Abst. # 413

CLINICAL UTILITY OF THE CELLSERACH™ SYSTEM TO PREDICT RADIOLOGICALLY CONFIRMED FAILURE OF SYSTEMIC THERAPY IN PATIENTS WITH METASTATIC BREAST CANCER: A SINGLE-INSTITUTION EXPERIENCE

CHRISTIAN M. KURBACHER[1,2,4], SUSANNE DEXEL[2], ULRIKE SCHWEEN[2], JUTTA A. KURBACHER[1], WOLFGANG J. NAGEL[1,2], R. REICHELT[2]

[1]Gynecologic Center Bonn-Friedensplatz; [2]L.a.n.c.e. Inc., Bonn, Germany
[3]Faculty of Medicine, University of Cologne, Cologne, Germany

BACKGROUND

During the last decade, an increasing body of evidence have been emerged suggesting the occurrence of circulating tumor cells (CTCs) is a negative prognostic factor in patients (pts) with various epithelial neoplasms including metastatic breast cancer (MBC). Moreover, serial CTC measurements have also been successfully used to monitor various antineoplastic therapies (Tx) in pts suffering from MBC. Currently, the CellSearch™ technology (CS, Veridex, Raritan, NJ, USA) is the only FDA-approved technique for CTC detection in pts with either MBC or other metastatic carcinomas (i. e. colorectal and prostate cancer). Its high acceptance is mainly due to the fact that Tx monitoring by CS allows to discriminate potential responders and no-responders earlier than any other diagnostic means. Therefore, CS has already been incorporated in many phase II and phase III for both BC and PC. CS is an immunomagnetic technique which uses the epithelial cell-adhesion molecule (EpCAM) as the primary target to capture CTCs which are then further characterized by expression of cytokeratines (CK), and various cell surface antigens such as HER-2/neu and others. Contrasting competing methods, CS is characterized by both a high specifity and a high positive predictive value offering an exceptionally low rate of false positive results. In pts with metastatic CRC, > 3 CTCs found in 7.5 µL peripheral venous blood is considered a positive result which is > 5 CTCs in all other indications. However, studies with CS in primary BC pts subjected to both neoadjuvant and adjuvant chemotherapy showed that the detection of only 1 CTC in 7.5 mL blood was associated with an impaired survival. Despite unequivocal prognostic and predictive merits, there is only limited information published so far, elucidating the value of CS in the routine oncologic setting. We hereby report on our single institution experience with the CS system used to monitor the clinical course of unselected MBC pts subjected to various antineoplastic treatments within an non-interventional study (NIS). A special focus of this study was laid on the ability of CS to early predict a Tx failure subsequently confirmed by radiological tumor imaging.

METHODS

In this study, both isolation and counting of CTCs were performed by using CS. This technology provides the immunomagnetic selection, fluorescence staining, concentration, and enrichment of CTCs. A total of 7.5 mL peripheral venous blood was collected in a CellSave™ Preservation Tube (Veridex), prefilled with an optimized EDTA-based preservative that stabilizes cells at room temperature for up to 96 hours. Another 7.5 mL sample which was taken simultaneously served as a backup. Immunomagnetic enrichment was performed automatically by using the anti-EpCAM Ferrofluid™ (Veridex). Isolated cells were then labeled fluorescently with the 4′,6-diamidino-2-phenylindole (DAPI) nucleic acid dye and with monoclonal antibodies detecting CKs 8/18/19 and CD45. CTCs were identified as cells with the appropriate morphology as cytokeratin positive, DAPI positive, and CD45 negative. CTC-negativity and –positivity were distinguished using a threshold of > 5 CTCs in 7.5 mL blood peripheral blood.
Until now, 51 pts systemically treated for MBC have been included into this NIS. Patients´ characteristics can be obtained from Table 1. Generally, this non-selected group of pts represents the whole population of MBC pts normally seen in Western Europe quite well apart from the fact that premenopausal pts were found to be somewhat over-represented. In brief, 24 pts (47,1%) were pre- or perimenopausal whereas the remainder (52,9%) were postmenopausal. A total of 36 cases (70,6%) were considered to have estrogen dependent disease. In 11 pts (21,6%), HER2 overexpression and/or amplification was detected by either immunohistochemistry or FISH. All pts on study were subjected to various systemic treatments, including endocrine Tx (HTx; n=17), chemotherapy (CTx; n=13), and targeted Tx (TTx; n=3). Multimodal Tx (MTx) was given to another 18 pts with 16 cases receiving combined Ctx and TTx and another 2 pts getting combined HTx+TTx. All systemic antineoplastic treatments were monitored by two subsequent CS analyses performed prior to and 6-8 weeks after Tx initiation. Accordingly, radiological tumor imaging was performed prior to and 12 weeks after start of Tx. The radiological response status was determined according to RECIST. In pts free from progression, additional radiological examinations were performed every three months, or at any other time upon the physician´s discretion (Figure 1). Treatment failure by CS was assumed in pts others than those showing throughoutly normal CTC counts or who did normalize 6-8 weeks after the start of therapy. Treatment failure by imaging was considered in all pts showing a clear evidence disease progression according to RECIST at time of the first radiolical reevaluation. The ability of CS to predict radiologically confirmed treatment failure was determined by calculating sensitivity, specifity, positive and negative predictive value.

