In order to assess the progression free-survival (PFS) time, miRNA expression levels were categorized in quartiles. In analogy to their relationship with clinical benefit, the same three miRNAs were also associated with longer PFS: hsa-miR-30a-3p (hazard ratio [HR]: 0.51, 95% CI: 0.34−0.76; P = 0.001), hsa-miR-30c (HR: 0.47, 95% CI: 0.31−0.70; P < 0.001), and hsa-miR-182 (HR: 0.57, 95% CI: 0.37−0.86; P = 0.008). Global testing using available global gene expression data significantly associated the 3 predictive miRNAs with differential gene expression of HER-2, Rac-1 and Ceramide signaling pathways.
Conclusion: This study shows associations between hsa-miR-30c, hsa-miR-30a-3p and hsa-miR-182 expression levels and clinical benefit to treatment with first-line tamoxifen for recurrent BC and describes pathways putatively involved in these associations. Assessment of these miRNA levels and their pathways in primary tumors could help to improve treatment strategies for patients with recurrent ER+ breast cancers.

PP122

Intraoperative tissue fluorescence using 5-aminolevolinic acid (ALA) is more sensitive than contrast-MRI or amino acid (FET)-PET guided glioblastoma (GBM) surgery

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Background: The ability of 5-ALA to visualize white matter infiltration zones of GBM compared to MRI contrast or [18F]fluoroethyltyrosine positron emission tomography (PET) was investigated.
Materials and Methods: Fluorescence tissue margins were mapped intraoperatively by neuronavigation and compared to pre- and postoperative MRI and FET-PET scans in 3 glioblastoma patients (2 temporal, 1 fronto-central tumor).
Results: In all patients, the intraoperatively detected 5 ALA fluorescence exceeded the MRI contrast tumor areas and FET-PET uptake, verified by intraoperative neuronavigation. Furthermore, all patients received complete resection of contrast affine tumor parts, which was verified by contrast MRI scans within 24 hours postoperatively. Although intraoperative fluorescence tissue was generously left in place, because it was estimated as tissue at risk for neurological deterioration, no contrast affine tissue could be detected by postoperative MRI. Additionally, postoperative FET-PET uptake was demonstrated only in one patient as a small residual spot. FET-PET did not show any uptake at the intraoperatively mapped large marginal areas of 5 ALA fluorescence, left in place in account of neurological preservation
Conclusion: Our findings demonstrate that 5 ALA fluorescence is more sensitive than FET-PET and MRI contrast uptake in detecting glioblastoma multiforme white matter infiltration zones.

PP127

INHANCE (Iressa™ Novel Head and Neck Chemotherapy Evaluation) randomised phase II trial: clinical findings and associated translational research into EGFR-related biomarkers in tumour and skin biopsies

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Background: The INHANCE randomised phase II trial (1839IL0544) explored the feasibility and benefits of adding an EGFR tyrosine kinase inhibitor (gefitinib, AstraZeneca, Macclesfield, UK) to induction chemotherapy with cisplatin and 5-fluorouracil in patients with newly diagnosed squamous cell carcinoma of the head and neck. Associated translational research enabled the unique investigation of EGFR-related signalling changes induced by chemotherapy and a comparison with those elicited by chemotherapy plus gefitinib in randomised therapy-naïve patients.
Materials and Methods: Patients were openly randomised to two cycles (q21 days) of cisplatin (100 mg/m^2 day 1) and 5-fluorouracil (1g/m^2 days 1−4) with or without oral daily gefitinib (250 mg days 1−42). Tumour and skin biopsies were collected pre-treatment and after 14 days of therapy. Given the limitations of immunohistochemistry, we analysed EGFR-related signalling by western blotting and a novel electrochemiluminescent immunoassay (Meso Scale Discovery, Gaithersburg, MA, USA) that we have previously validated in vitro and in vivo.
Results: Thirty-eight patients were randomised. The combination was well tolerated. 53% and 71% of patients donated paired tumour and skin biopsies respectively. Each biopsy yielded sufficient lysate for two western blots and three immunoassays. Using the two independent techniques, there was good concordance between expression and activation of EGFR and AKT in 80% and 86% of tumour biopsies respectively. Signalling changes in skin biopsies only reflected those seen in tumour biopsies in 50% of patients. Two of three patients treated with chemotherapy plus gefitinib who developed a rash demonstrated a reduction in EGFR phosphorylation in tumour, but not skin, biopsies.
Conclusion: Correlation with western blotting shows that the electrochemiluminescent immunoassay is a useful method for quantifying signalling changes in small volume clinical samples but skin was not a reliable surrogate tissue for tumour. Cytotoxic chemotherapy alone elicited changes in EGFR phosphorylation that confounded the interpretation of gefitinib-induced alterations. We conclude that biomarkers optimised for the evaluation of targeted therapies as single agents may be compromised when combined with conventional therapy.

