(Japanese tumor regression grade 2 or more). Post-CRT SUVmax in the responders was significantly lower than that of non-responders (median value 2.9 vs 6.2, respectively). Five-year overall survival was significantly better in patients with lower Post-CRT SUVmax (88% vs 47%, respectively, p = 0.036). Five-year local re-recurrence free survival was significantly better in patients with higher SUVRR(80% vs 24%, respectively, p = 0.035).
Conclusion: Metabolic response assessed by PET/CT is useful for predicting tumor response and prognosis. The response might be utilized for post-operative adjuvant chemotherapy.

PP 9

Men and women display different proteomic diagnostic profiles in non small cell lung cancer

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Background: Plasma biomarker-based screening for lung cancer could provide substantial survival benefits in properly targeted high-risk populations.
Materials and Methods: Fifty-nine circulating proteins were analyzed using multiplexed immunoassays in plasma of patients diagnosed with non-small cell lung cancer (NSCLC; 245 men, 114 women), asthma (AST; 67 men, 112 women) and normal controls (NOR; 122 men, 165 women). Samples were split randomly into training (N = 402) and test (N = 389) data sets. A support vector machine (SVM) was used to identify discriminatory biomarkers in NSCLC and AST taking into account patients' gender. Mass spectrometry (MS) followed by data analysis using Mascot software was employed for biomarker discovery; validation of select biomarkers was achieved by immunodetection of target proteins in plasma. Pathway analysis was applied to characterize pathology- and gender-specific patterns of biomarker expression.
Results: We developed seven SVM models that classified subjects to NSCLC, AST or NOR for all 59 markers or subsets thereof, for both genders or single gender only, and for both pathologies and NOR or NSCLC and NOR only. When all biomarkers and genders were accounted for, SVM classified subjects to NSCLC, AST with an accuracy of 0.94 (SE: 0.012). Restricting to NSCLC versus NOR produced 4 markers [EGF, sCD40 ligand, IL-8 and MMP-8; sensitivity (SE) 0.93 (0.014), specificity (SP) 0.87 (0.02)]. Best subset of 5 variables for men (EGF, IL-8, sFAS, MMP-9 and PAI-1) and 3 variables for women (EGF, sCD40 ligand, IL-8) yielded SE and SP of 1 (0). MS identified 11 differentially expressed proteins including 3 putative gene products and yet unnamed proteins, a protein corresponding to chromosome X open reading frame 38, and several known proteins (syntaxin 11, cAMP-specific, rolipram-insensitive phosphodiesterase 7B, and interleukin-25), whose presence was independently confirmed by immunobloting. Diagnostic biomarkers are products of genes residing on multiple chromosomes and are not limited to sex chromosomes.
Conclusion: The NSCLC-specific biomarkers and combinations thereof identified in this study warrant additional clinical validation to determine their role in screening targeted high-risk populations. The novel method for data mining is widely applicable to development of test kits for detecting biomarkers and combinations of biomarkers.

PP 88

C4.4A as a biomarker for poor prognosis in non-small cell lung cancer patients with adenocarcinomas

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Background: Lung cancer is the most common cancer form in the world with a 5-year survival rate of only 15%. It is consequently relevant to search for and characterize new prognostic and predictive factors, providing a better basis for treatment decisions in this disease, ultimately leading to higher patient survival. The glycolipid-anchored membrane protein C4.4A, which is a structural homolog of the urokinase-type plasminogen activator receptor, is such a potential candidate. C4.4A is absent in the normal healthy lung, but it is induced in early precursor lesions of non-small cell lung cancer (NSCLC).
Materials and Methods: In the present study, we have undertaken an immunohistochemical, retrospective study on the expression of C4.4A in 229 cases of NSCLC. For each patient, one tissue section from the periphery and one from the center of the tumor were stained with our well-characterized polyclonal anti-C4.4A antibody. C4.4A levels were scored semi-quantitatively for intensity and frequency of positive tumor cells (range 0−16) and statistically correlated to survival.
Results: Expression of C4.4A was more pronounced in squamous cell carcinomas (SCC) compared to adenocarcinomas (AC), with median tumor center scores of 8.0 and 1.3, respectively. Consequently, statistical analysis of survival was performed separately for 88 AC and 104 SCC patients. In addition to pathological stage, C4.4A score for the tumor center was a highly significant prognostic factor in the AC group both in univariate (p-value = 0.004; Hazard ratio (95% CI) = 1.44 (1.12–1.85)) and multivariate analysis (p-value = 0.0005; Hazard ratio (95% CI) = 1.65 (1.24–2.19)), demonstrating decreasing survival with increasing score. Only pathological stage was significant for the SCC group. These results consolidate earlier observations, now in a larger and independent patient cohort.
Conclusion: High expression of C4.4A is a significant, independent prognostic factor in AC of the lung and is also expressed in a fraction of atypical adenomatous hyperplasias, the putative precursor lesion of this histological subtype. Although the TNM classification still represents the gold standard for the management of NSCLC patients, C4.4A has a potential clinical value as a prognostic marker in pulmonary AC, which might be useful e.g. in decision-making regarding adjuvant radio- or chemotherapy in early stage patients.

