CIRCULATING TUMOR DNA AS A BIOMARKER TO DETECT MINIMAL RESIDUAL DISEASE FOR COLORECTAL AND BREAST CANCER

by

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Abstract

Minimal residual disease (MRD) is a key contributor to cancer recurrence, often escaping detection by conventional imaging and biomarker techniques. Circulating tumor DNA (ctDNA), a component of cell-free DNA shed by tumor cells into the bloodstream, has emerged as a promising biomarker for the non-invasive detection and monitoring of MRD in solid tumors. This review critically examines current evidence on the application of ctDNA for MRD detection in colorectal cancer (CRC) and breast cancer, two of the most common malignancies worldwide. It compares ctDNA detection platforms, including digital droplet PCR (ddPCR) and next-generation sequencing (NGS), emphasizing differences in sensitivity, specificity, and clinical relevance. The review also discusses the use of tumor-informed versus untargeted ctDNA assays and highlights key biomarkers such as KRAS, BRAF, TP53, and PIK3CA. Although ctDNA holds substantial potential to enhance surveillance strategies, challenges such as assay variability and tumor heterogeneity must be addressed to enable broader clinical implementation.

Keywords

Minimal residual disease (MRD), circulating tumor DNA (ctDNA), colorectal cancer, breast cancer, digital droplet PCR (ddPCR), next-generation sequencing (NGS), precision oncology

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List of Abbreviations

ACT	Adjuvant Chemotherapy
AI	Artificial Intelligence
AJCC	American Joint Committee on Cancer
BC	Breast Cancer
BRAF	B-Raf Proto-Oncogene
CA 15-3	Cancer Antigen 15-3
CEA	Carcinoembryonic Antigen
cfDNA	Cell-Free DNA
cCR	Clinical Complete Response
CRC	Colorectal Cancer
CRLM	Colorectal Liver Metastasis
CTC	Circulating Tumor Cell
ctDNA	Circulating Tumor DNA
DFS	Disease-Free Survival
ddPCR	Droplet Digital Polymerase Chain Reaction
DTC	Disseminated Tumor Cell
EBC	Early Breast Cancer
EDTA	Ethylenediaminetetraacetic Acid
EGFR	Epidermal Growth Factor Receptor
ER	Estrogen Receptor
FAP	Familial Adenomatous Polyposis
FDA	Food and Drug Administration
FISH	Fluorescence In Situ Hybridization
FGFR	Fibroblast Growth Factor Receptor
GATA3	GATA Binding Protein 3
HER2	Human Epidermal Growth Factor Receptor 2
HNPCC	Hereditary Nonpolyposis Colorectal Cancer
HR	Hazard Ratio / Hormone Receptor
IHC	Immunohistochemistry
KEGG	Kyoto Encyclopedia of Genes and Genomes
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog
LB	Liquid Biopsy
MRI	Magnetic Resonance Imaging
MRD	Minimal Residual Disease
MSI	Microsatellite Instability
MPS	Massively Parallel Sequencing
NGS	Next-Generation Sequencing
NRAS	Neuroblastoma RAS Viral Oncogene Homolog
NPV	Negative Predictive Value
OS	Overall Survival
PCR	Polymerase Chain Reaction
PET	Positron Emission Tomography
PICO	Patient, Intervention, Comparison, Outcome

PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
pCR	Pathologic Complete Response
PR	Progesterone Receptor
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PS	Performance Status
pTRG	Pathologic Tumor Regression Grade
RCB	Residual Cancer Burden
SNV	Single Nucleotide Variant
TP53	Tumor Protein p53
VAF	Variant Allele Frequency

Introduction

1.1 Cancer Overview

Cancer arises from a series of genetic and epigenetic alterations that disrupt normal cell growth and regulation. These changes not only promote uncontrolled proliferation but also enable the spread of cancerous cells throughout the body, leading to the formation of malignant tumors (1). Cancer remains the second leading cause of death globally, accounting for approximately one in every six deaths. According to the International Agency for Research on Cancer (IARC), approximately 19.3 million new cases and 10 million cancer-deaths were reported in the year of 2020, figures that are expected to be increased to 23.8 million new cases and 13.0 million deaths by 2030 (1). Globally, cancer is the first or second leading cause of death before age 70 in 112 of 183 countries, and it ranks third or fourth in another 23 countries, highlighting its role as a major contributor to premature mortality (2). These statistics depicts the urgent need for the development of more effective strategies for prevention, early detection, and treatment.

In response to this significant public health issue, researchers developed the hallmark theory of cancer, originally proposed in 2000 and updated in 2011, offering a foundational framework for understanding the molecular mechanisms driving tumorigenesis. The hallmarks outline ten core characteristics that cancer cells must possess: (1) sustaining proliferative signaling, (2) evading growth suppressors, (3) resisting cell death, (4) enabling replicative immortality, (5) inducing angiogenesis, (6) activating invasion and metastasis, (7) genome instability and mutation, (8) tumor-promoting inflammation, (9) deregulating cellular energetics, and (10) avoiding immune destruction. These hallmarks have proven valuable in interpreting large-scale genomic and transcriptomic data, allowing researchers to compare extensive datasets across entire cancer genomes and identify key biomarkers.

1.2 Colorectal and Breast Cancer: A Focused Perspective

Malignancies such as pancreatic cancer, leukemia, and brain tumors are relatively rare compared to more common cancers like breast, colorectal, and lung cancer. However, these cancers are often aggressive, and their typical late-stage detection leads to poorer prognoses. This presents significant challenges in oncology, highlighting that cancer incidence alone is not the only concern — the stage at diagnosis and the availability of effective treatment options are equally critical factors (1). Breast cancer is the most frequently diagnosed malignancy among women, while lung and prostate cancers are most common among men. Certain cancers, such as colorectal, stomach, and liver cancers, are diagnosed at high rates across both sexes. Some malignancies also show gender-specific patterns, with cervical and thyroid cancers primarily affecting women and prostate cancer affecting men. In terms of mortality, lung cancer remains the leading cause of cancer-related deaths globally, followed by colorectal, stomach, liver, and breast cancers. Given their high incidence and significant contribution to global cancer mortality, colorectal and breast cancers were selected as the focus of this thesis (1).

1.2.1 Colorectal Cancer (CRC)

Colorectal cancer (CRC) is considered as a global threat due to its ranking as the third most diagnosed cancer and the second leading cause of cancer-related deaths worldwide. A report on cancer statistics in Vietnam indicated that CRC accounted for approximately 9% of all cancer cases in both women and men, with 16,426 new diagnoses and 8,203 deaths reported in 2020 alone. These statistics highlight the urgent need to develop new and improved detection and treatment strategies to better control both the incidence and mortality rates. Limited access to genetic testing remains a major barrier to advancing CRC care, despite the transformative impact of technologies like next-generation sequencing (NGS) in enabling data-driven decision-making in clinical oncology. In developing countries such as Vietnam, high costs and a shortage of specialized laboratories restrict access to genetic testing, leaving the mutational landscape of CRC in this population largely unexplored (3).

Although the precise molecular triggers of CRC have not been fully defined, extensive research has established a multifactorial risk profile. Non-modifiable risk factors include age (particularly over 50 years) and hereditary conditions such as familial adenomatous polyposis (FAP) and hereditary nonpolyposis colorectal cancer (HNPCC). Modifiable risk factors, including inflammatory bowel diseases like ulcerative colitis and Crohn's disease, along with lifestyle factors such as high-fat and red meat diets, low physical activity, smoking, excessive alcohol consumption, and obesity, are strongly associated with increased CRC risk (4).

Despite its high prevalence, the diagnosis of CRC is often delayed, partly due to inadequate screening and inconsistent staging practices. Data from the Oxford Cancer Registry (1995–2003) illustrate several challenges in tumor characterization and classification. For example, Dukes staging a critical determinant of prognosis was recorded in only 81% of colon cancer and 69% of rectal cancer cases, with staging data less frequently available for patients over 75. While the distribution of tumor stages remained relatively stable over time, these gaps may reflect inconsistencies in pathology reporting or limitations in diagnostic capacity. Similarly, tumor grade was documented in approximately three-quarters of colon cancers and 81% of rectal cancers, with moderately differentiated tumors being the most common. Histological classifications were available in most cases, with adenocarcinomas comprising the dominant subtype, followed by mucinous adenocarcinomas and less common variants (5).

Together, these findings underscore the importance of enhancing diagnostic accuracy, staging consistency, and genomic profiling in CRC care particularly in under-resourced settings. The central question, then, is how to bridge the gap between the technological advancements of high-income countries and the practical realities faced by healthcare systems in developing nations. To address this concern will require not only investment in infrastructure and workforce training but also international collaboration aimed at building equitable, data-driven cancer care systems. In doing so, the potential to improve early detection, personalize treatment, and ultimately reduce CRC mortality across diverse populations becomes a tangible goal (5).

1.2.2 Breast Cancer

Breast cancer remains the most diagnosed cancer in women worldwide and the second leading cause of cancer-related deaths among women. Looking into the stats, in the United States alone, approximately 246,660 new cases were reported in 2016, reflecting a persistent and widespread health burden (6). Approximately 95% of women are diagnosed at an early stage, often without any macroscopic signs of metastasis. For the early-stage breast cancer (ESBC) patients, surgical excision is the cornerstone of treatment, often resulting in long-term recurrence. However, despite successful initial treatment, a major clinical challenge remains, as approximately 25% of ESBC patients eventually develop metastatic recurrence, which is typically incurable (6,7). One of the most elusive aspects of breast cancer management is the presence of micro-metastatic disease that is known as minimal residual disease (MRD) that remains undetected after surgery and systemic therapy. Although not evident at diagnosis, these microscopic cancer cells can lie dormant for years, later giving rise to overt metastatic relapse. This issue is particularly critical for patients with hormone receptor-positive (HR+)/HER2-negative breast cancer, the most prevalent molecular subtype. Despite significant advancements in therapeutic strategies over the past 30 years, recurrence continues to pose a major barrier to curative outcomes.

Recent research has focused on identifying molecular markers that can predict MRD status and inform personalized treatment plans. Notably, alterations in the fibroblast growth factor receptor (FGFR) family particularly FGFR1 have emerged as potential prognostic indicators in HR+/HER2− breast cancer. FGFR1 amplification appears to be enriched in patients who are MRD-negative post-treatment, suggesting a paradoxical role: while FGFR aberrations are generally associated with poor outcomes and increased risk of brain metastases, they may also signify a lower likelihood of residual disease in certain molecular contexts (8). Interestingly, FGFR1 expression has been linked to distant metastasis in luminal B tumors, but not in luminal A (HR+/HER2−) subtypes, underscoring the nuanced interplay between tumor biology and metastatic potential. Although these findings are promising, they are preliminary. The current evidence base lacks long-term survival data, limiting the ability to draw definitive conclusions about the clinical implications of FGFR1 alterations. Future studies will need to include survival analyses and broader molecular profiling to determine whether FGFR1 and related markers can be reliably used to stratify patients by recurrence risk and guide adjuvant therapy decisions.

1.3 Treatment Options and Therapeutic Resistance

The treatment landscape for recurrent or metastatic colorectal cancer (mCRC) has undergone significant evolution, reflecting broader shifts in oncology from generalized treatment approaches to precision-guided interventions. Despite the advanced stage of disease in mCRC, approximately

20% of patients are candidates for curative-intent procedures such as definitive surgery and/or radiotherapy, aimed at achieving complete radiographic remission (9). Among these patients, long-term survival is possible, with roughly 30% remaining alive a decade after intervention. These outcomes offer a glimmer of hope in a setting often associated with poor prognosis, but they also raise important questions about how best to select therapies to optimize benefit. A key area of ongoing debate centers around the management of oligo-metastatic CRC, particularly the role and timing of systemic therapy in relation to surgical resection. Clinicians face difficult decisions, like should treatment rely solely on surgery, or can chemotherapy be added preoperatively, postoperatively, or both? The absence of robust, predictive biomarkers makes these decisions highly individualized and often empirically driven (10). Perioperative chemotherapy is one approach used to address potential micro-metastatic disease beyond what is surgically resected. Although a meta-analysis involving 1,896 patients undergoing liver resection for CRC metastases demonstrated an improvement in disease-free survival (DFS) with perioperative chemotherapy, this did not translate into a significant overall survival (OS) benefit (9). This discrepancy underscores the complexity of balancing short-term disease control with long-term outcomes in metastatic settings.

These challenges in mCRC management reflect a broader transformation in cancer treatment over recent decades. The beginning with basic approaches like surgery and radiotherapy has expanded to include chemotherapy breakthroughs, followed by the emergence of targeted therapies and immunotherapy. The late 20th century saw the advent of monoclonal antibodies such as Rituximab and Trastuzumab, followed by tyrosine kinase inhibitors like Imatinib that revolutionized treatment paradigms for hematologic and solid malignancies (11). The 2000s brought further innovation with targeted agents for non-small cell lung cancer (NSCLC) and renal cell carcinoma, as well as angiogenesis inhibitors like Bevacizumab. Perhaps the most transformative leap has been the rise of immunotherapy. Checkpoint inhibitors such as Pembrolizumab and Nivolumab, CAR-T cell therapy for hematologic malignancies, and oncolytic viruses like T-VEC for melanoma have redefined what is possible in cancer treatment. Most recently, the development of KRAS inhibitors like Sotorasib and combinational strategies for hepatocellular carcinoma highlight the accelerating shift toward precision medicine, where treatments are tailored to the molecular and immunologic profiles of individual tumors (11).

1.4 Circulating Tumor DNA (ctDNA)

ctDNA analysis has emerged as a transformative tool in oncology, offering a noninvasive, dynamic approach to monitor treatment response, and guiding personalized therapeutic strategies. By identifying tumor-specific mutations through high-sensitivity sequencing techniques, ctDNA profiling enables real-time surveillance of disease progression and recurrence, especially in patients with solid tumors. This approach not only enhances diagnostic precision but also facilitates timely clinical decision-making, with the potential to significantly improve patient outcomes (12). In early-stage breast cancer (ESBC), particularly in hormone receptor-positive (HR+) and HER2-negative subtypes, ctDNA-guided MRD detection marks a significant advancement in personalizing treatment strategies. Despite curative-intent surgery and standard adjuvant endocrine therapy, a substantial subset of patients remains at risk of recurrence of cancer, often due to undetected micro-metastatic disease. Genomic technologies now allow for patient-informed ctDNA assays that can identify those with persistent MRD who may benefit from intensified therapy. One such development includes the U.S. Food and Drug Administration’s approval of adding abemaciclib for two years to standard endocrine therapy in high-risk ER+, HER2− ESBC patients. Emerging evidence suggests that preselecting these patients based on MRD status could improve therapeutic efficacy while minimizing overtreatment (13,6,14).

Now on the other hand, mentioning about colorectal liver metastases (CRLM), where no validated biomarkers currently guide post-surgical therapy or imaging-based surveillance, ctDNA offers a promising alternative. Recent longitudinal studies have demonstrated that serial ctDNA monitoring after curative resection can serve multiple purposes like distinguishing true progression from indeterminate CT (chemotherapy) findings, prompting early initiation of chemotherapy even when imaging shows no recurrence, and refining the use of follow-up imaging (15). These findings mark an important shift in post-operative management by introducing a molecular layer of surveillance that may decrease the radiological or symptomatic relapse. The prognostic value of MRD detected via ctDNA has been consistently observed across cancer types. For example, in stage II colon cancer, a prospective clinical trial demonstrated that ctDNA-guided adjuvant chemotherapy reduced the use of unnecessary treatments without compromising survival which is actually an outcome with major implications for patient's quality of life and healthcare costs (16).

Ongoing clinical trials are expanding this framework, evaluating how ctDNA can be used to stratify patients by recurrence risk and tailor adjuvant therapy intensity. This evolving field also opens the door to a more refined understanding of disease dynamics and therapeutic resistance. On the other hand, in breast cancer, for instance, the presence of specific genetic mutations in MRD identified through ctDNA could inform the selection of targeted agents for either consolidative or preventive treatment. Such personalization may not only delay progression but alter the natural course of the disease. Serial sampling, which captures molecular progression ahead of clinical relapse, could allow oncologists to intervene cautiously and prevent the metastatic distribution (6).

Briefing about triple-negative breast cancer (TNBC), a particularly aggressive subtype characterized by large tumors and high proliferation rates, neo-adjuvant chemotherapy (NCT) is standard practice to reduce tumor burden and eliminate disseminated tumor cells. However, current radiologic and biologic assessments fall short in reliably detecting MRD at distant sites. Here, ctDNA profiling shows unique promise. Its ability to quantify tumor burden and detect MRD at the molecular level addresses a critical gap in post-treatment surveillance and may significantly influence long-term survival outcomes. As ctDNA technologies continue to advance in sensitivity and affordability, their integration into standard oncology practice could redefine how treatment efficacy, disease recurrence, and patient risk are assessed. The central challenge now lies in validating these tools across diverse cancer types and clinical contexts and ensuring their accessibility and cost-effectiveness for broader populations. If successful, ctDNA-based MRD detection stands poised to become a cornerstone of personalized oncology.

