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REVIEW

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From residual risk to precision intervention: the evolving role of minimal residual disease in breast cancer management

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ABSTRACT Breast cancer mortality is driven predominantly by metastasis, which affects 20–30% of patients with early-stage disease despite guideline-directed therapies. Because conventional imaging modalities currently lack sensitivity to identify residual disease, molecular-level monitoring must be developed. Circulating tumor DNA (ctDNA) profiling currently enables transformative minimal residual disease (MRD) detection and can quantify tumor burden at low variant allele frequencies. This review provides a comprehensive overview of MRD in breast cancer, including its definition, detection technologies, positivity thresholds, pathophysiology, clinical applications in adjuvant and neoadjuvant settings, ongoing clinical trials, challenges, and future directions. ctDNA-defined MRD has potential as a precision tool for adaptive therapy, and might facilitate post-adjuvant interception, whereby targeted therapies are administered to eradicate micro-metastases before radiographic recurrence. Persistent challenges include MRD assay standardization, subtype-specific MRD thresholds, tumor heterogeneity, and positioning MRD as a potentially valuable tool for precision management in breast cancer.

KEYWORDS Circulating tumor DNA; minimal residual disease; breast cancer; tumor-informed MRD; precision management

Introduction

Breast cancer (BC) is well documented to be the most prevalent malignancy and the leading cause of cancer-related mortality among women worldwide. According to GLOBOCAN 2023 data, more than 2.3 million new cases and approximately 670,000 deaths have been reported annually. Currently, developing countries exhibit higher age-standardized incidence rates (ASR, 34.8 per 100,000) than developed nations (ASR, 34.4 per 100,000), and concerning trends toward earlier age at onset and higher relapse ratios have been observed[1]. Although multidisciplinary therapies combining surgery, radiotherapy, chemotherapy, endocrine therapy, and targeted agents have significantly improved outcomes, 30%–40% of patients with early-stage BC have elevated recurrence risk, and the 5-year overall survival rate for metastatic BC remains below 30%[2,3]. Two recurrence peaks have been observed at 2–3 years and 5–7 years in hormone receptor (HR)-positive BC, whereas recurrence peaks generally occur at 2–3 years in triple-negative BC (TNBC) and human epidermal growth factor receptor 2 (HER2)-positive BC with visceral and brain metastases[4]. The combination of novel CDK4/6 inhibitors (e.g., abemaciclib and ribociclib) combined with endocrine therapy decreases the 5-year recurrence risk in clinical high-risk HR+/HER2– patients[2,5-7], and the prognosis of HER2-positive BC has markedly improved in patients treated with dual-targeted regimens and antibody-drug conjugates[8,9]. Nevertheless, these breakthroughs require precise recurrence risk stratification tools for individualized and precision management[9-11]. Current post-operative monitoring relies primarily on imaging modalities (ultrasound, mammography, and CT/MRI)[12] and serum biomarkers (CEA and CA15-3), which have low sensitivity and specificity[13,14]. Although tissue biopsy remains

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the gold standard, its invasive nature limits repeatability and cannot capture tumor spatiotemporal heterogeneity[14,15]. Minimal residual disease (MRD) testing *via* circulating tumor DNA (ctDNA) addresses these gaps by enabling ultra-sensitive detection of residual tumor cells and prognostic stratification, and providing a lead-time advantage. MRD can further guide precision interventions: ctDNA clearance correlates with therapeutic efficacy, and rising levels prompt regimen adjustments. Plasma *ESR1* and *PIK3CA* mutations in BC are required to guide ESR1-proteolysis targeting chimeras (PROTACs)[16], oral selective estrogen receptor degraders (SERDs)[17], PI3K/AKT inhibitors[18], and other targeted treatments. MRD detection, by bridging the critical divide between population-based adjuvant therapy and individualized management, has transformed relapse monitoring from reactive diagnostics to proactive intervention.

Evolution and challenges in recurrence risk assessment

Contemporary risk stratification integrates clinicopathological prognostic models (e.g., ADJUVANT!) and RNA assay-based molecular subtypes (PAM50). Multigene assays (Oncotype DX and MammaPrint) analyzing 21-/70-gene expression profiles enable chemotherapy de-escalation in selected low-risk patients, yet have demonstrated limited benefits for patients with HR-positive, HER2-negative BC and low tumor burden in axillary lymph nodes[19,20]. Consequently, their applicability is restricted to a specific subset of patients. Tissue-based biomarkers (e.g., Ki-67 and TP53 mutations) provide insights into tumor proliferation and aggressiveness but are limited by sampling bias and heterogeneity-induced variability[21]. Through multi-omics analysis (genomics, transcriptomics, metabolomics, and proteomics) of 579 patients, the first Fudan Classification (SNF subtypes) was proposed for luminal BC with varying sensitivity to targeted therapies or immune checkpoint inhibitors[22]. An AI model using digital pathology (H&E slides) has been developed for rapid subtyping, and clinical trials to validate these precision strategies are ongoing. Spatial heterogeneity in immune cell topology has been identified to predict responses to SHR-A1811 (accuracy: 86%)[10]. Antigen-presenting mast cells play a critical role in immune therapy resistance[23]. Nonetheless, current approaches rely solely on tissue-based multi-omics analysis and have not yet incorporated liquid biopsy for multidimensional classification-guided precision therapy[3,24,25].

Breakthroughs and bottlenecks in liquid biopsy

Liquid biopsy has revolutionized non-invasive MRD monitoring through ctDNA[26], circulating tumor cells (CTCs)[27], cell-free RNA[28], tumor-educated platelets[29], and exosomes[30-32]. ctDNA demonstrates superior clinical utility to CTCs and exosomes in post-operative recurrence monitoring, because of its enhanced sensitivity[33], temporal resolution, and analytical robustness[34]. Although CTC detection remains constrained by low abundance in early-stage disease and technical challenges in cell capture/viability, ctDNA assays can identify tumor-specific mutations even at MRD levels through highly sensitive droplet digital polymerase chain reaction (ddPCR)[30,35-37] or next-generation sequencing (NGS)-based methods[38]. Similarly, exosome-based profiling faces biological heterogeneity and standardization challenges in isolation/analysis. In contrast, the relatively short half-life of ctDNA enables near real-time monitoring of tumor dynamics and can capture early molecular relapse weeks to months before radiographic recurrence[39]. This temporal advantage, combined with standardized quantification of tumor-derived genomic alterations (e.g., methylation signatures and somatic mutations)[40], positions ctDNA as a dynamic biomarker for longitudinal risk stratification and therapy response assessment[41]. Moreover, its compatibility with high-throughput sequencing facilitates comprehensive tumor evolution analysis, which remains unattainable *via* single-cell CTC characterization or fragmented exosomal RNA profiling. Consequently, ctDNA might potentially be the most promising liquid biopsy tool for guiding personalized adjuvant interventions. ctDNA analysis provides a molecular snapshot of the tumor's genetic landscape. This method reveals specific mutations and alterations, which are crucial for identifying targetable genomic changes and monitoring the emergence of resistance during targeted therapies. CTCs are intact, viable cancer cells that can be enumerated to assess tumor burden, and to conduct protein expression analysis and even *ex vivo* culturing for drug susceptibility testing. The combined application of ctDNA and CTCs transcends a single diagnostic lens by providing multidimensional characterization of the disease, thereby enhancing the accuracy of prognosis, monitoring, and treatment management (**Figure 1**).

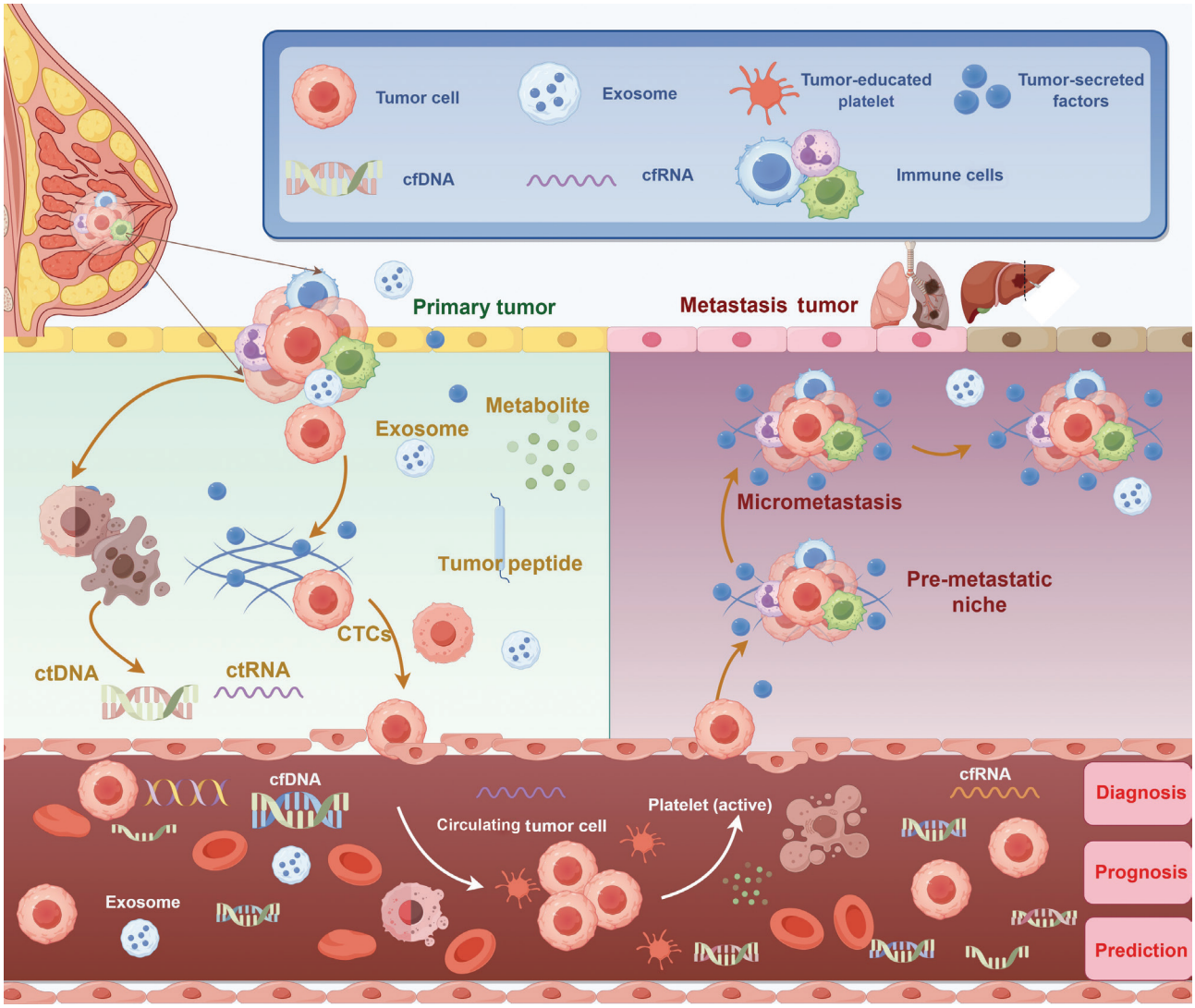

Figure 1 Multi-component liquid biopsy in primary breast cancer. This figure illustrates the key components of liquid biopsy released from primary breast cancer lesions into the circulation and their clinical significance. Liquid components included CTCs, ctDNA, cfRNA, exosomes, TEPs, tumor-associated peptides, and metabolites. Detection of these components in the blood offers a non-invasive approach for early diagnosis, monitoring, prognosis, and prediction in breast cancer. CTCs, circulating tumor cells; ctDNA, circulating tumor DNA; cfRNA, cell-free RNA; TEPs, tumor-educated platelets.

Importantly, ctDNA detection based on whole-exome sequencing (WES) or target NGS panels enables recurrence prediction 7.9–11 months earlier than imaging techniques. NGS-based personalized MRD panels can monitor clonal evolution and resistance mutations (*ESR1* and *PIK3CA*), and CTC phenotyping can aid in predicting metastatic potential. Meta-analyses have demonstrated a 40% reduced disease-free survival in patients with ≥5 CTCs than those with <5 CTCs per 7.5 mL of blood. Prospective trials (c-TRAK-TN, PADA-1, and SERENA-6) have concluded that ctDNA-guided therapeutic intensification (e.g., pembrolizumab augmentation) or strategy switching (replacing aromatase inhibitors with fulvestrant or camizestrant) in metastatic BC extends the median progression-free survival[42]. These advancements have marked a paradigm shift from population-based approaches to MRD-driven precision intervention in BC management.

MRD detection technologies: tumor-informed *vs.* tumor-agnostic approaches

Recent advances in high-throughput sequencing and bioinformatics have made MRD detection clinically useful, and ctDNA-based methods have emerged as critical tools for monitoring residual cancer burden. Two main methods, tumor-informed (patient-specific) and tumor-naive strategies, each with distinct operational frameworks and clinical implications, are used for contemporary MRD testing[43]. The tumor-agnostic or strategy, under the tumor-naive umbrella, refers to assays designed to detect a pre-defined, fixed set of genomic alterations (e.g., mutations or methylation patterns) that are common across many cancer types (**Figure 2**).

