Detection, significance and potential utility of circulating tumor cells in clinical practice in breast cancer (Review)

DOMINIKA ŠMIČKOVÁ RUSNÁKOVÁ[1], RAMADAN AZIRI[1], PETER DUBOVAN[1], MIROSLAV JURÍK[1], MICHAL MEGO[2] and DANIEL PINĎÁK[1]

[1]Department of Surgical Oncology, Faculty of Medicine, Slovak Medical University and National Cancer Institute, 833 10 Bratislava, Slovak Republic; [2]Second Department of Oncology, Faculty of Medicine, Comenius University and National Cancer Institute, 833 10 Bratislava, Slovak Republic

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Abstract. Although advances in diagnostic techniques, new therapeutic strategies and personalization of breast cancer (BC) care have improved the survival for a number of patients, BC remains a major cause of morbidity and mortality for women. The study of circulating tumor cells (CTCs) has significant potential in translational oncology since these cells represent promising biomarkers throughout the entire course of BC in patients. CTCs also have notable prognostic value in early BC as well as metastatic BC. Based on current knowledge, it seems that the dynamics of CTCs that change during therapy reflect therapy response, and CTCs could serve as a tool for risk stratification and real-time monitoring of treatment in patients with BC. The question of how to use this information in everyday clinical practice and how this information can guide or change therapy to affect the clinical outcome of patients with BC remains unanswered. The present review aims to discuss current completed and ongoing trials that have been designed to demonstrate the clinical significance of CTCs, offer insights into treatment efficacy and assess CTC utility, facilitating their implementation in the routine management of patients with BC.

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Correspondence to: Dr Dominika Šmičková Rusnáková, Department of Surgical Oncology, Faculty of Medicine, Slovak Medical University and National Cancer Institute, Klenová 1, 833 10 Bratislava, Slovak Republic
E-mail: dr.dominika.rusnakova@gmail.com

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1. Introduction

In 2020, breast cancer (BC) became the most prevalent cancer, ahead of lung cancer, for the first time in history. BC accounts for 15.5% of all cancer-related deaths in women worldwide and therefore is a leading cause of cancer mortality within the female population globally (1,2). The early detection of BC with advancements in therapy effectiveness has led to a decrease in mortality over the past two decades (3). The 5-year relative survival rate for early-stage BC is generally high (80-92%), but it significantly declines to <25% for advanced BC (4).

It is necessary to understand the mechanisms underlying the metastatic process and the complex tumor-host interactions causing the progression of the disease, as 25% of patients with non-metastatic BC will eventually develop distant metastases, although initial treatment was successful (5). It was discovered that 10-50% of patients without nodal involvement at the time of curative surgery subsequently developed distant metastatic lesions (6-8). Metastatic BC (MBC) is widely considered an incurable medical condition, although the application of systemic therapies has improved its prognosis. The median overall survival (OS) time of MBC is ~2 years, while survival varies from a couple of months to a few years, depending on the type of treatment used and the molecular and patient characteristics (9). For patients with $HER2^+$ MBC, an OS of >5 years is now common in developed countries, whereas individuals with triple negative BC (TNBC) have the shortest median OS time of ~10.2 months (10).

Available data indicate that distant metastases belong to the most significant cause of cancer mortality in clinical practice (11,12) and are related to the aggressive phenotype of small heterogeneous tumor cells, termed circulating tumor cells (CTCs), which spread from the primary tumor and circulate in the bloodstream. CTCs have a crucial role in tumor dissemination and progression, making them a key component of the metastatic cascade. Numerous trials have consistently demonstrated the prognostic value of CTCs in

both metastatic and primary BC (PBC) (13-17). The role of CTCs in treatment failure and disease progression is linked to biological processes such as the epithelial-to-mesenchymal transition (EMT) and 'self-seeding', which refers to the re-infiltration of the primary tumor or established metastases by more aggressive CTCs (18,19). Although CTCs originate from the primary tumor, with EMT properties, dissemination in clusters and/or exhibition of stemness features, they differ from primary tumor cells (20). Tumor evolution represents an important dynamic situation, illustrating modifications in tumor heterogeneity along the temporal axis. This process can be affected by applied therapeutic approaches (21). However, a single tumor biopsy provides information about the specific area sampled but may not capture the entire complexity and heterogeneity of the tumor. Taking into consideration the ease of blood sampling, a 'liquid biopsy' repeatedly during disease progression to assess the pool of CTCs may provide evaluation of the tumor heterogeneity, prediction of treatment response or resistance and thus provide valuable information for novel anticancer strategy.

It took over a century to develop the appropriate methods for CTC isolation and detection necessary for subsequent in-depth analysis. Over the past two decades, a number of methods for capturing CTCs have been proposed. However, the CellSearch system is the only platform approved by the U.S. Food and Drug Administration (FDA) and therefore it has been the most utilized (9). The increasing number of advanced and sensitive technologies for CTC detection has enabled detailed investigation into the characteristics, behavior and molecular profile of CTCs and support their clinical implementation.

Numerous investigations in the CTC field have been performed, mostly in BC, due to their potential role in cancer diagnosis, prognosis and treatment monitoring, and although CTCs have not been encompassed in clinical guidelines, their results have projected the potential of CTCs in clinical practice (13-17,22,23).

There are numerous problems and unanswered questions concerning the application of CTCs in clinical practice. These issues are related to the technologies used for CTC detection, which still primarily rely on analyzing cell count and molecular phenotype. Additionally, there is no standardized, evidence-based guideline for subsequent intensive or prolonged treatment based on CTC (23). The sensitivity and specificity of detection methods present another challenge. A number of clinical studies lack external validation and are based on small, single-center, case-control studies with diverse patient characteristics (24). The instability and uncertainty of CTC test results can influence diagnostic and treatment decisions. The primary focus of clinical trials has been on assessing CTC count rather than their biology (23,25). Most notably, data on the clinical utility of CTCs remains limited. In the present review, the introduction of numerous CTC detection techniques is summarized to provide a suitable framework for CTC-related technologies. Herein, the published investigations on the role of CTCs in predicting prognosis, their clinical importance and utility in early-stage as well as advanced-stage BC are also reviewed. Since the CellSearch system is one of the most widely used methods for detecting CTCs, most of the publications included in the present review focused almost exclusively on the CTC status assessed by this system.

2. Detection of CTCs

CTC detection, as a non-invasive method, has the potential to be used for prognosis and treatment monitoring; however, the identification and isolation of CTCs among blood cells is challenging, since they are very rare, with 1 CTC in 10^9 nucleated blood cells (26). CTCs also present a heterogeneous cell population, including CTCs with partial as well as complete EMT phenotypes with different clinical and biological properties (27). Nonetheless, technological development has facilitated the detection and characterization of these cells (26).

Immune isolation. The most accepted approach used for CTC isolation is immune isolation. Based on the principle of immunoaffinity, CTCs can be identified by two different methods: positive and/or negative selection (28,29).

Positive selection is based on immunorecognition of cancerous-related markers that are not expressed by leukocytes. CellSearch from Veridex (USA), the only U.S. FDA-approved technique for CTC detection, is a semi-automated immunological method based on immunomagnetic isolation using antibodies specific for epithelial adhesion molecule (EpCAM) and cytokeratin markers (CK8, CK18 and CK19), with anti-CD45 antibody (anti-leukocyte) for negative selection (30,31). Although this method is efficient and highly reproducible, it is limited to recognizing a subset of CTCs and may miss those with downregulated or lost markers due to EMT (29,32-35). Another example of a positive enrichment system is the reverse transcription-PCR-based AdnaTest™. This technology can identify CTCs expressing EMT-associated genes, but the kit still includes a step to enrich EpCAM-expressing cells (36-38). In addition, it is worth mentioning several improved alternative immunobead technologies providing higher purity and fidelity such as MagSweeper (39), IsoFlux™ (40) and CTC-μChip (41).