1.5 Minimal Residual Disease (MRD) in Cancer

MRD has emerged as a pivotal concept in modern oncology, referring to the presence of residual cancer cells that persist after initial treatment but remain undetectable using conventional imaging or laboratory methods. These residual lesions are believed to represent drug-resistant or dormant tumor cells that survive therapeutic pressure whether from chemotherapy, radiotherapy, or targeted therapies and may later give rise to recurrence or distant metastasis (17). MRD is increasingly recognized as a major driver of post-treatment relapse, particularly in cancers such as CRC and BC, where recurrence can occur despite apparent radiological remission. In colorectal cancer, the presence of MRD following curative-intent surgery is strongly associated with poor prognosis. Recent studies have demonstrated that ctDNA can serve as a sensitive and specific biomarker for detecting MRD in the postoperative setting. Patients with detectable ctDNA after surgery have a significantly higher risk of recurrence compared to those with undetectable levels. Furthermore, serial monitoring of ctDNA allows for earlier detection of relapse than standard imaging, providing clinicians with a window of opportunity to intervene before overt clinical progression (18). These findings emphasize the prognostic utility of ctDNA and suggest that integrating ctDNA surveillance into clinical workflows could refine adjuvant treatment decisions and improve patient outcomes.

This approach addresses a key limitation in current clinical trials and postoperative care. Traditional adjuvant trials often enroll a broad population of patients, many of whom may already be cured, thereby diluting the observed effect of therapy. By identifying the subset of MRD-positive patients those truly at risk of recurrence ctDNA-guided approaches offer a more targeted and efficient strategy. This could enhance trial design, reduce unnecessary treatment, and help direct resources toward those who stand to benefit the most (19). Advances in methylation profiling of tumor-derived DNA offer additional promise for improving MRD detection. One study utilizing cfMeDIP-seq a technique for identifying differentially methylated regions in cell-free DNA revealed distinct methylation patterns in breast cancer patients with BRCA1 or BRCA2 mutations compared to wild-type controls. These patterns may serve not only in early cancer detection but also in the monitoring of MRD and therapy response. Such minimally invasive assays hold significant potential for improving risk stratification and tailoring treatment strategies based on individual tumor biology (20). The clinical relevance of MRD detection is particularly clear in HER2-positive early breast cancer. A recent study underscored the value of ctDNA analysis in this subgroup, showing that MRD status after completion of all treatment could help differentiate patients at low risk who may avoid overtreatment from those requiring intensified surveillance or therapy (1.51). Repeated ctDNA testing during long-term follow-up further enhances the ability to predict recurrence and allows for therapeutic intervention before clinical signs of relapse become evident. This paradigm shift moves oncology toward a proactive rather than reactive model of care.

However, detecting ctDNA in the postoperative setting remains technically challenging. MRD may be present at extremely low allelic fractions, requiring highly sensitive assays to distinguish true signals from background noise. Tumor-informed assays customized to each patient's specific tumor mutations have been developed to increase detection sensitivity and reduce false positives caused by phenomena such as clonal hematopoiesis of indeterminate potential (CHIP) (22). These assays represent a crucial refinement in the effort to establish ctDNA as a standard tool in MRD assessment. Beyond ctDNA, other liquid biopsy components, such as circulating tumor cells (CTCs), are also under investigation for MRD detection, particularly in breast cancer. However, their rarity often occurring at a frequency of one per million mononuclear cells makes them difficult to isolate and quantify. Current enrichment techniques rely on either the physical properties (e.g., size, density) or immunological features (e.g., cell surface antigens) of these cells, but reliable integration into clinical practice remains limited (23).

Ultimately, the persistence of MRD after curative-intent therapy represents a critical determinant of long-term survival. Conventional radiological and biological tools lack the sensitivity to detect these residual threats, which is why the development of molecularly precise, minimally invasive diagnostics like ctDNA is so transformative. These technologies not only enable the detection of residual disease but also offer insight into tumor biology, treatment response, and recurrence risk which forms the cornerstone of personalized cancer management. As sensitivity improves and validation progresses across cancer types, ctDNA-based MRD monitoring is poised to reshape the future of post-treatment oncology care (24).

1.6 Current MRD Monitoring Approaches in CRC and Breast Cancer

MRD detection has become a critical focus in the postoperative management of CRC, offering a more precise and personalized approach to assessing recurrence risk and guiding adjuvant therapy decisions. Traditionally, MRD has been evaluated using conventional imaging methods such as magnetic resonance imaging (MRI), computed tomography (CT), or positron emission tomography CT (PET/CT), alongside serum tumor markers like carcinoembryonic antigen (CEA) and carbohydrate antigen 19-9 (CA 19-9). However, these tools suffer from limited sensitivity and specificity, often failing to detect microscopic disease. For example, serial CEA measurements detect recurrence with only 69% sensitivity and 64% specificity, making them unreliable for high-stakes decisions like the administration of adjuvant chemotherapy (25, 26, 27).

In contrast, ctDNA has emerged as a highly sensitive and specific biomarker for detecting MRD in CRC. Unlike traditional markers, ctDNA reflects the direct presence of tumor-derived genetic material in the bloodstream, making it a real-time and molecularly accurate indicator of residual disease. This distinction is significant: while conventional risk stratification relies on clinicopathologic features such as tumor size or grade, ctDNA offers a binary, actionable measure of MRD yes or no ushering in a new paradigm of precision oncology. Recent studies have demonstrated the transformative potential of ctDNA-guided therapy in stage II and III CRC. For patients with undetectable ctDNA after surgery, adjuvant chemotherapy (ACT) may be safely omitted without compromising survival outcomes. One analysis described that withholding chemotherapy in ctDNA-negative patients resulted in an 83% cure rate equivalent to standard treatment while sparing 13% of patients from unnecessary toxicity and healthcare costs (25).

Conversely, ctDNA-positive patients exhibit markedly poorer outcomes, with significantly lower recurrence-free survival (RFS) rates, making them ideal candidates for intensified or altered therapeutic regimens (28, 29, 30).

This molecular approach to decision-making offers a multitude of clinical advantages. For ctDNA-positive patients post-surgery, ACT can be promptly initiated to target MRD, regardless of other clinico-pathological risk factors. Treatment efficacy can then be monitored via serial ctDNA testing, allowing for dynamic adjustments in therapeutic strategy. In cases where ctDNA remains detectable despite standard ACT, escalation of treatment or a shift to alternative regimens guided by mutational analysis of the ctDNA may be warranted. Additionally, because ctDNA often shows molecular progression ahead of radiologic evidence, its presence could inform the intensified use of imaging modalities, such as PET-CT, to enable earlier detection of lesions and increase the window for secondary curative interventions (31, 32). Importantly, the implications of ctDNA-guided therapy extend beyond clinical benefit to include psychological and economic advantages. By identifying patients unlikely to benefit from further treatment, clinicians can avoid exposing them to the burdens of chemotherapy side effects, repeated hospital visits, and emotional stress thereby improving quality of life and reducing unnecessary strain on healthcare systems such as the NHS (33).

Despite the promise of ctDNA, challenges remain detecting ctDNA in the postoperative setting is difficult due to the low allelic fraction of circulating tumor material. Highly sensitive, tumor-informed assays have been developed to distinguish true MRD from background noise, including artifacts caused by clonal hematopoiesis of indeterminate potential (CHIP), further enhancing test reliability (22). Histopathological evaluation of resected tumors continues to serve as the gold standard for assessing treatment response and surgical margin status. Complete tumor resection with negative margins remains a key prognostic factor, while positive margins correlate with increased recurrence risk (34). Similarly, in breast cancer, pathological complete response (pCR) after neoadjuvant chemotherapy (NAC) is a critical endpoint. Although imaging modalities such as MRI or minimally invasive biopsies have been explored to predict pCR, current evidence suggests that these methods lack sufficient accuracy to replace surgical pathology. A recent meta-analysis concluded that image-guided biopsy techniques are not reliable enough to rule out residual disease, reaffirming the necessity of surgery in post-NAC management (35).

Nevertheless, ctDNA is reshaping how oncologists approach treatment across multiple cancer types. In breast cancer, ctDNA is increasingly being integrated into MRD surveillance, with studies exploring its use in risk stratification, therapy intensification, and the early detection of recurrence. This is particularly relevant for aggressive subtypes like triple-negative breast cancer, where conventional imaging and biomarkers often fail to detect disseminated disease. In summary, ctDNA-based MRD assessment represents a paradigm shift in oncology offering a powerful, noninvasive tool for tailoring postoperative care, reducing overtreatment, and improving long-term outcomes. As technologies become more refined and accessible, ctDNA has the potential to become a routine component of cancer surveillance and therapeutic decision-making, bringing precision medicine closer to everyday clinical practice (36).

1.7 Circulating Tumor DNA (ctDNA): A Promising Biomarker

The detection and monitoring of MRD have become essential components in the evolving landscape of cancer management, particularly in early-stage solid tumors such as CRC and BC. MRD refers to the presence of residual cancer cells after curative-intent therapy, often undetectable by conventional imaging or serum biomarkers. Recent advances in ctDNA analysis have positioned this biomarker at the forefront of precision oncology, offering a noninvasive, dynamic, and highly specific means to detect and track MRD. In localized CRC, ctDNA has emerged as a particularly reliable indicator of MRD following surgery. Numerous studies have demonstrated its high specificity and prognostic power: the presence of ctDNA post-operatively increases the risk of relapse by as much as 40-fold and precedes radiographic detection by a median of four months sometimes up to eleven months (8, 37). This lead time provides a valuable opportunity for early intervention, especially in patients who may otherwise appear disease-free by conventional methods. These findings have influenced expert guidelines, such as those from the National Cancer Institute's Colon and Rectal-Anal Task Force, which now endorse next-generation sequencing (NGS) based ctDNA assays for MRD detection after surgery or adjuvant therapy (8).

Importantly, ctDNA has proven superior to traditional blood-based markers like carcinoembryonic antigen (CEA), which has long been used to monitor systemic therapy response in metastatic CRC (mCRC). However, CEA suffers from limited sensitivity and specificity, with up to 34% of CRC patients being CEA non-producers rendering it unreliable in a significant proportion of cases (38). Unlike CEA, ctDNA offers a binary and highly tumor-specific signal, and its dynamic changes have been shown to correlate closely with treatment efficacy. In mCRC, ctDNA levels after just two cycles of chemotherapy better predicted radiologic response than CEA levels, highlighting its value as an early treatment response marker. The predictive power of ctDNA extends to assessing the efficacy of adjuvant chemotherapy (ACT). In patients with detectable ctDNA after surgery, the subsequent clearance of ctDNA following ACT has been associated with improved prognosis, suggesting effective eradication of MRD (37). Conversely, patients who remain ctDNA-positive may benefit from escalation or alteration of therapy. This personalized, ctDNA-guided approach is especially valuable in CRC, where overtreatment with ACT particularly with agents like oxaliplatin can result in long-term toxicities such as peripheral neuropathy. A ctDNA-based risk stratification model offers the potential to safely spare low-risk patients from unnecessary treatment while intensifying therapy in those with molecular evidence of residual disease (39).

Despite its promise, several challenges limit the widespread adoption of ctDNA in clinical practice. These include uncertainties around the optimal timing for ctDNA sampling, variations in assay sensitivity and specificity, and the lack of standardized methodologies. Tumor-informed assays personalized to a patient's unique tumor mutations are currently the most sensitive approaches but are complex and time-intensive. Broadly applicable, high-throughput platforms and real-world evidence are needed to refine ctDNA's role in both surveillance and therapeutic decision-making. Ongoing clinical trials will be critical in addressing these gaps and validating ctDNA's utility in improving long-term survival outcomes (29). Similar challenges and opportunities exist in breast cancer, the most common malignancy among women globally. Approximately 30% of patients diagnosed with early-stage breast cancer will eventually develop metastatic disease, underscoring the importance of early MRD detection (40). While circulating tumor cells (CTCs) have been approved for prognostic use, their clinical utility is hampered by low sensitivity and technical complexity. ctDNA offers a more practical and scalable alternative. It can be sampled repeatedly over time, enabling dynamic assessment of tumor burden, treatment response, and recurrence risk. In estrogen receptor positive (ER+) BC, where recurrence can continue steadily from five to twenty years after initial therapy, real-time ctDNA monitoring provides a powerful tool to identify residual disease and adjust follow-up care accordingly.

ctDNA represents a promising biomarker for MRD detection in both colorectal and breast cancers, offering high specificity, early prognostic insight, and real-time monitoring capabilities. Its utility in tailoring adjuvant therapy and guiding surveillance strategies could transform cancer care, minimizing overtreatment and improving outcomes. However, the successful translation of ctDNA from research to routine practice will depend on continued technological refinement, standardization of methodologies, and robust clinical validation through prospective trials. As evidence accumulates, ctDNA may become an indispensable tool in the precision management of early-stage solid tumors (41).

To comprehensively evaluate the clinical performance of ctDNA assays for minimal residual disease detection, several key analytical and clinical variables must be considered. Table 1 summarizes the critical components that determine assay effectiveness, including sensitivity, specificity, false positive and false negative rates, and predictive values. Additional parameters such as lead time, variant allele frequency (VAF), turnaround time, concordance with tissue biopsy results, and the utility of longitudinal monitoring are also outlined. Regarding VAF, this variable refers to the proportion of DNA fragments carrying a tumor-specific mutation relative to the total number of DNA fragments at that genomic position. In ctDNA analysis, VAF is a crucial metric because it reflects the burden of tumor-derived DNA within the bloodstream. Techniques such as next-generation sequencing (NGS) and digital droplet PCR (ddPCR) are commonly used to quantify VAF with high sensitivity, enabling the detection of minimal residual disease even when ctDNA is present at very low levels. Taken together, these variables collectively influence the ability of ctDNA-based approaches to accurately detect MRD, guide treatment decisions, and predict patient outcomes in both colorectal cancer and breast cancer. Understanding these factors is essential for interpreting ctDNA assay results and for assessing their role in clinical practice.

Table 1. Key analytical and clinical performance metrics used to evaluate ctDNA assays for MRD detection. This table defines commonly reported variables in ctDNA-based MRD studies, including their clinical relevance, typical or acceptable ranges, and contextual notes. Metrics such

as sensitivity, specificity, predictive values, variant allele frequency (VAF), and lead time are critical for assessing assay accuracy and utility.

Variable	Definition	Typical/Acceptable Range	Notes
Sensitivity	Ability to detect MRD when it is truly present	70–95%	Higher in personalized/trackable assays (e.g., Signatera)
Specificity	Ability to rule out MRD when it is truly absent	90–99%	Essential for avoiding false positives
False Positive Rate (FPR)	% of positive ctDNA results in patients without MRD	1–10%	Lower is better; often <5% in validated assays
False Negative Rate (FNR)	% of negative ctDNA results in patients who do have MRD	5–30%	Influenced by assay sensitivity and tumor shedding
Positive Predictive Value (PPV)	Likelihood that a positive result reflects true MRD	80–100%	Higher values in CRC, especially when recurrence risk is high
Negative Predictive Value (NPV)	Likelihood that a negative result reflects absence of MRD	85–100%	Often used to justify withholding adjuvant therapy
Lead Time	Time between ctDNA positivity and radiological/clinical recurrence	3–12 months (mean ~5–6 months)	Demonstrated in both CRC and BREAST CANCER studies
Variant Allele Frequency (VAF)	Proportion of ctDNA fragments with tumor-specific mutations	0.01%–5%	Lower detection limits (~0.01–0.1%) critical for MRD detection
Turnaround Time	Time from sample collection to result	7–21 days	Important for clinical decision-making
Concordance with Tissue	Agreement between ctDNA mutations and tissue biopsy mutations	>90% for targeted assays	Personalized assays typically show higher concordance
Longitudinal Monitoring	Ability to track ctDNA changes over time to reflect treatment response or relapse	Qualitative measure (trends)	ctDNA clearance post-treatment is associated with better outcomes

1.8 Study Objective and Hypothesis

1.8.1 Hypothesis

This study hypothesizes that ctDNA is a more efficient biomarker for cancer detection compared to standard techniques. It is expected to offer higher sensitivity and specificity in identifying MRD in patients. By enabling early detection of recurrence, ctDNA has the potential to guide timely interventions and improve treatment outcomes. Its non-invasive nature makes it a promising alternative to traditional methods like tissue biopsies and imaging. Ultimately, ctDNA could play a crucial role in preventing disease progression through proactive monitoring.

1.8.2. Objective

The objective of this literature review is to critically evaluate existing evidence on the utility of ctDNA as a biomarker for detecting MRD in CRC and breast cancer. This review focuses on assessing reported sensitivity and specificity across studies to determine ctDNA's effectiveness in identifying residual disease following treatment. Additionally, it explores the potential of ctDNA-guided MRD detection to inform personalized therapeutic decisions, particularly regarding the administration of adjuvant therapy. Furthermore, this review aims to highlight current limitations in ctDNA-based MRD detection and identify opportunities for future research and clinical application.

