ErbB receptor interactions were tested by coimmunoprecipitation, western blot and by FRET analysis.
Results: Analyses of ErbB receptor indicated that ErbB2 and ErbB 3 were expressed in the four cell lines studied.
These two receptors dimerize upon ligand binding, and this ligand-mediated interaction was long lasting. Overexpression of ErbB2 did not cause stable Her2-Her3 dimers.
The action of six different TKI on receptor activation and dimerization was analyzed. We observed that irreversible drugs more potently inhibited ErbB phosphorylation. Unexpectedly, we saw that some TKI interfere with oligomerization whereas others do not.
To gain insights into the mechanism by which distinct TKI differently affect ErbB dimerization, we compared crystal structures of EGFR tyrosine kinase domain, available at the Protein database (PDB) bound to four of the drugs. We found that drugs interfering with ErbB dimerization, bind to the close conformation of the tyrosine kinase domain.
Conclusions: NRG stimulation is compulsory for ErbB2/3 dimerization in breast cancer cell lines.
Some TKIs are able to interfere with ErbB2/3 dimerization while others do not, and this disparity is not due to their reversibility.
TKIs capable of disrupting ErbB dimerization can only bind to the closed ErbB tyrosine kinase domain conformation. Thus, ErbB kinase domain, plays a key role on ErbB dimerization.
In addition to offer information about the impact of distinct TKI on ErbB receptor dimerization, our results demonstrate a role of the open/close states of ErbB receptors in regulating stabilization of receptor-receptor complexes.

338 POSTER

Preclinical profile of novel and potent c-Met kinase inhibitors

S. Vakkalanka[1], M.P. Muthuppalaniappan[2], G. Babu[2], S. Kuppireddi[3], S. Viswanadha[3], S. Veeraraghavan[4], K.K.V.S. Varanasi[4]. *[1]Incozen Therapeutics Pvt. Ltd, General Management, Hyderabad Andhra Pradesh, India; [2]Incozen Therapeutics Pvt. Ltd, Medicinal Chemistry, Hyderabad Andhra Pradesh, India; [3]Incozen Therapeutics Pvt. Ltd, Biological Research, Hyderabad Andhra Pradesh, India; [4]Incozen Therapeutics Pvt. Ltd, Pharmacokinetics, Hyderabad Andhra Pradesh, India*

Background: *c-Met* is a proto-oncogene that encodes the protein Met with intrinsic tyrosine kinase activity. Aberrant Met kinase activity triggers a series of unwarranted phosphorylation events and signalling processes that ultimately lead to the development of cancer. Alteration of the Met kinase signalling cascade represents an attractive approach aimed at blocking invasion and metastasis of cancer cells. Herein, we describe the biological and pharmacokinetic properties of representative molecules from a series of novel and small molecule c-Met kinase inhibitors with scope to be further developed as clinical candidates for various cancers.
Methods: Met Kinase activity of the test compounds was determined using using an HTScan® recombinant human c-Met Kinase Assay Kit (Cell Signaling Technology, Beverly, MA) with modifications. Hepatocyte growth factor (HGF) induced cell proliferation assay (MTT) was conducted to determine the growth inhibitory effect of the compounds on the high Met kinase expressing sk-LMS-1 cell line. Inhibition of HGF induced Met kinase phosphorylation in LMS-1 cells was measured in an ELISA assay. Metabolic stability of the compounds was evaluated in microsomes obtained from mouse, rat, dog, monkey, and human. Pharmacokinetic behaviour of compounds in plasma after single dose oral administration or IV injection was determined in female Balb/c mice.
Results: Among the compounds evaluated, RP1088 and RP1101 demonstrated remarkable potency against the purified Met kinase by inhibiting enzyme activity at low nanomolar concentrations (K_i <6 nM). In addition, the compounds caused a significant reduction in HGF-stimulated proliferation (IC_{50} <100 nM) and phosphorylation (IC_{50} <50 nM) in sk-LMS-1 cells. Pharmacokinetic studies in female Balb/c mice indicated good oral absorption with peak plasma concentrations reaching above 2 μM. Further, the compounds were metabolically stable across the species studied.
Conclusions: Our findings demonstrate that RP1088 and RP1101 are potent Met kinase inhibitors with a favourable pharmacokinetic profile and IC_{50} values comparable to existing Met kinase inhibitors in development. Besides Met kinase, these compounds have the potential to inhibit the anaplastic lymphoma tyrosine kinase (ALK) and are currently being evaluated in relevant cell assays. The compounds are also being tested for *in vitro* and *in vivo* efficacy across various cancer cell lines and xenograft models besides selectivity against other receptor tyrosine kinases.