RESULTS

In all individuals included in this trial, both subsequent CS analysis were found to be evaluable, resulting in an assay success rate of 100%. In regard to the result of the first radiological tumor re-evaluation performed 12 weeks after the start of Tx, 29 pts responded or experienced at least disease stabilization. In contrast, the ramaining 22 pts failed to benefit from Tx by showing disease progression. As shown in Figure 2, all but one progression-free pts. showed constantly normal or declining CTC counts during Tx. In only three pts out of this group, the CS results did not completely normalize although throughoutly improving. One patient without progression had a slight CTC increase within the normal range. In contrast to these findings, 16 of 22 pts with progressive disease had increasing CTC counts. Additionally, 3 pts out of this group did not normalize although showing declining CTC counts during Tx. Notably, a CTC increase within the normal range indicated progression in 6 cases whereas a decrease within the normal range was associated with response to Tx in 10 individuals. Analyzing the ability of CS to predict disease progression, the sensitivity was 63.8%, the specifity was 89.7%. The positive-predictive value was 82.4% and the negative-predicitve value was 76.5% resulting in a predictive accuracy of 78.4% (Table 2). It seems to be of particular interest, that 4 pts with HER2-negative disease were found to have HER2-positive CTCs prior to start Tx. All these pts were subjected to anti-HER2 TTx with either Trastuzumab or Lapatinib and all of them were found to be free from progression at time of the first radiological re-evaluation. All these pts experienced a decline of CTC counts while being on Tx. Moreover, the proportion of HER2-positive CTCs decreased during the course of Tx in all 4 individuals strongly arguing in favor of a therapeutic effect on the anti-HER2 drugs in these cases.

CONCLUSIONS

- The FDA-approved CellSearch™ technology is a valuable and robust tool to determine circulating tumor cells in the peripheral blood of cancer patients in the routine setting
- The assay evaluability rate in this study was 100%, demonstrating the methological robustness of CellSearch™
- CellSearch™ offers a high specifity and, accordingly, a high positive-predictive value, suggesting that a cell under suspicion detected by this technology is most likely a real tumor cell
- In this unselected population of patients with metastatic breast cancer, serial CellSearch™ measurement were able to identify individuals who are unlikely to respond to the systemic treatment given
- In patients treated for metastatic breast cancer, constantly normal or declining CTC counts were associated with tumor response whereas increasing CTC counts indicated tumor progression in the majority of cases
- Changes of CTC counts within the normal were of particular interest. In our population of patients, an increase of CTCs within the normal range was associated with disease progression in six patients whereas a decrease within the normal range indicated response to therapy whatsoever in another ten individuals
- HER2 determination on CTCs may help to identify individuals with HER2-negative perimary tumors who are likely to benefit from anti-HER2 therapy
- In regard to both the high specifity and the high predictive accuracy of the CellSearch™ system and our own experiences reported herein, we conclude that the appearance of any CTC detected should be taken seriously because it may indicate subclinical metastatic tumor burden

Table 1: Patients´ characteristics

	N (%)
Menopausal status	
Pre-/Perimenopausal	24 (47.1%)
Postmenopausal	27 (52.9%)
Hormone-receptor status	
ER+ and/or PR+	36 (70.6%)
ER- and PR-	15 (29.4%)
HER2 status	
HER2-positive	11 (21.6%)
HER2-negative	36 (78.4%)
HER2-positive (CTCs only)	4 (7.8%)
Treatment	
Endocrine therapy	17 (33.3%)
Chemotherapy	13 (25.5%)
Targeted therapy	3 (5.9%)
Multimodal therapy	18 (35.3%)

