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Clinical Utility of Circulating Tumor DNA (ctDNA) in the Bloodstream as Predictive and Prognostic Biomarkers in Breast Cancer Management

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Breast cancer remains one of the most prevalent malignancies among women worldwide, with early detection and individualized treatment significantly improving patient outcomes. In recent years, biomarkers have emerged as pivotal tools in enhancing diagnostic precision, predicting therapeutic response, and monitoring disease progression. Among these, circulating tumor DNA (ctDNA) and liquid biopsy techniques have gained substantial attention due to their non-invasive nature and potential to provide real-time insights into tumor dynamics.
Objective: This review aims to evaluate the clinical utility of liquid biopsy and ctDNA as predictive and prognostic biomarkers in breast cancer management.
Methods: A comprehensive literature review was conducted using peer-reviewed publications from databases such as PubMed, Scopus, and Web of Science. Studies were selected based on

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relevance, recency, and clinical significance, focusing on techniques for ctDNA extraction, quantification, and mutational analysis, including digital PCR and next-generation sequencing.
Results/Findings: Emerging evidence highlights that ctDNA can reflect tumor heterogeneity, detect minimal residual disease (MRD), and predict relapse earlier than traditional imaging methods. Liquid biopsy offers a less invasive alternative for molecular profiling and treatment monitoring, especially in metastatic settings. Mutations in genes such as PIK3CA, ESR1, and TP53 detected through ctDNA have been correlated with resistance to endocrine therapy and targeted agents, aiding in treatment planning.
Conclusion: Liquid biopsy and ctDNA analysis represent a transformative approach in the precision medicine landscape of breast cancer. Their integration into clinical practice may optimize patient stratification, therapeutic decision-making, and surveillance. However, standardization of methods and validation through large-scale clinical trials are essential for routine implementation.

Keywords: Breast cancer; liquid biopsy; ctDNA; predictive biomarker; prognostic biomarker.

1. INTRODUCTION

Breast cancer is the most commonly diagnosed cancer and a leading cause of cancer-related mortality among women worldwide. According to recent global cancer statistics, breast cancer accounts for over 2.3 million new cases and approximately 685,000 deaths annually, underscoring its significant public health burden. Despite advancements in screening, diagnosis, and therapeutic interventions, challenges persist in the early detection, real-time monitoring, and individualized treatment of breast cancer, particularly in cases of recurrence or metastasis.

Traditionally, tissue biopsy has been the gold standard for tumor characterization, guiding diagnosis and therapeutic decisions. However, tissue biopsies are invasive, often limited by tumor accessibility, and May not adequately capture the genetic heterogeneity or dynamic evolution of the disease. Furthermore, repeated biopsies to monitor treatment response or emerging resistance are often impractical and carry procedural risks.

To overcome these limitations, there is a growing interest in non-invasive biomarkers that can provide comprehensive and real-time insights into tumor biology (Zhang & Yuan, 2025). Liquid biopsy, a technique that analyzes tumor-derived components in body fluids, particularly blood, has emerged as a promising alternative. One of the most studied components of liquid biopsy is circulating tumor DNA (ctDNA), which consists of fragmented DNA shed into the bloodstream by cancer cells through apoptosis, necrosis, or active secretion.

ctDNA analysis offers a unique opportunity to detect specific genetic alterations, monitor treatment response, identify minimal residual disease (MRD), and predict disease recurrence with high sensitivity and specificity. As such, liquid biopsy represents a transformative shift toward precision oncology in breast cancer.

1.1 Objective

This article aims to explore the clinical utility of liquid biopsy and ctDNA as predictive and prognostic biomarkers in breast cancer, highlighting their advantages, technological advancements, and current challenges in clinical implementation.

2. MOLECULAR BASIS OF CIRCULATING TUMOR DNA (CTDNA)

2.1 Origin of ctDNA in the Bloodstream

Circulating tumor DNA (ctDNA) refers to fragmented DNA released into the bloodstream by cancer cells through several biological processes, including apoptosis, necrosis, and active secretion. Unlike normal cell-free DNA (cfDNA), ctDNA originates specifically from malignant cells and contains tumor-specific genetic and epigenetic alterations. The quantity and characteristics of ctDNA in circulation often correlate with tumor burden, stage, and biological aggressiveness. In breast cancer, ctDNA can be detected across all disease stages, including early diagnosis, treatment monitoring, and detection of minimal residual disease (MRD) (Chen, Geng, & Lucci, 2025).