Negative selection uses white blood cell markers to deplete the leukocytes from the blood sample (42). CD45 is the most prevalent antigen used in negative selection, which is often supplemented by a combination of additional techniques, including density gradient centrifugation (42). A classic example of this method is RosetteSep™, which has a higher recovery rate than the density gradient approach (43,44). Another bimodal selection technique is Cyttel, which has a high detection rate (45). Immunomagnetic methods, such as DynaBeads® and EasySep, use magnetic beads with attached antibodies recognizing cell surface antigens to remove unwanted cells (46).

EMT-inducing transcription factors (TFs)-based technique. CTCs that undergo EMT lose epithelial markers and conventional methods may not identify CTCs with EMT characteristics. Therefore, Mego *et al* (47) performed a translational study to test an innovative approach for CTC detection, focusing on the mRNA expression of EMT-inducing TFs in the blood of study participants with PBC. Despite certain limitations such as a small sample size (n=52), this was the first study to investigate a novel CTC detection technique based on the detection of EMT-TFs, demonstrating that EMT-CTCs may occur in the blood of patients with PBC who have undergone

neoadjuvant chemotherapy (NAC) without any correlation between the expression levels of EMT-TFs gene and tumor size, grade or type (47).

Physical features-based isolation. The alternative methodologies for recognizing CTCs independently of surface markers are isolation platforms based on physical features such as size, deformability, density and electrical properties (28).

Size-based selection systems such as the Metacell filtration instrument, ISET®, ScreenCellCyto and Parsotrix™, utilize filtration to isolate individual tumor cells from other smaller peripheral blood cells (48-51). For example, Parsotrix™ has a trapezoid shape that gradually narrows to trap the target cells, and its separation channel is lengthened thus allowing the counterflow (52,53). Although size-based CTC isolation devices utilize quite easy high-throughput methods, they have certain limitations, such as low recovery efficiency after deformation caused by the filtration resistance, lack of specificity and poor purity due to the heterogeneity of CTCs in terms of size (28-29).

Another technique for CTC separation is density gradient centrifugation based on the specific density of red blood cells, leukocytes and malignant cells (54). The OncoQuick® system is an example of a density-dependent technique, which also uses a porous membrane to improve tumor cell enrichment (55,56).

CTCs can also be sorted using electric charge-based technology. The ApoStream® device uses di-electrophoresis, an electrokinetic method, to exploit the electrical characteristics of suspended cells for discrimination and separation with a unique level of precision (57-59). Changes in surface charges are also applied for precise CTC isolation. Studies have shown that malignant cells display the so-called 'Warburg Effect', which is linked to the negative charge of cancer cells (60-62). A high glycolysis rate and robust lactate acid production belong to the most distinguishable metabolic characteristics of all cancer cells. The malignant cell surface negative charge is regulated by glycolysis, which is facilitated by sufficient glucose concentration (63). Based on these exclusive metabolic characteristics, positively charged magnetic nanoparticles have been designed for the detection and acquirement of CTCs from the blood samples of patients. A previous study has shown that charged nanoparticles provide exclusive CTC capturing with high sensitivity and without any protein biomarkers (60).

Microfluidic techniques. Recent advancements in the field of CTC detection have seen the establishment of new techniques, including those based on microfluidics and nanotechnology elements. Microfluidic platforms use 'intrinsic' vs. 'extrinsic' forces to separate cells and capture target cells through different methods, such as utilizing epithelial cell markers as antigens, the physical and biological characteristics of malignant cells and other methods (64,65). A powerful microfluidic platform termed 'CTC-chip' captures CTCs using molecular marker-coated micro-posts (66). Additionally, a modified chip-based platform utilizes a chemical ligand-exchange reaction that involves gold nanoparticles on a herringbone chip (67). Zhang *et al* (68) designed an automated microfluidic device for size-based cell isolation with high-throughput and efficient recovery. They successfully utilized the device to sort human BC cell lines from blood samples suggesting potential applications in the isolation of CTCs. Wang *et al* (69) proposed another integrated microfluidic platform equipped with automation capabilities for effective CTC capture and identification within 90 min. Furthermore, Lee and Kwak (70) showcased a microfluidic instrument that employed differences in magnetic field gradient and immunofluorescence to achieve on-chip separation and simultaneous CTC characterization. This novel microfluidic DEVICE can isolate CTCs with >99% efficiency and can differentiate eight different subtypes of heterogenic CTCs based on the statuses of the HER2, estrogen receptor (ER) and progesterone receptor (PR) biomarkers guiding BC diagnosis and prognosis. Additionally, a previous study presented the OPENchip platform for a single CTC examination. Using this platform, Lee *et al* (71) were able to concurrently analyze both the gene activity and genetic mutations in CTCs circulating in the blood. This was achieved using a chip-based technology (microfluidics) combined with techniques that analyze molecules directly in their original location (*in situ* molecular profiling). Molecular analyses of single CTCs from patients with MBC (expression of HER2 and PIK3CA mutations) or metastatic pancreatic cancer (KRAS mutations) were demonstrated without any off-chip procedures, proposing its possible application of early molecular uncovering of cancer metastasis (71).

Nanotechnology-based techniques. With progress in the development of nanoscale materials and structures, nanotechnology-based techniques offer unique advantages for real-time cancer diagnosis and detection in terms of cost and simplicity (72). The success of nanoparticles lies in their large surface-to-volume ratio, which allows the adsorption of numerous targeting ligands with the capability to bind and identify explicit cancer molecules. Due to this property, nanomaterials offer specific benefits such as precision of CTC-separation and highly sensitive CTC-recognition (72). Recently, a variety of nanomaterials, including gold nanoparticles, magnetic nanoparticles, polymer dots, nano-fibers, nanorod arrays and nanoparticle-coated silicon beads have been reported for CTC detection (73). Although there have been great expectations from the progress in the field of nano-biotechnology, only certain nanotechnology-based techniques have progressed to clinical trials (74). There are still a number of challenges and limitations to be solved before their translation into clinical applications. Namely, their reliability and reproducibility, which can be affected by several factors such as the interaction of nanoparticles with unintended targets, their aggregation, unfit detection settings, possible toxicity of the nanoparticles and the fact that most were prepared in academic laboratories (75).

Current challenges in CTC analysis. To sum up, CTC analysis has great clinical value and the underlying basis for its subsequent application in clinical practice is the development of reliable and reproducible technologies for CTC detection. As aforementioned, various CTC-related techniques have been showcased; however, further optimization is necessary. The main goal of ongoing research is to enhance the capabilities of these methods such as their specificity, sensitivity and overall performance. Despite great effort, an ideal device that can isolate a pure and viable population of CTCs is still missing.

Enhanced detection efficiency and contaminant removal are indeed crucial for the success of CTC detection. Several studies have highlighted this necessity (23,25,29,34,44). For instance, due to the low abundance of CTCs in whole peripheral blood, the ability to distinguish CTCs from the vast majority of non-tumor blood cells is essential for the effectiveness of CTC capture technologies. Most of the existing methods for CTC detection involve a two-step process of cell enrichment followed by subsequent detection. However, the low concentration of CTCs in the bloodstream, coupled with the heterogeneity observed among them, renders the high-precision detection process demanding and time-consuming. Achieving high specificity and efficiency in these processes remains a significant challenge to ensure that CTCs can be accurately detected and analyzed without significant contamination. These issues necessitate the development of advanced techniques and meticulous procedures to improve the reliability of CTC detection (29). The current CTC detection technologies are summarized in Table I.

3. Significance of CTCs

Constant advancement of the methods enabling CTC detection has expanded the application of CTCs in predicting prognosis and outcomes in BC. In the next sections, the latest research and the ongoing efforts to include CTC analysis as part of regular medical care will be explored. As aforementioned, throughout the review particular attention will be paid to studies that almost exclusively examine CTCs detected by the CellSearch system.

