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Liquid Biopsy and Circulating Tumor DNA for Monitoring Minimal Residual Disease in Hormone Receptor-Positive Breast Cancer: Current Evidence, Technologies, Clinical Outcomes, and Future Directions

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Abstract. *Background. Minimal residual disease (MRD) drives late relapse and mortality in hormone receptor-positive (HR+) breast cancer. Conventional surveillance modalities—serum tumor markers and cross-sectional imaging—lack sufficient sensitivity to detect MRD at a clinically actionable stage. Objective. This review synthesizes evidence on liquid biopsy and circulating tumor DNA (ctDNA) for MRD monitoring in HR+ breast cancer, covering assay technologies, clinical trial findings, methodological limitations, and future research priorities. Evidence Base. Evidence derives from systematic reviews, meta-analyses, prospective and retrospective cohorts, and interventional trials (PADA-1, SERENA-6) published between 2018 and 2026, as identified through the Scopus AI analytical platform. Key Findings. ctDNA-based MRD assays detect recurrence a median of 5–14 months before clinical or radiographic manifestation, with individual cases reporting a lead time of up to five years. Tumor-informed mutation-based assays achieve sensitivity of 50–79% with specificity approaching 100%. Methylation-based assays demonstrate sensitivity of 62.5% and specificity of 100%, outperforming mutation-based approaches in early-stage, low-shedding HR+ tumors. ctDNA dynamics correlate with progression-free and overall survival. The SERENA-6 trial demonstrated improved PFS and quality of life with ctDNA-guided early endocrine switching; the PADA-1 trial showed ctDNA dynamics predict PFS and OS, with ctDNA-based risk models outperforming clinical parameters. Conclusion. Despite robust prognostic evidence, assay standardization deficits, clonal hematopoiesis-related false positives, and cost barriers preclude routine clinical adoption. Large-scale, prospective, randomized trials are urgently required.*

Keywords: *liquid biopsy, circulating tumor DNA (ctDNA), minimal residual disease (MRD), hormone receptor-positive (HR+) breast cancer, tumor-informed assay, methylation-based assay, ESR1 mutation, clonal hematopoiesis, PADA-1, SERENA-6*

Introduction

Hormone receptor-positive (HR+) breast cancer is the most prevalent molecular subtype of breast malignancy. Despite substantial advances in adjuvant endocrine therapy, a significant proportion of patients experience disease relapse, often years or decades after the completion of primary treatment (Sears & Davis, 2023; Chen et al., 2025a). The primary biological driver of this late recurrence is minimal residual disease (MRD): the persistence of microscopic, dormant tumor cells following curative-intent therapy that remain entirely undetectable by conventional clinical and radiological surveillance (Chen et al., 2025a; Xu et al., 2025).

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The unique biology of HR+ tumors—characterized by comparatively low proliferative activity and correspondingly diminished ctDNA shedding into the bloodstream—imposes particular analytical demands on MRD detection platforms (Elliott et al., 2025b).

Traditional surveillance tools lack the sensitivity to identify residual disease at a stage when therapeutic intervention could be most effective, leaving the majority of patients undiagnosed until macroscopic metastatic disease is established (Xu et al., 2025; Abdo et al., 2026). Conventional surveillance methods, including imaging and serum tumor markers, lack the sensitivity to detect MRD at a stage when intervention could be most effective (Pfister et al., 2025b). This diagnostic gap has catalyzed intense scientific interest in non-invasive, molecularly sensitive tools for MRD monitoring in real time.

Liquid biopsy—the non-invasive analysis of tumor-derived biomarkers in peripheral blood—has emerged as a transformative paradigm in oncological diagnostics (Malik & Zaheer, 2025; Wu & Chu, 2022). Circulating tumor DNA (ctDNA) consists of small DNA fragments released into the bloodstream from apoptotic and necrotic tumor cells, carrying the somatic mutations and epigenetic alterations characteristic of the originating tumor (Sears & Davis, 2023). These features render ctDNA a real-time, dynamic reflection of tumor burden, treatment response, and molecular evolution.

