Breast Cancer: Targets and Therapy

Dovepress
Taylor & Francis Group

Open Access Full Text Article

REVIEW

Clinical Management of Circulating Tumor DNA in Breast Cancer: Detection, Prediction, and Monitoring

Yongqu Lu[1,*], Lehao Ren[2,*], Meng Yang[1], Jun Liu[1]

[1]Department of Breast and Thyroid Surgery, China-Japan Friendship Hospital, Beijing, 100029, People's Republic of China; [2]Department of Critical Care Medicine, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan, 430022, People's Republic of China

*Yongqu Lu and Lehao Ren contributed equally to this work

Correspondence: Meng Yang; Jun Liu, Department of Breast and Thyroid Surgery, China-Japan Friendship Hospital, Beijing, 100029, People's Republic of China, Email mengyang7012@163.com; docljun@126.com

Abstract: Despite substantial progress in the diagnosis and treatment of breast cancer, current therapeutic regimens exhibit limitations, necessitating the identification of more robust biomarkers to optimize personalized strategies. Circulating tumor DNA (ctDNA), as a non-invasive liquid biopsy modality, overcomes the inherent constraints of biopsies in capturing tumor heterogeneity. Accumulating evidence from prospective cohort studies demonstrates the clinical utility of ctDNA in risk stratification, guidance of therapeutic decision-making, recurrence surveillance and other clinical applications. Furthermore, ctDNA profiling enhances real-time pharmacodynamic monitoring and accelerates drug development by identifying molecular responders. The methodical requirements and challenges inherent in implementing liquid biopsy assessments in the clinic are examined. These encompass critical pre-analytical variables, the need for highly sensitive and specific analytical techniques, standardization of assays and bioinformatics pipelines across laboratories and the complexities of interpreting results. This review synthesizes current evidence supporting ctDNA integration into breast cancer management frameworks and systematically addresses its methodological challenges and clinical limitations.

Keywords: breast cancer, circulating tumor DNA, minimal residual disease, neoadjuvant therapy

Introduction

Breast cancer as a common malignancy among the world remains the leading morbidity and cause of cancer death bringing about significant burden and economic expenditure.[1–3] Currently there are still persistent challenges for the diagnosis and therapy of breast cancer. Conventional screening has reduced breast cancer mortality while Breast Imaging Reporting and Data System classification (BI-RADS) based on ultrasonography or mammography has wide-ranging potential for prediction.[4] In resource-constrained healthcare systems where advanced diagnostic modalities remain prohibitively resource-intensive or impractical, breast cancer patients frequently present with advanced-stage disease at initial diagnosis. Current clinical practice guidelines establish multimodal therapy as the cornerstone for resectable breast cancer management, combining definitive surgical intervention with evidence-based adjuvant strategies including chemotherapy, molecularly targeted agents and endocrine therapy. Furthermore, neoadjuvant systemic therapy has been integrated into standard protocols, demonstrating efficacy in downstaging tumors to facilitate breast-conserving surgical approaches or optimize radical resection outcomes.[5,6] While the sensitivities of patients to different medicines exhibit diverse responses as the result of individual or tumor heterogeneity.[7] Drug resistance and disease recurrence pose critical impediment to long-term survival. The investigation of biomarkers has been advancing rapidly, with the goal of effectively applying them to various aspects of cancer clinical management, including early tumor diagnosis, precise identification of optimal patient populations for specific therapies, evaluation of treatment response post-intervention, and longitudinal disease monitoring.[8] Among these, circulating tumor DNA (ctDNA) has gained substantial momentum in

Received: 25 May 2025
Accepted: 19 September 2025
Published: 25 September 2025

molecular indicators and clinical implementation due to its non-invasive detection methodology, streamlined sample accessibility, and dual advantages of high analytical sensitivity and specificity, which positions it as a transformative tool in precision oncology.[9]

The Origination and Detection of ctDNA

Circulating cell-free DNA (cfDNA) and its tumor-derived subset, circulating tumor DNA (ctDNA), have emerged as pivotal biomarkers in oncology due to their non-invasive accessibility and dynamic reflection of tumor biology.[10] The cfDNA comprises fragmented extracellular DNA and released into the bloodstream or other body fluid through apoptosis, necrosis or active secretion of both normal and pathological cells. In plasma, cfDNA typically consists of double-stranded DNA fragments of around 140–170 base pairs (bp).[11] While ctDNA specifically originates from malignant cells, carrying tumor-specific genomic alterations such as mutations, copy number variations and methylation patterns.[12,13] The developed ctDNA detections of end-motifs and fragmentomics are newly found to provide comprehensive information in breast cancer.[14,15]

The ctDNA exhibits distinct physicochemical properties that underpin its clinical utility in oncology (Figure 1). The clinical utility of ctDNA lies in its ability to mirror tumor heterogeneity, monitor minimal residual disease (MRD), and detect early molecular relapse, often preceding radiographic evidence by months. MRD refers to the presence of trace amounts of cancer cells persisting after curative-intent therapy, which are undetectable by conventional imaging or histopathological methods but may drive disease relapse.[16] MRD detection employs highly sensitive technologies to identify residual malignant clones. In breast cancer, MRD monitoring enables early detection of molecular relapse, often months to years before clinical manifestation. Clinically, MRD status serves as a dynamic prognostic biomarker, stratifying patients into distinct risk categories for recurrence and guiding adjuvant therapy escalation or de-escalation.[17] Emerging strategies based on ctDNA to refine MRD-driven interception trials, positioning MRD detection as a cornerstone of precision oncology in the era of liquid biopsy.[18]

The rapid methodological evolution in sequencing technologies and bioinformatic frameworks for ctDNA analysis has promoted a proliferation of distinct analytical platforms, which are taxonomically stratified into two principal methodological paradigms: tumor-agnostic approaches, utilizing fixed genomic panels or epigenetic signatures for hypothesis-free screening, and tumor-informed strategies, requiring prior whole profiling of matched tumor tissue to design patient-specific mutational tracking assays.[19,20] This dichotomy reflects fundamental differences in sensitivity

