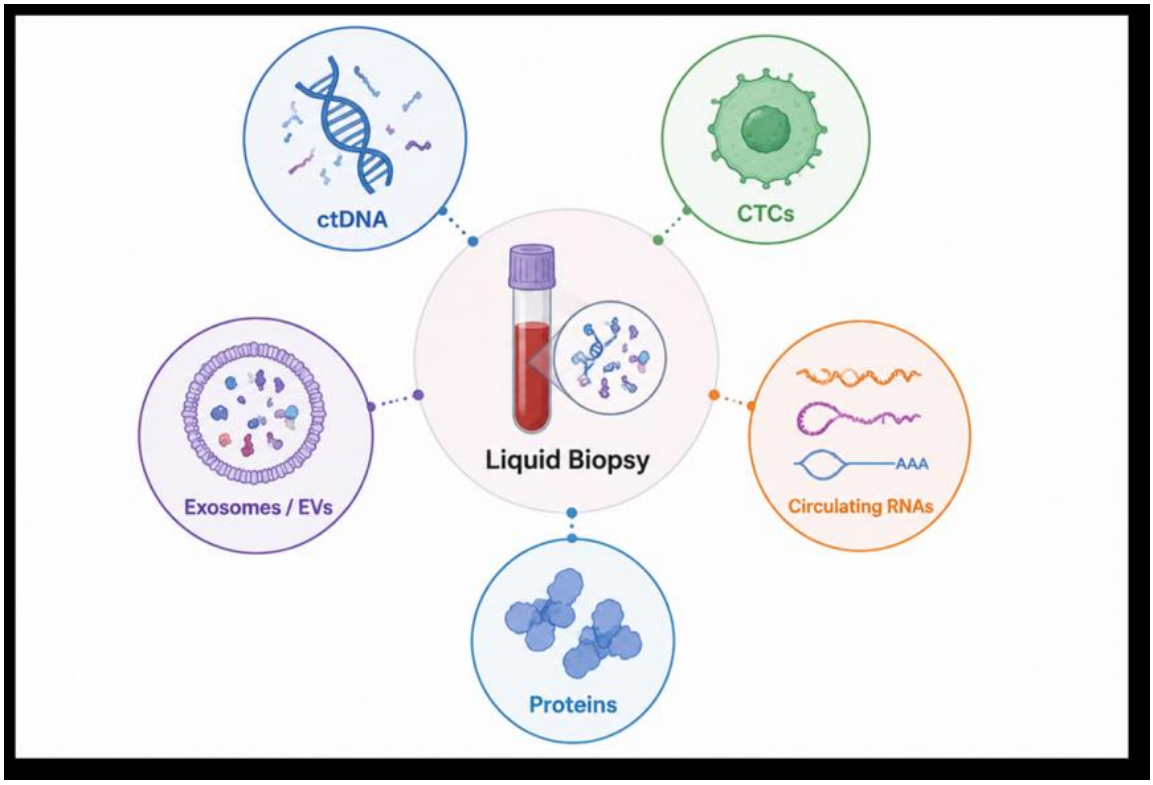


Figure 3 Overview of liquid biopsy biomarkers in colorectal cancer, including ctDNA, circulating tumor cells, exosomes, circulating RNAs, and proteins.

6. Challenges and Limitations of ctDNA-Based Liquid Biopsy

ctDNA is becoming a promising new biotechnology platform for the diagnosis, monitoring, and precision oncology of CRC, but a number of challenges remain in the quest to bring this new tool into routine clinical practice (Figure 4). The utility of ctDNA to provide real-time molecular information relies on many factors, such as the quantity of tumor DNA released in the blood, tumor biology, analytical sensitivity of detection platforms, and standardization of clinical workflows. These limitations need to be overcome for the success of ctDNA methods in terms of reliability, reproducibility, and clinical use (75).