Tumor-informed approach: precision through personalization

The tumor-informed strategy starts with deep sequencing of the patient's primary tumor tissue, typically *via* custom-targeted multiple panels or WES, to identify somatic mutations unique to the individual's malignancy. A personalized ctDNA assay is subsequently designed to monitor these tumor-derived variants in serial blood samples through ultra-deep sequencing (≥ 30,000× coverage or ≥ 100,000× coverage)[44,45]. Notably, this approach achieves exceptional sensitivity (detection limits as low as 0.001% variant allele frequency, VAF) by minimizing background interference from clonal hematopoiesis or germline polymorphisms. Moreover, its adaptability to tumor evolution enables monitoring of clonal dynamics during therapy and provides critical insights into emerging resistant subpopulations[46]. However, because this method is limited by its dependency on high-quality tumor tissue, it has limited applicability in cases with inadequate or degraded biopsy specimens. The requirement for WES analysis and custom panel design poses challenges in rapid clinical deployment, because it extends turnaround times (typically 2–4 weeks) and elevates costs.

In the context of ultra-low ctDNA abundance monitoring after early-stage tumor resection, transitioning from WES to a tumor-informed whole-genome sequencing (WGS) baseline strategy significantly enhances MRD detection performance. WGS achieves this enhancement through comprehensive genomic coverage, which captures additional individualized variant sites (e.g., non-coding region mutations and structural variations) and consequently increases the number of personalized monitoring targets. Concurrently, by integrating high-depth sequencing with noise suppression algorithms, it effectively attenuates background noise and increases the signal-to-noise ratio for low-frequency signals. These synergistic effects elevate detection sensitivity to the parts per million (ppm) level (1–8 ppm) and therefore enable precise early detection and dynamic tracking of post-operative trace MRD[47]. However, because WGS generates 50–100 times more raw data than WES, it requires substantial analytical resources and significantly higher costs; as a result, its widespread clinical adoption is limited.

The NeXT Personal MRD platform has provided a major advancement in BC monitoring, through its innovative use of WGS to detect ctDNA at unprecedented sensitivity and specificity. This ultrasensitive approach, which enables identification of residual disease at levels as low as 1 ppm, significantly expands the window for early intervention and improves patient outcomes. By leveraging tumor-informed ctDNA panels, the platform ensures high specificity, thus minimizing false positives and enabling evaluation of each patient's disease status with enhanced precision. Studies have highlighted the prognostic relevance of ctDNA detection, by demonstrating its strong association with relapse risk and survival outcomes. In an early-stage non-small cell lung cancer (NSCLC) cohort, NeXT Personal MRD, compared with the WES-based > 100-plex PCM™ monitoring strategy and the 16-plex Signatera strategy (179 d *vs.* 119 d *vs.* 70 d), significantly prolonged the lead time before radio-relapse. The NeXT Personal MRD method was used to stratify the preoperative ctDNA-negative population (below 0.008%) in the TracerX cohort (100+ sites) monitored by Invitae PCM into 2 groups (ultra-low MRD+ and ctDNA-negative populations) with different overall survival. The recurrence risk and mortality in ctDNA-negative patients approached those in the cured post-operative population but were significantly higher in patients with ultra-low MRD positivity [between 0.0001% and 0.008%; RFS: P = 4.4e-5; overall survival (OS): P = 0.0017]. In patients with early BC, the NeXT Personal WGS-powered ultrasensitive ctDNA MRD assay detected ctDNA in 98% of patients at diagnosis, and significantly outperformed exome-based and dPCR-based assays. This outstanding sensitivity enabled earlier detection of MRD and improved the lead time to clinical relapse (median lead time of 15 months with the WGS-based MRD method). The assay's prognostic value was also demonstrated: ctDNA detection was strongly associated with

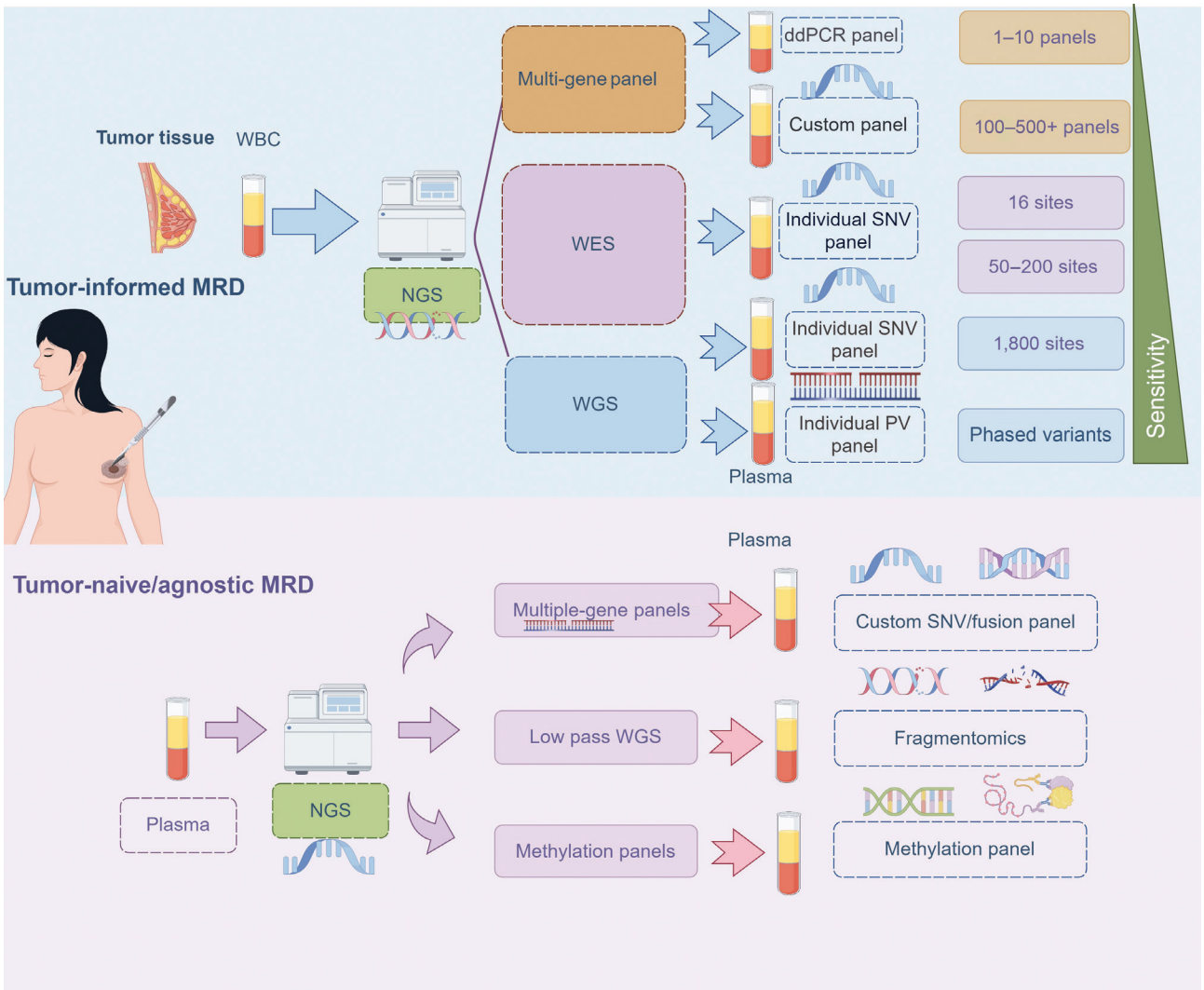

Figure 2 MRD detection strategies in liquid biopsy: tumor-informed MRD and tumor-naive/agnostic MRD. This schematic illustrates two core strategies for MRD detection *via* liquid biopsy. Tumor-informed analysis requires baseline profiling of tumor tissue (e.g., large-panel sequencing, WES, or WGS) to identify patient-specific mutations and enable the design of personalized panels for longitudinal MRD monitoring. Tumor-naive/agnostic analysis bypasses tumor tissue sampling by using predefined fixed panels targeting population-level recurrent mutations or epigenetic markers (e.g., methylation) and consequently is suitable for pan-cancer screening. Low-pass WGS fragmentomics can also be performed by analyzing fragment length distribution, end motifs, and other genomic features of cfDNA, thus offering a non-invasive approach for early-stage MRD screening. cfDNA, cell-free DNA; MRD, minimal residual disease; WES, whole-exome sequencing; WGS, whole-genome sequencing.

shorter RFS and OS than those without ctDNA detection. The assay identified a subset of patients with low-level MRD who cleared ctDNA and did not relapse during long-term follow-up (64/64), thus highlighting its potential for risk stratification and guiding treatment decision-making[45]. Overall, the NeXT Personal MRD platform has the potential to transform BC management, by enabling earlier detection of relapse, more accurate assessment of disease status, and improved patient outcomes through personalized treatment approaches.

The PhasED-seq method leverages the unique characteristics of phased variants (PVs), defined as 2 or more mutations occurring within a single DNA fragment, located on the same DNA strand, in tumors[48]. Through design of a specific sequencing panel, PhasED-seq enriches and detects these PVs, and therefore achieves ctDNA detection with enhanced sensitivity. PVs exhibit highly consistent genomic distribution in B-cell malignancies, probably because of physiological and aberrant somatic hypermutations. PhasED-seq uses a

sequencing panel targeting PVs, which can capture PVs from tumor or plasma samples for monitoring residual disease. Its advantages include its low background signal and enhanced DNA molecule recovery efficiency. Compared with traditional single-nucleotide variant (SNV)-based methods, PhasED-seq achieves a superior detection sensitivity capable of identifying ctDNA at the one-in-a-million level[48]. Consequently, PhasED-seq can detect low-burden residual disease. In clinical settings, PhasED-seq has demonstrated remarkable results in patients with B-cell lymphoma. Specifically, PhasED-seq has detected ctDNA that could not be identified by Cancer Personalized Profiling by Deep Sequencing (CAPP-Seq) and was associated with poor prognoses. PhasED-seq is applied to solid tumors through a personalized approach. WGS of tumor and healthy tissues is conducted to identify patient-specific PVs, and a personalized sequencing panel targeting these PVs is designed and used to sequence plasma samples for residual disease monitoring. Notably, PhasED-seq has also demonstrated promising performance in solid tumors. In lung adenocarcinoma and BC, PhasED-seq has detected ctDNAs that CAPP-Seq could not identify and provided more accurate prediction of disease progression.

Structural variations (SVs), prevalent cancer genome features that include breakpoints and rearrangements, are tumor-specific and reflect tumor biology. Given the high inter-individual variability of SV breakpoints, this assay detects low ctDNA levels on the basis of SVs from tumor tissue WGS, and the design of a personalized panel effectively decreases the false positives commonly encountered in SNV-based detection approaches[49,50]. In one study, ctDNA was detected in blood samples from 96% of participants at baseline, with a median VAF of 0.15%. All participants with detectable ctDNA post-surgery or during follow-up experienced recurrence, and the median lead time was 417 days[49]. On the basis of tumor WGS, Signatera Genome MRD has revealed a strong association between ctDNA negativity and improved RFS and OS among 126 patients tested within the MRD window period (within 3 months after surgery and before the initiation of adjuvant therapy). The hazard ratios for RFS were 10.0 in the overall patient population and 17.0 in patients with extracranial recurrence[51]. The MONSTAR-SCREEN-3 study, using ultra-sensitive WGS combined with ctDNA analysis, has demonstrated that ctDNA is cleared in patients achieving pathological complete response (pCR) but persists in patients without pCR. Patients who were landmark MRD negative after surgery had no recurrence within 6 months, thus indicating excellent short-term prognosis, whereas MRD-positive patients who did not receive adjuvant therapy faced an 80% recurrence risk within 6 months; these findings confirmed MRD as a valid predictor of treatment efficacy and prognosis[52].

The tumor-informed WGS-based MRD strategy is considered a highly sensitive ctDNA detection method offering enhanced precision for disease monitoring and prognosis assessment.

Tumor-agnostic approach: accessibility through standardization

The tumor-agnostic approach uses fixed, predefined gene panels targeting recurrent driver mutations, methylation signatures, or fragmentomic patterns to detect ctDNA directly from plasma without prior tumor sequencing. Techniques such as multiplex PCR or hybrid capture-based NGS enable standardized workflows, deliver results within 7 days, and are ideal for large-scale screening. This approach broadens applicability to patients with inaccessible tumors (inoperable cases or long-interval samples) and integrates multi-omic enhancements. Moreover, use of methylation markers improves sensitivity in low-mutation-burden cancers by leveraging dense epigenetic alterations.

Despite operational efficiency, tumor-agnostic assays exhibit lower sensitivity (typical limit of detection: 0.1% VAF) than tumor-informed methods, because of restricted genomic coverage. The use of fixed panels increases the risk of false positives arising from clonal hematopoiesis of indeterminate potential (CHIP)-derived mutations and might result in suboptimal performance in tumors with low mutation burdens[53]. Platforms such as Guardant Reveal and FoundationOne Liquid CDx have demonstrated utility for early relapse prediction in NSCLC and colorectal cancer (CRC)[54,55], although their prognostic accuracy remains inferior to that of tumor-informed assays.