Clinical value of CTCs in early BC (EBC). A tumor is typically comprised of $>10^9$ cancer cells before it can be visualized by conventional imaging modalities (23). Findings from mouse model studies suggest that early dissemination of tumor cells can occur in cancer, such as in breast (76,77) and pancreatic carcinogenesis (78,79). This implies that cancer cells may be present in the circulatory system early in BC progression even before the primary tumor reaches an invasive stage (80). A study comparing the quantitative assessment of CTCs with breast imaging modalities, such as ultrasonography, mammography and contrast-enhanced magnetic resonance imaging, in screening for BC demonstrated that CTC tests perform comparatively with widely used imaging modalities for early and mid-stage BC (81). Additionally, the study also revealed that single CTCs were detected in 19% (n=12) of participants with negative breast disease and 43% (n=3) of participants with benign breast disease. CTCs may appear during the premalignant phase or very early stages of cancer development; however, long-term follow-up studies are needed to determine the clinical significance of detecting CTCs in individuals with negative or benign imaging results. These findings highlight the diagnostic potency of CTC detection rate/counts and suggest that CTC detection could serve as a valuable complementary screening tool for BC, potentially increasing the overall detection rate and identifying BC at an earlier stage (22).

Despite these findings, the clinical utility of CTCs in the EBC setting is constrained by their scarcity. A study that enrolled >70 participants with carcinoma *in situ* showed that 1 CTC per 22.5 ml was detected in 4.1% of cases using the CellSearch system (82). Non-metastatic BC typically shows <1 CTC per 10 ml of blood (83), with rare instances of detecting ≥5 CTCs in this volume (1-5.9%) (84). CTCs are identified in 20-25% of individuals with non-metastatic BC at the point of initial diagnosis using a lower threshold (≥1 CTC per 7.5 ml blood) compared with MBC (≥5 CTCs per 7.5 ml blood) (16,84-86). Nevertheless, extensive research on the prognostic value of CTCs indicates that their detection in the initial diagnosis of BC is an independent prognostic factor (84,87-97).

In 2008, during the annual American Society of Clinical Oncology (ASCO) meeting, Rack *et al* (85) showcased the results of the randomized trial, SUCCESS-A. The study, involving >2,000 patients with EBC, highlighted the significance of CTCs as an independent prognostic marker in both pre- and post-adjuvant chemotherapy settings. CTC positivity was associated with poorer outcomes, disease-free survival [DFS; hazard ratio (HR)=2.28] and OS (HR=3.95). Participants exceeding a threshold of 5 CTCs per 30 ml of blood exhibited the most unfavorable disease course and outcome (85). Furthermore, the ECOG-ACRIN study (E5103) along with SUCCESS-A revealed the adverse prognostic implications of CTC persistence post neoadjuvant or adjuvant chemotherapy (98,99). The analysis of 1,087 patients enrolled in the SUCCESS-A trial showed that continued CTC presence after 2 years was linked to a 2.3-fold higher recurrence risk and a 3.9-fold higher mortality risk (100). A 6-fold elevated likelihood of recurrence was noted in the analysis of 206 patients, with available CTC status after 5 years (99). Additionally, Sparano *et al* (98) associated a ~13-fold elevated risk of late recurrence (>5 years from the initial diagnosis) in participants with hormone receptor$^+$ BC in which CTCs were detected compared with participants without CTCs, suggesting the potential utility of CTC-positivity in the context of long-term prognosis and risk assessment in these individuals.

In a pooled analysis of >3,000 patients with stage I-III BC, Janni *et al* (86) demonstrated that the presence of CTCs (specifically ≥1 CTC per 7.5 ml) before treatment was a marker predicting a significantly poorer outcome (the incidence of mortality doubled). This large-scale analysis demonstrated the significance of CTC presence in predicting various clinical outcomes, including DFS [HR, 1.82; 95% confidence interval (CI), 1.47-2.26], distant DFS (DDFS; HR, 1.89; 95% CI, 1.49-2.40), breast cancer-specific survival (BCSS; HR, 2.04; 95% CI, 1.52-2.75), and overall survival (OS; HR, 1.97; 95% CI, 1.51-2.59) (86). This supported the results of other studies, emphasizing that CTC detection holds independent prognostic value and provides valuable insights into both DFS and OS outcomes, notwithstanding traditional factors (87,88,101,102). Studies evaluating the prognostic impact of CTCs in the NAC or adjuvant chemotherapy setting are summarized in Table II.

Clinical value of CTCs in the neoadjuvant setting. The successful attainment of a complete pathologic response (pCR) after NAC is linked to a more favorable prognosis (103). Nevertheless, to the best of our knowledge, no association between the attainment of a pCR and the presence of CTCs has been revealed so far (104).

Table I. CTC detection technologies.

A, Based on cell surface markers (label-dependent technologies)-Immunomagnetic assays

Device	Technology	Limitations	Merits	Cancer type	(Refs.)
CellSearch® system	Uses ferrofluids coated with. EpCAM for positive selection of CTCs. Confirmation by IF staining for CK8, 18, and 19 but an absence of CD45	EpCAM dependence, large blood volumes required and low purity of captured CTCs.	FDA approval and the most clinically validated technique.	Breast, colorectal and prostate	(28,29,34,35)
AdnaTest®	Utilizes magnetic beads coated with an antibody mixture for positive selection and detects CTCs via RT-PCR assay for various gene panels.	High contamination with WBCs.	Evaluates blood and bone marrow samples with high sensitivity.	Breast, prostate, ovarian and colon	(28,29,35, 33-38)
MagSweeper	Uses immunomagnetic beads with antibodies against EpCAM and cell surface markers. Whole transcriptome analysis with RNA-Seq.	Expensive and uses fixed or lysed cells.	Almost 100% purity of captured CTCs and high-throughput processing (9 ml/h)	Breast, prostate and colorectal	(28,29,39)
IsoFlux™ Rare Cell Access System	A microfluidic dual platform-flow control and immunomagnetic trapping.	Analysis of 12 samples per day and biomarker heterogeneity of CTCs.	Capacity to detect genetic alterations	Breast, prostate and colorectal	(29,40)
RosetteSep™	Utilizes tetrameric antibody complexes for recognition of WBCs and RBCs for negative selection followed by flow cytometry.	Low recovery rate.	Sensitive	Pancreatic, breast and colorectal	(28,29,44,46)

B, Based on biophysical properties (label-independent technologies)-size-based

Device	Technology	Limitations	Merits	Cancer type	(Refs.)
ISET®	Uses a filter-based isolation and enrichment.	Misses CTCs due their morphological and size heterogeneity; damage due to multi-step cell processes.	High efficiency	Breast, hepatocellular and lung	(28,29,51)
Parsotrix™	A semi-automated commercial microfluidics system with programmable fluidics and pneumatics designed for special Parsortix cassettes.	CTC loss caused by different size ranges.	Allows reverse flow	Ovarian and prostate	(28,29,44, 52,53)

Table I. Continued.

C, Based on biophysical properties (label-independent technologies)-density-based

Device	Technology	Limitations	Merits	Cancer type	(Refs.)
OncoQuick®	Uses porous membrane filtration for separation of blood cells, then density-grade centrifugation for CTC collection.	Low purity, loss of sample and additional techniques are needed.	Reliable, high- throughput and inexpensive.	Colorectal and MBC	(28,29,56)

D, Based on biophysical properties (label-independent technologies)-electric charge-based

Device	Technology	Limitations	Merits	Cancer type	(Refs.)
ApoStream®	Utilizes dielectrophoretic technology in a microfluidic flow chamber.	Cellular damage	High precision, linearity of recovery, independent of EpCAM expression level	Lung, breast and ovarian	(29,58,59)
SPPCN	Uses serum protein-coated electrically charged nanoparticles.	Loss of cells	Can detect diverse CTC subpopulations	Colorectal	(29,60)

CK, cytokeratin; CTCs, circulating tumor cells; EpCAM, epithelial adhesion molecule; FDA, Food and Drug Administration; IF, immunofluorescence; MBC, metastatic breast cancer; RBCs, red blood cells; RT-PCR, reverse transcription polymerase chain reaction; SPPCN, serum protein-coated electrically charged nanoparticles; WBCs, white blood cells.

