2010), BO18279 MERIT (Tan et al. 2010), BO18602 TITAN (Ciuleanu et al. 2010), MO18109 TRUST (Heigener et al. 2011)).
Results: In total EGFR mutation status could be obtained from 970 samples out of the 4 studies. 26 (3%) of those had 'other' mutations, 6 in exon 18, 4 in exon 19, 10 in exon 20 and 7 in exon 21. One sample had a double mutation in exon 18 and 21. A detailed breakdown of the single mutations with clinical outcome will be shown and discussed.
Conclusion: The clinical implications of 'other' EGFR mutations cannot be categorized easily mainly due to low incidence rates of each single mutation. However, some patients respond well to Tarceva treatment, others have long PFS and OS which seems not necessarily linked to treatment, but rather to the molecular status of the underlying disease. Collection of more clinical data on 'other' EGFR mutations is warranted.

PP 3

S-100B concentrations predict disease specific survival in AJCC Stage III melanoma patients

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Background: S100-B is a tumor marker used in melanoma patients but its role in AJCC stage III melanoma patients is still undefined. Elevation of S-100B in AJCC stage III can be highly specific indicator for recurrence. The role of S-100B was evaluated as a pre-operative tumor marker in FDG-PET staged stage III melanoma patients undergoing a therapeutic lymph node dissection.
Materials and Methods: All patients with melanoma Stage III between January 2004 and August 2010 were included. There were 53 males and 47 females with a median age 54.5 (range 21.8–90.6). S-100B was measured pre-operative and recorded as elevated when S-100B $\geqslant 0.15\,\mu g/l$. Univariate and multivariable Cox Proportional Hazard Models were used to assess the association of S-100B with Disease Free Period (DFP) and Disease Specific Survival (DSS).
Results: Overall, 100 patients were included. S-100B was elevated in 50% of the patients. Patients with a normal S-100B value had a 5-years DFP of 40.6% (23.0–57.5) versus 15.3% (5.6–29.4) in patients with an elevated S-100B (Hazard Ratio (HR) 2.3 (95% CI 1.4−4.0); p = 0.002). S-100B was an independent prognostic factor (HR 2.6; p = 0.002). Patients with a normal S-100B had a 5-years DSS of 46.2% (27.1–63.3) while patients with an elevated S-100B had a 5-years DSS of 28.1% (14.9–42.9); HR 2.4 (95% CI 1.3−4.3; p = 0.003). In multivariable analysis, S-100B was an independent prognostic factor (HR 2.2 (95% CI 1.2−4.0); p = 0.01).
Conclusion: Preoperative elevated S-100B is strongly correlated with a reduced survival. S100-B should be used as a prognostic marker in the stratification in trials for adjuvant systematic treatment and should be considered to be added to the AJCC melanoma staging system.

PP 21

Gene expression module biomarkers to stratify multiple clinical and therapeutic endpoints for universal breast cancer companion diagnostic

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Background: Gene expression patterns are increasingly capable of stratifying patients based on prognosis and response to therapy. Given the limited availability of sample tissue, however, it is not feasible to run many tests, suggesting the need for a universal companion diagnostic assay that is informative with respect to multiple clinical and therapeutic endpoints. Key challenges are identification of appropriate gene expression biomarkers, translation of biomarkers to clinical assays, and development of reliable gene expression profiling of formalin-fixed clinical specimens. Here, we describe a meta-analysis approach that identifies novel biomarker modules that results in multiple clinical and therapeutic read-outs.
Materials and Methods: A co-expression meta-analysis of 5,339 breast tumors from 56 microarray datasets identified highly co-expressed sets of genes (modules) across multiple datasets. And these module based biomarkers were tested for their ability to associate with prognostic and predictive targets in published datasets. In addition, each module was reduced from 10–1000 genes to top performing 2−3 genes based on degree of co-expression across the meta-analysis and validation by quantitative PCR in an independent panel of FFPE tumor samples.
Results: This study demonstrates that a single 96 gene qPCR test utilizing multiple module biomarkers is not only capable of stratifying patients by standard histopathological parameters (ER, PR and Her2), but also stratifies by other diverse elements of the disease (cell lineage, dysregulated core biological functions, factors of cell growth, underlying genomic aberrations and the tumor microenvironment). Taken together, these biological variables represent the major biological diversity present within the breast cancer population. A series of retrospective analyses demonstrated that different single module and combinations of modules were capable of predicting a variety of clinical endpoints, including 5-year survival, neoadjuvant chemotherapy response in ER− patients and targeted therapy response in model systems.
Conclusion: The molecular heterogeneity of breast cancer can be summarized by discrete gene expression modules that individually represent distinct biological pathways, and that collectively can be represented by as few as 96 genes. These breast cancer modules, together with outlier genes, allow for summation of the entire transcriptional program and provide a universal assay with broad application to companion diagnostics development.