CAPP-Seq, an ultrasensitive tumor-agnostic ctDNA detection technology developed in 2014, was introduced in response to the urgent clinical need for dynamic monitoring of solid tumors[35]. Conventional ctDNA detection faced limitations arising from insufficient sensitivity to capture trace DNA released by early-stage tumors or residual disease (typically accounting for less than 0.01% of cell-free DNA). The technological breakthrough, achieved by integrating cancer gene databases such as COSMIC to design cancer-specific

"selectors" targeting high-frequency mutation regions, achieves a sensitivity of 0.02% mutant allele frequency, equivalent to detecting one tumor DNA molecule among 10,000 normal DNA molecules. This method enables early diagnosis and staging, as evidenced by a detection rate of 50% patients with stage I NSCLC and 100% of patients with stage II–IV NSCLC, with a specificity of 96%. Furthermore, ctDNA levels significantly correlate with tumor volume ($R^2 = 0.89$, $P = 0.0002$), and the assay can assist in residual lesion identification[56]. For treatment monitoring and prognosis, ctDNA dynamics change earlier than radiographic responses, as illustrated in a stage IIIB lung cancer case in which ctDNA elevation during apparent radiographic remission preceded clinical recurrence by 7 months. Postoperative ctDNA positivity is associated with a significantly shorter progression-free survival than that with ctDNA-neagtive, and provides a mean recurrence warnings 5.2 months earlier than imaging modalities. Key applications of CAPP-Seq include early diagnosis (detecting mutations with allele frequencies as low as 0.02% to improve early-stage detection), treatment monitoring (dynamic ctDNA quantification enabling timely intervention), prognosis assessment (postoperative ctDNA positivity identifying high-risk patients requiring intensive therapy), and resistance management (detecting resistance mutations to optimize targeted therapies)[57].

Challenges and converging innovations

The core trade-off between sensitivity (favoring tumor-informed methods) and accessibility (favoring tumor-naive methods) emphasizes the need for context-specific implementation. Standardization gaps persist, and the consensus recommendation is ultra-deep sequencing (> 30,000× coverage for agnostic panels) to mitigate technical noise[35,58]. Emerging solutions include integrating multi-modal biomarkers (e.g., methylation or fragmentomics)[55] and leveraging machine learning algorithms to enhance specificity[59]. Tumor-informed and tumor-naive MRD detection are complementary paradigms[60]. Some Chinese companies have integrated tumor-informed and tumor-agnostic MRD strategies to balance sensitivity and specificity, and have used fusion mutation monitoring to further optimize the detection of treatment-driven alterations. As multi-omic refinements mature and prospective data accumulate, these technologies are anticipated to play decisive roles in guiding therapy de-escalation, recurrence surveillance, and drug development across oncology.

Cross-platform MRD detection: thresholds and positivity standards

Positivity criteria vary across various MRD detection technologies. Signatera® (Natera), a tumor-informed multiplex PCR assay, customizes a personalization panel of as many as 16 somatic mutations, with a median of 11 (range 8–16), derived from WES of tumor tissue. MRD positivity is defined as the detection of ≥ 2 tumor-specific mutations, with a LoD95 of 17 ppm[61,62]. In contrast, the Signatera Genome assay uses WGS to identify and monitor as many as 64 patient-specific somatic mutations from tumor tissue, a number representing an expansion beyond its original 16-mutation panel. The assay uses mPCR with a sensitivity of low single-digit ppm levels and maintains the same validated chemistry as the original Signatera platform. With customized primers, serial blood-based ctDNA detection achieves MRD positivity determination *via* a proprietary sample-calling algorithm that compares target confidences against sample-level noise and demonstrates a LoD95 of 5–9 ppm[51].

PCM (Invitae) uses a similar tumor-informed multiplex PCR approach targeting a maximum of 50 variants and a median of 33 personalized mutations (range: 11–50). Positivity is defined with a Poisson distribution-based statistical model ($P < 0.001$) to distinguish tumor-derived signals from background noise[63,64]. Likewise, RaDaR™ (Inivata) customizes as many as 48 tumor-specific variants (median 48) and requires either an estimated VAF > 0.001% or detection of ≥ 2 tumor-specific mutations to indicate MRD positivity[65,66]. ArcherDx's AMP assay, used in the TRACERx cohort, simultaneously monitors 150 clonal and subclonal mutations (median 197 variants) and applies a Poisson model ($P < 0.01$) to differentiate tumor signals from noise[67]. CAPP-Seq uses a fixed panel design covering 95% of NSCLC driver genes, coupled with integrated digital error suppression (iDES) technology to minimize sequencing background noise. The iDES system, by suppressing average noise levels from 0.03% to 0.0015%, enables reliable detection of true low-frequency mutations. The presence or absence of ctDNA has been assessed with a Monte Carlo-derived detection algorithm and a threshold value of ≤ 0.05 (validated in previous research) as the criterion for positivity. Specimens exhibiting values

exceeding this threshold were designated as ctDNA-negative, whereas those meeting or falling below the cutoff were classified as ctDNA-positive[35].

Novel technologies include MAESTRO (Broad Institute), which enriches thousands of low-frequency mutations (< 0.1% VAF) for 10-fold signal-to-noise enhancement. Because MRD positivity is defined by the detection of > 1 tumor-specific mutation, false negatives are minimized while reasonable specificity is maintained. To further enhance accuracy, use of the duplex sequencing consensus (DSC)/single-strand consensus (SSC) ratio as a noise-filtering metric ensures specificity in chimeric mutation detection. This ratio represents the proportion of DSC reads supporting a mutation relative to the SSC reads. Empirical evidence has demonstrated that at ratios below 0.15, non-mutant libraries display poor reproducibility during replicate capture, thereby indicating high background noise. A DSC/SSC ratio threshold of ≥ 0.15 is applied to exclude low-confidence variants[68,69].

The PhasED method implements personalized hybrid capture panels targeting PVs to analyze plasma-derived cell-free DNA (cfDNA) and achieves high unique molecular depth through ultra-deep sequencing. The tumor fraction is calculated as the ratio of sequencing reads containing predefined PVs to the total reads covering PV genomic positions. A sample is classified as ctDNA-positive when at least one predefined PV is detected above the established threshold, and all calls require statistical significance ($P < 0.01$) after background noise correction[48].

The tumor-informed NeXT Personal MRD platform uses WGS to achieve ultra-sensitive detection at single-molecule resolution. The ctDNA burden is quantified by integration of variant allele signals across all patient-specific genomic targets after rigorous noise filtration. Sample-level positivity is established through a stringent statistical framework in which tumor-derived molecular counts are aggregated to surpass background noise expectations with Poisson-distributed significance ($P \leq 0.001$)[45].

The determination of optimal MRD positivity thresholds requires careful consideration of the specific MRD monitoring strategy, the number and characteristics of personalized genomic variant targets, and the assay's background noise suppression ability (**Table 1**). When properly balanced, these parameters enable enhanced detection of MRD-positive cases without compromising test sensitivity or specificity, thereby improving the identification of patients at high recurrence risk. This optimized approach provides clinicians with substantially longer lead times and consequently facilitates timely therapeutic intervention. However, indiscriminate lowering of positivity thresholds to maximize detection rates might increase the false-positive results, and expose patients to unnecessary psychological distress and anxiety without conferring clinical benefit[70,71].

The sensitivity, specificity, cost, and clinical utility of various MRD monitoring strategies significantly vary across technologies and applications (**Table 2**). Tumor-informed personalized panels based on WGS have demonstrated the highest sensitivity and specificity. However, this approach is costly because of the high-depth sequencing, and the need for tumor tissue for baseline WGS profiling limits its accessibility. Tumor-agnostic methods offer moderate sensitivity and lower specificity than personalized panels, but are more affordable and widely available. Cost-effectiveness analyses have suggested that NGS might offset expenses through treatment de-escalation in MRD-negative cases, although the upfront costs and tissue dependency remain barriers. Overall, sensitivity and clinical utility are maximized by tumor-informed WGS-based or WES-based methods, whereas tumor agnostic fixed panels balance affordability and broad applicability.

Clinical applications of MRD detection in early-stage BC

ctDNA-based MRD detection has emerged as a transformative tool in early-stage BC management, by enabling dynamic risk stratification and personalized therapeutic interventions[72] (**Figure 3**). Unlike traditional imaging, ctDNA analysis provides molecular-level insights into residual tumor burden. Postoperative ctDNA positivity has been demonstrated to confer a 35.8-fold higher recurrence risk and significantly shorter disease-free survival (DFS) than that with ctDNA-negative[73,74]. This prognostic power is subtype-dependent: ctDNA positivity rates correlate with biological aggressiveness, as observed in 28.6% of HR+ tumors, 18.8% of TNBC, and 5.0% of HER2+ tumors[73]. In a study by Shaw et al.,[62] Signatera™ testing every 6 months in high-risk patients achieved a sensitivity and specificity of 88% and 95%, and a median lead time exceeding 10 months. In another study, RaDaR™ monitoring every 6–12 months has shown 100% sensitivity for metastatic recurrence (median lead time 12.4 months), although one localized recurrence was missed (overall sensitivity 85.7%)[62]. In contrast, Guardant

Table 1 MRD detection technologies and their positivity criteria

Technology name	Design approach	Variant panel size	Positivity criteria	Statistical method	Ref
Signatera (Natera)	Tumor-informed mPCR	WES-based Up to 16	≥ 2 Tumor-specific mutations detected	Proprietary calling algorithm	55
Signatera Genome (Natera)	Tumor-informed mPCR	WGS-based Up to 64	Proprietary sample calling score (compared target confidence *vs.* noise)	Sample-level noise modeling	61
PCM (Invitae)	Tumor-informed mPCR	WES-based Up to 50, median 33	Poisson model ($P < 0.001$) to distinguish tumor signals	Poisson distribution	62
RaDaR™ (NeoGenomics)	Tumor-informed mPCR	WES-based Up to 48	VAF > 0.001% OR ≥ 2 tumor-specific mutations	ctDNA score	63
ArcherDx AMP	Tumor-informed mPCR	Multiple regions WES-based Up to 200	Poisson model ($P < 0.01$) TRACERx-validated	Poisson distribution	65
CAPP-seq (Roche)	Fixed panel hybrid capture	125 kb genomic regions	Monte Carlo algorithm (threshold ≤ 0.05) iDES: ultra-low background noise (0.0015%)	Statistical modeling ($P < 0.01$)	35
MAESTRO (Broad Institute)	Low-frequency mutation enrichment	10,000 SNVs	Not specified	Signal-to-noise enhancement (10×)	66
PhasED	Personalized hybrid capture	WGS-based PVs	≥ 1 Predefined PV with $P < 0.01$	Statistical significance ($P < 0.01$)	48
NeXT Personal MRD	Tumor-informed hybrid capture	WGS-based Up to 1,800	Aggregate tumor counts > background ($P \leq 0.001$)	Poisson distribution ($P \leq 0.001$)	49

Reveal™ detected 85% of metastatic relapses but only 14% of local recurrences in early BC, and single-timepoint testing at approximately 2 years post-chemotherapy identified 34% of distant recurrences. Guardant Infinity™ methylation analysis of 83 patients with TNBC/luminal BC further corroborated a strong association between postoperative ctDNA positivity and recurrence. The PCM study confirmed that ctDNA detection preceded imaging by 11.7 months and achieved a specificity of 100%, thus highlighting its potential for early intervention[64]. Technologies such as WGS-based NeXT Personal MRD can achieve 98% sensitivity at diagnosis and track ultra-low ctDNA levels as low as 2.19 ppm[45].

Dynamic MRD monitoring in the adjuvant setting

The detection of MRD in the adjuvant setting can aid in identifying patients at high recurrence risk who might benefit from more aggressive adjuvant therapies, additional chemotherapy, targeted therapy, or prolonged treatment duration. In contrast, patients with undetectable MRD might be considered for treatment de-escalation, to decrease the risk of unnecessary toxicity. Moreover, longitudinal MRD monitoring can provide valuable insights into the effectiveness of adjuvant treatment. Undetectable MRD or achieving ctDNA clearance might indicate a favorable treatment response, whereas the emergence or persistence of MRD might suggest therapeutic resistance and poor prognosis.

Personalized multiple-mutation sequencing (e.g., Signatera or RaDaR), compared with ddPCR, significantly extends lead times and improves sensitivity. The MRD positive ratio has been found to be significantly higher in the TNBC population than the HR+ population, regardless of post-surgery time[62,73]. In TNBC, combining baseline ctDNA thresholds with post-operative dynamic monitoring optimizes risk stratification: longitudinal MRD monitoring has achieved 88%–100% sensitivity and 100% specificity for distant metastasis, and a median lead time of 8.9 months[73,75]. For HR+ BC in the late adjuvant phase, the MRD detection rate was 10% with a median lead time of 12.4 months, and all distant metastatic recurrences were preceded by MRD detection[76].

