10 and 30 mg/kg doses. For pERK assessments in blood, BalbC mice were dosed at 1, 10 and 30 mg/kg and blood samples were taken at the same time points for PK and PD measurements. pERK was measured via flow cytometry in CD3+ lymphocytes after whole blood ex-vivo stimulation with phorbol myristate acetate. We applied a linear compartmental PK framework to describe plasma PK. The time course of tumor PD in-vivo was described by an indirect response model. A sigmoidal E_{max} model was used to describe the dose response relationship of blood PD. Fitted PK−PD models were then used to simulate the PD time course in plasma and tumor at efficacious doses in the A375 model. The models were further extended to simulate human PK−PD profiles.
Results: Simulations of blood and tumor PD profiles upon repeat dosing suggest that continuous and substantial inhibition of both tumor and blood PD is associated with drug response. The EC50s of tumor and blood PD were in broad agreement indicating biological relevance of measuring pERK inhibition in the blood. Simulations of the PD profile demonstrated that the trough PD response has better dynamic range than peak PD response, suggesting sampling strategies of blood PD should focus on trough levels.
Conclusions: A continuous and substantial inhibition of blood p-ERK level is expected to associate with TAK-733 response. Sampling time for PD response at trough levels offers an advantage to peak levels because of higher dynamic range.

601 POSTER

Comparative tissue distribution of the HDAC inhibitor JNJ-26481585

H. Bohets[1], K. Van Uytsel[1], M. De Meulder[1], P. King[2], I. Hickson[2], A. Forslund[3], P. Palmer[2], S. McClue[2]. *[1]Johnson & Johnson Pharmaceutical R&D a Division of Janssen Pharmaceutica N.V., Global Preclinical Development, Beerse, Belgium; [2]Ortho Biotech Oncology R&D a Division of Janssen Pharmaceutica N.V., Oncology, Beerse, Belgium; [3]Ortho Biotech Oncology R&D a Division of Janssen Pharmaceutica N.V., Oncology Biomarkers, Beerse, Belgium*

Background: The histone deacetylase inhibitor JNJ-26481585 has been shown in preclinical testing to have significant efficacy against a number of solid tumour xenografts and improved potency when compared to other HDAC inhibitors such as Vorinostat (Arts et al, Clin Can Res 15, 6841, 2009). We hypothesised that this might be due to a combination of intrinsic potency against target HDACs and also improved tissue distribution. In this study we determined the comparative tissue distribution of JNJ-26481585 and compared it to that of other hydroxamic acid HDAC inhibitors.
Material and Methods: Male nude mice were dosed once per day by the oral route for up to 7 days with 40 mg/kg of Vorinostat, Panobinostat and JNJ-26481585. Dosed animals were sacrificed at 0, 0.5, 1, 2, 4, 7 and 24 hours (3 animals per timepoint), either for single or repeat dose, and plasma and tissues were prepared for compound analysis by LC-MS/MS. Tissues sampled included bone marrow, brain, heart, kidney, large intestine, liver, lung, muscle, prostate, skin and fat. In addition, tissue samples from skin, liver, lung and bone marrow were selected for immunohistological examination for markers of HDAC inhibition.
Results: Comparative exposures (AUC) showed highest levels of all compounds in the large intestine. After this, exposures were highest in the kidney, lung, prostate skin and heart. In all cases JNJ-26481585 showed superior tissue distribution to that of Vorinostat and Panobinostat, reaching levels up to 6 times those of Vorinostat in lung and up to 3 times in prostate, skin and kidney. JNJ26481585 showed better tissue penetration than Panobinostat particularly in brain, liver, muscle and skin. No tissue accumulation was noted after multiple dosing. Tissue levels of JNJ-26481585 exceeded those of plasma levels, with T/P ratios being in excess of 100 in large intestine, kidney and lung and over 50 in heart, prostate and skin.
Preliminary analysis of pharmacodynamic changes in tissues in response to the HDAC inhibitors showed a significant increase in histone acetylation concurrent with a significant reduction in the Ki67 marker of proliferation. Further comparative analysis will be presented.
Conclusions: JNJ-26481585 shows excellent tissue penetration in nude mice, superior to that of Vorinostat and Panobinostat. This property may make JNJ-26481585 an attractive candidate for clinical trials in solid tumours.

