[GI_{50} value = 130 nM] and SNU-16 [GI_{50} value = 690 nM]). Concentration-dependent inhibition of phosphorylation of downstream FGFR signals (FRS, MEK, ERK, and AKT) is evident in response to ARQ 087 treatment. In addition, growth of SNU-16 gastric carcinoma, AN3CA endometrial cancer, and FGFR2-transfected Ba/F3 tumor xenografts in athymic mice was markedly suppressed after daily oral administration. Finally, ARQ 087 shows favorable pharmaceutical properties that warrant its consideration as a candidate for future clinical development.

120 POSTER

Primary tumor derived preclinical model mimics human colon cancer: a novel platform to study cancer biology and to evaluate anti-cancer drugs

M. Sundaram[1], D. Pinto[1], B. Majumder[1], K. Ameer[1], P. Majumder[1].
[1]*Mitra Biotech Pvt. Ltd, Oncology, Bangalore, India*

Background: Colon cancer is a major cancer in developed and developing nations for which, the underlying mechanism of initiation, maintenance and progression is relatively unknown. The preclinical models used to understand the biology of colon cancer were, till recently, less valuable largely due to lack of consistency in maintaining intra-tumor heterogeneity and tumor microenvironment. At present, established cancer cell lines and cell line based allograft or genetically engineered mouse models are being used for testing personalized therapeutics. However, these models often fail to mimic the real disease and therefore have had limited success as predictive platforms. In order to understand the biology of cancer pathways and testing novel anti-cancer agents, we have developed a novel organotypic explant culture and xenograft models using primary tumors from treatment naïve patients.
Materials and Methods: In this organotypic culture we used paracrine growth factors or ligands for receptors that were derived from the same patient. Extensive profiling of these tumors was performed using transcriptomics and phospho proteomics to provide a mechanistic insight of this system. As proof of concept, advanced stage colorectal cancer explants were treated with oxaliplatin at different concentrations for 4 days. Further freshly isolated treatment naïve primary tumors from patients was propagated in immune compromised mice and treated with oxaliplatin. Anticancer effect of oxaliplatin was evaluated by immuno-histochemical and biochemical analysis.
Results: Data indicate that presence of autologous human ligands significantly enhance the survival and viability of tumor cells due to signal induced activation of key oncogenic pathways. Dose and time dependent effect of oxaliplatin was observed in these models. Molecular profiling and histological data from both primary and xenografted tumor maintained in mice are very similar which suggests that these models preserve the pathological characteristics of primary tumors.
Conclusion: Our data indicate that signaling pathways responsible for tumor growth require human ligands for the activation of downstream signaling network in ex vivo setting. Anti-tumor efficacy of oxaliplatin in explant and primary human derived xenograft models correlates with the clinical outcome which suggest that these models might be useful to predict the treatment options for patients.

121 POSTER

Functional role of CD133 in glioblastoma multiforme

G. Bandopadhyay[1], A. Grabowska[1], B. Coyle[2], S. Watson[1]. [1]*University of Nottingham, Division of Pre-Clinical Oncology, Nottingham, United Kingdom;* [2]*University of Nottingham, Children's Brain Tumour Research Centre, Nottingham, United Kingdom*