Table II. Selected studies/trials/analyses evaluating the prognostic impact of CTCs in adjuvant/neoadjuvant setting.

First author/s, year	No. of patients	Independent prognostic relevance of CTCs	Setting	(Refs.)
Pierga *et al*, 2008	118	DMFS	NAC	(87)
Bidard *et al*, 2009	115	DMFS and OS	Only before NAC	(95)
Bidard *et al*, 2013	115	DMFS, OS	Only before NAC	(96)
Rack *et al*, 2014	2,026	DFS, OS	Adjuvant	(85)
Hall *et al*, 2015	57	RFS, OS	NAC	(88)
Janni *et al*, 2016	3,173	DFS, DDFS, BCSS and OS	Adjuvant	(86)
Hall *et al*, 2016	509	RFS and OS	NAC	(102)
Pierga *et al*, 2017	137	DFS and OS	Only before NAC	(93)
Riethdorf *et al*, 2017	213	DFS and OS	Only before NAC	(95)
Janni *et al*, 2018	206	RFS	Adjuvant	(99)
Bidard *et al*, 2018	2,156	DDFS, OS and LRRFS	Only before NAC	(84)
Sparano *et al*, 2018	547	Recurrence rate	Adjuvant	(98)
Trapp *et al*, 2019	1,087	DFS and OS	Adjuvant	(100)

BCSS, breast cancer-specific survival; DDFS, distant DFS; DFS, disease-free survival; DMSF, distant metastasis-free survival; LRRFS, locoregional recurrence-free survival; OS, overall survival; RFS, recurrence free survival; NAC, neoadjuvant chemotherapy; CTC, circulating tumor cell.

The results of the REMAGUS02 neoadjuvant treatment study highlight that the prechemotherapy CTC count has a significant effect on survival outcome, whereas no significant impact of post-chemotherapy CTC detection was observed (96). As with the phase II randomized REMAGUS02 trial, Riethdorf *et al* (97) examined samples from participants with BC treated in the GeparQuattro trial and revealed that the incidence of CTC was 21.6% before treatment, which decreased to 10.6% after NAC, but no association between the detection of CTCs and pCR was revealed. In addition, the presence of ≥1 CTC per 7.5 ml and ≥2 CTCs per 7.5 ml in the pre-NAC setting was significantly related to reduced survival outcomes, specifically DFS (P=0.031) and OS (P=0.0057), with a dose-dependent effect (≥2 CTCs per 7.5 ml had a more notable impact). However, no such correlation was observed in the post-NAC setting (94). The lack of correlation may be caused by CTC viability after completing adjuvant therapy (77).

A large meta-analysis, the IMENEO study, using individual patient data from 16 centers and 21 studies showed that CTC detection in the pre-NAC setting had an unfavorable impact on OS, DDFS and the locoregional relapse-free interval (LRRFI) (all P<0.001), but was not correlated with the pCR. Participants with varying CTC numbers (1 to ≤5 CTCs) detected in the pre-NAC setting showed a different HR of mortality [specifically, 1.09 (95% CI, 0.65-1.69) to 6.25 (95% CI, 4.34-9.09)], illustrating a dose-dependent impact. This finding emphasizes the quantitative nature of CTC counts as a marker. Patients achieving pCR had improved outcomes in terms of OS, DMFS and LRRFI. However, the attainment of pCR did not significantly correlate with the detection of CTCs (84).

Clinical value of CTCs in advanced BC. A number of investigations have demonstrated the prognostic value of CTCs in MBC (17,105,106). In 2005, Cristofanilli *et al* (31) demonstrated for the first time that the detection of ≥5 CTCs per 7.5 ml blood (by CellSearch) in previously untreated participants with MBC was linked to a significantly poorer outcome in terms of progression-free survival (PFS) and OS. These convincing data played a key role in obtaining FDA approval (30,31). Subsequent validation studies (107-109), including the study by Bidard *et al* (110), further reinforced the independent prognostic effect of CTCs in MBC on the PFS (HR, 1.92; P<0.0001) and OS (HR, 2.78; P<0.0001).

Lv *et al* (111) presented a meta-analysis based on 24 clinical studies (35,107-109,112-131) with 3,701 patients with MBC, which assessed the prognostic value and clinical relevance of CTCs. All analyzed studies used the CellSearch system and CTCs were identified as CD45-cytokeratin$^+$ or CD45-EpCaM$^+$ cells. The cut-off value for positive CTC status was 5, excepting 1 study that considered 1 CTC as positive. The results of this meta-analysis showed that CTC detection was more frequent with HER2$^+$ primary tumors [pooled risk ratio (RR)=0.73; 95% CI, 0.63-0.84]. In addition, a higher CTC count indicated a worse treatment response (RR=0.56; 95% CI, 0.40-0.79) as well as a decreased PFS (RR=0.64; 95% CI, 0.56-0.73) and OS (RR=0.69; 95% CI, 0.64-0.75) in participants with MBC (116). CTCs studies included in this meta-analysis (35,107-109,112-131) are summarized in Table III.

A retrospective pooled analysis of >2,000 patients with MBC, categorizing participants into CTC-indolent (<5 CTCs per 7.5 ml) and CTC-aggressive (≥5 CTCs per 7.5 ml) groups, indicated a longer median OS time for all participants that were Stage IV indolent compared with those that were Stage IV aggressive (36.3 vs. 16.0 months), and likewise in the case of participants with *de novo* MBC. The observed differences were statistically significant with P<0.0001 and were consistent across all disease subtypes (hormone receptor$^+$, HER2$^+$ and TNBC). This study suggested that the CTC count should

Table III. CTC studies in metastatic breast cancer.

First author/s, year	Country	No. of patients	Study design	NOS	(Refs.)
Hayes *et al*, 2006	USA	177	Prospective, double-blind, multi-institutional clinical trial	8	(112)
Budd *et al*, 2006	USA	138	Retrospective	8	(107)
Cristofanilli *et al*, 2007	USA	151	Retrospective	7	(113)
Bidard *et al*, 2008	France	37	Prospective	7	(114)
Yagata *et al*, 2008	Japan	38	Prospective	6	(115)
De Giorgi *et al*, 2009	USA	115	Retrospective	8	(116)
Liu *et al*, 2009	USA	72	Prospective, longitudinal, clinical study	7	(117)
Mego *et al*, 2009	USA	149	Retrospective	6	(118)
De Giorgi *et al*, 2010	USA	195	Retrospective	7	(119)
Consoli *et al*, 2011	Italy	53	Multi-center prospective	5	(120)
Giuliano *et al*, 2011	USA	235	Retrospective	6	(108)
Hartkopf *et al*, 2011	Germany	58	Retrospective	5	(121)
Tokudome *et al*, 2011	Japan	28	Prospective	6	(122)
De Giorgi *et al*, 2012	USA	195	Retrospective	7	(123)
Giordano *et al*, 2012	USA	517	Retrospective	6	(109)
Hayashi *et al*, 2012	USA	52	Prospective	6	(124)
Müller *et al*, 2012	Germany	254	Prospective, open-label, non-randomized	7	(35)
Pierga *et al*, 2012	France	257	Prospective trial	7	(125)
Weissenstein *et al*, 2012	Switzerland	59	Retrospective	6	(126)
Jiang *et al*, 2013	China	294	Multicenter, double-blind, prospective	7	(127)
Liu *et al*, 2013	China	71	Retrospective	5	(128)
Tarhan *et al*, 2013	Turkey	30	Prospective	6	(129)
Turker *et al*, 2013	Turkey	22	Prospective trial	5	(130)
Wallwiener *et al*, 2013	Germany	486	Prospective multicenter	8	(131)

CTC, circulating tumor cell; NOS, Newcastle-Ottawa scale.

be used as a valuable tool for staging and stratification of MBC, emphasizing its role in prospective clinical trials (132).

