and AAbs may considerably increase the efficacy of SERPA method to identify relevant cancer biomarkers.

PP 91

MicroRNA-mediated breakage of tumor cell differentiation

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Background: Tumor growth is tightly associated with regular shifts in microRNA (miRNA) expression pattern. Expression of several miRNAs, e.g. miR-21, miR-23a/b, miR-100, miR-146a, miR-155, mir-181, miR-206, miR-221 and miR-222, is up-regulated in leukemia cells. This investigation aims to identify how abnormalities in miRNA network contribute to the arrest of tumor cell differentiation.
Materials and Methods: miRNA targets within gene transcripts were predicted in silico using TargetScan software.
Results: miRNA mir-21 silences genes encoding transcription factors Meis1 and Sox2 (as well as nuclear factor NFIB that inhibits NF-kappaB, a key element of antiapoptotic pathway). miR-125b targets transcript of genes encoding NFIB, transcription factors Stat3, IRF4, Ets1 and IL-6 receptor. Transcripts of genes encoding transcription factors EBF1, CEBPB, Ets1, Meis1 and PU.1 carry miR-155 binding sites. miR-181 can target transcripts of genes encoding transcription factors Ets1, Foxp1, Runx1, MITF, Bcl6 and Blimp1. Also, mir-23a/b can suppress genes encoding MITF and Blimp1. miR-150 and miR-23a/b can target transcript of gene encoding transcription factor IRF8. miR-29b suppresses gene encoding T-bet (TBX-21). miR-29b, miR-146a, miR-206 and miR-219−5p silence gene encoding transcription factor Bcl11a. miR-206 targets transcripts of genes encoding transcription factors EBF1 and Lef1 as well as retinoic acid receptor beta RARB. miR-221 and miR-222 silence gene encoding receptor c-Kit, transcription factors Ets1 and Fos.
Conclusion: Leukemia cells up-regulate expression of miRNAs that silences genes encoding key elements of cell differentiation network. EBF1 is a master regulator for B-cell development, as well as T-bet is for Th1-cell differentiation. Transcription factors Bcl11a, Ets1, Foxp1, Runx1, MITF, Bcl6, IRF4, IRF8, Blimp1 and IL-6 receptor are responsible for some stages of lymphoid cell differentiation and for recombination in immunoglobulin gene loci. Factors CEBPB and Meis1 are required for myelopoiesis. Illegitimate miRNA expression can directly repress these stage-specific genes; thereby leukemia cells can lose the normal cytokine susceptibility. As a result, the course of cell specialization proves to be complicated, requiring a high concentration of cytokines, or appears to be impossible at all, and transformed cells proliferate and accumulate, forming a tumor.

PP 46

In vivo imaging of modulation of IGF-1R expression in breast cancer models

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Background: The insulin-like growth factor 1 receptor (IGF-1R) is a new target for breast cancer treatment. In vitro studies have shown that IGF-1R expression can predict response to IGF-1R targeted therapy. In vivo, other factors affect targeting of antibodies to tumors, such as vascular density, vascularity and interstitial pressure. Therefore, uptake of anti-IGF-1R antibodies in a tumor may be a better predictor for response to IGF-1R targeted treatment than immunohistochemical analysis of IGF-1R expression. The aim of the study was to determine whether immunoSPECT with radiolabeled R1507, an antibody directed against IGF-1R, can be used to measure IGF-1R expression and accessibility in vivo.
Materials and Methods: BALB/c nude mice with MCF-7 xenografts, were implanted subcutaneously with estradiol pellets. Three days later, mice were injected with 20 MBq 111In-R1507. Alternatively, mice were treated with tamoxifen and after seven days of treatment, 111In-R1507 was administered. In a third experiment, mice with SUM149 tumors were treated with a single dose of bevacizumab. Four days after treatment, mice received 111In-R1507. In all experiments, three days after injection of 111In-R1507, SPECT images were acquired and the biodistribution was determined ex vivo. IGF-1R expression was analyzed with immunohistochemistry.
Results: Uptake of 111In-R1507 in the tumor was significantly higher in the estradiol treated mice compared to non-treated mice (14.2 versus 10.9%ID/g ($p = 0.016$)). Differences in tumor uptake were visualized with immunoSPECT and correlated with IGF-1R expression as determined immunohistochemically. Tamoxifen did not affect tumor uptake of 111In-R1507, although on immunohistochemistry membranous IGF-1R expression was decreased. Bevacizumab treatment significantly decreased tumor uptake of 111In-R1507 (19.9 versus 26.6%ID/g for treated versus non-treated mice($p = 0.002$)), while immunohistochemically IGF-1R expression was unaltered.
Conclusion: ImmunoSPECT with 111In-R1507 is a sensitive method to measure modulations in IGF-1R expression caused by estradiol treatment. However, as illustrated by the results of tamoxifen and bevacizumab treatment, tumor uptake of 111In-R1507 does not necessarily correlate with IGF-1R expression. These data underscore that target availability also affects tumor targeting by antibodies. Therefore, immunoSPECT of IGF-1R expression with 111In-R1507 could be a better predictor of response to anti-IGF-1R antibodies than immunohistochemical analysis of IGF-1R expression.

