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Vandetanib, a Dual Inhibitor of Vascular Endothelial Growth Factor Receptor (VEGFR) and Epidermal Growth Factor Receptor (HER1), Potentiates Anti-Tumor Effects of Combined Endocrine and Trastuzumab Treatment in Estrogen Receptor-Positive (ER+)/HER2-Overexpressing Xenografts.

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Background: Compelling evidence suggests that cross-talk between the pathways of ER and HER1/HER2 contributes to endocrine resistance. Using a xenograft model of human ER+ breast cancer cells engineered to overexpress HER2 (MCF-7/HER2-18), we have shown that tamoxifen (Tam) stimulates tumor growth resulting in de novo resistance. Anti HER1/HER2 targeted treatment overcomes this Tam resistant phenotype, though these effects are transient using single agents like the anti-HER2 antibody trastuzumab (T), thus suggesting that a more potent anti-HER therapy or simultaneous inhibition of alternative pathways is required to reverse resistance. Both ER and HER2 are known to promote angiogenesis. Vandetanib (V) (ZD6474, ZACTIMA) is an orally active, VEGFR/HER1 tyrosine kinase inhibitor that has shown both anti-angiogenic and anti tumor cell activities in different models. We thus hypothesize that in ER+/HER2+ tumors, the addition of V to the combination regimen of Tam+T would increase antitumor effect and delay onset of resistance due to inhibition of tumor angiogenesis and delivery of a more complete blockade of the HER network.

Methods: Mice bearing MCF-7/HER2-18 tumors were randomized and treated with Tam given alone or in combination with T (10 mg/Kg, bi-weekly), V (50 mg/Kg, 5 d/week), or T+V. Tumor volumes were followed weekly. Mean tumor regression (M-TR) ± SD and median time to tumor progression (M-TTP, defined by tumor doubling from its size at randomization) and 95% CIs were analyzed by Wilcoxon Rank Sum and survival analysis methods (Kaplan-Meier estimates and generalized Wilcoxon test), respectively. All statistical tests were two-sided and adjusted (Holm) for multiple comparisons.

Results: Tam monotherapy had minimal effect on tumor growth (M-TR 22%±15 SD) after which all tumors progressed rapidly [M-TTP 42 (35-56) days, 95% CI]. The addition of either T or V to Tam significantly improved tumor regression compared to Tam monotherapy (M-TR 66%±27, P=0.0012, and 67%±25, P=0.001, respectively) and significantly delayed time to tumor progression [M-TTP 91 (70-119) days, P=0.012, and 77 (63-98) days, P=0.02, respectively]. However, the combined regimen of T+V with Tam was significantly more effective than either T or V added separately to Tam, resulting in further significant tumor regression (M-TR 81%±29, P=0.0086 vs. T and 0.0012 vs. V) and a prolonged M-TTP (>154 d, P=0.02 vs. T and 0.01 vs. V).

Conclusions: This study supports the concept that the dual VEGFR/HER1 inhibitor vandetanib can improve efficacy of combined endocrine and anti-HER trastuzumab therapy in ER+/HER2+ tumors, probably due to angiogenesis inhibition and to a more potent blockade of HER family signaling and its cross-talk with ER. Clinical studies to examine this approach are warranted.

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Circulating Epithelial Tumor Cell (CETC) Monitoring during SERM and Aromatase Inhibitor Treatment for Early Detection of Patients Resistant to Tamoxifen.

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The observation, that part of breast cancer patients benefit from ovarectomy led to the realization that in these patients the estrogen receptor crucially contributes to the growth potential of malignant tumors of the female reproductive system. This has led to the development of compounds specifically binding to the estrogen receptor but without activating activity. Although this has led to an impressive improvement in relapse free survival a considerable part of patients some of which being carriers of the CYP2D6 polymorphism will not respond to this targeted treatment. Therefore, in addition to defining the subpopulation with a high probability of responding to the targeted therapy only an immediate control of the response to treatment will ultimately improve outcome.

Epithelial tumor cells circulating in peripheral blood are easily accessible and have been shown to reflect the response of the tumor to therapy. They can, therefore, be used to monitor the effect of therapy also in the adjuvant situation when the tumor has been removed by surgery.

Here we report on the application of therapy monitoring using CETCs during adjuvant hormone blocking therapy. ER positive breast cancer were prospectively analysed for the number of CETC before therapy, before each new cycle of chemotherapy and during maintenance therapy with tamoxifen of aromatase inhibitor or during switch from one principle to the other. 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.

178 patients were followed initially at quarterly intervals and later at annual intervals for their numbers of CETCs. 131 patients remained disease free during the observation interval of up to 7 years and 47 (26%) suffered distant relapse. Of the patients in complete continuous remission, 58% showed decreasing or stable CETCs whereas in the patients with relapses 73% showed increasing CETCs.

45 patients were subsequently switched to aromatase inhibitor treatment, 17 relapsed patients and 28 patients without evidence of disease. Of the relapsed patients 35% have remained progress free all with a re-decrease in CETCs during AI treatment.

Thus, gauging of CETCs can help to monitor hormone treatment and in the future contribute to early switch patients to AI if an increase in CETCs indicates resistance to tamoxifen.

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miRNA Profiling of Endocrine-Resistant Breast Tumours.

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Rationale

miRNAs are a relatively recently discovered class of molecules with major implications in cellular biology, including tumorigenesis; yet little is known about their possible involvement in the development of endocrine resistance in breast cancer. The aim of this study was to identify sub-groups of endocrine-resistant tumours with distinctive miRNA profiles.

Methods and Results

Frozen biopsies were obtained from a series of 51 breast cancers, which were either progressing primary tumours, local recurrences or contra-lateral breast lesions developed while patients received endocrine treatment. Hormonal treatment included aromatase inhibitors or tamoxifen, most of the patients reached post-menopausal age, miRNA profiles were obtained using Illumina DASL® Sentrix Array Matrix.

Un-supervised hierarchical clustering split 51 studied endocrine resistant tumours into two major groups, distinctive in their miRNA profiles. Several of miRNAs discriminating between the clusters have already been implemented in breast cancer (mir7, mir10a, mir205, mir206, mir 210). Most of the other miRNAs associated with the main tumour clusters are yet to be studied in relation to breast cancer biology (e.g. mir192, mir625, mir941, mir145 and mir512 among others). The miRNA-based tumour clusters were not clearly associated with a specific treatment or type of resistance. The miRNAs most clearly able to discriminate between tumours resistant to AIs and Tamoxifen included mir186, mir196a, mir196b and miR-594:9.1 ($p<0.05$, fold change >1.2).

Discussion

The results show heterogeneity of miRNA profiles in endocrine resistant breast cancers, for the first time suggesting that endocrine resistant tumours can be split into two major molecular sub-groups basing on their miRNA profiles. The results suggest that (i) miRNA-mediated gene expression regulation may be involved in the mechanisms of endocrine resistance and (ii) miRNA profiling allows different molecular sub-classes of endocrine-resistant tumours to be distinguished.

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Down Regulation of EZH2 Is Associated with ESR1 Upregulation and Response to Endocrine Therapy in Breast Cancer.

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Background: We have previously identified a gene signature for resistance to first-line tamoxifen therapy in advanced breast cancer. One of these genes is Enhancer of Zeste Homolog 1 (EZH1), a member of the EZH family of which EZH2 has been identified as being prognostic. Both genes are involved in transcriptional control and epigenetic memory maintenance and act as