Results: Five patients were found heterozygous IVS14+1GA (DPYD*1/*2A) and one patient was homozygous mutant IVS14+1AA (DPYD*2A/*2A). The homozygous patient was initially tested with a reduced 5-FU test dose and showed diarrhea grade 2, mucositis grade 3, anemia grade 1, pistrinopenia grade 3, febrile neutropenia grade 4, complete alopecia and Staphylococcus aureus sepsis. This patient required 20 days of hospitalization and was managed with antibiotics, platelet transfusion, port removal, G-CSF administration and parenteral nutrition.
Conclusion: Although the frequence of DPYD*2A allele is low, the screening for DPD mutation is clinically relevant to avoid the severe toxicities or death in patients trated with fluoropyrimidine-containing regimens.

PP 25

Preclinical efficacy of a dual PI3K-mTOR inhibitor, BEZ235 in triple negative breast cancer

N. Dey, H. Wu, Y. Sun, P. De, B. Leyland-Jones. *Emory University, School of Medicne, Atlanta, USA*

Background: Pathway-targeted therapy has not been established for the treatment of Triple Negative (TN) subset of breast cancer (BC). Studies demonstrated that the frequent activation of the PI3K pathway is part of the natural history of ER-negative BC, and PTEN protein/function is down regulated in ~40% of breast tumors (BT) including TNBT (Saal et al., 2007, 2008). We hypothesize that the inhibition of PI3K/mTOR pathway by BEZ235 will have anti-proliferative, anti-angiogenic, and anti-migratory effects on TNBT cells.
Materials and Methods: The effects of BEZ235 were studied on: (a) the cell survival/proliferation (MTT, SRB, & cell titer-GLO assay), (b) IGF-induced upregulation of HIF-1α, (c) the cellular signals for proliferation and apoptosis, (d) fibronectin-directed migration (scratch-assay), and (e) the organization of polymerized-actin (confocal microscopy) in TNBT cell lines.
Results: The results show that, (1) the effect of BEZ235 was pronounced only after 96 hrs of the treatment in TNBT cell lines (HCC70, HCC1937, MDA-MB231, SUM149), in contrast to HER2+ cell lines wherein the EC50s can be determined as early as 48 hrs, (2) the range of EC50s in TNBT cells varied from 1−5 uM as compared to 10−70 nM in HER2+ cells, (3) PTEN-null and ATM kinase mutated MDA-MB468 cell line exhibited 200 uM EC50 (72 hrs), (4) BEZ235 treatment decreased cellular-ATP levels within 48 hrs, (5) IGF-induced HIF-1α expression was abrogated by BEZ235 in MDA-MB468 cells, (6) BEZ235 treatment (50nM) decreased pAKT-S473 and pP70S6K after 1 and 3 hrs, (7) the decrease in pAKT-S473 was reversed after 48 hrs while the decrease in pP70S6K was reversed partially after 48 hrs, (8) treatment with BEZ235 time dependently increased cleaved-caspase9 and cleaved-PARP, and (9) BEZ235 treatment dose dependently inhibited fibronectin-directed migration and altered organization of actin-cytoskeleton in TNBT cells.
Conclusion: BEZ235 has anti-proliferative/pro-apoptotic, anti-angiogenic and anti-migratory effects on TNBT cells. We are currently pursuing studies to, (a) delineate the relationship between the anti-proliferative effects (3D-ON-TOP clonogenic assay) of BEZ235 and the status of the PI3K-PTEN-mTOR pathway using PIK3CA-mutated and PTEN-null cell lines, (b) demonstrate the effect of BEZ235 on integrin-directed real-time migration of live TNBT cells, and (c) find out the effect of BEZ235 on vascular-mimicry in TNBT cells; the results of which will be presented in the meeting.

PP 80

Identification of translocations involving the PRDM16 locus in hematological malignancies with 1p36 alterations

F.P. Duhoux, G. Ameye, C.P. Montano-Almendras, K. Bahloula, D. Latinne, J-M. Libouton, J-B. Demoulin, H.A. Poirel. *Cliniques Universitaires Saint-Luc, La Hulpe, Belgium*