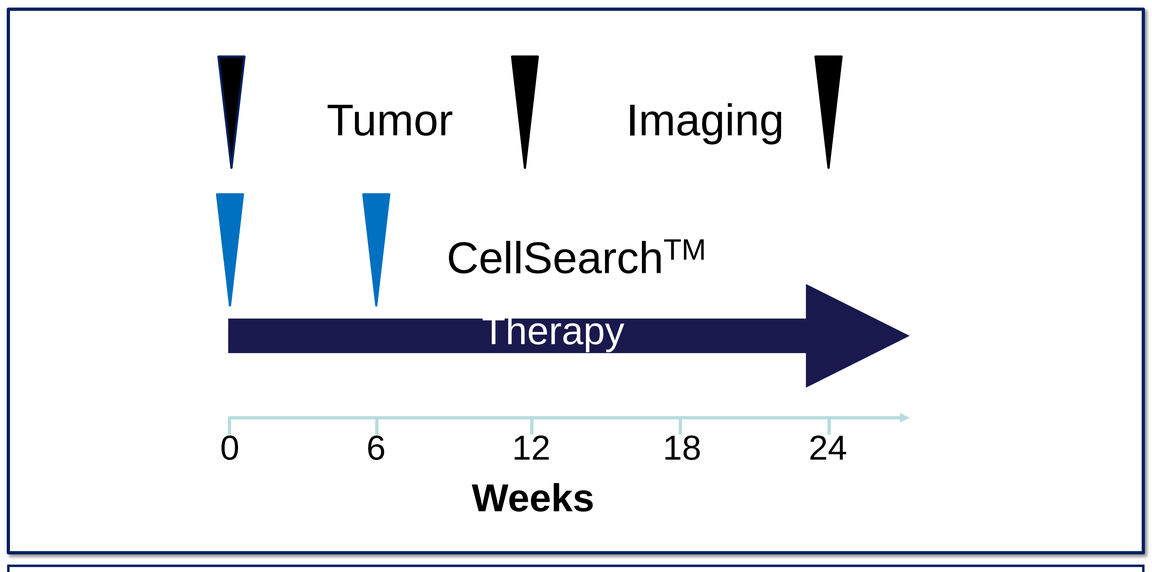

Figure 1: Evaluation scheme applied in 51 patients systemically treated for overt metastatic breast cancer

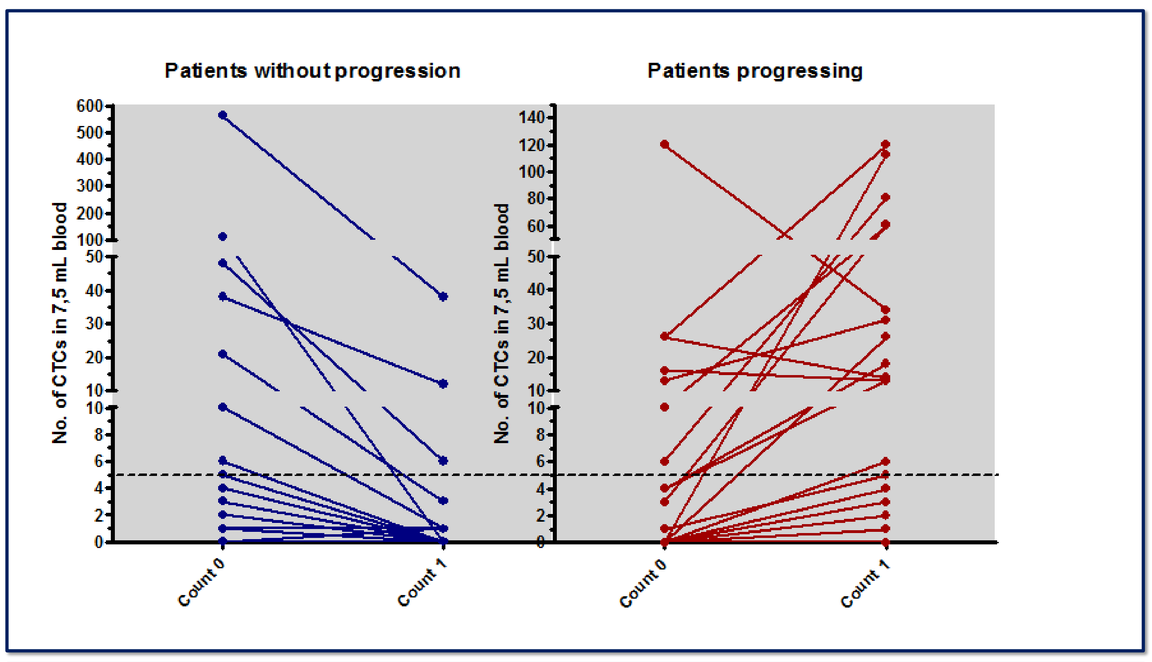

Figure 2: Results of sequential CTC counts performed by CellSearch™ in patients with metastatic breast cancer exposed to antineoplastic therapy

Table 2: Biostatistic parameters for serial CTC measurements by CellSearch™ to predict radiologically confirmed failure of systemic treatment in metastatic breast cancer

Biostatistic parameter	%
Sensitivity	63.8
Specificity	89.7
Positive-predictive value	82.4
Negative-predictive value	76.5
Predictive accuracy	78.4

Corresponding author: Christian M. Kurbacher, MD PhD, Medical Director, Head of the Department of Gynecology, Obstetrics and Gynecologic Oncology, Medical Center Bonn-Friedensplatz, Friedensplatz 16, 53111 Bonn, Germany, Phone: +49 228 22720515; Fax: +49 228 22720112; e-mail: sekretariat@medizinisches-zentrum-bonn.de