Background: Glioblastoma multiforme (GBM) is a cytologically malignant tumour of the central nervous system, associated with poor prognosis and fatal outcome (5 year survival, <6%). Such tumours are believed to be initiated and maintained by a subpopulation of cells, which resemble normal adult stem cells. Cancer stem cells (CSCs), may contribute to the chemo-/radio-resistance exhibited by these tumours and can be identified using the immunocytochemical marker CD133. This pentaspan membrane protein is associated with increased tumorigenicity, chemo-/radio-resistance and poor prognosis. In this study we investigated the functional role of CD133 in the progression of GBM to elucidate any therapeutic benefits of modulating CD133 expression.
Materials and Methods: CD133-specific siRNA and siPORT-Amine transfection reagent were used to achieve knockdown of CD133 in GBM cell lines. Gene and protein expression were measured over time using real-time PCR and FACS, respectively. GBM cell lines were cultured in 1% oxygen to induce hypoxia. Transient knockdown of hypoxia-inducible factors (HIF) was achieved using HIF-specific siRNA, in hypoxic conditions. Biological functions of CD133 were assessed by performing wound-healing assay to investigate migration; MTT to measure the rate of proliferation; neurosphere formation to assess tumorigenicity; and etoposide drug challenge to assess chemo-resistance.
Results: Hypoxia upregulated CD133 expression by 4-fold ($p < 0.001$; $n = 3$) compared to cells cultured in normoxia. Knockdown of hypoxia inducible factors resulted in the downregulation of CD133 in hypoxia. For example, in GBM cell line U251, HIF2-α knockdown resulted in a significant reduction in CD133 gene expression (60% downregulation; $p < 0.01$; $n = 5$). CD133-specific siRNA successfully knocked down gene expression of CD133 (85% knockdown; $p < 0.0001$; $n = 3$) leading to significantly reduced migration ($p < 0.001$; $n = 3$); increased susceptibility to chemotherapeutic agent etoposide ($p < 0.05$; $n = 3$); and reduced neurosphere forming potential ($p < 0.05$; $n = 3$) in GBM cell lines. No change in cell proliferation was noted.
Conclusions: Hypoxia, via HIF-2a, increases CD133 expression in GBM cells. CD133 expression alters important biological properties of GBM cells with CD133 knockdown reducing their migration ability, tumorigenicity and sensitivity to chemotherapeutics. Therefore, using CD133 targeted therapies, in combination with established standards-of-care may improve GBM patient outcome.

122 POSTER

Novel class I PI3K inhibitor CH5132799: potential clinical application in rational combination with molecular targeted therapeutics

H. Tanaka[1], M. Yoshida[1], H. Tanimura[1], T. Yamazaki[1], J. Ohwada[1], H. Ebiike[1], Y. Yoshimura[1], N. Ishii[1], O. Kondoh[1], Y. Aoki[1]. [1]*Chugai Pharmaceutical Co. Ltd., Research Division, Kamakura, Japan*

Background: The phosphatidylinositol 3-kinase (PI3K) pathway regulates various cellular processes, such as proliferation and apoptosis. Class I PI3K is a heterodimer, consisting of a regulatory and a p110 catalytic subunit, which transduces signals from receptor tyrosine kinases (RTKs). One of four p110 isoforms, p110α is known to be actively mutated in various human cancers. CH5132799 is a potent class I PI3K inhibitor with a novel structure, which will be presented in an accompanying poster. We will also present data showing that PI3K pathway-activated tumors, particularly the PIK3CA-mutated tumors, are sensitive to CH5132799. Here, we describe the preclinical efficacy in combination with current standard therapeutics, including RTK-targeted drugs.
Results: The trastuzumab-insensitive breast cancer cell line KPL-4, which harbors Her2 amplification and PIK3CA mutation (H1047R), showed tumor regression by CH5132799 monotherapy. The combination of CH5132799 with trastuzumab induced remarkable antitumor efficacy, resulting in the disappearance of the xenografted tumors. This suggests that CH5132799 can overcome trastuzumab insensitivity in PIK3CA mutants through PI3K inhibition. With lapatinib, *in vitro* cell growth inhibition and apoptosis was enhanced in Her2-amplified breast cancer BT-474 cells. Consistently, this combination enhanced tumor growth inhibition in the BT-474 xenograft model. These data indicate potent compatibility of CH5132799 with Her2-targeted drugs.
Combined administration of CH5132799 with erlotinib was also examined. In NSCLC NCI-H292 cells, erlotinib treatment suppressed EGFR-driven Erk phosphorylation with weak suppression of Akt phosphorylation, whereas CH5132799 could completely suppress Akt phosphorylation. When the drugs were combined, phosphorylation of Erk and Akt were efficiently suppressed concomitantly. Using this rationale, the combination of CH5132799 and erlotinib achieved enhanced antitumor efficacy in a H292 xenograft model.
In addition to RTK-targeted drugs, CH5132799 combined with paclitaxel induced more prolonged tumor growth inhibition than each alone.
Conclusions: CH5132799 showed enhanced efficacy when combined with current therapeutics including RTK-targeted drugs and paclitaxel. These results suggest potential clinical applications of CH5132799 in combination therapy with molecular targeted agents and cytotoxics. CH5132799 is progressing toward phase I clinical trials.