2.2 Molecular Features of ctDNA

ctDNA harbors key molecular hallmarks reflective of the tumor genome. These include:

- **Somatic mutations:** Single nucleotide variants (SNVs), insertions/deletions (indels), and copy number alterations in cancer-related genes such as *TP53, PIK3CA, ESR1,* and *BRCA1/2.*

- **Epigenetic modifications:** Aberrant DNA methylation patterns, particularly in promoter regions of tumor suppressor genes, serve as highly specific markers for malignancy.

- **Fragmentomics:** The analysis of ctDNA fragment size, end motifs, and nucleosomal footprints reveals that ctDNA is typically shorter (~90–150 base pairs) than non-tumor cfDNA and carries distinct fragmentation profiles that can aid in its identification (Hadd et al., 2025).

2.3 Detection Methods

The sensitive and specific detection of ctDNA from blood samples remains a technical challenge due to its low abundance amidst high background cfDNA. However, several advanced technologies have been developed to address this:

- **Digital PCR (dPCR):** A highly sensitive method that partitions a DNA sample into thousands of micro-reactions, enabling precise quantification of specific mutations. It is cost-effective and suitable for known hotspot mutations but limited in multiplexing capacity.

- **Next-Generation Sequencing (NGS):** NGS allows for broad genomic profiling of ctDNA across multiple genes or entire exomes. Techniques such as targeted panels (e.g., Cancer SEEK, Foundation One Liquid) can detect a wide range of mutations, fusion genes, and copy number alterations. Ultra-deep sequencing with error-correction methods increases sensitivity and accuracy (Janni et al., 2025).

- **BEAMing (Beads, Emulsion, Amplification, and Magnetics):** This combines emulsion PCR and flow cytometry to detect and quantify mutations in ctDNA with high sensitivity. It is especially effective for monitoring specific known mutations during treatment.

Each method has its own advantages and limitations in terms of sensitivity, cost, turnaround time, and clinical applicability. A combination of methods is often used to optimize ctDNA analysis for clinical use.

3. CLINICAL APPLICATIONS OF CTDNA IN BREAST CANCER

The application of circulating tumor DNA (ctDNA) in the clinical management of breast cancer represents a major advancement in personalized oncology. ctDNA enables non-invasive, real-time monitoring of tumor biology, which can guide clinical decision-making across several stages of disease management.

A. Diagnostic Utility

Early detection of breast cancer is critical for improving survival outcomes. Traditional imaging methods (e.g., mammography) have limitations, including low sensitivity in dense breast tissue and false-positive results. ctDNA offers a promising alternative as a non-invasive biomarker for early detection due to its ability to reflect tumor-specific mutations and epigenetic changes in plasma even before clinical symptoms arise.

Emerging studies have explored ctDNA methylation signatures as sensitive markers for breast cancer screening. However, the routine use of ctDNA for population-wide screening is still under investigation, as sensitivity in early-stage, low-burden disease remains a challenge.

B. Predictive Biomarker

ctDNA is increasingly recognized for its role as a predictive biomarker, aiding in the selection of targeted therapies and monitoring therapeutic response.

- **Treatment Selection:** ctDNA analysis can detect actionable mutations such as ESR1 mutations, which are associated with resistance to aromatase inhibitors in hormone receptor-positive breast cancer. Similarly, detection of HER2 amplifications in ctDNA can guide anti-HER2 therapy in HER2-positive disease, even in cases of tumor heterogeneity.

- **Monitoring Treatment Response:** Quantitative changes in ctDNA levels during chemotherapy, endocrine therapy, or targeted therapy correlate with treatment efficacy. A rapid decline in ctDNA during treatment often reflects a good response, while persistently high or

rising levels may indicate resistance or progressive disease.

C. Prognostic Biomarker

ctDNA serves as a powerful **prognostic tool**, providing insights into disease progression, relapse risk, and survival outcomes.