6.1 Biological Challenges

Low ctDNA Concentration

The low detection levels of ctDNA in the blood are one of the biggest challenges in the study of ctDNA, especially for patients with early-stage colorectal cancer (76). The fraction of ctDNA within the total circulating cell-free DNA (cfDNA) is dependent on various tumor characteristics, including tumor size, vascularization, metastatic burden and the biology of individual tumors (11). For localized CRC, the concentration of the ctDNA in the bloodstream might not be high enough to detect with conventional molecular methods.

Because of the low concentration of ctDNA, detection issues arise when trying to differentiate the signals from the tumor DNA from background DNA from normal cells. The challenge is especially pertinent for early detection and screening applications, where the tumor burden is small and the mutation frequency may be too low for current detection and screening technologies to detect. Advanced techniques, such as digital droplet PCR (ddPCR), unique molecular identifier (UMI)-based sequencing, and ultra-deep next generation sequencing (NGS) have helped to increase analytical sensitivity, but detecting very low frequency mutations is still difficult (9).

Further, biological processes like inflammation, tissue damage, and normal cellular attrition may also affect the levels of circulating DNA, and impact the specificity of the ctDNA-based assays. As a result,

there is a continued need for the development of better ways to enrich the DNA obtained from tumors, as well as for the development of more sensitive detection platforms.

Tumor Heterogeneity

Another major drawback to ctDNA-based liquid biopsy is tumor heterogeneity. Colorectal cancer is a genetically heterogeneous disease with various molecular changes found in different regions of the tumor and in metastatic sites (77). Within one tumor, there are several subclones that contain different genetic variants, sensitivity to therapy and metastatic potential. While the ctDNA will give a more comprehensive picture of the genetic material from a tumor than a single tissue sample biopsy, it will not always reflect the full molecular complexity of a tumor. DNA may be shed into the circulation by some subclones of tumors, therefore underrepresentation of certain mutations. Also, over time there can be changes in the composition of the tumor cells due to the selective pressure of chemotherapy and/or targeted therapy, which may lead to the emergence of new resistant clones, and necessitates ongoing monitoring (7).

The study of tumor evolution from longitudinal changes in ctDNA is therefore crucial but difficult because tumor genetics evolve dynamically, and there are processes of clonal selection.

Early-Stage Disease Detection Limitations

One of the most enticing uses of ctDNA is also one of the most challenging, namely early detection. However, the release of ctDNA is typically greater in cases of advanced cancers due to increased tumor mass, necrosis and vascular invasion (78). By contrast, the amount of circulating DNA in early stage CRC tumors is generally low and makes the assay less sensitive. Increasing the detection potential in the early stages of disease has been possible by methylation-based strategies, fragmentomic analysis and multi-omics strategies but the ability to obtain high sensitivity and specificity for population screening has proven challenging. In addition to the possibility of false-negative results (when the level of ctDNA is too low to be detected), false-positive results can also result from non-tumor-associated genetic alterations or clonal hematopoiesis that happens with aging (79). Further studies are needed to identify whether ctDNA screening can be used to supplement or replace current CRC screening programs in the future.

