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# **The Clinical Relevance of Circulating Tumor Cells in Early Breast Cancer**

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## **Abstract**

Circulating tumor cells (CTCs) are considered to be evading cancer cells that have been shed or actively invaded from the primary tumor into the blood circulation or lymphatic system and which may finally extravasate to found metastases. CTCs as “liquid biopsy” hold great promise to be a powerful non-invasive real-time measurable biomarker for predicting clinical outcomes and cancer treatment response. Several studies evaluated the role of CTC presence and count in the neoadjuvant and adjuvant setting of early breast cancer (EBC) and revealed their significant prognostic value. In this chapter, we highlight the clinical relevance of CTCs in early breast cancer (EBC) and state the urgency for further research in this field to definitely translate this marker from bench to bedside.

**Keywords:** early breast cancer, circulating tumor cells, liquid biopsy, survival, clinical relevance

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## **1. Introduction**

Circulating tumor cells (CTCs) are deemed to be evading cancer cells that have been shed or actively invaded from the primary tumor into the blood circulation or lymphatic system and which may finally extravasate to found metastases. CTCs as “liquid biopsy” hold great promise to be a powerful non-invasive real-time measurable biomarker for predicting clinical outcome and cancer treatment response [1]. Several studies evaluated the role of CTC presence and count in the neoadjuvant and adjuvant setting of breast cancer (BC) and revealed their significant prognostic value. In this chapter, we highlight the clinical relevance of CTCs in early

BC and state the urgency for further research in this field to definitely translate this marker from bench to bedside.

## 2. CTCs as a screening tool

Several studies observed the presence of CTCs in patients with no clinically detected metastatic lesion [2, 3]. Illie et al. revealed association of CTC presence with early carcinogenesis and risk of cancer [4]. However, the use of CTC presence as a screening tool to diagnose early breast cancer (EBC) is challenged by the low sensitivity of current CTC detection methods. Current CTC detection platforms such as the FDA-approved CellSearch™ system can only detect CTCs in about 70% of patients with metastatic breast cancer [5]. One of the solutions to improve sensitivity may be the previous performance of leukapheresis. Compared to 20–30% of CTC detection by the CellSearch™ system in early breast cancer, the combination of leukapheresis and the CellSearch™ system has revealed to be able to identify CTCs in 90% of patients with early breast cancer [6]. Respectively, rise of sensitivity due to enrichment techniques may pave the way to use CTCs for early breast cancer screening or diagnosis. An ongoing trial is currently enrolling patients who do not have a prior history of invasive breast carcinoma or clinically apparent metastatic disease to investigate the potential role of CTCs as a screening tool (NCT01322750).

## 3. CTCs for prediction of prognosis

Cancer cells may leave the primary tumor and enter blood circulation long before the disease becomes clinically detectable and are considered a potential source of metastatic spread. Accordingly, numerous studies reported that early BC patients with detectable CTCs have significantly worse clinical outcomes than CTC-negative patients (**Table 1**). Among these, the largest data set was provided by the German SUCCESS trial (EUDRA-CT No. 2005-000490-21, NCT02181101). Briefly, blood samples from over 2000 average-to-high risk non-metastatic BC patients before chemotherapy and nearly 1500 patients after chemotherapy were examined [8]. Patients with CTCs at baseline had significantly shorter disease-free and overall survival. Further, the trial explored the relationship between CTC counts and prognosis in order to determine the optimal cut-off (i.e., no CTCs vs.  $\geq 1$ ; 0–1 vs.  $\geq 2$ ; 0–4 vs.  $\geq 5$  CTCs in 30 ml blood). A statistically significant impact on the clinical outcome was demonstrated for all cut-offs while patients with  $\geq 5$  CTCs had the highest relapse risk. Results from the SUCCESS trial are in accordance with smaller studies with longer follow-up [11, 14].

Janni et al. performed a large, multicenter pooled analysis of the available trials and confirmed CTC presence in early BC as an independent predictor of shorter disease-free, overall, breast cancer-specific, and distant disease-free survival [7]. Interestingly, CTC positivity was not associated with survival in low-risk, small node-negative tumors, suggesting that early-stage

Author	Number of patients	Patients	Method	CTC positivity n (%)	Follow up (median, months)	Prognostic significance
Janni pooled analysis [7]*	3173	Stage I–III	CellSearch	641 (20%)	63	DFS, DDFS, BCSS, OS
Rack, SUCCESS trial [8]	2026	Stage I–III, node-positive or high risk node-negative, all pts. received chemotherapy	CellSearch	435 (21%)	36	DFS, DDFS, BCSS, OS
Molloy [9]	733	Stage I–II	qRT-PCR (CK19, p1B, EGP-2, PS2, MmGI)	58 (8%)	91	MFS, BCSS
Ignatiadis [10]	444	Stage I–III, all pts. received adjuvant chemotherapy	RT-PCR (CK19)	181 (41%)	54	DFS, OS
Franken [11]	404	Stage I–III	CellSearch	76 (19%)	48	DDFS, BCSS
Lucci [3]	302	Stage I–III	CellSearch	73 (24%)	35	DFS, OS
Kuniyoshi [12]	167	Stage I–III	RT-PCR (CK19, c-erbB-2)	n.a.	n.a.	None
Hwang [13]	166	Stage I–IIIa	RT-PCR (CK20)	37 (22%)	100	MFS, OS
REMAGUS02 trial [14]	95	Neoadjuvant trial, Stage II–III, ineligible for breast conserving surgery at diagnosis or high-risk	CellSearch	22 (23%)	70	DDFS, OS

n.s.: not significant; BCSS: breast cancer-specific survival; DDFS: distant disease-free survival; DFS: disease-free survival; OS: overall survival; MFS: metastasis-free survival.  
 \*.including data from five centers, some previously published as [3, 8, 11, 14].