339 POSTER

Fibroblast growth factor receptor 4 (FGFR4) G388R polymorphism in colorectal cancer

C. Heinzle[1], M. Hunjadi[1], Z. Erdem[1], S. Stättner[2], M. Klimpfinger[3], B. Grasl-Kraupp[1], K. Holzmann[1], M. Grusch[1], W. Berger[1], B. Marian[1]. *[1]Institute Of Cancer Research, Medicine 1, Wien, Austria; [2]sozialmedizinisches Zentrum Süd, Surgery, Wien, Austria; [3]sozialmedizinisches Zentrum Süd, Pathology, Wien, Austria*

Introduction: Fibroblast Growth Factors (FGFs) and their receptors (FGFRs) play a crucial role for cell proliferation, differentiation, and migration. In tumorigenesis their expression and activity is frequently deregulated. A genetic polymorphism has been described in the transmembrane domain of FGFR4 (G388R) and has been correlated with enhanced tumor aggressiveness in several tumour types. In colon cancer its role is under dispute.
Materials and Methods: Tissue specimens of human colon cancer patients were collected and allelic expression of FGFR4 was measured. In addition the expression of FGFR4 in different colon cancer cell lines were analyzed. Cell lines specifically overexpressing FGFR4-G388 (G388) and FGFR4-R388 (R388) were constructed and the biological impact of transgene expression on cell viability, proliferation, clonogenicity, migration, and anchorage independent growth was tested in vitro. The transfected cells were injected subcutaneously into SCID-mice and tumor growth was measured during a period of 4−9 weeks. Tumors and lungs of the mice were harvested and evaluated by immunohistochemistry. Furthermore the consequences of FGFR4 knock down on the biological characteristics of the tumor cells were assessed.
Results: Presence of the R388 allele was predominant in higher grade human tumors and metastatic lesions suggesting a role for this allele in invasion and metastasis. In vitro data support this assumption. R388 overexpression strongly stimulated cell migration but decreased clonogenicity while G388 had the reverse effect. In clonogenicity and anchorage independent growth G388 demonstrated a strong stimulatory effect.Tumorigenicity in vivo was differentially affected by the G388 and R388 alleles with G388 enhancing local tumor growth, while R388 overexpressing cells had a higher tendency of metastasis to the lung. SiRNA mediated knock down showed downregulation of viability, migration and colony formation in all tested cell lines.
Conclusion: Based on the results of this study both forms of FGFR4 have to be regarded as oncogenes and relevant targets for therapy in colorectal cancer. While R388 overexpression was correlated with higher tumor aggressiveness in vivo, mediated by upregulation of cell migration, overexpression of G388 stimulated malignant cell growth in vitro and enhanced local tumor growth in vivo.

340 POSTER

Inhibition of aldehyde dehydrogenase (ALDH) reduces chemotherapy and radiation resistance of stem-like ALDHhiCD44+ breast cancer cells

A. Croker[1], A.L. Allan[2]. *[1]The University of Western Ontario, Anatomy and Cell Biology, London Ontario, Canada; [2]The London Regional Cancer Program, Anatomy and Cell Biology, London Ontario, Canada*

Breast cancer is a leading cause of death in women, due primarily to the ineffective treatment of metastatic disease. In order to reduce mortality from breast cancer, it is essential to learn more about the biology of the metastatic process, specifically what makes metastases so resistant to current cancer treatments. Recently, we discovered stem-like $ALDH^{hi}CD44^{+}$ cells in several different breast cancer cell lines that demonstrated significantly increased metastatic behavior both *in vitro* and *in vivo* when compared to $ALDH^{low}CD44^{-}$ cells. The objectives of the current study were to determine the response of $ALDH^{hi}CD44^{+}$ stem-like breast cancer cells to standard cancer therapy and to test the hypothesis that differentiation therapy with All-*trans* Retinoic Acid (ATRA) would sensitize these cells to therapy. $ALDH^{hi}CD44^{+}$ (stem-like) and $ALDH^{low}CD44^{-}$ (non stem-like) populations were isolated by FACS from MDA-MB-231 and MDA-MB-468 breast cancer cells lines and were plated onto 24-well dishes. The cells were then exposed to vehicle, chemotherapy (doxorubicin [0.1−0.5 μM] or paclitaxel [0.1−0.4 μM]) or radiation [2 × 3−5 Gy] in the presence or absence of ATRA. After 72 hours of treatment, cells were harvested and viable cells were quantified using trypan blue exclusion, or 1000 viable cells were re-plated into 6-well dishes and colonies were counted after 2 weeks. Significantly more $ALDH^{hi}CD44^{+}$ cells survived chemotherapy when compared to $ALDH^{low}CD44^{-}$ cells ($p < 0.001$). Western blots were performed in order to identify proteins that may be contributing to this therapeutic resistance. Glutathione-*s*-Transferase pi (GSTpi) and p-glycoprotein (Pgp) were found