In HR+ breast cancer, ctDNA-based MRD monitoring offers several clinically distinct advantages: early detection of residual disease months to years before clinical recurrence, enabling pre-emptive therapeutic intervention; dynamic risk stratification based on ctDNA kinetics rather than static clinical parameters; non-invasive, longitudinal assessment of tumor evolution and acquired resistance; and the potential to guide adaptive, personalized treatment strategies (Elliott et al., 2025a; Oliveira et al., 2026; Sears & Davis, 2023).

This review provides a critically appraised synthesis of ctDNA-mediated MRD monitoring in HR+ breast cancer. It addresses: (1) biological rationale for ctDNA as an MRD biomarker; (2) principal assay technologies and their comparative analytical performance; (3) clinical trial evidence from PADA-1 and SERENA-6; (4) the role of ESR1 mutations and resistance monitoring; (5) technological innovations including machine learning and multi-omic integration; (6) current methodological challenges; and (7) priority future research directions.

Theoretical and Biological Framework

Residual tumor cells following curative treatment in HR+ breast cancer may enter a state of prolonged biological dormancy—metabolically quiescent but genomically intact—shielded from immune surveillance and pharmacological pressure (Chen et al., 2025a; Sears & Davis, 2023). This dormancy underlies the characteristically protracted recurrence pattern of HR+ disease, with late relapses documented years to decades after initial diagnosis.

The low-shedding phenotype of dormant HR+ tumor cells means ctDNA concentrations in plasma may be extremely low—often below the detection threshold of standard sequencing pipelines (Elliott et al., 2025b). This demands assays of exceptional analytical sensitivity and has driven the development of methylation-based and multi-omic approaches as alternatives to mutation-based detection in early-stage and post-treatment surveillance settings.

ctDNA fragments are distinguished from non-tumor cell-free DNA (cfDNA) by somatic alterations: point mutations, copy number variations, structural variants, and cancer-specific epigenetic modifications (Sears & Davis, 2023; Widman et al., 2024). The fraction of ctDNA within total cfDNA—the variant allele fraction (VAF)—is proportional to tumor burden and provides a quantitative index of residual disease.

Beyond mutation detection, DNA methylation patterns and fragmentomic features (fragment length distribution and preferred end-sequence motifs) provide complementary or superior biomarker signals in low-shedding contexts (Elliott et al., 2025b; Janni et al., 2025). Cancer-specific methylation signatures are detectable at lower ctDNA concentrations than somatic mutations and are substantially less susceptible to confounding by clonal hematopoiesis—the primary source of false-positive results in mutation-based liquid biopsy.

A coherent clinical framework for ctDNA-based MRD monitoring integrates three dimensions. First, the detection modality: somatic mutation, DNA methylation, fragmentomics, or multi-omic integration. Second, the assay architecture: tumor-informed (personalized, patient-specific), tumor-agnostic (fixed panel or methylation signature, no tumor tissue required), or tumor-naive multi-omic (plasma-only). Third, the clinical application: early relapse prediction, risk stratification, therapy escalation or de-escalation, or resistance monitoring (Abdo et al., 2026; Sears & Davis, 2023).

Technologies and Methodologies

Next-generation sequencing (NGS) enables comprehensive mutational profiling across targeted gene panels or the entire tumor genome. Ultra-deep sequencing employing molecular barcoding and error-correction algorithms achieves the sensitivity required for low-frequency variant detection characteristic of MRD settings (Janni et al., 2025; Semenkovich et al., 2023). Tumor-informed NGS panels—designed around patient-specific somatic variants from primary tumor sequencing—represent the current analytical benchmark for ctDNA MRD detection (Panet et al., 2024; Qiu et al., 2025). Parsons et al. (2020) established the foundational proof-of-concept that tracking multiple patient-specific mutations simultaneously significantly enhances sensitivity compared to single-mutation approaches. The MAESTRO-Pool methodology further extended this principle to highly parallel cohort-level implementation (Blewett et al., 2024).