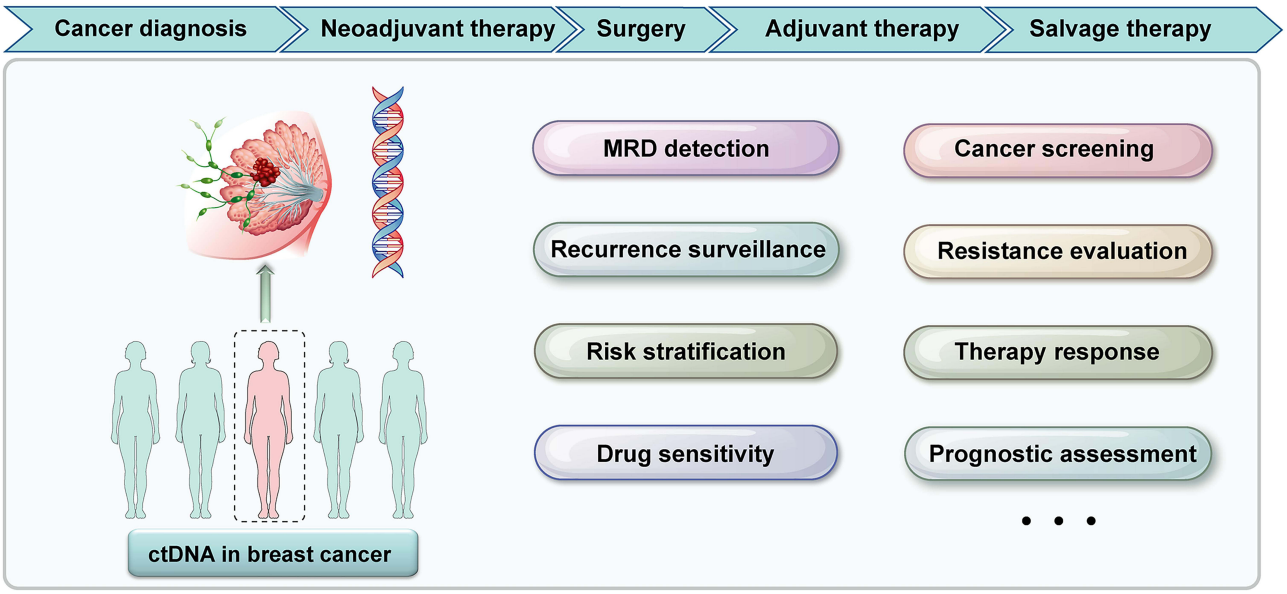

Figure 1 Clinical application of ctDNA analysis in breast cancer.
Abbreviation: MRD, minimal residual disease.

thresholds, clinical applicability and requirements for tumor biospecimen availability. Advanced sequencing technologies enable high-sensitivity detection of ctDNA even at low concentrations, facilitating applications in early cancer screening, treatment response assessment, and personalized therapeutic targeting.[21–23] Tumor-agnostic approaches do not need prior tumor profiling using the technologies like next-generation sequencing (NGS) for genomic variants and methylation analysis.[24,25] NGS allows simultaneous analysis of multiple cancer-associated genes, including single-nucleotide variants (SNVs), copy number variations (CNVs), indels and fusion events. The ultra-deep sequencing detects low-frequency mutations within ctDNA. This multiplex capability provides a holistic view of tumor heterogeneity and evolution. While tumor-informed methodologies demonstrate enhanced analytical sensitivity for detecting low-frequency tumor-derived variants, this approach imposes significant logistical constraints due to its prerequisite sequential workflow: initial tumor sequencing, followed by computationally intensive bioinformatic clonal selection and patient-specific multiplex panel design with personalized NGS, methylation-specific assays and droplet digital PCR (ddPCR).[26,27] This multistep process typically creates operational bottlenecks in time-sensitive clinical contexts compared to tumor-agnostic fixed panels.

ctDNA in Early Screening of Breast Cancer

The traditional screening modalities for breast cancer must balance economic consideration and equipment accessibility. Ultrasound and mammography consequently demonstrate limitations in sensitivity, particularly in the detection of malignancies within dense breast, while the interpretation of results also exhibits significant subjectivity.[28–30] The occult biological behavior of early-stage breast cancer poses significant diagnostic challenges. However, latent malignant features can be detected through low concentrations of ctDNA. Leveraging the advantages of non-invasive sampling and procedural convenience, ctDNA analysis has emerged as a highly feasible modality for breast cancer screening and enables the detection of breast malignancies at subclinical stage.[31,32] The AmpliSeq, a multiplex PCR method, and hybridization-based capture of 102 patients with early-stage breast cancer and 50 individuals with benign breast tumors demonstrated that preoperative ctDNA mutations analysis reliably mirrored tumor heterogeneity while achieving 74.2% (49/66) detection sensitivity and the blood-derived tumor mutational burden (TMB) strongly correlated with tissue TMB. Integrating ctDNA with BI-RADS imaging enhanced the positive predictive value to 92% (49/53), suggesting clinical utility in reducing unnecessary surgeries.[33] The methods used in this study were based on NGS but tumor-informed with the large gene panels designed. And blood-derived TMB represents a dependable biomarker for ctDNA-based cancer studies.

The application of ctDNA in pan-cancer screening has emerged as a prominent research focus in the field of early cancer detection.[34–36] Investigators are rigorously assessing the diagnostic performance of ctDNA with particular focus on optimizing sensitivity and specificity across heterogeneous screening in both localized and metastatic settings. In a study with a total 12,337 pan-cancer patients including 1178 breast cancer using parallel sequencing between plasma and white blood cells, ctDNA sensitivity was correlated with disease stage: lower in localized disease than in metastatic cases and 39.1% of breast cancer samples harbored prevalent *PIK3CA* mutations. While ctDNA detection sensitivity in breast cancer was less than 80%, reflecting its moderate utility in identifying tumor-derived genomic alterations compared to other cancers like non-small cell lung cancer, which was 91.1%.[37] Garcia-Murillas et al[38] demonstrated diagnostic sensitivity across breast cancer subtypes, achieving 100% sensitivity in HER2-positive (25/25) and triple-negative breast cancer (TNBC, 16/16) cohorts, with 88% sensitivity (7/8) observed in hormone receptor-positive/HER2-negative (HR+/HER2−) disease, though limited by small sample size in the latter subgroup. And this study used dual-profiled methods showing whole genome sequencing (WGS)-powered assay outperformed both digital PCR and whole exome sequencing (WES). The application of ctDNA staging remains insufficient in sensitivity and accuracy, particularly for early-stage regional lesions, may be attributed to low tumor shedding.

Beyond the most utilized plasma sample, researchers are actively investigating the diagnostic potential of ctDNA derived from alternative biofluids to optimize screening efficacy across diverse clinical scenarios.[39,40] Saura et al[41] collected ctDNA from the breast milk of 15 patients which contains 90-fold higher cfDNA concentrations resulting in more available genomic equivalents compared to plasma (751.7 ng/mL of breast milk vs 8.3 ng/mL of blood) with superior fragment integrity in pregnancy-associated and postpartum breast cancer. ctDNA-specific variants like *PIK3CA*/p.E545K and *AKT1*/p.E17K were detected breast milk via ddPCR and NGS. Breast milk-derived ctDNA was collected

6–18 months before clinical diagnosis, preceding imaging detection in some pre-diagnostic cases. This study pioneers a novel approach to sample sourcing for ctDNA. As a biofluid unique to mammary physiology, breast milk from specific populations such as pregnant and lactating women provides biologically proximate insights into mammary gland status offering a noninvasive window for breast screening.