PP 12

Evidence of Galectin-1 involvement in glioma pro-angiogenic and pro-migratory effects and chemoresistance

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Background: Despite the advances in the management of malignant gliomas of which glioblastomas represent the ultimate grade of malignancy, they remain characterized by dismal prognoses. Glioblastoma patients have a median survival expectancy of 14 months on the current standard treatment of surgical resection to the extent feasible, followed by radiotherapy plus temozolomide chemotherapy, given concomitantly with and after radiotherapy. This prognosis can be at least partly explained by the fact that glioma cells diffusely infiltrate the brain parenchyma making them elusive targets for effective surgical management and exhibit decreased levels of apoptosis and are thus resistant to cytotoxic drugs [1]. We have previously reported that progression of malignancy in patients bearing astrocytic tumors correlates with increased tumor levels of Galectin-1 [2], that Galectin-1 is involved in the modulation of the migration of tumor astrocytes [3] and that Galectin-1, the expression of which is stimulated by hypoxia [4], is also a pro-angiogenic molecule [4,5].
Materials and Methods: We investigated whether decreasing Galectin-1 expression (by means of a siRNA approach) in human Hs683 glioblastoma cells increases their chemosensitivity.
Results: Temozolomide increases Galectin-1 expression in the Hs683 glioblastoma model both in vitro and in vivo [6]. Consequently, reducing Galectin-1 expression in this model increases the anti-tumor effects of various chemotherapeutic agents, in particular temozolomide [5,6]. Reducing Galectin-1 expression in glioblastoma cells does not induce apoptotic or autophagic features, but rather modulates p53 transcriptional activity and decreases p53-targeted gene expression. The decrease in Galectin-1 expression also impairs the expression levels of several genes implicated in chemoresistance: ORP150, HERP, GRP78/Bip, TRA1, BNIP3L, GADD45B and CYR61 [6].
Conclusion: The involvement of Galectin-1 in different steps of glioma malignant progression [7], such as migration, angiogenesis or chemoresistance, makes it a potential target for the development of new drugs to combat these malignant tumors [8].

References

[1] Lefranc et al. J Clin Oncol, 2005.
[2] Camby et al. Brain Pathol, 2001.
[3] Camby et al. J Neuropathol Exp Neurol, 2002.
[4] Thijssen et al. PNAS, 2006.
[5] Le Mercier et al. J Neuropathol Exp Neurol, 2008.
[6] Le Mercier et al. Toxicol Appl Pharmacol, 2008.
[7] Le Mercier et al. Brain Pathol, 2010.
[8] Camby et al. Drug of the Future, 2008.

PP 10

Biomarker discovery by pharmacological studies in a population based tumor model for VEGFR inhibitors

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Background: VEGF pathway inhibitors have been shown to elicit broad activity in traditional preclinical models, yet clinical development thus far has met with significant variation in response due to the complexity of human genetics and tumor microenvironment. Since empirical biomarker discovery in the clinic is both challenging and time consuming, preclinical models that provide variation of genetic context and complex microenviroment and therefore variation in drug response will greatly facilitate predictive biomarker discovery, especially for drugs in development.
Materials and Methods: Using chimeric murine model technology, we generated over one hundred primary breast tumors driven by HER2