Digital droplet PCR (ddPCR) partitions the PCR reaction into thousands of individual droplets, enabling absolute quantification of target variants with high analytical sensitivity. It is particularly well-suited to longitudinal monitoring of known hotspot mutations, including *ESR1* ligand-binding domain variants associated with acquired endocrine resistance (Lu et al., 2025). Its principal limitation is the requirement for prior knowledge of the target mutation, restricting utility to hypothesis-directed monitoring.

Methylation-based ctDNA detection exploits cancer-specific DNA methylation signatures at loci differentially methylated in breast cancer relative to normal tissue (Elliott et al., 2025b). These assays require no prior knowledge of patient-specific mutations and are substantially less susceptible to clonal hematopoiesis interference. In HR+ breast cancer, methylation-based assays have demonstrated superior performance compared to tumor-agnostic mutation-based approaches in early-stage, low-shedding disease.

Single-cell sequencing platforms enable detailed molecular characterization of circulating tumor cells (CTCs), revealing intratumoral heterogeneity and clonal resistance mechanisms at single-cell resolution (Chen et al., 2023; Xu et al., 2021). Microfluidic technologies improve CTC isolation efficiency (Velpula & Buddolla, 2025; Li et al., 2021b). CTC and ctDNA analyses produce largely non-overlapping detection profiles, providing complementary biological information that—in combination—enhances MRD detection sensitivity, risk stratification, and resistance monitoring (Bortolini Silveira et al., 2021; Gerratana et al., 2021).

Tumor-informed assays employ whole-genome or whole-exome sequencing of primary tumor tissue to design a personalized tracking panel. Commercial platforms such as Signatera and NeXT Personal simultaneously interrogate dozens to hundreds of patient-specific loci (Chen & Zhou, 2023; Chen et

al., 2021). Tumor-agnostic assays apply fixed gene panels or methylation signatures across patients without tumor tissue, improving logistical feasibility at some cost to sensitivity in low-shedding tumors (Nguyen et al., 2025). Tumor-naive multi-omic assays integrate methylation and genomic features from plasma alone, entirely eliminating the tumor tissue requirement and reducing turnaround time (Janni et al., 2025). Overall, tumor-informed assays outperform tumor-agnostic alternatives, though the latter offer greater accessibility (Panet et al., 2024).

Critical Analysis of Evidence

The most clinically consequential attribute of ctDNA-based MRD monitoring is its capacity to detect impending relapse substantially in advance of conventional methods. ctDNA MRD detection precedes clinical or radiographic disease recurrence by a median of 5–14 months, with individual cases reporting a lead time of up to five years (Elliott et al., 2025a; Nader-Marta et al., 2024; Pfister et al., 2025a). This pre-clinical window provides a therapeutic opportunity for pre-emptive intervention before macroscopic metastatic disease is established.

ctDNA positivity in the post-adjuvant period is strongly associated with increased risk of recurrence and inferior survival outcomes in HR+ breast cancer (Lipsyc-Sharf et al., 2022; Ajjawi et al., 2025). Conversely, ctDNA clearance during or after treatment is strongly correlated with longer survival and prolonged time to treatment failure (Fuentes-Antras et al., 2025; Elliott et al., 2024). These bidirectional associations establish ctDNA dynamics as a robust real-time surrogate for treatment efficacy and residual disease burden.

The comparative analytical performance of principal ctDNA assay architectures is summarized in Table 1, based on data reported by Elliott et al. (2025b) and Nguyen et al. (2025). Tumor-informed mutation-based assays demonstrate sensitivity of 50–79% and specificity of approximately 100%, with a detection lead time of 5–14 months, but require primary tumor tissue and incur higher costs. Methylation-based assays achieve sensitivity of 62.5% and specificity of 100%, with a lead time exceeding five months; they outperform mutation-based tumor-agnostic assays in early-stage, low-shedding HR+ tumors and are less susceptible to clonal hematopoiesis interference (Elliott et al., 2025b). Tumor-agnostic multi-omic assays achieve sensitivity of approximately 54.5% and specificity of 98.8% but demonstrate lower sensitivity in early-stage disease (Nguyen et al., 2025).

Table 1
Comparative performance of ctDNA assay architectures in HR+ breast cancer MRD monitoring. Source: Elliott et al. (2025b); Nguyen et al. (2025).