ctDNA in Neoadjuvant Therapy

The neoadjuvant therapy strategies for breast cancer can downstage advanced breast cancer to achieve a clinical status more suitable for radical surgery or to attain goals such as breast-conserving surgery and avoidance of axillary lymph node dissection, while simultaneously enabling early drug sensitivity testing.[42,43] However, due to the significant heterogeneity among individuals and tumors, there exists substantial variability in therapeutic responses to neoadjuvant treatment. Patients with HR+/HER2− breast cancer exhibit a significantly lower incidence of pathological complete response (pCR) to neoadjuvant chemotherapy compared to other subtypes.[44–46] Such variability may potentially delay subsequent therapeutic interventions and even influence tumor progression. TNBC represents a clinically aggressive subtype with distinct molecular heterogeneity. Given the absence of druggable targets inherent to its biological profile, specifically the lack of hormone receptor expression and HER2 amplification, therapeutic options remain constrained to cytotoxic regimens.[47] Current therapeutic paradigms for TNBC prioritize neoadjuvant chemotherapy followed by definitive surgical resection with adjuvant capecitabine consolidation for residual disease.[48,49] These two subtypes have emerged as predominant research priorities in neoadjuvant therapeutic investigations due to their distinct biological behaviors and differential responses to preoperative systemic therapies. Beyond assessing therapeutic sensitivity, we propose to investigate the prognostic risk stratification through longitudinal monitoring of ctDNA dynamics during neoadjuvant therapy, leveraging its capacity to reflect real-time tumor evolution and residual disease burden (Table 1).[50–63]

Li et al[64] demonstrated the utility of ctDNA in risk stratification and prognostic assessment for 130 stage II–III TNBC patients undergoing neoadjuvant chemotherapy, with dynamic ctDNA analysis enabling early identification of high-risk subgroups and informing personalized therapeutic strategies. Post-neoadjuvant chemotherapy ctDNA positivity was strongly correlated with inferior event-free survival (EFS) and distant recurrence-free survival (DRFS). A baseline maximum variant allele frequency (MVAF) threshold of 1.1% effectively stratified high-risk patients, validated internally

Table 1 Clinical Trials Based on ctDNA Detection in Neoadjuvant Therapy

Author	Country	Cancer Stage	Cancer Subtype	Number of Patients	Method for ctDNA Detection	Median Follow-up
Cailleux et al[50]	Belgium	II–III	ALL	44	Signatera	3.03 years
Cavallone et al[51]	Canada, USA	I–III	TNBC	26	ddPCR	63 months (post-diagnosis), 55 months (post-surgery)
Ciriaco et al[52]	Spain	I–III	HER2+, TNBC	20	SafeSEQ	NA
Kjær et al[53]	Denmark	NA	All	78	ddPCR	4.41 years
Liu et al[54]	China	I–III	All	56	NGS	898 days
Magbanua et al[55]	USA	I–III	TNBC, HR +/HER2−	283	Signatera	3.12 years (TNBC), 3.10 years (HR+/HER2−)
Magbanua et al[56]	USA	II–III	All	84	Ultradeep Sequencing	4.8 years
McDonald et al[57]	USA, UK	I–III	All	22	TARDIS	NA
Ortolan et al[58]	Italy	II–III	TNBC	26	ddPCR	NA
Parsons et al[59]	USA	II–III	TNBC	38	MAESTRO	NA
Riva et al[60]	France	I–III	TNBC	38	ddPCR	24 months
Rothe´ et al[61]	multicenter	NA	HER2+	69	ddPCR	NA
Takahashi et al[62]	Japan	II–III	All	87	one-step methylation-specific PCR	23 months
Zhou et al[63]	Austria	I–III	TNBC, HR +/HER2−	145	SiMSen-Seq	NA

Abbreviations: TNBC, triple-negative breast cancer; HR+/HER2−, hormone receptor-positive/HER2-negative; ddPCR, droplet digital PCR; NGS, next-generation sequencing; NA, not available.

and externally, while a systemic tumor burden model integrating baseline and post-surgery ctDNA status independently predicted relapse risk. Longitudinal ctDNA surveillance during follow-up identified MRD with 100% negative predictive value (NPV) for DRFS, though sensitivity for post-surgery MRD detection was limited (50%). Another TNBC study, TBCRC 030, evaluating neoadjuvant paclitaxel plus cisplatin and residual cancer burden (RCB) was utilized as a reference standard for evaluating treatment response.[59] MAESTRO mutation enrichment sequencing was used to track variants across 114 plasma samples from three timepoints and ctDNA positivity rates were 100% at baseline, 79% at 3 weeks and 55% at 12 weeks (end of treatment). From baseline to 3 weeks, responders exhibited a 285-fold reduction in median tumor fraction, compared to a 24-fold reduction in non-responders, highlighting ctDNA dynamics as a robust predictor of therapeutic response in TNBC. Data from BRE12-158 trial also confirmed ctDNA in patients with early-stage TNBC after neoadjuvant chemotherapy was independently associated with disease recurrence.[65] The forementioned studies demonstrate the validated utility of ctDNA in predicting pathological response and stratifying prognostic risk during neoadjuvant therapy for TNBC.

From the I-SPY2 trial, 283 HER2-negative breast cancer patients (145 HR+/HER2− and 138 TNBC cases) were evaluated for the predictive and prognostic value of ctDNA using the Signatera assay. Serial blood samples (n=1024) were collected at predefined timepoints: pretreatment (T0), 3 weeks post-treatment initiation (T1), 12 weeks post-treatment (mid-therapy, T2) and post-neoadjuvant therapy/pre-surgery (T3). Results indicated ctDNA positivity at T0-T3 in HER2-negative patients was significantly associated with inferior DRFS. Early clearance of ctDNA during T1 served as a critical predictive biomarker for therapeutic response in TNBC, enabling the prediction of pCR (odds ratio=13.06; 95% confidence interval [CI], 3.54–57.95) and RCB (odds ratio=19.00; 95% CI, 4.98–89.06). Notably, ctDNA clearance at T3 correlated with improved DRFS. Compared to pCR and RCB indices, ctDNA status at T3 provided superior risk stratification and prognostic accuracy, highlighting its potential as a dynamic biomarker for guiding post-neoadjuvant management strategies.[55]

The application of ctDNA in neoadjuvant therapy will focus on enhancing its role in dynamic risk stratification and precision-guided therapeutic escalation. Key advancements will involve optimizing standardized protocols for longitudinal ctDNA monitoring to detect MRD with higher sensitivity and specificity, particularly in TNBC and HR+/HER2− subtypes. Prospective trials are needed to validate ctDNA-driven adaptive strategies, such as tailoring adjuvant therapies based on post-neoadjuvant ctDNA clearance or persistence.

ctDNA in Postoperative Monitoring

Patients with resectable breast cancer require regular postoperative surveillance following surgery and adjuvant therapy. Conventional serological biomarkers such as CA15-3 exhibit suboptimal sensitivity and specificity in long-term follow-up for detecting tumor recurrence.[66,67] Accumulating evidence has demonstrated the superior performance of ctDNA in dynamic monitoring for distant recurrence. Serial ctDNA quantification allows noninvasive tracking of tumor burden dynamics, reflecting therapeutic response or recurrence risk in real time.

