Journal of Biosciences and Medicines, 2026, 14(5), 160-174
https://www.scirp.org/journal/jbm
ISSN Online: 2327-509X
ISSN Print: 2327-5081

Dynamic Monitoring and Prognostic Value of Circulating Tumor Cells in Neoadjuvant Therapy for Breast Cancer: Research Progress

Keliang Ren, Dengfeng Chen*

Breast Department, Jingzhou Hospital Affiliated to Yangtze University, Jingzhou, China
Email: 2280792556@qq.com, *cdf320412@163.com

How to cite this paper: Ren, K.L. and Chen, D.F. (2026) Dynamic Monitoring and Prognostic Value of Circulating Tumor Cells in Neoadjuvant Therapy for Breast Cancer: Research Progress. *Journal of Biosciences and Medicines*, **14**, 160-174.
https://doi.org/10.4236/jbm.2026.145013

Received: March 23, 2026
Accepted: May 9, 2026
Published: May 12, 2026

Abstract

Circulating tumor cells (CTCs), which detach from primary or metastatic lesions and enter the peripheral blood, serve as crucial biomarkers in liquid biopsy for breast cancer. In the context of neoadjuvant therapy, the dynamic changes of CTCs offer a real-time and minimally invasive window to evaluate treatment response, predict pathological complete response, and forecast long-term prognosis. Despite advances in detection technologies and growing evidence supporting their clinical utility, challenges remain in standardizing methodologies, understanding molecular heterogeneity, and integrating CTC analysis into routine clinical practice. This review systematically summarizes current detection techniques for CTCs, baseline and dynamic characteristics during neoadjuvant treatment, molecular subtyping and heterogeneity, as well as their predictive and prognostic significance. Furthermore, it discusses existing limitations and future research directions aimed at optimizing neoadjuvant therapeutic strategies in breast cancer. By providing a comprehensive overview, this article aims to enhance the understanding of CTCs' role in personalized treatment and improve patient outcomes.

Keywords

Circulating Tumor Cells, Breast Cancer, Neoadjuvant Therapy, Liquid Biopsy, Treatment Response Monitoring, Prognosis

1. Introduction

Breast cancer remains the most prevalent malignancy among women worldwide, with a significant proportion of patients presenting with locally advanced disease at diagnosis. Neoadjuvant chemotherapy (NAC) has become a standard therapeu-

tic approach for locally advanced breast cancer (LABC) and certain molecular subtypes, aiming to reduce tumor burden, downstage disease to facilitate breast-conserving surgery, and provide an *in vivo* assessment of tumor chemosensitivity (The treatment approach in this article primarily involves neoadjuvant chemotherapy). Despite advances in imaging and pathological evaluation, conventional methods for monitoring treatment response are limited by their inability to capture real-time tumor dynamics and biological heterogeneity during therapy. This gap underscores the urgent need for minimally invasive biomarkers that can dynamically reflect tumor behavior and predict therapeutic outcomes.

Circulating tumor cells (CTCs), defined as malignant cells shed from primary or metastatic lesions into the peripheral blood, have emerged as a promising "liquid biopsy" component that can be repeatedly and noninvasively sampled to provide insights into tumor biology and treatment response. The detection and characterization of CTCs offer a unique opportunity to overcome the limitations of tissue biopsies, which are invasive, often limited by tumor heterogeneity, and impractical for serial monitoring. Technological advances in CTC isolation and molecular profiling have enabled the identification of heterogeneous CTC populations, including epithelial, mesenchymal, and stem-like phenotypes, which are implicated in metastasis and drug resistance [1]. These phenotypic diversities reflect the dynamic epithelial-mesenchymal transition (EMT) processes and cancer stem cell (CSC) properties that contribute to tumor progression and therapeutic evasion.

Recent clinical studies have demonstrated that CTC enumeration and molecular features correlate with response to NAT and prognosis in breast cancer patients. For instance, persistence of CTCs after NAT is associated with poorer distant metastasis-free survival in patients with residual triple-negative breast cancer after NAC. Moreover, dynamic changes in CTC numbers during NAT provide supplementary prognostic information beyond conventional imaging criteria such as RECIST, enhancing early prediction of treatment efficacy. Notably, the presence of specific CTC subpopulations expressing EMT markers has been linked to increased risk of recurrence in stage III breast cancer, highlighting their potential as biomarkers for risk stratification.

Molecular characterization of CTCs further refines their clinical utility. Studies have revealed discordance in molecular subtypes between primary tumors and CTCs, with subtype conversions often toward more aggressive phenotypes during NAC, which may underlie treatment resistance and metastatic potential [2]. Additionally, expression of therapeutic targets such as HER2 on CTCs can differ from the primary tumor, influencing response to targeted therapies and underscoring the value of CTC-based phenotyping for personalized treatment decisions. Integration of CTC enumeration with circulating tumor DNA (ctDNA) analysis enhances sensitivity and prognostic accuracy, as these complementary liquid biopsy components capture distinct aspects of tumor heterogeneity and burden.

Despite these promising findings, challenges remain in standardizing CTC detection methodologies, interpreting heterogeneous CTC phenotypes, and validating their predictive and prognostic roles in large prospective trials. The rarity of CTCs in peripheral blood and technical variability in isolation platforms necessitate continued refinement of detection technologies, including antibody-independent enrichment methods and multi-omic profiling approaches. Furthermore, the biological significance of CTC subtypes and their interactions with the tumor microenvironment and immune system requires deeper investigation to elucidate mechanisms of metastasis and therapeutic resistance [3].

