Liquid Biopsy in Metastasized Breast Cancer as Basis for Treatment Decisions

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Summary

According to current guidelines, the additional biopsy of breast cancer metastases to analyze the receptor status for phenotype assessment is recommended. However, due to clinical difficulties in performing biopsies of metastatic lesions, the phenotype of the primary tumor most often determines the treatment decisions in metastatic breast cancer. Liquid biopsy allows the analysis of several circulating biomarkers like circulating tumor cells (CTCs) or circulating tumor DNA (ctDNA) in peripheral blood samples of cancer patients. Thus, it is an elegant and easily practicable technique that delivers information on the current disease status. Determination of the CTC phenotype regarding the hormone receptor and human epidermal growth factor receptor 2 (HER2) status might replace additional tissue biopsy for planning further therapy strategies. Liquid biopsy is a crucial step towards a more individualized cancer therapy. In contrast to the conventional concept of tissue biopsy, it offers an easy, less invasive acquisition of biomaterial. In addition, it allows multiple repetitions and real-time monitoring of metastasized disease in the clinical routine. However, the clinical utility of liquid biopsy still needs to be evaluated.

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Background

Metastatic breast cancer (MBC) is still a challenge for clinicians as it is the cause of death in about 20% of patients initially diagnosed with localized disease [1]. As in the curative setting, treatment decisions in MBC are based on the hormone receptor (HR) and/or human epidermal growth factor receptor 2 (HER2) status. Based on the knowledge that the expression profile of breast cancer may change in the course of disease, national and international guidelines advise an additional biopsy of metastatic lesions for further assessment of the breast cancer phenotype in MBC [2, 3]. However, due to limited tissue accessibility and invasiveness of the procedure, repeated sampling of metastases is often not feasible and the current treatment decisions in MBC are still mostly based on the biological characteristics of the primary tumor. Moreover, since MBC represents a heterogeneous disease, reevaluation of the tumor characteristics founded on the biopsy of 1 metastatic lesion may not sufficiently reflect the tumor burden [4, 5].

In this context, a simple and noninvasive blood analysis for solvent biomarkers like circulating tumor cells (CTCs) or free circulating tumor DNA (ctDNA) as a 'liquid biopsy' may represent an attractive alternative allowing the real-time monitoring of disease progression and therapy response.

CTCs as a Prognostic and Predictive Tool in MBC

CTCs can be detected in 40–80% of patients with MBC. Cristofanilli et al. [6] in 2004 were the first to report that CTC counts above the cut-off value of 5 cells per 7.5 ml of blood indicate an unfavorable clinical outcome. The prognostic role of CTCs in MBC patients has been further demonstrated by several studies [7–13]. A recent pooled analysis on 1,944 MBC patients confirmed the influ-

ence of CTC detection on progression-free survival (PFS) and overall survival (OS) with the highest level of evidence [14].

Beyond the prognostic impact of CTC detection in MBC patients, dynamic changes in CTC counts during therapy have been reported to reflect treatment response: In the study by Hayes et al. [9], a decrease in CTC levels under the cut-off value of 5 cells predicted better PFS and OS. Moreover, therapy response assessed by evaluation of the CTC dynamics might be more suitable for therapy monitoring than standard radiological imaging [8]. In a prospective multicenter trial by Budd et al. [8], persistently high levels of CTCs predicted worse clinical outcome despite radiological therapy response.

Additionally, several studies have shown that the phenotypes and genotypes of the primary tumor, the metastatic lesion, and CTCs often differ, especially with regard to the HR and HER2 status [15–19]. Since CTCs in MBC possibly most adequately represent the current dominant clone of tumor cells, their expression profile may predict therapeutic response [20]. It has been demonstrated in small experimental trials that targeted therapy guided by the CTC phenotype is able to eliminate persistent tumor cells from the blood and/or bone marrow of breast cancer patients [21–23]. The clinical significance of the CTC phenotype (in particular, the HER2 status) for guiding therapeutic decisions and evaluating treatment response is being investigated within the DETECT studies. Furthermore, recent research reported the possibility to provide an analysis of CTCs on the DNA, RNA, and protein level including next-generation sequencing (NGS) [24]. CTC characterization on the molecular level might help to identify resistance mechanisms of tumor cells: an important step for the optimization of systemic treatment [20]. In this context, one of the main goals of the recently initiated PRAEGNANT trial (NCT02338167) is to correlate liquid biopsy-based tumor assessments with actual tumor characteristics in order to facilitate comprehensive tumor profiling and evaluate new blood-based prognostic and predictive biomarkers in MBC patients [25].

