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## HER2 as marker for the detection of circulating tumor cells

Volkmar Müller · Klaus Pantel

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### Background and potential clinical impact of the study

In breast cancer patients, the onset of subclinical tumor cell spread is the putative precursor stage of solid metastases and can be assessed with the detection of disseminated tumor cells (DTC) in bone marrow samples or circulating tumor cells (CTC) in the blood by immunocytochemical and molecular techniques. Sequential peripheral blood analyses should be more acceptable than bone marrow aspirations and many research groups are currently assessing CTC with different methods. In patients with no clinical signs of overt metastases, detection rates range from 10 to 100% [1], (Table 1). The detection of CTC is of potential clinical relevance in the context of a growing number of therapeutic options, especially in the adjuvant setting when no tumor is present. In contrast to patients with metastatic disease, less information about the prognostic relevance of CTC in the blood of patients with early-stage disease is available [2]. In this context, the study published by Apostolaki and coworkers in this issue of Breast Cancer Research and Treatment is of importance [3]. The authors use a nested RT-PCR assay for HER2 mRNA to

detect CTC in patients with primary breast cancer after surgery and before the start of systemic treatment. With this method, they detect CTC in 24.5% of 216 patients. The detection of a positive mRNA signal for HER2 is an independent prognostic factor for disease free and overall survival. No correlation of CTC detection by HER2 mRNA and HER2 status of the primary tumors was observed.

In metastatic patients, it has been demonstrated that patients with HER2 negative primary tumors show HER2 positive CTC. Moreover, a subset of these patients were treated with trastuzumab and showed clinical response [4, 5]. Since the number of patients included in these studies were small, no conclusive data have been obtained. The intriguing perspective of this study is on one hand the potential clinical impact since HER2 is a prominent therapeutic target in breast cancer and on the other hand the biologic hypothesis supported by this study: HER2-positive cells are more frequently disseminated from the primary tumors. When looking at the seemingly high frequency of HER-positive CTC here one has to bear in mind that HER2 is usually not overexpressed or amplified in all cells of a primary tumor, even in those tested as “positive”. In addition, the applied RNA-based test is not able to detect a heterogeneity in HER2 expression between different CTC (i.e., the concomitant presence of HER2-positive and HER2-negative CTC in one sample), which was, however, observed by the authors of this study when performing an immunocytochemical or FISH analysis in a small subset of patients tested HER2 positive by RNA.

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V. Müller  
Department of Gynecology, University Medical Center  
Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg,  
Germany  
e-mail: vmueller@uke.uni-hamburg.de

K. Pantel (✉)  
Institute of Tumor Biology, University Medical Center  
Hamburg-Eppendorf, Martinistrasse 52, 20246 Hamburg,  
Germany  
e-mail: pantel@uke.uni-hamburg.de

### Open questions and further perspectives of the study

Despite adding important new information to the field of CTC detection, several questions arise from this study.

**Table 1** Examples of studies for CTC detection in early breast cancer

Author, Reference	CTC detection rate	Clinical relevance	Marker	Method
Apostolaki et al. [3]	53/216 (24.5%)	DFS, OF	HER2	RT-PCR
Stathopoulou et al. [7]	44/148 (30%)	DFS, OS	CK19	RT-PCR
Jotsuka et al. [14]	30/100 (30%) preoperative 14/100 (14%) postoperative	RFS	CEA	RT-PCR
Ignatidis et al. [15]	CK19: 63/185 (34%) CK19+HER2: 33/185 (18%)	DFS; CK19+HER2; OS: CK19	CK19– and HER2, CK19, HER2	RT-PCR
Ignatidis et al. [16]	181/444 (41%)	DFS; OS	CK19	RT-PCR
Ignatidis et al. [17]	72/175 (41%) CK19 14/175 (8%) mammaglobin 50/175 (29%) HER2	DFS, CK19 mammaglobin; HER2; OS, CK19, mammaglobin	CK19, mammaglobin A and HER2	RT-PCR
Wiedswang et al. [18]	34/341 (10%)	DFS; BCSS	Pan-CK	ICC
Wülfing et al. [19]	17/35 (49%)	DFS; OS	CK+HER2	ICC
Pierga et al. [11]	32/118 (27%) pre and/or post PST	DMSF	Pan-CK	ICC
Pachmann et al. [13]	91/91 (100%)	RFS	EpCAM	LSC

