against the extracellular domain of HER-2, which inhibits proliferation and survival of the tumour. Not all HER-2+ patients respond to Trastuzumab, although the patients have the HER-2 amplicon; over half of them become resistant to this treatment or show no response at all. Activation of the PI3K/Akt pathway has been suggested to play a role in HER-2+ patients without Trastuzumab-response. Searching for other drugs inhibiting cancer proliferation are therefore of vital importance to identify novel and combinatorial treatment strategies for HER-2+ patients.
Materials and Methods: Thirteen HER-2+ breast cancer cell lines (5 responsive and 8 non-responsive to Trastuzumab) were screened using 22 compounds targeting HER-2, the EGFR family, or HER-2 downstream signaling pathways for 5 days. The compounds were printed in 7 different concentrations with two replicates in 384 well plates, and the screenings for each cell line were done with two biological replicates. Cell viability using the CellTiter-Glo® Luminescent Viability Assay (Promega), detecting the ATP-levels, was used as an endpoint. The luminescence was measured with a MicroBeta LumiJET (Perkin Elmer). miRNA and mRNA profiling data together with copy number changes and PIK3CA mutation status are available for the same cell lines and will be used for integrative data-analysis.
Results: Drug inhibition data from four replicates for each compound were used to obtain EC_{50} (half maximal effective concentration)-values and growth inhibition curves for each cell line. Preliminary screening data show that several compounds inhibited growth of the cell lines that did not respond to Trastuzumab. Interestingly, several drugs were more efficient than Trastuzumab also for the Trastuzumab responding cell lines. Integration of the drug data together with PIK3CA mutation status and genomic profiling data from the same cell lines are ongoing.
Conclusions: Compound screening of HER-2+ breast cancer cell lines revealed that several compounds targeting the HER-2, the EGFR family, or HER-2 downstream signaling pathways are efficient for inhibiting the growth of these cancer cells. Therefore, we suggest alternative compounds for the treatment of the cells that do not respond to Trastuzumab. Finally, we believe that the integration of the genomic profiling data together with the compound screening data will lead to increased knowledge about the mechanisms of action of these drugs.

5004 ORAL

HER2-HER3 Signaling Pathway Regulates NK Cell-mediated Cytotoxicity via MHC Class I-related Chain A/ B in Human Breast Cancer Cells

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Background: HER2 and HER3 are frequently expressed in several types of cancer including breast cancer and their over-expression is associated with poor prognosis. HER2 targeting therapies are already in clinical practice since more than a decade and therapies targeting HER3 are in clinical trials. The role of HER2-HER3 signaling in tumour escape from the host immune system is however poorly understood. We previously reported that the HER2 oncogene down-regulated the expression of MHC class I, resulting in a phenotype promoting tumour escape from adaptive immunity. Here we demonstrate that HER2-HER3 signaling in breast cancer cell lines increases the expression of MHC class I-related chain A and B (MICA/B) molecules of the NK group 2 member D (NKG2D) ligand in breast cancer cell lines, resulting in enhanced sensitivity to NK cell-mediated recognition.
Material and Methods: A possible influence of HER2-HER3 signaling on MICA/B expression in human breast cancer cell lines (MDA-MB231, MDA-MB453, and T47D) was investigated. In order to assess the effect of blocking the HER2-HER3 signaling pathway, cells were either treated with siRNA of HER2 or HER3 or with inhibitors of the HER2-HER3 signaling pathways. To assess the consequences of HER2-HER3 activation, cells were either transfected with the HER3 oncogene or stimulated with the HER3 ligand NRG1-beta. NK cell-mediated cytotoxicity against tumour cells was assessed using ^{51}Cr release assay.
Results: The siRNA-mediated silencing of HER3 down-regulated MICA/B expression while transfection with a plasmid expressing the HER3 oncogene enhanced MICA/B in cell lines with high and low HER3 expression respectively. Treatment of HER3 positive tumour cells with the HER3 ligand NRG1-beta enhanced MICA/B. Among the major pathways activated by HER2-HER3 signaling, the expression of MICA/B was mainly regulated by the PI3K pathway. As expected, HER2-HER3 signaling-regulated MICA/B induced NK cell cytotoxicity in a NKG2D dependent manner.
Conclusions: We conclude that while signaling via the HER2-HER3 pathway may lead to decreased sensitivity to CTL mediated tumour elimination, this may instead lead to an enhanced recognition of the innate immune system mediated via MICA/B.