**Table 1.** The prognostic relevance of CTC presence in patients with non-metastatic BC.

BC can be treated successfully despite the presence of minimal residual disease in the blood. In high-risk patients, the strong prognostic value of CTCs underlines the necessity to establish new treatment strategies for this particular patient group. Further, CTCs predicted clinical outcomes in women with triple-negative and luminal (i.e., hormone-receptor positive, including luminal B HER2-positive subtype) tumors, but no association was found in case of patients with HER2-positive, hormone receptor-negative disease [7]. This observation is in contrast with the previous study by Ignatiadis et al. who reported that CTCs were highly predictive of clinical outcomes in the triple-negative and HER2-subtype but not in luminal tumors [10, 15]. Similar findings were reported by others [13, 16, 17]. Possibly, the relatively short follow-up may contribute to these partly contradictory results since none of the abovementioned trials reported a follow-up longer than 100 months. Longer follow-up might be necessary to fully understand the relevance of CTCs in patients with luminal tumors who are more at risk for a late relapse compared to women with more aggressive subtypes [18].

## 4. Therapy monitoring

Gold standard for evaluation of therapy response involves clinical examination, measurement of tumor markers, and radiologic imaging. CTCs provide a blood biomarker for early carcinogenesis, cancer progression, and treatment effectiveness. The identification of circulating tumor cells under therapy correlates with poor prognosis in metastatic breast cancer, but there are few data describing the importance of circulating tumor cells in patients with early breast cancer.

Regarding adjuvant treatment modalities of patients with early breast cancer, the SUCCESS trial and a trial by Xenidis et al. are the only trials in which CTCs were monitored [8, 19]. In the SUCCESS trial, CTCs were analyzed in 1492 patients with early breast cancer before adjuvant chemotherapy and post-chemotherapy using the CellSearch™ system [8]. The 36-month OS was 92.8% for persistently CTC-positive patients and 97.6% for persistently CTC-negative patients. Regarding the DFS, the Kaplan-Meier estimate was 85.9% for persistently CTC-positive patients and 93.9% for persistently CTC-negative patients. This large prospective trial of patients with early breast cancer suggests the independent prognostic relevance of CTCs both before and after adjuvant chemotherapy. In line, the presence of persistent CTCs 2 years after completion of adjuvant chemotherapy in clinically disease-free patients predicted worse clinical survival [20]. Xenidis et al. analyzed blood samples of 237 patients who were initially positive before start of taxane-based or taxane-free adjuvant chemotherapy [19]. After a median follow-up of 71 months, patients treated with taxane-based regimen had a longer DFS compared to patients receiving taxane-free regimen. Positive effects on median survival in the taxane group were reflected by a shift toward CTC-negative status: 50% of patients in the taxane-treated group turned CTC negative compared to only 33% of patients in the taxane-free arm [19]. In the phase III SUCCESS C trial (NCT00847444), 3547 patients with HER2-negative early breast cancer were randomized to either six cycles of docetaxel and cyclophosphamide (DOC-C) or to epirubicin, 5-fluorouracil, and cyclophosphamide followed by three cycles of docetaxel (FEC-DOC). Data on CTC prevalence after adjuvant chemotherapy between both treatment arms were available for 1766 patients. First results revealed no significant difference of CTC prevalence at the time of last chemotherapy cycle between patients randomized to FEC-DOC or DOC-C (11.5 vs. 13.6%). The comparable prevalence of CTCs may indicate that anthracycline-free chemotherapy is equally effective to anthracycline-containing chemotherapy in HER2-negative, hormone receptor-positive early breast cancer. However, this interpretation needs to be confirmed by data of the final survival analysis [21].

In the neoadjuvant setting, four studies explored the association of CTCs and clinical outcomes. In a small study of Hall et al. focusing on 57 patients with triple negative breast cancer, CTC persistence after neoadjuvant treatment was an independent predictor of worse clinical outcomes [22]. The study showed a significant correlation between CTC presence and shorter relapse-free and overall survival after completion of neoadjuvant therapy. This is in contrast to other studies in which conflicting results were reported in the neoadjuvant setting [14, 23, 24]. These studies also aimed to explore the signatures of CTC dynamics and pathological changes in the primary tumor during neoadjuvant chemotherapy. In several clinical trials pathological complete response is used as an endpoint because of its ability to predict long-term survival.