In summary, the dynamic monitoring of circulating tumor cells during neoadjuvant chemotherapy represents a rapidly evolving field with significant potential to improve breast cancer management. By providing real-time, minimally invasive insights into tumor biology, CTC analysis may enable early prediction of treatment response, identification of residual disease, and personalized therapeutic adjustments. This review will comprehensively examine the technological advances, biological underpinnings, and clinical evidence supporting the prognostic and predictive value of CTCs in breast cancer neoadjuvant therapy, as well as discuss future directions for their integration into clinical practice.

2. CTC Detection Technologies in the Context of Neoadjuvant Therapy: Application and Optimization

2.1. Mainstream Detection Platforms Based on Physical Properties and Immunoaffinity

The identification of circulating tumor cells (CTCs) in breast cancer patients receiving neoadjuvant therapy has advanced considerably. Various platforms now employ either biophysical characteristics or immunoaffinity-based methods for CTC enrichment and analysis. The CellSearch system, which uses immunomagnetic enrichment directed at the epithelial cell adhesion molecule (EpCAM) on tumor cells, continues to be the sole FDA-cleared platform for CTC detection and enumeration. In studies of neoadjuvant chemotherapy, this system has been utilized for quantitative CTC assessment, revealing both prognostic and predictive significance in locally advanced breast cancer (LABC) and demonstrating prognostic value for recurrence in early-stage triple-negative breast cancer (TNBC). Nonetheless, the dependence of CellSearch on EpCAM restricts its capacity to isolate CTCs undergoing epithelial-mesenchymal transition (EMT). During EMT, epithelial markers are downregulated while mesenchymal characteristics are acquired, resulting in an underestimation of CTC heterogeneity and the potential omission of subpopulations linked to metastasis and treatment resistance. To address these constraints, microfluidic chip technologies like the CTC-iChip have been developed. These platforms leverage the size, deformability, and other physical properties of CTCs for label-free or hybrid antibody-mediated capture. Such methods improve sensitivity and allow for the isolation of a wider array of CTC phenotypes, including those exhibiting EMT and cancer stem cell (CSC)-like fea-

tures, which are essential for tracking phenotypic changes induced by treatment throughout NAT. Additional enrichment techniques, such as membrane filtration and density gradient centrifugation, provide benefits in terms of simplicity and cost-effectiveness but differ in their capture efficiency and purity. Recent initiatives are centered on combining multiple capture principles into integrated platforms to enhance the reliability and thoroughness of CTC isolation across sequential samples during NAT. This approach tackles the dynamic variability and phenotypic adaptability of CTC populations. Together, these technological developments support more precise and sensitive CTC detection, thereby enabling improved prognostic classification and real-time assessment of therapeutic efficacy in breast cancer patients undergoing neoadjuvant treatment.

2.2. Single-Cell Analysis Technologies and Downstream Molecular Characterization

Advances in single-cell isolation and molecular profiling technologies have revolutionized the characterization of circulating tumor cells (CTCs), providing new perspectives for understanding tumor heterogeneity, clonal evolution, and resistance mechanisms in the context of neoadjuvant therapy. Techniques such as size-exclusion membrane capture enable the isolation of individual CTCs, allowing for subsequent single-cell transcriptomic analysis [4]. Performing single-cell genomic sequencing of CTCs before and after neoadjuvant therapy enables the tracking of clonal dynamics and the identification of acquired resistance mutations, including variants in ESR1 and PIK3CA associated with endocrine therapy resistance and targeted therapy failure. Evaluation of individual CTCs for hormone receptor status, HER2 expression, and proliferation markers such as Ki-67 reveals molecular subtype switching and phenotypic transitions that may differ from primary tumor biopsy results during treatment [5] [6]. These molecular profiling studies indicate that CTCs often differ from the primary tumor, including transformation into more aggressive or treatment-resistant subtypes, further underscoring the importance of liquid biopsy in dynamic treatment monitoring. Moreover, an integrated analysis of single-cell data and clinical outcomes demonstrated that early apoptotic CTCs expressing at least two stem cell markers are associated with poor neoadjuvant therapy response and adverse prognosis [7]. Longitudinal single-cell analysis of CTCs during neoadjuvant therapy provides a minimally invasive window into tumor evolution, facilitating personalized treatment adjustments and early detection of resistance. Therefore, single-cell technologies represent a key approach for optimizing neoadjuvant treatment strategies and improving the accuracy of breast cancer prognosis.