- **Prediction of DFS and OS:** Elevated ctDNA levels after surgery or during systemic therapy have been associated with shorter disease-free survival (DFS) and overall survival (OS), particularly in triple-negative and HER2-positive subtypes.
- **Minimal Residual Disease (MRD) and Relapse Prediction:** ctDNA is increasingly used to detect MRD following curative-intent therapy. ctDNA can reveal the presence of residual microscopic disease not visible on imaging, often predicting relapse months before clinical symptoms or radiologic findings. This allows for earlier therapeutic intervention.

D. Resistance Monitoring

One of the most impactful clinical applications of ctDNA is in tracking the development of treatment resistance.

- **Detection of Resistance Mutations:** Acquired mutations such as PIK3CA, ESR1, and HER2 mutations can be identified via ctDNA, offering insight into evolving resistance mechanisms. For example, ESR1 mutations are associated with endocrine therapy resistance, while PIK3CA mutations may confer resistance to certain kinase inhibitors.
- **Therapy Adjustment in Real-Time:** Longitudinal ctDNA monitoring enables oncologists to adjust treatment strategies dynamically based on molecular evolution. This includes switching to alternative targeted therapies or enrolling patients in mutation-specific clinical trials, thereby improving patient outcomes through a precision medicine approach.

Conclusion of Section: The integration of ctDNA into clinical practice is reshaping the management of breast cancer. From diagnosis to resistance monitoring, ctDNA offers a minimally invasive, dynamic, and highly informative approach to personalize treatment and improve prognosis. Ongoing clinical trials and technological advancements will further enhance its clinical utility and standardization.

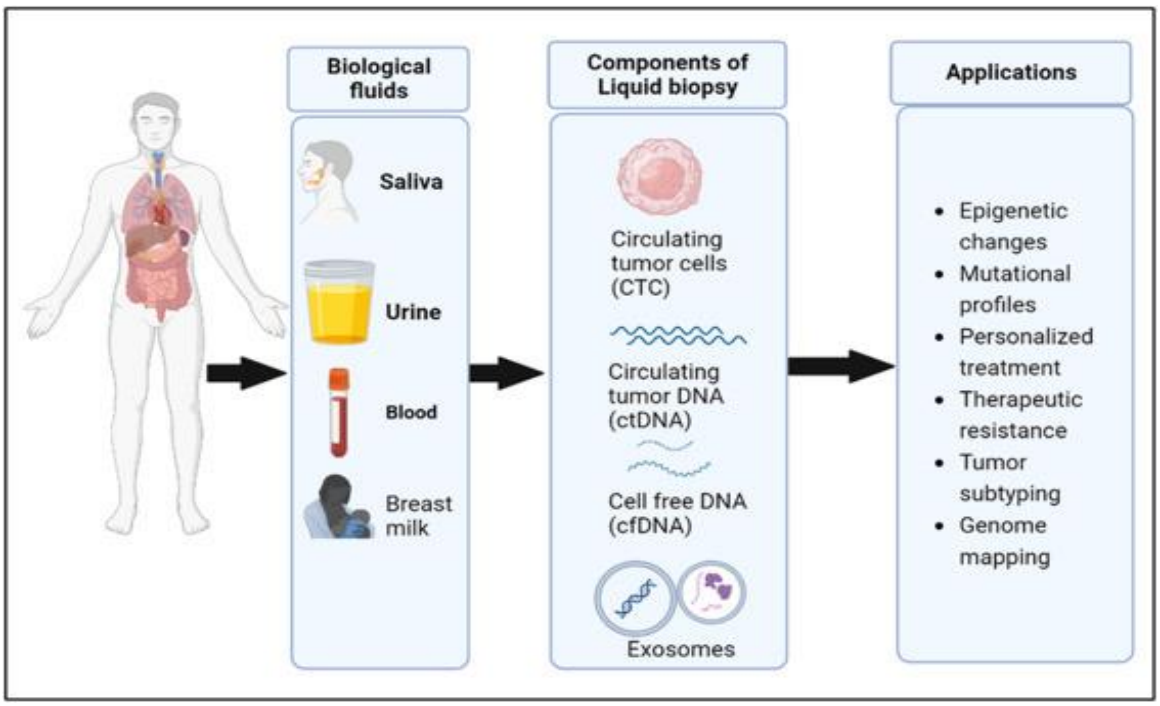

Fig. 1. Workflow of liquid biopsy in clinical practice

4. COMPARATIVE ADVANTAGES OF LIQUID BIOPSY

Liquid biopsy, particularly through the analysis of circulating tumor DNA (ctDNA), has emerged as a valuable tool complementing or, in some instances, replacing traditional tissue biopsy. While tissue biopsy remains the gold standard for initial tumor diagnosis and histopathological evaluation, it has several limitations that can be effectively addressed by liquid biopsy.