PP63

Predictive markers in patients with upper gastrointestinal (GI) cancers treated with erlotinib and bevacizumab in a multicenter phase II trial

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Background: We investigated the role of several growth factors, growth factor receptors, and markers of ischemia as predictors of response and survival in patients treated with VEGF and EGFR targeted therapy.
Materials and Methods: This exploratory study evaluated the predictive value of plasma levels of vascular endothelial growth factor A (VEGF-A), platelet derived growth factor AB and BB (PDGF-AB and PDGF-BB), soluble Fms-related tyrosine kinase 1 (sFlt-1 or sVEGFR-1), growth differentiation factor 15 (GDF15), hepatocyte growth factor (HGF), high sensitivity troponin T (hsTnT), and pro brain natriuretic peptide (proBNP) in patients with advanced upper GI cancer in progression after chemotherapy. Patients were treated with drugs targeting angiogenesis (bevacizumab) and the EGFR pathway (erlotinib) in a multicenter phase II trial (ASCO GI 2009, abstract #170). Plasma was collected at baseline and weekly during the first 4 weeks. Plasma was analysed using quantitative immunoassays. Results of baseline samples and changes in plasma levels of the markers were correlated to progression-free survival (PFS) and clinical benefit (CB), defined as stable disease (SD) or partial response (PR).
Results: Baseline plasma was available in 79 out of 100 patients (median age 62 [25−78]) with carcinoma in esophagus (36%) (adeno [30%], squamous [6%]), stomach (12%), pancreas (33%), and biliary tract (19%). Three patients had PR, 28 SD, 22 PD, and 26 were not evaluable. Patients with baseline PDGF-AB in the upper quartile had significantly longer PFS (HR: 0.45, 95% CI: 0.23–0.85). Patients with proBNP below median had significantly better PFS than patients with levels above (HR: 1.73, 95% CI: 1.06–2.83). The remaining markers failed to predict CB or PFS.
We observed a decrease of VEGF-A (11.36, 95% CI: 6.16–32.41), HGF (0.07, 95% CI: 0.01–0.28), and GDF15 (0.33, 95% CI: 0.08–1.31), and a trend towards decrease of proBNP (22.34, 95% CI: −0.68–159.29) between baseline and after 1 week of therapy. However, VEGF-A was in most cases undetectable in plasma after start of therapy.
Conclusion: Decrease in plasma levels of VEGF-A, HGF, proBNP, and GDF15 was observed during EGFR/VEGF-targeted therapy. The high frequency of undetectable VEGF-A after initiation of therapy could be attributed to its binding to bevacizumab.
High levels of PDGF-AB and low levels of proBNP seem to predict longer PFS. These findings are of biological and therapeutic relevance and warrants further investigations.