3. Dynamic Patterns of CTC Changes during Neoadjuvant Therapy and Their Significance

3.1. Correlation between Baseline CTC Counts and Treatment Response

The enumeration of circulating tumor cells (CTCs) at baseline prior to Neoadju-

vant chemotherapy (NAC) has become a crucial prognostic and predictive biomarker in the management of breast cancer. Numerous prospective studies indicate that the presence of CTCs in peripheral blood before starting NAT, especially when one or more CTCs are detected per 7.5 mL of blood, is significantly associated with lower rates of pathological complete response (pCR). This correlation implies that baseline CTC positivity may indicate a more aggressive tumor phenotype or a subtype resistant to chemotherapy. For example, in locally advanced breast cancer (LABC), patients with elevated baseline CTC counts frequently present with larger tumor volumes or early micrometastatic spread, which can lead to suboptimal responses to conventional chemotherapy and may require more intensive or personalized treatment strategies [8]. The predictive utility of baseline CTCs also differs across molecular subtypes of breast cancer. In particular, CTC positivity (Completion of all cycles of neoadjuvant chemotherapy (NAC), peripheral blood collection prior to surgery, and detection of ≥1 circulating tumor cell (CTC) in 7.5 mL of peripheral blood). Following neoadjuvant chemotherapy in triple-negative breast cancer (TNBC) is linked to treatment outcomes, emphasizing the heterogeneity of CTC biology among subtypes. Additionally, molecular analysis of CTCs has uncovered subtype transitions and phenotypic adaptability that may not be evident from primary tumor analysis alone, adding complexity to predictive assessments [9]. Baseline CTC positivity is also associated with poorer long-term prognosis and can serve as an independent adverse predictor for disease-free survival (DFS) and overall survival (OS). In locally advanced breast cancer, elevated baseline CTCs are correlated with an increased risk of micrometastasis, potentially indicating a higher probability of distant recurrence. Together, these observations underscore the value of baseline CTC enumeration as a non-invasive biomarker capable of risk stratification and informing neoadjuvant treatment planning (Sampling time points: Baseline before treatment).

3.2. Dynamic Monitoring Value of Early and Mid-Treatment CTC Clearance

Variations in circulating tumor cell (CTC) levels during the initial and intermediate phases of neoadjuvant treatment offer vital insights for assessing therapeutic effectiveness and tumor dynamics. The early clearance of CTCs, characterized by a shift from detectable to undetectable CTC counts following one or two cycles of Neoadjuvant chemotherapy (NAC), has emerged as a promising indicator for forecasting ultimate pathological outcomes. This clearance phenomenon is strongly correlated with increased rates of pathological complete response (pCR) and extended disease-free survival (DFS), acting as an early biological indicator of favorable treatment response. In contrast, sustained high CTC levels after neoadjuvant chemotherapy are associated with reduced progression-free survival and overall survival. Early clearance of CTCs after 1 - 2 treatment cycles was significantly associated with a higher pCR rate, suggesting that early CTC kinetics can be used to predict therapeutic efficacy. Failure to achieve CTC clearance during early treat-

ment was associated with shorter disease-free survival (DFS) and overall survival (OS), indicating that persistent CTC positivity may serve as an early marker of poor long-term prognosis. [8]. Combining CTC kinetic data with other biomarkers, such as circulating tumor DNA (ctDNA) and immunological markers, can improve its predictive accuracy, allowing for a more holistic evaluation of tumor response [10] [11]. Importantly, the phenotypic heterogeneity of CTCs, encompassing epithelial-mesenchymal transition (EMT) and cancer stem cell (CSC) traits, affects their detectability and prognostic value, underscoring the necessity for sophisticated detection techniques that extend beyond EpCAM-based approaches [1] [3]. Ongoing CTC evaluation throughout the NAT course provides a non-invasive, real-time surveillance strategy for patients, enabling therapeutic modifications and enhancing clinical results (Sampling time point: After 1 to 2 treatment cycles).

3.3. CTC Status after Completion of Neoadjuvant Therapy and before Surgery

The presence of circulating tumor cells (CTCs) after neoadjuvant treatment and before surgery is an independent predictor of residual disease and metastatic potential. Even in patients who exhibit radiological or pathological responses, the detection of CTCs at this stage signals a substantially higher likelihood of disease recurrence, suggesting the existence of minimal residual disease or therapy-resistant tumor populations. Molecular profiling indicates that these remaining CTCs often possess unique genetic and phenotypic characteristics, such as subclones with resistance to treatment, which can aid in determining postoperative adjuvant strategies, including the choice of targeted drugs or escalation of systemic therapy. In triple-negative breast cancer, CTC positivity following neoadjuvant chemotherapy correlates with a greater risk of recurrence. CTC positivity after NAC is associated with the presence of residual disease before surgery, suggesting its potential as a predictive marker for minimal residual disease [5]. For instance, identifying HER2 expression changes in CTCs after neoadjuvant therapy can inform decisions regarding HER2-targeted treatments, highlighting tumor adaptation under therapeutic selection pressure [12]. Moreover, integrating CTC and ctDNA analysis at this time point improves prognostic precision and may help select patients for more intensive monitoring or innovative therapeutic interventions. Consequently, assessing CTCs prior to surgery is a vital element of personalized breast cancer care, delivering clinically relevant insights that extend beyond standard imaging and pathological evaluation (Sampling time point: After NAC and before surgery).