A. Safety and Minimally Invasive Nature

Tissue biopsy procedures, such as core needle or surgical biopsies, are inherently invasive and associated with risks, including bleeding, infection, pain, and potential complications, especially in anatomically challenging or inaccessible tumor sites (e.g., lung, brain, or bone metastases). These procedures are often not feasible in frail or elderly patients or those with comorbid conditions.

In contrast, liquid biopsy requires only a simple venipuncture (blood draw), which significantly reduces procedural risks and discomfort. This makes it a safer and more acceptable option for patients, particularly for repeated sampling during treatment and follow-up (Chen et al., 2025).

B. Repeatability and Real-Time Monitoring

A major limitation of tissue biopsy is its inability to be repeated frequently. Repeated tissue sampling is not only invasive but may be logistically and ethically impractical. This restricts its use in dynamic disease monitoring.

Liquid biopsy, being non-invasive, enables serial sampling at multiple time points, allowing clinicians to monitor tumor evolution, treatment response, and emergence of resistance mutations in real time. This temporal tracking enhances the ability to adjust treatment strategies promptly, thereby improving clinical outcomes (Elliott et al., 2025).

C. Assessment of Tumor Heterogeneity

Tissue biopsy samples represent only a snapshot of a localized portion of the tumor, often missing intratumoral and intertumoral heterogeneity—particularly relevant in metastatic or multifocal breast cancer. This can result in an underestimation of molecular diversity, leading to suboptimal therapeutic choices.

Liquid biopsy overcomes this limitation by capturing ctDNA shed from multiple tumor sites, including primary and metastatic lesions. This provides a more comprehensive molecular profile of the entire tumor burden, reflecting both clonal and sub clonal populations. It is especially valuable in detecting emergent resistance mutations that may arise in metastatic sites not sampled by tissue biopsy (Dang Cao et al., 2025; Juric et al., 2019).

Conclusion of Section: While tissue biopsy remains indispensable for initial cancer diagnosis and histopathological assessment, liquid biopsy offers a safer, repeatable, and more comprehensive approach for monitoring tumor dynamics and guiding personalized therapy. Its integration into clinical workflows enhances precision oncology, particularly in metastatic breast cancer and in contexts requiring real-time molecular insights.

5. CURRENT LIMITATIONS AND CHALLENGES

Despite the promising clinical utility of liquid biopsy and circulating tumor DNA (ctDNA) analysis in breast cancer management, several limitations and challenges hinder its widespread adoption and integration into routine clinical practice. These challenges span across technical, clinical, and regulatory domains.

Table 1. Summary of advantages

S.No	Feature	Tissue Biopsy	Liquid Biopsy
1	Invasiveness	Invasive (needle/surgical)	Minimally invasive (blood sample)
2	Safety	Risk of complications	Safe and well-tolerated
3	Repeatability	Limited	High—can be repeated frequently
4	Tumor Heterogeneity	Limited snapshot	Reflects total tumor burden
5	Turnaround Time	Days to weeks	Often faster, hours to a few days
6	Feasibility in Metastasis	Often limited or inaccessible	Accessible even in metastatic cases

A. Technical Sensitivity and Specificity

One of the major technical challenges in ctDNA analysis is its low abundance in early-stage cancers or in patients with minimal residual disease (MRD). ctDNA often constitutes a very small fraction of total circulating cell-free DNA (cfDNA)—sometimes <0.1%—making detection and accurate quantification technically demanding (Talarico et al., 2025).