This model of classification was further improved by Magbanua *et al* (133), who analyzed the CTC trajectory (tCTC) during first-line treatment. By assessing changes in CTC levels throughout the course different tCTC patterns were exposed: Undetectable CTCs (56.9%), low (23.7%), intermediate (tCTCmid; 14.5%) or high (tCTChi; 4.9%), which were highly prognostic for PFS and OS. Comparative analysis using Akaike Information Criterion manifested that the tCTC model outperformed other static models based on CTCs, specifically in the prediction of OS and PFS. Their report, including 2,202 samples from >700 participants, remains the most extensive single study on a serial analysis of CTCs to date. In this study it was also demonstrated that baseline CTC analysis is not only informative in the present but it is also able to provide insights into the likely course of CTC levels during treatment, highlighting its prognostic value. In addition, it was found that individuals belonging to the tCTCmid and tCTChi groups (19.4% of patients) exhibited poor responses to first-line chemotherapy. These individuals could benefit from a more targeted treatment, in which first-line chemotherapy could theoretically be omitted. To sum up, this new prognostic classification system may refine the precision of risk assessment, enhance treatment evaluation and contribute to therapeutic development in the context of MBC (133).

4. Clinical utility of CTCs

In clinical settings, CTCs are applied as surrogate biomarkers in various aggressive solid tumors such as breast, lung, prostate, liver, gastric and pancreatic cancer (134). A summary of selected trials that have evaluated the clinical utility of CTCs or trials that are still on-going are shown in Tables IV and V, respectively.

Monitoring treatment response. There has been significant investment in evaluating the effectiveness of evaluating CTCs in the monitoring and management of patients with MBC. The estimation of CTCs appears to offer some advantages over imaging methods in the follow-up of patients with MBC (107). A study by Hayes *et al* (112) demonstrated that elevated CTC levels, not only at the baseline but also at any stage of treatment, serve as valuable indicators of prognosis, specifically indicating rapid disease progression and increased mortality in the MBC context. Determining that CTC recognition has an unfavorable effect on clinical outcomes and that changes in CTC levels during therapy may reflect treatment response was

Table IV. Selected trials evaluating clinical utility of CTCs.

First author/s, year	Trial name	Trial number	Study design	Purpose	No. of patients	Major findings	(Refs.)
Bidard *et al*, 2013	TREAT-CTC	NCT01548677	Randomized phase II trial	To assess CTC detection as a liquid biopsy to test a novel BC treatment strategy-trastuzumab in HER2 non-amplified disease.	1,317	Trastuzumab did not reduce the detection rate of CTCs in $HER2^-$ BC leading to the recommendation to halt additional accrual by an Independent Data Monitoring Committee.	(101)
Smerage *et al*, 2014	SWOG S0500	NCT00382018	Randomized phase III trial	To study treatment decision making based on blood levels of cancer cells in MBC individuals receiving CT.	624	The prognostic significance of CTCs in participants with MBC receiving first-line CT was confirmed. Early switching to an alternative chemotherapeutic regimen was not effective in prolonging OS or PFS for participants with MBC and persistently increased CTCs.	(138)
Bidard *et al*, 2021	STIC-CTC	NCT01710605	Randomized, open-label, non-inferiority phase III trial	To compare the efficacy of a clinician-driven choice vs. a CTC-driven choice for first-line treatment. The main medical objective was to demonstrate the non-inferiority of the CTC-based strategy for the PFS.	800	Primary objective was reached-no overt survival difference between the two arms was reported. CTC count may be a reliable biomarker method for guiding the choice between single-agent ET and CT as first-line treatment of hormone $receptor^+$, HER^- MBC.	(139)
Jacot *et al*, 2019	CirCe T-DM1	NCT01349842	Prospective phase II trial	To assess the effectiveness of T-DM1 in $HER2^-$ MBC participants with $HER2^+$ CTC.	155	HER2-amplified CTC represented a fraction of the total CTC detected in participants with $HER2^-$ MBC with detectable CTC. Treatment with single-agent T-DM1 resulted in a partial response in only 1 patient.	(140)
Fehm *et al*, 2021	DETECT III	NCT01619111	Randomized phase III trial	To evaluate the efficacy of HER2-targeted therapy with the tyrosine kinase inhibitor, lapatinib, in individuals with MBC who initially had $HER2^-$ primary tumors but $HER2^+$ CTCs. To analyze the significance of CTC as an early predictive marker for treatment response.	105	CTC clearance rate at the time of the final visit did not differ significantly between the standard arm and lapatinib arm. PFS was numerically but not significantly better in the lapatinib arm. However, HER2-directed therapy with lapatinib showed significantly improves OS in those patients. $HER2^+$ CTCs might serve as a suitable biomarker to predict clinical benefit in this cohort.	(141)

Table IV. Continued.

First author/s, year	Trial name	Trial number	Study design	Purpose	No. of patients	Major findings	(Refs.)
Paoletti *et al*, 2017	COMETI phase 2	NCT01701050	Prospective, multi-institutional, and multi-national phase II trial.	To determine a CTC-ETI in individuals with ER^+, $HER2^-$ MBC before the initiation of a new ET for the identification of individuals who will progress rapidly.	121	CTC record at initiation of ET as well as at other subsequent time points was prognostic in patients with ER^+ MBC who started second-line or later ET. The outcomes of the trial failed to reveal that the phenotypic-based CTC-ETI algorithm adds to enumeration alone, at baseline or during serial follow-up time points.	(155)

BC, breast cancer; CTC-ETI, CTC-endocrine therapy index; CTCs, circulating tumor cells; ET, endocrine therapy; MBC, metastatic BC; OS, overall survival; PFS, progression-free survival; tCTC, CTC trajectory; T-DM1, trastuzumab-emtansine.

an incentive to perform certain translational research projects. The main goal of these projects was to examine the potential utilization of monitoring and treatment decisions guided by CTC in the context of MBC. Despite promising data, only a limited number of studies have consistently used CTC detection for real-time monitoring during therapy (110,112,135,136) and some of them failed to demonstrate the clinical utility of CTC monitoring (137). The current evidence is mixed, and its clinical utility remains to be fully established. This gap highlights the need for more comprehensive research to validate and standardize CTC-based monitoring protocols.

Change in therapy according to CTC kinetics. A prospectively randomized study by Smerage *et al* (138) (SWOG S0500), addressed whether CTC monitoring could offer early insights into treatment response and thus influence treatment decisions. The study specifically assessed the utility of CTC levels as a biomarker for evaluating the effectiveness of chemotherapy in real-time. This clinical trial enrolled 595 patients from which a total of 123 patients (43% of patients with CTC evaluation completed) exhibited persistently >5 CTCs per 7.5 ml at the baseline and first follow-up after one cycle of chemotherapy. Those patients were randomly assigned to continue initial treatment (n=64) until disease progression or to switch to alternative therapy (n=59). The findings of the study reinforced the impact of CTCs in the prognosis of patients with MBC, demonstrating the poor prognosis (OS, 13 months; P<0.001) of patients with persistently elevated CTC levels. Unfortunately, the approach of early change in therapy for patients with persistently high CTCs did not disclose an improvement of either OS or PFS for such patients. These observations suggest that this persistence might indicate a population of cancer with relative, if not absolute resistance, to several commonly used chemotherapeutic agents. Thus, alternative therapeutic approaches are required (138).