PP 38

A fully automated molecular diagnostic system capable of point-of-care for personalized cancer treatment

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Background: KRAS, BRAF and PIK3CA mutations are strong predictors for efficacy of molecularly targeted agents such as cetuximab and panitumumab in metastatic colorectal cancer (mCRC). For mutation analysis, the current methods are costly, time-consuming, and not commonly available to clinicians. We have developed a novel, simple, sensitive and fully automated DNA mutation detection system (Toppan Genetic Analyzer, TGA) based on the Invader Plus technology for molecular diagnostics. This system includes the DNA extraction process from homogenized tissue sample. Here we report the results of comparison study between our detection system and direct sequencing (DS) in the detection of KRAS, BRAF and PIK3CA mutations. The effect of DNA purification/extraction process on mutation detection was also compared between the TGA system and the use of commercial kits.
Materials and Methods: Detection of KRAS, BRAF and PIK3CA mutations in mCRC samples were conducted by TGA and DS in a double-blind manner. DNA was extracted from a slice of either frozen tissue (n = 89) or formalin-fixed and paraffin-embedded (FFPE) tissue (n = 70) by using QIAamp DNA Micro Kit and EPICENTRE QuickExtract kit, respectively, and then used for TGA and DS experiments. For automated DNA extraction and mutation detection by TGA, a small slice (<1mg) of frozen tissue (n = 5) was homogenized in a glass homogenizer. The supernatant was then transferred to TGA for mutation detection.
Results: In the experiments with DNA extracted by commercial kit, all mutations (n = 41 among frozen and 27 among FFPE samples) detected by DS were also successfully (100%) detected by the TGA. However, 8 frozen and 10 FFPE samples detected as wild-type in the DS analysis were shown as mutants in the TGA analysis. In the experiment testing for the automated DNA extraction and mutation analysis, TGA detected all mutations directly compared to the use of kit-extracted DNA samples. The fully automated reaction can be finished in 80 min.
Conclusion: We have developed a novel fully automated mutation detection system. Our data suggest that this system has the same accuracy as the DS but a higher sensitivity in mutation analysis. The system also has an excellent capacity of mutation detection in both frozen and FFPE samples. Meanwhile, TGA can rapidly detect mutations with simply crashed small amount of frozen tissue in a fully automated mode. These features highlight the great potential of our system for molecular diagnosis in personalized cancer treatment at the point of care.

PP 71

'Other' (non-activating, non-T790M) EGFR mutations and their clinical implications collected from various Tarceva trials in NSCLC

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Background: EGFR activating mutations (exon 19 in-frame deletions and exon 21 point mutation L858R) in patients with NSCLC have been established as selection marker for 1st line treatment with TKIs like erlotinib and gefitinib (Azzoli et al 2009). The T790M mutation is characterized as resistance mutation (Pao et al. 2005). In many studies only EGFR activating and resistance mutations are assessed, e.g. OPTIMAL, EURTAC, IPASS. However, 'other' EGFR mutations could also contribute to clinical benefit from TKI treatment (Xu et al. 2009).
Materials and Methods: Exons 18−21 of the EGFR gene were amplified by polymerase chain reaction (PCR) using nested primers, and multiple independent products were directly sequenced on both strands. Data were collected from various Tarceva trials (BO18192 SATURN (Cappuzzo et al.

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].

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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