4. Molecular Heterogeneity of CTCs and Their Role in Neoadjuvant Therapy Resistance Mechanisms Research

4.1. Epithelial-Mesenchymal Transition and Stem Cell Properties

Neoadjuvant chemotherapy (NAC) exerts selective pressure on circulating tumor

cells (CTCs) in breast cancer, frequently promoting epithelial-mesenchymal transition (EMT). This process involves the downregulation of epithelial markers and the acquisition of mesenchymal characteristics, enhancing the invasive potential and chemoresistance of CTCs. Research indicates that during NAC, CTCs undergo EMT, as shown by reduced levels of epithelial markers like EpCAM and cytokeratins and elevated expression of mesenchymal markers such as vimentin and β-catenin, which boost migratory and invasive abilities. EMT-positive CTCs are more common after NAC and are associated with a lower pathological complete response (pCR) and unfavorable prognosis, emphasizing their contribution to therapy resistance. Additionally, a fraction of CTCs displays cancer stem cell (CSC) features, identified by markers including $CD44^{+}CD24^{-}$ and high aldehyde dehydrogenase 1 (ALDH1) activity. These CSC-like CTCs possess intrinsic chemoresistance and act as "seed" cells for metastasis and disease recurrence. For example, the detection of CSC-CTCs during or following NAC has been connected to treatment inefficacy and early metastatic relapse. Dynamic tracking of these subpopulations throughout NAC demonstrates that their increase correlates with adverse clinical outcomes, highlighting their prognostic relevance. Mechanistically, EMT and stemness are controlled by signaling pathways such as TGF-β and Wnt, which become activated in CTCs under chemotherapeutic stress. Studies employing CTC models reveal that the stimulation of these pathways encourages EMT and stem-like phenotypes, aiding survival and spread despite chemotherapy. Moreover, the apoptotic status of CTCs affects their stemness and therapeutic response; early apoptotic CTCs expressing stemness markers like CD44 and CD133 are linked to poor NAC response and reduced metastasis-free survival [8]. Currently, conclusions regarding the association between EMT phenotypes and stem-like CTC subgroups with pathological complete response (pCR) are inconsistent across different studies. This discrepancy may be specific to breast cancer molecular subtypes and influenced by the dependence on CTC detection platforms, and a unified consensus has not yet been reached. The interaction among EMT, stemness, and apoptotic regulation in CTCs under NAC pressure forms a cellular foundation for chemoresistance. Consequently, thorough molecular profiling of CTCs, encompassing EMT and CSC markers, provides a critical understanding of NAC resistance mechanisms and could inform the creation of targeted treatments to eliminate these persistent cell groups, thereby enhancing patient outcomes.

4.2. Receptor Status Discordance and Targeted Therapy Resistance

Discordance in receptor status between circulating tumor cells (CTCs) and primary or residual tumors represents a key determinant of targeted therapy resistance in breast cancer patients undergoing neoadjuvant chemotherapy (NAC). The expression profiles of hormone receptors (HR) and HER2 on CTCs frequently diverge from those observed in the primary tumor, with such discrepancies oc-

curring more commonly in patients treated with neoadjuvant chemotherapy. This divergence becomes especially evident following NAC, where CTCs in HER2-positive patients may exhibit loss of HER2 expression, thereby contributing to resistance against anti-HER2 treatments. Existing evidence does not indicate that HER2-negative patients develop HER2 amplification in CTCs, which could otherwise reveal novel therapeutic targets. For instance, research indicates that HER2-positive breast cancer patients whose CTCs transition to a HER2-negative state during NAC demonstrate reduced responsiveness to trastuzumab-based therapies, suggesting the emergence of acquired resistance. Alterations in molecular subtypes within CTCs can introduce complexity to treatment planning, as a mismatch between the evolved subtype and the chosen therapeutic regimen is linked to decreased metastasis-free survival. Serial molecular profiling of CTCs throughout NAC allows for real-time tracking of receptor status evolution, offering a dynamic perspective on tumor heterogeneity and potential therapeutic targets. This methodology holds promise for enhancing the prediction of treatment response and facilitating individualized therapeutic modifications. Furthermore, the identification of heterogeneity in estrogen receptor (ER) expression among CTCs—including the detection of ER-negative CTCs in patients with ER-positive primary tumors—points to potential mechanisms of endocrine therapy evasion [13]. Combining longitudinal molecular characterization of CTCs with clinical parameters improves the capacity to pinpoint patients at elevated risk of resistance and disease recurrence. Consequently, receptor status discordance in CTCs constitutes a fundamental mechanism driving targeted therapy resistance during NAC, and its investigation through sequential liquid biopsy presents a viable strategy for refining personalized treatment approaches in breast cancer.

5. Clinical Validation of CTC as a Surrogate Endpoint Marker and Prognostic Assessment Tool

5.1. Predictive Performance Analysis for Pathological Complete Response

The dynamic monitoring of circulating tumor cells (CTCs) during neoadjuvant chemotherapy (NAC) has emerged as a promising approach to predict pathological complete response (pCR) in breast cancer patients. Integrating CTC dynamics, such as early clearance or reduction, with imaging modalities and serum tumor markers enables the construction of multimodal predictive models that significantly enhance the accuracy of pCR prediction. In locally advanced breast cancer receiving NAC, patients in the high-response group showed an initial slight increase in CTC counts followed by a return to baseline [14]. CTC dynamic changes (early clearance, decline) can be used to predict pCR; combining imaging with tumor markers can improve predictive accuracy. Existing evidence suggests that CTC counts and early kinetic changes are associated with pCR, but they are not yet sufficient as a decisive basis for adjusting interventional therapy [2] [13]. The combination of CTC enumeration with imaging assessment based on RECIST al-