oncogene. The tumors were characterized for molecular characteristics, histologic phenotypes and angiogenesis, and expended in vivo for multiple in vivo pharmacological studies. Like human tumor populations, this population based tumor model exhibited significant inter-tumor variation in gene expression and tumor microvasculature. We therefore deployed the model for drug response biomarker discovery.
Results: Treatment of a subset of the tumors with the potent selective VEGFR inhibitor tivozanib which is currently in phase 3 clinical trial for kidney cancer revealed significant variation in response. Pharmacogenomic analysis using a novel coherence based bioinformatics approach revealed the association of a specific biological phenotype comprising tumor myeloid infiltration with drug resistance. A multi-gene signature derived from the biological phenotype has been used to query genetic data of human tumor populations. The result suggests that the drug resistance phenotype is represented in a subset of all solid tumor types examined but frequencies vary. We have subsequently developed a single, semi-quantitative immunohistochemistry (IHC) marker that represents the drug resistance phenotype. In a retrospective analysis of RCC patients that have received tivozanib monotherapy a significant correlation between the IHC biomarker and drug response (by RECIST) has been observed.
Conclusion: These results provide a promising candidate predictive biomarker for tivozanib activity in patients and indicate the utility of tumor population based models for biomarker discovery.

PP 55

Serum levels of the extracellular domain of HER-2 receptor in osteosarcoma patients

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Background: The prognostic role of HER-2 expression in osteosarcoma is controversial. The aim of the study was to asses a clinical value of the serum extracellular domain of HER-2 receptor (ECD/HER-2) level and its correlation with disease progression in patients with osteosarcoma.
Materials and Methods: 33 patients (aged 6−18 years, median 15 years) with primary osteosarcoma were analyzed; 13 were metastatic at presentation. Follow up period was 12−44 months; median 22 months. ECD/HER-2 levels were analyzed by ELISA method four times: at the time of diagnosis (1), before surgery of primary site (2), after surgery (3) and at the end of treatment 6−9 month since diagnosis (4). In 12 patients, tumor biopsy samples were available for assessment of the immunohistochemical HER-2 expression. The disease progression was confirmed by the CT scan and bone scan. The poor result of ECD/HER-2 value was calculated as higher than 5.5 ng/mL and/or elevated more than +1 ng/mL.
Results: Disease progression was observed in 10 patients, 7 of them died. 8/10 patient who progressed during treatment, had elevated ECD/HER-2 in serum. In 19/23 patient with SD/PR decreasing levels of ECD/HER-2 was observed; $P = 0.001$. 6/7 patients who died had the ECD/HER-2 elevated during treatment or at the end of treatment. There was no relationship between ECD/HER-2 serum level and HER-2 expression in biopsy samples. Immunohistochemical expression of HER-2 did not correlate with treatment results.
Conclusion: This pilot study shows the possible clinical value of ECD/HER-2 assessment in serum of patients with osteosarcoma.

PP 68

Cytokines' profiles to predict chemotherapy outcome in castration resistant prostate cancer (CRPC)

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Background: Chemotherapy improves symptoms and survival in advanced CRPC, however, around 50% of patients have chemoresistant disease. Early markers of chemoresistance will allow cessation of ineffective therapy and avoid toxicity. While MIC-1 and IL-6 may be markers of response, comprehensive cytokine evaluation has not been performed. This study examined whether early changes in cytokine levels can predict chemoresistance and clinical outcome.
Materials and Methods: Plasma/serum samples pre and post cycle 1 chemotherapy were collected from 59 men with metastatic CRPC (55 docetaxel, 7 mitoxantrone). Levels of 27 cytokines were measured using a bead-based immunoassay. MIC-1 levels were measured by ELISA. Patients were grouped as clinical responders (PR + SD) and non-responders (PD) based on PSA response criteria (PD >25% PSA increase over treatment course if no PSA response). The associations between cytokine levels, response groups and overall survival (OS) were assessed by non-parametric tests and Cox regression survival analysis.
Results: After one cycle of chemotherapy, non-responders had significantly greater increases in 10 cytokines from baseline compared with those who responded to treatment (MIC-1 $p = 0.003$; IL1ra $p = 0.004$; IL1b $p = 0.01$; IL4 $p < 0.001$; IL5 $p = 0.041$; IL6 $p = 0.003$; IL7 $p = 0.009$; IL8 $p = 0.017$; IL12 $p = 0.004$; IFN gamma $p = 0.001$). At a median survival of 15 months with 49 deaths, increases in IL8 (HR 2.4, 95% CI 1.1−5.0; $p = 0.02$) and IFN gamma (HR 2.6, 95% CI 1.3−5.2; $p = 0.009$) levels by more than 40% from baseline after one chemotherapy cycle were associated with poorer OS. Higher baseline MIC-1 levels also predicted shorter OS (HR 1.6, 95% CI 1.3−2.0; $p < 0.0001$). In a multivariate model including presence of visceral metastases ($p = 0.02$), Gleason score ($p = 0.1$), baseline ALP ($p = 0.2$), baseline Hb ($p = 0.02$) and baseline PSA ($p = 0.76$), baseline MIC-1 levels were an independent predictor of survival (HR 2.0, 95% CI 1.3−3.1; $p = 0.002$).
Conclusion: Early changes in circulating levels of cytokines were associated with chemoresistance in men with CRPC. Additional associations between OS, baseline MIC-1 levels and changes in IL8 and IFN gamma levels suggest inflammation influences outcomes in men with CRPC treated with chemotherapy.