Table 2 MRD detection technologies' sensitivity, specificity, cost, and clinical utility

Technology Name	Platform	Sensitivity	Specificity	Cost	Clinical utility
Signatera (Natera)	Tumor-informed WES-based Up to 16	★★☆☆☆	★★★★☆	★★★★☆	★★★★☆
Signatera Genome (Natera)	Tumor-informed WGS-based Up to 64	★★★☆☆	★★★☆☆	★★★★☆	★★☆☆☆
PCM (Invitae)	Tumor-informed WES-based Up to 50	★★☆☆☆	★★★☆☆	★★☆☆☆	★★☆☆☆
RaDaR™ (NeoGenomics)	Tumor-informed WES-based Up to 48	★★☆☆☆	★★★☆☆	★★★☆☆	★★★☆☆
PROPHET (Rock)	Tumor-informed WES-based Up to 50	★★★☆☆	★★★☆☆	★★★★☆	★★☆☆☆
ArcherDx AMP	Tumor-informed multiple regions WES-based Up to 200	★★★☆☆	★★★☆☆	★★★☆☆	★☆☆☆☆
CAPP-seq (Roche)	Tumor-agnostic 125 kb genomic regions	★☆☆☆☆	★★☆☆☆	★☆☆☆☆	★★☆☆☆
MAESTRO (Broad Institute)	Tumor-informed WGS-based Up to 10,000	★★★☆☆	★★★☆☆	★★☆☆☆	★☆☆☆☆
PhasED	Tumor-informed WGS-based PVs	★★★★☆	★★★☆☆	★★★★☆	★★☆☆☆
NeXT Personal	Tumor-informed WGS-based Up to 1,800	★★★★☆	★★★★☆	★★★★☆	★★★☆☆

The MonarchE study has evaluated MRD status *via* Signatera ctDNA testing in patients with HR+/HER2– high-risk early BC after adjuvant therapy[72]. The results revealed a strong association between ctDNA positivity and relapse risk: in a subgroup of 910 patients, baseline ctDNA-positive patients had an 80% relapse rate (*vs.* 23% in the negative group), whereas ctDNA detection at any time during treatment was predictive of poor prognosis. Among persistently ctDNA-positive patients, 87% experienced invasive DFS events, as compared with only 15% of persistently ctDNA-negative patients[77]. Interestingly, patients with ctDNA conversion from negative to positive during treatment experienced a high relapse risk of 93%, and ctDNA positivity preceded radiographic relapse by 9–19 months, thus providing a critical window for therapeutic intervention.

The PADA-1 trial pioneered ctDNA ESR1 mutation-guided therapy switching in HR+ metastatic disease. Patients switching to fulvestrant + palbociclib after *ESR1* mutation detection had a median PFS of 11.9 months, as compared with 5.7 months in the control group[78]. The SERENA-6 trial pioneered ctDNA-guided dynamic monitoring of *ESR1* mutations to direct therapy switching to oral SERD camizestrant while maintaining the original CDK4/6 inhibitor before radiographic progression. The median progression-free survival was significantly extended (16.0 *vs.* 9.2 months; HR = 0.44), and the safety profiles were manageable[17,42]. Similar strategies are being evaluated in early-stage disease. For example, the c-TRAK-TN trial has leveraged ctDNA to guide adjuvant therapy intensification. This prospective phase II study evaluated ctDNA-based MRD monitoring to guide immunotherapy intensification with pembrolizumab (PD-1 inhibitor) in early-stage TNBC[79]. The study enrolled 208 patients with high-risk TNBC (non-pCR after neoadjuvant therapy or tumors > 2 cm with nodal positivity) and used personalized digital PCR for ctDNA surveillance every 3–12 months. The 12-month ctDNA positivity rate was 27.3% (44/161), and the baseline rate was 14.3% (23/161). ctDNA positivity strongly predicted relapse: 23 of 32 ctDNA-positive patients (71.9%) had concurrent clinical recurrence at detection, thus confirming MRD as a robust prognostic biomarker. None of the 5 ctDNA-positive patients without recurrence receiving

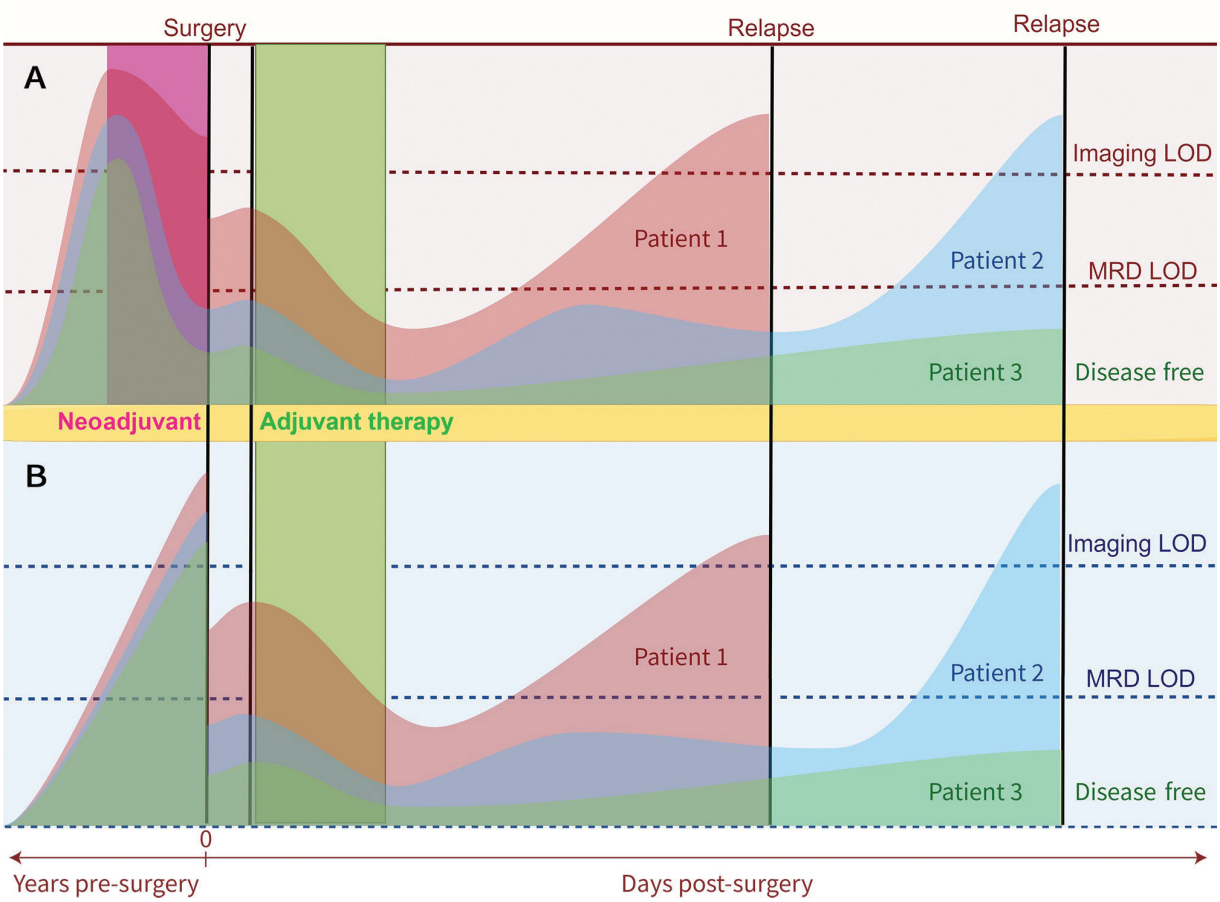

Figure 3 Clinical applications of MRD monitoring in breast cancer: lead time advantage of ctDNA-based detection and intervention opportunities. This schematic demonstrates the clinical utility of two MRD monitoring strategies, high-sensitivity customized panels and standard fixed panels, across the breast cancer management continuum. A. The lead time advantage of high-sensitivity MRD assays enables molecular relapse detection earlier than radiographic imaging, thereby establishing a critical window for therapeutic intervention. During the neoadjuvant therapy phase, dynamic ctDNA changes predict pCR. ctDNA clearance correlates with treatment efficacy, whereas persistent positivity signals resistance, thus guiding timely regimen modifications. B. For adjuvant therapy stratification, MRD-positive status triggers intensified intervention, whereas MRD-negative status supports de-escalation strategies to mitigate overtreatment toxicity. Postoperative landmark testing (4–8 weeks post-surgery) has revealed that ctDNA positivity predicts a significantly elevated recurrence risk in negative patients. Three representative patient trajectories further illustrate these principles. Patient A (red curve), with persistent landmark positivity and rising ctDNA levels, experiences radiographic relapse (e.g., bone metastasis) 11 months after initial MRD detection; intensified therapy delays disease progression. Patient B (blue curve) exhibits transient MRD positivity followed by clearance, thus indicating tumor dormancy; absence of radiographic relapse enables de-escalated management and sustained remission. Patient C (green curve) is persistently MRD-negative during surveillance. ctDNA, circulating DNA; MRD, minimal residual disease; pCR, pathological complete response.

pembrolizumab (200 mg IV every 3 weeks for 1 year) achieved ctDNA clearance, and all eventually relapsed. These findings indicated the limited efficacy of pembrolizumab monotherapy in these patients. Irreversible micrometastases were likely to have been present in most ctDNA-positive cases (23/32), thereby narrowing the intervention window. MRD assay sensitivity requires improvement (current specificity 96%–100%, sensitivity 19%–93%), and the monitoring frequency should be optimized (e.g., every 3 months) to enable earlier intervention[79]. Although c-TRAK-TN prospectively validated ctDNA-MRD as a reliable tool for identifying patients with high-risk TNBC, pembrolizumab monotherapy did not improve the outcomes. Therefore, earlier intervention or combination therapies must be investigated in future studies.

Clinical applications of MRD detection in the neoadjuvant setting

In the neoadjuvant setting, dynamic MRD assessment serves as a real-time biomarker of therapeutic response. Baseline ctDNA detection can further identify patients with inferior DFS and OS, and therefore has a role in upfront risk stratification[80]. Among patients with early BC, the prevalence of pre-neoadjuvant therapy (pre-NAT) ctDNA positivity is higher in hormone receptor-negative (HR-negative) than HR-positive cancers. Overall, 47.7%–78.7% of patients exhibited MRD positivity[32,64,81-86] (**Table 3**). Notably, within the TNBC subgroup, ctDNA positivity rates ranged from 63.4% to 91%[80,87,88]. WGS-based MRD detection demonstrated a positivity rate as high as 98% at diagnosis, with a median lead time of 15 months, and no recurrence was observed in patients with undetectable ctDNA[45]. MRD detection with a tumor-agnostic approach yielded a relatively lower positivity rate but was associated with a shorter lead time[53,59,89]. Persistent ctDNA detection mid-therapy correlates with residual cancer burden (RCB) and poor outcomes, whereas ctDNA clearance predicts pCR and improved survival, particularly in HER2-negative disease[75,82]. Therapeutic decision-making is increasingly informed by ctDNA kinetics. During neoadjuvant therapy, the I-SPY2 trial used Signatera ctDNA testing to dynamically monitor 283 patients with high-risk early-stage HER2-negative BC (HR+/HER2−: 145; TNBC: 138). The ctDNA positivity at baseline, and during the treatment (3/12 weeks) and pre-surgery (T3) timepoints, significantly correlated with shorter distant relapse-free survival (DRFS) ($P = 0.02$ to $P < 0.0001$)[85]. Patients with ctDNA-negative status at T3 exhibited markedly improved DRFS, particularly among non-pCR patients. The HR+/HER2- group exhibited a 5.89-fold higher relapse risk in patients with ctDNA-positive than those with ctDNA-negative (95% CI: 2.68-12.98), while the TNBC group displayed a 3.79-fold higher risk in patients with ctDNA-positive than those with ctDNA-negative (95% CI: 1.87-7.68). In patients with TNBC, ctDNA clearance at 3 weeks (T1) was strongly predictive of pCR (OR = 13.06; 95% CI: 3.54–57.95) and low residual cancer burden (RCB-0/I) (OR = 19.00; 95% CI: 4.98–89.06). In contrast, this association was absent in HR+/HER2− subtypes[90]. Of note, ctDNA status can refine risk stratification beyond pCR/RCB. One patient with TNBC who achieved pCR but remained ctDNA-positive relapsed at 21 months, whereas ctDNA-negative non-pCR patients experienced favorable outcomes, thus supporting ctDNA integration into post-treatment evaluation[87]. In a study by Elliott et al.[44,49] ($n = 119$ patients with early BC receiving neoadjuvant therapy), tumor-informed RaDaR™ monitoring of 48 mutations demonstrated that failure to clear ctDNA after neoadjuvant therapy predicted inferior outcomes, particularly in HER2-negative patients (HR = 14.4). ctDNA dynamics strongly correlated with pathological response, and patients achieving pCR (RCB-0) did not experience clinical recurrence, regardless of mid-treatment ctDNA status. Among patients with suboptimal response (RCB 1/2/3), those with ctDNA clearance during therapy showed significantly superior RFS to those with non-clearance ($P = 0.011$)[87]. Postoperative or longitudinal ctDNA detection demonstrated even stronger prognostic relevance and was associated with significantly shorter RFS than that with ctDNA undetection ($P < 0.0001$)[85]. Early ctDNA clearance in TNBC might therefore be used to guide de-escalation, whereas post-surgery positivity warrants intensified therapy.