PP103

RT-PCR-based UCA1 expression detection in urine samples as non-invasive reliable method for urothelial cancer diagnosis

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Background: Bladder cancer is among the five most common malignancies in industrialized countries. There are currently no satisfactory markers

for bladder cancer available in clinics. UCA1 (urothelial cancer associated 1 gene) is a non-protein-coding RNA reported as up-regulated in bladder carcinoma, influencing cell growth and promoting invasion. It is a very sensitive and specific unique marker for bladder cancer. A growing body of data suggests the outstanding clinical utility of early non-invasive molecular diagnostics. In this research, we report the setting up and validation of UCA1 identification in urine samples, along with its successful use in clinical diagnosis.
Materials and Methods: We used a standard RT-PCR analysis with specifically designed primers for UCA1 identification in routinely obtained urinary samples. Validation assays were based on the assessment of TBP (TATA Box Binding Protein) expression. Primer and test specificity were demonstrated by sequencing of amplified UCA1 and TBP fragments.
Results: Test specificity was assessed on 20 negative samples (with no tumor cells detected by standard cytology evaluation) and perfect matching (100% of correlation) was obtained (i.e. no UCA1 expression in urinary samples devoid of tumor cells). Test repeatability and reproducibility were demonstrated on matching independent triplicates from samples with both high and low UCA1 expression. The assay sensibility was demonstrated by correlation of the results of UCA1 expression (samples with high and low UCA1 expression) with the results obtained by standard microscopy diagnosis. Finally, 30 samples were compared by the standard cytology approach with the results obtained with the RT-PCR-based method. Four parameters were assessed for concordance: sensitivity (concordance between two tests: 100%), specificity (67%), positive predictive value (75%) and negative predictive value (100%).
Conclusion: Our results demonstrated very good correlation of this non-invasive assay with the widely used invasive cytology analysis evidencing thus the reliability and interest of use of UCA1 testing for urothelial cancer diagnosis. Our ongoing large-scale study will (i) help in better understanding the clinical signification of low UCA1 expressing samples, (ii) enable validation of potential use of this assay for predicting of bladder cancer recurrence and (iii) support to further acknowledge the standardization of this diagnostic approach.

PP24

CXCR4: a predictive marker of bone metastases in breast cancer patients

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Background: CXCR4, a chemokine cell receptor, is expressed by several tumor histotypes and, together with its ligand CXCL12, is involved in tumor growth, angiogenesis, and homing of cancer cells to distant sites. Our objective was to investigate the possible predictive role of this biological marker in bone metastatization in breast cancer patients.
Materials and Methods: CXCR4 expression was evaluated by immunohistochemical staining in paraffin-embedded tissue sections of primary breast cancers from 40 individuals: 11 disease-free (DS) at 110 months (83–138), median age 61 years (range 48−78) and 29 with relapsed disease, median age 67 years (range 42−87). In the latter group, 10 had visceral metastases (VM), median age 68 years (range 52−86) and 19 had bone metastases (BM), median age 66 years (range 42−87).
Results: CXCR4 was detected in the cytoplasm and/or nucleus of tumor cells. 13% of all samples showed strong nuclear staining and 25% strong cytoplasmic staining. In particular, cytoplasmic expression was observed in 9% of samples from DF patients, in none of the samples from those with VM ($p = 0.048$), and in 47% of sections from BM patients ($p = 0.011$). Considering either nuclear or cytoplasmic CXCR4 expression, sensitivity was observed in 18% of DF patients, 10% of VM patients (n.s.) and 53% of BM patients ($p = 0.044$). However, no relation was observed between CXCR4 expression and disease-free or overall survival in the last subgroup.
Conclusion: Our preliminary results suggest that cytoplasmic CXCR4 expression in the primary tumor could be a predictive marker of bone metastases in breast cancer patients. A larger study is ongoing to confirm these results.