lows for a more comprehensive evaluation of tumor response to NAC in locally advanced breast cancer, overcoming limitations inherent to imaging assessment alone. Moreover, the heterogeneity of CTCs, including phenotypic subpopulations undergoing epithelial-mesenchymal transition (EMT) or expressing stemness markers, provides additional layers of information that can refine predictive models. Existing studies have not reached a consensus on the association between EMT and stem-like CTC subpopulations with pCR; some studies have not observed a significant correlation between the two, resulting in heterogeneous conclusions. CTC is a promising biomarker in the neoadjuvant setting that can be used to monitor metastatic risk and assess prognosis. CTC phenotypes (such as EMT and stem-like subpopulations) showed no clear association with pCR, suggesting that CTC count alone may be a more suitable predictor of pCR than phenotype [10]. However, the clinical adoption of CTC-based predictive models faces challenges, including variability in detection technologies, lack of standardized time points for sampling, and inconsistent cutoff values for defining response. Large-scale, multicenter prospective studies are needed to validate optimal CTC assessment protocols and establish clinically relevant thresholds that reliably predict pCR across diverse patient populations and breast cancer subtypes. Additionally, integrating CTC molecular characterization of HER2 status may further personalize prediction models and guide targeted therapies. In summary, the dynamic monitoring of CTCs combined with imaging and serum markers holds substantial promise for improving the prediction of pathological complete response in breast cancer patients undergoing neoadjuvant therapy, but requires rigorous clinical validation and standardization before routine clinical implementation.

5.2. Long-Term Prognostic Value for Disease-Free and Overall Survival

Baseline positivity of circulating tumor cells (CTCs) and their persistence following neoadjuvant chemotherapy (NAC) have been consistently identified as independent adverse prognostic factors impacting disease-free survival (DFS) and overall survival (OS) in breast cancer patients. Multiple studies have demonstrated that patients with detectable CTCs prior to NAC or those with residual CTCs after completion of therapy exhibit significantly shorter progression-free intervals and higher rates of relapse compared to patients with undetectable or cleared CTCs. Notably, follow-up CTC assessment can predict overall survival independent of tumor subtype and treatment [15]. Baseline CTC positivity and persistent CTCs after NAC were significantly associated with shorter DFS and OS, serving as independent adverse prognostic indicators. Persistent CTC negativity correlates with excellent long-term outcomes, identifying a low-risk patient subset that may benefit from de-escalated adjuvant treatment, thereby minimizing overtreatment and associated toxicities. Models integrating post-NAC CTC changes with other established parameters, including stromal tumor-infiltrating lymphocyte level and

Ki-67 expression, have demonstrated improved accuracy for predicting pathological complete response to NAC, facilitating more tailored therapeutic strategies [16]. Post-treatment CTC negativity indicates a favorable prognosis and may support de-escalation of adjuvant therapy in low-risk patients; however, this inference remains based on correlative evidence and has not yet been confirmed by interventional trials.. Furthermore, combining CTC status with circulating tumor DNA (ctDNA) analyses enhances prognostic precision, as these complementary biomarkers capture distinct aspects of tumor biology and residual disease burden [17]. The combination of CTCs and ctDNA can further improve the accuracy of prognostic assessment, suggesting its utility in recurrence risk stratification. The dynamic changes in CTC counts during and after NAC also provide valuable prognostic insights; for example, patients exhibiting a decline in CTCs during treatment are more likely to achieve pathological complete response [18]. A declining trend in CTCs during treatment is associated with better prognosis, but existing evidence is mostly observational and does not yet support directly changing treatment regimens based on this finding. Despite these promising findings, challenges remain in standardizing CTC detection methodologies, defining clinically meaningful cutoff values, and integrating CTC monitoring into routine clinical workflows. New randomized trials are needed to validate the utility of CTC-guided risk stratification and to determine whether therapeutic modifications based on CTC status can improve survival outcomes [19]. In conclusion, persistent CTC positivity before and after neoadjuvant therapy serves as a robust prognostic biomarker for adverse long-term outcomes in breast cancer, and its integration into prognostic models holds potential to refine postoperative treatment decisions and improve personalized patient management.

6. Current Challenges, Clinical Application Bottlenecks, and Future Research Directions

6.1. Technical Standardization and Clinical Translation Barriers

The clinical application of circulating tumor cell (CTC) detection in breast cancer, particularly in the neoadjuvant setting, faces significant challenges rooted in methodological heterogeneity and lack of standardization. Various detection platforms employ differing enrichment and identification techniques, such as antibody-based positive selection (e.g., CellSearch), size-based filtration (e.g., MetaCell), or antibody-independent methods (e.g., ApoStream), each with distinct sensitivities and specificities. This methodological diversity leads to discrepancies in CTC enumeration and phenotypic characterization, complicating direct comparisons across studies and impeding the establishment of universally accepted clinical thresholds for prognostic or predictive use [20]. The absence of standardized operating procedures and quality control measures further exacerbates this issue, limiting the reproducibility and reliability of CTC assays in routine clinical practice. Moreover, the high cost and technical complexity of current CTC detection methods, which often require specialized laboratory infrastructure and trained

personnel, restrict their accessibility, especially in resource-limited settings and primary care hospitals. This economic and logistical barrier hinders widespread adoption and integration into standard breast cancer management pathways. Another critical bottleneck is the lack of large-scale, phase III randomized controlled trials providing robust evidence that dynamic CTC monitoring can effectively guide neoadjuvant chemotherapy (NAC) decision-making and improve patient outcomes. While several studies, including a prospective secondary analysis of a randomized clinical trial, have demonstrated the prognostic value of CTC counts and their changes during NAC, most of these findings remain exploratory or retrospective from single-center studies, with limited large-scale prospective validation. Without such high-level evidence, CTC analysis remains primarily a research tool rather than a clinical decision-making aid. Additionally, the heterogeneity of CTC phenotypes, including epithelial, mesenchymal, and stem-like subpopulations, complicates the interpretation of CTC data and necessitates more sophisticated detection and characterization methods that are not yet standardized. Addressing these challenges requires concerted efforts to harmonize detection protocols, develop cost-effective and automated platforms, and conduct rigorous clinical trials to establish the clinical utility of CTC monitoring in guiding NAC and other therapeutic interventions in breast cancer.