Methodology

A comprehensive literature search was conducted using the PubMed database on November 12, 2024, to identify peer-reviewed research articles relevant to ctDNA and MRD in CRC and breast cancer. The search strategy was developed using the PICO framework, and study selection adhered to PRISMA guidelines. Search terms included: circulating tumor DNA (ctDNA), minimal residual disease (MRD), breast cancer, and colorectal cancer (CRC). The specific search string used was: "((ctDNA[Title/Abstract]) OR (circulating tumor DNA[Title/Abstract])) AND ((MRD[Title/Abstract]) OR (minimal residual disease[Title/Abstract])) AND ((breast cancer[Title/Abstract]) OR (colorectal cancer[Title/Abstract]) OR (CRC[Title/Abstract])) NOT (Review[Publication Type])."

A total of 89 articles were retrieved and screened for eligibility. Studies were included if they: (1) focused on CRC and/or breast cancer, (2) investigated ctDNA in the context of MRD, and (3) were original research articles. Studies were excluded if they addressed other cancer types or lacked data on ctDNA or MRD. Eligible studies were extracted and organized into structured data tables, categorized by cancer type to facilitate comparative analysis of CRC (Table 2, Appendix) and breast cancer (Table 3, Appendix).

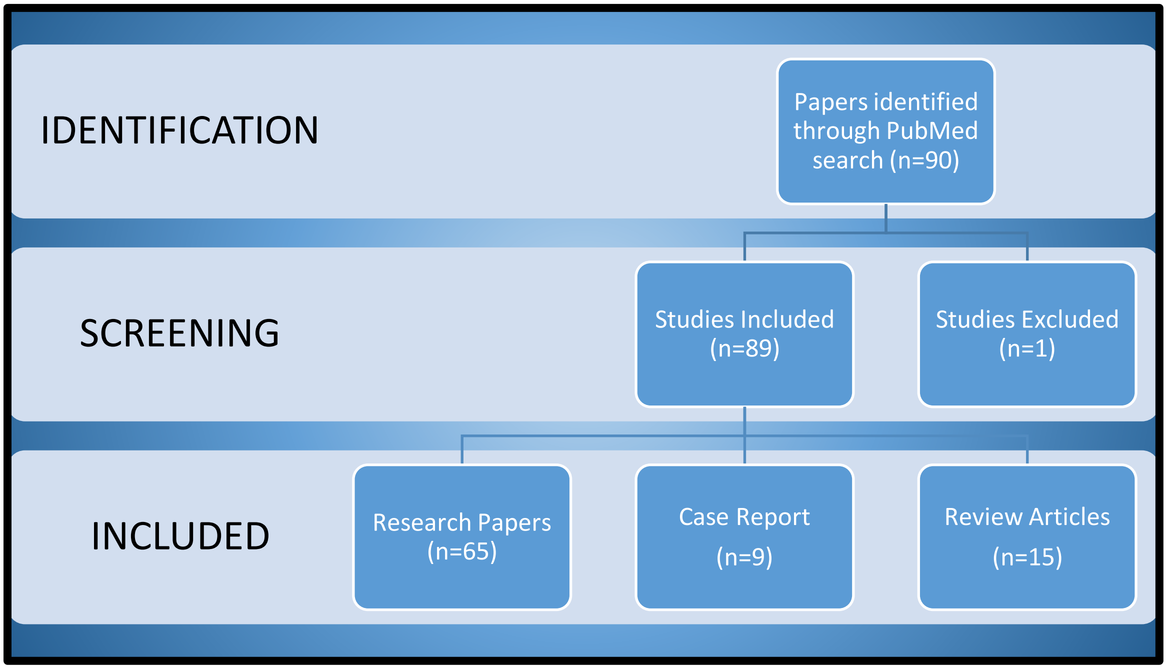

Figure 1. PRISMA diagram outlining the literature search and study selection process.

Results

3.1 Colorectal Cancer (CRC) Studies

3.1.1 Study Characteristics

Table 2 (Appendix) summarizes the key characteristics of the CRC studies included in this review, focusing on patient demographics, geographic distribution, study types, and ctDNA detection methodologies. The studies featured a wide range of patient sample sizes, with 26 studies enrolling between 4 and 215 patients, and 7 studies analyzing larger cohorts ranging from 342 to 3,002 patients. Patient age demographics varied, with 16 studies focusing on patients aged 25 to 85 years, 4 studies including older populations above 85 years, and 8 studies incorporating younger adults aged 18 to 20 years. The studies were conducted across diverse geographic regions, including Asia (Japan, China, Pakistan, Taiwan, Vietnam), Europe (Denmark, Italy, Netherlands, United Kingdom), Australia, and North America (Canada, United States), reflecting a broad international effort in CRC research. Most studies were original research articles (32 papers), supplemented by 7 case reports, 1 roundtable discussion, 1 clinical trial protocol, and 2 reviews. ctDNA detection methodologies varied, with 13 studies employing personalized multiplex NGS assays, 12 studies utilizing digital droplet PCR (ddPCR) or conventional PCR, and 4 studies applying panel-based assay approaches. Additional detection methods reported included liquid biopsy platforms, blood- and stool-based testing, immunohistochemistry (IHC), and commercial assays such as the Guardant Reveal test.

3.1.2 Biomarkers and Detection Methods

The detection methods and biomarkers evaluated across the included studies varied considerably. Fourteen studies focused on the KRAS gene, thirteen studies targeted TP53, and twelve studies analyzed the APC gene. BRAF was examined in ten studies, PIK3CA in nine studies, and NRAS in seven studies. Notably, sixteen studies did not focus on specific gene mutations when assessing ctDNA.

Panel-based tests were used in four studies; although these assays are advantageous because they can be performed on plasma without requiring tumor tissue, they are associated with a higher rate of false positives and higher costs. In contrast, personalized ctDNA assays were employed in nineteen studies, offering higher sensitivity and specificity but limited to known tumor-specific mutations.

Regarding sample types, plasma was the predominant biological material used, reported in thirty studies. Plasma is preferred due to the ease of blood collection compared to tissue sampling and its greater stability and reliability relative to serum. Timing of sample collection varied: eighteen studies collected plasma samples post-surgery to detect minimal residual disease, while five studies collected samples pre-surgery to guide treatment planning based on ctDNA findings.

3.1.3 Diagnostic Performance

Numerous studies have reported varied sensitivity and specificity for different diagnostic and monitoring assays in CRC. For instance, (31) highlighted that mononucleotide markers and ctDNA-based testing are highly sensitive and specific, whereas (39) found 92.3% sensitivity and 87% specificity using the Cologuard test (a non-invasive, stool based DNA screening approach to detect CRC or precancerous lesions). (42) reported high sensitivity with ddPCR and used mass spectroscopy for specificity. (43) noted 67–100% sensitivity and 84–87% specificity through ctDNA analysis. Across other studies, sensitivities ranged from as low as 29.8% (44) to nearly 100% (18), while specificities varied similarly, with several studies achieving up to 100%

specificity for example, (10 & 45). Techniques such as ctDNA, NGS, ddPCR, and tumor-informed assays consistently demonstrated strong diagnostic performance. However, differences in assay types, timing (e.g., landmark vs longitudinal), and patient context influenced these metrics significantly across the studies. Based on the available data, two studies specifically discussed false positive and false negative results in the context of ctDNA analysis for CRC. (29) reported that ctDNA detection post-surgery showed a significant hazard ratio, indicating strong prognostic power; however, they noted that the specificity was characterized by a higher proportion of true negatives and fewer false positives, suggesting reliable discrimination between recurrence and non-recurrence. Conversely, (46) highlighted challenges in accurately assessing specificity and the positive predictive value due to selection bias and exclusion of false negatives. In their surveillance analysis, sensitivity was reportedly inflated from 55.6% to 90.9% after excluding seven false negative cases, emphasizing the impact such exclusions can have on assay performance metrics. These findings underscore the importance of accounting for false positive and false negative rates when evaluating the clinical utility of ctDNA-based monitoring.

In the study conducted by (17), a direct comparison was made between ctDNA and the conventional tumor marker CEA for predicting tumor recurrence in colorectal cancer. The results demonstrated that ctDNA exhibited a substantially higher detection performance than CEA, with a reported sensitivity of 98% compared to CEA's 69%, and a specificity of 64% for ctDNA versus 88% for CEA. These findings suggest that while CEA remains a valuable clinical marker, ctDNA provides superior sensitivity in recurrence detection, making it a more effective tool for early monitoring and potentially improving patient outcomes through timely intervention. Among the reviewed studies, only a few explicitly reported the use of VAF as a quantitative metric in ctDNA analysis. (43) included VAF alongside specific genetic alterations such as TP53, KRAS, and

PIK3CA in their ctDNA profiling, demonstrating 67% sensitivity and 87% specificity in recurrence detection using landmark analysis. This reflects the role of VAF in refining mutation tracking and recurrence prediction. Similarly, (47) reported a sensitivity greater than 95% for their ctDNA assay at a VAF threshold as low as 0.01%, with a corresponding specificity of 99.7%, showcasing the high analytical sensitivity enabled by incorporating VAF. These findings highlight how leveraging VAF enhances the precision of ctDNA-based assays, allowing for earlier detection of MRD and more accurate assessment of recurrence risk in colorectal cancer patients.

3.1.4 Clinical Relevance

Several studies have explored the application of ctDNA analysis across different stages of CRC. (29) assessed ctDNA detection post-surgery in a cohort primarily composed of stage II and III patients, reporting significant sensitivity and specificity for recurrence prediction. (46) also focused on early-stage CRC, particularly in a surveillance setting, noting how false negatives influenced the apparent sensitivity (which increased from 55.6% to 90.9% upon exclusion of such cases). (12) highlighted the effectiveness of ctDNA-based MRD detection even in early-stage tumors, achieving nearly 100% sample-level sensitivity when three positive sites were detected. While some techniques like ultra-deep sequencing and PCR-based methods showed promise across all stages, the consistent performance of ctDNA assays in early-stage CRC reflects their growing role in detecting minimal disease and guiding adjuvant therapy decisions. Collectively, the data supports the utility of ctDNA not only in advanced cancer monitoring but also in early detection and post-treatment surveillance in earlier-stage CRC. Recurrence determined by ctDNA analyses was 77 days after definitive treatment, compared to 236 days determined by radiological imaging, resulting in a ctDNA median lead time of 159 days. In the 3 recurrence cases where plasma samples were available at the time of radiological detection of relapse, ctDNA remained positive with an increase in the VAF of the detected mutations in all patients at the time of radiological recurrence (mean 10.1 folds, range: 2.9–16.8 folds) (43). Lastly, ctDNA has demonstrated superior sensitivity to the conventional blood tumor marker (CEA) and can offer median lead times of up to 11 months for radiographic detection of recurrence during the surveillance of resected, stage I-III colorectal cancer (38).

Several studies in this dataset demonstrated the integration of ctDNA analysis with treatment planning and therapeutic response assessment in colorectal cancer. (29) evaluated ctDNA levels post-surgery, finding strong sensitivity and specificity in recurrence detection, thereby supporting its role in guiding postoperative management. (48) reported that ctDNA assays achieved 81% sensitivity and 98.2% specificity in post-treatment samples, emphasizing its potential for monitoring treatment efficacy. Similarly, (49) applied a tumor-naïve strategy for detecting MRD after treatment, further illustrating the feasibility of ctDNA in non-invasive surveillance. (42) also incorporated ctDNA assessment in the context of surgery and other localized treatments such as radiofrequency ablation and stereotactic radiotherapy. These studies collectively indicate that ctDNA is being increasingly leveraged not only for recurrence surveillance but also for evaluating treatment response and informing post-treatment decision-making, highlighting its growing relevance in personalized oncology care.

3.2 Breast Cancer Studies

3.2.1 Study Characteristics

Table 3 (Appendix) summarizes the key characteristics of the breast cancer studies included in this review, highlighting patient demographics, geographic distribution, study designs, and ctDNA-based MRD detection methodologies. A total of 23 studies were reviewed, encompassing 2,632 patients. Sample sizes varied across studies, ranging from 7 to 300 patients.

The studies captured a broad age range among participants, typically spanning from early adulthood to older age. Most studies included patients aged from their late 20s to late 70s, with median ages commonly reported in the early to mid-50s. Specific examples include ranges such as "32–73 years," "38–88 years (median = 57)," and "28–75 years (median = 51)." Some studies categorized patients into groups such as "<35, 35–50, and >50 years" or broadly included all patients "18 years and older." Menopausal status was variably reported: one study described a "46-year-old pre-menopausal female," while others grouped patients as "pre- and post-menopausal" or stratified them by age as a proxy (e.g., "≤50 years and >50 years").

Overall, the data reflect broad age inclusion with a notable emphasis on middle-aged women, encompassing both pre- and post-menopausal patients where specified. The studies also demonstrated substantial geographic diversity, with patient populations drawn from North America (United States, Canada), Europe (United Kingdom, Spain, France, Italy, Greece, Ireland, Netherlands), Asia (China, Vietnam, Japan, Philippines), and collaborative efforts across regions such as Australia, Singapore, and France, or the United Kingdom and United States. This international representation enhances the generalizability of the findings.

In terms of study design, the dataset included a mix of original research articles, case reports, review articles, and commentaries. Research articles formed the majority, providing a strong foundation of primary clinical and laboratory investigations, while case reports and reviews offered focused clinical insights and broader contextual analysis.

3.2.2 Biomarkers and Detection Methods

Across the reviewed studies, several molecular targets consistently emerged as key focuses in breast cancer research. PIK3CA and TP53 were the most frequently studied genes, appearing in eight and seven studies, respectively. Both are well-established oncogenes and tumor suppressors implicated in breast cancer progression and therapeutic response. Other commonly investigated biomarkers included GATA3, a transcription factor associated with luminal breast cancer subtypes, and ctDNA itself, each reported in five studies as critical tools for monitoring tumor dynamics non-invasively. Additional targets such as CDH1, FGFR1, and ERBB2 (HER2) were also highlighted, reflecting the ongoing interest in pathways related to cell adhesion, growth signaling, and targeted therapy. The repeated investigation of these biomarkers underscores their clinical significance as diagnostic, prognostic, and therapeutic indicators in breast cancer management. A clear predominance of untargeted ctDNA platforms over tumor-informed approaches was observed across the studies.

Detection methods commonly employed included nNGS, tagged targeted deep sequencing (tTDS), and broad-panel assays, which were reported in eight studies. These untargeted methods are valuable for detecting a wide range of mutations without requiring prior knowledge of individual tumor profiles. In contrast, tumor-informed ctDNA platforms such as *Signatera* and *RaDaR*, which involve sequencing the primary tumor to design personalized assays, were only reported in one study. While tumor-informed approaches offer superior sensitivity and specificity for monitoring MRD, they are less frequently used, likely due to their greater complexity and higher costs. This imbalance suggests that although tumor-informed ctDNA technologies hold significant promise for precision oncology, untargeted approaches remain more commonly implemented in current breast cancer research.

Approximately one-third of the studies incorporated longitudinal monitoring of patients, reflecting the growing importance of tracking disease evolution over time. These studies often focused on detecting MRD, monitoring recurrence, or evaluating treatment response, typically through repeated blood sampling or other serial assessments. Some studies employed personalized, tumor-informed ctDNA assays to enhance monitoring accuracy. Although longitudinal tracking was not universally performed, the studies that incorporated it highlighted its potential value in enabling earlier interventions and guiding more informed treatment decisions.

3.2.3 Diagnostic Performance

Many of the included studies reported diagnostic performance metrics, focusing primarily on sensitivity and specificity. Sensitivity measures the assay's ability to correctly identify true cases of cancer, while specificity reflects its accuracy in minimizing false positives. Of the 23 studies reviewed, sensitivity was reported in 18 studies and specificity in 13 studies, emphasizing the strong research focus on validating assay effectiveness. Several assays demonstrated high sensitivity, with some exceeding 95%, indicating a strong ability to detect MRD. Tests such as Signatera also exhibited high specificity, often approaching 100%, suggesting a low rate of false positive results. These findings highlight the potential of ctDNA-based assays not only for early cancer detection but also for minimizing unnecessary interventions.

When comparing assay types, personalized tests such as *Signatera* and *RaDaR* appeared in four studies, while standard, untargeted tests like NGS-based platforms were employed in nine studies. Personalized assays, which are designed based on a patient's unique tumor mutation profile, demonstrated superior sensitivity and specificity for detecting MRD and identifying early recurrence. However, their use was less frequent, likely due to greater complexity, longer setup times, and higher costs. In contrast, standard untargeted tests are more widely adopted due to their broader applicability and ease of implementation, despite offering lower individualized precision compared to personalized assays.