PP 45

In vivo isolation of circulating tumor cells

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Background: Circulating tumor cells (CTC) from cancers of epithelial origin frequently give rise to metastasis responsible for most cancer-related deaths. In addition, they can also serve as biomarker source to improve the management of cancer treatment. However, current technologies for isolation of these extremely rare cells are limited by their capability to detect sufficient cell numbers in the majority of cancer patients. In order to increase the sensitivity of CTC detection, GILUPI has developed a functionalized and structured medical wire (FSMW) that allows CTC isolation directly from the patients' blood stream.
Materials and Methods: In a clinical trial with 30 breast cancer patients, CTC were isolated by the medical wire that has been inserted into the patients' vein for 30 minutes. The medical wire mediates target CTC isolation by antibodies directed against the epithelial cell adhesion molecule (EpCAM). To confirm that the target CTC are bound to the wire, immunocytochemical staining against EpCAM or cytokeratin is performed as well as staining against CD45 for negative cell selection. In addition, 6 further patients are scheduled for two subsequent medical wire applications to evaluate the reliability of this method to detect comparable CTC numbers on the same day.
Results: Analysis of the breast cancer patients regarding the performance of the medical wire indicates besides very good biocompatibility and the absence of any side effects substantially higher CTC detection rates compared to the FDA-approved CellSearch method. This result proves the in vivo application of the medical wire technology with access to the whole blood stream being superior to methods isolating CTC from relatively small blood samples in vitro.
Conclusion: Increased CTC detection rates of the medical wire may serve to improve early detection, prognosis, and therapy monitoring of cancer patients in future. As this technology is an efficient method for tumor cell enrichment, subsequent molecular analysis of these cells have been initiated in collaboration with Bayer HealthCare and Prometheus to eventually establish more personalized treatment regimes.

PP 6

The metabolic response using FDG/PET for predicting tumor response and prognosis after pre-operative chemoradiotherapy (CRT) in patients with locally recurrent rectal cancer (LRRC)

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Background: Local recurrence is the most common type of recurrence after resection of advanced low rectal cancer. Radical resection of recurrent tumor including adjacent tissues such as bladder, sacral bone is the only means of cure. Even R0 resection, incidence of local re-recurrence is 20 to 60%. In order to reduce the incidence of local re-recurrence, we have employed pre-operative CRT. The aim of the study was to predict tumor regression in pre-operative CRT and prognosis after radical resection using 18F-fluorodeoxyglucose-positron emission tomography/computed tomography (PET/CT) and serum carcinoembryonic antigen (CEA) in patients with LRRC.
Materials and Methods: Fourteen males and 6 females with median age of 61 (range 36 to 70) who had preoperative CRT and underwent R0 resection were evaluated. PET/CT was performed before and after three weeks of pre-operative CRT in all patients. Histological diagnosis was made based on resected specimen. The metabolic response of the tumor was assessed by determining the maximal standardized uptake value (SUVmax), absolute difference [ΔSUV(max)], and SUV reduction ratio (SUVRR) on pre- and post-CRT PET/CT scans. The serum CEA, absolute difference, and the CEA reduction ratio were also determined.
Results: Median pre- and post-CRT SUVmax was 7.8 and 3.1, respectively. The median serum pre- and post-CRT CEA was 12 ng/ml and 3.5 ng/ml, respectively. Ten patients (50%) were classified as responders

(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.