EBLIS study enrolled patients with early-stage breast cancer after surgery and adjuvant therapy, of whom 156 completed comprehensive ctDNA-based MRD analysis with Signatera assay including 16 somatic mutations and underwent up to 12 years of follow-up with blood sampling every 6 months for MRD detection, with a total of 1136 plasma samples.[68] This MRD detection demonstrated a median lead time of 10.5 months (range up to 38 months) ahead of clinical or radiologic relapse, achieving a sensitivity of 88.2% (30/34). Patients with ctDNA-MRD positivity exhibited significantly shorter relapse-free survival (RFS) and overall survival (OS) compared to MRD-negative counterparts, regardless of hormone receptor status or HER2 subtype (RFS hazard ratio [HR]=52.98, 95% CI: 18.32–153.20; OS HR=53.69, 95% CI: 7.01–411.49). Multivariate analysis identified ctDNA-MRD status as the most significant independent predictor of both RFS and OS. Another ctDNA-based MRD research enrolled 103 patients diagnosed with high-risk stage II–III HR+ breast cancer five years prior and 83 patients comprised the analytic cohort, all without clinical evidence of recurrence.[69] WES was performed on primary tumor tissues to design personalized and tumor-informed ctDNA assays targeting 12–51 somatic mutations for MRD monitoring. Plasma samples were collected at enrollment and during routine follow-up visits every 6–12 months. Despite no clinical evidence of metastatic recurrence at the first plasma sampling, 10% of patients detected positive for MRD at 5 years post-diagnosis. The ctDNA assay showed 85.7% sensitivity and

98.7% NPV for detecting any clinical recurrence. For predicting distant metastatic recurrence, the assay achieved 100% sensitivity and NPV. Specificity was 97.4% for all recurrences and distant metastases with a positive predictive value of 75%. ctDNA monitoring shows significant clinical utility in the long-term follow-up of high-risk breast cancer. There exists a window period during which MRD can be detected via ctDNA prior to the manifestation of overt symptoms of advanced breast cancer recurrence. This approach provides critical clinical insights to inform liquid biopsy-guided personalized therapeutic strategies aimed at preventing or delaying disease recurrence.

ctDNA in Advanced Breast Cancer

Systemic therapy for advanced breast cancer continues to confront numerous challenges, including treatment resistance and disease dissemination.[70–72] The primary therapeutic objective in advanced breast cancer management centers on sustaining treatment sensitivity through strategic interventions aimed at delaying or preventing acquired resistance mechanisms. ctDNA plays a critical role in clinical management by enhancing real-time monitoring of tumor progression and guiding dynamic treatment regimen adjustments in advanced patient. The MONALEESA-3 Phase III trial investigated the efficacy and safety of the CDK4/6 inhibitor ribociclib combined with various endocrine therapies as first- or second-line treatment for HR+/HER2− advanced breast cancer.[73] The study sequenced 1674 baseline plasma ctDNA samples, analyzing 1045 patients with ctDNA fraction ≥1%. Compared to placebo, mutations in the most frequently altered genes like *FAT3, FRS2, MDM2, SFRP1* and *ZNF217* were associated with greater progression-free survival (PFS) benefit from ribociclib. Patients with ctDNA fraction <1% generally exhibited more favorable overall responses, lower rates of prior chemotherapy and endocrine therapy and higher frequency of de novo metastatic disease. Baseline ctDNA fraction <1% correlated with smaller sum of target lesion diameters, while elevated ctDNA fraction predicted inferior PFS outcomes regardless of treatment modality.

The therapeutic efficacy of numerous agents in advanced breast cancer correlates with biomarker status, including estrogen receptor (ER) and HER2 profiles. This pathophysiological association provides a conceptual framework for investigating ctDNA as a dynamic predictor of drug sensitivity, informing precision therapeutic strategies through longitudinal genomic surveillance. Some findings demonstrate liquid biopsy-driven ctDNA may match advanced breast cancer patients harboring rare actionable mutations to therapies with enhanced clinical efficacy. The plasmaMATCH clinical trial analyzed ctDNA from the blood of over 1000 breast cancer patients who experienced recurrence or metastasis following prior therapies.[74] ctDNA testing was done with ddPCR and targeted sequencing. Researchers focused on three targetable rare mutations, *HER2, AKT1* and *ESR1*, known to drive breast carcinogenesis. Among 142 patients with detectable mutations, matched targeted therapies were administered based on molecular profiles. Those with *ESR1* mutations received fulvestrant while *HER2*-mutated patients received neratinib either as monotherapy or combined with fulvestrant. For *AKT1*-mutated cases, patients were stratified by ER status, receiving capivasertib plus fulvestrant (ER-positive) or capivasertib alone (ER-negative). After a median follow-up of 14.4 months, objective response rates were observed in 25% (5/20) of *HER2*-mutant and 22% (4/18) of ER-positive *AKT1*-mutant cohorts, indicating tumor regression or stabilization. The targeted inhibition of cyclin-dependent kinases 4/6 (CDK4/6), when combined with first-line endocrine therapy, has significant improvement in progression-free survival (PFS) and overall survival (OS) among patients with HR+/HER2− advanced breast cancer, as evidenced by landmark trials including MONALEESA and PALOMA series.[75,76] MONARCH trials suggested baseline ctDNA genomic alterations could serve as prognostic biomarkers for endocrine therapy outcomes but they lack predictive utility in determining response to the CDK4/6 inhibitor like abemaciclib.[77] Therefore, the monitoring of therapeutic sensitivity in advanced breast cancer reflects obvious site-specific heterogeneity, particularly in loci associated with treatment response warranting further investigation into these spatially divergent biological characteristics.

Conclusion

From early detection through comprehensively clinical management to advanced-stage treatment, ctDNA analysis offers a minimally invasive, real-time window into breast cancer biology, enabling earlier detection of recurrence and resistance, guiding personalized treatment decisions, monitoring response dynamically and improving prognostic stratification, ultimately aiming to enhance patients' outcomes.[78] The non-invasive nature of ctDNA via liquid biopsy

overcomes limitations of repeated tissue biopsies. It enables highly sensitive early detection of recurrence and MRD monitoring, often preceding clinical or imaging findings.[79] It allows real-time assessment of treatment response and dynamic tumor evolution, facilitating timely therapeutic adjustments. Critically, it identifies emerging biomarkers of resistance to guide the selection of targeted therapy. ctDNA analysis also provides valuable prognostic information on recurrence risk and overall survival. Furthermore, it captures tumor heterogeneity more comprehensively than single-site biopsy.