While highly sensitive technologies like digital PCR and ultra-deep next-generation sequencing (NGS) have improved detection limits, issues such as false positives (due to sequencing errors or clonal hematopoiesis) and false negatives (from insufficient ctDNA quantity) still persist. Therefore, technical sensitivity and analytical accuracy remain critical barriers, especially in early detection or MRD settings (Sheng et al., 2025; Wang et al., 2025).

Another challenge is that tumor heterogeneity and clonal evolution complicate ctDNA interpretation, as mutations detected in blood may represent only subsets of tumor clones. This phenomenon has been well documented in other cancers, where longitudinal and multiregion sequencing studies have revealed extensive genomic diversity and dynamic tumor evolution (Jamal-Hanjani et al., 2017; Gerlinger et al., 2014).

B. Lack of Standardization

There is currently no universally accepted standard for ctDNA analysis in terms of:

- Sample collection (e.g., blood tube type and processing time)
- DNA extraction methods
- Assay platforms (e.g., dPCR, NGS, BEAMing)
- Data interpretation and reporting

This lack of assay standardization and harmonization across laboratories and platforms creates variability in test results, complicating clinical interpretation and limiting reproducibility. Moreover, the absence of established reference materials and quality control metrics makes validation and comparison across studies difficult.

C. Regulatory and Clinical Integration Challenges

Although the U.S. FDA and other regulatory bodies have approved certain ctDNA-based assays (e.g., *Guardant360, Foundation One Liquid CDx*) for specific indications, broad clinical adoption remains limited due to:

- **Insufficient clinical validation:** Many ctDNA applications are still under investigation in clinical trials and lack large-scale, prospective evidence of improved patient outcomes.
- **Cost and reimbursement issues:** ctDNA assays are often expensive, and insurance coverage is inconsistent, posing a financial burden to healthcare systems and patients.
- **Clinical decision-making uncertainty:** Clinicians may hesitate to base major treatment decisions solely on liquid biopsy results, particularly in the absence of concordant tissue data or standardized guidelines.

Additionally, the integration of liquid biopsy into existing clinical workflows requires multidisciplinary coordination among oncologists, pathologists, and molecular diagnostics experts, which can be challenging in resource-limited or non-specialized settings.

Conclusion of Section: While ctDNA-based liquid biopsy holds transformative potential in breast cancer care, significant hurdles including limited technical sensitivity, lack of assay standardization, and regulatory complexities must be addressed. Future efforts should focus on large-scale clinical validation, international guideline development, and technological refinement to enable robust, reliable, and accessible implementation in everyday oncology practice.

6. ONGOING CLINICAL TRIALS AND RECENT STUDIES

The clinical application of circulating tumor DNA (ctDNA) in breast cancer has gained substantial momentum, with several landmark trials currently investigating its role in early detection of recurrence, treatment stratification, and therapy monitoring. These trials aim to validate the clinical utility of ctDNA in real-world settings and

lay the foundation for its integration into standard oncology practice.

A. c-TRAK TN (Circulating Tumor DNA-Guided Therapy in Triple-Negative Breast Cancer)

- **Sponsor:** Cancer Research UK.
- **Design:** Phase II, multicenter, prospective trial.
- **Objective:** To determine whether ctDNA-guided intervention improves outcomes in early-stage triple-negative breast cancer (TNBC).

Study Summary:

- Enrolled patients with stage II–III TNBC who had completed standard surgery and adjuvant chemotherapy.
- Serial blood samples were collected post-treatment to monitor for minimal residual disease (MRD) using ctDNA.
- Patients with ctDNA-positive status (indicating molecular relapse before clinical recurrence) were offered immunotherapy with atezolizumab, a PD-L1 checkpoint inhibitor.

Key Findings (interim):

- ctDNA was able to detect molecular relapse months before radiologic recurrence.
- Early immune intervention in ctDNA-positive patients may delay or prevent overt metastasis.
- Demonstrated feasibility of surveillance-guided therapeutic intervention, laying groundwork for personalized post-treatment monitoring.

B. DARE Trial (Detection of Asymptomatic Recurrence Using ctDNA in Breast Cancer)

- **Sponsor:** Dana-Farber Cancer Institute / Translational Breast Cancer Research Consortium.
- **Design:** Prospective, multicenter observational study
- **Objective:** To evaluate whether ctDNA can detect recurrence in high-risk breast cancer patients before clinical or radiologic signs appear.

