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