Background: The PRDM16 gene on chromosome 1p36 is rearranged in acute myeloid leukemias (AML) and myelodysplastic syndromes (MDS) with t(1;3)(p36;q21). PRDM16 rearrangements are currently not explored in clinical practice.
Materials and Methods: We studied 120 cases of hematological malignancies (74 myeloid, 44 lymphoid and 2 undifferenciated malignancies) with karyotypic 1p36 rearrangements. We used a contig of bacterial artificial chromosomes (BAC) clones to study the 1p36 region by fluorescence in situ hybridization (FISH). Using TaqMan® gene expression assays, we studied the expression of PRDM16 in 8 cases with available RNA.
Results: The PRDM16 locus was the most frequently rearranged locus, as 39 out of the initial 120 cases harbored a translocation involving PRDM16. The various breakpoints were clustered within a region of less than 400 kb in or 5× of the PRDM16 locus. BAC probes RP11–181G12 and RP11−22L13 allowed the identification of all cases. PRDM16 rearrangements were more frequent in myeloid than in lymphoid cases (37/2), with an overrepresentation of therapy-related myeloid malignancies in this series. We found PRDM16 to be rearranged with the RPN1 locus (3q21) in 30 cases and with other loci in 9 cases. We describe novel translocation partners, including transcription factors ETV6 and IKZF1. There was an overexpression of PRDM16 in all studied cases (range of $2^{-\Delta\Delta C_t}$: 4.8 to 737). Survival data of the 32 patients with available data interestingly suggest that patients with AML/MDS and PRDM16 translocations have a poor prognosis whatever the partner gene, RPN1 versus others, as the median overall survival (OS) was 18 months [95% CI, 6 to 31 months] and 5-year OS was 25.7% [95% CI, 8.4–43.0%].
Conclusion: Our data support the proposal for the addition of a "PRDM16"-entity in the World Health Organization classification of acute myeloid leukemias, as is already the case for the "EVI-1"-entity. In our series, PRDM16 is constantly overexpressed in cases where PRDM16 is rearranged by FISH. Given the apparent bad prognosis associated with this finding, we propose to screen hematological malignancies with karyotypic 1p36 alterations by FISH, using BAC probes RP11–181G12 and RP11−22L13. As 95% of positive cases arose from the myeloid lineage, screening for PRDM16 alterations could be restricted to myeloid malignancies. Before implementing this screening into clinical practice, survival data should be confirmed prospectively in a clinical trial.

PP 8

The role of StarD13 in astrocytoma malignancy: tumor suppressor or oncogene

M. El-Sibai, S. El-Sitt, B. Khalil, J. Backer. *Lebanese American University, Beirut, Lebanon*

Background: Astrocytomas are tumors occurring in young adulthood. Astrocytic tumors can be classified into four grades according to histologic features: grade I, grade II, grade III and grade IV. Malignant tumors, those of grade III and IV, are characterized by uncontrolled proliferation, which is known to be regulated by the family of Rho GTPases. StarD13, a GAP for Rho GTPases, has been described as a tumor suppressor in hepatocellular carcinoma.
Materials and Methods: In the present study, we used immunohistochemistry on tissues taken from human patients of different grade astrocytomas. We also used astrocytoma cell lines. We knocked down Stard13 by transfecting the cells with StarD13 siRNA and we overexpressed Stard13 by transfecting the cells with a GFP-Stard13 construct. We measured cell proliferation and cell death using the MTT and WST kits and doing cell cycle analysis by flow cytometry.
Results: In the present study, IHC analysis on Grade I-IV brain tissues from patients showed StarD13 to be overexpressed in grade III and IV astrocytoma tumors when compared to grade I and II. However, when we mined the REMBRANDT data, we found that the mRNA levels of StarD13 are indeed higher in the higher grades but much lower than the normal tissues. The overexpression of a GFP-StarD13 construct in astrocytoma cells led to the increase in cell death and a decrease of cell viability. Knocking down StarD13 using siRNA led to a decrease in cell death and an increase in cell viability. When looking at the mechanism, we found that the tumor suppressor effect of StarD13 is through the inhibition of the cell cycle and not through the activation of apoptosis. When knocking down StarD13, we also saw an increase in p-ERK, uncovering a potential link between Rho GTPases and ERK activation.
Conclusion: In conclusion, we found StarD13 to be a tumor suppressor in astrocytoma. It is underexpressed in comparison to normal brain and when knocked down in astrocytoma cells, this leads to a decrease in cell proliferation.

PP 56

Suitability of advanced non-small cell lung cancer biopsies for prospective, multiple molecular analyses in clinical trials

D. Fennell, M. Nicolson, D. Ferry, K. O'Byrne, S. Moore, S. McErlean, L. Kennedy, N. Murray, M. Das, K. Kerr. *Queen's University Belfast, Belfast, Northern Ireland, UK*

Background: Accessing somatic molecular data from cancer tissues is a critical requirement underpinning the development of novel personalised therapy. Presently, there is a lack of clarity on the amount of tumour tissue that is sufficient to support prospective exploratory research in clinical trials. We describe the feasibility of multiple laboratory assessments including array-based analyses on routine archival specimens in a clinical trial setting.
Materials and Methods: An open-label, single-arm, phase II, multicentre study in the UK/Ireland was conducted (with appropriate approvals/informed consents) to correlate thymidylate synthetase (TS) expression and progression free survival. Enrolled patients (n = 70, ECOG PS 0−1) with stage IIIB/IV non-squamous non-small cell lung cancer (NSCLC) received pemetrexed (pem)/cisplatin induction followed by pem maintenance

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

K. Gill, A.K. Singh, R. Pandey, R. Kumar, B.K. Mohanti, S. Dey. *All India Institute of Medical Sciences, New Delhi, India*

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)

D. Gonzalez de Castro, B. Angulo, B. Gomez, D. Mair, R. Martinez, F. Shieh, M. Velez, V.H. Brophy, H.J. Lawrence, F. Lopez-Rios. *The Institute of Cancer Research, Surrey, United Kingdom*

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

M. Grandjean, F. Defresne, C. Guilbaud, M. Raes, M. Dieu, A. Sermeus, M. De Ridder, O. Feron. *Université Catholique de Louvain, Brussels, Belgium*

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