Some studies also addressed breast cancer subtypes, including HER2-positive and triple-negative breast cancer (TNBC). Approximately 20 studies mentioned these subtypes, often in relation to specific biomarkers such as HER2, TP53, and BRCA genes. While HER2 amplification and BRCA mutations were frequently reported in association with their respective subtypes, most studies did not systematically evaluate or compare assay performance across different breast cancer subtypes. As a result, although there is growing awareness that subtype-specific differences may influence ctDNA assay performance, additional focused research is needed to fully understand these variations and optimize testing strategies across diverse breast cancer populations.

3.2.4 Clinical Relevance

The detection of ctDNA in the plasma of cancer patients is emerging as a very sensitive and specific prognostic biomarker. Previous studies with ctDNA have focused on the ability of ctDNA detection to predict micro-metastatic and eventual clinical metastatic relapse. There are few data on the role of ctDNA in monitoring response to local therapy. (50) reports the case of a patient with early-stage lobular breast cancer, with a detectable ctDNA test which resolved with local radiotherapy to the breast. This case suggests that ctDNA is sensitive enough to detect the response of MRD, localized in the breast, to radiation therapy, and thus may assist in providing indications for local breast cancer treatment ctDNA analysis is emerging as a valuable prognostic biomarker in breast and other solid tumors. Studies thus far have shown excellent specificity of ctDNA, enabling clinical trials evaluating the impact of additional therapy for ctDNA positive patients (NCT03285412, NCT04567420, NCT04985266, NCT04849364, NCT05512364 and NCT05388149 – these are the current ongoing clinical trial identification numbers undergoing for the ctDNA as a biomarker for MRD).

Currently available ctDNA tests have lacked sufficient sensitivity to reliably guide therapeutic decision-making, such as escalating or altering treatment for patients who remain ctDNA-positive after standard therapy or safely withholding additional treatment for ctDNA-negative patients. ctDNA dynamics were recently evaluated in 84 patients with high-risk early-stage breast cancer enrolled in the I-SPY2 trial (51), where patients received standard neoadjuvant chemotherapy (paclitaxel followed by anthracycline) with or without the addition of the AKT inhibitor MK-2206. Blood samples were collected for ctDNA analysis at four time points: T0 (pre-treatment), T1 (three weeks after initiating paclitaxel), T2 (between paclitaxel and anthracycline), and T3 (prior to surgery). At T0, ctDNA was detectable in 61 patients (73%). Persistence of ctDNA at T1 was associated with a significantly lower likelihood of achieving a pathological complete response (pCR) compared to those who cleared ctDNA ($p = 0.012$). Among patients who did not achieve pCR, detectable ctDNA was associated with a significantly higher risk of metastatic recurrence. However, many patients with residual disease after neoadjuvant therapy had undetectable ctDNA, limiting the clinical actionability of a negative result and highlighting the need for more sensitive ctDNA detection methods.

The c-TRAK-TN prospective clinical trial identified patients with MRD following treatment for early-stage moderate to high-risk triple-negative breast cancer (TNBC) and assessed the potential activity of further adjuvant therapy with pembrolizumab after MRD detection. Other clinical trials with a similar design are ongoing, such as IMvigor011 (NCT04660344), which aims to identify and treat patients with MRD following therapy for high-risk muscle-invasive bladder cancer. There was a higher rate of metastatic disease at the point of MRD detection with dPCR in the c-TRAK TN clinical trial than anticipated, emphasizing the need to assess whether ctDNA assays with better sensitivity may lengthen the lead time from MRD detection to clinical relapse and facilitate clinical trials designed to improve patient outcomes from interventions at the point of MRD detection (52). Several clinical trials are underway to investigate the efficacy of potential interventions after MRD detection. For example, in the ongoing TRAK-ER (NCT04985266), DARE (NCT04567420), and LEADER (NCT03285412) trials, patients with high-risk HR+/HER2– early-stage breast cancer who are ctDNA-positive in the adjuvant setting are treated in the intervention arm with endocrine therapy (ET) and a CDK 4/6 inhibitor compared with ET alone in the control arm (53).

Research across the reviewed studies demonstrates that ctDNA tracking could significantly enhance the ability of clinicians to predict breast cancer recurrence and guide post-surgical treatment decisions. For instance, one study utilizing the *RaDaR* assay reported 88% sensitivity and 91% specificity for detecting MRD, indicating a high level of accuracy in identifying true cases while minimizing false positives. Another study employing targeted deep sequencing achieved 100% specificity, successfully avoiding false positive results among healthy patients. Importantly, these ctDNA-based methods were able to detect molecular signs of recurrence several months before clinical or radiological evidence emerged, providing an opportunity for earlier therapeutic intervention. ctDNA analysis also played a role in personalizing therapy; in one study involving 141 patients, treatment plans were adapted based on ctDNA findings, including the use of pembrolizumab, an immune checkpoint inhibitor. This approach highlights the potential to spare patients from unnecessary treatments when ctDNA is absent, or to escalate therapy when there is a heightened risk of recurrence. With emerging data from international studies conducted in regions such as Montreal, Spain, Italy, the United Kingdom, and the United States, ctDNA-based liquid biopsies are increasingly recognized as promising tools for routine follow-up care in breast cancer, supporting more personalized and timely clinical decision-making.

Discussion

4.1 Summary of Key Findings

A total of 82 studies were included in the final analysis. Out of these, 23 studies specifically investigated outcomes related to breast cancer, while the remaining 58 studies specifically focused on CRC. Across both CRC and breast cancer studies, ctDNA has emerged as a prominent tool for MRD detection, with diverse methodologies reflecting advancements in assay sensitivity and specificity. In CRC, the predominant techniques included ddPCR, deep targeted sequencing, and multiplex genomic profiling, often targeting biomarkers such as MSI, RAS, BRAF, and TP53. These approaches demonstrated high analytical sensitivity (e.g., up to 85%) and were frequently integrated with immunohistochemistry (IHC) or NGS platforms for enhanced detection accuracy. In breast cancer studies, the use of ctDNA also focused on targeted gene panels commonly TP53, PIK3CA, and GATA3 detected via NGS-based assays, tagged targeted deep sequencing (tTDS), or commercial platforms like *Signatera*. Sensitivity in this context reached up to 93%, with reported specificity as high as 100%, underscoring ctDNA's potential in early relapse detection and treatment monitoring. Overall, the trend indicates a shift toward personalized, molecularly driven surveillance strategies leveraging ctDNA as a non-invasive biomarker for MRD in both malignancies. The included studies revealed notable differences in study design, biomarker selection, and ctDNA detection technologies between colorectal and breast cancer research. CRC studies frequently involved large patient cohorts and prospective designs, often integrating standard-of-care therapies with ctDNA monitoring.

Breast cancer studies tended to feature smaller sample sizes, including several case reports and early-phase observational studies. Biomarker panels in breast cancer were more diverse, often including TP53, PIK3CA, GATA3, and NTRK fusions, with a heavier reliance on NGS-based platforms such as tTDS and commercially available assays like *Signatera*. The specificity and sensitivity of these technologies varied across studies, with breast cancer research often reporting higher specificity despite more limited cohort sizes. These discrepancies underscore differing levels of clinical integration and technological standardization between the two cancer types, reflecting the evolving landscape of ctDNA-based MRD detection.

The overall performance metrics of ctDNA-based MRD detection across the included studies demonstrated promising sensitivity, specificity, and lead time advantages, though these varied by cancer type and assay used. In CRC studies, sensitivity values were commonly reported in the range of 80–85%, with some assays achieving higher accuracy when multiple biomarkers or longitudinal sampling strategies were employed. Specificity was also high, with several studies reporting true negative rates exceeding 90%, particularly when using targeted deep sequencing or ddPCR. Breast cancer studies, although fewer in number and often involving smaller cohorts, demonstrated comparable or superior sensitivity, with some assays achieving up to 93.3% sensitivity and 100% specificity in detecting MRD. Importantly, several studies across both cancer types highlighted a significant lead time advantage of ctDNA over conventional imaging or clinical recurrence detection ranging from several weeks to months enabling earlier intervention opportunities. Collectively, these findings support the utility of ctDNA as a sensitive and specific tool for MRD surveillance, with the added benefit of earlier relapse prediction compared to standard monitoring approaches. Based on the dataset, several high-performing assays and platforms have been utilized for ctDNA-based MRD detection in CRC and breast cancer, each offering varying strengths in sensitivity, specificity, and clinical applicability.

4.2 Interpretation of Findings in CRC

4.2.1 Effectiveness of ctDNA in Detecting MRD

Several studies have reported high sensitivity and specificity values exceeding 85% for various diagnostic or monitoring approaches in CRC. For instance, (39) observed 92.3% sensitivity and 87% specificity using the Cologuard test, while (43) reported perfect sensitivity (100%) alongside 87% specificity through landmark ctDNA analysis. Other notable examples include (47), who reported sensitivity and specificity as high as 95% and 99.7% respectively, and (12), who demonstrated 100% sensitivity with 98.9% specificity. These high-performance figures, observed across diverse techniques like PCR, NGS, and ctDNA assays, highlight the growing accuracy of biomarker-driven diagnostics in CRC management. Also, several studies have highlighted the ability of ctDNA to detect recurrence of CRC months before it becomes radiologically visible. This early detection capability is particularly important in post-operative surveillance, where timely intervention could significantly influence outcomes. In (38), ctDNA monitoring provided a median lead time of up to 11 months ahead of radiographic detection in patients with resected stage I–III CRC, clearly underscoring its value in early relapse identification. Similarly, (43) reported a median lead time of approximately 5 months, where longitudinal ctDNA analysis detected recurrence before it was visible on imaging, offering clinicians a critical window for early intervention. These findings collectively demonstrate the clinical utility of ctDNA in providing an early warning system for CRC recurrence, with lead times ranging from 5 to 11 months substantially ahead of conventional imaging modalities. Several studies in the dataset underscore the association between ctDNA analysis and both the benefit of adjuvant therapy and recurrence risk stratification in CRC.

For example, (10) demonstrated that ctDNA MRD detection achieved a sensitivity of 95% and a specificity of 100%, suggesting strong potential for identifying patients who could benefit from additional therapy post-surgery. Similarly, (27) showed that ctDNA positivity after surgery was a significant indicator of recurrence risk, with implications for guiding adjuvant chemotherapy decisions. (45) and (35) both employed sequencing-based approaches to track ctDNA levels before and after neo-adjuvant therapies, showing how dynamic changes could predict response and inform risk-adapted treatment strategies. Collectively, these findings highlight ctDNA's emerging role in personalizing post-operative care by pinpointing which patients may need intensified treatment and which may safely avoid it.

4.2.2 Biomarker Diversity and Assay Variation

A wide array of studies in the dataset illustrate the extensive use of key molecular markers like – MSI, KRAS, NRAS, BRAF, and methylation-based signatures in CRC research and clinical applications. These biomarkers are central to patient stratification, treatment selection, and disease monitoring. For instance, (31) and (39) employed comprehensive panels incorporating RAS, BRAF, and MSI for guiding therapy decisions. (42) and (54) utilized KRAS, NRAS, and BRAF in post-treatment surveillance to detect minimal residual disease via ctDNA. Moreover, methylation-based markers such as those used in (29) and (55) add a layer of sensitivity, particularly in plasma-only assays. These markers are analyzed using a variety of techniques ranging from PCR and droplet digital PCR to NGS and personalized tumor-informed assays underscoring their broad integration across different molecular platforms. This widespread adoption reflects the biomarkers' robust utility in advancing precision oncology for CRC. The dataset reveals substantial variability in the techniques employed for biomarker detection in CRC, reflecting the evolving landscape of precision diagnostics. ddPCR, as seen in studies like (56) and (44), offers high sensitivity for detecting low-frequency mutations such as KRAS and BRAF, making it a preferred choice for MRD monitoring. In contrast, NGS platforms used in studies like (6), (45), and (10) enable broader genomic profiling, capturing a wide array of mutations across panels of hundreds of genes.

Meanwhile, stool-based testing, as incorporated in (39), provides a non-invasive alternative for early detection and screening, often using methylation and mutation markers. This diversity in methodologies ranging from targeted, high-sensitivity approaches to comprehensive genomic scans highlights both the technological advancements and the tailored application of each method based on clinical objectives such as screening, recurrence surveillance, or treatment planning. The dataset highlights how both sample type and collection timing significantly influence the accuracy and clinical relevance of test results in CRC management. Blood-based assays using plasma for ctDNA analysis are prominently featured in studies like (6) & (10), where serial sampling post-surgery or post-therapy allowed for dynamic monitoring of disease recurrence and treatment response. In contrast, (39) utilized both blood and stool samples, showcasing how sample type can determine the practicality and sensitivity of detection, particularly for early-stage disease. Timing of collection is also critical in studies such as (45) and (35), which emphasized the predictive value of ctDNA measured immediately after neo-adjuvant therapy or surgery, with early post-treatment sampling serving as a strong indicator for recurrence risk and the need for adjuvant therapy. This underscores that not only the technology used, but also when and how samples are collected, and can profoundly impact diagnostic precision and patient outcomes.

4.2.3 Clinical Integration and Challenges

ctDNA has emerged as a valuable biomarker for guiding adjuvant therapy decisions and tailoring surveillance intensity in CRC management. Studies such as (6) have demonstrated that ctDNA detection post-surgery correlates with MRD and recurrence risk, allowing for stratified follow-up strategies. With a reported sensitivity of 67% and specificity of 87% in landmark ctDNA analysis, this approach offers a non-invasive means to identify patients who might benefit from intensified adjuvant therapy or, conversely, be spared from unnecessary treatment. Moreover, ctDNA-guided strategies provide dynamic, real-time insights compared to static pathological risk factors, enabling personalized care that adapts to disease progression or clearance. Despite the promise of ctDNA as a biomarker in CRC, significant limitations persist due to tumor heterogeneity and low-shedding tumors. For instance, (6) highlighted variability in ctDNA detectability, which may stem from differences in tumor biology, including genetic heterogeneity and anatomical location.

Tumors that shed minimal DNA into the bloodstream especially those at early stages or with certain molecular subtypes can lead to false negatives, reducing the sensitivity of ctDNA assays. Techniques like NGS and ddPCR, while highly sensitive, still face challenges in consistently capturing ctDNA from all patients, particularly when tumor burden is low. This inherent variability underscores the need for multimodal approaches or complementary biomarkers to ensure accurate assessment and avoid under-treatment due to undetected residual disease. The evolving landscape of CRC management emphasizes the importance of personalized assay design and real-time adaptation of therapeutic strategies. Studies like (8) and (6) highlight the use of tumor-informed assays and targeted panels that account for individual mutational profiles, such as TP53, KRAS, APC, and others commonly detected through NGS. Given the diverse genetic landscape and dynamic nature of CRC, standardized assays may overlook patient-specific variants or emerging resistance mutations. Personalized ctDNA assays enable precise tracking of relevant alterations over time, enhancing the ability to detect MRD and adapt treatments accordingly. This real-time monitoring facilitates a more responsive approach, allowing clinicians to escalate or de-escalate therapy based on molecular evidence rather than relying solely on static clinical or pathological indicators.

4.3 Interpretation of Findings in Breast Cancer

4.3.1 Effectiveness of ctDNA in MRD Monitoring

The reviewed studies consistently demonstrated high sensitivity and specificity in detecting breast cancer biomarkers, particularly when personalized assays were employed. For instance, (52) reported that personalized sequencing assays achieved notable sensitivity in detecting ctDNA for monitoring disease progression, with a specificity of 91.0% using the *RaDaR* platform. Similarly, (50) achieved an impressive 88% sensitivity in detecting MRD via ctDNA, alongside a specificity of 99.9% using the *Signatera* assay. These results underscore the reliability of patient-specific techniques in capturing tumor-specific genetic alterations with minimal false positives or negatives, supporting their growing utility in precision oncology.

Some studies highlight the promising utility of ctDNA and sequencing technologies in early-stage breast cancer and in monitoring therapeutic response. (40) applied tTDS to early invasive breast cancer patients, demonstrating its effectiveness in detecting ctDNA and key mutations such as TP53 and PIK3CA. (50) illustrated how ctDNA dynamics correlated with treatment response in a single pre-menopausal patient, with ctDNA levels decreasing post-therapy, thereby reflecting therapeutic efficacy. Likewise, (34) monitored multiple biomarkers in a cohort of early-stage patients, demonstrating that serial measurements could inform disease progression and response. These findings affirm the value of ctDNA analysis not just for initial detection but as a dynamic biomarker to guide real-time clinical decisions throughout the treatment journey. Emerging evidence from the reviewed studies supports the potential of ctDNA analysis in guiding therapy de-escalation and enabling early relapse detection. (50) demonstrated that ctDNA levels could serve as an early indicator of MRD, with high sensitivity (88%) and near-perfect specificity (99.9%) using the *Signatera* assay, suggesting its utility in identifying patients who may not require extended treatment. Similarly, (50) showed that personalized ctDNA assays effectively tracked molecular relapse, preceding radiological detection, which could allow for earlier therapeutic intervention. These findings indicate that ctDNA monitoring can provide actionable insights for tailoring treatment intensity and timing, potentially reducing overtreatment while ensuring prompt response to recurrence.

