

## Identification of a stem cell marker and markers involved in epithelial-mesenchymal transition on circulating tumor cells in patients with metastatic breast cancer

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**Background:** Stem cell like tumor cells have been suggested to be the active source of metastatic spread in primary tumors. Furthermore, these cells may undergo phenotypic changes, known as epithelial-mesenchymal transition (EMT), which allows them to travel to the site of metastasis formation without getting affected by conventional treatment. Assuming that metastasis requires a dissemination of tumor stem cells or tumor cells showing EMT, it was the purpose of this study to identify these cells among circulating tumor cells (CTC) in blood samples of 28 metastatic breast cancer patients. **Materials and Methods:** 5 ml blood was analyzed for EpCAM, MUC-1 and HER-2 transcripts with the *AdnaTest BreastCancer* (AdnaGen AG). The recovered c-DNA was additionally multiplex tested for three EMT markers (Twist, Akt2, PI3K) and separately for the tumor stem-cell marker ALDH1. The analytical sensitivity was determined by the detection of a low number of target cells (5 IGROV cells spiked into 5 ml blood of healthy donors) using the *AdnaTest BreastCancer* procedure. The identification of EMT markers was considered positive if at least one marker was detected in the sample. Healthy donor samples without spiked tumor cells were used to determine the specificity of the test. The resulting PCR fragments were separated and quantified using the Agilent Bioanalyser 2100. **Results:** Applying an amplicon cut-off value of 0.2 ng/μl for Akt2, 0.15 ng/μl for Twist, 0.25 ng/μl for PI3K and 0.15 ng/μl for ALDH1, 97% of 30 healthy donor samples investigated were negative for any of the transcripts analyzed. The spiking experiments revealed a 80% recovery of the IGROV cells. CTC were detected in 12/28 (43%) cancer samples. All samples were further examined for tumor stem cell or EMT markers. In the CTC(+) group 50% were positive for at least one of the EMT markers (Twist, Akt2 or PI3K) and 42% for ALDH1. In the CTC(-) group the percentages were 19% and 12%, respectively. 3% of healthy donors tested positive for EMT and 5% for ALDH1. The expression of EMT and/or stem cell markers in CTC was correlated with the clinical follow up results: It could be shown that in CTC(+) patients the expression of TAP and ALDH1 was found in 5/12 (40%) samples of patients diagnosed as non-responders. Only 1/12 (8%) sample of the responder group expressed TAP and no positive case was found for ALDH1. In the CTC(-) group 1/14 (7%) of the non-responders was TAP/ALDH1 positive and 2/14 of the responders (14%) were positive for TAP and 1/14 (7%) for ALDH1, respectively. It remains to be seen if the sources of these markers in the CTC(-) group are actually CTC not detected by the preceding expression analysis for EpCAM, MUC-1 and HER-2.

**Conclusion:** A major proportion of CTC found in the blood of metastatic breast cancer patients shows EMT and tumor stem cell characteristics supporting the hypothesis that these markers are an indication for therapy resistant tumor cell populations and, therefore, for an inferior prognosis.