Challenges persist and a key obstacle is the low abundance of ctDNA in early-stage breast cancer with necessitating ultrasensitive detection platforms. While tumor-informed personalized assays like Signatera enhance sensitivity for MRD monitoring, they introduce logistical burdens, including prolonged turnaround times and dependency on archived samples which may be unavailable or degraded.[80,81] Ongoing innovations, such as signal amplification technologies, aim to overcome sensitivity barriers, positioning ctDNA analysis as a cornerstone of precision oncology. Nevertheless, integrating multi-analyte approaches, like combining genetic and epigenetic signatures, holds promise for enhancing clinical specificity.[82] Combining ctDNA mutation profiling with methylation markers or fragmentomic patterns improves the sensitivity of detection. Integration of multi-omics approaches, such as combining ctDNA with transcriptomic, proteomic, and radiomic data, will refine predictive models for treatment response and recurrence risk.[83]

Standardization hurdles persist across pre-analytical variables and analytical protocols, complicating cross-trial comparisons. The standardization of ctDNA detection faces significant challenges across multiple technical and procedural aspects, like the conditions for sample collection, processing, storage; DNA extraction efficiency; sequencing depth and coverage; bioinformatics analysis pipelines and the thresholds for result reporting. ctDNA has a short half-life, requiring strict control over collection timing and immediate processing to avoid degradation. Clinical samples vary in composition, necessitating tailored protocols for different fluid types. Improper storage can compromise ctDNA integrity. Standardized protocols for plasma separation, anticoagulant use and long-term storage are lacking, leading to inter-laboratory variability. Influence of external factors like physiological states (inflammation, pregnancy) and treatments (chemotherapy, radiation) dynamically alter ctDNA levels, complicating consistent sample collection timing. ctDNA constitutes less than 10% of total cfDNA, requiring high-efficiency extraction methods. Traditional silica or magnetic beads-based methods often fail to recover ultra-low concentrations, leading to false negatives. Commercially available kits vary in recovery rates and novel platforms like microfluidics are not yet standardized. Insufficient sample volume or low tumor burden exacerbates extraction inefficiency, demanding optimized protocols for minimal input. For tumor-agnostic approach, high sequencing depth increases background noise from PCR errors, oxidative damage or clonal hematopoiesis. Targeted panels range from small gene sets like *EGFR*-only to large panels complicating cross-study comparisons. Coverage uniformity and capture efficiency differ between hybridization-based and amplification-based methods. Variant-calling tools and error-suppression strategies like duplex sequencing and molecular barcoding differ in sensitivity and specificity. Distinguishing tumor-derived mutations from clonal hematopoiesis or germline variants requires matched white blood cell sequencing, which is not universally adopted. Clinical relevance thresholds for mutation detection are context-dependent and lack universal guidelines. Low ctDNA levels or technical limitations result in false negatives, while clonal hematopoiesis or sequencing artifacts cause false positives. There is a critical lack of both direct comparisons between different technological platforms under identical clinical contexts and universally accepted cutoff values to determine ctDNA positivity. And parameters for filtering artifacts such as minimum variant allele frequency and read depth vary across platforms, leading to inconsistent mutation reporting. We need to adopt uniform guidelines for sample collection timing, tube types, and storage conditions. The validate extraction kits and sequencing platforms can use reference materials for the convenience to establish open-source pipelines and cross-platform benchmarking initiatives. For clinical thresholds, context-specific reports through multicenter studies and regulatory collaboration are confoundedly recommended.

From a clinical perspective, the biological heterogeneity of breast cancer subtypes, particularly the divergent ctDNA shedding rates between HR+/HER2− and TNBC, challenges uniform biomarker application.[84] The conventional view holds that variations in ctDNA levels are primarily associated with the extent of tumor cell release. Therefore, the related factors such as tumor size, stage, extent of necrosis and treatment pressure can all contribute to the heterogeneity in ctDNA content. Although the underlying reasons for differences in ctDNA among different molecular subtypes remain to

be clarified, the implementation of highly sensitive ctDNA detection methods ensures a reliable lower limit of detection. Cost barriers further restrict accessibility, disproportionately affecting low-resource settings. Although ctDNA demonstrates far superior sensitivity and specificity compared to conventional serum tumor biomarkers such as carcinoembryonic antigen (CEA) and cancer antigen 125 (CA125) in breast cancer detection, its detection cost substantially exceeds that of traditional testing methods.[85] For instance, Signatera MRD product can cost USD 1000–3000. Ultra-deep sequencing and complex experimental procedure is resource-intensive, limiting accessibility and hindering standardization in clinical settings. While cost-reduction strategies, including in silico panel optimization and centralized high-throughput sequencing, are lowering prices.

The innovations collectively position ctDNA as a transformative tool for dynamic risk stratification and precision intervention in breast cancer care. Ultimately, ctDNA is poised to revolutionize therapy paradigms by enabling real-time monitoring, early intervention for high-risk cohorts, and reducing overtreatment in low-risk patients, thereby improving long-term survival and quality of life. Although doctors and researchers are actively exploring multiple approaches to validate the clinical utility of ctDNA and enhance the detection reliability, the standardization of pre-analytical variables and analytical processes remain imperative.

Funding

This work was supported by Beijing Natural Science Foundation (No. 7244406), National High Level Hospital Clinical Research Funding and Elite Medical Professionals Project of China-Japan Friendship Hospital (NO. ZRJY2023-QM13).

Disclosure

Yongqu Lu and Lehao Ren contributed equally to this work and share first authorship. The authors report no conflicts of interest in this work.