However, changes in CTC count generally did not correlate with tumor's response to neoadjuvant chemotherapy. In the REMAGUS02 trial, the CTC count of 85 patients was analyzed after neoadjuvant chemotherapy [25]. No correlation between CTC dynamics and pathological response was found after neoadjuvant treatment. Analog results were shown in the Gepar Quattro trial [26]. Riethdorf et al. analyzed blood samples from 213 non-metastasized breast cancer patients before and after preoperative chemotherapy. Interestingly, in 22% of patients, CTCs could be detected by CellSearch™ before neoadjuvant treatment, whereas positivity rates decreased to 11% after chemotherapy. However, neither CTC count before nor after preoperative chemotherapy was predictive to pathological response of the primary tumor.

## 5. Treatment decisions based on CTCs

Although there are several ongoing trials investigating the role of CTCs as a decision tool in metastatic breast cancer, there are only few studies investigating the clinical utility of isolated tumor cells encountered in the blood stream in early breast cancer. This might be due to technical challenges of CTC research in early breast cancer. Up to date, treatment decisions in early breast cancer are still based on the phenotype of the primary tumor without considering the disease evolution. Nevertheless, features of minimal residual disease may differ from those of the primary tumor. Riethdorf et al. examined the HER2 status of CTCs in HER2-negative primary breast cancer [26]. In 19% of patients with HER2-negative BC, CTCs expressing the HER2 receptor were detected in peripheral blood [26]. Anti-HER2-targeted treatment is not eligible for these patients, which might result in undertherapy and higher risk for relapse. Georgoulis et al. showed an increased DFS and reduced number of relapses among patients with persistent HER2-positive CTCs detected after completion of adjuvant therapy and administration of trastuzumab [27]. In this small Phase II trial (n = 75), additional therapy with trastuzumab resulted in a 75% reduction of patients with detectable CTCs in the trastuzumab arm compared to 17.9% in the control group. Based on these results, this therapeutic approach is currently investigated in the TREAT CTC randomized trial (NCT01548677) [28]. Patients with HER2 negative early breast cancer with persistent CTCs after (neo) adjuvant chemotherapy were randomized concerning additional trastuzumab treatment. HER2 status of CTCs was assessed; nevertheless, treatment decisions were only based on CTC presence. However, the TREAT CTC trial was closed for patient recruitment. To date, there are no published results yet.

Concerning treatment decisions, additional molecular profiling of CTCs may provide important additional information to CTC count. Several studies revealed intra- and intertumoral heterogeneity and demonstrated differences in phenotypes and genotypes between CTCs and primary tumors [29]. Therefore, detection and molecular characterization of CTCs are of great interest for selection of proper medical treatments and prevention of therapeutic resistance. In metastatic breast cancer, clinical significance of CTC subtype for guiding treatment decisions and evaluating therapy response is currently investigated within the German DETECT trials (NCT01619111).

6. Limitations of current methods for CTC detection

In this context, one needs to keep the limitations of current methods for CTC detection in mind. Epithelial cell adhesion molecule (EpCAM)-dependent enrichment techniques are the most widely used with the CellSearch™ system being so far the only FDA-approved system [30]. However, detection of CTCs is limited by the CellSearch™ system to cells with expression of EPCAM and cytokeratin 8/18/19. Respectively, the CellSearch system can certainly miss the detection of subpopulations of CTCs with decreased epithelial marker expression as a result of CTCs that have undergone epithelial-mesenchymal transition (EMT) [31]. It was observed that tumor cells which already initiated EMT are correlated with worse prognosis and therapy resistance [32]. Therefore, many EpCAM-independent methods are currently being developed and tested for CTC characterization. Translation into clinical routine practice of these new methods seems to be currently difficult. Multicenter assessment studies are lacking, and thus their reproducibility, sensitivity and specificity remain to be evaluated.

7. Conclusions

Circulating tumor cells are currently considered one of the most promising biomarkers for prediction of survival and monitoring of therapy in solid malignancies. While their prognostic significance has long been proven in early and metastatic breast cancer, further research is urgently needed to examine the possibility of guiding treatment decisions based on the presence and phenotype/genotype of CTCs (Table 2).

Potential	Early BC	Metastatic BC
Prognostication	Yes; CTCs are significantly associated with disease-free and overall survival	Yes (level I evidence); high CTC levels correlate with shorter progression-free and overall survival (cut-off: 5 CTCs/7.5 ml PB)
Therapy monitoring	Unclear; presence of CTCs 2 years after completion of chemotherapy predicts worse survival; contradictory results with regard to association between CTC changes and response to neoadjuvant treatment	Possibly relevant; High CTC levels after start of first-line chemotherapy can adequately predict progression; however, patients do not benefit from a switch to another regimen (clinical trials: SWOG 0500, ongoing: CirCe01)
Treatment selection based on CTCs	Possibly relevant; evidence pending (clinical trials: TREAT CTC, active, closed to patient entry)	Possibly relevant; evidence pending (ongoing clinical trials: STIC CTC METABREAST, DETECT III/IVa/IVb/V)

Modified after Ref. [33].

Table 2. Clinical role of CTCs in early and metastatic breast cancer.

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