6.2. Deepening Research from Enumeration to Functional Analysis

Future research in the field of circulating tumor cells (CTCs) must transcend mere enumeration to embrace comprehensive functional analyses that elucidate the biological roles and therapeutic vulnerabilities of these cells. Establishing patient-derived CTC in vitro culture systems or organoid models represents a promising avenue to directly assess drug sensitivity and resistance mechanisms, thereby realizing the concept of "functional liquid biopsy" [21]. Such models enable ex vivo testing of chemotherapeutic agents and targeted therapies on CTCs, potentially guiding personalized treatment regimens in real time. Furthermore, integrating CTC analyses with circulating tumor DNA (ctDNA), another liquid biopsy component, can provide a more holistic view of tumor heterogeneity and genomic alterations [22]. This integrative strategy enhances the sensitivity and specificity of disease monitoring and may uncover novel biomarkers predictive of treatment response or resistance. Another critical research frontier involves dissecting the interactions between CTCs and peripheral blood components, which can facilitate immune evasion and metastatic dissemination. Understanding these interactions at the molecular and cellular levels could reveal mechanisms underlying CTC survival in circulation and resistance to neoadjuvant immunotherapies, informing the development of combinatorial therapeutic strategies. Additionally, characterizing the epithelial-mesenchymal transition (EMT) status and stemness properties of CTCs is essential, as these phenotypic states influence metastatic potential and treatment resistance. Advanced single-cell sequencing and proteomic technolo-

gies can unravel the heterogeneity within CTC populations, identifying subclones with distinct functional attributes. Collectively, these deep functional analyses will transform CTC research from a prognostic tool into a platform for precision oncology, enabling dynamic treatment adaptation based on real-time tumor biology.

6.3. Guiding Personalized Treatment and Intervention Strategies

The molecular characterization and dynamic monitoring of circulating tumor cells (CTCs) hold substantial promise for guiding personalized neoadjuvant treatment strategies in breast cancer. Real-time assessment of CTC molecular features, such as hormone receptor status, HER2 expression, and actionable mutations, can facilitate early identification of therapeutic resistance, enabling timely modification of treatment regimens. For instance, CTC-HER2 status can predict the efficacy of anti-HER2 therapy, but current evidence has not detected HER2-positive CTCs in patients with HER2-negative primary tumors. Moreover, including post-NAC CTC changes in prediction models can improve the accuracy of predicting pathological complete response. Beyond conventional therapies, novel interventions targeting CTCs themselves are emerging. Nanotechnology-based approaches, such as multifunctional nanotheranostics, have been developed to selectively capture and eradicate circulating tumor clusters, potentially preventing metastatic seeding [23]. Similarly, immunotherapeutic strategies employing bispecific antibodies or nanoparticle-mediated delivery systems aim to eliminate CTCs, complementing systemic neoadjuvant treatments. Integrating CTC monitoring into comprehensive treatment management pathways necessitates the establishment of standardized clinical decision algorithms that incorporate CTC enumeration, molecular profiling, and functional assays. Such algorithms would enable dynamic treatment optimization, real-time response evaluation, and early detection of resistance, ultimately improving patient outcomes. However, translating these strategies into routine clinical practice requires validation through prospective clinical trials demonstrating improved survival and quality of life. In summary, leveraging CTC-based biomarkers for personalized therapy guidance and developing CTC-targeted interventions represent critical steps toward precision neoadjuvant treatment in breast cancer.

7. Conclusions

Circulating tumor cells (CTCs) have emerged as a pivotal biomarker in the neoadjuvant treatment of breast cancer, offering a dynamic and minimally invasive "liquid biopsy" that reflects tumor biology in real time. From an expert perspective, the integration of CTC analysis into clinical practice represents a significant advancement in personalized oncology, bridging the gap between molecular insights and therapeutic decision-making. The evidence consistently demonstrates that baseline CTC positivity, failure to clear CTCs early during treatment, and persistent CTC presence post-therapy are robust predictors of poor pathological response and diminished long-term survival. This prognostic value underscores

the clinical relevance of CTC enumeration as a non-invasive surrogate for tumor burden and treatment efficacy.

Despite the compelling data, the translation of CTC monitoring into routine clinical use is still hindered by technical and validation challenges. Variability in detection methods, lack of consensus on cutoff values, and limited prospective interventional trials have slowed widespread adoption. From an expert standpoint, addressing these issues requires concerted efforts to standardize CTC detection technologies and to validate their clinical utility through well-designed, prospective studies. Such trials should aim not only to confirm prognostic significance but also to evaluate whether CTC-guided therapeutic adjustments can improve patient outcomes. This approach will help reconcile differing research perspectives by providing high-level evidence to support clinical decision-making.