602 POSTER

Identification of HSP105 as a novel non-Hodgkin lymphoma restricted antigen

S.M. Pupa[1], R. Zappasodi[2], G.C. Ghedini[1], L. Castagnoli[1], P. Aiello[1], F. Miccichè[1], A.D. Cabras[3], I. Bongarzone[1], A.M. Gianni[2]. *[1]Fondazione IRCCS Instituto Nazionale Tumori, Experimental Oncology, Milano, Italy; [2]Fondazione IRCCS Instituto Nazionale Tumori, Medical Oncology, Milano, Italy; [3]Fondazione IRCCS Instituto Nazionale Tumori, Pathology, Milano, Italy*

Background: We reported that vaccination of relapsed indolent non-Hodgkin lymphoma (NHL) patients using dendritic cells loaded with killed autologous tumor achieved clinical benefits associated with humoral immunity. To identify novel NHL-restricted antigens (ags), we exploited the antibody (Ab) repertoire of responder patients (R) compared to that of non-R (NR), using both pre- and post-vaccine serum samples.
Methods: Purified pre- and post-vaccine Abs from R and NR were biotin-conjugated and tested by immunohistochemistry (IHC), flow cytometry (FC) and western blot (WB) both on autologous and allogeneic NHL specimens and cell lines. Ag discovery was performed applying a modified serological proteomic-based approach (SERPA) followed Mass Spectrometry (MS) analysis. MS-identified cancer-related proteins were further investigated for their role in lymphomagenesis.
Results: By IHC and FC, we found that post-vaccine Abs from R reacted not only on autologous but also on allogenic NHL biopses and cell lines at significantly higher levels than matched pre-vaccine R samples or NR pre- and post-vaccine Abs, respectively. Furthermore, Abs from post-vaccine R serum significantly impaired NHL cell line growth when added for 72 hours in culture as compared to Abs from normal human serum ($p = 0.001$). Towards the identification of novel potential targets for NHL, WB analyses of the follicular lymphoma (FL) cell line DOHH2, tested either as total cell lysate or acidic protein fractions, revealed one differential band migrating at about 100 kDa only when post-vaccine samples from R was used. MS analysis identified the heat shock protein (HSP) 105 as possible ag candidate. By FC, we observed that HSP105 was expressed both on the tumor cell surface and in the cytoplasm of a panel of B-NHL cell lines and, at lower levels, in normal B cells. On the other hand, no reactivity was found following FC analysis of normal T cells or T- lymphoma cell lines. In addition, by IHC on 50 lymphoma specimens, we determined that HSP105 expression levels increased at the increasing of tumor aggressiveness. Accordingly, in vitro blocking assays using a commercial anti-HSP105 rabbit serum revealed a higher anti-tumor activity directed to Burkitt's lymphoma than diffuse large B cell lymphoma or FL cell lines, respectively.
Conclusions: Our preliminary results suggest that HSP105 may represent a novel B-NHL-restricted ag that could be exploited as potential immunotherapeutic target. In vivo studies are ongoing to corroborate our working hypothesis to target HSP105 for the treatment of B-cell lymphoma.

603 POSTER

Baseline circulating tumor cell (CTC) counts enhance the performance of the Royal Marsden Hospital (RMH) Prognostic Score and improve patient selection for phase 1 clinical trials

S. Sandhu[1], C. Massard[1], D. Papadatospastos[1], T. Yap[1], D. Olmos[1], R. Baird[1], J.S. De-bono[1]. *[1]The Royal Marsden NHS Foundation Trust, Drug Development Unit, London, United Kingdom*

Background: CTCs are prevalent in many patients with advanced cancer with higher CTC counts portending a worse prognosis. The use of the RMH Prognostic Score for patient selection for phase 1 clinical trials has been previously validated in prospective analyses. (Arkenau et al, JCO 2009). We evaluated the incorporation of baseline CTC counts to further improve the utility of this prognostic score and enhance patient selection in phase I trials at the RMH.
Methods: We performed a retrospective analysis on the patients who had CTC enumeration as part of their phase 1 trial between January 2006 and December 2009. Blood samples were collected at baseline, during and post therapy for CTC counts and analysed using the CellSearch system (Veridex). Patient characteristics and baseline CTC counts were correlated with the RMH Phase 1 Prognostic Score, which is based on 3 objective markers (albumin <35 g/dL, lactate dehydrogenase [LDH] > upper limit of normal [ULN], and >2 sites of metastases).
Results: Data from 128 patients, male:female ratio (1.1:1), median age 60.5 years (range, 17.5–79.1 years) were collected. The most frequent tumor sites were genitourinary ($n = 31$), gastrointestinal ($n = 30$) and breast ($n = 18$). Median CTC count was 1 (range 0–134). Multivariate analysis indicated that both higher baseline CTC counts and RMH Prognostic Score were independent prognostic factors (HR 1.014, $p = 0.006$). The addition of baseline CTC count enhanced the performance of the RMH Prognostic Score and classified patients eligible to participate in Phase 1 clinical

trials into 3 prognostic groups ($p < 0.0001$): (A) Good prognosis (score 0−1, median overall survival (OS) 63.7 weeks); (B) Intermediate prognosis (score 2−3, median OS 37.3 weeks) and (C) Poor prognosis (score 4, median OS 13.4 weeks).
Conclusion: Pre-treatment CTC counts provide important prognostic data for the selection of patients for phase 1 clinical trials; prospective studies are now needed in unscreened patients to determine the clinical utility of CTC testing in this population.

