

## REVIEW

# Circulating tumor cells in metastatic breast cancer: ready for clinical practice?

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**Abstract**

Circulating tumor cells (CTCs) have emerged as a promising new biomarker in breast cancer and various settings in which liquid biopsy may improve diagnostics are currently under discussion. In patients with metastatic disease, high CTC counts have been shown to predict poor prognosis with level I evidence. Persistence of elevated CTC numbers after begin of systemic treatment is associated with higher risk of progression. This review discusses current evidence on the clinical use of CTC diagnostics in metastatic breast cancer.

**Keywords**

circulating tumor cells; CTCs; liquid biopsy; breast cancer; metastatic breast cancer; therapy interventions

## 1. Introduction

Breast cancer (BC) is the most common malignant tumor in women worldwide with an estimated 2.09 million new cases in 2018, and over 600,000 deaths from the disease yearly [1, 2]. In the metastatic situation imaging techniques such as ultrasound, computed tomography, bone scintigraphy and/or magnetic resonance imaging are commonly used in clinical practice for staging and therapy monitoring. However, imaging methods lack treatment relevant information about tumor expression profiles (e.g., Her2 receptor and hormone receptor status) as well as other prognostic and predictive biomarkers (such as newer targetable oncogenic biomarkers like PIK3CA, AKT1 or ESR1 mutations).

In BC research, circulating tumor cells (CTCs) and cell-free tumor cell-specific nucleic acids (ctDNA) are currently being discussed as promising biomarkers to close the former – and timely delayed – diagnostic and therapeutical gap in order to move a step forward towards a more individualized and personalized medicine. Detection of hematogenous cell dissemination and characterization of isolated tumor cells for optimized prognostic assessment and development of targeted therapeutic approaches are currently the main focus of oncology research discussions.

In early and metastatic BC (MBC) detection of CTCs in the peripheral blood and disseminated tumor cells in the bone marrow indicates a poor clinical prognosis [3–5]. CTCs can be detected through a simple noninvasive blood aspiration and its detection and characterization is usually referred to as liquid biopsy. Further, nucleic acid fragments (such as DNA, RNA, non-coding RNA [ncRNA]) are continuously

washed out into the blood circulation and can also be detected with molecular methods. The characteristics and dynamics of these biomarkers may improve the assessment of therapy response, a change of expression profiles, and detection of therapy resistance as well as prediction of prognosis.

In MBC, CTC counts and their expression profiles are assumed to reflect the current tumor burden and the dominant cell population of distant metastatic sites. Unlike the current gold standard of tissue sampling (by e.g., puncture and/or surgery which only reflects one metastatic site at a single time point) CTC evaluation can potentially access multiple metastatic lesions and can be performed in a repeatable manner, thus serving as a “real-time” biopsy.

In the following review, we will highlight the current clinical potential of CTC-based liquid biopsy in the metastatic setting.

## 2. Prognostic Relevance of CTCs

In a large proportion of non-metastasized patients tumor cells are already detectable outside the primarius at the time of diagnosis. Disseminated tumor cells (DTC) are found in the bone marrow in 31% of patients and CTCs in the peripheral blood in 20% of patients [3, 6]. Most of these cells are not capable of proliferation and perish, and only few (<0.02%) are able to survive. Whether survival advantages of this small selected subpopulation are due to their stem cell-like features, remains matter of debate [7]. The detection of DTC/CTC has a strong and independent prognostic significance: their presence roughly doubles the risk of metastasis and death for the individual patient [3, 6].