References

1. Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2024;74(3):229–263. doi:10.3322/caac.21834
2. Thomas HR, Hu B, Boyraz B, et al. Metaplastic breast cancer: a review. *Crit Rev Oncol Hematol*. 2023;182:103924. doi:10.1016/j.critrevonc.2023.103924
3. Giaquinto AN, Sung H, Miller KD, et al. Breast Cancer Statistics, 2022. *CA Cancer J Clin*. 2022;72(6):524–541. doi:10.3322/caac.21754
4. Loibl S, Poortmans P, Morrow M, Denkert C, Curigliano G. Breast cancer. *Lancet*. 2021;397(10286):1750–1769. doi:10.1016/S0140-6736(20)32381-3
5. Asselain B, Barlow W, Bartlett J, Early Breast Cancer Trialists' Collaborative G. Long-term outcomes for neoadjuvant versus adjuvant chemotherapy in early breast cancer: meta-analysis of individual patient data from ten randomised trials. *Lancet Oncol*. 2018;19(1):27–39. doi:10.1016/S1470-2045(17)30777-5
6. Yang C, Liu H, Feng X, et al. Research hotspots and frontiers of neoadjuvant therapy in triple-negative breast cancer: a bibliometric analysis of publications between 2002 and 2023. *Int J Surg*. 2024;110(8):4976–4992. doi:10.1097/JS9.0000000000001586
7. Onkar SS, Carleton NM, Lucas PC, et al. The great immune escape: understanding the divergent immune response in breast cancer subtypes. *Cancer Discov*. 2023;13(1):23–40. doi:10.1158/2159-8290.CD-22-0475
8. Britt KL, Cuzick J, Phillips KA. Key steps for effective breast cancer prevention. *Nat Rev Cancer*. 2020;20(8):417–436. doi:10.1038/s41568-020-0266-x
9. Alix-Panabieres C, Pantel K. Liquid biopsy: from discovery to clinical application. *Cancer Discov*. 2021;11(4):858–873. doi:10.1158/2159-8290.CD-20-1311
10. Cohen SA, Liu MC, Aleshin A. Practical recommendations for using ctDNA in clinical decision making. *Nature*. 2023;619(7969):259–268. doi:10.1038/s41586-023-06225-y
11. Tivey A, Church M, Rothwell D, Dive C, Cook N. Circulating tumour DNA - looking beyond the blood. *Nat Rev Clin Oncol*. 2022;19(9):600–612. doi:10.1038/s41571-022-00660-y
12. Nikanjam M, Kato S, Kurzrock R. Liquid biopsy: current technology and clinical applications. *J Hematol Oncol*. 2022;15(1):131. doi:10.1186/s13045-022-01351-y
13. Herberts C, Wyatt AW. Technical and biological constraints on ctDNA-based genotyping. *Trends Cancer*. 2021;7(11):995–1009. doi:10.1016/j.trecan.2021.06.001
14. Nguyen VTC, Nguyen TH, Doan NNT, et al. Multimodal analysis of methylomics and fragmentomics in plasma cell-free DNA for multi-cancer early detection and localization. *Elife*. 2023. doi:10.7554/eLife.89083
15. Mouliere F, Chandrananda D, Piskorz AM, et al. Enhanced detection of circulating tumor DNA by fragment size analysis. *Sci Transl Med*. 2018;10(466). doi:10.1126/scitranslmed.aat4921
16. Medford AJ, Moy B, Spring LM, Hurvitz SA, Turner NC, Bardia A. Molecular Residual Disease in Breast Cancer: detection and Therapeutic Interception. *Clin Cancer Res*. 2023;29(22):4540–4548. doi:10.1158/1078-0432.CCR-23-0757

17. Zhang S, Jiang Y, Zhou L, et al. Genomic characteristics and its therapeutic implications in breast cancer patients with detectable molecular residual disease. *Cancer Res Treat*. 2024;56(2):538–548. doi:10.4143/crt.2023.1059
18. Coakley M, Villacampa G, Sritharan P, et al. Comparison of circulating tumor DNA assays for molecular residual disease detection in early-stage triple-negative breast cancer. *Clin Cancer Res*. 2024;30(4):895–903. doi:10.1158/1078-0432.CCR-23-2326
19. Fu Y, Xu Y, Liu W, et al. Tumor-informed deep sequencing of ctDNA detects minimal residual disease and predicts relapse in osteosarcoma. *EClinicalMedicine*. 2024;73:102697. doi:10.1016/j.eclinm.2024.102697
20. Santonja A, Cooper WN, Eldridge MD, et al. Comparison of tumor-informed and tumor-naive sequencing assays for ctDNA detection in breast cancer. *EMBO Mol Med*. 2023;15(6):e16505. doi:10.15252/emmm.202216505
21. Stadler JC, Belloum Y, Deitert B, et al. Current and future clinical applications of ctDNA in immuno-oncology. *Cancer Res*. 2022;82(3):349–358. doi:10.1158/0008-5472.CAN-21-1718
22. Vellanki PJ, Ghosh S, Pathak A, et al. Regulatory implications of ctDNA in immuno-oncology for solid tumors. *J Immunother Cancer*. 2023;11(2). doi:10.1136/jitc-2022-005344
23. Verschoor N, Bos MK, Oomen-de Hoop E, et al. A review of trials investigating ctDNA-guided adjuvant treatment of solid tumors: the importance of trial design. *Eur J Cancer*. 2024;207:114159. doi:10.1016/j.ejca.2024.114159
24. Hai L, Li L, Tong Z, Liu Z, Sun Y. Whole-genome circulating tumor DNA methylation landscape reveals sensitive biomarkers of breast cancer. *MedComm*. 2022;3(3):e134. doi:10.1002/mco2.134
25. Iams WT, Mackay M, Ben-Shachar R, et al. concurrent tissue and circulating tumor dna molecular profiling to detect guideline-based targeted mutations in a multicancer cohort. *Jama Network Open*. 2024;7(1):e2351700. doi:10.1001/jamanetworkopen.2023.51700