Study Summary:

- Focused on hormone receptor-positive (HR+) and HER2-negative breast cancer survivors at high risk of late recurrence.
- Blood samples collected every 6 months for up to 5 years post-primary treatment.
- ctDNA detection was correlated with subsequent clinical relapse.

Key Insights:

- ctDNA was detectable well before imaging or symptom onset, with a median lead time of several months.
- Provided critical insight into the lead-time advantage of ctDNA for relapse detection.
- Created a platform for future intervention-based trials, in which treatment is initiated at molecular relapse rather than clinical recurrence.

C. Additional Notable Trials

1. IMMray™ PanCan-d Trial

- While focused more broadly on solid tumors, components of this trial evaluate ctDNA and methylation signatures in early breast cancer.

2. MONALEESA-7 and PALOMA-3 Sub studies

- Retrospective analyses of ctDNA in patients receiving CDK4/6 inhibitors have shown that PIK3CA and ESR1 mutations detected in ctDNA correlate with treatment resistance.

3. SIGNAL Trial (NCT03728361)

- Evaluates the use of ctDNA-based MRD monitoring to guide escalation or de-escalation of therapy in early-stage breast cancer.

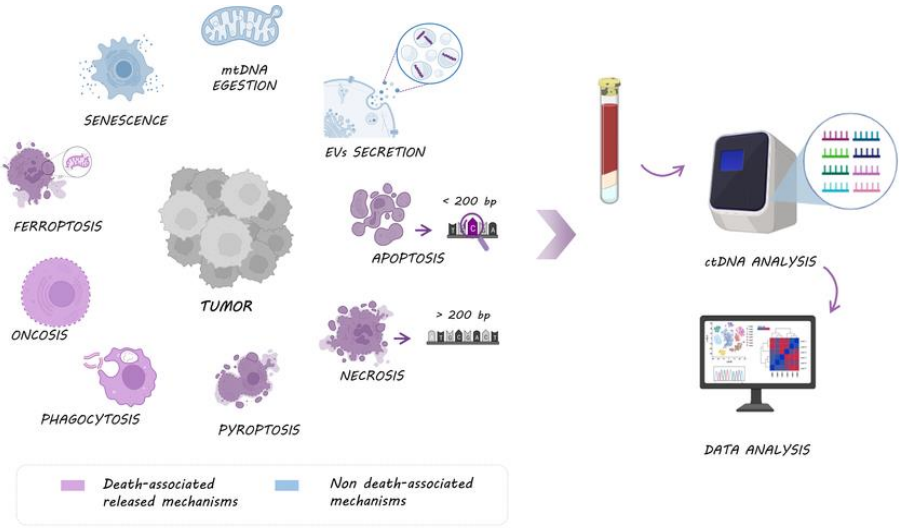

Fig. 2. Mechanism of ctDNA release

Clinical Impact and Implications

These studies are redefining how breast cancer is monitored and managed:

- Early relapse detection via ctDNA allows for proactive intervention.
- Treatment stratification based on ctDNA mutations enables personalized therapy.
- Longitudinal monitoring supports dynamic, real-time decision-making.

While results are promising, the full clinical integration of ctDNA will require:

- Completion of these and similar trials.
- Robust outcome data linking ctDNA-based decisions to survival benefits.
- Standardization of protocols across institutions.

Conclusion of Section: Ongoing clinical trials such as c-TRAK TN and DARE represent critical steps toward validating ctDNA as a transformative tool in breast cancer care. The ability to detect recurrence early, tailor treatment strategies, and monitor disease in real time offers a paradigm shift from reactive to proactive oncology. The next decade is likely to see ctDNA move from experimental to routine clinical practice, particularly with successful trial outcomes and regulatory approval.