PP 96

NHERF1 in advanced colorectal cancer: its interaction with HIF-1α and TWIST1 plays an important role in synchronous lymph node and liver metastases

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Background: Colorectal cancer (CRC) is one of the major malignancies worldwide and recurrence and metastasis is the leading cause of death in CRC patients. Despite recent advances, characterization of new potential biomarker may be useful for the outcome prediction and fundamental to correct management of the disease. NHERF1, a potential candidate of clinical relevance for breast cancer, has been shown to be a new player in CRC progression, promoting Wnt signaling pathway by direct interaction with β-catenin. TWIST, a basic helix-loop-helix transcription factor, is essential in mediating cancer metastasis through the epithelial–mesenchymal transition (EMT) and, recently, a new finding showed that TWIST is critical for hypoxia-mediated EMT. Aim of this study was to investigate the correlation among the expression of NHERF1, HIF-1α, and TWIST1 in metastatic CRC.
Materials and Methods: We assayed NHERF1, HIF-1α, and TWIST1 protein expressions by immunohistochemistry in 51 patients diagnosed with Stage IV CRC, whose distant non-tumoral tissues (DNT), primary tumor (T) and adjacent non-tumoral tissue (NT), lymph node (LnM) and liver metastases (LM) were available.
Results: A shift of NHERF1 from the apical membrane of DNT and NT, to cytoplasmic and nuclear compartments of T, LnM and LM was noticed. Cytoplasmic NHERF1 expression was statistically higher in T, LnM and LM than DNT and NT ($P < 0.0001$). Nuclear NHERF1, such as HIF-1α, was found significantly overexpressed in T and metastatic compartments compared to DNT ($P < 0.0001$). TWIST1 is upregulated in tumor compared with normal tissue and protein expression was significantly higher in T than LnM and LM ($P < 0.0001$). Spearman's rank test showed that nuclear NHERF1 was directly correlated to TWIST1 in T ($P = 0.015$, $r = 0.339$), and, a significant association between HIF-1α and TWIST1 was also noticed in T ($P = 0.016$, $r = 0.365$).
Conclusion: These results indicate that NHERF1 expression results strongly related to the hypoxic microenvironment of metastatic CRC. Tumor progression and metastasis are mediated by a key signaling pathway involving nuclear NHERF1 together with HIF-1α and TWIST1. Thus, we propose nuclear NHERF1 as a new diagnostic marker of advanced malignancy.

PP 11

Correlation between both serum osteopontin/osteonectin and bone remodelling parameters, inflammatory/metabolic variables and survival in metastatic cancer patients with tumors at different sites

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Background: Osteopontin (OPN) is a secreted, integrin-binding phosphoprotein that has been correlated with tumor grade and stage and disease progression in several tumor types. High OPN levels have been clinically correlated with metastatic bone disease and bone resorption in cancer patients. The secreted protein, acidic and rich in cystein (SPARC) is closely related to progression, invasion, angiogenesis and metastatic process of several malignant tumors. The aim of the study was to verify in a population of advanced cancer patients with tumors at different sites whether there is a correlation between circulating levels of OPN and SPARC