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.
Study supported by funding from Novartis.

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

min intravenously on days 1 and 8 of a 21-day cycle or TPC (cytotoxic, hormonal, or biologic monotherapy, or supportive care only). Exploratory OS sub-analysis was carried out.
Results: Of the 254 pts enrolled in the TPC arm, 156 received a class of agent they had not previously received, and 98 had been re-challenged with a therapy of the same type. Re-challenged pts were in the following TPC groups: taxanes (n = 38/41; 92.7%), anthracyclines (n = 24; 100%), vinorelbine (n = 5/65; 7.7%), capecitabine (n = 4/45; 8.9%), hormonal therapy (n = 4/8; 50%), and other (n = 23/25; 92.0%); no-one receiving gemcitabine (n = 46) was re-challenged. When the re-challenged pts were excluded from the analysis, eribulin significantly improved OS vs TPC (HR 0.74; 95% CI 0.58, 0.94; nominal p = 0.014) with median OS of 13.1 and 10.5 months, respectively. Analysis of eribulin vs the 98 re-challenged pts in the TPC arm showed median OS of 13.1 vs 10.7 months, respectively (HR 0.92; 95% CI 0.68, 1.23; nominal p = 0.556).
Conclusions: Eribulin demonstrated OS improvement for pts with locally recurrent or MBC, even when re-challenged pts were removed from the TPC arm.

5007 ORAL

The MicroRNAs-30 Family Interferes With the Formation of Breast Cancer Bone Metastases by Targeting Osteomimetic Genes

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MicroRNAs (miRNAs), a class of endogenous small non coding RNAs have been involved in tumorigenesis and metastatic dissemination through their activity as negative regulators of gene expression. Alterations of miRNA expression have been reported in the different steps of initiation, progression and dissemination of breast tumours. Breast cancer cells preferentially invade and grow as secondary tumours in bone. To adapt and thrive in this environnement metastatic breast cancer cells express genes normally expressed by the osteoblastes and acquire a bone cell pseudo-phenotype named osteomimicry.
To investigate if miRNAs interfere with this specific steps of bone metastasis formation, we compared the expression profiles of the human breast cancer cell line MDA-MB-231 with that of a subpopulation (MDA-BO2) that metastasize to bone with high efficiency, using TaqMan Low Density Array analysis. Then, target genes of the differentially expressed miRNAs within the two cell lines were predicted by looking at sequence complementarities of the 5′ seed regions of the miRNAs within the 3′UTR of the genes using miRanda, PicTAR and TargetScan softwares. By doing so, it appeared that the miRNAs-30 family which is composed of 5 members: miRNA-30a, 30b, 30c, 30d and 30e, was substantially downregulated in MDA-BO2 cells. Therefore, the expression of these miRNAs-30 in MDA-BO2 cells were restored through retroviral transduction using the pmiR-Vec plasmid vector to perform functional studies. The mRNA expression of connective tissue growth factor (CTGF), connexin 43, integrin β3 and transcription factor runx2, which all harbor phylogenetically conserved miRNAs-30 binding sites in their 3′-UTR, was decreased in MDA-BO2 overexpressing miRNAs-30 (miRNAs-30-BO2), when compared to pmiR-Vec-BO2 clones. The expression of cadherin-11, for which we found a perfect base pairing of the miRNAs-30 “seed region” in the coding sequence, was also decreased. MDA-BO2 modified clones were inoculated into the tail vein of nude mice and bone metastases were radiographically detected and enumerated. A 50% decrease in the extent of osteolytic lesions was observed in animals bearing miRNAs-30-BO2 tumours versus mice inoculated with pmiR-Vec-BO2 clones or with MDA-BO2 wild-type clones.
These results strongly suggest that miRNAs-30 act as a regulator of breast cancer bone metastases formation.