to be over-expressed in the $ALDH^{hi}CD44^{+}$ population compared to the $ALDH^{low}CD44^{-}$ population ($p < 0.05$), indicating that these proteins may play a significant role in the therapy resistance in these cells. Pre-treatment of cell populations with ATRA or diethylaminobenzaldehyde (DEAB, an ALDH inhibitor) had no effect on $ALDH^{low}CD44^{-}$ cells, but resulted in a significant sensitization of $ALDH^{hi}CD44^{+}$ cells to doxorubicin, paclitaxel, and radiation initially ($p < 0.01$); however, only cells that were pre-treated with DEAB maintained this sensitization over the long term and resulted in fewer colonies being formed after 2 weeks in culture compared to the cells only control ($p < 0.01$). In summary, the results of this study demonstrate that stem-like $ALDH^{hi}CD44^{+}$ cells may play an important role in therapy resistance, potentially via increased expression of Pgp and GSTpi. In addition, pre-treatment with the differentiation agent ATRA appears to convey an initial sensitization of $ALDH^{hi}CD44^{+}$ cells to both chemotherapy and radiation; however, DEAB was able to sensitize the $ALDH^{hi}CD44^{+}$ cells for much longer, indicating that ALDH may play a more important role in therapy resistance than was first thought. Ongoing experiments are aimed at clarifying the mechanisms behind this sensitization.

341 POSTER

Targeting NEK2 kinase in drug resistant multiple myeloma with small molecule inhibitors

B. Stephens[1], F. Xiao[2], M. Zangari[2], H. Xu[2], G. Tricot[2], F. Zhan[2], A. Mollard[1], H. Vankayalapati[1], S. Sharma[1], D. Bearss[1]. *[1]Huntsman Cancer Institute, Center Inv. Therap, Salt Lake City, USA; [2]University of Utah, Hematology, Salt Lake City, USA*

Background: Drug resistance is a major obstacle in cancer therapy. The molecular mechanisms of drug resistance still remain largely elusive. Microarray analyses on paired primary myeloma samples at baseline and after therapy or at relapse showed that NEK2 was one of the most up-regulated genes in myeloma cells after high-dose chemotherapy or at relapse. Additionally, high expression of NEK2 was associated with a shorter event-free and overall survival in multiple cancer types.
Materials and Methods: Three-dimensional models of Nek2 were generated using the crystal structures of Nek2 (PDB ID: 2JAV and 2W5A). High throughput virtual screening was carried out using ICM and GOLD docking programs. A biochemical kinase assay was performed using a TR-FRET approach.
Results: Our studies indicate that over-expressing NEK2 in cancer cells resulted in enhanced cell proliferation and drug resistance, whereas knockdown of NEK2 by RNAi induced significant cancer cell death and growth inhibition. In order to identify novel small molecule inhibitors of NEK2, we utilized three-dimensional models of the NEK2 crystal structure and computer-aided, structure-based drug design. These tools allowed us to virtually screen 2 million compounds in our computational database, which we followed up by screening just 18 compounds in a biochemical kinase assay. These efforts yielded a chemical lead containing a substituted benzo[*d*]imidazole scaffold with sub-micromolar activity (IC50 = 0.6 microM) in a NEK2 biochemical kinase assay and good cell-based activity in drug resistant myeloma cell line models. Lead optimization around this chemical series is ongoing in efforts to generate a drug-like compound that can be advanced into myeloma animal studies and eventually into clinical evaluation.
Conclusion: We conclude that NEK2 represents a predictor for drug resistance and poor prognosis in cancers and could be a potential target for cancer therapy. Furthermore, small molecule NEK2 kinase inhibitors based on the benzo[d]imidazole scaffold show promise as therapeutic agents targeting treatment-refractory myeloma.

342 POSTER

Characterization of cancer cell killing mechanisms of targeted hybrid peptide that binds to transferrin receptor

M. Kawamoto[1], T. Horibe[1], M. Kohno[1], K. Kawakami[1]. *[1]Kyoto University, Pharmacoepidemiology Graduate School Of Medicine And Public Health, Kyoto, Japan*