Assay Architecture	Sensitivity	Specificity	Lead Time	Key Advantage	Primary Limitation
Tumor-informed (mutation-based)	50–79%	~100%	5–14 months	Highest per-patient sensitivity	Requires tumor tissue; higher cost
Methylation-based	62.5%	100%	>5 months	Superior in low-shedding tumors; resistant to clonal hematopoiesis	Requires further large-scale validation

Assay Architecture	Sensitivity	Specificity	Lead Time	Key Advantage	Primary Limitation
Tumor-agnostic (multi-omic)	~54.5%	~98.8%	Moderate	No tumor tissue required; broad applicability	Lower sensitivity in early-stage disease

The PADA-1 trial is a landmark investigation of ctDNA-guided therapeutic decision-making in advanced HR+/HER2− breast cancer. Longitudinal analysis by Mamann et al. (2025) demonstrated that early on-treatment evolution of ctDNA—including emergence of *ESR1* mutations and other resistance-associated alterations—was significantly associated with shorter progression-free survival (PFS) and overall survival (OS). Critically, ctDNA-based risk models outperformed conventional clinical parameters in prognostic discrimination (Mamann et al., 2025).

SERENA-6 evaluated ctDNA-guided early endocrine therapy switching in HR+/HER2− metastatic breast cancer. The trial demonstrated improved progression-free survival (PFS) and quality of life in patients managed with ctDNA-guided early therapy switching compared to imaging-based standard management (Oliveira et al., 2026). This demonstrates that ctDNA-based therapeutic decisions can be enacted weeks to months earlier than radiographic progression would otherwise mandate.

Oliveira et al. (2026) reviewed the combined evidence from both PADA-1 and SERENA-6 in the context of molecularly-driven early switch therapy, affirming that ctDNA-guided strategies represent an advance over conventional imaging-based monitoring. Fuentes-Antras et al. (2025) confirmed that ctDNA clearance during early treatment cycles was strongly correlated with prolonged time to treatment failure and longer overall survival in patients receiving endocrine therapy combined with CDK4/6 inhibitors. Elliott et al. (2024) further showed that ctDNA metrics of response and progression in the same therapeutic context provided clinically informative prognostic data. Collectively, these trials indicate that ctDNA-guided therapy adaptation is associated with improved outcomes, but definitive survival benefit requires further large-scale validation (Oliveira et al., 2026).

Mutations in the ligand-binding domain of the estrogen receptor gene (*ESR1*) represent the most clinically significant mechanism of acquired resistance in HR+ breast cancer patients receiving aromatase inhibitors. ctDNA-based longitudinal monitoring of *ESR1* mutational status enables real-time surveillance for emerging resistance before clinical progression, guiding timely therapy adaptation (Allouchery et al., 2018; Li et al., 2020; Maloberti et al., 2025). Different *ESR1* mutation subtypes confer heterogeneous resistance profiles and may be associated with distinct patterns of metastatic dissemination (Reinert et al., 2019). The systematic review by Najim et al. (2019) established that *ESR1* mutations arise predominantly under aromatase inhibitor pressure and associate with inferior clinical outcomes. CDK4/6 inhibitors have demonstrated capacity to partially overcome *ESR1* mutation-mediated resistance (Crucitta et al., 2023), reinforcing the value of ctDNA-based resistance monitoring for informing combination therapy decisions. The ctDNA profile and its clinical significance in patients with HR+/HER2− disease has been further characterized by Tang et al. (2022), confirming the importance of serial ctDNA monitoring for resistance management.

CTC and ctDNA analyses produce non-overlapping detection profiles. While ctDNA reflects the bulk genomic landscape through DNA shedding, CTCs represent intact disseminated tumor cells amenable to detailed phenotypic characterization (Bortolini Silveira et al., 2021). Their combination enhances early detection, risk stratification, and resistance monitoring beyond either modality alone (Gerratana et al., 2021; Park et al., 2024). Machine learning-driven integration of CTC and ctDNA data has been

shown to improve endocrine resistance profiling accuracy (Gerratana et al., 2025). Stergiopoulou et al. (2023) confirmed the clinical value of comprehensive multimodal liquid biopsy for early MRD detection in breast cancer.