Oral Presentations (Sat, 24 Sep, 11:15–13:20)
Breast Cancer – Early Disease

5008 ORAL

Long Term Results of Video-assisted Breast Surgery (VABS)

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Background: The breast conserving surgery and the sentinel node (SN) biopsy became to be recognized as the standard treatment for early breast cancers. We have reported about cosmetic effectiveness and lower infestation of the video-assisted breast surgery (VABS) for the breast diseases. We devised the trans-axillary retro-mammary (TARM) approach of VABS. It needs only one skin incision in the axilla and can treat any tumour even in the medial or lower side of the breast without making any injuries on the breast skin. And it can preserve skin touch sensation. We evaluated the aesthetic results and the curability of this surgical method.
Methods: We have performed VABS on 300 patients since December, 2001. The newly devised trans-axillary retromammary-route approach (TARM) was performed on 120 patients of early breast cancer, stage I and II. After endoscopic sentinel node biopsy, we elongated the axillary skin incision to 2.5 cm. We marked the surgical margin 2 cm apart from the tumour edge by injecting blue dye into subcutane and retromamma. We dissected major pectoral muscle fascia to detach retromammary tissue under the tumour. The working space was made by lifting traction sutures through the gland. We cut the proximal side of the gland vertically at dye marking points, and dissect skin flap over the tumour by tunnel method. Then we cut each sides of the gland vertically and removed it through the axillary port by using the tumour collection bag. The breast reconstruction was done by filling absorbable fiber cotton. The postoperative aesthetic results were evaluated by our original score system, ABNSW.
Results: Breast conserving surgery was performed on 286 patients (26 after preoperative chemotherapy) and skin-sparing total mastectomy on 14. We do not use the special disposable apparates. The operative cost is very low as the conventional one. There was no significant difference in operational infestation. There was no serious complication after surgery. Surgical margin was minimally positive in 2 patients. The original shapes of the breast were preserved well. All patients expressed great satisfaction. The follow-up time is 112 months at maximum and 72 months on average. There is two locoregional recurrence and 12 distant metastases (brain: 4 with 2 cancer deaths, lung: 3, liver: 3, bone: 5). 5-year survival rate is 97.3%. With regard to TARM, The skin incision was made only in the axilla without any wound on the breast. It could be applied for any tumour existing in the medial or caudal side of the breast (A and B regions). The reconstruction by filling absorbable fiber cotton need no excessive detachment of the skin beyond the surgical margin of the mammary gland. The postoperative esthetic results were excellent and good. The sensory disturbance was minimal, observed only in the detached area within the surgical margin.
Conclusions: VABS can be considered as a surgical procedure with good locoregional control and can provide aesthetic advantages for patients with breast disease.

5009 ORAL

Near-infrared Fluorescence Sentinel Lymph Node Detection in Breast Cancer Patients – the GREEN LIGHT Studies

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Introduction: Detection of the sentinel lymph node is important in the staging and treatment of breast cancer. Near-infrared (NIR) fluorescence imaging is a technique that can be used to visualize lymph nodes during surgery, several centimeters into the living tissue, in real-time. Currently, indocyanine green (ICG) is the only clinical approved NIR fluorescent contrast agent. Premixing of ICG with human serum albumin (HSA) improved the fluorescence and sentinel lymph node retention in preclinical experiments. The current studies focus on optimizing the use of NIR fluorescence imaging for the sentinel lymph node procedure in 3 clinical trials with a total of 64 patients.
Material and Methods: In all studies, the Mini-FLARE intraoperative imaging system (Frangioni Lab, Boston, USA) was used. First (study 1), the optimal dose of ICG:HSA was studied in 24 consecutive patients. These patients received the standard of care sentinel lymph node procedure (blue dye and ^{99m}Tc-nanocoll) and were injected with 1.6 mL of ICG:HSA (dose groups 50 to 1000 μM).
The potential advantage of premixing of ICG with HSA was then studied in a randomized, double-blind study (study 2), with 18 consecutive patients. Patients were injected with 1.6 mL of 500 μM ICG alone or ICG:HSA. Subsequently, sentinel lymph node mapping was performed using 500 μM ICG in 24 patients, with randomization between the use or omission of patent blue (study 3).
Results: The sentinel lymph node was successfully detected in all patients. In the dose finding study (study 1), a total of 35 lymph nodes were detected (average 1.45), all of which were radioactive, 30 nodes were blue. The optimal dose was between 400 and 800 μM.
No differences were observed when premixing ICG with HSA (study 2), in the number of sentinel lymph nodes identified (average of 1.4 per patient), nor in fluorescence intensity (P = 0.18). No difference in fluorescence was