4.3.2 Biomarker and Platform Considerations

According to the reviewed studies, TP53, PIK3CA, and GATA3 emerged as some of the most frequently targeted genetic alterations in breast cancer ctDNA analysis. These mutations were consistently included in assay panels due to their high prevalence and clinical relevance. For instance, (40) utilized tTDS to detect ctDNA mutations in early breast cancer, with a focus on TP53, PIK3CA, and GATA3 among others. (34) similarly reported these genes as part of a broader panel, reflecting their diagnostic and prognostic utility. Their recurrence across multiple studies highlights their significance in tumor biology and underscores the rationale for their inclusion in both personalized and standardized molecular assays aimed at improving detection and monitoring strategies. Tumor-informed assays such as *RaDaR* and *Signatera* demonstrate superior performance in ctDNA-based breast cancer monitoring by leveraging personalized mutation profiles. (52) reported that the *RaDaR* assay achieved a specificity of 91.0% in detecting ctDNA, enabling precise tracking of disease status and early relapse.

Similarly, a tumor-informed blood test was used by (50) to detect MRD, achieving a high sensitivity of 88% and an exceptional specificity of 99.9% in identifying normal and non-cancerous DNA. These results shows the test's strong capability in distinguishing tumor-derived signals from background noise. These tumor-informed platforms, which are designed based on each patient's unique tumor mutation signature, significantly enhance the accuracy of ctDNA detection, making them highly effective tools for personalized disease monitoring and management. While ctDNA assays show considerable promise, some limitations are evident, particularly in case reports and smaller studies. For example, (50) presented data from a single-patient case report, which, while demonstrating high specificity (99.9%) with a tumor-informed blood test, limits broader applicability due to its anecdotal nature. Additionally, several studies, including (57), lacked specificity data entirely, underscoring a common gap in early reports. These limited sample sizes reduce statistical power and generalizability, making it difficult to draw firm conclusions about assay performance across diverse patient populations. As such, while the findings are encouraging, larger, well-controlled studies are necessary to validate these results and establish consistent benchmarks for clinical use.

4.3.3 Clinical Relevance and Adoption Barriers

The molecular heterogeneity of breast cancer poses significant challenges for the development and standardization of ctDNA assays. The reviewed studies illustrate a wide array of targeted mutations including TP53, PIK3CA, GATA3, and various receptor markers highlighting the diversity of genomic alterations across patients. For instance, (57) reported a broad biomarker panel, including NTRK1, HER2/ERBB2, and GRM3, to capture this variability, while (57) combined multiple genes in their ctDNA analysis to account for inter-patient differences. This variability complicates assay design, as a one-size-fits-all approach may miss critical mutations in certain subtypes. As a result, tumor-informed or highly multiplexed strategies are often required, increasing complexity, cost, and turnaround time factors that must be addressed to make these assays more scalable and clinically accessible. The data suggest strong potential for integrating ctDNA monitoring into follow-up care and clinical trial frameworks for breast cancer. Several studies, including those by (14) and (57), demonstrated that ctDNA levels can provide early indicators of treatment response or molecular relapse, often before radiographic evidence appears. This real-time molecular insight positions ctDNA as a powerful tool for longitudinal surveillance, potentially guiding personalized follow-up schedules and informing decisions about therapy escalation or discontinuation. Furthermore, ctDNA's sensitivity to dynamic tumor changes makes it well-suited for incorporation into clinical trials, where it can serve as a non-invasive biomarker for response evaluation and early endpoint determination.

As the technology matures, its integration into routine clinical pathways and trial protocols could significantly enhance precision in post-treatment monitoring. The current landscape of ctDNA research in breast cancer reflects a pressing need for methodological harmonization and validation in larger, more diverse cohorts. The studies reviewed vary widely in assay types, target gene panels, and reporting metrics for sensitivity and specificity. For instance, while some employed highly personalized platforms like *RaDaR* or *Signatera* with robust performance data, others lacked clear specificity reporting or were limited to small case studies with single-patient data. This inconsistency hinders cross-study comparisons and limits the generalizability of findings. To advance clinical adoption, standardized protocols for sample collection, assay design, and reporting outcomes are essential. Additionally, large-scale validation studies are critical to confirm clinical utility across varied patient populations and breast cancer subtypes, ensuring that ctDNA technologies can be reliably implemented in both research and routine care settings.

4.4 Comparative Reflections: CRC vs. Breast Cancer

Based on the CRC studies presented in the data table, detection performance in CRC research demonstrates a slight edge compared to other cancer types, primarily due to the relatively larger sample sizes and increased standardization in biomarker usage. Several studies, such as (58) with 747 patients and (59) with over 180 patients, exemplify this trend. These larger cohorts enable more statistically robust analyses and validation of detection methods. Furthermore, the repeated use of standardized biomarkers – like MSI, KRAS, BRAF, and methylation markers facilitates consistency across different studies, enhancing both sensitivity and specificity. Techniques such as ddPCR and NGS, commonly applied across CRC studies, also contribute to improved detection accuracy. Overall, this focus on methodological rigor and standardized biomarker panels gives CRC studies a detection performance advantage within the broader field of cancer diagnostics. The breast cancer studies in the data table highlight a notable trend toward the use of personalized, tumor-informed assays for detection and monitoring, distinguishing this cancer type from others. Assays such as *Signatera* and *RaDaR*, which tailor their design based on individual tumor profiles, are frequently employed in these studies to enhance the precision of MRD detection.

For instance, (52) reported the use of personalized sequencing assays, achieving high sensitivity and specificity (91% specificity with *RaDaR*, while (50) demonstrated 88% sensitivity and 99.9% specificity using the *Signatera* assay. These tumor-informed approaches offer greater accuracy by targeting patient-specific mutations, allowing for real-time, non-invasive monitoring through ctDNA. The preference for such advanced, customized assays underscores a broader trend in breast cancer research toward precision medicine, enhancing early detection and treatment response tracking. The data table reveals a clear contrast in biomarker complexity between CRC and BREAST CANCER studies. CRC research tends to employ fewer, more standardized biomarkers, with markers like MSI (microsatellite instability), KRAS, BRAF, and NRAS appearing consistently across multiple studies. This streamlined approach supports reproducibility and allows for more uniform comparisons between patient cohorts and treatment responses. In contrast, breast cancer studies showcase a broader and more diverse biomarker landscape. Markers such as TP53, PIK3CA, GATA3, HER2/ERBB2, and various epithelial and hormone-related proteins were reported, often in combinations tailored to individual patient profiles. This complexity reflects the molecular heterogeneity of breast cancer and the growing reliance on personalized medicine, where multi-marker panels are used to guide diagnosis, prognosis, and therapy decisions. Consequently, while CRC benefits from biomarker standardization, breast cancer research embraces a more intricate and individualized biomarker strategy.

The data table highlights that while both CRC and BREAST CANCER studies demonstrate strong potential for clinical utility, CRC research appears to be further along in integrating liquid biopsy technologies into clinical practice. This is largely due to influential clinical trials such as CIRCULATE-Japan and DYNAMIC, which provide robust evidence supporting the use of ctDNA to guide adjuvant therapy decisions. For instance, CRC studies frequently refer to the use of ctDNA to stratify patients post-surgery, enabling more tailored treatment approaches and reducing unnecessary chemotherapy. In contrast, BREAST CANCER studies, though rich in technological innovation and diverse biomarker use, are still in earlier stages of clinical translation, focusing more on assay development and exploratory research. While the promise is evident in both fields, the structured integration of ctDNA monitoring into treatment pathways gives CRC a clinical edge, marking a significant step toward routine implementation in oncology care.

4.5 Comparison of ctDNA Detection Methods

Signatera is a tumor-informed, multiplex NGS assay specifically designed for personalized ctDNA monitoring. The process begins by sequencing a patient's tumor tissue to identify a set of individual somatic mutations unique to their cancer. These selected mutations are then used to design personalized multiplex PCR assays, which amplify the corresponding regions in plasma-derived ctDNA, followed by next-generation sequencing for highly sensitive detection. Signatera consistently demonstrates high specificity (up to 99.9%) and strong sensitivity, making it particularly effective for minimal residual disease (MRD) detection.

ddPCR is another commonly used method, particularly in CRC studies. ddPCR partitions a DNA sample into thousands of nanoliter-sized droplets, with each droplet serving as an individual PCR reaction compartment. This partitioning enables highly precise quantification of low frequency ctDNA mutations with excellent accuracy and reproducibility. The method is considered valuable due to its cost-effectiveness, superior precision, and higher sensitivity for detecting rare genetic variants compared to broader sequencing-based approaches.

Beyond *Signatera* and ddPCR, other notable platforms included the *Guardant Reveal* assay, a plasma-only ctDNA test that integrates both genomic and epigenomic signatures using NGS of cell-free DNA extracted directly from blood. By focusing exclusively on plasma-derived DNA, this approach reduces the need for tumor tissue samples, offering a less invasive yet highly sensitive method for MRD detection. tTDS also emerged as a reliable technology, particularly in breast cancer studies, with several reports citing 100% specificity. Additionally, the *Safe-Sequencing System* (Safe-SeqS) and *AVENIO* targeted NGS platforms were utilized for their high-depth, error-corrected sequencing capabilities, enhancing detection accuracy in low-abundance ctDNA scenarios. In addition, techniques such as tumor-informed whole-exome sequencing (WES) and multiplex PCR combined with ultra-deep NGS further highlighted the trend toward highly personalized and sensitive ctDNA assays. Collectively, these platforms represent a significant shift toward precision oncology, providing clinicians with robust tools for earlier MRD detection and more informed treatment guidance.

4.6 Common ctDNA Biomarker Genes in CRC & Breast Cancer

The most commonly identified ctDNA biomarker genes in CRC and breast cancer studies include TP53, KRAS, APC, BRAF, and PIK3CA. These genes are critical molecular drivers of tumorigenesis and are frequently mutated across a range of malignancies, making them important targets for diagnostic and therapeutic strategies.

TP53 encodes a tumor suppressor protein that plays a pivotal role in maintaining genomic stability, regulating cell cycle arrest, and promoting apoptosis in response to DNA damage. Mutations in TP53 are among the most prevalent in human cancers and lead to loss of tumor suppressive functions, allowing cells with genomic abnormalities to survive and proliferate. TP53 mutations are extensively observed in both CRC and BREAST CANCERand are associated with aggressive disease and poorer prognosis.

KRAS encodes a small GTPase that functions as a key regulator of the RAS/MAPK signaling pathway, which controls cell proliferation, differentiation, and survival. Mutations in KRAS result in constitutive activation of downstream signaling pathways, driving uncontrolled cell growth. KRAS mutations are particularly common in CRC and have also been implicated in certain subtypes of BC, contributing to treatment resistance and disease progression.

APC is another crucial tumor suppressor gene involved in the Wnt signaling pathway and regulation of cell adhesion and migration. Loss-of-function mutations in APC are a hallmark of CRC and lead to dysregulated cellular proliferation and tumor initiation. Although less frequent in BC, APC pathway disruptions have been noted in certain breast cancer contexts, contributing to tumor progression.

BRAF encodes a serine/threonine-protein kinase involved in the MAPK/ERK signaling cascade. Activating mutations, particularly BRAF V600E, result in persistent activation of this pathway, promoting cell division and survival independent of external growth signals. BRAF mutations are observed in subsets of CRC and, less commonly, in BC, and are often associated with poor clinical outcomes.

PIK3CA encodes the catalytic subunit of phosphoinositide 3-kinase (PI3K), a key component of the PI3K/AKT signaling pathway that regulates cell growth, metabolism, and survival. Mutations in PIK3CA lead to hyperactivation of the pathway, conferring growth advantages and resistance to apoptosis. PIK3CA mutations are frequently detected in BC, especially in hormone receptor-positive subtypes, and are also present in CRC, making them attractive targets for therapeutic intervention.

Rather than targeting each mutation individually in separate protocols, these commonly altered genes present an opportunity to develop multiplexed or panel-based ctDNA assays. Detecting multiple relevant mutations simultaneously can improve the efficiency of screening, enhance the precision of MRD monitoring, and refine post-treatment management strategies across multiple cancer types.

Implementing integrated molecular diagnostics based on these shared genetic alterations could accelerate the adoption of personalized oncology by standardizing ctDNA testing platforms, lowering diagnostic costs, and streamlining clinical workflows. Ultimately, focusing on these common biomarker genes offers a promising avenue to develop universal guidelines and robust ctDNA assays capable of accurately detecting and monitoring tumor dynamics, potentially benefiting a broader population of cancer patients beyond CRC and BC.

4.7 Limitations of the Reviewed Literature

Multiple limitations in the detection and clinical application of ctDNA were identified across the dataset. A significant number of studies were based on small sample sizes, with some relying on single-case reports (e.g., studies 25, 50, and 8) or cohorts of fewer than five patients (e.g., 38). Such limited sample sizes constrain statistical power and reduce the generalizability of findings. Additionally, insufficient long-term follow-up was frequently observed. For instance, in study (43), patient monitoring extended only up to 16 months post-ctDNA detection, potentially leading to underestimation of recurrence rates due to delayed confirmation by imaging. In study (25), early ctDNA detection preceded imaging-based recurrence identification, yet no extended follow-up was conducted to assess long-term outcomes. Although study (59) implemented a longer follow-up period (3–4 years), variability in pre-analytical conditions and inconsistent durations across patients may have affected ctDNA detection sensitivity. Methodological heterogeneity further complicates interpretation. A wide range of ctDNA analysis platforms were employed, including ddPCR, tTDS, and NGS with proprietary technologies such as *Signatera*. However, standardization in assay sensitivity, specificity, and reporting practices was lacking. While some studies reported quantitative metrics, others provided only methodological descriptions without performance indicators, hindering cross-study comparisons and reproducibility.

On the other hand, a few studies conducted direct comparisons between ctDNA analysis and established diagnostic methods such as imaging or serum biomarkers. This absence of head-to-head evaluations limits the ability to assess the relative value of ctDNA in clinical settings. Finally, a lack of demographic and geographic diversity was noted. Most studies were conducted in high-income countries with patient populations drawn from urban academic centers. Underrepresented ethnic groups and individuals from low-resource settings were largely excluded, raising concerns about the broader applicability of findings given the potential differences in genetic backgrounds, environmental exposures, and healthcare access. Collectively, these limitations underscore the need for larger, more diverse cohorts, standardized methodologies, extended follow-up, and comparative studies to fully establish the clinical utility of ctDNA.

4.8 Future Directions

This section explores potential avenues for future research and clinical application of ctDNA-based MRD detection, emphasizing areas where current evidence is limited, and further investigation is warranted. First, testing should not be performed in the immediate postsurgical period, to avoid false positives, which may create unnecessary anxiety for patients. In this regard, the group notes that timing of blood collection following resection is an especially important consideration. A recent study that included 453 patients with stage I–III CRC undergoing elective surgery found an approximately three-fold increase in the level of ctDNA following surgery ($p \leq .0001$), and this elevation was noted to persist for approximately 4 weeks. Therefore, it is suggested that later sampling would reduce the potential for contamination with wild type (non-tumor) ctDNA. The group suggests 4 weeks post-surgery as a reasonable time frame for plasma sampling. There was also an agreement that serial ctDNA testing further improves sensitivity. (15) found an improvement in sensitivity with two tests versus one, and sensitivity was further improved with three tests. Therefore, if a patient is found to be ctDNA positive in the early post-surgery period, the group thought it would be reasonable to consider a second test before treatment is given. Although the time between blood draw and initial ctDNA test result takes longer, a second blood draw (which takes less time than the initial) could be performed immediately following receipt of the first. Most in the group felt that MRD testing is more commonly ordered in stage II cases and less often in stage III CRC, as it is not expected to change management in the latter until more data in this setting are available. It was also agreed that, outside the setting of a clinical trial, ctDNA testing would generally not be ordered if it would not impact or otherwise change their treatment decision based on clinicopathologic factors.

The group also notes a recent publication of the National Cancer Institute (NCI) Task Force on the application of ctDNA testing; this report noted multiple potential applications of ctDNA testing in CRC, including the detection of MRD, management of rectal cancer, and as a means to monitor clonal evolution of the tumor and response to targeted therapies and other systemic treatments. Notably, the NCI task force called for harmonization around time-points for the collection of ctDNA, standardization of sample collection and clinical validation of testing, a need for patient and provider education on the applications of the technology, and the incorporation of ctDNA testing into treatment guidelines (39). This study should ideally be expanded to fully test the performance of different assays targeting the same number of structural variants (SVs), single nucleotide variants (SNVs), and somatic copy number alterations (SCNAs) in ctDNA in a larger cohort of patients within specific clinical settings to assess their utility. Furthermore, the assays used were not developed for clinical diagnostic use and need to undergo full analytical validation prior to clinical application to assess the relative sensitivity and specificity of each assay type for detection of different mutation classes.