Looking forward, the future of CTC research lies in its evolution from a prognostic biomarker to a dynamic decision-making tool that can guide individualized treatment strategies in real time. Advancements in single-cell sequencing, functional assays, and integration with other liquid biopsy components, such as circulating tumor DNA, will likely enhance the resolution and predictive power of CTC analyses. Moreover, deepening our understanding of CTC biology through functional studies will be critical to unlocking their full potential as therapeutic targets and biomarkers. Ultimately, the goal is to establish CTC monitoring as a standard component of neoadjuvant breast cancer management, enabling clinicians to tailor therapies based on tumor evolution and treatment response dynamically. The development of CTC analysis in the context of neoadjuvant breast cancer therapy marks a transformative step toward precision oncology. While challenges remain in standardization and clinical validation, the accumulating evidence supports the integration of CTC monitoring into personalized treatment paradigms. By balancing the diverse research findings and focusing on rigorous clinical evaluation, the field is poised to harness CTCs not only as prognostic indicators but also as actionable biomarkers that can optimize therapeutic outcomes and improve patient survival. Continued multidisciplinary collaboration and innovation will be essential to realize the full clinical impact of CTCs, ultimately translating molecular insights into tangible benefits for breast cancer patients undergoing neoadjuvant therapy.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Le Du, F., Fujii, T., Kida, K., Davis, D.W., Park, M., Liu, D.D., *et al.* (2020) EpCAM-independent Isolation of Circulating Tumor Cells with Epithelial-to-Mesenchymal Transition and Cancer Stem Cell Phenotypes Using Apostream® in Patients with Breast Cancer Treated with Primary Systemic Therapy. *PLOS ONE*, **15**, e0229903. https://doi.org/10.1371/journal.pone.0229903

[2] Wang, J., Wang, X., Chen, R., Liang, M., Li, M., Ma, G., *et al.* (2022) Circulating Tumor Cells May Serve as a Supplement to RECIST in Neoadjuvant Chemotherapy of Patients with Locally Advanced Breast Cancer. *International Journal of Clinical Oncology*, **27**, 889-898. https://doi.org/10.1007/s10147-022-02125-9

[3] Du, K., Wu, S., Ma, X. and Liu, Y. (2024) Circulating Tumor Cell Phenotype Detection Based on Epithelial-Mesenchymal Transition Markers Combined with Clinicopathological Risk Has Potential to Better Predict Recurrence in Stage III Breast Cancer Treated with Neoadjuvant Chemotherapy: A Pilot Study. *Breast Cancer Research and Treatment*, **207**, 517-527. https://doi.org/10.1007/s10549-024-07430-7

[4] Grigoryeva, E.S., A.Tashireva, L., Alifanov, V.V., Savelieva, O.E., Vtorushin, S.V., Zavyalova, M.V., *et al.* (2022) Molecular Subtype Conversion in CTCs as Indicator of Treatment Adequacy Associated with Metastasis-Free Survival in Breast Cancer. *Scientific Reports*, **12**, Article No. 20949. https://doi.org/10.1038/s41598-022-25609-0

[5] Wang, M., Liu, Y., Shao, B., Liu, X., Hu, Z., Wang, C., *et al.* (2022) HER2 Status of CTCs by Peptide-Functionalized Nanoparticles as the Diagnostic Biomarker of Breast Cancer and Predicting the Efficacy of Anti-HER2 Treatment. *Frontiers in Bioengineering and Biotechnology*, **10**, Article ID: 1015295. https://doi.org/10.3389/fbioe.2022.1015295

[6] Godoy-Ortiz, A., Alba-Bernal, A., Pascual, J., Comino-Méndez, I. and Alba, E. (2022) Unveiling the Potential of Liquid Biopsy in HER2-Positive Breast Cancer Management. *Cancers*, **14**, Article 587. https://doi.org/10.3390/cancers14030587

[7] Radovich, M., Jiang, G., Hancock, B.A., Chitambar, C., Nanda, R., Falkson, C., *et al.* (2020) Association of Circulating Tumor DNA and Circulating Tumor Cells after Neoadjuvant Chemotherapy with Disease Recurrence in Patients with Triple-Negative Breast Cancer. *JAMA Oncology*, **6**, 1410-1415. https://doi.org/10.1001/jamaoncol.2020.2295

[8] Keup, C., Suryaprakash, V., Hauch, S., Storbeck, M., Hahn, P., Sprenger-Haussels, M., *et al.* (2021) Integrative Statistical Analyses of Multiple Liquid Biopsy Analytes in Metastatic Breast Cancer. *Genome Medicine*, **13**, Article No. 85. https://doi.org/10.1186/s13073-021-00902-1

[9] Brechbuhl, H.M., Vinod-Paul, K., Gillen, A.E., Kopin, E.G., Gibney, K., Elias, A.D., *et al.* (2020) Analysis of Circulating Breast Cancer Cell Heterogeneity and Interactions with Peripheral Blood Mononuclear Cells. *Molecular Carcinogenesis*, **59**, 1129-1139. https://doi.org/10.1002/mc.23242

[10] Welter, L., Zheng, S., Setayesh, S.M., Morikado, M., Agrawal, A., Nevarez, R., *et al.* (2023) Cell State and Cell Type: Deconvoluting Circulating Tumor Cell Populations in Liquid Biopsies by Multi-Omics. *Cancers*, **15**, Article 3949. https://doi.org/10.3390/cancers15153949