Treatment decision guided by CTCs

Quantitative assessment of CTCs as a tool for guiding treatment decisions. Similarly, the STIC CTC III phase study (NCT01710605) aimed to explore the role of CTCs in first-line treatment management of patients with hormone receptor$^+$HER2$^-$ MBC. Participants were randomized into two groups: i) Physicians chose between hormone therapy (HT) or chemotherapy according to current guidelines, without disclosure of CTC count; and ii) treatment choice was guided by CTC count (administration of HT for participants with <5 CTCs per 7.5 ml of peripheral blood or chemotherapy for those with ≥5 CTCs per 7.5 ml of peripheral blood). This trial showed non-inferiority of the CTC-driven arm in terms of PFS, with an HR of 0.98 (90% CI, 0.84-1.13). Analyzing the discordance between a priori and CTC-based decision in the chosen therapy demonstrated that switching to chemotherapy in participants with elevated baseline CTC counts (≥5 CTC per 7.5 ml) led to improved PFS compared with participants in the standard arm whose treatment was clinically-driven, with statistical significance. For those patients with a high CTC count in the clinically-driven arm treated by HT, the median PFS time was 10.5 vs. 15.5 months in the CTC-driven arm receiving chemotherapy. The findings of this study are encouraging and demonstrate that CTC count could be used as a guiding

Table V. Ongoing CTC-related trials assessing their clinical utility[a].

Trial name	Trial no.	Study design	Purpose	No. of patients	Intervention	Status
Breast-CTC-HER1	NCT05834699	Observational prospective study	Evaluation of the predictive and prognostic value of HER2 expression of CTCs in individuals with HER2-low ABC treated with ADC.	50	Detection of HER2 expression of CTCs in participants with HER2-low ABC treated with ADC.	Recruiting
Breast-CTC-HER2	NCT05834686	Observational prospective study	Evaluation of the predictive and prognostic value of HER2 expression of CTCs in individuals with $HER2^+$ ABC treated with ADC.	50	HER2 expression of CTCs in participants with $HER2^+$ ABC treated with ADC.	Recruiting
CA 111359	NCT01048918	Observational prospective study	To identify CTCs in patients with locally advanced or MBC.	280	Not provided.	Active, not recruiting
ACT-MBC	NCT05662345	Prospective Observational Impact Study	Assessment of the impact of CTCs on treatment decisions, response assessment and prognosis in MBC.	65	CellSearch CTCs and serial CTC enumeration.	Recruiting
Shengjing-LJY04	NCT04065321	Non-inferiority randomized controlled clinical trial	To spot the differences between detection of CTCs and standard imaging examination in the postoperative management of individuals with luminal A^- BC without metastatic involvement of the lymph nodes.	500	All participants in the control group undergo PET-CT. Blood samples of all participants in the trial group undergo CTC detection. Both at each follow-up-after operation, once every 4 months in 24 months, every half year till 5 years and then once a year. PET-CT examination in case of CTCs ≥2 or CD133 ≥1.	Recruiting
HER2Cell	NCT04993014	Interventional Phase II unicentric randomized trial	To compare the DFS between trastuzumab and trastuzumab + ertuzumab arms. To compare the DFS between patients with $HER2^+$ CTCs at baseline vs. $HER2^-$ CTCs.	80	Individuals will be divided into two cohorts. Cohort 1, $HER2^+$ CTCs: randomization to adjuvant trastuzumab vs. trastuzumab + pertuzumab in participants with pCR; Cohort 2, $HER2^-$ CTCs: randomization to adjuvant trastuzumab vs. trastuzumab + pertuzumab in participants with pCR	Recruiting
DETECT IV	NCT02035813	Prospective, multicenter, open-label, Phase II study	To evaluate CTC clearance rate within the everolimus/ribociclib cohort and assess PFS defined as time interval from date of recruitment until progressive disease within the eriubulin cohort.	116	Experimental Ribociclib arm: Postmenopausal participants with HR^+, $HER2^-$ MBC with $HER2^-$CTCs will be treated by standard ET in combination with CDK4/6 inhibitor. Experimental Eriubulin arm: participants with TNBC or HR^+, $HER2^-$ MBC indicated for CT, each with detected CTCs lacking HER2 expression.	Active, not recruiting
CTCSFBC	NCT05633680	Observational prospective study	To compare the differences in CTCs in peripheral blood between the case group (BC) and the control group (non-BC), and to conclude statistical analysis.	200	Diagnostic Test: Peripheral blood sampling for CTCs.	Active, not recruiting

Table V. Continued.

Trial name	Trial no.	Study design	Purpose	No. of patients	Intervention	Status
CTCNeoBC-E	NCT05360290	Prospective, multicenter study	To evaluate the prognostic value of CTCs in patients with BC who completed surgery after neoadjuvant treatment.	484	Use of GILUPI CellCollector® to detect CTCs.	Recruiting
Shengjing-LJY03	NCT04059003	Observational prospective study	To investigate the relationship between dynamics of CTCs changes and the treatment efficacy of NAC in TNBC. To attempt the development of predictive markers for treatment response in patients with TNBC.	200	Taxanes and/or anthracycline-based therapy applied every 3 weeks. Regularly (after each two-course cycle of chemotherapy), the efficacy of treatment assessed and after 6th application the participants undergo mastectomy.	Recruiting
KYJJ-2021-186	NCT05326295	Observational retrospective study	To investigate the utility of CTC surveillance in predicting treatment responses, iDFS, OS and metastasis in participants with BC undergoing neo/adjuvant chemotherapy and surgical intervention.	1,000	To assess the prognostic value of CTC number, cell phenotypes, as well as the expression of PD-L1 and FOXC2 in participants with EBC.	Recruiting
178-2018	NCT03709134	Observational prospective study	To identify genomic markers (CTCs, ctDNA and transcriptomic markers) as biomarkers of response to NAC among patients with invasive BC.	100	Diagnostic Test: Genomic Markers (CTC/ctDNA). This is a non-interventional study.	Recruiting
NEO-R-IPC 2019-041	NCT04504747	Observational prospective study	To provide valuable insights into drug resistance mechanisms in patients with BC undergoing NAC using the integration of molecular analyses, *in vitro* PDO models and CTC marker assessments to identify common candidates for treatment resistance.	150	Molecular analysis of samples (blood and tumor), RNA-seq and DNA-seq.	Recruiting
Concordance	NCT04241237	Observational prospective study	To determine whether blood factors (CTCs or ctDNA) and other biomarkers can serve as reliable indicators for BC recurrence in comparison with tissue biopsy.	120	Blood and as tissue samples will be collected to verify the results.	Recruiting
CTC-SMMiL-E	NCT03979339	Prospective interventional single-site research	Evaluation of the performance of the new CTC isolation technology in selected patients with cancer.	76	Blood sample (total of 32 ml) will be taken from every study participant, then distributed into four EDTA vacutainer tubes.	Recruiting

Table V. Continued.

Trial name	Trial no.	Study design	Purpose	No. of patients	Intervention	Status
IMAGE-II	NCT02965755	Prospective interventional single-site research	To determine if personalized genetic information can be obtained from a subject's blood sample which is similar to that obtained from a tumor tissue sample, and if that information can be used to make treatment suggestions.	200	A panel of Johns Hopkins investigators will interpret the molecular and genetic profiling results in order to identify any actionable mutations	Recruiting
09-056	NCT00941759	Observational prospective study	To study patients presenting with stage IV BC. To collect information about the patient and their treatment. To collect blood and tissue samples for laboratory studies.	100	Not Provided	Active, not recruiting
Detect V/ CHEVENDO	NCT02344472	Multicenter, randomized Phase III Study	To observe the differences between effectiveness of CT vs. combination of ET and dual HER2-targeted therapy in patients with $HER2^+$ and HR^+ MBC. Moreover, the assessment of CTCs may demonstrate their potential role as indicators for therapy success.	270	Drugs: Pertuzumab, Trastuzumab, Paclitaxel, Capecitabine, Vinorelbine, Exemestane, Docetaxel, Anastrozole, Letrozole, Fulvestrant, Ribociclib, Eribulin, Nab-Paclitaxel, Goserelin, Leuprorelin	Recruiting
PCS-001	NCT04962529	Observational prospective study	To investigate whether blood samples can be an alternative to the tissue biopsy sample in the evaluation of MBC. To explore the accuracy and reliability of liquid biopsy in capturing molecular and genetic information by comparison of Epic Sciences' DefineMBC liquid biopsy with standard-of-care pathology.	450	Blood and tissue samples will be collected to compare the results.	Recruiting

[a]The table summarizes the list of ongoing clinical trials dealing with CTC detection, clinical significance and potential utility of CTCs in clinical practice in patients with breast cancer (according to https://clinicaltrials.gov/). ABC, advanced breast cancer; ADC, antibody-drug conjugates; BC, breast cancer; CTCs, circulating tumor cells; ctDNA, circulating tumor DNA; DFS, disease-free survival; iDFS, invasive-DFS; MBC, metastatic BC; NAC, neoadjuvant chemotherapy; OS, overall survival; PDO, patient-derived organoids; PFS, progression-free survival; TNBC, triple-negative BC.

factor in choosing the first-line treatment for discrepant cases of hormone receptor$^+$HER2$^-$ MBC and thus improve patient outcomes. However, this study had some limitations such as a lack of standardized clinical criteria for chemotherapy in the clinically-driven arm. In addition, treatment with CDK4/6 inhibitors was not used as the study was conducted after the implementation of a new endocrine therapy (139).