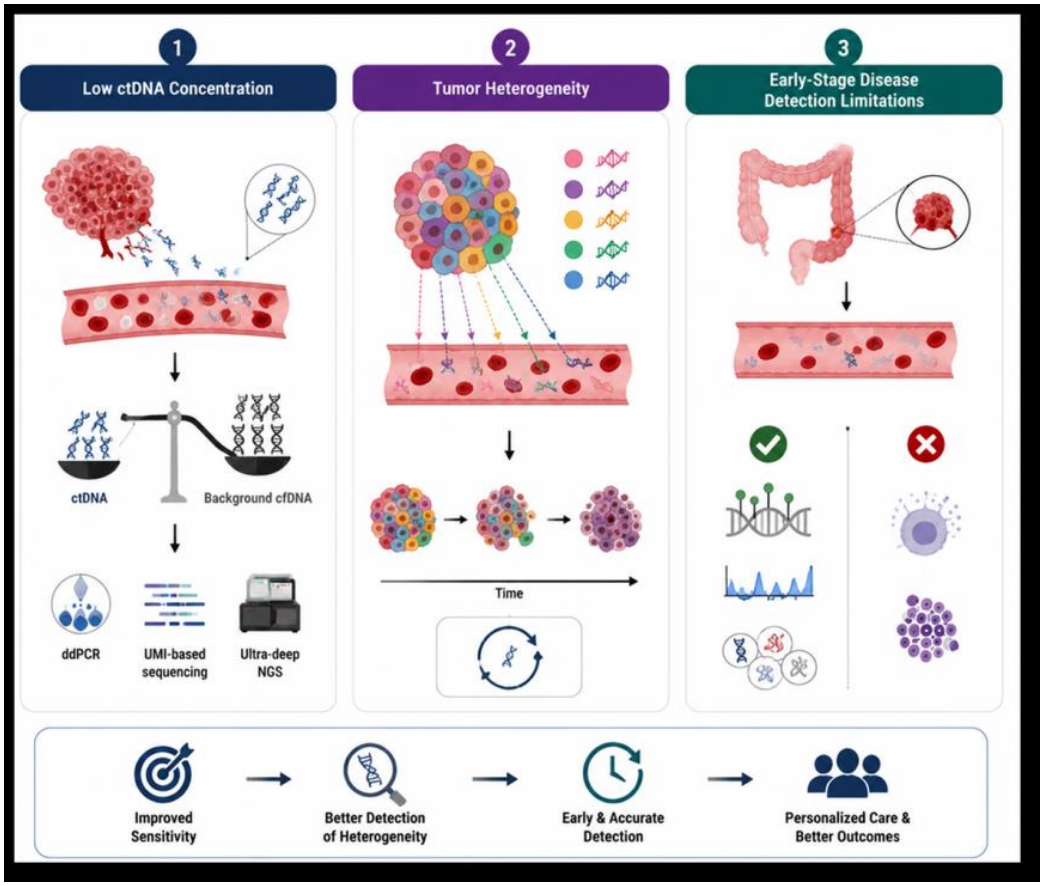


Figure 4 Biological challenges limiting ctDNA-based liquid biopsy in colorectal cancer, including low ctDNA concentration, tumor heterogeneity, and limitations in early-stage disease detection.

6.2 Technical Challenges

Sample Preparation Variability

One of the biggest technical challenges for the implementation of ctDNA is standardization of sample collection and processing. Pre-analytical conditions, such as blood collection tubes, the storage conditions, the processing time, plasma separation techniques, etc., and the DNA extraction techniques may have a significant impact on the quality and quantity of the ctDNA (80). For instance, plasma that is processed late can be more contaminated with the genomic DNA from lysed blood cells and less amount of plasma can be detected as tumor-derived DNA. Laboratory methods may result, therefore, in varying results from study to study and clinical center to clinical center. For increasing assay reproducibility, it is necessary to have internationally agreed upon guidelines for blood collection, cfDNA extraction, quality control and reporting standards.

Sequencing Errors

Next generation sequencing-based ctDNA analysis demands a very high sensitivity in detecting rare genetic variants, typically occurring at less than 1% of all the DNA molecules (81). Technical errors, however, are unavoidable with sequencing technologies and could be confused with real tumor mutations. These errors can have a negative effect on assay specificity and are especially impactful for low frequency variants. The recent advances such as error corrected sequencing, molecular barcoding and better bioinformatics algorithms have brought down false positive results considerably (82). These approaches, however, are complex, add to the sequencing effort and its expense. It is always a challenge to balance the analytical correctness with clinical usefulness.

Detection Sensitivity Limitations

ctDNA detection technologies have made significant strides, but there is still a challenge with achieving sufficient sensitivity in various clinical settings. The sensitivity of different platforms varies according to

the type of assay, sequencing depth, targeted regions and thresholds of analysis (83). PCR-based assays like ddPCR are very sensitive for known mutations, but aren't comprehensive genomic. NGS, on the other hand, can provide more comprehensive molecular profiling, and may need to be sequenced more deeply to identify rare variants. The choice of the best technology is determined by the clinical use, early detection, minimal residual disease monitoring, or assessment of treatment resistance.

