# Editorial on "Gene expression signatures in circulating tumor cells correlate with response to therapy in metastatic breast cancer"

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Breast cancer recurrences, especially in patients with estrogen receptor (ER)-negative disease occur within the first 5 years after diagnosis (1). Several therapeutic options are available for the treatment of metastatic breast cancer, such as surgery, radiotherapy, hormone treatment, chemotherapy as well as targeted therapies (2). However, it would be very important to predict which patients will benefit from each treatment, since several patients experience significant treatment-related adverse events without any clinical benefit.

Analysis of CTC represents a powerful and diagnostic real-time liquid biopsy tool that provides to the physicians the assessment of the tumor burden and the efficacy of treatment (3). The frequency of CTC in whole blood is very low and their isolation and detection still remains a great challenge. Most of the isolation procedures of CTC are mainly based on the expression of EpCAM protein on the surface of the cells. Until now, the Cell Search is the only FDA-cleared technology for the isolation of EpCAM(+) CTCs and their subsequent enumeration (4). Other isolation procedures depend on the expression of specific cancer markers or the size of the cells and their deformability or, finally, based on cellular functions (5).

The published RT-qPCR assays for the quantification of the mRNA of specific cancer genes and, thus the detection of CTCs, suffer from the lack of consensus of their performance (6). According to the MIQE Guidelines of Real-Time PCR Experiments, better experimental practice allows more reliable and unequivocal interpretation of qPCR results (7). However, the availability of guidelines has done very little to improve the quality of published data based on the use of RT-qPCR and raise the question whether the situation must be like for the much more complex technologies based on it (8).

In this issue of *Clinical Chemistry*, Bredemeier *et al.* described a 46 multimarker gene PCR assay to characterize CTCs from MBC patients undergoing palliative therapy and predict treatment response on the basis of gene expression at 2 consecutive clinical time points (TP1 and TP2) compared to their expression at baseline. The multimarker RT-PCR assay was developed in order to evaluate whether it might have better prognostic accuracy, since simple RT-PCR may have limitations because the used markers might be expressed not only in CTCs but also in normal blood cells. The pre-analytical and analytical conditions of the assay were carefully controlled by using the AdnaCollectTM tubes (QIAGEN) for the blood collection, which stabilizes blood cells in a blood sample at 4–10 ℃ for 24 h, allowing the transportation or the storage of the sample as well as 2 different algorithms to find the most optimal normalization gene among 5 candidates control genes and selected the ACTB gene. These are very critical points for the calculation of the relative RNA amount and the validity of an assay (9-11). Bredemeier *et al.*

defined a sample as CTC if at least one of the used markers (EpCAM, ERBB2, MUC1, or KRT19) was expressed. Most of the CTC+ samples expressed two of these genes, while *KRT19* was uniquely expressed in some cases. KRT19 is a member of the keratin family, which is intermediate filament protein responsible for the structural integrity of epithelial cells (12). The expression of the mRNA of *KRT19* of CTC has been shown to be associated with predictive (13) and prognostic implications (14) in breast cancer patients. Moreover, the authors have shown that *KRT19* is highly expressed in patients who failed to respond (NR and ONR) to treatment. These results are consistent with previous findings showing that CTC expressing *KRT19* after adjuvant chemotherapy is chemotherapy-resistant (15). Xenidis *et al.* has shown that the elimination of *KRT19* mRNA-positive CTCs during taxane-based chemotherapy seems to be an efficacy indicator of treatment and is related to a better clinical outcome (16). Concerning the specificity of the expression of the mRNA of *KRT19*, the authors tested 20 healthy donors (HD) and detected *KRT19* expression in 2 of them. This fact raises an issue, about the design of the primers used for evaluation of the *KRT19*-mRNA expression in the RT-qPCR, since *KRT19* presents high alignment with 2 pseudo genes. Stathopoulou *et al.* (17) has shown that the design of the primers and probes is very important for the accurate detection of the *KRT19*-mRNA (+) CTCs.

An interesting finding of the Bredemeier's study concerns the observation that both epithelial and EMT or stemness markers were always co-expressed in CTCs. This is in agreement with studies from several research groups, which have demonstrate that the pool of CTCs is consisted from different CTC sub-population which may be hybrids with epithelial and mesenchymal characteristics (18,19). Nevertheless, it should be mentioned that in late metastatic cancers, the isolation of CTC based on the expression of EpCAM protein, could not be feasible, because of the prevalence of the mesenchymal phenotype (20). It has been previously reported that CTCs bearing both an EpCAM- and EMT-associated phenotype have been associated with metastatic disease (21). This clinical correlation clearly validate the importance of the developed 46 multimarker gene PCR assay for the detection of CTCs and emphasizes the need of capturing CTCs not only based on EpCAM expression but also in combination with other markers.

The Bredemeier's *et al.* study also revealed that 14 genes (*KRT19*, *FLT1*, *EGFR*, *EPCAM*, *GZMM*, *PGR*, *CD24*, *KIT*, *PLAU*, *ALDH1A1*, *CTSD*, *MKI67*, *TWIST1*, and *ERBB2)* were differentially expressed between the CTC+ and CTC– samples but only the *ADAM17* and *ABCC1*genes were differentially expressed in the NR or ONR groups. Although this finding could be related to the fact that some samples were CTC–, based on the expression of the 4 genes, CTCs might expressed other epithelial cancer markers. Therefore, the emerged interesting predictive value of the ADAM17- and ABCC1-expressing CTCs merits to be further evaluated in a prospective and larger cohort of patients with metastatic breast cancer in order to validate their clinical relevance.

The heterogeneity of CTC constitutes the cornerstone of their successful isolation and detection. Molecular characterization of CTC provides their biological specificity and, thus, their characterization based on mRNA gene profile (22), DNA mutation status (23), DNA methylation (24) and chromosomal aberrations (25) of extreme biological and clinical importance. Therefore, the combination of the phenotypical and molecular identity of CTCs could enlighten us about the aggressive and malignant nature of these cells.

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## Footnote

*Conflicts of Interest:* The authors have no conflicts of interest to declare.

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