7. FUTURE DIRECTION

As the field of oncology continues to advance toward precision medicine, the future of circulating tumor DNA (ctDNA) and liquid biopsy in breast cancer holds immense promise. Although significant progress has been made in understanding the molecular utility of ctDNA, several key areas remain pivotal for its full clinical integration

A. Integration into Clinical Guidelines

One of the foremost goals is the formal integration of ctDNA testing into national and international clinical practice guidelines for breast cancer management. At present, most professional organizations (e.g., ASCO, NCCN, and ESMO) recognize ctDNA primarily in the context of investigational or emerging use. However, ongoing clinical trials and accumulating evidence are expected to provide the necessary data to support routine ctDNA testing for minimal residual disease (MRD) and surveillance, establish thresholds for intervention based on ctDNA positivity, recommend standardized testing intervals post-treatment, and inform regulatory approval and reimbursement policies (Amir et al., 2011; Simmons et al., 2009).

In the future, ctDNA testing could be included in adjuvant therapy decision-making and surveillance algorithms, particularly for high-risk subtypes like triple-negative or HER2-positive breast cancer (Fan et al., 2025; Zhang et al., 2025).

Table 2. Comparison of detection techniques for ctDNA in breast cancer

S.NO	Detection Technique	Principle	Sensitivity	Specificity	Advantages	Limitations	Common Use Cases
1	qPCR (Quantitative PCR)	Amplifies known DNA sequences	Moderate (~0.1%)	High	Fast, cost-effective	Limited to known mutations	Mutation-specific detection
2	Digital PCR (dPCR)	Partitioned PCR to detect rare alleles	High (~0.01%)	High	High precision, quantifies low-level mutations	Low multiplexing, limited throughput	Monitoring specific mutations, MRD detection
3	BEAMing	Combines emulsion PCR and flow cytometry	Very high (~0.01%)	High	Sensitive and specific	Labor-intensive, expensive	Rare mutation detection
4	NGS (Next-Generation Sequencing)	Massively parallel sequencing	High (0.01–1%)	High	Broad mutation coverage, can detect unknown variants	Expensive, complex data analysis	Comprehensive mutation profiling
5	CAPP-Seq (Cancer Personalized Profiling by Sequencing)	Targeted NGS approach	Very high (~0.02%)	High	High depth, customizable panels	Requires design of patient-specific panels	Tumor burden tracking, personalized profiling
6	Whole Genome Sequencing (WGS)	Sequencing entire genome	Moderate (~5%)	Moderate	Detects structural variants, CNVs	Low sensitivity for ctDNA, costly	Research, genome-wide alterations
7	Methylation Analysis	Detects DNA methylation patterns	Variable	High	Epigenetic insights, early detection potential	Standardization still evolving	Early detection, subtype differentiation

Table 3. Clinical trials involving ctDNA in breast cancer

Trial Name / ID	Phase	Objective	Method of ctDNA Use	Patient Population	Status	Key Findings / Notes
BRE12-158 (NCT03145961)	II	Guide treatment post-neoadjuvant chemo using ctDNA	Detection of minimal residual disease (MRD)	HER2-negative early BC	Completed	ctDNA-positive patients had worse outcomes; suggests MRD utility
Circulating Tumor DNA-Stop Trial (NCT04567420)	III	Stop therapy based on undetectable ctDNA	ctDNA monitoring for treatment discontinuation	Hormone receptor-positive BC	Recruiting	Aims to minimize overtreatment via ctDNA-informed decisions
PlasmaMATCH (NCT03182634)	II	Match targeted therapies to ctDNA-detected mutations	ctDNA genotyping for treatment allocation	Advanced/metastatic BC	Completed	Demonstrated feasibility of ctDNA for genomic profiling
AURORA (BIG 14-01)	Observational	Understand metastatic evolution	Serial ctDNA profiling	Metastatic breast cancer	Ongoing	Integrates ctDNA to track clonal evolution
NATALEE (NCT03701334)	III	Evaluate ribociclib in early BC	Exploratory ctDNA endpoints	HR+/HER2- early BC	Ongoing	Will inform prognostic role of ctDNA in adjuvant setting
cTRAK-TN (NCT03145961)	II	Detect relapse using ctDNA	ctDNA for early detection of recurrence	Triple-negative breast cancer	Completed	Early relapse detection feasible with ctDNA surveillance
SOLAR-1 (NCT02437318)	III	Assess PIK3CA mutation status from ctDNA	ctDNA as companion diagnostic	HR+/HER2- advanced BC	Completed	ctDNA PIK3CA mutations predicted response to alpelisib

B. Personalized Treatment planning

ctDNA offers a unique opportunity for real-time, individualized treatment planning:

- **Treatment stratification:** Identifying actionable mutations (e.g., *PIK3CA, ESR1*) can guide the use of targeted therapies like alpelisib or fulvestrant. This approach reflects how molecular profiling has been used in other cancers to guide personalized therapy (Xu et al., 2012).

