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Frequent detection of *PIK3CA* mutations in single circulating tumor cells of patients suffering from HER2-negative metastatic breast cancer

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ABSTRACT

Modern technologies enable detection and characterization of circulating tumor cells (CTC) in peripheral blood samples. Thus, CTC have attracted interest as markers for therapeutic response in breast cancer. First studies have incorporated CTC analyses to guide therapeutic interventions and stratification of breast cancer patients. Aim of this study was to analyze characteristic features of CTC as biomarker for predicting resistance to HER2-targeted therapies. Therefore, CTC from metastatic breast cancer patients with HER2-negative primary tumors screened for the prospective randomized phase III trial DETECT III were explored for their HER2 status and the presence of *PIK3CA* mutations. Detection and characterization of HER2 expression of CTC were conducted with the CellSearch® system. Fifteen of 179 CTC-positive patients (8.4%) contained ≥1 CTC with strong HER2 expression. Genomic DNA from individual CTC isolated by micromanipulation was propagated by whole genome amplification and analyzed for *PIK3CA* mutations in exons 9 and 20 by Sanger sequencing. One or more CTC/7.5 mL were detected in 179/290 patients (61.7%). In 109 patients (34.8%), ≥5 CTC/7.5 mL were found. We detected at least one CTC with the mutation p.E542K, p.E545K, p.H1047R, p.H1047L or p.M1043V in 12/33 patients (36.4%). Thirty six of 114 CTC (31.6%) harbored one of these mutations. CTC in individual patients exhibited heterogeneity concerning *PIK3CA* mutations and HER2 expression. In

Abbreviations: CTC, circulating tumor cells; mBC, metastatic breast cancer; FISH, fluorescence in situ hybridization; *PIK3CA*, phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha; WGA, whole genome amplification.

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conclusion, clinically relevant genomic aberrations such as mutations in the hotspot regions of exon 9 and 20 of the *PIK3CA* gene can be detected in single CTC and might provide insights into mechanisms of resistance to HER2-targeted therapies.

1. Introduction

To improve cancer therapy, current research efforts are directed to develop targeted therapies to ensure highly efficient personalized treatment without severe side effects. In this context, strategies influencing e.g., epidermal growth factor receptor HER2-and hormone-dependent tumor growth have already become part of standard treatment schedules for breast cancer patients (Van Poznak et al., 2015).

Currently, treatment decisions are guided by evaluating primary tumor tissues or biopsies from single metastases for target expression. However, choosing the best suitable targeted therapy is hampered by the necessity to identify the aggressive, treatable tumor cells and the heterogeneity of target expression within a given tumor tissue. Moreover, primary tumors and recurrences or metastases can differ in the expression of these target molecules (Fehm et al., 2010; Van Poznak et al., 2015). Thus, in patients with accessible newly diagnosed metastases, also metastatic tumor tissue should be evaluated. If discrepancies in the expression of the target between primary tumors and metastasis are measured, consistent with the clinical situation and the patient's goal for care, preferentially the situation in the metastases should be used to guide therapy (Van Poznak et al., 2015). However, since it is not always possible to access metastases for biopsies and also a heterogeneity of different metastatic sites might occur, several ongoing studies investigate whether detecting and characterizing CTC in peripheral blood as a "liquid biopsy" can improve clinical care (Alix-Panabieres and Pantel, 2014; Joosse et al., 2015).

During the last decade, HER2-targeted therapies have significantly improved the outcome of breast cancer patients with HER2-positive primary tumors and metastases (Rimawi et al., 2015). As clinical relevant evidence has been provided that the HER2 status can change during breast cancer progression and thereby differ between primary tumors and metastases (Van Poznak et al., 2015), the DETECT study was aimed to re-evaluate the HER2 status by assessing HER2 expression of CTC in patients with metastatic disease (Fehm et al., 2010). This and other studies concordantly showed that HER2-positive CTC can be detected in relevant numbers of patients with HER2-negative primary tumors (Fehm et al., 2010; Wallwiener et al., 2015). Moreover, Georgoulias et al. (2012) showed for the first time that administration of trastuzumab in patients with HER2-negative primary tumors could eliminate keratin 19-positive/HER2-positive CTC, thereby prolonging disease-free survival of these patients.

Thus, several prospective large interventional studies were initiated to find out whether CTC detection and characterization from "liquid" biopsies might improve treatment strategies (Bidard et al., 2013; Schramm et al., 2016).

As one of the first interventional trials based on the assessment of CTC phenotypes, the still ongoing German multicentric phase III trial DETECT III aims to evaluate the efficacy of a HER2-targeted therapy in metastatic breast cancer (mBC) patients with HER2-negative primary tumors, but HER2-positive CTC (Schramm et al., 2016). Here, patients are treated randomized with the HER2-targeted therapy lapatinib, in combination with standard therapy versus standard therapy alone (https://clinicaltrials.gov/ct2/show/NCT01619111).

It is important to note that overcoming resistance against HER2-targeted therapy that is frequently observed during the course of treatment has become a major challenge in tumor research (Ibrahim et al., 2015; Rexer and Arteaga, 2012; Wilks, 2015). To date, there is no routine biomarker available to predict resistance to HER2-targeted therapies and to help therapy decision making when resistance occurs. However, mutations or silencing of the *PTEN* (*phosphatase and tensin homolog*) gene found in about 40% of HER2-positive breast cancers have been described to induce disease progression and resistance against HER2-targeting therapies (Burnett et al., 2015; Nagata et al., 2004; Sansal and Sellers, 2004). Moreover, there is a growing body of evidence that activation of the phosphoinositide-3 (PI3) kinase pathway, e.g., by mutations in the *PIK3CA* (*phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha*) gene plays a pivotal role in this process (Ibrahim et al., 2015; Mukohara, 2011, 2015).

Schneck et al. analyzed the entire CTC pools from patients enrolled in the DETECT III study using the SNaPshot (Hurst et al., 2009) method to search for *PIK3CA* mutations. Here, mutations in 7/44 (15.9%) patients were found (Schneck et al., 2013). Other authors investigated single CTC from mBC patients and reported strong heterogeneity in the *PIK3CA* mutational status even among CTC from individual patients (Neves et al., 2014; Pestrin et al., 2015; Polzer et al., 2014). These single CTC analyses resulted in higher detection rates of *PIK3CA* mutations; however, more information about the occurrence of these mutations in the context of therapeutic interventions is urgently needed.

Despite a considerable number of studies dealing with the analysis of HER2 expression of CTC, there is still a controversial debate about immunocytochemical approaches for the detection of HER2 overexpression in CTC. Based on the situation in tumor tissues, where FISH is the gold standard for the determination of the *HER2* status, we further aimed to analyze single CTC for *HER2* gene amplification by FISH. To test the hypothesis that mutational analysis of single CTC might be a meaningful tool to predict resistance and influence therapeutic decision making, the present study was intended to explore the