Multiple studies are currently exploring the potential of using patient-specific assays to identify patients at high risk of relapse, who may benefit from adjuvant therapy, or to de-escalate treatment in patients with no residual ctDNA detected post-treatment, thereby avoiding unnecessary side-effects. Currently, the development of tumor-informed assays is challenging in the clinical setting given the high cost, the mandatory requirement for tumor and germline samples, and the time required for patient-specific assay development. In the future, it is expected that tumor sequencing will become more affordable and routine in the clinic, enabling tumor-informed assays to be more readily developed. A tumor-naïve assay would be ideally used in the clinical setting given they avoid some of the operational challenges associated with accessing tumor and developing assays within a clinically relevant timeframe. However, tumor-naïve assays are currently not sufficiently sensitive for detection of low-burden disease. Further research is required to improve the sensitivity of tumor-naïve assays to the required levels of sensitivity, through incorporation of additional features or biomarkers (60).

Because patients with tumors with low mutational burden may be less likely to relapse, larger studies are needed to determine the efficacy and utility of personalized cancer monitoring in patients with lower numbers of tumor-informed DNA variants. Thus, additional studies are needed to determine how long-term ctDNA monitoring predicts relapse, especially in HR+/HER2- breast cancer. A number of clinical trials, such as TRAK-ER (ClinicalTrials.gov identifier: NCT04985266), which will determine whether palbociclib and fulvestrant can defer or prevent relapse in patients with MRD, are underway. Additional prospective trials in larger cohorts and across multiple cancer types will be essential to refine and validate the clinical utility of ctDNA-based monitoring strategies (14). Despite development of continuous monitoring strategies utilizing advanced modalities (CT/MRI or PET-CT), or a repertoire of tumor biomarkers in blood (e.g., CEA, CA-199), detection of MRD or micro-recurrence, remains elusive.

The assessment of ctDNA in combination with improved imaging modalities may improve the prediction and identification of micro-metastatic disease. Furthermore, serial ctDNA monitoring provided a comprehensive view of the patient's clinical and pathologic status, in a manner that is more precise than the methods currently used. The ctDNA detection could clarify equivocal imaging and/or CEA findings to enhance disease monitoring accuracy in gastric cancer. In stages II to III CRC, postoperative ctDNA inferred tumor recurrence ahead of radiological imaging. The result confirmed that ctDNA has superior sensitivity and specificity in detecting MRD compared to traditional radiographic or laboratory analyses. Therefore, this case highlights the value of ctDNA monitoring to gain further insight into the evolution of a patient's response over time (61).

Customized ctDNA detection by ddPCR achieved a 75% detection rate at baseline. During neoadjuvant chemotherapy, ctDNA levels decreased quickly and MRD was not detected after surgery. However, a slow decrease of ctDNA level during neoadjuvant chemotherapy was strongly associated with shorter survival. Triple-negative breast cancers (TNBCs) represent 15%–20% of invasive breast cancers. TNBCs are often treated by neo-adjuvant chemotherapy followed by surgery. In addition to breast tumor shrinkage, NCT aims at eradicating any disseminated tumor cell (also known as micro-metastasis) that may have spread throughout the body. The persistence of a MRD at distant sites after the treatment of a localized breast cancer is a key parameter for post treatment survival but cannot be reliably assessed by the current biological or radiological tools. In that context, the detection and quantification of ctDNA is a very promising tool that can assess tumor burden, response to therapy, and minimal residual disease (24).

Considering the cost and invasiveness of monitoring postoperative MRD of CRC after adjuvant chemo-radiotherapy, a study developed a favorable approach based on methylated circulating tumor DNA to detect MRD after radical resection. Analyzing the public database, this study identified the methylated promoter regions of the genes FGD5, GPC6, and MSC. Using ddPCR, the 'amplicon of methylated sites using a specific enzyme' assay as 'AMUSE' was developed (62). The AMUSE assay addresses the clinical need for accurate MRD monitoring with universal applicability, minimal invasiveness, and cost-effectiveness, thereby enabling the timely detection of recurrences. This assay can effectively evaluate the efficacy of adjuvant chemo-radiotherapy in patients with stage III CRC following curative resection. This study strongly recommends reevaluating the clinical application of adjuvant chemo-radiotherapy using the AMUSE assay (1.62). The effectiveness of neoadjuvant chemotherapy treatment regimens for BREAST

CANCER has improved during recent years and has achieved high rates of pCR, particularly in HER2+ and TN tumors. These observations have prompted the notion that patients that achieve cCR following neoadjuvant chemotherapy might be spared of breast surgery, as forgoing surgery would potentially reduce surgical complications, improve quality of life, and decrease healthcare costs. This idea of de-escalation of breast surgery in breast cancer patients with an apparent complete response to neoadjuvant chemotherapy is gaining recognition as an alternative approach to patient management (34).

Conclusion

ctDNA has emerged as a transformative biomarker for detecting MRD in both CRC and breast cancer. Unlike traditional imaging or tissue biopsy techniques, ctDNA analysis offers a minimally invasive, repeatable, and highly sensitive method to monitor disease burden and detect recurrence at an earlier stage. Numerous studies reviewed in this literature review demonstrate that ctDNA outperforms conventional biomarkers like CEA in both sensitivity and specificity, while also offering significant lead times ahead of radiological detection. The ability to identify patients with molecular evidence of MRD enables more precise risk stratification, guiding the intensity and timing of adjuvant therapies. This personalized approach has the potential to reduce unnecessary treatments in ctDNA-negative patients and intensify therapy in those at higher risk, ultimately improving clinical outcomes and resource efficiency in oncology care.

The integration of ctDNA into clinical workflows represents a major advancement in precision oncology, especially in managing CRC and breast cancer. As demonstrated by recent studies and clinical trials, ctDNA not only facilitates early detection of relapse but also assists in tailoring individualized treatment strategies based on real-time molecular dynamics. This approach aligns with the evolving paradigm of personalized medicine, where therapies are increasingly guided by molecular profiling rather than solely by histopathological staging. Although technical, economic, and standardization challenges remain, the compelling evidence supporting ctDNA's prognostic and predictive value underscores its growing clinical relevance. With continued validation in large-scale, real-world studies, ctDNA-based MRD detection is poised to become an indispensable tool in optimizing patient surveillance and enhancing survivorship in both CRC and breast cancer populations. There is now a compelling body of evidence showing that ctDNA plays a powerful role in catching cancer recurrence earlier than ever before. In both CRCs and breast cancer, studies have shown that ctDNA can pick up traces of MRD months before a recurrence becomes visible through standard imaging. This early insight gives doctors a head start, allowing them to adjust treatments, begin adjuvant therapies sooner, or reduce unnecessary interventions when the risk of recurrence is low. For patients, this means more personalized care: fewer side effects from overtreatment, greater peace of mind, and potentially better chances of long-term survival. The promise of ctDNA isn't just in its technical accuracy, it's in how it helps shift cancer care from a reactive to a proactive approach, improving outcomes and the patient experience as a whole.

As this review shows, ctDNA is more than just a biomarker; it's becoming a clinical tool that helps doctors make smarter, more individualized decisions. By tracking ctDNA levels in the blood over time, clinicians can get a real-time picture of whether treatment is working or if cancer may be coming back. That information is incredibly powerful, it can mean escalating therapy when it's needed most or holding back when the risks of treatment outweigh the benefits. Most importantly, ctDNA helps return a sense of control to both patients and providers. It supports more timely interventions, less guesswork, and a more tailored path forward, all of which are crucial for improving outcomes and quality of life in patients facing colorectal or breast cancer. Despite the ongoing technological and methodological hurdles, ctDNA is rapidly emerging as a cornerstone of precision oncology. Current limitations, such as inconsistent assay sensitivity, variability in detection platforms, and the complexity of interpreting results across diverse tumor types, do pose real challenges to routine clinical implementation. However, as highlighted in this review, the progress made in recent years is nothing short of remarkable. Advances in NGS, ddPCR, and personalized assay development have significantly improved the accuracy and feasibility of ctDNA testing. More importantly, ctDNA offers an unprecedented opportunity to move beyond traditional, one-size-fits-all approaches in cancer care. By providing real-time, tumor-specific information from a simple blood draw, ctDNA has the potential to guide more precise and timely treatment decisions, whether that means escalating therapy in high-risk patients or sparing low-risk individuals from unnecessary toxicity.

With continued validation and standardization, ctDNA could soon become a routine part of cancer management, making precision oncology not just a goal, but a reality. While the integration of ctDNA into clinical oncology is not without its obstacles, the potential benefits far outweigh the current limitations. One of the most significant challenges lies in the lack of standardized protocols across laboratories, leading to variability in test sensitivity, specificity, and interpretation. Additionally, some tumors shed DNA at lower rates, making detection more difficult in certain patient populations. Yet, the promise of ctDNA lies in its ability to capture the molecular fingerprints of cancer in real time, allowing for early detection of recurrence, dynamic treatment monitoring, and even the identification of emerging resistance mutations. These capabilities are central to the vision of precision oncology: treating the right patient, at the right time, with the right therapy. As technology evolves and clinical trials continue to demonstrate the utility of ctDNA in both colorectal and breast cancers, we are moving closer to a future where ctDNA is seamlessly integrated into everyday oncology practice. The road ahead will require collaboration between researchers, clinicians, and regulatory bodies, but the destination is a more personalized, proactive approach to cancer care is well within reach.

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Appendix

Table 2. Summary of key studies investigating ctDNA as a biomarker for MRD in CRC. This table presents a comprehensive overview of selected studies evaluating the use of ctDNA in detecting MRD. Key characteristics of each study are listed, including publication details, therapeutic interventions, patient demographics, geographic locations, sample sizes, specific biomarkers evaluated, and reported sensitivity and specificity values. Techniques used for ctDNA detection and analysis (e.g., ddPCR, NGS, personalized assays) are also included. The data are categorized to highlight the diversity in study design, methodology, and performance metrics across different cohorts and technologies.

Paper Name	DOI	Drug	Age group	Patient location	# of patients	Specific biomarker	Sensitivity	Specificity	Technique
Andersen L. et al, 2024	10.1016/j.ejca.2024.114186	NA	63 - 78 years (-ve - 70 years, +ve - 72 years)	Treated at Aarhus University Hospital, Denmark	747	MSI	sensitivity was found to be 85%	True negative patients: 261, False positive patients: 21	Droplet Digital PCR (ddPCR), Deep Targeted Sequencing, Signatera ultradeep multiplex PCR sequencing
Bando H. et al, 2023	10.1111/cas.16039	Cetuximab and panitumumab (Anti-EGFR antibodies), BRAF inhibitors, combination of pertuzumab and trastuzumab (HER2-targeted), Pembrolizumab (Immune checkpoint inhibitors), combinations	NA	Japan	NA	RAS, BRAF V600E, HER2, MMR, MSI	Mononucleotide markers are more sensitive and specific than dinucleotide markers for detecting MSI, NGS-based testing is highly sensitive and specific	IHC testing is used to detect specific proteins in tissue samples, such as HER2 and MMR proteins.	RAS testing, BRAF testing, HER2 testing, MSI testing, IHC testing, NGS based testing, tissue based CGP, liquid biopsy

		such as FOLFIRI (irinotecan, 5-FU, leucovorin), FOLFOX (oxaliplatin, 5-FU, leucovorin), and FOLFOXIRI (oxaliplatin, irinotecan, 5-FU, leucovorin) with FOLFOXIRI combined with bevacizumab (Chemotherapy regimens)					for detecting genetic mutations and alterations in colorectal cancer, ctDNA-based testing is highly sensitive for detecting minimal residual disease (MRD)		
Baraniskin A. et al, 2023	10.3389/fonc.2023.1141833	FOLFOX4 (a combination of folic acid, fluorouracil, and oxaliplatin) and bevacizumab	47 year old	NA	1	NRAS, TP53	ddPCR	NGS	ddPCR
Benhaim L. et al, 2021	10.1016/j.ejca.2021.09.004	NA	30.1 - 86.1 (mean = 66.7)	France	187 (3 without plasma)	WIF1, NPY	Before surgery (D0) = 50%, After 5 days of surgery (D5) = 27%, by ddPCR	D0 = 77%, D5 = 93%, by ddPCR	ddPCR, targeting mutation & methylation markers
Bonilla A. et al, 2021	10.1002/onco.13738	FOLFOX, FOLFIRI, FOLFOXIRI, CAPOX, Cetuximab, Bevacizumab	50 - 84	NA	NA	KRAS, BRAF, MSI, NDRG4 & BMP3	92.3% for detecting CRC & 42.4% for detecting advanced	Overall = 87%, by Cologuard test	PCR, NGS, Blood / Stool based testing

							precancerous lesions, by Cologuard test, High grade dysplasia = 69.2%, Serrated sessile Polyps (≥1cm) = 42.4%		
Boysen A. et al, 2020	10.1080/0284186X.2020.1806357	NA	Older than 18	Aarhus University Hospital, Denmark	35	KRAS, NRAS, BRAF	ddPCR with limit of detection of 0.1%	Mass Spectroscopy (MassARRAY (MA) Agena Bioscience)	Surgery, Radiofrequency Ablation (RFA) & Stereotactic Body Radiotherapy (SBRT)
Chan H. et al, 2023	10.3389/fonc.2022.1055968	NA	42 - 88 (median = 66)	Tokyo	38	ctDNA, TP53, KRAS, PIK3CA, APC and VAF	By Landmark ctDNA analysis:- 67% sensitivity for recurrence detection & By Longitudinal ctDNA analysis:- 100%	By Landmark ctDNA analysis:- 87% specificity for recurrence detection & By Longitudinal ctDNA analysis:-	NGS using Oncomine Pan-Cancer Cell-Free Assay, DNA Extraction, Sequencing Data Analysis by using Torrent Suite Software and Ion Reporter, Statistical Analysis

							sensitivity for recurrence detection.	84% specificity for recurrence detection	performed using Kaplan-Meier method and Cox proportional hazards regression analysis.
Chen G. et al, 2021	10.1186/s13045-021-01089-z	NA	19 - 84 (median = 60)	China	276	SMAD4, PTEN, and PKHD1	Preoperative - 64.2% in plasma, 652.% in stage 2 & 63.3% in stage 3, Postoperative - At day 3-7, hazard ratio of 10.98 (95% Cl, 5.31 - 22.72; P<0.001) was observed, Post-adjuvant Chemotherapy - After ACT, the patients were observed to be 12 times more at risk for	Preoperative - 1005 in both stage 2 & 3, Postoperative - At day 3 - 7 found specificity of 91.7%, Post-ACT - Specificity of 94.1% is found, Surveillance Specificity - the specificity was 94.1%	Targeted sequencing, Variant Identification, ctDNA detection

							recurrence (HR, 12.76; 95% Cl, 5.39 - 30.19; P<0.001), Surveillance Sensitivity:- Found high risk of recurrence (HR, 32.02; 95% Cl, 10.79 - 95.08; P<0.001).		
Eslinger C. et al, 2024	10.21037/jgo-24-81	Pembrolizumab	75 year old (male)	Phoenix, Arizona	1	MSH6, MLH1, MSH2, MSH6, PMS2, ACVR2A, APC, B2M, CDK12, CDKN2A, FANCE, FBXW7, KEAP1, KMT2B, KMT2C*, MAP3K1, MSH3, PIK3CA, PIK3R1, RASA1,	NA	NA	Tumor-informed assays

						SMARCA4, SOX9, TCF7L2, TSC2, ARID1A, ARID2, ASXL1, ATM, EP300, ERBB2, ERBB3, GNAS, HRAS, RNF43, GLYR1, KMT2C* & ZBTB7A.			
Fan W. et al, 2024	[10.1177/17588359231220607](https://doi.org/10.1177/17588359231220607)	NA	18 years & older	China	309	ZFP36L2, GAB2, CTNNB1, MLL3 & ATR	Sensitivity of ctDNA based MRD was found to be 64.6%.	Specificity of ctDNA based MRD was found to be 94.8%	NGS & ctDNA based MRD assay
Faulkner L. et al, 2022	[10.1038/s41416-022-02017-9](https://doi.org/10.1038/s41416-022-02017-9)	NA	55 - 73	North America, Europe, Asia & Australia	3002	KRAS, TP53, APC, PIK3CA, BRAF, CTNNB1, hypermethylated genes like SEPT9,	Sensitivity of ctDNA detection post-surgery was found to be significant with HR of 6.92 (Cl: 4.49 -	Specificity of ctDNA detection was found with more true negative & less false positive	PCR-based methods, NGS