Limitations and Challenges

Since CTCs are a rare event, despite easy achievement of the blood sample, their detection remains challenging, requiring complex enrichment procedures [26]. Thus, the use of liquid biopsy for the assessment of the breast cancer pheno- or genotype is limited to patients with relevant CTC counts. Moreover, most of the CTC detection methods are based on their epithelial character, missing the cells that underwent epithelial-mesenchymal transition (EMT). In this process, assumed to be responsible for cancer resistance to therapy, CTCs lose their epithelial features and express mesenchymal or stem cell markers [27, 28]. Finally, the impact of CTC-driven clinical decisions still needs to be evaluated.

ctDNA as a Biomarker in MBC

ctDNA consists in degraded DNA fragments released into the blood circulation from tumor cells undergoing necrosis or apopto-

sis [29]. These small DNA fragments have to be differentiated from other cell-free DNA (cfDNA) in peripheral blood (PB) based on the identification of tumor-specific mutations. Detection and genomic analysis of ctDNA, including NGS, may represent a promising noninvasive tool for the characterization of tumor material circulating in the PB and for monitoring the efficacy of anti-cancer treatment.

Several studies till date have demonstrated that ctDNA can be detected in the PB of early-stage and MBC patients [30–32]. In the study by Bettegowda et al. [30], ctDNA was detected in 82% of the patients with metastatic malignancies and in 55% of the patients with localized tumors, also in breast cancer. Rothe et al. [32] demonstrated in their pilot trial using NGS that ctDNA can serve as a biomarker for cancer monitoring and as a serious alternative to metastatic biopsy; in 13/17 (76%) patients examination of plasma samples and metastatic tissue showed concordant results, whereas in 2/17 (12%) patients ctDNA analysis revealed additional information. Moreover, several groups have shown that the load of ctDNA adequately reflects the tumor burden and that high levels of ctDNA are associated with poor prognosis [29, 31, 33–35]. In the study by Dawson et al. [31], ctDNA has been detected in 29/30 (97%) MBC patients, showing higher sensitivity and higher correlation with the tumor burden than cancer antigen (Ca) 15-3 or CTCs. In contrast, in their study investigating the dynamic range of ctDNA in MBC patients, Heidary et al. [36] demonstrated unexpectedly low frequencies of ctDNA in patients with tumor progression, which neither reflected the tumor burden nor the dynamics of the disease. Moreover, in the recently published trial by Madic et al. [37] analyzing TP53 mutation in primary tumors and corresponding plasma samples of triple-negative MBC patients, ctDNA was detected in 81% of the patients with TP53-positive tumors whereas only 52% of these patients had ≥ 5 CTCs in the PB. However, the ctDNA levels have shown no prognostic impact on the time to progression (TTP) or OS whereas CTCs counts were associated with a shorter OS ($p = 0.04$).

Beyond ctDNA detection in the plasma of cancer patients in order to monitor disease dynamics, some groups were able to trace ctDNA mutations relevant to anticancer treatment resistance [38–40]. Murtaza et al. [38] demonstrated in their study on 6 patients with advanced ovarian, breast and lung cancers that a high incidence of specific ctDNA alterations is associated with acquired drug resistance: A truncating mutation in the gene coding for mediator complex subunit 1 (MED1) was found in an MBC patient with progressive disease following treatment with tamoxifen and trastuzumab, and a splicing mutation in the growth arrest-specific 6 (GAS6) gene was identified in the same MBC patient following subsequent treatment with lapatinib. Sefrioui et al. [39] demonstrated that the mutation in the estrogen receptor 1 (ESR1) gene that is responsible for resistance to endocrine therapy in breast cancer patients can be traced in ctDNA and possibly predicts disease progression.