Emerging therapies and clinical trials of MRD in BC

The relationship between ctDNA positivity rates and biological aggressiveness is particularly evident in the comparison between TNBC and HER2+ subtypes. TNBC demonstrates higher ctDNA positivity rates pre-, intra-, and post-NAC than HR+/HER2− disease, given its aggressive biology, including rapid proliferation and a propensity for early metastasis. In contrast, HER2+ tumors (with a positivity rate of ~5% in some cohorts) potentially show lower ctDNA shedding because of targeted therapy efficacy or different biological drivers, although this finding does not diminish their potential aggressiveness. Technologies such as tumor-informed PCR or NGS assays (e.g., Signatera) are optimal for TNBC, because of their high sensitivity and ability to track rapid dynamics, whereas HR+ tumors, with their lower shedding rates, might benefit from broader panel-based NGS to capture heterogeneous mutations over time. Practical recommendations should also consider clinical context: for NAC-treated high-risk patients, we advise baseline, mid-treatment, pre-surgery, and post-surgery time points, with subtype-guided adjustments; e.g., TNBC might require additional testing every 3–6 months for the first 3 years. Ultimately, the testing frequency should balance prognostic value against patient burden. We propose standardized windows based on risk (e.g., high-risk TNBC

Table 3 Clinical performance comparison of MRD monitoring technologies in breast cancer neoadjuvant/adjuvant therapy

N Stage	Subtype	Monitoring strategy	ctDNA+	MRD performance	Ref
N = 295 (II–III)	HR+/HER2− (35%) HER2+ (23%) TNBC (43%)	Tumor-informed Signatera Up to 16 variants	Pre-NAT 73.0% Pre-surgery 9.0%	pCR 17% *vs.* 48% (C3D1 ctDNA+ *vs.* ctDNA−) HR for DRFS 10.4	53
N = 283 (II–III)	HR+HER2− (51.2%) TNBC (48.8%)	Tumor-informed Signatera Up to 16 variants	HR+ Pre-NAT 69.0% Pre-surgery 12.0%	HR for DRFS 3.79 pCR 12.5% *vs.* 18.5% (pre-NAT ctDNA-high *vs.* ctDNA-low)	32
			TNBC Pre-NAT 91.0% Pre-surgery 22.0%	HR for DRFS 5.89 pCR 27.9% *vs.* 23.4% (pre-NAT ctDNA-high *vs.* ctDNA-low)	
N = 196 (II–III)	TNBC	Tumor-informed Foundation one liquid (62/70 Variants)	Pre-NAT 63.4%	DDFS 32.5 months *vs.* NR (ctDNA+ *vs.* ctDNA−) RFS 22.8 months *vs.* NR (ctDNA+ *vs.* ctDNA−) HR for DDFS 2.99 HR for RFS 2.67 HR for OS 4.16	84
N = 170 (I–III)	ER+/ERBB2− (30%) ERBB2+ (32.4%) TNBC (22.4%)	Tumor-informed PCR Up to 150 variants	Pre-NAT 51.2%	Lead time 10.7 months RFS 20.8 months *vs.* NR (ctDNA+ *vs.* ctDNA−) HR for RFS 16.7	81
N = 168 (I–III)	HR+/HER2− (56.0%) HR+/HER2+ (16.6%) HR−/HER2+ (17.9%) HR−/HER2− (9.5%)	Tumor tissue 95 Genes Plasma tumor-informed mPCR 1–9 variants (depth 100,000×)	Pre-surgery in high-risk 56.5% Landmark 12.5% Longitudinal 7.8%	Landmark 2 y DFS 73.3% *vs.* 95.4% (ctDNA+ *vs.* ctDNA−) Longitudinal Sensitivity 90.9% Specificity 98.8% Lead time 9.7 months HR for DFS 207.05 2 y DFS 20.2% *vs.* 99.2% (ctDNA+ *vs.* ctDNA−)	79
N = 130 (II–III)	TNBC	Tumor-informed 227 Genes (depth > 10,000×)	Pre-NAT 78.7% Pre-surgery 23.0% Post-surgery 16.9% Longitudinal 46.34%	Lead time 3.4 months Pre-surgery HR for EFS 4.34 Post-surgery HR for EFS 4.63 Pre-surgery HR for DRFS 4.34 Post-surgery HR for DRFS 6.18 pCR 15% *vs.* 39% (ctDNA+ *vs.* ctDNA−) Sensitivity 97.85% Specificity 98.3%	85
N = 95 (I–III)	ER+/HER2− (43.2%) HER2+ (30.5%) TNBC (26.3%)	Tumor-informed QIAseq targeted DNA panel 14 Variants	Pre-NAT 63.0% Pre-surgery 33.0%	Specificity 100% pCR 15.4% *vs.* 35.4%(ctDNA+ *vs.* ctDNA−) RFS 1.19 year (ctDNA+)	83
N = 78 (I–III)	HR+/HER2− (23.1%) HER2+ (44.8%) TNBC (29.5%)	Tumor-informed NeXT personal MRD Up to 1,451 variants	Pre-NAT 98.0% Pre-surgery 33.3% Post-surgery 5.1%	Lead time 15 months Sensitivity 100% Specificity 95% 5 y RFS 41.67% *vs.* 100% (ctDNA+ *vs.* ctDNA−)	45
N = 61 (II–III)	HR+ (34%) HER2+ (33%) TNBC (26%)	Tumor-informed Invitae Personalized Cancer Monitoring™ PCM Up to 50 variants	Pre-NAT 67.8% Pre-surgery 0.0% Post-surgery 0.0% Longitudinal 16.4%	Lead time 11.7 months Sensitivity 76.9% Specificity 100% HR for RFS 37.16 pCR 36.8% *vs.* 23.1% (pre-NAT ctDNA+ *vs.* ctDNA−) pCR 30.8% *vs.* 27.3% (pre-surgery ctDNA + *vs.* ctDNA−)	51

Table 3 Continued

N Stage	Subtype	Monitoring strategy	ctDNA+	MRD performance	Ref
N = 52 (I–III)	HR+/HER2− (40.4%) HR+/HER2+ (15.4%) HR−/HER2+ (17.3%) TNBC (11.5%)	Tumor-informed 227 Genes	Pre-NAT 47.7%	ORR 47.6% *vs.* 73.9% (ctDNA+ *vs.* ctDNA−) HR for RFS 5.72 HR for OS 11.27	64
N = 44 (I–III)	HR+HER2 (45%) TNBC (30%) HER2+ (25%)	Tumor-informed Signatera Up to 16 variants	Pre-NAT 58.0% Pre-surgery 5.0% Longitudinal 13.0%	Pre-surgery HR for DRFS 53 Longitudinal HR for DRFS 31	80
N = 42 (I–III)	TNBC	Tumor-informed Ion Ampliseq comprehensive cancer panel (409 variants)	Pre-NAT 77.0% Pre-surgery 43.0% Post-surgery 16.7%	Lead time 6.5 months HR for RFS 2.65 2 y EFS 57.1% *vs.* 66.7% (pre-NAT ctDNA+ *vs.* ctDNA−) 2 y EFS 40.0% *vs.* 83.9% (pre-surgery ctDNA+ *vs.* ctDNA−)	86
N = 26 (I–III)	TNBC	Tumor-informed ddPCR (121 variants)	Pre-NAT 96.0% Pre-surgery 69%	pCR 11.8% *vs.* 57.1% (pre-NATctDNA+ *vs.* ctDNA−) pCR 13.3% *vs.* 55.6% (pre-surgery ctDNA+ *vs.* ctDNA−) HR for RFS 0.29 HR for OS 0.27	17
N = 141 (II–III)	TNBC (22.7%) HR+ (40.9%) HER2+ (36.4%)	Tumor-informed RaDaR Up to 48 variants	Longitudinal 39.7%	Lead time 6.1 months Sensitivity 95.7% Specificity 91.0%	72
N = 119 (II–III)	TNBC	Tumor-agnostic Guardant reveal 74 Genes	Pre-NAT 78.0% Pre-surgery 3.6% Post-surgery 8.9%	Sensitivity 83% Specificity 99.5% pCR 52.3% *vs.* 27.8% (pre-NAT ctDNA+ *vs.* ctDNA−) pCR 0% *vs.* 49.1% (pre-surgery ctDNA+ *vs.* ctDNA−)	57
N = 95 (II–III)	ER+ (53.7%) TNBC (46.3%)	Tumor-agnostic Guardant reveal 74 Genes	Pre-NAT 72.5%	Lead time 152 d Sensitivity 62.5% Specificity 100% HR for EFS 17.0	51
N = 38 (II–III)	TNBC	Tumor-agnostic Oncomine research panel (134 variants)	Pre-surgery 12.0%	HR for RFS 12.6	87
N = 156 (I–III)	HR+/HER2− (57.75%) HR+/HER2+ (22.4%) TNBC (14.7%) HER2+ (5.1%)	Tumor-informed Signatera Up to 16 variants	Longitudinal 23.1%	Lead time 10.5 months Sensitivity 88.2% HR for RFS 52.98 HR for OS 53.69	60
N = 103 (IIA–IIIC)	HR+	Tumor-informed RaDaR Up to 36 variants	Pre-surgery 5.0% Longitudinal 10%	Lead time 12.4 months Sensitivity 100% Specificity 97.4%	73
N = 49 (I–III)	HR+/HER2− (69.4%) HR+/HER2+ (16.3%) TNBC (14.3%)	Tumor-informed Signatera Up to 16 variants Depth > 100,000×	Longitudinal 32.7%	Lead time 8.9 months Specificity 100% Sensitivity 89% HR for RFS 11.8 (Landmark) HR for RFS 35.8 (Longitudinal)	71

Table 3 Continued

N Stage	Subtype	Monitoring strategy	ctDNA+	MRD performance	Ref
N = 11 (III)	TNBC	Tumor-informed LiquidSCANv2-PanCang GENINUS Up to 38 variants	Landmark 27.2%	4 y DFS 67% *vs.* 100% (Landmark ctDNA+ *vs.* ctDNA−)	62

Table 4 Emerging MRD-guided clinical trials in breast cancer adjuvant therapy

Trial name/NCT No.	Population	ctDNA Assay	Intervention	Primary endpoint	Unique design features	Ref.
TRAK-ER NCT04985266	HR+ Intermediate to high risk	RaDaR	CDK4/6i + ET *vs.* ET alone	RFS	Early intervention window; ctDNA-guided therapy escalation	92
TREAT ctDNA NCT05512364	ER+/HER2− stage II–III, MRD+	Signatera	Standard ET *vs*. elacestrant (oral SERD)	DMFS	Targets ESR1 mutations; novel SERD in MRD+ setting	91
DARE NCT04567420	HR+/HER2− High risk	Signatera	Switching to fulvestrant + palbociclib (arm A) or to continuation of adjuvant therapy (arm B) in MRD+ patients	ctDNA positivity Delayed recurrence	Patients with serially ctDNA-results during surveillance had 99% RFS after a median follow-up of 27.4 months	93
LEADER NCT03285412	HR+ high risk early stage	ctDNA	Ribociclib + ET *vs*. ET alone	ctDNA clearance; DFS; AE	ctDNA clearance after 12 cycles of adjuvant ribociclib + ET *vs*. ET alone	95
ZEST NCT04915755	gBRCAm and MRD+ HR+/HER2− or TNBC	Signatera	Niraparib (PARPi) *vs*. placebo	TEAE; DFS	Defined germline BRCAm and MRD detection as high-risk population	96

and HER2+: 0, 3, 6, 12, 18, or 24 months; HR+: 0, 6, 12, 18, or 24 months annually thereafter). Future trials should validate these intervals prospectively.

Emerging interventional clinical trials investigating the clinical efficacy of MRD in BC adjuvant and neoadjuvant settings are also underway. The TRAK-ER, DARE, and LEADER trials were aimed at determining the clinical utility of MRD-directed therapies and assisting in the development of more effective treatment strategies to eliminate MRD in HR+ BC[8,91]. The TRAK-ER trial (NCT04985266) dynamically monitors ctDNA every 3 months in patients with intermediate-to-high-risk HR+ cancers post-neoadjuvant therapy. Those with ctDNA positivity are randomized to either standard endocrine therapy or an intensified regimen, and an early intervention window (11.7 months ahead of imaging) is being explored[92]. The DARE trial assessed intensified therapy with CDK4/6 inhibitors plus endocrine therapy for ctDNA-positive high-risk HR+/HER2− postoperative patients and validated targeted escalation to delay recurrence in the MRD-positive high-risk population[93]. The TREAT ctDNA trial (NCT05512364)[91] randomized patients with ER+/HER2− stage II–III cancers with MRD positivity to standard endocrine therapy or elacestrant (a novel oral SERD)[30]. Using Signatera tumor-informed assays (30% higher sensitivity), elacestrant targets *ESR1* mutations in high risk patients with MRD-positivity, with distant metastasis-free survival as the primary endpoint. The LEADER part trial adapts the PADA-1 model by switching to elacestrant plus CDK4/6 inhibitors upon detection of MRD positivity in patients with early HR+ high-risk cancers, to optimize sequential adjuvant therapy[8,94,95]. Regarding PARPi-targeted therapy, the ZEST trial evaluates the efficacy and safety of niraparib *vs.* placebo in 800 patients with HR+/HER2− germline *BRCA*