						WIF1, and NPY	10.64, P<0.00001)		
Frydendahl A. et al, 2024	10.3390/ijms25084252	NA	NA	NA	364	categories like Single-Nucleotide Variants (SNVs), Insertions and Deletions (INDELs), Multi-Nucleotide Variants (MNVs), Phased Variants	Sensitivity of UMIseq was found to be 80% in training cohort & 70% in the validation cohort	Specificity of UMIseq was found to be 95%	UMIseq
Gao Z. et al, 2023	10.1186/s12967-023-03884-3	NA	29 - 75 (median = 61)	Beijing, China	124	733-gene panel for tumor tissue and a 127-gene panel for circulating tumor DNA (ctDNA) detection	Predicting recurrence after 24 months was found to be 49.6%	Predicting recurrence after 24 months was found to be 86.5%	NGS
Gong J. et al, 2022	10.3892/mco.2022.2533	Regorafenib & Pembrolizumab	55 - 74	United States	4	ctDNA & mSEPT9	NA	NA	modified Epi proColon® 2.0 CE assay for ctDNA testing on the methylated SEPTIN9 gene (mSEPT9)

Henriksen T. et al, 2022	[10.1158/1078-0432.CCR-21-2404](https://doi.org/10.1158/1078-0432.CCR-21-2404)	adjuvant chemotherapy (ACT) treatment used was fluoropyrimidine combined with oxaliplatin	18 years and older	Danish	168	patient-specific somatic single-nucleotide variants (SNVs)	sensitivity of ctDNA detection postoperatively was a strong predictor of recurrence with a hazard ratio (HR) of 7.0	specificity of ctDNA detection postoperatively was also high, with a specificity rate of 80%	multiplex-PCR and next-generation sequencing to profile patient-specific somatic single-nucleotide variants
Henriksen T. et al, 2023	[10.1016/j.annonc.2023.11.009](https://doi.org/10.1016/j.annonc.2023.11.009)	NA	64 - 77 (median - 71)	Danish	851	patient-specific clonal mutations	sensitivity of post-operative ctDNA detection was 35%	specificity of post-operative ctDNA detection was 98%	digital PCR (dPCR)
Jain S. et al, 2024	[10.3389/fonc.2024.1426941](https://doi.org/10.3389/fonc.2024.1426941)	NA	17 - 90 (median - 59)	Asian countries	215 patients with 279 samples	ctDNA	current version of the assay reported 81% sensitivity with serial samples for colon cancer	Post-treatment sample level specificity of 98.2%	Next-generation sequencing (NGS)
Jiang H. et al, 2023	[10.3389/fonc.2023.1153685](https://doi.org/10.3389/fonc.2023.1153685)	NA	NA	China	67	AKT1, APC, BRAF, CTNB1, EGFR, ERBB2, FBXW7, GNAS, KRAS, MAP2K1,	sensitivity of the tumor-naïve strategy for detecting postoperative molecular residual disease	NA	Next-generation sequencing (NGS) panels containing 15 colorectal cancer hotspot mutated genes

						NRAS, PIK3CA, SMAD4, TP53	(MRD) was 37%		
Kalil J. et al, 2024	10.3389/fonc.2024.1418696	NA	44 - 80 (median - 61)	Canada	29	KRAS and PIK3CA mutations	81% of patients with detectable ctDNA at the time of liver resection developed disease recurrence	46% of patients with undetectable ctDNA at the time of liver resection developed disease recurrence	Digital PCR (dPCR)
Kasi P. et al, 2021	10.1136/bmjopen-2020-047831	NA	18 years of age or older	United States	2000 patients with stage I–IV colorectal cancer	tumor-specific clonal variants	The study also showed disease recurrence up to 16.5 months ahead of radiological imaging (average 8.7 months) with serial ctDNA analysis (sensitivity 88% and specificity 98%). A follow-up study by the	The study also showed disease recurrence up to 16.5 months ahead of radiological imaging (average 8.7 months) with serial ctDNA analysis (specificity 98%).	personalised ctDNA assay (Signatera bespoke mPCR-NGS)

							same group enrolled 198 patients with stage I–III CRC and showed an HR of 14 (95% CI 6.1 to 30), Our most recent study stage IV patients with CRC found that postresectio n ctDNA status was predictive of disease progression, with a sensitivity of 72% and a positive predic tive value of 96.7%. MRD-positivity was associated with a marked reduction in disease-free survival		

							(DFS) (HR: 5.8; 95% CI 3.5 to 9.7; p<0.001) and overall survival (OS) (HR: 16.0; 95% CI 3.9 to 68.0, p<0.001).		
Kasi P. et al, 2022	10.7759/cureus.26648	Larotrectinib	43 year old woman	United States & Pakistan	1	MLH1, PMS2, MSH2, MSH6, KRAS, NRAS, BRAF, HER2, NTRK1	NA	NA	Next-generation sequencing (NGS) and immunohistochemistry (IHC)
Kataoka K. et al, 2024 AR&C	10.23922/jarc.2024-025	modified-FOLFOXIRI (mFOLFOXIRI), which includes irinotecan, oxaliplatin, l-leucovorin, and 5-fluorouracil	20 - 75	Japan	85	ctDNA	NA	NA	clinically validated, personalized, tumor-informed, 16-plex PCR-NGS assay (Signatera™, Natera, Inc.)
Kataoka K. et al, 2024 ESMO	10.1016/j.annonc.2024.08.2240	adjuvant chemotherapy (ACT) with drugs such as 5-fluorouracil (5-FU) and oxaliplatin	34 - 85	Japan	190	ctDNA	sensitivity of ctDNA detection in the MRD window was 32.1%	NA	personalized, tumor-informed 16-plex polymerase chain reaction-next-generation sequencing

									(mPCR-NGS) assay
Kopetz S. et al, 2022	10.1158/1078-0432.CCR-21-2181	NA	NA	United States	NA	SEPT9, WIF1 and NPY	sensitivity of the plasma-only MRD assay was 55.6%, which improved to up to 91% by integrating epigenomic signatures and incorporatin g serial longitudinal and surveillance samples	specificity of the plasma-only MRD assay was 100%	plasma-only ctDNA assays and methylation signatures
Li X. et al, 2024	10.1038/s44321-024-00115-0	NA	NA	Shangh ai, Beijing, and New Haven	57	BRAF, KRAS, TP53, APC & PIK3CA	sensitivity of AccuScan was 90% for colorectal cancer and 67% for esophageal cancer	specificity of AccuScan was 100% for both colorectal and esophagea l cancers	whole genome sequencing (WGS) with single-read error correction
Liu W. et al, 2022	10.1016/j.ebiom.2022.103945	neoadjuvant chemotherapy with capecitabine and oxaliplatin	29 - 68	China	60 with locally advance d rectal cancer (LARC)	KRAS, TP53, APC & SMAD4	post-NAT ctDNA fraction in predicting recurrence was 76.47%	post-NAT ctDNA fraction in predicting recurrence	targeted sequencing of a panel of 509 genes or exomes

								was 97.67%	
Lonardi S. et al, 2022	10.3390/ijms231911529	NA	median - 60.1	Italy & United States	69	KRAS, NRAS, APC, TP53, PIK3CA, BRCA1, EGFR, ROS1, KMT2D, POLE, and ABL1	overall sensitivity at the follow-up time point was 69%	overall specificity at the follow-up time point was 100%	tissue comprehensive genomic profiling (CGP)-informed ctDNA assay
Loupakis F. et al, 2021	10.1200/PO.21.00101	NA	median - 60.1	Italy	112	KRAS mutations: G12A, G12C, G12D, G12R, G12S, G12V, and G13D	assay showed a sensitivity of 72%	assay showed a specificity of 93.3%	personalized and tumor-informed ctDNA assay (bespoke multiple PCR, next-generation sequencing assay)
Luo J. et al, 2023	10.1097/MD.0000000000034330	: The patient received systemic therapy and local treatment, including FOLFOX, XELOX, and a combination therapy of bevacizumab plus FOLFIRI	45 year old man	China	1	KRAS (particularly the G12V mutation), APC, TP53, TCF7L2	NA	NA	next-generation sequencing-based ctDNA profiling
Lutfi A. et	10.7759/cureus.43391	Pembrolizumab (PD-1 blockade)	50 year	United States	1	ctDNA & POLE	NA	NA	Next-generation

al, 2023 CURE US		and FOLFOXIRI with bevacizumab	old male						sequencing (NGS) and ctDNA assay using SignateraTM
Lutfi A. et al, 2023 KARGER	10.1159/000535174	Glecaprevir/pibrentasvir, a combination of HCV NS3/4A protease inhibitor and an HCV NS5A inhibitor	62 year old male	United States	1	ctDNA	NA	NA	Liquid biopsy assays
Nagarajan A., 2022	10.1016/j.suronc.2022.101738	chemoradiotherapy (CRT), CAPOX, FOLFOX4, and modified FOLFIRINOX	NA	United States	1206 patients receiving total neoadjuvant therapy (TNT) and 1210 patients treated with CRT	ctDNA	NA	NA	Liquid biopsy, Ultradeep sequencing, Serial ctDNA analysis, Epigenomic-based ctDNA assay
Nakamura Y. et al, 2024 AACR	10.1158/1078-0432.CCR-24-1651	NA	20 years and older	Japan	342	ctDNA	sensitivity for recurrence detection was 81% for colon cancer and 60% for rectal cancer	specificity was 98.2% among posttreatment samples	Guardant Reveal assay, which involves next-generation sequencing and bioinformatic analysis

Nakamura Y. et al, 2024 Nature	10.1038/s41591-024-03254-6	NA	28 - 95 (median - 69)	Japan	2240	RAS mutations, BRAF V600E mutation, Microsatellite instability (MSI), KRAS G12C mutation, ERBB2 amplification, TP53 Y220C mutation, NTRK1/2/3 fusion, RET fusion	sensitivity of the ctDNA assay is reported as greater than 95% at 0.01% mean variant allele frequency	specificity of the assay is reported to be 99.7%	Tumor-informed ctDNA testing was performed using a personalized, 16-plex polymerase chain reaction (PCR) next-generation sequencing (NGS) assay (Signatera, Natera, Inc.)
Nakamura Y. et al, 2024 Text	10.1158/1078-0432.CCR-24-2396	NA	37 - 89 (median - 67.7)	Japan & Taiwan	80	ctDNA	clinical sensitivity at the landmark timepoint (LMT) was 61.1%, and the longitudinal sensitivity was 83.3%	clinical specificity at the landmark timepoint (LMT) was 87.9%, and the longitudinal specificity was 89.5%	tumor-naive ctDNA assay

Nakano T. et al, 2023	10.1111/cas.16149	Capecitabine plus oxaliplatin (CAPOX) as adjuvant chemoradiotherapy (ACT)	NA	Japan	173	FGD5, GPC6 & MSC	sensitivity of the AMUSE assay was 78.6%	specificity of the AMUSE assay was 89.5%	Digital polymerase chain reaction (dPCR) using the AMUSE assay
Nguyen H. et al, 2022	10.3389/fonc.2022.1069296	Cetuximab and Panitumumab	27 - 85 (median - 60)	Vietnam	103	APC, TP53, and KRAS. Additionally, genes such as FAT4, NRAS, PIK3CA, and TRRAP are highlighted	ctDNA detection rate in pre-operative plasma samples was 90.5%	specificity of the ctDNA detection assay was reported to be over 99%	personalized multiplex PCR and ultra-deep sequencing
Ohta R. et al, 2021	10.1016/j.ejso.2021.07.017	NA	over 20 years	Japan	200	KRAS mutation sites in exon-2: G12A, G12C, G12D, G12R, G12S, G12V, and G13D	sensitivity of detection using ctDNA was 29.8%, and using trtDNA was 33.3%	specificity of detection using ctDNA was 94.8%, and using trtDNA was 93.1%	Droplet digital PCR (ddPCR) was used to detect KRAS mutations
Parikh A. et al, 2021 Dec	10.1158/1078-0432.CCR-21-3306	NA	NA	United States	NA	ctDNA	landmark 30-day sensitivity of 41%	specificity of 95.4% for all patients on the study	plasma-only circulating tumor DNA (ctDNA) assay

Parikh A. et al, 2021 Oct	10.1158/1078-0432.CCR-21-0410	NA	35 - 84 (median - 60)	United States	103	ctDNA	sensitivity for recurrence prediction was 55.6% at the landmark timepoint, which improved to 69% with longitudinal plasma monitoring and 91% with surveillance draws	specificity for recurrence prediction was 100%	plasma-only ctDNA assay integrating genomic and epigenomic cancer signatures
Parikh A. et al, 2024	10.1158/1078-0432.CCR-23-3660	NA	39 - 75 (median - 56)	United States	52	ctDNA	sensitivity of ctDNA analysis at the primary time point was 58.1% for any future recurrence	specificity of ctDNA analysis at the primary time point was 90.9%	the Guardant Reveal assay
Pellini B. et al, 2020	10.1200/PO.20.00276	neoadjuvant chemotherapy	median - 57	United States	24	TP53, APC, KRAS, PIK3CA, FBXW7, SOX9, and NRAS	sensitivity of plasma ctDNA MRD detection was 95%	specificity of plasma ctDNA MRD detection was 100%	AVENIO targeted next-generation sequencing
Pretta A. et	10.5306/wjco.v13.i12.980	5-fluorouracil, oxaliplatin, and	NA	Italy	80	ctDNA	NA	NA	liquid biopsy

al, 2022		folinic acid (FOLFOX) in the PePiTA trial							
Pu W. et al, 2024	10.21147/j.issn.1000-9604.2024.04.07	NA	NA	China	NA	ctDNA	ctDNA had a substantially higher detection performance than CEA (88% and 69%, respectively, with sensitivity of 98%) in predicting tumor recurrence.	ctDNA had a substantially higher detection performance than CEA (88% and 69%, respectively, with specificity of 64%) in predicting tumor recurrence.	methylation-specific polymerase chain reaction (PCR), droplet digital PCR (ddPCR), microarray, and next-generation sequencing (NGS)
Qi Z. et al, 2023	10.1002/cam4.6286	NA	29 - 79 (medain - 60)	China	127	ctDNA	detection of three positive sites was sufficient to achieve nearly 100% overall sample level sensitivity	specificity of the ctDNA MRD detection was 98.9%	ultradeep sequencing of plasma-free DNA using a tumor-informed assay
Reinert T. et al, 2021	10.1158/1078-0432.CCR-21-2735	NA	NA	Denmark	NA	ctDNA	sensitivity of the ctDNA assay in the	challenges in assessing the true	plasma-only circulating tumor DNA (ctDNA) assay

AARC Letter							surveillance analysis was artificially inflated from 55.6% to 90.9% due to the exclusion of 7 false negative patients	specificity and positive predictive value (PPV) due to selection bias and other factors	
Reinert T. et al, 2021	10.1002/ijc.33924	NA	45.1 - 89.4 (median - 67.7)	Denmark	115	APC, BRAF, KRAS, NRAS, PIK3CA & TP53	sensitivity for detecting recurrence during surveillance was 72.3%	specificity for detecting recurrence during surveillance was 100%	Droplet digital PCR (ddPCR)
Ryoo S. et al, 2023	10.1038/s41416-023-02300-3	NA	median - 67	Korea	98	KRAS & BRAF	sensitivity of postoperative 3-week MRD positivity predicting recurrence within 3 years was 61.9%	specificity of postoperative 3-week MRD positivity predicting recurrence within 3 years was 83.9%	tumour-informed, hybrid-capture ctDNA sequencing MRD assay
Schøler L. et al, 2017	10.1158/1078-0432.CCR-17-0510	NA	NA	Denmark	45	KRAS: Specific focus on mutations in codons	sensitivity of ctDNA analysis for detecting	specificity of ctDNA analysis for detecting	massively parallel sequencing

						12, 13, and 61. BRAF: Highlighted during mutational profiling alongside KRAS.	relapse was 100%	relapse was also 100%	
Schraa S. et al, 2020	10.1186/s12885-020-07252-y	Capecitabine plus oxaliplatin	18 years and older	Netherlands	1320 stage II colon cancer patients	APC, TP53, KRAS & BRAF	NA	NA	Tissue-informed detection of plasma ctDNA using the PGDx elio™ platform
Siravegna G. et al, 2017	10.1093/annonc/mdx095	Entrectinib	53 year old	Italy	1	CAD-ALK gene rearrangement, TP53 mutation & Emerging ALK kinase mutations	sensitivity of the assays developed to detect the CAD-ALK rearrangement in urine tr-DNA was high, as it anticipated radiological confirmation of disease progression	specificity of the assays was validated through TOPO TA cloning and Sanger sequencing, confirming the presence of the genomic rearrangement	Droplet digital PCR (ddPCR) and end-point PCR