6.3 Clinical Translation and Regulatory Challenges

Although the research results are promising, some clinical and regulatory hurdles must be addressed before ctDNA-based liquid biopsy can be used in routine clinical practice. A significant problem is the lack of commonly agreed standards for validation, interpretation and clinical decision making of assays (84). The platforms, thresholds and analytical pipelines vary between different laboratories, making it difficult to compare studies. There is also a need for evidence in large-scale prospective trials on the benefits of ctDNA guided interventions in clinical practice. There are multiple studies demonstrating very strong correlations between the level of postoperative ctDNA positivity and the risk of recurrence, but more evidence is necessary to determine if modifying treatment based on ctDNA will have an impact on survival.

The cost of the assay and access to it are also challenges, especially in countries with weak molecular diagnostic facilities within the health system (85). Advanced sequencing platforms can be limited in low resource areas due to requiring specialized equipment, training, and support from bioinformatics specialists. Another point to consider is regulatory approval as ctDNA assays need to prove analytical validity, clinical validity, and clinical utility before they are widely adopted. Regulatory agency and professional organization provided standardized frameworks will be needed to guarantee patient security and dependable interpretation (86).

Finally, although the liquid biopsy is a revolution in the management of CRC, there are still a lot to be done before it can become a clinical standard. To fully realize the potential of ctDNA in precision oncology, further studies are needed to advance assay sensitivity, technology standardization, clinical validation, and regulatory frameworks.

7. Future Perspectives and Emerging Directions

In the near future, the clinical application of liquid biopsy based on ctDNA is anticipated to continue to grow in CRC, as the fields of biotechnology, computational sciences and molecular oncology continue to evolve rapidly (Table 4) (87). While the current applications have proven to be valuable in diagnostics, minimal residual disease (MRD) detection and monitoring of treatment, the future will be characterized by increasing analytical sensitivity, combining various molecular data sources, decreasing costs and personalized real-time cancer management. The future of ctDNA analysis is poised to be a revolution, with emerging technologies such as artificial intelligence (AI), multi-omics integration, the use of advanced sequencing platforms and digital diagnostic systems set to transform it from a specialized research tool into a standard practice in precision oncology.

Table 4 Emerging Directions in ctDNA-Based Liquid Biopsy for Colorectal Cancer

Emerging Approach	Application in ctDNA Analysis	Potential Clinical Impact
Artificial Intelligence (AI) and Machine Learning	Analysis of complex genomic, methylation, and fragmentomic patterns	Improved early detection, recurrence prediction, and treatment decisions
Multi-omics Integration	Combination of genomic, epigenomic, transcriptomic, and proteomic data	Enhanced tumor characterization and personalized precision medicine
Advanced Sequencing Technologies	Ultra-deep sequencing, single-molecule sequencing, and error-corrected methods	Increased sensitivity for rare mutations and MRD detection
Digital Diagnostic Platforms	Automated ctDNA extraction, analysis, and clinical interpretation	Faster, standardized, and accessible molecular testing

Personalized ctDNA Surveillance	Patient-specific monitoring of tumor mutations and methylation signatures	Early recurrence detection and risk-adapted follow-up strategies

7.1 Artificial Intelligence and Machine Learning in ctDNA Analysis

There is a growing trend towards the application of AI and machine learning (ML) in cancer genomics to enhance the understanding of complex molecular data from the analysis of ctDNA (88). Traditional ctDNA methods are primarily used to identify specific mutations or predefined markers, but the genomic, epigenetic and fragmentomic patterns associated with cancer evolution are very complex and are not as easily identified in traditional analytical methods. By using AI based algorithms to analyze the vast amount of sequencing information available to them, they can detect molecular fingerprints that are otherwise hidden with respect to the presence of a cancer, its progression, treatment response and the risk of recurrence.