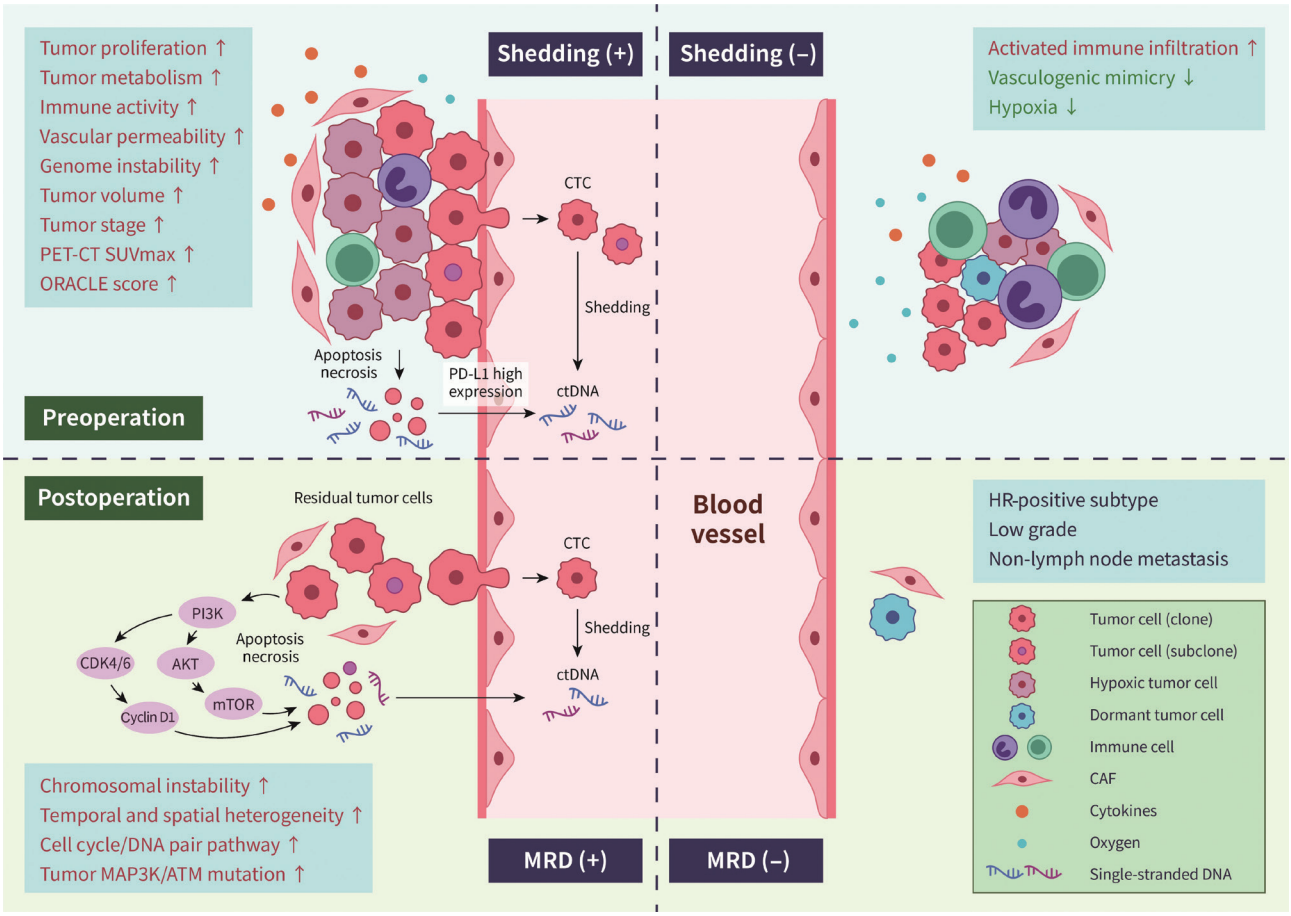

Figure 4 Tumor characteristics associated with preoperative and postoperative ctDNA detection. This schematic illustrates key tumor features stratified by ctDNA detection status. In the preoperative phase, shedders (ctDNA-positive patients) exhibit high invasiveness (large tumor volume, solid nodules, and high histological grade), high metabolic activity (elevated SUVmax on PET imaging), and high genomic instability (chromosomal abnormalities and upregulated proliferation signaling). Tumors display vascular disruption with elevated permeability and suppressive immune infiltration. In contrast, non-shedders (ctDNA negative) demonstrate diminished aggressiveness, metabolic activity, and genomic stability. In the postoperative phase, MRD-positive tumors maintain persistent genomic instability and elevated proliferation indices. ctDNA, circulating DNA; MRD, minimal residual disease; SUVmax, maximum standardized uptake value; ORACLE, outcome risk associated with clonal lung expression; PET, positron emission tomography; CAF, cancer-associated fibroblasts.

mutation or high-risk TNBC, and uses the Signatera assay to detect MRD and evaluate the optimal frequency of ctDNA monitoring, independently of relapse timing[30,96] (**Table 4**).

Pathophysiological mechanisms underlying ctDNA shedding in BC

The pathophysiological mechanisms of MRD in BC are complex and elusive. Tumor cells that persist after treatment might have unique metabolic and regulatory characteristics. Elucidating these pathophysiological mechanisms is crucial for developing effective strategies to target and eliminate MRD[97,98] (**Figure 4**). Exploratory analysis of pre-treatment gene expression profiles has revealed both shared and subtype-specific patterns potentially associated with ctDNA shedding[61]. Preoperative ctDNA-positive patients (also referred to as "shedders") exhibit a distinct profile characterized by 3 defining features: high invasiveness, manifesting as larger tumor volume, predominance of solid nodules, and high histologic grade; high metabolic activity, evidenced by significantly elevated PET-CT SUVmax values, correlating with aggressive tumor proliferation and high Ki67 index[99]; and high genomic instability, which positively correlates with ctDNA shedding driven by chromosomal abnormalities, and upregulated proliferation and cell cycle/DNA repair pathway signaling[61,67].

Tumor heterogeneity can lead to false-negative MRD results if the resistant cells responsible for recurrence originate from a sub-clone not captured by the original biopsy, because of sampling bias, or from a sub-clone that acquired new mutations after treatment. Although the power to detect differentially expressed genes in TNBC was limited by a sample size imbalance between the ctDNA-positive and ctDNA-negative groups, protein-protein interaction enrichment analysis of common differentially expressed genes in both the HR+/HER2− and TNBC subtypes identified cell cycle genes associated with ctDNA shedding, thus suggesting that tumor cell proliferation plays a key role in ctDNA release[85]. In the HR+/HER2− subtype, the association between cell cycle-associated gene sets and ctDNA shedding remained statistically significant even after adjustment for MammaPrint status[100]. Transcriptomic analysis of urothelial tumors from ctDNA-positive patients further revealed upregulation of cell cycle genes and hypoxia-related pathways. Additionally, gene expression profiling implicated specific immune response pathways in ctDNA release, in agreement with preclinical evidence indicating that immune cells might facilitate DNA shedding into the circulation. The tumor microenvironment, including interactions with immunosuppressive cells, stromal cells, the extracellular matrix, and elevated vascular permeability, can influence the fate of ctDNA release[101].

Genomically, landmark MRD profiling is predictive of poor prognosis and a potential need for intensive anti-cancer therapy. Tumor-informed sequencing has identified actionable targets (e.g., *PIK3CA*, *ESR1*, and DNA repair defects) in 77.8% of post-operative MRD-positive patients with therapies matched *via* databases such as OncoKB. Patients with detectable post-operative MRD exhibited greater enrichment in somatic mutations and amplifications of genes such as *MAP3K1*, *ATM*, *FLT1*, *GNAS*, *POLD1*, *SPEN*, and *WWP2* in tumor tissue than those with undetectable MRD[102,103]. GSEA of RNA sequencing data from tumors in MRD-positive patients indicated elevated frequencies of cell cycle-related pathway mutations, DNA repair disorder, and AID-mediated somatic hypermutation signatures, thus implying potential mechanistic links to MRD persistence in BC[102,104]. Non-Tie I/II mutations (variants of unknown clinical signature) or subclonal mutations from the tumor tissue were associated with a significantly higher recurrence risk than observed in MRD-negative patients. These findings highlight the prognostic value of monitoring these traditionally overlooked non-driver mutations during MRD surveillance[46].

Challenges and future trajectories of MRD detection in BC

Standardizing MRD detection and interpretation of the findings remains a major challenge. A universal consensus regarding the optimal detection methods is lacking, including the selection between tumor-informed and tumor-agnostic approaches, as well as the specific assays within each approach. This lack of standardization poses challenges in comparing results across studies and translating research findings into clinical practice. Heterogeneity in MRD methods, encompassing blood sample collection, storage, transport, sequencing depth, variant-calling algorithms, and positivity thresholds, compromises the reproducibility of results across laboratories. Discrepancies in detection techniques (e.g., PCR *vs.* NGS) further exacerbate inter-laboratory variability and can undermine clinical decision-making. Tumor-agnostic panels targeting recurrent driver genes (e.g., *TP53*, *PIK3CA*, and *ESR1*) are particularly vulnerable, and the reported false-positive rates have exceeded 15% in some cohorts[42]. The integration of fragmentomics, methylation signatures, and CHIP mutation databases has been used to filter background noise[72,98]. To address the potential interference of CHIP with MRD detection, the investigators explored its underlying mechanisms and proposed corresponding solutions. The study revealed that the infiltration of CHIP-associated mutations (e.g., in DNMT3A, TET2, ASXL1) into tumors, a phenomenon known as TI-CH, is not only associated with an unfavorable prognosis but may also interfere with ultra-sensitive ctDNA testing by contributing to background noise. To address this, the researchers employed tumor-normal paired sequencing. This approach allowed them to rigorously distinguish true somatic tumor mutations from CHIP-derived mutations by using the normal tissue sample as a genetic baseline. Furthermore, they applied tumor purity correction to exclude low-frequency CHIP signals and integrated filtering based on known CHIP-associated gene databases (e.g., COSMIC CHIP tags) for accurate mutation annotation[105]. Finally, ethical considerations in MRD research and treatment cannot be overlooked. The high cost of MRD detection, particularly tumor-informed approaches requiring WES or WGS of primary tumors, remains a major impediment to widespread use[106]. Economic evaluation of MRD monitoring has revealed a paradigm shift in cost-effectiveness by enabling risk-stratified treatment decisions, particularly through decreasing overtreatment in low-risk populations while maintaining

clinical outcomes. Key studies such as the DYNAMIC trial in colorectal cancer have demonstrated that MRD-guided strategies can significantly decrease unnecessary chemotherapy (from 28% under conventional surveillance to 15% in MRD-positive patients) without compromising 5-year RFS[107]. This precision approach not only lowers direct treatment costs (e.g., chemotherapy agents, administration, and hospitalization) but also mitigates indirect costs associated with treatment-related adverse events (e.g., toxicity management and productivity loss). However, the cost-benefit balance hinges on MRD testing expenses, which vary by method: tumor-informed assays have higher initial costs because of WES and personalized panel designs, whereas tumor-agnostic assays eliminate tissue biopsy costs but might sacrifice sensitivity in low-VAF situations. The design of MRD intervention trials must incorporate strategies to address participants' comprehension of complex diagnostic information, particularly by validating the informed consent process. Patients must be explicitly provided with a clear understanding of both the technical capabilities and inherent limitations associated with advanced MRD detection technologies. Such clarity is critical, given the evolving clinical implications of MRD status to guide therapy escalation/de-escalation. Large-scale clinical trials are warranted to validate the clinical utility of MRD-directed therapies and to establish standardized treatment algorithms. By following this strategic management, MRD can be effectively integrated into BC management, thereby leading to improved patient outcomes.

Conclusions

As liquid biopsy technologies evolve, MRD-guided strategies have potential to transform the paradigm of BC management from empirical approaches to dynamic, biomarker-driven precision oncology. Despite its transformative potential, MRD integration in BC faces challenges in cost, standardization, and biological specificity. Tumor-informed and agnostic strategies each offer distinct advantages. Future adaptive trials based on MRD are highly anticipated to serve as a cornerstone for precision oncology.

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Conflict of interest statement

No potential conflicts of interest are disclosed.

Author contributions

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References

1. Zhang Y, Vaccarella S, Morgan E, Li M, Etxeberria J, Chokunonga E, et al. Global variations in lung cancer incidence by histological subtype in 2020: a population-based study. Lancet Oncol. 2023; 24: 1206-18.
2. Tolaney SM, Guarneri V, Seo JH, Cruz J, Abreu MH, Takahashi M, et al. Long-term patient-reported outcomes from monarchE: a bemaciclib plus endocrine therapy as adjuvant therapy for HR+, HER2−, node-positive, high-risk, early breast cancer. Eur J Cancer. 2024; 199: 113555.
3. Meattini I, Coles CE, Tramm T, Borghesi S, Krug D, Montero A, et al. Biomarker-directed radiotherapy in breast cancer: a narrative review. JAMA Oncol. 2025; 11: 329-39.
4. Lloyd MR, Jhaveri K, Kalinsky K, Bardia A, Wander SA. Precision therapeutics and emerging strategies for HR-positive metastatic breast cancer. Nat Rev Clin Oncol. 2024; 21: 743-61.
5. Rastogi P, O'Shaughnessy J, Martin M, Boyle F, Cortes J, Rugo HS, et al. Adjuvant abemaciclib plus endocrine therapy for hormone receptor-positive, human epidermal growth factor receptor 2-negative, high-risk early breast cancer: results from a preplanned monarchE overall survival interim analysis, including 5-year efficacy outcomes. J Clin Oncol. 2024; 42: 987-93.
6. de Boniface J, Appelgren M, Szulkin R, Alkner S, Andersson Y, Bergkvist L, et al. Completion axillary lymph node dissection for the identification of pN2-3 status as an indication for adjuvant CDK4/6 inhibitor treatment: a post-hoc analysis of the randomised, phase 3 SENOMAC trial. Lancet Oncol. 2024; 25: 1222-30.
7. Slamon D, Lipatov O, Nowecki Z, McAndrew N, Kukielka-Budny B, Stroyakovskiy D, et al. Ribociclib plus endocrine therapy in early breast cancer. N Engl J Med. 2024; 390: 1080-91.
8. Spring LM, Scarpetti L, Medford AJ, Niemierko A, Comander A, Mulvey T, et al. Adjuvant endocrine therapy with cyclin-dependent kinase 4/6 inhibitor, ribociclib, for localized hormone receptor-positive/HER2- breast cancer (LEADER). NPJ Breast Cancer. 2025; 11: 2.