Qualitative assessment of CTCs as a tool for guiding treatment decisions. In this subsection, it is emphasized that historically BC research predominantly focused on the enumeration of CTCs rather than their biology. For a deeper understanding of CTC biology and more comprehensive insights into BC, moving beyond simple counting to explore the biomolecular properties of CTCs is of growing importance (23).

At present, available data on treatment adjusting guided by CTC are promising but still very limited. Ongoing prospective trials are expected to provide valuable insights. Considering the ease of blood sampling, CTCs offer the potential for real-time liquid biopsy, enabling repeated assessments of tumor evolution and response to treatment. This facilitates timely and appropriate therapy adjustments. The expression of predictive biomarkers such as ER, PR and HER2 may evolve during the disease course, and consequent reassessment of these markers via CTCs at the time of disease progression could optimize treatment decisions, making CTCs a valuable tool for personalized treatment strategies (25,140-152). In the CirCe T-DM1 trial, which was the first clinical trial using the phenotype of CTCs as a decision criterion, the efficacy of trastuzumab-emtansine (T-DM1) in women with HER2$^-$ MBC who exhibited HER2$^+$ CTC was assessed. However, the application of T-DM1 resulted in a partial response in only 1 patient (140). The DETECT study, including DETECT III, IV and V, is a comprehensive trial investigating the effectiveness of treatment decisions guided not only by the presence but also by the phenotype of CTCs in women with MBC who exhibit various biological characteristics (153). The phase 3 DETECT III study (NCT01619111), which enrolled individuals initially diagnosed with HER2$^-$ MBC but exhibited HER2$^+$ CTCs, showed that application of lapatinib had a positive impact on OS, suggesting the potential acceptance of HER2$^+$ CTCs as a biomarker to predict clinical benefit in these individuals. Such findings could be clinically significant as other HER2 drugs become accessible (141).

Reinforcing the biological role of HER2 expression on CTCs, data from another analysis, including only participants with HER2$^-$ MBC screened for enrollment in DETECT III and IV with the exclusion of survival results of participants who receiving HER2-directed treatment with lapatinib, has been published (154). This large multicenter analysis with nearly 2,000 patients showed that CTC status in these patients is a strong prognostic factor and that CTC positivity is associated with worse clinical outcomes. In this study, ~15% of participants with HER2$^-$ MBC harbored ≥1 CTC with strong HER2 staining. The presence of CTCs with strong HER2 staining ranged between 0.06-100% among all CTCs (mean, 15.8%). Participants with at least 1 CTC displaying strong HER2 staining had a reduced OS time compared with those with CTCs showing only moderate HER2 staining or none at all, with OS time of 9.7 vs. 16.5 months, respectively (P=0.013). Moreover, multivariate analysis identified hormone receptor status, CTC status, age, Eastern Cooperative Oncology Group performance status and the therapy line as independent predictors of OS (154).

CTCs in predicting treatment success. In the DETECT V trial, the quantity or characterization of CTCs is not included in the therapeutic decision-making; however, one of the objectives of translational research projects is to develop the 'endocrine responsiveness score' (ERS), focusing on the expression of ER and HER2 in CTCs (153). The findings from the COMETI-2 study indicates that tumors expressing ER often respond well to endocrine therapy, whereas those with upregulated HER2 are linked to a poorer response (155). The successful establishment of the ERS could help identify individuals who are sensitive to endocrine therapy, thereby optimizing treatment approaches (153).

In addition, the comprehensive translational research project, 'DETECT-CTC', is currently being conducted. This project aims to utilize novel biomarkers and assays concentrated on the molecular features of CTCs and circulating nucleic acids to examine their potential in the clinical practice of patients with advanced BC (153). Several different DETECT-CTC subprojects are examining: i) The genetic and epigenetic characteristics of CTCs, circulating free DNA and microRNA; ii) the genomic alterations associated with DNA damage response pathways; iii) the resistance mechanism to endocrine therapy; iv) the expression of specific biomarkers across different stages of cancer progression and dissemination; and v) genetic and phenotypic changes occurring at the single-cell level within CTC populations over time (153).

5. Limitations of CTC test results and future directions

It is important to highlight the limitations concerning current CTC test results, which include the specificity and sensitivity of the detection methods, the lack of standardization of biomarkers for identifying CTCs and the biological heterogeneity CTC that could impact CTC tests results.

The evaluation of HER2 and hormone receptors on CTCs is critical for personalized treatment in BC. Discrepancies between primary tumor and metastatic tissue or CTCs reported by numerous studies are often explained as a change in the biology of BC during the disease (142-151) (Figs. 1 and 2). However, technical issues such as limited CTC count, variability of utilized staining protocols as well as utilization of various CTC enrichment techniques, contribute to this discordance (156). CTCs have potential for monitoring disease progression and guiding therapy; however, the clinical utility of CTC counts and molecular phenotyping has not yet been fully validated. This hinders the ability to develop uniform guidelines for intensive or prolonged treatment based on CTC findings (157).

Another cause of reported receptor discrepancies is heterogeneity within CTCs subpopulations, which can be further complicated by processes such as EMT and mesenchymal-epithelial transition. This limitation could lead to treatment failure. In addition, current CTC detection methods may not capture all subpopulations with the highest clinical validity. The lack of established cut-off values for predictive markers on CTCs further complicates their clinical application. Another crucial limitation is the low level of effectiveness

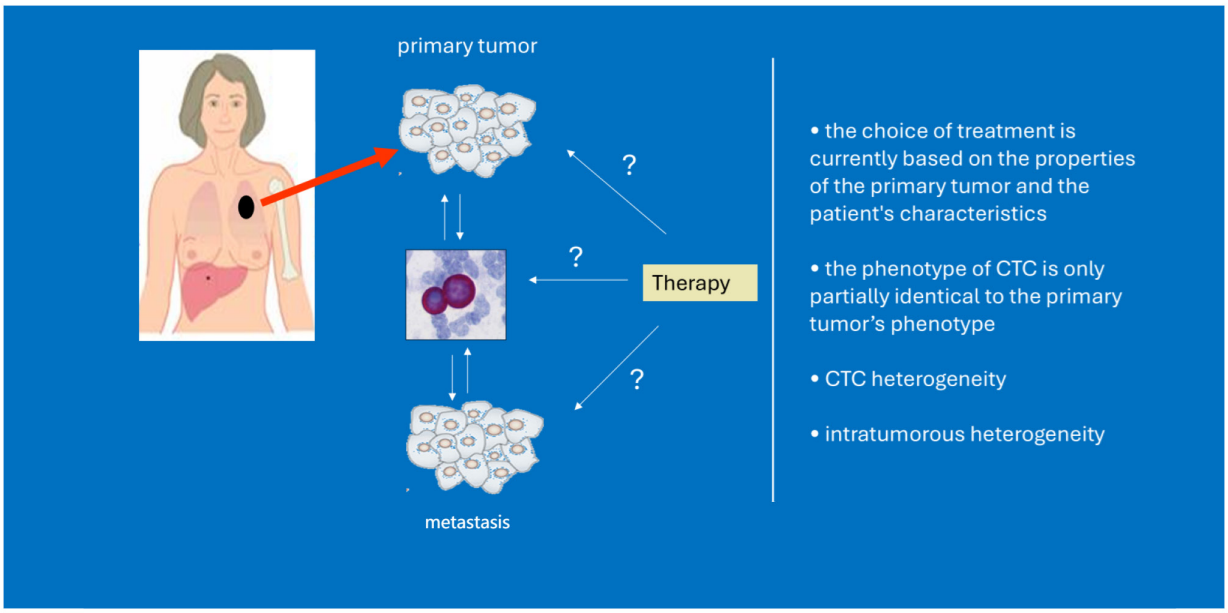

Figure 1. Limitations of the clinical utility of CTC. CTC, circulating tumor cell.