123 POSTER

Anti-tumor activity of CXR1002, a novel anti-cancer clinical phase compound that induces ER stress and inhibits PIM kinases: Human tumor xenograft efficacy and in vitro mode of action

A. Barnett[1], S. Ding[1], C. Murray[1], M. Chamberlain[1], S. Plummer[1], T.R.J. Evans[2], I. MacPherson[2], D. Bissett[3], C.R. Elcombe[1], C.R. Wolf[1]. [1]*CXR Discovery, CXR Discovery, Dundee, United Kingdom;* [2]*Beatson West of Scotland Cancer Centre, Clinical Oncology, Glasgow, United Kingdom;* [3]*Aberdeen Royal Infirmary, Clinical Oncology, Aberdeen, United Kingdom*

Summary: CXR1002 is an ammonium salt of perfluorooctanoic acid. It has a unique pharmacokinetic, pharmacologic and toxicity profile and induces cell death in a wide range of human tumor cells *in vitro* and *in vivo*. CXR1002 causes an ER stress response, acts as a fatty acid mimetic,

and is an inhibitor of PIM kinases. The aim of this study was to explore further its mechanism of action *in vitro*, and to determine its cytotoxicity both *in vitro* and *in vivo*.
Methods: *In vitro* cytotoxicity of CXR1002 was determined using an ATP depletion assay, alone and in combination with 8 other drugs in a panel of tumor cell lines. The mode of action was examined using microarray analysis (followed by analysis using the Ingenuity system) and western blots. Five CXR1002 xenograft studies (25 mg/kg *p.o*. 3x per week) were performed in nu/nu mice.
Results: CXR1002 was cytotoxic to a wide range of human tumor cells, including pancreatic and ovarian carcinoma and sarcoma. Cell lines derived from hematological malignancies were the most sensitive to CXR1002. The IC50 value was lower after 7 days vs 2 days exposure (range 100−590 μM vs 175−>1000 μM), suggesting potency was linked to duration of exposure. IC50 values for the most sensitive cell lines were substantially lower than the plasma concentrations achieved with a non-toxic dose in an on-going phase I trial. Drug combination studies with 8 anti-cancer drugs in 11 human cancer cell lines indicated that CXR1002 was synergistic with other anti-tumor drugs, particularly gemcitabine. Microarray studies in the pancreatic cell line PANC-1 treated with an IC15 dose of CXR1002 showed 4996 gene changes. Representation analysis of the 4996 signature list identified a number of pathways that were over-represented, in particular, genes in the ER stress pathway, including the ATF family of transcription factors. Western blot analysis of PANC-1 cells using PCNA, cleaved PARP and caspase antibodies showed that PCNA was reduced and cleaved PARP and cleaved caspases 3 & 7 were increased 24 hr after treatment with 300 μM CXR1002, suggesting a pro-apoptotic/anti-proliferative outcome. CXR1002 was active in all 5 human xenograft (*i.e*. pancreatic, liver, prostate, lung and colon) models examined with best absolute tumor volume as a % of control of 50.13%, 77.05%, 19.14%, 75.04% and 49.22% respectively.
Conclusions: CXR1002 is a unique potential anti-cancer therapy that exhibits unique pharmacokinetics and a wide spectrum of biological activities. CXR1002 appears to act in part by its ability to induce ER stress. A phase I human trial is on-going.