The machine learning models have shown promise in early cancer detection using multiple features of ctDNA, such as mutation profile, DNA methylation pattern, DNA fragment size distribution, and DNA instability signatures (89). For instance, AI technologies have been developed to analyze genome wide methylation patterns to identify cancer-derived DNA from the normal circulating DNA in the bloodstream, thereby enhancing diagnostic accuracy. In the same way, deep learning models can detect the intricate molecular structures that could be the signs of a tumor, even at very low levels of ctDNA (90).

For colorectal cancer, AI-powered ctDNA analysis could help refine the ability of recurrent cancer detection after surgery by leveraging both longitudinal molecular and clinical, imaging, and pathological signatures. These predictive models would assist in making individual treatment decisions by helping to identify patients who are more likely to suffer from disease recurrence and might require more aggressive monitoring or more treatment (91).

The use of AI-driven ctDNA analysis is not without its challenges, however, as it is vital to have high-quality datasets, standardized experimental protocols, clear algorithms, and independent clinical validation of these techniques. Furthermore, data privacy, algorithm bias, and interpretability will be key challenges before the widespread adoption of AI-assisted ctDNA diagnostics.

7.2 Integration of ctDNA With Multi-Omics Precision Medicine

This future is likely to extend beyond the use of a single biomarker, and will rely on integrated multi-omics approaches, which integrate genomic, epigenomic, transcriptomic and proteomic information (92). Although ctDNA can offer significant insights into the genetic changes unique to a tumor, using multiple molecular layers together could give a more comprehensive picture of the tumor biology and aid in the more accurate diagnosis.

The genome may be analyzed for mutations or the epigenome may be analyzed for DNA methylation signatures for early tumor development, such as KRAS, NRAS, BRAF, APC and TP53. Furthermore, the incorporation of circulating RNA profiles, extracellular vesicle markers and protein markers could also offer complementary information about the activity of the tumor and the immune response (93).

The ability of multi-omics approaches to overcome the limitations of low ctDNA levels and tumor heterogeneity is especially beneficial. These have the potential to combine molecular signals and thus enhance the sensitivity of early detection and minimize false-negative rates. Additionally, incorporating ctDNA with patient specific clinical data may lead to more precise prediction of the response to treatment and disease progression (89).

Multi-omics ctDNA platforms could enable adaptive treatment plans in precision oncology by tracking the changes in tumor molecular make-up over time. Real-time molecular information can be used to fine-tune the therapy based on changing tumor features, rather than relying on the initial biopsy report.

7.3 Advanced Sequencing Technologies and Digital Diagnostics

The sensitivity and accessibility of ctDNA-based assays can further be enhanced through technological advancements related to sequencing and digital diagnostics (83). New DNA sequencing methods are

improving the ability to detect extremely rare DNA fragments found in the blood that are derived from tumors, such as ultra-deep sequencing, single-molecule sequencing, and error-corrected sequencing. More sophisticated methods of sequencing with the use of unique molecular identifiers (UMIs) and improved error correction can distinguish true tumor mutations from sequencing errors. In such applications as early-stage detection of CRC or MRD monitoring, ctDNA levels can be very low, making these developments particularly significant.

Digital PCR technologies are also continually improving which offers highly sensitive and rapid detection of specific mutations. The potential to integrate digital PCR with automation of the sample processing and portable diagnostic platforms could facilitate decentralized testing and enhance access in clinical contexts (94).

Moreover, liquid biopsy based digital diagnostic systems can enable the incorporation of molecular testing into the mainstream of healthcare. Routine, fully automated platforms that prepare samples, sequence, analyze bioinformatics and interpret clinically may decrease complexity in the lab and speed up decision making.