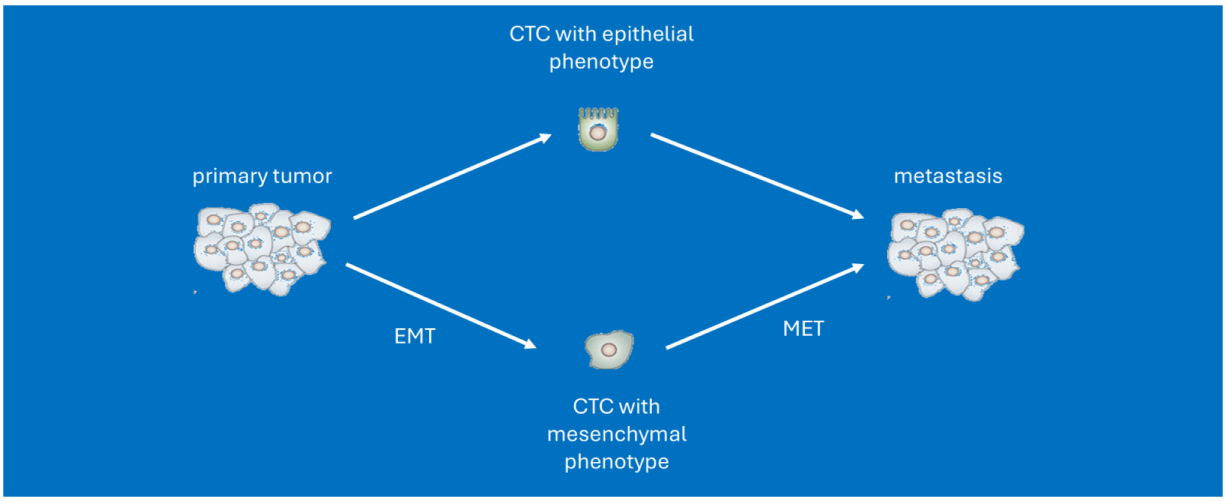

Figure 2. CTC phenotype could be different from the phenotype of the primary tumor and metastases. CTC, circulating tumor cell; EMT, epithelial-to-mesenchymal transition; MET, mesenchymal-to-epithelial transition.

of CTC detection, which significantly affects the number of CTCs detected and the subsequent clinical decisions that can be made based on their characterization (23,25).

Addressing these limitations, enhancing the detection methods for capturing CTC subpopulations with the highest clinical utility is a major future research direction. Incorporating new platforms and standardizing biomarker expression on CTCs are critical steps toward achieving this goal. Prospective clinical trials are essential to validate these approaches and determine their impact on patient outcomes. By solving these tasks, the clinical application of CTCs as predictive markers can be significantly improved, leading to more personalized and effective treatment strategies (25).

Another limitation of CTC research is that a number of clinical studies lack external validation, and the majority of data are based on small, single-center, case-control studies with widely varying patient characteristics. However, due to the robust evidence provided by large-scale studies and meta-analyses such as the study by Zhang *et al* (17), the clinical relevance of CTCs has been significantly supported, leading to their incorporation into the 2010 edition of the TNM staging manual as cM0 (i+) (158), indicating the presence of isolated tumor cells in the blood, bone marrow or lymph nodes without clinical signs of overt metastasis (23). The integration of CTCs into the TNM staging system was a critical step forward underscoring their importance in clinical oncology and marking their broader application in oncology.

Despite the significant evidence supporting the prognostic value of CTCs, they have not been incorporated into clinical guidelines, such as those of the ASCO. Clinical guidelines

require high-quality evidence that a biomarker not only predicts outcomes but also facilitates therapeutic decision-making. The aim of future and ongoing studies is to bridge these gaps by evaluating the impact of CTC-based interventions on patient outcomes (23). Larger, multicenter prospective studies based on homogeneous populations are particularly needed to determine whether CTC detection can change clinical practice and clarify their clinical utility (17,159).

6. Conclusion

In conclusion, current therapeutic management is based on the samples taken from primary tissues, despite the heterogeneity that characterizes BC. Advances in the development of detection platforms for CTCs and molecular technologies that upgrade knowledge of the biological properties of CTCs generate the optimism that CTCs can routinely participate in personalizing treatment for patients with cancer. Although numerous detection methods are available, the sensitivity and specificity of these approaches require further enhancement and the detection of cells escaping EpCAM selection is crucial.

It seems that characterizing CTCs is pivotal for gaining insights into cancer prognosis as two fundamental phenomena associated with CTCs are cancer stem cells (CSCs) and EMT. A deeper understanding of the signaling pathways associated with EMT and the characteristics of CSCs are critical for addressing the limitations of CTC enumeration. The ongoing exploration of these aspects has the potential to overcome tumor heterogeneity, drug resistance and thus to develop targeted therapeutic strategies.

CTCs have already shown their prognostic value in a number of clinical studies, not only in early but also in advanced BC; however, there are still numerous challenges to be conquered before CTC examination can be extensively utilized in clinical practice. Although clinical utility of CTCs remains uncertain, being easily accessible, CTCs offer an opportunity for dynamic monitoring and thus provide valuable insights into real-time changes in tumor characteristics and the identification of specific mechanisms associated with resistance to treatment. Molecular characterization of CTCs revealing the mutational profile of BC could be beneficial to avoid 'over/undertreatment'. Additionally, other potential markers related to CTCs besides those already established should be further identified.

The study of CTCs is attractive as they have promising potential for a wide range of clinical applications. Due to the minimally invasive nature of their sampling, CTCs offer dynamic and real-time information, facilitating longitudinal monitoring and may contribute to more personalized and adaptive treatment strategies. However, the inclusion of CTC-based assays in clinical guidelines and subsequent full integration of CTCs into daily practice requires more clinical and molecular studies with large cohorts of patients. Bridging the gap between CTC research findings and clinical implementation of liquid biopsy requires standardization, validation and collaboration between researchers and healthcare professionals.

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DŠR substantially contributed to the conception and design of the manuscript; RA, PD and MJ contributed to drafting the manuscript and the acquisition, analysis and interpretation of the studies; MM and DP contributed to critical revisions on the intellectual content of the manuscript. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

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Competing interests

The authors declare that they have no competing interests.

Authors' information

Dominika Šmičková Rusnáková, ORCID: 0009-0006-3638-9101; Peter Dubovan, ORCID: 0000-0002-0853-4045; Miroslav Jurík, ORCID: 0000-0002-0290-1748; Michal Mego, ORCID: 0000-0002-6511-6827; Daniel Pinďák, ORCID: 0000-0003-0413-8907.

Use of artificial intelligence tools

During the preparation of this work, artificial intelligence tools (ChatGPT by OpenAI) were used to improve the readability and language of the manuscript, and subsequently, the authors revised and edited the content produced by the artificial intelligence tool as necessary, taking full responsibility for the ultimate content of the present manuscript.

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