3014

Effect of Trastuzumab and Chemotherapy on Circulating Tumor Cells in Patients with Poor Prognosis Metastatic Breast Cancer.

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Background. Trastuzumab has significant activity in HER2 neu amplified metastatic breast cancer (MBC). We hypothesized that it may selectively act against circulating tumor cells (CTC) in HER2-positiveMBC. We assessed the effect of trastuzumab-based regimens on CTC in HER2-positive MBC with poor prognosis (≥ 5 CTC).

Patients and Methods. We retrospectively evaluated patients with poor prognosis MBC (baseline ≥ 5 CTC) treated with a first-line regimen consisting of trastuzumab+antimitotic agents in 11 HER2-positive patients not previously pretreated with trastuzumab, chemotherapy with antimitotic agents in 24 HER2-normal patients, and other chemotherapeutic drugs (mainly capecitabine) in other 16 HER2-normal patients. CTC were detected and enumerated using the CellSearch system (Veridex LLC, Warren NJ, USA). We evaluated the effect on CTC counts and on progression-free survival (PFS) and overall survival (OS).

Results. At a median follow-up of 16 months (range, 4 to 48), 24 patients (47%) died. All 11 HER2-positive patients treated with trastuzumab+antimitotic agents had <5 CTC during the treatment. Only 16 (67%) with HER2-normal MBC had <5 CTC with antimitotic agents (p = 0.037), and 28 (70%) with other chemotherapeutic regimens (p = 0.048). No statistically significant difference was observed between patients treated with antimitotic agents and those treated with other chemotherapeutic agents (p = 0.73). There was no difference between patients receiving polychemotherapy and monochemotherapy (p = 0.73). The median PFS was 12 months in HER2-positive patients treated with trastuzumab+antimitotic agents compared with 7 months for those with HER2-normal (p = 0.09). The median OS was not reached (>20 months) and 19 months (p = 0.034), respectively. The median PFS was 8 months in HER2-normal patients with ≥5 CTC and 4 months in those with <5 CTC (p = 0.01). The median OS was not reached (>17 months) and 9 months (p = 0.01), respectively.

Conclusions. Trastuzumab is highly effective in patients with HER2-positive MBC with poor prognosis (≥ 5 CTC). Antimitotic agents and other chemotherapy agents did not show a similar effect in HER2-normal MBC. CTC might be useful in the monitoring of poor prognosis MBC patients undergoing therapy with trastuzumab.

3015

Implementation of a Composite Score System for Evaluation of Circulating Tumor Cells in Blood of Breast Cancer Patients.

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Background: We present a highly specific immunomagnetic separation technique in combination with an improved multimarker gene panel for molecular identification and characterization of circulating breast tumor cells in blood. Here we describe the creation of a new composite score in order to manage results and avoid false positives.

Methods: Blood from breast cancer patients with primary or metastatic disease was drawn into two 10mL EDTA-tubes. The high affinity antibodies BM7 (MUC1) and VU1D9 (EpCAM) were used for immunomagnetic tumor cell enrichment. Separated cells were lysed and used for mRNA isolation and c-DNA synthesis (Qiagen®). A real-time quantitative RT-PCR approach using MESA FAST SYBR Assay (Eurogentec®) and primers selected from the UniversalProbeLibrary system (Roche AG®) for the epithelial markers cytokeratin 19 (CK19), mammaglobin 1 (MG1), epithelial cell adhesion molecule (EpCAM), baculoviral IAP repeat-containing 5 (Sur), immunosuppressive CD276 (CD276), carcinoembryonic antigen-related cell adhesion molecule 5 (CEA), HER-2, aldehyde dehydrogenase 1 family, member A1 (ALDH1), hypoxia inducible factor (HIF-1alpha) and CD44 molecule (CD44) was used for tumor cell identification and characterization. The ß-actin transcript was used for internal control and matched calibrator probes containing 2 or 10 tumor cells were used for quantitative expression analysis of tumor associated genes present in blood.

Results: Positivity rate was based on a score that consists of 2 different characteristics: Marker detection (ranking according to the number of positive markers, varies from 0 – negative - to 1 – positive - for each marker) in combination with the marker expression level (ranking according to the C(t) values and varies from 0 – less then 2 tumor cells – to 1 – more then 2 tumor cells). This composite score is based on the expression of CK19, MG1, EpCAM, Sur and CD276 and has a ranking from 0 to 10. Negative cases are classified with score 0 and 1, cases ranked with score 2 need retesting and finally scores above 2 indicate clearly positive patients. The additional surrogate markers CEA, HER-2, ALDH, HIF and CD44 are analysed in positive patients in order to obtain further information. In our group of metastatic breast cancer patients, 53% were classified as positive, 42% of the patients were negative and in 5% a retesting is needed.

Conclusion: The results of the composite score clearly show an increase of sensitivity and specificity for this assay. The implementation of this test in the routine monitoring of patients should help us to evaluate treatment response and create individualized treatment schedules.

3016

Circulating Epithelial Cells as a Tool for Monitoring Treatment Success of Primary Chemotherapy with Simultaneous or Sequential and without Trastuzumab.

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In spite of ample prognostic markers available in breast cancer, still a considerable proportion of patients with good prognostic markers suffers relapse whereas patients with poor prognostic markers may remain disease free. It would, therefore, be desirable to control, at the individual patient level, whether the applied therapy is effective. Our previous work indicates, that in cancer patients most of the epithelial cells circulating in peripheral blood (CETC) are part of the tumor and that the response of these cells reflects the response of the tumor to the applied therapies.

Therefore monitoring the decrease or increase in numbers of these cells providing a unique tool for therapy surveillance was used to monitor neoadjuvant chemotherapy in 26 her2/neu positive breast cancer patients with either IHC3+ or FISH confirmed Her2/neu positive breast cancer. Patients were prospectively analysed for the number of CETC before therapy, before each new cycle of chemotherapy and during maintenance therapy at each visit initially every three months and subsequently at more extended intervals. 1ml of blood was drawn into EDTA vials, red blood cells lysed and the white blood cell pellet stained with FITC-labelled anti-Epcam. Green fluorescent cells were detected by image analysis and dead cells excluded due to red PI fluorescence.

After an initial variable reduction of CETC during neoadjuvant chemotherapy, tightly connected to tumor reduction we regularly observed a massive release of cells from the shrinking tumor. Although these cells may not be able to settle in tumors with low metastatic potential, in 4/4 patients with Her2/neu positive tumors who did not receive trastuzumab the number of CETCs further increased after termination of therapy and surgery followed by rapid distant relapse indicating that the cells released in these patients may be highly aggressive with a high potential to settle and grow into metastases.

It is, however not clear, whether sequential or simultaneous addition of trastuzumab to chemotherapy is preferential. In our hands 65% of the patients receiving simultaneous trastuzumab showed increasing CETCs and all have suffered relapse whereas all patients with decreasing CETCs are still in complete remission indicating that in the neoadjuvant situation during maintenance therapy an increase in CETC is the earliest indicator of imminent relapse.

6/6 patients who received trastuzumab sequentially to the neoadjuvant treatment all showed decreasing numbers with all of them still without signs of disease after 4 years of follow up. Thus, trastuzumab was highly effective in this treatment and even if the tumor cells were not eliminated immediately, trastuzumab contributed to prevent them from settling and growing into metastases and CETC monitoring favours sequential trastuzumab.