PP33

Functional characterization of CYP2C8 promoter polymorphisms

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Background: The objective of the present study was (1) to determine polymorphic variations in the CYP2C8 gene in three distinct healthy Asian subjects (Chinese: N = 101, Malay: N = 91 and Indian: N = 90, populations), and (2) to functionally characterize high frequency CYP2C8 promoter polymorphisms.
Materials and Methods: Screening for genetic variations in the promoter, exons and exon-intron junctions of CYP2C8 gene was performed by PCR followed by direct DNA sequencing. Functional characterization of promoter polymorphisms were studied by using various combinations of plasmid constructs containing the identified promoter polymorphic variants. The different constructs were cloned in pGL3 expression vector and investigated for their activity in driving reporter gene expression in transfected HepG2 cells under optimized conditions.
Results: Seven polymorphisms were identified and their allelic frequencies were as follows: 5′-UTR: g.−411C>T (C:0.33;T:0.67), g.−370T>G (T:0.72;G:0.28) and g.−271C>A (C:0.88;A:0.12); intron 2: I.−64A>G (A:0.50;G:0.50), −13insT (Wt:0.87;insT:0.13); intron 7: +49T>A (T:0.47;A:0.53) and 3′-UTR: 24C>T (C:0.62;T:0.38). Haplotype analysis revealed fourteen different haplotypes in Chinese, eighteen in Malays, and twenty one in the Indian population. Two haplotype blocks were inferred in each ethnic group based on the solid-spline algorithm. The promoter construct harboring the single g.−411C>T variant showed approximately 2-fold higher luciferase activity compared with the reference construct ($P = 0.002$). The construct harboring the combined g.−411C>T and g.−271C>A polymorphic variants showed a severe reduction (44-fold) in luciferase activity compared with the construct containing the reference sequence ($P = 0.006$).
Conclusion: Future studies should be done to investigate the influence of CYP2C8 g.−411C>T and g.−271C>A polymorphisms on the disposition CYP2C8 drug substrates.

PP89

Upregulated p38 MAPK signaling in circulating pancreatic cancer cells

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Background: Hematogenous cancer cell dissemination is the most important route of metastasis in pancreatic ductal adenocarcinoma. Our aim was to identify gene expression profiles of circulating tumor cells (CTC) immediately after resection of the primary tumor.
Materials and Methods: CTC were isolated from whole blood by density centrifugation (Oncoquick®) followed by negative selection fluorescence activated cell sorting combining a dump channel, anti-CD45, anti-CD34 and 7-AAD viability staining to exclude all hematologic (G) and non-viable cells. Four subgroups were obtained for each patient: two sorted fractions (CTC and G), the original tumor (T) and non-tumor pancreatic control tissue (P). RNA was isolated from all samples. After double linear amplification of RNA, microarrays (whole genome affymetrix genechip HG-U133_Plus_2) were run. The robust multi-array (RMA) analysis was run on the probes that had at least 4 out of 6 detection calls. On this list of probe sets, a filter was applied selecting genes a 2-fold up- or down-regulation in the comparisons of CTC versus T, AND in CTC versus P, AND CTC versus G, using uncorrected p-values ($p < 0.001$). Resulting data were analyzed with 'Ingenuity Pathways Analysis' software.
Results: In 6/10 patients the samples from all four subgroups reached the RNA quality standards set for microarray analysis. From 46,467 probes a set of 8,152 probe sets were retained. After application of the filter, 1,059 probe sets were retained, of which 572 were eligible for function and pathway analysis. Most molecules were involved in genetic diseases, inflammatory response, cancer, cell-to-cell signaling and cellular movement. The pathway with the highest ratio of molecules that met cut-off criteria was p38 MAPK signaling. In this pathway transforming growth factor beta 1 (TGFβ1) and MYC associated factor X (MAX) were significantly upregulated in the CTC fraction compared to the T, P and G groups. S100A8 was found to be strongly upregulated in CTC.
Conclusion: Gene expression profiles can be obtained from circulating tumor cells without a priori selection markers. S100A8, TGFβ1 and MAX are upregulated in CTC of patients with PDAC. These genes are involved in p38 MAPK signaling which is responsible for increased CTC motility.

PP67

Early alpha-fetoprotein response predicts treatment efficacy of anti-angiogenic therapy in combination with metronomic chemotherapy for advanced hepatocellular carcinoma

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Background: Sorafenib and other molecular-targeted agents with anti-angiogenic activity have shown moderate clinical benefit in patients with advanced hepatocellular carcinoma (HCC). However, the biomarkers