until tumour progression. Histological diagnosis of FFPE samples obtained by bronchoscopy was available. Tissue was primarily used to assess TS IHC. Additional tumour molecular profiling was performed to further understand the TS IHC results and correlative clinical response. Pathologist assessment of tissue in a central laboratory (ALMAC, UK) determined the quality/quantity of samples. A pre-specified prioritised assay list (TS IHC>RNA>DNA) was implemented using standard operating procedures in an ISO17025 accredited laboratory. RNA was extracted for transcriptome analyses, qPCR of TS expression, array and qPCR-based microRNA analysis. DNA was extracted for array SNP profiling. RNA was extracted from $3\times10\mu M$ sections (primary extraction) and $2\times10\mu M$ sections (back-up) and for DNA $4\times10\mu M$ sections were cut and extracted for downstream assays.
Results: Tumour tissue samples from 67 of 70 patients were evaluated and average 9 sections/sample were prepared. TS IHC was assessed in 59 samples (88%). Eight yielded no tumour/tissue. RNA and DNA were extracted from 64 samples (96%) with majority of the samples assessed successfully [gene expression on lung DSA − 59 (88%), TS qPCR − 61 (91%), miRNA-array − 9 (13%)/qPCR − 61 (91%) and SNP array − 28 (42%)]. Nine samples (13%) were processed on all six platforms (3 passed array QC). 49 (73%) of blocks contained sufficient samples for additional correlative work. Correlation with clinical response data is awaited.
Conclusion: NSCLC biopsy yields are frequently low in the advanced disease setting, but multiple molecular genetic assessments are feasible.

PP 27

Evaluation of expression levels of p38α, a signalling protein in Head and Neck Squamous Cell Carcinoma and design of peptide inhibitors against the same

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Background: Head and Neck Squamous Cell Carcinoma (HNSCC) is one of the leading causes of high mortality rate in the present world involving the sequential activation of Mitogen Activated Protein (MAP) kinase pathways. Among them the p38 MAP Kinase pathway is responsible for production of cytokines during the progression of inflammation and malignancies. The p38 consists of four isoforms-α, β, γ and δ. This study quantifies the p38α level in serum of HNSCC patients indicating it as a prognostic marker thereby establishing its correlation with radiation therapy (RT) and to inhibit p38α pathway by structure based designed peptide inhibitors.
Materials and Methods: In the case–controlled study, 81 HNSCC (oral and oropharyngeal) patients and 45 controls (Healthy subjects) were enrolled which were statistically analysed. The primary endpoints were clinical response and experimental p38α level assessment. The p38α estimation was done at presentation, during-RT and post-RT using a real time Surface Plasmon Resonance (SPR) technology BIAcore 2000 and ELISA. The peptide inhibitors were designed using the Glide 4.5 protocol utilizing the ATP binding site, synthesised by SPPS and screened biochemically using SPR technology and competitive ELISA methods.
Results: The HNSCC patients exhibited a higher circulating levels of p38α at pre-RT period (0.61 ng/μl, 95% CI:0.53–0.69)) as compared to the controls (0.23 ng/μl, 95% CI:0.21–0.25, p< 0.0001). The p38α further declined significantly at during-RT (0.35ng/μl, 95% CI:0.31–0.38) and post-RT periods (0.30 ng/μl, 95% CI:0.26–0.33). The p38α levels evaluated by ELISA were 0.11 μg/μl (95% CI:0.10–0.12), 0.60 μg/μl (95% CI:0.59–0.61), 0.43 μg/μl (95% CI:0.42–0.44) and 0.30 μg/μl (95% CI:0.27–0.33)($p < 0.0001$) for the control and the HNSCC group at the pre, during and post RT, respectively. Out of 20 peptides, a tetrapeptide K11 was found to be comparable to that of standard SB203580. The KD determined by SPR analysis was $10 \times 10{-}9$ M and $7.22 \times 10{-}9$ M for SB203580 and K11, respectively. The inhibitory efficacy (IC50) determined by ELISA technique using ATF-2 as a substrate for p38α was 0.9 μM (SB203580), 0.3 μM (K11).
Conclusion: The p38α expression was elevated at diagnosis and significantly declined with the radiation therapy. Hence, it can be used as a prognostic serum marker in HNSCC. Further, the biochemical assays supports the candidature of K11 as a future therapeutic agent.