[11] Ma, G., Wang, J., Fu, J., Chen, R., Liang, M., Li, M., *et al.* (2023) Heterogeneous Circulating Tumor Cells Correlate with Responses to Neoadjuvant Chemotherapy and Prognosis in Patients with Locally Advanced Breast Cancer. *Breast Cancer Research and Treatment*, **201**, 27-41. https://doi.org/10.1007/s10549-023-06942-y

[12] Ningsi, R., Elazezy, M., Stegat, L., Laakmann, E., Peine, S., Riethdorf, S., *et al.* (2022) Detection and Characterization of Estrogen Receptor α Expression of Circulating Tumor Cells as a Prognostic Marker. *Cancers*, **14**, Article 2621. https://doi.org/10.3390/cancers14112621

[13] Grigoryeva, E.S., Tashireva, L.A., Alifanov, V.V., Savelieva, O.E., Vtorushin, S.V., Zavyalova, M.V., *et al.* (2022) The Novel Association of Early Apoptotic Circulating Tumor Cells with Treatment Outcomes in Breast Cancer Patients. *International Journal of Molecular Sciences*, **23**, Article 9475. https://doi.org/10.3390/ijms23169475

[14] McCracken, A., Grafinger, O.R., Leong, H.S., Lohmann, A.E., Jerzak, K.J. and Das, S. (2026) Neoadjuvant Therapy and Translational Biomarkers for Metastatic Risk in Early Breast Cancer. *Cancer Treatment Reviews*, **144**, Article 103111. https://doi.org/10.1016/j.ctrv.2026.103111

[15] Janni, W., Friedl, T.W.P., Yab, T.C., *et al.* (2025) Clinical Validity of Repeated Circulating Tumor Cell Enumeration as an Early Treatment Monitoring Tool for Metastatic Breast Cancer in the PREDICT Global Pooled Analysis. *Clinical Cancer Research*, **31**, 2196-2209. https://pubmed.ncbi.nlm.nih.gov/40100138/

[16] Fang, S., Xia, W., Zhang, H., Ni, C., Wu, J., Mo, Q., *et al.* (2024) A Real-World Clinicopathological Model for Predicting Pathological Complete Response to Neoadjuvant Chemotherapy in Breast Cancer. *Frontiers in Oncology*, **14**, Article ID: 1323226. https://doi.org/10.3389/fonc.2024.1323226

[17] Zhou, J., Wu, J., Hao, X., Li, P., Zhang, H., Wu, X., *et al.* (2022) An Exploratory Study on the Checkout Rate of Circulating Tumor Cells and the Prediction of Efficacy of Neoadjuvant Therapy and Prognosis in Patients with Her-2-Positive Early Breast Cancer. *Frontiers in Oncology*, **12**, Article ID: 966624. https://doi.org/10.3389/fonc.2022.966624

[18] Munoz-Arcos, L.S., Nicolò, E., Serafini, M.S., Gerratana, L., Reduzzi, C. and Cristofanilli, M. (2023) Latest Advances in Clinical Studies of Circulating Tumor Cells in Early and Metastatic Breast Cancer. *International Review of Cell and Molecular Biology*, **381**, 1-21. https://pubmed.ncbi.nlm.nih.gov/37739480/

[19] Woo, H.J., Rademacher, P.N., Shin, H.Y., Lee, J., Intisar, A., Warkiani, M.E., *et al.* (2025) Robust Automated Separation of Circulating Tumor Cells and Cancer-Associated Fibroblasts for Enhanced Liquid Biopsy in Breast Cancer. *Analytical Chemistry*, **97**, 17452-17461. https://doi.org/10.1021/acs.analchem.5c02154

[20] Lozar, T., Jesenko, T., Kloboves Prevodnik, V., Cemazar, M., Hosta, V., Jericevic, A., *et al.* (2020) Preclinical and Clinical Evaluation of Magnetic-Activated Cell Separation Technology for CTC Isolation in Breast Cancer. *Frontiers in Oncology*, **10**, Article ID: 554554. https://doi.org/10.3389/fonc.2020.554554

[21] Chou, H., Che, T., Lee, K., Chen, S., Chen, J., Huang, Y., *et al.* (2026) Application of CTC-Derived Spheroid for Drug Screening toward Personalized Treatment in Patients with Breast Cancer. *Translational Oncology*, **63**, Article 102573. https://doi.org/10.1016/j.tranon.2025.102573

[22] Nel, I., Herzog, H. and Aktas, B. (2022) Combined Analysis of Disseminated Tumor Cells (DTCs) and Circulating Tumor DNA (ctDNA) in a Patient Suffering from Triple Negative Breast Cancer Revealed Elevated Risk. *Frontiers in Bioscience-Landmark*, **27**, Article No. 208. https://doi.org/10.31083/j.fbl2707208

[23] Dhandapani, R., Sethuraman, S., Krishnan, U.M. and Subramanian, A. (2023) Self-Assembled Multifunctional Nanotheranostics against Circulating Tumor Clusters in Metastatic Breast Cancer. *Acta Pharmaceutica Sinica B*, **13**, 1711-1725. https://doi.org/10.1016/j.apsb.2022.12.003