Technological Innovations

Machine learning and advanced bioinformatics have substantially improved ctDNA analytical performance. Widman et al. (2024) developed an ultrasensitive plasma-based monitoring system employing machine learning-guided signal enrichment, achieving tumor burden quantification at variant allele fractions previously below the detection threshold of standard pipelines, with significant improvement in MRD detection sensitivity without compromising specificity. Machine learning further enhances variant calling accuracy, reduces technical noise, and integrates multi-omic data for improved performance in low-shedding contexts (Widman et al., 2024; Abdo et al., 2026).

Multi-omic approaches integrating genomic, epigenomic, and fragmentomic features demonstrate additive improvements in ctDNA detection (Janni et al., 2025; Semenkovich et al., 2023). By combining mutation-based signals with methylation signatures and fragment length information, these platforms extract richer biological information from the same plasma specimen. This is of particular value in HR+ breast cancer, where single-signal sensitivity is constrained by low ctDNA shedding.

Tumor-naive, plasma-only ctDNA assays integrating methylation profiling and genomic data require no prior tumor tissue sequencing, improving clinical feasibility and reducing turnaround time (Janni et al., 2025). Their performance is approaching, but has not yet equalled, that of tumor-informed personalized approaches (Nguyen et al., 2025). Ongoing methodological development and prospective validation of these platforms are active research priorities.

Structural variant-based ctDNA detection—targeting chromosomal rearrangements and large deletions nearly absent in normal cfDNA—offers inherently high specificity and is less susceptible to sequencing error and clonal hematopoiesis interference (Elliott et al., 2025a). Mohiuddin (2025) reviewed the landscape of ultrarapid sensitivity innovations, including nanomaterial-based sensors and AI-driven error suppression systems, as further tools for MRD detection in low-shedding tumor contexts.

Methodological Challenges and Limitations

The absence of standardized assay protocols, analytical thresholds, and reporting frameworks is the foremost barrier to clinical translation (Lin et al., 2026; Zhu et al., 2023). Heterogeneity in pre-analytical variables—blood collection tube type, plasma processing time and temperature, storage conditions, and DNA extraction methods—introduces substantial inter-laboratory variability impeding cross-study comparability and clinical interpretation (Lu et al., 2025). International consensus protocols are urgently needed. The definition of clinically appropriate MRD positivity thresholds is an equally pressing challenge. Longitudinal ctDNA trajectory monitoring, rather than single time-point results, may provide more robust evidence of true MRD (Chin et al., 2019; Klimova et al., 2025; Deveson et al., 2021).

Clonal hematopoiesis of indeterminate potential (CHIP)—the age-related accumulation of somatic mutations in hematopoietic stem cells—is a major source of false-positive ctDNA results in cancer surveillance (Janni et al., 2025; Chin et al., 2019). CHIP-derived mutations in plasma cfDNA can mimic low-burden ctDNA signals, potentially misclassifying patients as MRD-positive. Recommended mitigation strategies include paired white blood cell sequencing to subtract CHIP-derived variants, longitudinal monitoring to distinguish persistent from transient signals, and advanced bioinformatic filtering (Janni et al., 2025; Chin et al., 2019). Methylation-based assays offer

an intrinsic advantage, as methylation signatures are less susceptible to CHIP-related interference (Elliott et al., 2025b).

False-negative results—arising from ctDNA concentrations below current assay detection limits—are particularly acute in HR+ breast cancer. Patients with small residual tumor burden or predominantly dormant micrometastatic deposits may harbor biologically significant MRD undetectable by available platforms (Chen et al., 2025a; Lin et al., 2026). A ctDNA-negative result cannot be interpreted as confirmation of complete pathological remission in HR+ disease, underscoring the need for complementary multimodal approaches and continued assay sensitivity improvements.

The cost of tumor-informed ctDNA assays constitutes a prohibitive barrier in many healthcare systems (Abdo et al., 2026; Lin et al., 2026). Limited reimbursement and restricted assay availability in lower-resource settings raise important health equity concerns. Most evidence derives from high-resource clinical trial settings, limiting generalizability to global patient populations (Xu et al., 2025). Cost-effectiveness studies and simplified, accessible assay formats are critical prerequisites for equitable implementation.

