UKE Paper of the Month October 2012

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Loss of Heterozygosity at Tumor Suppressor Genes Detectable on Fractionated Circulating Cell-Free Tumor DNA as Indicator of Breast Cancer Progression

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ABSTRACT: Purpose: LOH on circulating DNA may provide tumor-specific information on breast cancer. As identification of LOH on cell-free DNA is impeded by the prevalence of wild type DNA in blood of cancer patients, we fractionated plasma DNA, and determined the diagnostic and prognostic value of both fractions. Experimental design: Our cohort of 388 patients with primary breast cancer before chemotherapy was selected from a multicenter study (SUCCESS). Postoperative plasma was fractionated in low- and high-molecular weight DNA by two different column systems. In both fractions, LOH was determined by a PCRbased microsatellite analysis using a panel of 8 polymorphic markers. Circulating tumor DNA in plasma from 30 patients after chemotherapy was additionally analyzed. The significance levels were adjusted for multiple comparisons. Results: More patients (38%) had LOH at all markers in the fraction containing short DNA fragments than in the fraction containing the long DNA molecules (28%, P ¼ 0.0001). In both fractions 32.85% of LOH were concordant. LOH at the markers D3S1605, D10S1765, D12S1725, D13S218, and D17S855 significantly correlated with tumor stage, tumor size, and lymph node metastasis, positive progesterone, and HER2 receptor status. Most importantly, LOH at D12S1725 mapping to cyclin D2 correlated with shorter overall survival (P= 0.004). Conclusions: The improved detection of LOH on cell-free DNA provides important information on DNA losses of tumor suppressor genes TIG1, PTEN, cyclin D2, RB1, and BRCA1 in breast cancer. In particular, loss of the cyclin D2 gene might become an important prognostic marker easily detectable in the peripheral blood.

STATEMENT: *To advance the identification of DNA losses on circulating blood plasma DNA and to establish a new, non-invasive tumor marker, we optimized the DNA extraction method and fractionated this DNA from blood plasma of breast cancer patients. By our DNA fractionation, the exposure of DNA losses in the plasma samples could be improved. Validating our improved method in the context of the multicenter SUCCESS trial showed that the detection of DNA losses of a panel of tumor suppressor genes was associated with a more aggressive biology of breast cancer. Thus, the improved detection of circulating tumor DNA may provide clinically relevant information on the variable biology of breast cancer. In particular, loss of the cyclin D2 gene in the blood may become an important prognostic marker.*

BACKGROUND: This work was performed at the Institute of Tumor Biology in the group of the author Heidi Schwarzenbach who habilitated in 2009. The research of her laboratory (Co-authors Corinna Eichelser, Jolanthe Kropidlowski) has been focused on the characterization and quantification of cell-free nucleic acids in the blood of patients with different tumour types. Klaus Pantel, director of the Institute, has pioneered the field of micrometastasis research. The plasma samples were collected from breast cancer patients participating in a multicenter study (SUCCESS), which includes 251 German centers and is supervised by Wolfgang Janni and Brigitte Rack. This work was funded by the Deutsche Krebshilfe.