ICC immunocytochemistry, CK cytokeratin, OS overall survival, DFS disease-free survival, BCSS breast cancer-specific survival, DMFS distant metastasis-free survival, LSC laser scanning cytometry, EpCAM epithelial cell adhesion molecule, RFS relapse-free survival RT-PCR reverse-transcriptase polymerase chain reaction

Does systemic treatment eliminate CTC detected with this method? It would be of interest to see the clinical impact of CTC changes induced by treatment. The authors have published the results for CTC determination after treatment using the same method in this cohort and also demonstrated a prognostic impact [6]. Therefore, information about the effect of systemic treatment on CTC should be available.

Is this method suitable for monitoring of anti-HER2 treatment? None of the patients was treated with trastuzumab. Potentially, the method described here should be able to monitor the effects of treatment directed against HER2.

How does the detection of HER2 mRNA correlate with CK19 mRNA that was used by the group in earlier studies [7, 8]? It is of interest to see if the two markers correlate and their combined use could improve the clinical impact.

Is the method usable for multicenter studies? One requirement for the implementation of a test into clinical routine is the stability of samples during shipment and also the reproducibility of the test in different labs as previously shown for the CellSearch™ system [9]. To our knowledge, this has to be demonstrated for this test.

How do different tests for the detection of CTC currently available correlate with respect to their results and prognostic impact? The comparison of different tests on the same patient cohorts is in our opinion one of the major tasks for future research. Using the FDA-approved CellSearch™ system for CTC detection, blood from 1,500 patients before and after adjuvant chemotherapy was examined by immunocytochemistry in the German SUCCESS-trial. In 10% of patients more than one CTC was

detected before the start of systemic treatment. After completion of chemotherapy, 9% of patients presented with >1 CTC. While the presence of CTC before systemic treatment did not show prognostic relevance, persistence of CTC after chemotherapy was a significant predictor for reduced disease free and overall survival [10]. Pierga et al. [11] monitored CTC counts in 118 patients with large operable or locally advanced breast cancer before and after primary systemic chemotherapy, and showed that the presence of CTC after a short follow-up time of 18 months was an independent prognostic factor for shorter metastasis-free survival. Interestingly, they did not find a significant correlation for response of the primary tumor to chemotherapy, which is usually used as an indicator for long-term treatment outcome.

At present, there is no gold standard for the detection of CTC and no direct comparison of different techniques on a larger cohort of patients exists. A higher detection rate might improve the clinical application of a test. Recently, a microfluid platform called the “CTC-chip”, which consists of an array of anti-EpCAM antibody-coated microposts capable of capturing CTC from unfractionated blood under controlled laminar flow conditions, was presented, and revealed CTC in almost all cancer patients analyzed [12]. CTC counts 2–3 log units higher than those found by other groups were reported by Pachmann et al. in nearly 100% of breast cancer patients using the MAINTRAC™ assay [13]. However, the specificity of these “ultra-sensitive” techniques need to be further validated in ring experiments using international consortia specialized ob CTC/DTC detection such as the European DISMAL working group ([www.dismal-project.eu](http://www.dismal-project.eu)).

## Conclusion

This study demonstrates the clinical impact of HER2 as RNA-based marker for the detection of CTC in patients with early stage breast cancer. For the future implementation of this and other tests into clinical practice, clinical trials that are aimed to improve the treatment of breast cancer patients by guiding treatment decisions with CTC detection need to be initiated.

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