26. Fitzpatrick A, Iravani M, Mills A, et al. Assessing CSF ctDNA to improve diagnostic accuracy and therapeutic monitoring in breast cancer leptomeningeal metastasis. *Clin Cancer Res*. 2022;28(6):1180–1191. doi:10.1158/1078-0432.CCR-21-3017
27. Hrebien S, Citi V, Garcia-Murillas I, et al. Early ctDNA dynamics as a surrogate for progression-free survival in advanced breast cancer in the BEECH trial. *Ann Oncol*. 2019;30(6):945–952. doi:10.1093/annonc/mdz085
28. Uspst F, Nicholson WK, Silverstein M, et al. Screening for breast cancer: us preventive services task force recommendation statement. *JAMA*. 2024;331(22):1918–1930. doi:10.1001/jama.2024.5534
29. Henderson JT, Webber EM, Weyrich MS, Miller M, Melnikow J. Screening for breast cancer: evidence report and systematic review for the us preventive services task force. *JAMA*. 2024;331(22):1931–1946. doi:10.1001/jama.2023.25844
30. Keller L, Belloum Y, Wikman H, Pantel K. Clinical relevance of blood-based ctDNA analysis: mutation detection and beyond. *Br J Cancer*. 2021;124(2):345–358. doi:10.1038/s41416-020-01047-5
31. Papakonstantinou A, Gonzalez NS, Pimentel I, et al. Prognostic value of ctDNA detection in patients with early breast cancer undergoing neoadjuvant therapy: a systematic review and meta-analysis. *Cancer Treat Rev*. 2022;104:102362. doi:10.1016/j.ctrv.2022.102362
32. Panet F, Papakonstantinou A, Borrell M, Vivancos J, Vivancos A, Oliveira M. Use of ctDNA in early breast cancer: analytical validity and clinical potential. *NPJ Breast Cancer*. 2024;10(1):50. doi:10.1038/s41523-024-00653-3
33. Zhang X, Zhao W, Wei W, et al. Parallel analyses of somatic mutations in plasma circulating tumor DNA (ctDNA) and matched tumor tissues in early-stage breast cancer. *Clin Cancer Res*. 2019;25(21):6546–6553. doi:10.1158/1078-0432.CCR-18-4055
34. Hitchen N, Shahnam A, Tie J. Circulating tumor DNA: a pan-cancer biomarker in solid tumors with prognostic and predictive value. *Annu Rev Med*. 2025;76(1):207–223. doi:10.1146/annurev-med-100223-090016
35. Heidrich I, Ackar L, Mossahebi Mohammadi P, Pantel K. Liquid biopsies: potential and challenges. *Int J Cancer*. 2021;148(3):528–545. doi:10.1002/ijc.33217
36. Cutts R, Ulrich L, Beaney M, et al. Association of post-operative ctDNA detection with outcomes of patients with early breast cancers. *ESMO Open*. 2024;9(9):103687. doi:10.1016/j.esmoop.2024.103687
37. Zhang Y, Xu Y, Yao Y, et al. Pan-cancer circulating tumor DNA detection in over 10,000 Chinese patients. *Nat Commun*. 2021;12(1):11. doi:10.1038/s41467-020-20162-8
38. Garcia-Murillas I, Abbott CW, Cutts RJ, et al. Whole genome sequencing-powered ctDNA sequencing for breast cancer detection. *Ann Oncol*. 2025;36(6):673–681. doi:10.1016/j.annonc.2025.01.021
39. Bao Y, Zhang D, Guo H, Ma W. Beyond blood: advancing the frontiers of liquid biopsy in oncology and personalized medicine. *Cancer Sci*. 2024;115(4):1060–1072. doi:10.1111/cas.16097
40. Shuai Y, Ma Z, Ju J, et al. Liquid-based biomarkers in breast cancer: looking beyond the blood. *J Transl Med*. 2023;21(1):809. doi:10.1186/s12967-023-04660-z
41. Saura C, Ortiz C, Matito J, et al. Early-stage breast cancer detection in breast milk. *Cancer Discov*. 2023;13(10):2180–2191. doi:10.1158/2159-8290.CD-22-1340
42. Villacampa G, Navarro V, Matikas A, et al. Neoadjuvant immune checkpoint inhibitors plus chemotherapy in early breast cancer: a systematic review and meta-analysis. *JAMA Oncol*. 2024;10(10):1331–1341. doi:10.1001/jamaoncol.2024.3456
43. Turner NC, Swift C, Jenkins B, et al. Results of the c-TRAK TN trial: a clinical trial utilising ctDNA mutation tracking to detect molecular residual disease and trigger intervention in patients with moderate- and high-risk early-stage triple-negative breast cancer. *Ann Oncol*. 2023;34(2):200–211. doi:10.1016/j.annonc.2022.11.005
44. von Minckwitz G, Untch M, Blohmer JU, et al. Definition and impact of pathologic complete response on prognosis after neoadjuvant chemotherapy in various intrinsic breast cancer subtypes. *J Clin Oncol*. 2012;30(15):1796–1804. doi:10.1200/JCO.2011.38.8595
45. Johnston SRD, Harbeck N, Hegg R, et al. Abemaciclib combined with endocrine therapy for the adjuvant treatment of HR+, HER2-, node-positive, high-risk, early breast cancer (monarchE). *J Clin Oncol*. 2020;38(34):3987–3998. doi:10.1200/JCO.20.02514
46. Cantini L, Trapani D, Guidi L, et al. Neoadjuvant therapy in hormone Receptor-Positive/HER2-Negative breast cancer. *Cancer Treat Rev*. 2024;123:102669. doi:10.1016/j.ctrv.2023.102669
47. Chen JH, Addanki S, Roy D, et al. Monitoring response to neoadjuvant chemotherapy in triple negative breast cancer using circulating tumor DNA. *BMC Cancer*. 2024;24(1):1016. doi:10.1186/s12885-024-12689-6
48. Sharma P, Stecklein SR, Yoder R, et al. Clinical and biomarker findings of neoadjuvant pembrolizumab and carboplatin plus docetaxel in triple-negative breast cancer: neoPACT Phase 2 clinical trial. *JAMA Oncol*. 2024;10(2):227–235. doi:10.1001/jamaoncol.2023.5033