9. Bardia A, Pusztai L, Albain K, Ciruelos EM, Im S-A, Hershman D, et al. TROPION-Breast03: a randomized phase III global trial of datopotamab deruxtecan ± durvalumab in patients with triple-negative breast cancer and residual invasive disease at surgical resection after neoadjuvant therapy. Ther Adv Med Oncol. 2024; 16: 17588359241248336.
10. Ma D, Dai L-J, Wu X-R, Liu C-L, Zhao S, Zhang H, et al. Spatial determinants of antibody-drug conjugate SHR-A1811 efficacy in neoadjuvant treatment for HER2-positive breast cancer. Cancer Cell. 2025; 43: 1061-75.e7.
11. Davis AA, Hesse J, Pereira PMR, Ma CX. Novel treatment approaches utilizing antibody-drug conjugates in breast cancer. NPJ Breast Cancer. 2025; 11: 42.
12. Gao Y, Yin L, Duan X, Fu Z, Liu Q, Chen J, et al. HER2-targeted PET/CT imaging provides potential biomarkers for differentiating HER2-zero, -low, and -positive breast cancer. Eur J Nucl Med Mol Imaging. 2025; 52: 4525-38.
13. Paul ED, Huraiová B, Valková N, Matyasovska N, Gábrišová D, Gubová S, et al. The spatially informed mFISHseq assay resolves biomarker discordance and predicts treatment response in breast cancer. Nat Commun. 2025; 16: 226.
14. Li Y-W, Dai L-J, Wu X-R, Zhao S, Xu Y-Z, Jin X, et al. Molecular characterization and classification of HER2-positive breast cancer inform tailored therapeutic strategies. Cancer Res. 2024; 84: 3669-83.
15. Cheng T-C, Hung M-C, Wang L-H, Tu S-H, Wu C-H, Yen Y, et al. Histamine N-methyltransferase (HNMT) as a potential auxiliary biomarker for predicting adaptability to anti-HER2 drug treatment in breast cancer patients. Biomark Res. 2025; 13: 7.
16. Campone M, De Laurentiis M, Jhaveri K, Hu X, Ladoire S, Patsouris A, et al. Vepdegestrant, a PROTAC estrogen receptor degrader, in advanced breast cancer. N Engl J Med. 2025; 393: 556-68.
17. Bidard F-C, Mayer EL, Park YH, Janni W, Ma C, Cristofanilli M, et al. First-line camizestrant for emerging *ESR1*-mutated advanced breast cancer. N Engl J Med. 2025; 393: 569-80.
18. Turner NC, Im S-A, Saura C, Juric D, Loibl S, Kalinsky K, et al. Inavolisib-based therapy in *PIK3CA*-mutated advanced breast cancer. N Engl J Med. 2024; 391: 1584-96.
19. Licata L, Viale G, Giuliano M, Curigliano G, Chavez-MacGregor M, Foldi J, et al. Oncotype DX results increase concordance in adjuvant chemotherapy recommendations for early-stage breast cancer. NPJ Breast Cancer. 2023; 9: 51.
20. Rios-Hoyo A, Xiong K, Dai J, Yau C, Marczyk M, Garcia-Milian R, et al. Hormone receptor-positive HER2-negative/MammaPrint high-2 breast cancers closely resemble triple-negative breast cancers. Clin Cancer Res. 2025; 31: 403-13.
21. Zeng Z, Yi Z, Xu B. The biological and technical challenges facing utilizing circulating tumor DNA in non-metastatic breast cancer patients. Cancer Lett. 2025; 616: 217574.
22. Jiang Y-Z, Ma D, Jin X, Xiao Y, Yu Y, Shi J, et al. Integrated multiomic profiling of breast cancer in the Chinese population reveals patient stratification and therapeutic vulnerabilities. Nat Cancer. 2024; 5: 673-90.
23. Wu S-Y, Jin X, Liu Y, Wang Z-Y, Zuo W-J, Ma D, et al. Mobilizing antigen-presenting mast cells in anti-PD-1-refractory triple-negative breast cancer: a phase 2 trial. Nat Med. 2025; 31: 2405-15.
24. Yang S-Y, Jin M-L, Andriani L, Zhao Q, Ling Y-X, Lin C-J, et al. Kinesin-like protein KIFC2 stabilizes CDK4 to accelerate growth and confer resistance in HR+/HER2- breast cancer. J Clin Invest. 2025; 135: e183531.
25. Wu Y, Tian X, Ma J, Lin Y, Ye J, Wang Y, et al. Label-free discrimination analysis of breast cancer tumor and adjacent tissues of patients after neoadjuvant treatment using Raman spectroscopy: a diagnostic study. Int J Surg. 2025; 111: 1788-800.
26. Lucas O, Ward S, Zaidi R, Bunkum A, Frankell AM, Moore DA, et al. Characterizing the evolutionary dynamics of cancer proliferation in single-cell clones with SPRINTER. Nat Genet. 2025; 57: 103-14.
27. Kurzeder C, Nguyen-Sträuli BD, Krol I, Ring A, Castro-Giner F, Nüesch M, et al. Digoxin for reduction of circulating tumor cell cluster size in metastatic breast cancer: a proof-of-concept trial. Nat Med. 2025; 31: 1120-4.
28. Nesselbush MC, Luca BA, Jeon Y-J, Jabara I, Meador CB, Garofalo A, et al. An ultrasensitive method for detection of cell-free RNA. Nature. 2025; 641: 759-68.
29. In 't Veld SGJG, Arkani M, Post E, Antunes-Ferreira M, D'Ambrosi S, Vessies DCL, et al. Detection and localization of early- and late-stage cancers using platelet RNA. Cancer Cell. 2022; 40: 999-1009.e6.
30. 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: 50.
31. Galli E, Patelli G, Villa F, Gri N, Mazzarelli C, Mangoni I, et al. Circulating blood biomarkers for minimal residual disease in hepatocellular carcinoma: a systematic review. Cancer Treat Rev. 2025; 135: 102908.
32. Lin P-H, Wang M-Y, Lo C, Tsai L-W, Yen T-C, Huang TY, et al. Circulating tumor DNA as a predictive marker of recurrence for patients with stage II-III breast cancer treated with neoadjuvant therapy. Front Oncol. 2021; 11: 736769.
33. Zhong H-J, Zhen Y, Chen S, Shi W, Liang X, Yang G-J. Advances in CTC and ctDNA detection techniques: opportunities for improving breast cancer care. Breast Cancer Res. 2025; 27: 97.
34. Shuai Y, Ma Z, Ju J, Wei T, Gao S, Kang Y, et al. Liquid-based biomarkers in breast cancer: looking beyond the blood. J Transl Med. 2023; 21: 809.
35. Chaudhuri AA, Chabon JJ, Lovejoy AF, Newman AM, Stehr H, Azad TD, et al. Early detection of molecular residual disease in localized lung cancer by circulating tumor DNA profiling. Cancer Discov. 2017; 7: 1394-403.
36. Patel RP, Somasundram PM, Smith LK, Sheppard KE, McArthur GA. The therapeutic potential of targeting minimal residual disease in melanoma. Clin Transl Med. 2023; 13: e1197.
37. Zhong R, Gao R, Fu W, Li C, Huo Z, Gao Y, et al. Accuracy of minimal residual disease detection by circulating tumor DNA profiling in lung cancer: a meta-analysis. BMC Med. 2023; 21: 180.
38. Xi J, Ma CX, O'Shaughnessy J. Current clinical utility of circulating tumor DNA testing in breast cancer: a practical approach. JCO Oncol Pract. 2024; 20: 1460-70.

39. Wang S, Li M, Zhang J, Xing P, Wu M, Meng F, et al. Circulating tumor DNA integrating tissue clonality detects minimal residual disease in resectable non-small-cell lung cancer. J Hematol Oncol. 2022; 15: 137.
40. Chen K, Kang G, Zhang Z, Lizaso A, Beck S, Lyskjær I, et al. Individualized dynamic methylation-based analysis of cell-free DNA in postoperative monitoring of lung cancer. BMC Med. 2023; 21: 255.
41. Fan Y, Liu Y, Wang L, Cai Y, Cao W, Sun W, et al. bITH, a blood-based metric of intratumor heterogeneity, is associated with clinical response to immune checkpoint blockade in non-small cell lung cancer. EBioMedicine. 2023; 91: 104564.
42. Turner NC, Mayer EL, Park YH, Janni W, Ma CX, Cristofanilli M, et al. Camizestrant + CDK4/6 inhibitor (CDK4/6i) for the treatment of emergent *ESR1* mutations during first-line (1L) endocrine-based therapy (ET) and ahead of disease progression in patients (pts) with HR+/HER2– advanced breast cancer (ABC): phase 3, double-blind ctDNA-guided SERENA-6 trial. J Clin Oncol. 2025; 43: LBA4.
43. Santonja A, Cooper WN, Eldridge MD, Edwards PAW, Morris JA, Edwards AR, et al. Comparison of tumor-informed and tumor-naïve sequencing assays for ctDNA detection in breast cancer. EMBO Mol Med. 2023; 15: e16505.
44. Elliott MJ, Echelard P, Pipinikas C, Main S, Fuentes Antrás J, Dou A, et al. Longitudinal evaluation of circulating tumor DNA in patients undergoing neoadjuvant therapy for early breast cancer using a tumor-informed assay. Nat Commun. 2025; 16: 1837.
45. Garcia-Murillas I, Abbott CW, Cutts RJ, Boyle SM, Pugh J, Keough KC, et al. Whole genome sequencing-powered ctDNA sequencing for breast cancer detection. Ann Oncol. 2025; 36: 673-81.
46. Chen K, Yang F, Shen H, Wang C, Li X, Chervova O, et al. Individualized tumor-informed circulating tumor DNA analysis for postoperative monitoring of non-small cell lung cancer. Cancer Cell. 2023; 41: 1749-62.e6.
47. Widman AJ, Shah M, Frydendahl A, Halmos D, Khamnei CC, Øgaard N, et al. Ultrasensitive plasma-based monitoring of tumor burden using machine-learning-guided signal enrichment. Nat Med. 2024; 30: 1655-66.
48. Kurtz DM, Soo J, Co Ting Keh L, Alig S, Chabon JJ, Sworder BJ, et al. Enhanced detection of minimal residual disease by targeted sequencing of phased variants in circulating tumor DNA. Nat Biotechnol. 2021; 39: 1537-47.
49. Elliott MJ, Howarth K, Main S, Fuentes Antrás J, Echelard P, Dou A, et al. Ultrasensitive detection and monitoring of circulating tumor DNA using structural variants in early-stage breast cancer. Clin Cancer Res. 2025; 31: 1520-32.
50. Nguyen DD, Hooper WF, Liu W, Chu TR, Geiger H, Shelton JM, et al. The interplay of mutagenesis and ecDNA shapes urothelial cancer evolution. Nature. 2024; 635: 219-28.
51. George MA, Schwartz G, Mchayleh W, Margulis V, Tan A, Sudhaman S, et al. Clinical performance of Signatera Genome assay in a cohort of patients (pts) with solid tumors. J Clin Oncol. 2025; 43: 3142.
52. Hashimoto T, Kobayashi S, Oki E, Nakamura Y, Kinoshita T, Nonomura N, et al. Ultra-sensitive pan-cancer molecular residual disease assessment using whole-genome sequencing-based personalized ctDNA panel: initial results from the MONSTAR-SCREEN-3 project. J Clin Oncol. 2025; 43: 3007.
53. Elliott MJ, Kim J, Dou A, Fuentes Antrás J, Amir E, Nadler MB, et al. Comprehensive tumor-agnostic evaluation of genomic and epigenomic-based approaches for the identification of circulating tumor DNA in early-stage breast cancer. ESMO Open. 2025; 10: 105286.
54. Martín-Arana J, Gimeno-Valiente F, Henriksen TV, García-Micó B, Martínez-Castedo B, Gambardella V, et al. Whole-exome tumor-agnostic ctDNA analysis enhances minimal residual disease detection and reveals relapse mechanisms in localized colon cancer. Nat Cancer. 2025; 6: 1000-16.
55. Slater S, Bryant A, Aresu M, Begum R, Chen H-C, Peckitt C, et al. Tissue-free liquid biopsies combining genomic and methylation signals for minimal residual disease detection in patients with early colorectal cancer from the UK TRACC part B study. Clin Cancer Res. 2024; 30: 3459-69.
56. Moding EJ, Liu Y, Nabet BY, Chabon JJ, Chaudhuri AA, Hui AB, et al. Circulating tumor DNA dynamics predict benefit from consolidation immunotherapy in locally advanced non-small cell lung cancer. Nat Cancer. 2020; 1: 176-83.
57. Abbosh C, Birkbak NJ, Swanton C. Early stage NSCLC – challenges to implementing ctDNA-based screening and MRD detection. Nat Rev Clin Oncol. 2018; 15: 577-86.
58. Abbosh C, Hodgson D, Doherty GJ, Gale D, Black JRM, Horn L, et al. Implementing circulating tumor DNA as a prognostic biomarker in resectable non-small cell lung cancer. Trends Cancer. 2024; 10: 643-54.
59. Ademuyiwa FO, Ma CX, Weilbaecher K, Suresh R, Peterson LL, Bose R, et al. Detection of circulating tumor DNA using a tissue-free epigenomic assay is a highly prognostic biomarker in early-stage triple-negative breast cancer. Clin Cancer Res. 2025; 31: 2173-82.
60. Chidharla A, Rapoport E, Agarwal K, Madala S, Linares B, Sun W, et al. Circulating tumor DNA as a minimal residual disease assessment and recurrence risk in patients undergoing curative-intent resection with or without adjuvant chemotherapy in colorectal cancer: a systematic review and meta-analysis. Int J Mol Sci. 2023; 24: 10230.
61. Abbosh C, Birkbak NJ, Wilson GA, Jamal-Hanjani M, Constantin T, Salari R, et al. Phylogenetic ctDNA analysis depicts early-stage lung cancer evolution. Nature. 2017; 545: 446-51.
62. Shaw JA, Page K, Wren E, de Bruin EC, Kalashnikova E, Hastings R, 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.
63. Zhao J, Reuther J, Scozzaro K, Hawley M, Metzger E, Emery M, et al. Personalized cancer monitoring assay for the detection of ctDNA in patients with solid tumors. Mol Diagn Ther. 2023; 27: 753-68.
64. Garcia-Murillas I, Cutts RJ, Walsh-Crestani G, Phillips E, Hrebien S, Dunne K, et al. Longitudinal monitoring of circulating tumor DNA to detect relapse early and predict outcome in early breast cancer. Breast Cancer Res Treat. 2025; 209: 493-502.