**TABLE 1. The most important trials showing the prognostic relevance of circulating tumor cells (CTCs) in breast cancer (BC) with the CellSearch assay.**

Trial	Number of patients	Stage	Positivity rate (%)	Cut-off value CTCs per 7.5 mL blood	Correlation with prognosis
Cristofanilli <i>et al.</i> [8]	2436	Stage IV	1099 (45.1%)	5	OS
Janni <i>et al.</i> [3]	3173	Stage I–III	641 (20%)	1	DFS, DDFS, BCSS, OS
Müller <i>et al.</i> [12]	1933	Stage IV	1217 (63%)	1 and 5	OS
Bidard <i>et al.</i> [13]	1574	Stage I–III before neoadjuvant chemotherapy	398 (25.2%)	1	OS, DDFS, LRRFS, pCR rate higher in CTC positive patients (24.2% vs. 17.4%)

CTC, circulation tumor cells; OS, overall survival; BCSS, breast cancer specific survival; DFS, disease free survival; DDFS, distant metastasis free survival; LRRFS, locoregional recurrence-free survival; pCR, pathological complete remission.

In MBC, CTCs have also been shown to be as an independent strong prognostic factor, with the well-established cutoff of 5 CTCs per 7.5 mL of blood (sometimes referred to as CTC<sub>aggressive</sub>) [8–11] (Table 1, Ref. [3, 8, 12, 13]). Within the recently published analysis by Cristofanilli *et al.*, [8] blood samples of 2436 patients with metastatic breast carcinoma from 18 centers were analyzed, including 533 women with de novo metastatic disease. All patients underwent blood analysis using the CellSearch system. This assay uses immunomagnetic separation to isolate tumor cells with subsequent visualization by microscopy and counting of cells that express cytokeratin by immunofluorescence and has been cleared by the U.S. Food and Drug Administration (FDA) for use in patients with metastatic breast cancer. In the study by Cristofanilli *et al.*, [8] detection of at least 5 CTCs per 7.5 mL blood was associated with a significantly worse clinical outcome (median OS: 36.3 in CTC<sub>aggressive</sub> vs. 16 months in CTC<sub>indolent</sub> [i.e., <5 CTCs per 7.5 mL],  $p < 0.0001$ ). This effect was independent from the localization of the metastases (OS in the subgroup with visceral metastases: 13.2 vs. 29.9 months; in the subgroup with bone metastases alone: 23.8 vs. 46.9 months). In the group of patients with newly diagnosed metastasis, positive CTC status also predicted significantly shorter OS (18.7 vs. 41.4 months,  $p < 0.0001$ ). In multivariate analysis the number of CTCs was the strongest predictor of shorter OS (HR 2.71, 95% CI 2.35–3.12,  $p < 0.0001$ ). The prognostic impact of CTCs upon diagnosis was confirmed in all tumor subtypes [8].

### 3. Clinical Value of Therapy Monitoring

CTC detection can also differentiate between patients with a favorable and unfavorable course of disease and the detection of CTC at time of distant metastasis or progression has a high prognostic significance. Repeated blood samples may predict therapy response even before the first imaging restaging [14, 15]. Janni *et al.* [15] presented at the San Antonio Breast Cancer Symposium 2020 the results of a pooled analysis of individual data from 2761 MBC patients (Table 2, Ref. [14–18]). Blood samples were collected at baseline and at a follow up visit with a median time interval between the two CTC assessments of 35 days. Patients CTC-negative at both time points had longest OS (45.6 months), followed by patients initially CTC-positive who became CTC-negative during treatment (34.6 months), patients initially CTC-negative and CTC-

positive at follow up (26.1 months) and those CTC-positive at both time points (17.6 months). CTCs thus bear the potential to improve conventional staging methods in the future. The goal of current investigations is to avoid unnecessary diagnostics, therapies, and toxicities in order to improve the patients' quality of life through personalized therapy regimes.