604 POSTER

Farnesoid X receptor overexpression predicts breast cancer bone metastases through a Runx2-dependent mechanism

F. Journe[1], V. Durbecq[2], H. Boufker[1], D. Larsimont[2], G. Laurent[3], J.J. Body[4], A. Awada[5], G. Ghanem[1]. [1]*Institut Bordet, Lab Oncologie et Chirurgie Exp, Brussels, Belgium;* [2]*Institut Bordet, Département Pathologie, Brussels, Belgium;* [3]*Université de Mons, Service Histologie, Mons, Belgium;* [4]*CHU-Brugmann, Médecine Interne, Brussels, Belgium;* [5]*Institut Bordet, Oncologie Médicale, Brussels, Belgium*

Background: The skeleton is the most common site for breast cancer metastases. The bone matrix contains many growth factors, cytokines and lipids, which are released during osteolysis and may stimulate the proliferation of metastatic cells. Among lipids, bile acids have been recently reported to accumulate in bone tissue from serum and to promote the migration of human breast cancer cells. Bile acids act as physiological ligands for the farnesoid X receptor (FXR, NR1H4), a metabolic nuclear receptor endowed with ligand-dependent transcriptional activity. FXR is typically produced in the liver and the gastrointestinal tract, and we have demonstrated FXR protein expression in primary breast cancer.
Methods: We assessed FXR expression by IHC in primary breast tumors and correlated it with the site of metastasis. We also examined the possibility that FXR activation could induce the expression of bone-related factors in breast tumor cells.
Results: We found that FXR expression significantly correlated with the presence of bone metastases. Indeed, FXR was expressed in 98% of breast cancer samples ($n = 53$, median score = 6) of patients who developed bone metastases, while it was detected in only 68% of breast cancer specimens ($n = 28$, median score = 3) of patients with visceral metastases. Moreover, in the subgroup of patients with histological grade 3 tumors ($n = 18$), FXR was expressed at a high score (median = 7) in 100% breast cancer samples of patients who developed bone metastases, while it showed a much lower occurrence (50%) but also a lower score (median = 2) in patients who developed visceral metastases. Moreover, in the subgroup with high FXR expression, further analysis using a score cutoff at 5 gave a strong association between FXR and the development of bone metastases (positive predictive value of 91%). In parallel, we found that bile acids are able to stimulate the expression and binding to DNA of the transcription factor Runx2 as well as the extracellular structural protein osteopontin, at both the mRNA and protein levels, in the osteotropic MDA-MB-231 cells, but not in MCF-7 cells. The FXR antagonist guggulsterone significantly inhibited both effects.
Conclusions: Clinical and experimental data highly support a relationship between FXR overexpression in breast cancer and the propensity of the tumor cells to develop bone metastases, through a mechanism involving Runx2 stimulation and thus explaining the subsequent promotion of bone-related protein synthesis.

605 POSTER

Quantitative analyses of the impact of Akt inhibitor GDC-0068 on cell signaling and implications for clinical pharmacodynamic assessments

Y. Yan[1], V. Serra[2], D. Sampath[1], L. Prudkin[2], M. Scaltriti[2], B. Liederer[1], S. Murli[1], K. Lin[1], V. Ramakrishnan[1], J. Baselga[2]. [1]*Genentech, Research and Early Development (gRED), South San Francisco CA, USA;* [2]*Vall d'Hebron Institute of Oncology, Experimental Therapeutics Program and Pathology Department, Barcelona, Spain*