5005 ORAL

Exploratory Subgroup Analysis of the TAMRAD Phase 2 GINECO Trial Comparing Tamoxifen (TAM) Plus Everolimus (RAD) With TAM Alone in Patients With Hormone-receptor-positive, HER2-negative Metastatic Breast Cancer (mBC) With Prior Exposure to Aromatase Inhibitors (AIs): Implication for Research Strategies

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Background: In patients with mBC, resistance to hormonal therapy may be associated with activation of the PI3K/Akt pathway. RAD is an oral inhibitor of mammalian target of rapamycin (mTOR). In the previously reported TAMRAD phase 2 trial (N = 111), patients with prior exposure to AIs were randomized to receive TAM + RAD (TAM, 20 mg/d; RAD, 10 mg/d) or TAM alone (20 mg/d). Median time to progression (TTP) was 4.5 months with TAM and 8.6 months with TAM + RAD (hazard ratio [HR] = 0.53; 95% CI: 0.35–0.81). To gain insight as to which patient population may benefit the most from this strategy, unplanned exploratory subgroup analysis of this trial was performed.
Materials and Methods: HRs for TTP with TAM + RAD vs TAM alone were analyzed according to primary vs secondary hormone resistance, which was the study's only stratification variable besides the study site. Patients with primary hormone resistance were defined as having relapsed during adjuvant AI or <6 months after AI in the metastatic setting. Patients with secondary hormone resistance were defined as those who relapsed $\geqslant$6 months after adjuvant AI or responded for >6 months to AI in the metastatic setting. In addition, this analysis included the following factors: presence of liver or lung metastasis and TAM or previous chemotherapy for metastatic disease.
Results: Patients with secondary hormone resistance (n = 56) had an HR for TTP of 0.38 (95% CI: 0.21–0.71), whereas those with primary hormone resistance (n = 54) had a much smaller gain from the association (HR = 0.74; 95% CI: 0.42−1.3). HR for improvement in TTP in favor of the TAM + RAD arm was similar to the global HR in all other subgroups.
Conclusions: Patients with secondary hormone resistance may benefit more from the TAM + RAD combination than patients with primary hormone resistance. This result may have important implications for future clinical trial design.
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5006 ORAL

Eribulin Mesylate EMBRACE Study − Survival Analysis Excluding Patients Re-challenged With Therapies of the Same Class

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Background: Eribulin mesylate (Halaven™), a non-taxane microtubule dynamics inhibitor with a novel mode of action, has demonstrated prolonged overall survival (OS) in heavily pretreated patients (pts) with metastatic breast cancer (MBC) (EMBRACE study; NTC00388726; trial completed; sponsored by Eisai Ltd). It has been suggested that pts receiving treatment of physician's choice (TPC) may be less likely to gain benefit if they receive therapy with a class of agent they had previously been treated with (re-challenge), thereby favoring eribulin. This analysis excludes re-challenged pts in the TPC arm, allowing assessment of eribulin vs agents given for the first time. Eribulin vs re-challenged pts only was also assessed.
Methods: Pts (N = 762; 508 eribulin, 254 TPC) with locally recurrent or MBC who had received 2−5 prior chemotherapy regimens ($\geqslant$2 for advanced disease), including an anthracycline and a taxane (unless contraindicated) were randomized 2:1 to receive either eribulin mesylate 1.4 mg/m^2 2−5