- **Therapy adaptation:** Dynamic monitoring of ctDNA levels can signal emerging resistance or treatment failure, prompting timely modifications (Wang et al., 2025).

- **Therapy escalation/de-escalation:** MRD status from ctDNA may allow clinicians to de-escalate therapy in patients with no detectable disease, reducing overtreatment and toxicity. Conversely, early escalation may be warranted for ctDNA-positive patients (Park et al., 2025; Gómez-Trillos et al., 2025).

Future treatment pathways may be driven by molecular relapse rather than radiologic progression, enabling a shift from reactive to proactive oncology.

C. Combination with Other Liquid Biomarkers

To enhance sensitivity, specificity, and biological insight, ctDNA is increasingly being explored in combination with other circulating biomarkers, such as:

- **Exosomes:** Membrane-bound extracellular vesicles containing DNA, RNA, and proteins derived from tumor cells. Exosome profiling complements ctDNA by offering insight into gene expression and protein signaling pathways.

- **Circulating Tumor Cells (CTCs):** Intact cancer cells found in peripheral blood, CTCs provide cellular context and enable morphological, immunohistochemical, and functional analysis that ctDNA alone cannot.

- **Circulating microRNAs (miRNAs) and tumor-educated platelets (TEPs):** These novel biomarkers are also being investigated to further refine the liquid biopsy landscape.

The integration of these markers into multi-omic liquid biopsy platforms will allow for comprehensive tumor profiling, improving diagnostic accuracy, therapeutic targeting, and resistance tracking.

Conclusion of Section: The future of ctDNA in breast cancer is marked by its inevitable transition from research to routine clinical practice. Key developments such as clinical guideline inclusion, personalized therapy planning, and multi-modal liquid biopsy approaches will revolutionize how breast cancer is diagnosed, monitored, and treated. With continued innovation, validation, and collaboration across clinical and regulatory sectors, ctDNA is poised to become a cornerstone of precision oncology.

8. CONCLUSION

The landscape of breast cancer diagnostics and management is rapidly evolving, with circulating tumor DNA (ctDNA) emerging as a transformative tool in the era of precision oncology. As a core component of liquid biopsy, ctDNA offers a non-invasive, dynamic, and highly informative approach to understanding tumor biology at multiple stages of the disease continuum.

This review highlights the key clinical applications of ctDNA in breast cancer, including its utility in early detection, treatment selection, response monitoring, minimal residual disease (MRD) detection, and resistance tracking. Compared to traditional tissue biopsy, liquid biopsy provides significant advantages in terms of safety, repeatability, and the ability to capture tumor heterogeneity. Landmark clinical trials such as c-TRAK-TN and DARE underscore the potential of ctDNA to detect molecular relapse ahead of clinical symptoms, enabling earlier intervention and potentially improved outcomes.

However, the field must still overcome several challenges—including technical sensitivity, standardization, and regulatory integration—before ctDNA can be fully adopted into routine clinical practice. Continued research, multi-center trials, and consensus-building across oncology networks will be critical to validating its clinical impact.

Looking forward, the integration of ctDNA into clinical guidelines, combined with advances in personalized treatment planning and multi-analyte liquid biopsy platforms, holds immense promise. CtDNA is not just a biomarker—it represents a shift toward real-time, biology-driven cancer care that empowers clinicians and benefits patients.

In summary, ctDNA has the potential to revolutionize breast cancer management by enabling earlier detection, tailored therapies, and proactive disease monitoring ultimately contributing to improved patient survival and quality of life.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

The Author(s) Dr. Densingh Johnrose and Mr. Azaruddin Gohil hereby declare that no generative Artificial Intelligence (AI) technologies, including but not limited to Large Language Models (e.g., ChatGPT, Copilot) or text-to-image generators, were used in the preparation, writing, or editing of this manuscript. The entire content is the original work of the Author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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