Real-World Clinical Implications

The clinical implications of validated ctDNA-based MRD monitoring in HR+ breast cancer span several domains:

- *Personalized surveillance:* ctDNA monitoring enables individualized follow-up, reducing unnecessary imaging while allowing earlier intervention upon molecular evidence of relapse (Chen et al., 2025a; Sears & Davis, 2023).
- *Treatment adaptation:* Real-time ctDNA dynamics can inform decisions to escalate, de-escalate, or switch adjuvant therapy (Oliveira et al., 2026; Fuentes-Antras et al., 2025).
- *Resistance monitoring:* Detection of actionable mutations (e.g., *ESR1*, *PIK3CA*) via ctDNA supports timely therapy switching before overt clinical progression (Allouchery et al., 2018; Crucitta et al., 2023; Tang et al., 2022).
- *Multimodal integration:* Combining ctDNA with CTCs and advanced imaging may yield a composite MRD index with superior predictive accuracy (Bortolini Silveira et al., 2021; Stergiopoulou et al., 2023).
- *Implementation barriers:* Cost, assay accessibility, and standardization deficits currently limit widespread adoption (Abdo et al., 2026; Lin et al., 2026; Xu et al., 2025).

Future Research Directions

- *Large-scale randomized trials:* Prospective, adequately powered trials with survival endpoints are essential to definitively validate the clinical utility and survival benefit of ctDNA-guided therapeutic interventions (Abdo et al., 2026; Xu et al., 2025; Pfister et al., 2025b).
- *Consensus standardization:* International consensus on pre-analytical protocols, MRD positivity thresholds, and reporting standards is required (Klimova et al., 2025; Deveson et al., 2021; Lin et al., 2026).
- *Plasma-only multi-omic validation:* Tumor-naive plasma-only assays must be prospectively validated against tumor-informed standards (Janni et al., 2025; Nguyen et al., 2025).
- *Multi-modal biomarker integration:* Research combining ctDNA with CTCs, radiomics, and proteomic signatures may yield composite MRD indices with superior accuracy (Abdo et al., 2026; Stergiopoulou et al., 2023).

- *Implementation science and health economics:* Studies addressing workflow integration, cost-effectiveness, and implementation across diverse healthcare settings are critical (Abdo et al., 2026; Lin et al., 2026).

Conclusion

Liquid biopsy and ctDNA-based MRD monitoring represent a paradigm-shifting advance in the management of hormone receptor-positive breast cancer. The capacity to detect molecular evidence of residual disease months to years before clinical manifestation, and to dynamically monitor tumor evolutionary trajectories under therapeutic pressure, addresses fundamental limitations of conventional surveillance and opens substantive opportunities for pre-emptive, personalized intervention.

Current evidence demonstrates robust prognostic value for ctDNA positivity across early-stage and metastatic HR+ disease (Lipsyc-Sharf et al., 2022; Ajjawi et al., 2025). Tumor-informed and methylation-based assays lead in analytical performance (Elliott et al., 2025b). Clinical trial data from PADA-1 and SERENA-6 provide compelling evidence that ctDNA-guided therapeutic decisions can translate into measurable improvements in PFS and quality of life (Mamann et al., 2025; Oliveira et al., 2026), though definitive survival validation remains outstanding. Technological innovations in machine learning, multi-omic integration, and ultra-sensitive platforms are progressively expanding the sensitivity frontier (Widman et al., 2024; Elliott et al., 2025a).

The pathway to routine clinical adoption remains contingent on resolving outstanding challenges: pre-analytical standardization, validated MRD positivity thresholds, robust clonal hematopoiesis mitigation, and demonstrated cost-effectiveness across diverse healthcare systems (Lin et al., 2026; Xu et al., 2025; Abdo et al., 2026). The convergence of molecular diagnostics, computational analytics, and adaptive clinical trial design will determine the rate at which ctDNA-based MRD monitoring is translated into improved, equitably accessible care for the global population of patients with HR+ breast cancer.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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