In summary, ctDNA analysis has a great potential in monitoring disease dynamics and treatment response in MBC. However, the major challenge of this method is the low sensitivity, specificity,

Table 1. Potentials and limitations of liquid biopsy

Potentials of liquid biopsy	Limitations of liquid biopsy	
	CTCs	ctDNA
Noninvasive and simple tissue assessment	rare event	necrotic or apoptotic tumor cells
Easy repetition	epithelial cell-based detection methods	no standard detection method
Early detection of minimal residual disease	clinical validation required	
Blood-based tumor profiling on DNA, RNA, and protein levels	potential selection of irrelevant subclones	
Real-time disease monitoring	discordance between expression profiles of clinically predominant metastases and biomaterial in liquid biopsy	
Prediction of treatment response	financial effort for analysis (e.g. NGS)	
CTC = Circulating tumor cell, ctDNA = circulating tumor DNA, NGS = next-generation sequencing.		

and lack of standardization of existing approaches, which may lead to discordant results [41]. Moreover, the question why ctDNA, mostly released from dying tumor cells, should carry information crucial for understanding resistant tumor cell populations still needs to be answered [20]. The potentials and limitations of liquid biopsy are summarized in table 1.

Clinical Investigations Regarding the Utility of Liquid Biopsy for Treatment Decisions in MBC

While the clinical relevance of ctDNA diagnostics remains to be clarified, CTC detection has proven its prognostic significance in large clinical trials [7, 42]. Since CTC positivity predicts worse clinical outcome and changes in CTC counts seem to reflect therapy response, the question has been raised whether MBC patients can benefit from treatment decisions based on the CTC dynamics. The first study initiated in order to clarify this issue is the Southwest Oncology Group (SWOG) S0500 trial (NCT00382018). In this phase III randomized trial, patients with advanced breast cancer and persistently high CTC counts after 3 weeks of first-line chemotherapy (≥ 5 CTCs/7.5 ml of blood) were randomized between switching to an alternative treatment and continuing current therapy until the clinical evidence of disease progression [43]. First results of this trial were presented at the San Antonio Breast Cancer Symposium 2013 and showed no improvement in OS of patients who switched to a new treatment regime based on CTC persistence. However, the study confirmed the strong prognostic impact of CTCs: The median OS reached 35 months in patients with initially low CTC counts, 23 months in patients with CTC decrease under the therapy, and 13 months in patients with persistently high CTC counts. Thus, patients with CTC persistence under the cytotoxic treatment might represent a chemoresistant population that requires alternative treatment approaches [43]. Another, currently ongoing trial evaluating whether treatment decisions in MBC can be guided by CTC is the CirCe01 study by the Institut Curie, France (NCT01349842). In this multicenter, randomized, phase III trial, treatment response in CTC-positive MBC patients progres-

sive after 2 lines of chemotherapy is being evaluated by conventional clinical and radiological assessment versus by determination of CTCs. Patients without significant CTC decrease after the first cycle of a new chemotherapy will be switched to an alternative regime, which will also be evaluated by CTCs after the first cycle. Both studies attempt to demonstrate that patients with persistently high levels of CTCs under cytotoxic therapy should be switched from this treatment at an early time point in order to avoid inefficient and toxic chemotherapies. First results of CirCe01 are expected in 2018 [44]. The question whether the choice between chemotherapy and endocrine therapy in HR-positive MBC patients might be driven by CTC counts has been addressed by the STIC-CTC trial of the Institut Curie, France (NCT01710605). In the standard arm of this randomized, phase III study the treatment decision will be made by clinicians, whereas in the CTC arm the treatment decision will be driven by CTC counts: endocrine therapy if CTC count < 5 CTCs/7.5 ml PB or chemotherapy if CTC count ≥ 5 CTCs/7.5 ml PB.

The worldwide largest trial design for liquid biopsy using CTCs in MBC is the DETECT study concept. Treatment decisions based on the presence and phenotype of CTCs are investigated in this multicenter study. Women with HER2-negative MBC are screened for CTCs using the CELLSEARCH system (Janssen Diagnostics). In patients with at least 1 CTC, the HER2 status of each CTC is determined. Patients with 1 or more HER2-positive CTCs are included in the DETECT III trial (NCT01619111); women with only HER2-negative CTCs are eligible for DETECT IV (NCT02035813). DETECT V/CHEVENDO (NCT02344472), recently started in September 2015, completes the DETECT study program with a clinical trial for HER2-positive MBC patients.