Slater S. et al, 2023	10.1186/s12885-023-10699-4	adjuvant chemotherapy (ACT) with fluoropyrimidine (FP)-based chemotherapy, including capecitabine and oxaliplatin (CAPOX) or infusional 5-fluorouracil plus oxaliplatin (FOLFOX)	18 years and older	United Kingdom	1621	ctDNA	NA	NA	the Guardant Reveal plasma-only ctDNA assay
Slater S. et al, 2024	10.1158/1078-0432.CCR-24-0226	NA	≥18 years	United Kingdom	1,203 patients were enrolled, and plasma samples from 214 patients were analyzed with a new histologic diagnosis of stage I to III colorectal	RAS and RAF mutations, BRAF, Microsatellite instability-high (MSI-H) profiles, Genes involved in methylation patterns and epigenomic signals	Longitudinal sensitivity was 62.1% (95% CI, 42.2–79.3)	Longitudinal specificity was 85.9% (95% CI, 78.9–91.3)	The Guardant Reveal assay, which evaluates genomic and methylation signals using a multiomic next-generation sequencing (NGS) platform

					cancer (CRC)				
Tarazona N. et al, 2020	10.1136/esmoopen-2020-000847	NA	NA	Spain	150	CDX2 expression and circulating tumor DNA (ctDNA)	only positive ctDNA in plasma samples (HR 13.64; p=0.002) and lack of CDX2 expression (HR 23.12; p=0.001) were found to be independent prognostic factors for disease-free survival in the multivariable model.	CMS4 (37%) and CMS2 (28%), followed by CMS1 (20%) and CMS3 (15%) groups. CDX2-negative tumours were enriched in CMS1 and CMS4 subtypes.	ctDNA was tracked using droplet digital PCR, and CDX2 expression was analyzed by immunostaining
Tie J et al, 2019	10.1001/jamaoncol.2019.3616	Adjuvant chemotherapy, specifically oxaliplatin-based therapy and fluoropyrimidine	26n - 82 (median - 64)	Australia	100 patients were initially recruited, with 96 eligible patients included in the analysis	APC, TP53, KRAS, NRAS, PIK3CA, SMAD4, BRAF, FBXW7, TCF7L2, and PTEN	ctDNA was detectable in 20 of 96 (21%) postsurgical samples and 15 of 88 (17%) postchemotherapy samples	NA	Massively parallel sequencing and personalized Safe-Sequencing System (Safe-SeqS) assays

Tie J. et al, 2021	[10.1371/journal.pmed.1003620](https://doi.org/10.1371/journal.pmed.1003620)	NA	30.8 - 84.7 (median - 64)	Australia	54	SMAD4, TP53, AKT1, APC, BRAF, CTNNB1, ERBB3, FBXW7, HRAS, KRAS, NRAS, PIK3CA, PPP2R1A, RNF43, POLE	sensitivity of ctDNA detection was 85% at baseline and 24% after surgery	NA	Safe-SeqS assay was used to detect ctDNA
Wang Y. et al, 2021	[10.1371/journal.pmed.1003741](https://doi.org/10.1371/journal.pmed.1003741)	neoadjuvant chemoradiotherapy (nCRT) which included concurrent capecitabine and irinotecan chemotherapy	median - 57	China	119	TP53, APC, KRAS, BRAF, PTEN, and PIK3CA	sensitivity of ctDNA clearance during nCRT was associated with a low probability of non-pCR (odds ratio = 0.11, 95% confidence interval [95% CI] = 0.01 to 0.6, P = 0.04)	specificity of ctDNA detection for predicting pathological complete response (pCR) was improved when combined with MRI information, achieving an area under the curve	deep targeted panel sequencing of 422 cancer-related genes

								(AUC) of 0.886	
Yang J. et al, 2024	10.4143/crt.2023.1371	NA	57 - 71 (median - 67)	China	75 patients with resectable stage I-III colorectal cancer (CRC)	TP53, APC, KRAS, PIK3CA, SMAD4, BRCA2, POLE, ERBB2, & PTEN	tumor-informed fixed assay had a higher preoperative positive rate than the tumor-agnostic assay (73.3% vs. 57.3%)	tumor-informed fixed assay demonstrated a significant association with worse disease-free survival (DFS) (hazard ratio [HR], 20.74; 95% confidence interval [CI], 7.19 to 59.83; $p < 0.001$)	Whole-exome sequencing (WES) based tumor-informed assay and panel-based personalized assays

Table 3. Summary of key studies investigating ctDNA as a biomarker for MRD in breast cancer. This table presents a comprehensive overview of selected studies evaluating the use of ctDNA in detecting MRD. Key characteristics of each study are listed, including publication details, therapeutic interventions, patient demographics, geographic locations, sample sizes, specific biomarkers evaluated, and reported sensitivity and specificity values. Techniques used for ctDNA detection and analysis (e.g., ddPCR, NGS, personalized assays) are also included. The data are categorized to highlight the diversity in study design, methodology, and performance metrics across different cohorts and technologies.

Paper Name	DOI	Drug	Age group	Patient location	# of patients	Specific biomarker	Sensitivity	Specificity	Technique
Capella M. et al, 2024	10.3892/ol.2024.14415	NA	46 year old pre-menopausal female	Treated in Montreal	1	ctDNA	88% sensitivity was observed to detect MRD by Signatera assay with a limit of detection of 0.01% detected by VAF, and the analytical sensitivity assay gave result of high sensitivity above 0.03% tumor DNA concentrations.	99.9% in wild-type DNA detected by Signatera assay with 0.01% limit of detection which is detected by VAF, by analytical sensitivity, the tumor DNA showed the sensitivity above 0.03%.	Histological Analysis, Immunohistochemistry, Magnetic Resonance Imaging (MRI), Breast Biopsy, Partial Mastectomy, Circulating Tumor DNA (ctDNA) Analysis, Radiation Therapy, Tumor Marker Assessment, Tumor Whole-Exome Sequencing, Multiplex PCR (m-PCR), NGS

Ciriaco N. et al, 2022	10.1177/17588359221139601	NA	38 - 88 (median = 57)	Spain	29	TP53, PIK3CA, NTRK1, NTRK3, HER2/ERBB2, GRM3, AKT3, FGF10, and FANCF	Sensitivity of combination was foud to be 93.33%	Specificity of combination was found to be 75%	NGS (SureSeq technology from Oxford Gene Technology), for liquid biopsy (Sysmex SafeSeEQ)
Cirmena G. et al, 2020	10.1155/2020/8132507	NA	32 - 73	Genoa, Italy	38 (early invasive)	ctDNA, TP53, PIK3CA, GATA3	Sensitivity of tagged targeted deep sequencing (tTDS) for detecting minimal residual disease (MRD) in breast cancer patients was higher compared to droplet digital PCR (ddPCR)	tTDS demonstrated a specificity of **100%** in identifying non-relapsed cases, meaning that it accurately identified all patients who did not experience a relapse	tagged targeted deep sequencing (tTDS) and droplet digital PCR (ddPCR)
Coakley M. et al, 2024	10.1158/1078-0432.CCR-23-2326	Pembrolizumab	NA	United Kingdom	141	ctDNA	Sensitivity of personaliz	Specificity of the RaDaR	Tumor-informed digital PCR (dPCR) &

							ed sequencing assays (RaDaR) was found to be 95.7%.	was found to be 91.0%	personalized multimutaion sequencing
Forte V. et al, 2016	10.28092/j.issn.2095-3941.2016.0007	NA	NA	Los Angeles, CA	NA	Epithelial markers: E-cadherin and cytokeratins, Mesenchymal markers: Vimentin and fibronectin, Transcription factors linked to EMT (Epithelial to Mesenchymal Transition): TWIST1, SNAIL1, SLUG, ZEB1, and FOXC2, Other	NA	NA	NGS

						markers: CD146 and CD49f			
Grisolia P. et al, 2024	10.1186/s12967-024-05734-2	NA	34.5 to 50.1	Frattamaggiore	23 BC patients with germline mutation of BRCA1 and BRCA2 genes, 19 healthy controls without BRCA1/2 mutation, and 2 healthy individuals who carried BRCA1/2 mutations	BRCA1, BRCA2, RFC3, PARP4, RFC1, EYA4, ACTL6A, FANCA, TFPT, UBE2W, RAD50, SUMO1, RRM2B, ERCC4, UBR5, SHLD1, ERCC6, ATR, ETS1, ERG, FLI1 & RUNX1	sensitivity of the identified differentially methylated regions (DMRs) was 88%	specificity of the identified DMRs was 94.74%	cell-free Methylated DNA Immunoprecipitation and High-Throughput Sequencing (cfMeDIP-seq)
Guan G. et al, 2020	10.3233/CBM-190523	NA	three age groups: <35, 35-50, and >50 years old	China	300 early breast cancer patients and 50 healthy volunteers	PIK3CA and TP53	Hazard ratios for patients in this category was 1.65 (95% CI 1.26-2.16)	NA	droplet digital PCR (ddPCR)

							and 1.98 (95% CI 1.48-2.63) respectively		
Hassan F. et al, 2024	10.1016/j.suronc.2024.102060	NA	45 - 65 (median = 51.5)	Ireland	62	PIK3CA, E542K, E545K, E545Q on exon 9 and H107R, H107L on exon 20	PIK3CA gene mutations in ctDNA in postoperative period (hazard ratio (H.R: 18.05, p = 0.001) were a negative prognostic factor for recurrence free survival (RFS) and overall survival (OS) (H.R: 11.9, p = 0.01) in patients with breast cancer.	NA	High-resolution melting (HRM) PCR and allele-specific fluorescence probe-based PCR were used to detect PIK3CA gene mutations in ctDNA

Hoang V. et al, 2022	10.1002/1878-0261.13356	PIQRAY (alpelisib)	at least 18 years old	Vietnam	134 early stage breast cancer	TP53, PIK3CA, GATA3, AKT1, NOTCH2, KDM6A, and NCOR2	sensitivity of the ctDNA detection assay was 0.05%	specificity of the ctDNA detection assay was greater than 99%	multiplex polymerase chain reaction (mPCR) and ultra-deep next-generation sequencing (NGS)
Jongbloed E. et al, 2023	10.2147/BCTT.S387375	Abemaciclib	Pre- and post-menopausal patients with a mean age of 57 years	Netherlands	Hypothetical population of 1000 early breast cancer (EBC) patients	14 known driver breast cancer genes	NA	NA	patient-specific digital PCR assays
Le A. et al, 2018	10.3390/ijms19092553	NA	NA	Australia, Singapore, and France	NA	Epithelial-Expression-Associated Genes: GRHL2, MIR200C, CDH1, Mesenchymal-Expression-Associated Genes: TWIST1, DKK3, CRABP1, VIM, EGFR,	NA	NA	methylation-sensitive high-resolution melting (MS-HRM) and pyrosequencing

						EMP-Unassigned or Other Biomarker Candidates: AKR1B1, APC, RASSF1A, RARβ, SFRP2, GFRA1			
Majure M. et al, 2016	10.21037/atm.2016.11.77	NA	NA	United States	55 women	PIK3CA and HER-2	ctDNA was detected in 69% (95% CI, 53–82%) of baseline plasma samples	ctDNA was detected in 19% (95% CI, 8–35%) of post-surgical serum samples	digital PCR (dPCR) and massive parallel sequencing (MPS)
Murillas I. et al, 2015	10.1126/scitranslmed.aab0021	NA	NA	United Kingdom	55	PIK3CA, TP53, FGFR1, ESR1, RB1, GATA3, STAT3, SYNE1, CDH1, ERBB2, TBX3, XIRP2, ANK3,	sensitivity of ctDNA detection in predicting early relapse was high, with a hazard ratio (HR) of 25.1 for a single postsurgic	specificity of ctDNA detection was also high, with a concordance index (C-index) of 0.78 for a single postsurgi	personalized digital polymerase chain reaction (dPCR) assays and high-depth targeted massively parallel sequencing (MPS)

						APOBEC 1	al sample and 12.0 for serial samples	cal sample and 0.75 for serial samples	
Murillas I. et al, 2024	10.1007/s10549-024-07508-2	NA	28 - 75 (median - 51)	United Kingdom & United States	61 female patients	ctDNA	sensitivity to detect ctDNA in plasma from patients who relapsed during the monitoring phase was 76.9%	specificity and positive predictive values were both 100%	Next-generation sequencing (NGS)
Parsons H. et al, 2023	10.1016/j.annonc.2023.08.004	Neoadjuvant chemotherapy with cisplatin or paclitaxel	NA	United States	139	ctDNA	sensitivity of ctDNA detection was 100% at baseline, 79% at week 3, and 55% at week 12	NA	personalized ctDNA assay utilizing MAESTRO mutation enrichment sequencing
Radovich M. et al, 2020	10.1001/jamaoncol.2020.2295	NA	mean - 49.6	United States	196 female patients	ctDNA & CTCs	the sensitivity to detect recurrences increased from 79% with	inferior DDFS (median DDFS, 32.5 months vs not reached;	ctDNA was sequenced using the FoundationACT or FoundationOneLiquid Assay, and CTCs were

							ctDNA alone and 62% with CTC alone to 90% when combined.	hazard ratio [HR], 2.99; 95% CI, 1.38-6.48; P = .006). At 24 months, DDFS probability was 56% for ctDNA-positive patients compared with 81% for ctDNA-negative patients. Detection of ctDNA was similarly associated with inferior DFS (HR, 2.67; 95% CI, 1.28-	enumerated using an epithelial cell adhesion molecule–based, positive-selection microfluidic device

								5.57; P = .009) and inferior OS (HR, 4.16; 95% CI,1.66-10.42; P = .002).	
Riva F. et al, 2016	10.1373/clinchem.2016.262337	NA	18 years and older	France	46 patients with nonmetastatic TNBC	TP53, BRCA1 and BRCA2	sensitivity of the customized droplet digital PCR (ddPCR) assays was at least 0.1%	specificity of the ddPCR assays was determined to be 2 droplets per assay	Droplet digital PCR (ddPCR)
Santonja A. et al, 2022	10.15252/emmm.202216505	NA	NA	United Kingdom	breast cancer, including both early-stage (n = 4) and late-stage (n = 3), 7	Single Nucleotide Variants (SNVs), Structural Variants (SVs), Somatic Copy-Number Aberrations (SCNAs) & ALK and ROS1 gene fusions	hybrid capture sequencing targeting between 1,347 and 7,491 tumor-identified mutations at high depth was the most sensitive assay, detecting ctDNA	specificity of the assays was measured to be greater than 95%	multiplex PCR, hybrid capture, and different depths of whole-genome sequencing

							down to an allele fraction (AF) of 0.00024% (2.4 parts per million, ppm)		
Sharf M. et al, 2022	10.1200/JCO.22.00908	NA	29 - 71 (median - 53)	United States	103	PIK3CA, CDH1, TP53, RECQL4, KMT2C, MAP3K1, GATA3, NF1, PTEN, TBX3, ARID1A, PAK1, AKT1, CCND1, FGFR1, ERBB2, ESR1, GNAS, RARA, FOXA1, MYC, MCL1	sensitivity of ctDNA detection for identifying any clinical recurrence was 85.7%	specificity of ctDNA detection for identifying any clinical recurrence was 97.4%	whole-exome sequencing (WES) and personalized ctDNA assays
Stergiopoulou D. et al, 2023	10.1038/s41598-022-25400-1	NA	NA	Greece	13	CK-19 (Cytokeratin-19), CD24, CD44,	NA	NA	CTC enumeration using the CellSearch system,

						ALDH1, TWIST1, PIK3CA mutations (e.g., E545K, H1047R), ESR1 mutations (e.g., Y537C, Y537N, Y537S, D538G), DNA methylation of the ESR1 promoter.			phenotypic analysis using Immunofluorescence, gene expression analysis, mutation analysis, and DNA methylation analysis
Tokura M. et al, 2024	[10.1177/11782234241288671](https://doi.org/10.1177/11782234241288671)	Neoadjuvant chemotherapy (NAC) with anti-HER2 drugs	20 years or older	Japan and the Philippines	60	ERBB2 (HER2) and TP53	The minimum detection sensitivity of SNV/indel tested by the TOP using commercially available standard tissue DNA is approximately 5%.	NA	Todai OncoPanel (TOP) and DNA methylation analysis

							Furthermore, the minimum detection sensitivity for copy number is 4 copies.		
Yang Y. et al, 2022	10.21037/gs-22-634	NA	29 - 79 (median - 45)	China	82	BRCA2, SLX4, FGFR1, TP53, MYC, ERBB2, PIK3CA, GATA3, CDK12, PTEN, FAT1, CHEK2	NA	NA	next-generation sequencing (NGS) based on a 1,021-gene panel
Zhang X. et al, 2024	10.21037/gs-24-115	NA	≤50 years and >50 years	China	90 patients with early breast cancer and 24 healthy women	TP53 (12.3%), TERT (7%), FAT1 (7%), TSR2 (5.3%), SMARCA4 (5.3%), NF1 (5.3%), CDH23 (5.3%), PIK3CA	sensitivity of ctDNA detection was 67.9% among patients with invasive breast cancer	NA	targeted deep sequencing with a next-generation sequencing (NGS) panel of 1,021 cancer-related genes