7.4 Future Applications in Personalized Cancer Surveillance

The most promising future use of ctDNA technology is for individualized cancer monitoring. In the past, follow-up after CRC was primarily done using imaging and colonoscopy and markers like carcinoembryonic antigen (CEA) in blood. These techniques, however, do not have the ability to detect molecular recurrence early (95). Monitoring with ctDNA can offer the opportunity for treatment to be initiated earlier, as it may be detectable months in advance of radiological signs of recurrence. Personalized ctDNA assays, which can be created based on individual patient tumor-specific mutations or methylation signatures, may be a part of future surveillance programs. Patients might receive frequent blood tests to check for signs of recurrence, drug resistance or spread of the cancer (96).

Furthermore, ctDNA surveillance could aid in survivorship management by avoiding unnecessary imaging and having the ability to provide risk-adapted follow-up schedules. Those who remain negative for ctDNA would likely need less aggressive monitoring while those who are positive would be eligible for earlier intervention (97).

Sensitive detection of ctDNA together with AI-based prediction models, multi-omics analysis, and advanced sequencing technologies, could eventually form a comprehensive molecular monitoring system for patients with CRC. These strategies can change the way cancer is managed, to move from a 'reactive' approach to one of 'proactive' and 'personalized' disease prevention and control (98).

In conclusion, ongoing advancements in biotechnology and computational sciences will play a crucial role in addressing existing challenges and solidifying the role of liquid biopsy in precision management of CRC. The combination of molecular diagnostics, AI and personalized medicine is a significant milestone in advancing patient outcomes, therapeutic decisions and early diagnosis.

8. Conclusions

Despite the fact that there is a growing incidence, high mortality rate, and limitations in current approaches of diagnosis and monitoring, colorectal cancer remains a major health problem worldwide. While conventional techniques like colonoscopy, tissue biopsy, imaging, and serum biomarkers have made a great contribution to the management of CRC, these methods are invasive, have limited capacity to capture tumor heterogeneity, and do not provide immediate molecular information (99). The use of circulating tumor DNA (ctDNA) for liquid biopsy has become an effective biotechnology platform that could overcome most of these disadvantages, offering a molecularly informative and repeatable, minimally invasive evaluation of tumor characteristics. The molecular basis of the ctDNA technology is based on the ability to detect, in the bloodstream, tumor-specific genetic and epigenetic changes (like mutations in APC, KRAS, TP53, BRAF and DNA mismatch repair genes) (100). The sensitivity and clinical utility of ctDNA detection has been substantially enhanced by the development of new biotechnology platforms such as digital droplet PCR, next-generation sequencing, methylation-based assays, and multi-omics technologies. The use of these technologies has expanded the scope of ctDNA beyond diagnosis of cancer to assessment of prognosis, detection of minimal residual disease

(MRD), monitoring of treatment, identification of resistance mechanisms, and personalized therapeutic decision making.

Postoperative ctDNA detection has shown exceptional potential in the case of colorectal cancer in forecasting the risk of recurrence and tailoring adjuvant treatments. Moreover, serial ctDNA analysis offers live updates on the evolution of the tumor and on the clinical response to treatment, which is not possible with the classical tissue-based methods. Combining ctDNA with precision oncology strategies could allow for more precise strategies to deliver targeted therapies to patients, based on the evolving molecular composition of their cancer.

However, there are a number of challenges that need to be overcome before the use of ctDNA in liquid biopsy becomes a standard of care. These include low levels of ctDNA, intra- and inter-patient tumor heterogeneity, technical variation, non-standardized analytical procedures, high cost and the requirement for clinical validation in prospective patients. With the improvement of the assay sensitivity, the development of international standardization guidelines, and the integration of the ctDNA analysis with artificial intelligence, multi-omics and advanced sequencing platforms, future progress will rely on these advancements. In summary, liquid biopsy using ctDNA is a revolutionary strategy in colorectal cancer treatment and care that connects molecular pathology and biotechnology. As technology continues to advance and clinical trials validate it for use, ctDNA is poised to become a key element of cancer detection, monitoring, and precision medicine, potentially leading to earlier diagnosis, personalized treatment, and better long-term prognosis for CRC patients.

Declarations

Conflicts of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and publication of this article.

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