In DETECT III, patients are randomized 1:1 to standard therapy plus or minus additional treatment with lapatinib. The standard endocrine or standard chemotherapy is chosen according to the physician's choice. DETECT IV offers 2 different treatment cohorts, with everolimus and eribulin as study medication. Postmenopausal women with HR-positive tumors are treated with everolimus plus endocrine therapy (anastrozol, letrozol, tamoxifen, or exemestane). Patients with HR-positive tumors and the need for

Table 2. Current studies on CTC-based treatment decisions in MBC

Study	Status	Estimated enrollment	Condition	Intervention	Primary endpoint
SWOG S0500, NCT00382018 (phase III)	active, not recruiting	561	CT-resistant, CTC-positive MBC	treatment choice according to clinical and radiological criteria vs. CTC-driven treatment choice	OS
CirCe01, NCT01349842 (phase III)	recruiting	568	CT-resistant, CTC-positive MBC	treatment choice according to clinical and radiological criteria vs. CTC-driven treatment choice	OS
STIC-CTC, NCT01710605 (phase III)	recruiting	1,000	HR+/HER2- MBC	clinicians choice vs. CTC-driven choice between CT and ET	PFS
DETECT III, NCT01619111 (phase III)	recruiting	120	HER2- MBC, HER2+ CTCs	standard therapy ± lapatinib	CTC clearance
DETECT IV, NCT02035813 (phase II)	recruiting	520	HER2- MBC, HER2- CTCs	ET + everolimus (DIVa) or eribulin (DIVb)	PFS
NCT01975142 (phase II)	recruiting	480	HER2- MBC, HER2+ CTCs	T-DM1	tumor response rate

CT = Chemotherapy, CTC = circulating tumor cell, ET = endocrine therapy, HER2 = human epidermal growth factor receptor 2, HR = hormone receptor, MBC = metastatic breast cancer, OS = overall survival, PFS = progression-free survival, T-DM1 = trastuzumab-emtansine.

more aggressive treatment or patients with triple-negative tumors are included in the eribulin cohort. Patients with HR-positive, HER2-positive MBC are randomized 1:1 in DETECT V/CHEV-ENDO to a dual HER2-targeted therapy with pertuzumab and trastuzumab either in combination with chemotherapy (docetaxel, paclitaxel, capecitabine, vinorelbine) or endocrine therapy (fulvestrant, tamoxifen, letrozol, anastrozol, or exemestane).

In DETECT III and DETECT IV, the presence of CTCs is mandatory for study inclusion and changes in CTC counts during therapy are evaluated as study endpoints. As another objective, e.g., the benefit of an additional anti-HER2-targeted therapy in women with HER2-negative MBC and HER2-positive CTCs is analyzed in DETECT III, DETECT IV, and DETECT V/CHEV-ENDO. In contrast to previous evaluations of safety and tolerability, DETECT V/CHEV-ENDO emphasizes the importance of quality of life in MBC patients. A modified adverse event score and the 'quality-adjusted time without symptoms and toxicity' (Q-TWiST) method is used for assessing the value of life time.

The accompanying translational research projects try to generate additional knowledge of CTCs, their tumor biology and predictive value for cancer therapy, using methods like single-cell analysis, SNaPshot technology, and NGS.

In the DETECT III and DETECT IV trials, mutations of the phosphatidylinositol 3-kinase (PI3K)/Akt signaling pathway and the mutation status of ESR1 and the androgen receptor are analyzed. Solvent markers like tissue inhibitor of metalloproteinase 1 (TIMP-1) and carbonic dehydrase 9 (CA IX) or various circulating microRNAs (miRNA-125a/b, miRNA-18a/b) are also part of translational research projects. In addition, the roles of transcription fac-

tors in EMT and the value of DNA damage and repair markers for the prediction of treatment response to eribulin are assessed.

The value of CTC changes during therapy, determined by repeated sampling during therapy and interpretation of their dynamics, is assessed within the translational research program of all DETECT studies. Furthermore, the identification of potential targets for more individualized treatment options might improve the utility of CTCs in clinical routine. Especially in the DETECT V/CHEV-ENDO trial, research projects are mainly focused on CTCs and their use for the prediction of treatment response. Current studies on CTC-based treatment decisions in MBC are summarized in table 2.

Conclusion

Liquid biopsy in MBC represents an innovative technique for sampling circulating biomarkers. Information based on the detection and analysis of CTCs, ctDNA, and additional markers may provide crucial information for appropriate breast cancer treatment and individualized targeted therapy. Risk assessment and monitoring of treatment responses allow personalized therapy strategies. Further translational research is needed to obtain more detailed knowledge of circulating biomarkers and their clinical implications for daily routine.

Disclosure Statement

The authors declare no conflicts of interest.

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