124 POSTER

Mechanisms of action of histone deacetylase inhibitors (HDACi)

A. Newbold[1], M. Bots[1], L. Cluse[1], R. Lindemann[2], S. Lowe[3], V. Richon[4], P. Secrist[4], T. Miller[4], J. Hardwick[4], R. Johnstone[1]. *[1]PMCC, Cancer Immunology Research, East Melbourne Victoria, Australia; [2]Merck Serono TA Oncology, Merck KgaA, Darmstadt, Germany; [3]Cold Spring Harbor Laboratory, New York, USA; [4]MERCK, Boston, USA*

Background: Histone deacetylases (HDACs) are promising targets for anti-cancer drug development as evidenced by the rapid development of HDAC inhibitors (HDACi) as chemotherapeutics. While HDACi are now approved agents for the treatment of certain haematological malignancies, their mechanisms of action are not fully understood. New isoform-specific HDACi are being designed to target specific HDACs in the hope that a more tailored, less toxic approach to cancer therapy will be achieved. The purpose of this study is to determine whether an HDAC 1/2-specific HDACi will have more potent anti-cancer activities than an HDACi specific for HDACs 1/2/3 and 6.
Material and Methods: Two structurally different HDACi were used: vorinostat, an HDACi that inhibits class I and II HDACs (HDAC1/2/3 and 6) and MRLB223, a recently developed HDACi specific for HDAC1 and HDAC2 (class I). Eμmyc lymphomas were used to assess the biological activities of the two HDACi both in vitro and in vivo. Apoptosis readouts used were: propidium iodide staining to assess cell membrane permeabilisation; TMRE staining to assess mitochondrial function; histone acetylation and; TUNEL staining. C57BL/6 mice bearing Eμ-myc lymphomas were used for therapy studies. Anti-tumor efficacy was determined by assessing the tumor-free and overall survival of tumor-bearing, HDACi-treated mice.
Results: Both vorinostat and MRLB-223 killed Eμmyc lymphoma cells in vitro and engaged the same apoptotic pathways. Both HDACi induced histone hyperacetylation prior to cell death. The kinetics of apoptosis induced by MRLB-223 was slower than vorinostat and required significantly higher concentrations. In vivo, the survival of vorinostat- and MRLB-223-treated mice was significantly extended compared to vehicle-treated mice However, while MRLB-223 still provided a therapeutic benefit to the mice, the effect was not nearly as robust as that provided by vorinostat.
Conclusions: We have shown the HDAC1/2 inhibitor MRLB-223 can achieve similar anti-tumor activities as vorinostat in vitro and in vivo. However, MRLB223 was limited in its efficacy as higher concentrations were required to achieve the same effects as vorinostat. Interestingly, MRLB223 displayed increased toxicity in vivo compared to vorinostat. Therefore, while targeting the enzymes HDAC1 and HDAC2 may be sufficient to cause apoptosis in Eμmyc lymphomas, vorinostat was found to be the superior therapeutic agent.

125 POSTER

Targeting different conformations of BRAF kinase: efficacy of Omni-Raf inhibitors in NRAS and BRAF mutant tumors

C. Zhang[1], H. Cho[1], W. Spevak[1], Y. Zhang[1], B. Burton[1], B. Wong[1], J. Tsai[1], P. Ibrahim[1], P. Hirth[1], G. Bollag[1]. *[1]Plexxikon Inc., Research, Berkeley California, USA*