65. Gale D, Heider K, Ruiz-Valdepenas A, Hackinger S, Perry M, Marsico G, et al. Residual ctDNA after treatment predicts early relapse in patients with early-stage non-small cell lung cancer. Ann Oncol. 2022; 33: 500-10.
66. Sanz-Garcia E, Zou J, Avery L, Spreafico A, Waldron J, Goldstein D, et al. Multimodal detection of molecular residual disease in high-risk locally advanced squamous cell carcinoma of the head and neck. Cell Death Differ. 2024; 31: 460-8.
67. Abbosh C, Frankell AM, Harrison T, Kisistok J, Garnett A, Johnson L, et al. Tracking early lung cancer metastatic dissemination in TRACERx using ctDNA. Nature. 2023; 616: 553-62.
68. Sim ES, Rhoades J, Xiong K, Walsh L, Crnjac A, Blewett T, et al. Early postoperative minimal residual disease detection with MAESTRO is associated with recurrence and worse survival in patients with head and neck cancer. Clin Cancer Res. 2025; 31: 3494-502.
69. Gydush G, Nguyen E, Bae JH, Blewett T, Rhoades J, Reed SC, et al. Massively parallel enrichment of low-frequency alleles enables duplex sequencing at low depth. Nat Biomed Eng. 2022; 6: 257-66.
70. Pantel K, Alix-Panabières C. Minimal residual disease as a target for liquid biopsy in patients with solid tumours. Nat Rev Clin Oncol. 2025; 22: 65-77.
71. Kirk EP, Delatycki MB, Archibald AD, Tutty E, Caruana J, Halliday JL, et al. Nationwide, couple-based genetic carrier screening. N Engl J Med. 2024; 391: 1877-89.
72. Qiu P, Yu X, Zheng F, Gu X, Huang Q, Qin K, et al. Advancements in liquid biopsy for breast cancer: molecular biomarkers and clinical applications. Cancer Treat Rev. 2025; 139: 102979.
73. Coombes RC, Page K, Salari R, Hastings RK, Armstrong A, Ahmed S, et al. Personalized detection of circulating tumor DNA antedates breast cancer metastatic recurrence. Clin Cancer Res. 2019; 25: 4255-63.
74. Coakley M, Villacampa G, Sritharan P, Swift C, Dunne K, Kilburn L, 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: 895-903.
75. Lee TH, Kim H, Kim YJ, Park W-Y, Park W, Cho WK, et al. Implication of pre- and post-radiotherapy ctDNA dynamics in patients with residual triple-negative breast cancer at surgery after neoadjuvant chemotherapy: findings from a prospective observational study. Cancer Res Treat. 2024; 56: 531-7.
76. Lipsyc-Sharf M, de Bruin EC, Santos K, McEwen R, Stetson D, Patel A, 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: 2408-19.
77. Loi S, Johnston SRD, Arteaga CL, Graff SL, Chandarlapaty S, Goetz MP, et al. Prognostic utility of ctDNA detection in the monarchE trial of adjuvant abemaciclib plus endocrine therapy (ET) in HR+, HER2-, node-positive, high-risk early breast cancer (EBC). J Clin Oncol. 2024; 42: LBA507.
78. O'Leary B. PADA-1 trial: ESR1 mutations in plasma ctDNA guide treatment switching. Nat Rev Clin Oncol. 2023; 20: 67-8.
79. Turner NC, Swift C, Jenkins B, Kilburn L, Coakley M, Beaney M, 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: 200-11.
80. Cavallone L, Aguilar-Mahecha A, Lafleur J, Brousse S, Aldamry M, Roseshter T, et al. Prognostic and predictive value of circulating tumor DNA during neoadjuvant chemotherapy for triple negative breast cancer. Sci Rep. 2020; 10: 14704.
81. Nguyen ST, Nguyen Hoang V-A, Nguyen Trieu V, Pham TH, Dinh TC, Pham DH, et al. Personalized mutation tracking in circulating-tumor DNA predicts recurrence in patients with high-risk early breast cancer. NPJ Breast Cancer. 2025; 11: 58.
82. Cailleux F, Agostinetto E, Lambertini M, Rothé F, Wu H-T, Balcioglu M, et al. Circulating tumor DNA after neoadjuvant chemotherapy in breast cancer is associated with disease relapse. JCO Precis Oncol. 2022; 6: e2200148.
83. Garcia-Murillas I, Chopra N, Comino-Méndez I, Beaney M, Tovey H, Cutts RJ, et al. Assessment of molecular relapse detection in early-stage breast cancer. JAMA Oncol. 2019; 5: 1473-8.
84. Li S, Lai H, Liu J, Liu Y, Jin L, Li Y, et al. Circulating tumor DNA predicts the response and prognosis in patients with early breast cancer receiving neoadjuvant chemotherapy. JCO Precis Oncol. 2020; 4: PO.19.00292.
85. Magbanua MJM, Brown Swigart L, Ahmed Z, Sayaman RW, Renner D, Kalashnikova E, 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: 1091-102.e4.
86. Radovich M, Jiang G, Hancock BA, Chitambar C, Nanda R, Falkson C, 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: 1410-5.
87. Li S, Li Y, Wei W, Gong C, Wang T, Li G, et al. Dynamic ctDNA tracking stratifies relapse risk for triple negative breast cancer patients receiving neoadjuvant chemotherapy. Nat Commun. 2025; 16: 2786.
88. Ortolan E, Appierto V, Silvestri M, Miceli R, Veneroni S, Folli S, et al. Blood-based genomics of triple-negative breast cancer progression in patients treated with neoadjuvant chemotherapy. ESMO Open. 2021; 6: 100086.
89. Chen Y-H, Hancock BA, Solzak JP, Brinza D, Scafe C, Miller KD, et al. Next-generation sequencing of circulating tumor DNA to predict recurrence in triple-negative breast cancer patients with residual disease after neoadjuvant chemotherapy. NPJ Breast Cancer. 2017; 3: 24.
90. Magbanua MJM, Swigart LB, Wu H-T, Hirst GL, Yau C, Wolf DM, et al. Circulating tumor DNA in neoadjuvant-treated breast cancer reflects response and survival. Ann Oncol. 2021; 32: 229-39.
91. European Organisation for Research and Treatment of Cancer – EORTC. Elacestrant for treating ER+/HER2- breast cancer

patients with ctDNA relapse [Internet]. Report No.: NCT05512364, clinicaltrials.gov; 2025 Aug [cited 2025 Oct 23]. Available from: https://clinicaltrials.gov/study/NCT05512364.
92. Royal Marsden NHS Foundation Trust. A randomised trial of early detection of molecular relapse with circulating tumour DNA tracking and treatment with palbociclib plus fulvestrant versus standard endocrine therapy in patients with ER positive HER2 negative breast cancer [Internet]. Report No.: NCT04985266, clinicaltrials.gov; 2024 Dec [cited 2025 Oct 23]. Available from: https://clinicaltrials.gov/study/NCT04985266.
93. Criterium, Inc. A randomized, phase II trial of circulating tumor DNA-guided second line adjuvant therapy for high residual risk, estrogen receptor positive, HER-2 negative breast cancer (DARE) [Internet]. Report No.: NCT04567420, clinicaltrials.gov; 2025 Oct [cited 2025 Oct 23]. Available from: https://clinicaltrials.gov/study/NCT04567420.
94. Kindt CK, Alves CL, Ehmsen S, Kragh A, Reinert T, Vogsen M, et al. Genomic alterations associated with resistance and circulating tumor DNA dynamics for early detection of progression on CDK4/6 inhibitor in advanced breast cancer. Int J Cancer. 2024; 155: 2211-22.
95. Medford AJ. Phase II study of CDK 4/6 inhibitor, LEE011 (Ribociclib), in combination with adjuvant endocrine therapy at varying duration for ER-positive breast cancer (LEADER). [Internet]. Report No.: NCT03285412, clinicaltrials.gov; 2025 Aug [cited 2025 Oct 23]. Available from: https://clinicaltrials.gov/study/NCT03285412.
96. GlaxoSmithKline. A randomized phase 3 double-blinded study comparing the efficacy and safety of niraparib to placebo in participants with either HER2-negative BRCA-mutated or triple-negative breast cancer with molecular disease based on presence of circulating tumor DNA after definitive therapy (ZEST) [Internet]. Report No.: NCT04915755, clinicaltrials.gov; 2025 Jun [cited 2025 Oct 23]. Available from: https://clinicaltrials.gov/study/NCT04915755.
97. Würth R, Donato E, Michel LL, Saini M, Becker L, Cheytan T, et al. Circulating tumor cell plasticity determines breast cancer therapy resistance via neuregulin 1-HER3 signaling. Nat Cancer. 2025; 6: 67-85.
98. Lin H-Y, Chu P-Y. Mitochondrial calcium uniporter as biomarker and therapeutic target for breast cancer: prognostication, immune microenvironment, epigenetic regulation and precision medicine. J Adv Res. 2025; 70: 445-61.
99. Soto-Lanza F, Glick L, Chan C, Zhong L, Wilson N, Faiz S, et al. Long-term clinical, radiological, and mortality outcomes following pneumonitis in nonsmall cell lung cancer patients receiving immune checkpoint inhibitors: a retrospective analysis. Clin Lung Cancer. 2024; 25: 624-33.e2.
100. Corti C, Martin AR, Kurnia PT, Gómez Tejeda Zañudo J, Abravanel DL, Hughes ME, et al. Clinicopathological features and genomics of ER-positive/HER2-negative breast cancer relapsing on adjuvant abemaciclib. ESMO Open. 2025; 10: 105126.
101. Ayers M, Monteiro M, Kulkarni A, Reeser JW, Dykhuizen E, Roychowdhury S, et al. Growth factor receptor plasticity drives therapeutic persistence of metastatic breast cancer. Cell Death Dis. 2025; 16: 251.
102. Zhang S, Jiang Y, Zhou L, Xu J, Zhang G, Shen L, et al. Genomic characteristics and its therapeutic implications in breast cancer patients with detectable molecular residual disease. Cancer Res Treat. 2024; 56: 538-48.
103. Borea R, Cardinali B, Arecco L, De Luca G, Garuti A, Mazzitelli C, et al. 83P Circulating tumor cells (CTCs) and circulating tumor DNA (ctDNA) in eary high risk breast cancer: the CITUCEL trial update. Ann Oncol. 2024; 35: S247.
104. Yi Z, Feng K, Lv D, Guan Y, Shao Y, Ma F, et al. Genomic landscape of circulating tumor DNA in HER2-low metastatic breast cancer. Signal Transduct Target Ther. 2024; 9: 345.
105. Pich O, Bernard E, Zagorulya M, Rowan A, Pospori C, Slama R, et al. Tumor-infiltrating clonal hematopoiesis. N Engl J Med. 2025; 392: 1594-608.
106. Verschoor N, Smid M, Jager A, Sleijfer S, Wilting SM, Martens JWM. Integrative whole-genome and transcriptome analysis of HER2-amplified metastatic breast cancer. Breast Cancer Res. 2023; 25: 145.
107. Tie J, Wang Y, Lo SN, Lahouel K, Cohen JD, Wong R, et al. Circulating tumor DNA analysis guiding adjuvant therapy in stage II colon cancer: 5-year outcomes of the randomized DYNAMIC trial. Nat Med. 2025; 31: 1509-18.

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