PP 28

Analytical performance and workflow comparison study of three methods for detecting KRAS mutations in formalin-fixed paraffin-embedded tissue (FFPET) specimens of colorectal cancer (CRC)

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Background: With KRAS mutation testing now mandatory for the selection of patients with metastatic CRC to receive anti-EGFR antibodies, it is critical to have a well-validated, sensitive and robust assay. Although current guidelines recommend testing for codon 12 and 13 mutations, clinical data show that codon 61 mutations may also be predictive of non-responsiveness.
Materials and Methods: We conducted a two-site method comparison study of an investigational TaqMelt PCR assay, cobas® KRAS Mutation Test, which detects 19 mutations in codons 12, 13 and 61, vs. an ARMS-Scorpions assay covering 7 mutations in codons 12, 13 (TheraScreen® KRAS, Qiagen) and vs. Sanger sequencing. 120 FFPET specimens, selected from a bank of 525 vendor-purchased CRC specimens were tested in a blinded fashion with all 3 methods, with cobas® being performed at both sites. Positive (PPA) and negative (NPA) percent agreements were determined for the cobas® test vs. each of the other 2 methods. Specimens yielding discordant results between test methods were subjected to next-generation pyrosequencing (454 GS-Titanium). Plasmid DNA blends were tested to determine detection rates at 5% of mutant alleles.
Results: Repeatability of the cobas® test between the 2 sites was 98.2%. PPA between cobas® and Sanger was 98.2%; NPA was 89.7%. Of 6 specimens that were mutation-positive by cobas® and negative by Sanger, 454 testing resulted in 5 mutation positive calls and resulted in a composite PPA of 100% and NPA of 98.1%. PPA with TheraScreen was 100%; NPA was 86.7%. 454 testing indicated that out of 8 discordant cases (mutation positive by cobas® and negative by TheraScreen), 7 were mutation positive and 1 was negative, resulting in a 454-composite NPA of 98.1%. Three cases were positive for codon 61 and 3 were positive for codon 13 mutations that TheraScreen was not designed to detect. Detection rates with 5% mutant DNA blends were 100% for cobas®, 19.1% for Sanger, and 100% for TheraScreen for codon 12/13 mutations. Turnaround times for 24 samples were 1 day using cobas®, 5 days using Sanger, and 1 day for 12 samples using TheraScreen.
Conclusion: The cobas® KRAS Mutation test was highly reproducible across clinical testing sites, with a high level of agreement between cobas® and the 2 methods. The cobas® test has short turnaround times, software for automated analysis and interpretation of results, and offers a robust, fast and reliable method for routine clinical KRAS mutation analysis.

PP 63

Integrating hypoxia and native conditions for immune complex formation in the serological proteome analysis (SERPA) to improve the detection of autoantibodies as cancer biomarkers

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Background: The expression by tumor cells of proteins with aberrant structure, expression or distribution accounts for the development of a humoral immune response. Autoantibodies (AAbs) to tumor-associated antigens (TAAs) may thus be particularly relevant for early detection of cancer. Several proteomic approaches have been developed to identify circulating AAbs. One approach called SERPA is based on the immunoblotting with cancer patient serum, of 2DE-separated tumor cell proteins and the consecutive MS identification of reactive spots. This method has the advantage to use post-translationally modified proteins (contrary to methods using phage peptides or bacteria-produced proteins). Limitations are however the use of poorly relevant plastic-cultured tumor cells and the detection of AAbs reaction against denatured proteins.
Materials and Methods: Here, we propose an optimization of the SERPA method based on (i) the pre-exposure of tumor cells to hypoxia to allow the expression of a pattern of proteins closer to the in vivo conditions and/or (ii) the incubation of tumor cell extracts directly with purified seric IgG to allow interaction with TAAs in native conditions. Resulting immune complexes are consecutively purified via affinity chromatography before MS identification of the antigens. This modality also allows to deplete lysates of tumor-unspecific antigens by rounds of pre-incubation with IgG isolated from control sera.
Results: We used human breast cancer cells MDA-MB231 and human colorectal cancer cells HCT116 that we exposed for 48 hours to 1% O2. With the mammary cell line, only spots positive after immunoblotting of hypoxic cell lysates with the sera of tumor-bearing mice, were collected and identified by MS analysis. Specific ELISA were developed for 6 proteins and confirmed the presence of corresponding AAbs in the serum of tumor-bearing mice (vs healthy mice) ($P < 0.01$), the titer of which increasing with tumor growth. With the colorectal cancer cell line, we combined the strategy of hypoxia exposure to provide a more relevant repertoire of TAAs with LC-based isolation of IgG from patients with colorectal cancer (vs healthy volunteers). This led us to document the formation in native conditions, of immune complexes not detected by conventional SERPA.
Conclusion: In conclusion, this study provides evidence that integrating the hypoxia criteria and the interaction in native conditions between TAAs

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