Background: The RAS-RAF-ERK cascade is aberrantly activated in many types of cancer. RAF inhibitors such as PLX4032 have demonstrated clinical efficacy in melanoma patients carrying the oncogenic mutant form of BRAF. Tumors driven by RAS or other elevated upstream signaling can bypass mutant BRAF-targeted compounds by mechanisms that are currently under investigation. In particular, the induced or autonomous conformational changes of the RAF kinases potentially can dislodge a conformation-specific inhibitor and even turn an inhibitor-bound RAF molecule into an activator of other RAF molecules. By applying the scaffold-based discovery method, we have identified a new generation of RAF inhibitors that show potent inhibition of all RAF isoforms, including mutant BRAF. As revealed by X-ray co-crystallography, these compounds have the unique ability to bind in both the active and inactive states of the RAF kinases, thereby avoiding the conformational restraints of the activation loop. We call this class of compounds the Omni-Raf inhibitors (ORIs).
Material and Methods: This study used $BRAF^{V600E}$ cell lines and NRAS mutant cell lines from commercial sources. The sensitivities of these cell lines to ORIs and $BRAF^{V600E}$-specific inhibitor PLX4720 were determined by both growth and MTT assays. Balb/C nude, female mice were used for the xenograft studies. The treatment was started when mean tumor size reaches approximately 100 mm^3. On the last day of the efficacy studies, the blood samples were collected at different time points after dosing to determine the plasma exposures of the compounds.
Results: In cell culture, the ORIs not only showed improved activity against BRAF mutant cell lines, including some previously known to be resistant to $BRAF^{V600E}$-specific inhibitors, but also potently inhibit melanoma cell lines driven by mutated NRAS. In xenograft models using $BRAF^{V600E}$ and NRAS-driven cells, the ORIs demonstrated over 90% tumor growth inhibition, including significant tumor regression, whereas the $BRAF^{V600E}$-specific inhibitors showed no effect on NRAS-driven tumors.
Conclusion: These results show that RAF inhibitors structurally designed to target multiple conformations of the enzymes can prevent upstream signals from bypassing RAF inhibition.

126 POSTER

Impairment of S-nitrosothiol homeostasis and nitrosative stress modulate proliferation of breast cancer cells

A. Cañas[1], L.M. López-Sánchez[1], I. Porras[1], V. Hernández[1], G. Pulido[1], M.T. Cano[1], J.R. De la Haba-Rodríguez[1], C. López-Pedrera[2], E. Aranda[1], A. Rodriguez-Ariza[1]. *[1]Hospital Reina Sofía IMIBIC, Medical Oncology Department, Cordoba, Spain; [2]Hospital Reina Sofía IMIBIC, Rheumatology Department, Cordoba, Spain*

Background: Augmented nitric oxide (NO) levels in tumors have been usually detected compared to surrounding healthy tissue, and protein modifications induced by NO may constitute a significant regulating factor affecting both tumor progression and antitumoral treatment. S-nitrosothiol (SNO) formation is a cysteine modification, also referred to as S-nitrosation or S-nitrosylation, that controls the function of proteins in a manner similar to phosphorylation. One of the specific mechanisms governing protein de-nitrosylation is the system thioredoxin/thioredoxin reductase (Trx/TrxR). Manipulation or alteration of this enzymatic system may alter SNO homeostasis in tumor cells, providing new insights into the role of NO in cancer and its therapeutic significance.
Materials and Methods: Human breast cancer cells (MCF-7, MDA-MB-231 and BT-474) were pretreated or not with the specific TrxR inhibitor auranofin and exposed to different doses of S-nitroso-L-Cysteine (CSNO). Cell proliferation was measured using the XTT assay, and phosphorylation of Akt and Erk1/2 and cyclin D1 levels were determined by westernblot using the corresponding specific antibodies.
Results: Treatment with auranofin and 100 nM CSNO enhanced cell proliferation of MCF-7 (ER+), but not of MDA-MB-231 (ER-, mut p53), or BT-474 (ER+, mut p53) cells. The augmented rate of cell growth was associated with Akt and Erk1/2 phosphorylation and higher expression of cyclin D1. Significantly, this pro-proliferative effect was abolished by the estrogen receptor (ER) antagonist fulvestrant or the p53 specific inhibitor pythiphrin-alpha. In contrast, in all the three cell lines, a high CSNO dose (500 μM) reduced cell proliferation and this effect was potentiated by pretreatment with auranofin.
Conclusions: Impairment of SNO homeostasis modulated tumour cell growth depending on the grade of the subsequent nitrosative stress. A