Besides this, increasing CTC numbers under palliative chemotherapy indicate a higher risk of disease progression [4, 14]. So far there is no subsequent clear clinical consequence for this finding. The American SWOG-S0500 trial was initiated to address the question of the optimal approach for patients with persistently high CTC counts under palliative chemotherapy [14]. In 595 metastasized patients, with persistently high CTC counts after the first cycle of first-line cytotoxic treatment, therapy was either continued until radiological/clinical diagnosis of progression (standard arm) or switched early to another cytotoxic regime (CTC-based arm). Patients with low CTC counts at the beginning of therapy had the best prognosis (median OS: 35 months), followed by women whose CTC counts dropped to <5 CTCs after the first cycle of therapy (23 months). Patients with persistently high CTC numbers had the shortest OS of 13 months. However, in the group with persistently high CTC counts the progression-free (PFS) and OS were similar in both arms, i.e., early switch to another chemotherapy did not improve clinical outcomes. Therefore, it remains unclear which treatment should be recommended for this patient group. It is possible that a clear decline in CTC counts resembles chemotherapy response whereas the lack of CTC decline may indicate resistance to conventional cytotoxic treatment. Those patients might be most likely to benefit from targeted, immunologic, or experimental approaches.

Recently, another phase III multicenter, randomized, Phase III CirCe01 study (NCT01349842) compared early evaluation of the efficacy of palliative chemotherapy by determination of circulating tumour cells versus conventional clinical and radiological evaluation [16]. After two or more cycles of systemic chemotherapy patients were randomized between the standard arm and CTC-driven arm. In the CTC arm, changes in CTC counts were measured at the first cycle of each line of chemotherapy. An alternative subsequent chemotherapy regime was started if there was no decline in CTC levels. The CTC arm (n = 51) was completed in the third chemotherapy

**TABLE 2. Most important studies on circulating tumor cells in metastatic breast cancer.**

Study	Number of patients	Topic	Main results
Janni <i>et al.</i> [15]	2671	CTC dynamics during treatment	CTC-negativity as baseline and at first follow up visit was significantly associated with longest OS and CTC-positivity at both time points with shortest OS
SWOG-S0500 [14]	595	Randomized trial investigating therapy switch in case of CTC persistence	No benefit from early switch to another chemotherapy
STIC CTC [17]	755	Phase III randomized trial comparing physician's choice therapy to CTC-driven therapy in HR+ HER2- mBC in the first-line setting	CTC-driven therapy was not inferior to physician's choice; patients with elevated CTC counts and clinically low risk and those with low CTCs counts but clinically high-risk benefited from chemotherapy
CirCe01 [16]	204	Phase III randomized trial comparing early switch to another chemotherapy in case of CTC persistence with continuing chemotherapy	No benefit from early switch to another chemotherapy
DETECT III [18]	101	Phase III randomized trial comparing standard systemic therapy vs. standard therapy plus lapatinib in patients with tissue HER2-negative mBC and HER2-positive CTCs	Significant OS benefit in lapatinib-arm

line in 43 (83%) patients and in the fourth line in 18 (44%) patients. Unfortunately, 18 (42%) and 11 (61%) of patients had no adequate CTC decrease and OS did not differ between groups (hazard ratio = 0.95%, 95% CI = (0.6; 1.4),  $p = 0.8$ ). Interestingly, patients with no CTC response and switch of chemotherapy survived longer than those without switch of chemotherapy.

#### 4. CTC-based Therapy Interventions in the Metastatic Situation

Several studies are currently addressing the question of how the determination of CTC numbers or their characteristics can contribute to the individualization of therapy. At the 2018 San Antonio Breast Cancer Symposium, the results of the first positive study on CTC-based therapy interventions were presented [19]. In the French STIC-CTC phase III open-label noninferiority trial, 778 patients with metastatic HR-positive HER2-negative BC (chemotherapy-naive) were included [17]. In the standard arm (chemotherapy or endocrine therapy [ET] at the discretion of the patient's oncologist), patients received the therapy recommended by the physician; in the experimental CTC arm, the treatment decision was based solely on the result of the analyzed blood samples via the CellSearch system (i.e., patients with  $\geq 5$  CTCs per 7.5 mL blood received chemotherapy, and those with  $< 5$  CTCs per 7.5 mL blood were treated by ET). In 27% of the patients elevated CTC levels were detected (defined as the presence of at least 5 CTCs per 7.5 mL blood). In the standard arm, 73% of patients received ET, and 27% received chemotherapy, whereas in the CTC arm, the 63% of patients had  $< 5$  CTCs and were treated by ET, and 37%, i.e., those with  $\geq 5$  CTCs received chemotherapy. After a median follow-up of 30 months, PFS and OS were identical