49. Leon-Ferre RA, Goetz MP. Advances in systemic therapies for triple negative breast cancer. *BMJ*. 2023;381:e071674. doi:10.1136/bmj-2022-071674
50. Cailleux F, Agostinetto E, Lambertini M, et al. Circulating tumor DNA after neoadjuvant chemotherapy in breast cancer is associated with disease relapse. *JCO Precis Oncol*. 2022;6:e2200148. doi:10.1200/PO.22.00148
51. Cavallone L, Aguilar-Mahecha A, Lafleur J, et al. Prognostic and predictive value of circulating tumor DNA during neoadjuvant chemotherapy for triple negative breast cancer. *Sci Rep*. 2020;10(1):14704. doi:10.1038/s41598-020-71236-y
52. Ciriaco N, Zamora E, Escriva-de-Romani S, et al. Clearance of ctDNA in triple-negative and HER2-positive breast cancer patients during neoadjuvant treatment is correlated with pathologic complete response. *Ther Adv Med Oncol*. 2022;14:17588359221139601. doi:10.1177/17588359221139601
53. Kjaer IM, Kahns S, Timm S, et al. Phase II trial of delta-tocotrienol in neoadjuvant breast cancer with evaluation of treatment response using ctDNA. *Sci Rep*. 2023;13(1):8419. doi:10.1038/s41598-023-35362-7
54. Liu Z, Yu B, Su M, et al. Construction of a risk stratification model integrating ctDNA to predict response and survival in neoadjuvant-treated breast cancer. *BMC Med*. 2023;21(1):493. doi:10.1186/s12916-023-03163-4
55. Magbanua MJM, Brown Swigart L, Ahmed Z, et al. Clinical significance and biology of circulating tumor DNA in high-risk early-stage HER2-negative breast cancer receiving neoadjuvant chemotherapy. *Cancer Cell*. 2023;41(6):1091–1102e4. doi:10.1016/j.ccell.2023.04.008
56. Magbanua MJM, Swigart LB, Wu HT, et al. Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. *Ann Oncol*. 2021;32(2):229–239. doi:10.1016/j.annonc.2020.11.007
57. McDonald BR, Contente-Cuomo T, Sammut SJ, et al. Personalized circulating tumor DNA analysis to detect residual disease after neoadjuvant therapy in breast cancer. *Sci Transl Med*. 2019;11(504). doi:10.1126/scitranslmed.aax7392
58. Ortolan E, Appierto V, Silvestri M, et al. Blood-based genomics of triple-negative breast cancer progression in patients treated with neoadjuvant chemotherapy. *ESMO Open*. 2021;6(2):100086. doi:10.1016/j.esmoop.2021.100086
59. Parsons HA, Blewett T, Chu X, et al. Circulating tumor DNA association with residual cancer burden after neoadjuvant chemotherapy in triple-negative breast cancer in TBCRC 030. *Ann Oncol*. 2023;34(10):899–906. doi:10.1016/j.annonc.2023.08.004
60. Riva F, Bidard FC, Houy A, et al. Patient-specific circulating tumor DNA detection during neoadjuvant chemotherapy in triple-negative breast cancer. *Clin Chem*. 2017;63(3):691–699. doi:10.1373/clinchem.2016.262337
61. Rothe F, Silva MJ, Venet D, et al. Circulating tumor DNA in HER2-amplified breast cancer: a translational research substudy of the NeoALTTO phase III trial. *Clin Cancer Res*. 2019;25(12):3581–3588. doi:10.1158/1078-0432.CCR-18-2521
62. Takahashi H, Kagara N, Tanei T, et al. Correlation of methylated circulating tumor DNA with response to neoadjuvant chemotherapy in breast cancer patients. *Clin Breast Cancer*. 2017;17(1):61–69e3. doi:10.1016/j.clbc.2016.06.006
63. Zhou Q, Gampenrieder SP, Frantal S, et al. Persistence of ctDNA in patients with breast cancer during neoadjuvant treatment is a significant predictor of poor tumor response. *Clin Cancer Res*. 2022;28(4):697–707. doi:10.1158/1078-0432.CCR-21-3231
64. Li S, Li Y, Wei W, et al. Dynamic ctDNA tracking stratifies relapse risk for triple negative breast cancer patients receiving neoadjuvant chemotherapy. *Nat Commun*. 2025;16(1):2786. doi:10.1038/s41467-025-57988-z
65. Radovich M, Jiang G, Hancock BA, et al. Association of circulating tumor DNA and circulating tumor cells after neoadjuvant chemotherapy with disease recurrence in patients with triple-negative breast cancer: preplanned secondary analysis of the BRE12-158 randomized clinical trial. *JAMA Oncol*. 2020;6(9):1410–1415. doi:10.1001/jamaoncol.2020.2295
66. Seale KN, Tkaczuk KHR. Circulating biomarkers in breast cancer. clin breast cancer. *Clin Breast Cancer*. 2022;22(3):e319–e331. doi:10.1016/j.clbc.2021.09.006
67. Aristei C, Tomatis M, Antonio P, et al. Treatment and outcomes in breast cancer patients: a cross section study from the EUSOMA breast centre network. *Eur J Cancer*. 2024;196:113438. doi:10.1016/j.ejca.2023.113438
68. Shaw JA, Page K, Wren E, et al. Serial postoperative circulating tumor DNA assessment has strong prognostic value during long-term follow-up in patients with breast cancer. *JCO Precis Oncol*. 2024;8:e2300456. doi:10.1200/PO.23.00456
69. Lipsyc-Sharf M, de Bruin EC, Santos K, et al. Circulating tumor DNA and late recurrence in high-risk hormone receptor-positive, human epidermal growth factor receptor 2-negative breast cancer. *J Clin Oncol*. 2022;40(22):2408–2419. doi:10.1200/JCO.22.00908
70. Xiong X, Zheng LW, Ding Y, et al. Breast cancer: pathogenesis and treatments. *Signal Transduct Target Ther*. 2025;10(1):49. doi:10.1038/s41392-024-02108-4
71. Hanker AB, Sudhan DR, Arteaga CL. Overcoming endocrine resistance in breast cancer. *Cancer Cell*. 2020;37(4):496–513. doi:10.1016/j.ccell.2020.03.009
72. Ye F, Dewanjee S, Li Y, et al. Advancements in clinical aspects of targeted therapy and immunotherapy in breast cancer. *Mol Cancer*. 2023;22(1):105. doi:10.1186/s12943-023-01805-y
73. Andre F, Su F, Solovieff N, et al. Pooled ctDNA analysis of MONALEESA phase III advanced breast cancer trials. *Ann Oncol*. 2023;34(11):1003–1014. doi:10.1016/j.annonc.2023.08.011
74. Turner NC, Kingston B, Kilburn LS, et al. Circulating tumour DNA analysis to direct therapy in advanced breast cancer (plasmaMATCH): a multicentre, multicohort, phase 2a, platform trial. *Lancet Oncol*. 2020;21(10):1296–1308. doi:10.1016/S1470-2045(20)30444-7
75. Hortobagyi GN. Ribociclib for the first-line treatment of advanced hormone receptor-positive breast cancer: a review of subgroup analyses from the MONALEESA-2 trial. *Breast Cancer Res*. 2018;20(1):123. doi:10.1186/s13058-018-1050-7
76. Kish JK, Ward MA, Garofalo D, et al. Real-world evidence analysis of palbociclib prescribing patterns for patients with advanced/metastatic breast cancer treated in community oncology practice in the USA one year post approval. *Breast Cancer Res*. 2018;20(1):37. doi:10.1186/s13058-018-0958-2
77. Goetz MP, Hamilton EP, Campone M, et al. Landscape of baseline and acquired genomic alterations in circulating tumor DNA with abemaciclib alone or with endocrine therapy in advanced breast cancer. *Clin Cancer Res*. 2024;30(10):2233–2244. doi:10.1158/1078-0432.CCR-22-3573
78. Sant M, Bernat-Peguera A, Felip E, Margeli M. Role of ctDNA in Breast Cancer. *Cancers*. 2022;14(2). doi:10.3390/cancers14020310
79. Sabit H, Attia MG, Mohamed N, et al. Beyond traditional biopsies: the emerging role of ctDNA and MRD on breast cancer diagnosis and treatment. *Discov Oncol*. 2025;16(1):271. doi:10.1007/s12672-025-01940-6
80. Venetis K, Pepe F, Pescia C, et al. ESR1 mutations in HR+/HER2-metastatic breast cancer: enhancing the accuracy of ctDNA testing. *Cancer Treat Rev*. 2023;121:102642. doi:10.1016/j.ctrv.2023.102642

81. Tzanikou E, Lianidou E. The potential of ctDNA analysis in breast cancer. *Crit Rev Clin Lab Sci*. 2020;57(1):54–72. doi:10.1080/10408363.2019.1670615
82. Grisolia P, Tufano R, Iannarone C, et al. Differential methylation of circulating free DNA assessed through cfMeDiP as a new tool for breast cancer diagnosis and detection of BRCA1/2 mutation. *J Transl Med*. 2024;22(1):938. doi:10.1186/s12967-024-05734-2
83. Zhang W, Cao J, Liu K, et al. Non-invasive plasma testing for CD274 UTR structural variations by next-generation sequencing in cancer. *Cell Death Discov*. 2023;9(1):35. doi:10.1038/s41420-023-01316-1
84. Davis AA, Jacob S, Gerratana L, et al. Landscape of circulating tumour DNA in metastatic breast cancer. *EBioMedicine*. 2020;58:102914. doi:10.1016/j.ebiom.2020.102914
85. Chi Y, Su M, Zhou D, et al. Dynamic analysis of circulating tumor DNA to predict the prognosis and monitor the treatment response of patients with metastatic triple-negative breast cancer: a prospective study. *Elife*. 2023. doi:10.7554/eLife.90198