The transferrin receptor (TfR) is a cell membrane-associated glycoprotein involved in the cellular uptake of iron and the regulation of cell growth. Various studies have shown the elevated expression levels of TfR on cancer cells compared with normal counterparts, and the extracellular accessibility of this molecule make it an excellent antigen for the treatment of cancer. Recently, we generated series of molecularly-targeted drugs named hybrid peptide, which is chemically synthesized peptide composed of target-binding peptide and lytic peptide containing cationic-rich amino acids components that selectively disintegrates the cancer cell membrane. In this study, we designed TfR binding peptide connected with the newly designed lytic peptide and demonstrated the selective cytotoxic activity and the characterization of cancer cell killing mechanisms of this molecule.
Cytotoxic activity of TfR-lytic peptide to various cancer cell lines was as low as 4.0 to 9.3 mM of IC_{50}. On the other hand, normal cells were less sensitive to this TfR-lytic peptide (IC_{50} >30 mM). It was found that cytotoxic activity was correlated well with the expression levels of TfR on the cell as assessed by flow cytometry. Competitive assay using TfR antibody or knock down of this receptor by siRNA confirmed the specificity of TfR-lytic peptide to TfR. In addition, it was revealed that this molecule can penetrate cell membrane to make the pore on the T47D cancer cell surface within 10 min to effectively kill these cells. Finally, the cell death mechanism of TfR-lytic peptide assessed by annexin-V binding, caspase activity, and JC-1 staining were assessed, and it was found that this molecule induces approximately 80% of apoptotic cell death via caspase 3&7 activation and cytochrome C discharge but not to normal cells.
Taken together, we concluded that TfR-lytic hybrid peptide may provide potent and selective anticancer therapeutic options to TfR-expressing cancer.

343 POSTER

Targeting p21-activated kinase 1 (PAK1) to induce apoptosis of squamous NSCLC cells

K. Hoeflich[1], C. Ong[1], A. Jubb[2], P. Haverty[3], T. Truong[1], A. Harris[2], M. Belvin[1], L. Friedman[1], E. Blackwood[1], H. Koeppen[4]. *[1]Genentech, Cancer Signaling and Translational Oncology, South San Francisco CA, USA; [2]University of Oxford, Weatherall Institute of Molecular Medicine, Oxford, United Kingdom; [3]Genentech, Bioinformatics, South San Francisco CA, USA; [4]Genentech, Pathology, South San Francisco CA, USA*

Background: P21-activated kinases (PAKs) are serine/threonine protein kinases that serve as important mediators of Rac and Cdc42 GTPase function. PAK1 has been implicated in proliferation and survival signaling by growth factor and hormone receptors, in addition to morphogenetic processes that control cell polarity, invasion and actin cytoskeleton organization.
Results: To better understand the role of PAK1 in tumorigenesis, PAK1 expression was assessed via immunohistochemistry for approximately 600 tumors comprising multiple indications. Strong PAK1 expression was prevalent in specific tumor subtypes. For instance, elevated PAK1 expression was observed in 64% (cytoplasmic staining) and 30% (nuclear staining) of primary squamous cell lung carcinomas and prognostic significance was assessed using patient survival data. The functional requirement for PAK1 in tumor cell proliferation, survival and migration was further investigated via small molecule inhibitors and RNA interference, and in vivo anti-tumor efficacy was assessed for inhibition of PAK1 and PAK2 both individually and in combination. To better understand how PAK1 inhibition could be utilized for treatment of squamous NSCLC, a screen was performed testing a panel of compounds with well-characterized mechanism of action in isogenic lines with or without PAK1 knockdown. In accordance with our data for PAK1 effector signaling, strong combinatorial activity was observed for PAK1 knockdown and inhibitor of apoptosis (IAP) protein antagonists, a class of inhibitors that are currently in clinical development.
Conclusions: Taken together, our extensive preclinical validation of PAK1 inhibition renders this target attractive for future clinical investigation.

344 POSTER

Pharmacological and pharmacokinetic profile of AI1008, a novel and potent inhibitor of abl-kinase activity

R. Desai[1], G.S. Merikapudi[2], K.K.V.S. Varanasi[3], S. Viswanadha[4]. *[1]Atri Pharma Research (India) Pvt. Ltd., Medicinal Chemistry, Hyderabad, India; [2]Incozen Therapeutics Pvt. Ltd., Medicinal Chemistry, Hyderabad, India; [3]Incozen Therapeutics Pvt. Ltd., Pharmacokinetics, Hyderabad, India; [4]Incozen Therapeutics Pvt. Ltd., Biological Research, Hyderabad, India*

Background: Chronic Myelogenous Leukemia (CML) is a myeloproliferative disorder characterized by an increased proliferation and reduced apoptosis of malignant cells especially WBCs. The cancer involves a reciprocal translocation of the BCR and ABL genes resulting in a BCR-ABL fusion known as the *Philadelphia chromosome*. The objective of this study was to investigate the pharmacological and pharmacokinetic properties of AI1008, a novel small-molecule inhibitor of abl-kinase.
Methods: Abl-kinase activity of AI1008 was determined using using an HTScan® recombinant human abl Kinase Assay Kit (Cell Signaling Technology, Beverly, MA) with modifications. Viability assay (MTT) was conducted to determine the growth inhibitory effect of the compound on the *bcr-abl* overexpressing K562 cell line. Metabolic stability of the compound was evaluated in microsomes obtained from mouse, rat, dog, monkey, and human. Pharmacokinetic behaviour of AI1008 in plasma after single dose oral administration or IV injection was determined in female Balb/c mice.