in both arms, showing that the CTC-based therapy choice is not inferior to the decision of an experienced oncologist. Notably, patients with a discordant assessment (i.e., women with low CTC counts but clinically classified as "high risk" and women with high CTC counts but clinically classified as "low risk") benefited from chemotherapy (hazard ratio for OS: 0.65;  $p = 0.04$ ). Overall, PFS and OS showed a positive trend in patients treated with chemotherapy compared to those treated with ET indicating that CTC enumeration before start of treatment could be beneficial.

Although the STIC-CTC trial was the first positive study on CTC-based treatment interventions in breast cancer, the interpretation of these results remains challenging, since therapy regimes changed for most patients with HR-positive HER2-negative disease in the first-line setting to an endocrine-based combination therapy with a CDK 4/6 inhibitor. This treatment option was not included in the STIC-CTC trial initiated in 2012. It therefore remains unclear how the results can be implemented in clinical practice.

#### 5. Treatment Choices Based on CTC Pheno- and Genotypes

Tumor expression profiles are known to have important implications for response to targeted therapies in MBC, and CTCs are hypothesized to potentially serve as a real-time liquid biopsy to assess tumor expression profile changes. Although the data is limited regarding treatment decisions based on the CTC phenotype, it has been demonstrated that patients with HER2-negative MBC may harbor HER2-positive CTCs in the peripheral blood.

The aim of the German DETECT study program, the largest study program worldwide to investigate CTC-based therapy in-

terventions, is to examine treatment outcomes based on CTCs rather than tumor features assessed in the tissue. For patients with a discrepancy between the histologically examined tumor characteristics and the CTCs in the blood the DETECT III trial (NCT01619111) was initiated [20]. In this study, 105 patients with HER2-negative MBC and HER2-positive CTCs were enrolled in order to evaluate the potential benefit of the addition of the HER2-directed therapy with the tyrosine kinase inhibitor lapatinib to standard therapy [18]. The detection of CTCs and analysis of their HER2 expression were performed at regular intervals. Patients with no CTCs at the time of the first examination after the start of therapy, had a more favorable prognosis in terms of OS than patients with the evidence of CTCs. A decrease in CTC numbers was observed in both study arms (standard therapy versus standard therapy plus lapatinib), the direct comparison of both study arms however showed an improved OS in patients treated with lapatinib. Taking these findings into account, it might be hypothesized that expression profile of CTCs can be used to identify patients most likely to benefit from targeted therapy in the future. Further CTC-based therapeutic concepts are currently being investigated in other DETECT trials [21].

## 6. Conclusions

The investigation of relevant tumor characteristics, evaluation of disease progression and previous treatment response are of great importance for prognosis and further treatment decisions in MBC. However, invasive tissue sampling of the primary tumor and/or metastasis however is not always feasible. Furthermore, tissue samples may not necessarily reflect the intra- and interlesional heterogeneity of the tumor and thus the interpretation of results may be limited. By contrast, CTCs are assumed to reflect current state of the metastatic disease and their detection and characterization can provide unique insight into changes of the disease (“real-time liquid biopsy”). The potential of patient-friendly non-invasive complementary diagnostics might contribute to improved therapy decisions. The long-term goal of CTC-based analysis of MBC is to establish a personalized, targeted and thus efficient tumor therapy that, in addition to prolonging clinical outcomes, might also improve the quality of life by avoiding potentially harmful and ineffective therapies.

## AUTHOR CONTRIBUTIONS

LB, DGG, NK, MBP and PP analyzed the data and wrote the manuscript. MBP, NK, PP and AR designed the review. AR and TF provided help and advice on manuscript writing. LB, DGG, PP and NK constructed the tables. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

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## CONFLICT OF INTEREST

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