Background: Several classes of inhibitors of intracellular kinases are in preclinical and clinical development. Quantitative analyses of the effects of these inhibitors on signaling pathways would enable the use of mechanism-based biomarkers in clinical studies. Herein we describe the systematic analyses of serine and threonine phosphorylation events to generate a specific pharmacodynamic profile of GDC-0068, an inhibitor targeting the Akt node of the PI3 kinase pathway.
Methods: In order to dissect the impact of PI3 kinase pathway inhibitors on cell signaling, over 100 serine and threonine phosphorylation events were profiled using reverse-phase protein array (RPPA) at serial time points in five cell lines and three xenograft tumor models treated with varying concentrations of the Akt inhibitor GDC-0068 and rapamycin, an mTOR inhibitor. In addition, gene expression profiling and inhibitor concentration measurements in tumors were carried out in the tumor models.
Results: Phospho-protein profiling was used to demonstrate that the Akt inhibitor GDC-0068 suppressed tumor growth by down-regulating a selective set of phosphorylation events in the PI3K pathway, defining a profile corresponding to its main pharmacodynamic output. Interestingly, targeting Akt appeared to have distinct pharmacodynamic effects on signaling pathways *in vitro* and *in vivo* in tumor models that differed by PIK3CA, PTEN and HER2 status. Modeling the degree of signaling pathway inhibition in relation to tumor pharmacokinetics further refined the pharmacodynamic marker subset. Correlations with tumor growth measurements identified markers that correspond to tumor growth inhibition. The systematic analysis of signaling events revealed compensatory feedback loops that may relate to treatment escape mechanisms.
Conclusion: The quantitative pharmacodynamic analysis of protein phosphorylation in response to the Akt inhibitor GDC-0068 uncovered key signaling outputs that correlated with both tumor pharmacokinetics and tumor growth inhibition in preclinical models. This work defined mechanism-based biomarkers to assess the activity of the inhibitor in tumor biopsies of treated patients. In addition, this type of analysis has the potential to provide rationale to prioritize combination strategies to pursue in the clinic.

606 POSTER

Integrated analysis of genome-wide copy number and expression changes reveals novel genes in oesophageal adenocarcinoma

X.Y. Goh[1], J.R.E. Rees[1], A.L. Paterson[1], S.F. Chin[2], M. O'Donovan[3], D. Alderson[4], B. Ylstra[5], C. Caldas[2], R.C. Fitzgerald[1]. [1]*Hutchison-MRC Research Centre, MRC Cancer Cell Unit, Cambridge, United Kingdom;* [2]*Cambridge Research Institute, Cancer Research UK, Cambridge, United Kingdom;* [3]*Addenbrooke's Hospital NHS Trust, Department of Pathology, Cambridge, United Kingdom;* [4]*Queen Elizabeth Hospital, Academic Department of Surgery, Birmingham, United Kingdom;* [5]*VU University Medical Center, Department of Pathology, Amsterdam, The Netherlands*

Background: Genome-wide aberrations in oesophageal adenocarcinoma (OAC) are poorly characterised. We hypothesised that the discovery of putative important gene targets by integrating DNA and RNA profiles could enhance our understanding of the pathogenesis of OAC.
Method: Comparative genomic hybridisation (CGH) using an in-house 30K array was performed on 56 fresh frozen OAC resection samples from patients with long term-clinical follow-up. Common regions of aberrations (>5% samples) were called using swatCGH region detection algorithm. Matched gene expression microarray profiling data (median fold-change) was integrated with array CGH data ($\log_2$ ratios) to identify potential gene targets. Multiplex-nested PCR and quantitative fluorescence *in situ* hybridisation (qFISH) on tumour touch-imprints were used to confirm homozygous deletions (HDs). Immunohistochemistry (IHC) on OAC cores represented on tissue microarrays was used to validate targets with the most highly correlated copy number-expression changes on both internal ($n = 65$) and independent datasets ($n = 371$). Survival analyses were performed after unsupervised K-means clustering ($K = 5$, 50 iterations, reproducibility >50%) of array CGH data.
Results: 17 common regions of gains and 11 common regions of losses were identified. Integration of array CGH and expression data highlighted 6 potential gene targets (deletions of *p16* and *MBNL1*; gains of *EGFR*, *WT1*, *NEIL2*, *MTMR9*). Nested-multiplex PCR on microdissected tumour DNA and qFISH confirmed HD of tumour suppressor *p16*. IHC assays confirmed over-expression of EGFR (10% of tumours) and WT1 (20% of tumours), which was not restricted to tumours with gains. Survival analyses following clustering identified a group (32.1% of cohort) with significantly worse prognosis (median survival = 1.37 years; $p = 0.0149$). Modified T-test, with adjusted Bonferroni correction, identified 17 clones with different $\log_2$ ratios ($p < 4 \times 10^{-7}$), implicating 3 regions of gains (2p14, 7q22.1, 15q24.1) and including 5 novel genes (*ZMYND15*, *SYCP2L*, *PMP2*, *LYPD6* and *MEXD3*), between this group and all other groups combined.
Conclusion: Copy number gains with prognostic significance were identified using array CGH. Integration of array CGH and gene expression microarray data highlighted novel gene targets, including *WT1, NEIL2* and *MTMR9*. Overexpression of *EGFR* and *WT1* was observed in 10% and 20% of OAC respectively.Validation of novel genes *NEIL2* and *MTMR9* is currently underway. Functional validation will be required to determine the clinical relevance of these targets.