the T790M resistance mechanism is coexistent. The growing preclinical data in EGFR-mutated NSCLCs with acquired resistance to gefitinib or erlotinib has led to test novel MET inhibitors in combination with EGFR TKIs in different clinical trials. Efficacy of MET inhibitors to overcome T790M induced TKI resistance was previously demonstrated in vitro (Ref) and in vivo with cell line xenografts (Ref). Nevertheless information regarding patient derived NSCLC tumors in a *in vivo* setting is lacking. Here we show for the first time evidence in various patient-derived NSCLC xenografts (with known mutational status) that MET inhibition in combination with erlotinib treatment can overcome erlotinib resistance in vivo.

208 POSTER

The alkylating prodrug J1 inhibits ovarian cancer cell growth, activates proapoptotic signalling and potentiates gemcitabine responsiveness in vitro and in vivo in mice

K. Viktorsson[1], J. Gullbo[2], T. Juntti[3], M. Wickström[4], R. Larsson[5], R. Lewensohn[1], J. Spira[3]. *[1]Karolinska Institutet, Department of Oncology/Pathology Karolinska Biomics Center, Stockholm, Sweden; [2]Uppsala University, Department of Medical Sciences Division of Clinical Pharmacology, Uppsala, Sweden; [3]Oncopeptides AB, Karolinska Institute Science Park, Stockholm, Sweden; [4]Karolinska Institutet, Department of Women's and Children's Health Childhood Cancer Research Unit, Stockholm, Sweden; [5]Uppsala University, Department of Medical Sciences Division of Clinical Pharmacology, Stockholm, Sweden*

Background: Ovarian carcinomas are the second most common tumour malignancy of women worldwide. Although most of the patients respond with tumour regression to first line chemotherapy regimen relapses are frequent with few therapeutic options at hand. We have developed a prodrug of melphalan, J1, which has proven to have greater efficacy than melphalan in a number of solid tumor malignancies *in vitro* and *in vivo* in mice. J1 is currently undergoing phase I clinical trial. Here we have analyzed J1-induced cytotoxicity and efficacy in ovarian carcinoma cells *in vitro* and *in vivo*.
Material and Methods: The ovarian carcinoma cell line A2780 was profiled for J1 and melphalan sensitivity, alone or in combination with gemcitabine or doxorubicin, using MTT and FMCA cell viability assays. J1-induced apoptotic signalling was measured as caspase-3 activation in flow cytometry, PARP cleavage on western blot and assessment of J1-induced apoptotic morphology. J1-induced cell cycle effects were evaluated using PI-staining in flow cytometry. The effects of J1 *in vivo* either alone or in combination with gemcitabine or liposomal doxorubicin were examined on A2780 xenografts in SCID mice.
Results: J1 caused a dose and time dependent inhibition of cell growth and induced apoptotic cell death in A2780 cells, with a ten-fold higher potency compared to the parental drug melphalan. Profiling of cell cycle distribution of A2780 cells after J1 treatment revealed G2 accumulation, which preceded induction of cell death. The antitumor efficacy of J1 in combination with gemcitabine or doxorubicin in A2780 cells was found to be synergistic and additative, respectively. Finally we evaluated the effect of J1 or melphalan alone or in combination with gemcitabine or liposomal doxorubicin on A2780 xenografts in SCID mice. Whereas single treatment of either J1 (8 mg/kg) or melphalan (16 mg/kg) caused partial growth inhibition of A2780 tumour growth, a more prominent inhibition of growth was observed when J1 (4 mg/kg) was combined with gemcitabine (5 mg/kg) or liposomal doxorubicin (4 mg/kg).
Conclusion: In conclusion, our data demonstrate that the melphalan prodrug J1 significantly inhibits ovarian cancer cell growth *in vitro* or *in vivo,* either alone or in combination with conventional chemotherapy. Importantly, we show that J1 is more efficient in inhibiting ovarian carcinoma growth than the parental drug melphalan. Taken together these data suggests that J1 may be a good candidate for ovarian carcinoma treatment.

209 POSTER

Monoclonal antibody targeting of the anaplastic lymphoma kinase (ALK) receptor

A. Wellstein[1], J.J. LaConti[1], S.E. Kim[1], S. Flachmann[1], E. Tassi[1], H. Juhl[1], A.T. Riegel[1]. *[1]Georgetown University, Lombardi Cancer Center, Washington DC, USA*

Background: Pleiotrophin (PTN), a secreted growth factor, is upregulated in pancreatic cancer tissues and in patients' serum [1] and can drive proliferation of pancreatic cancer cells in vitro and of xenograft tumors in vivo [2]. PTN is a ligand for the Anaplastic Lymphoma Kinase (ALK) transmembrane tyrosine kinase receptor [3]. Here we report targeting of this ligand/receptor interaction in vitro and in vivo.
Material and Methods: Human pancreatic cancer cells (COLO357) were depleted of their endogenous ALK mRNA using ribozymes and tested for phenotypic alterations in vitro and in vivo. Also, the PTN/ALK interaction site was targeted using a monoclonal antibody to ALK. Antibody efficacy was assessed in vitro, on tumor growth in a syngeneic model of pancreatic cancer and on progression of pancreatic adenocarcinoma in a tissue-specific mutant Kras (G12D) model.
Results: PTN and ALK expression were increased in pancreatic duct adenocarcinoma. Ribozyme depletion of endogenous ALK from human COLO357 pancreatic cancer cells increased their apoptosis, decreased anchorage independent growth, and decreased subcutaneous xenograft tumor growth in athymic nude mice. ALK and PTN expression were increased during initiation and progression of a p48-Cre/LSL-KrasG12D transgenic mouse model of pancreatic duct adenocarcinoma. Systemic treatment of mice with a monoclonal antibody (anti-ALK IgG) targeted to the PTN binding site of ALK reduced the incidence of adenocarcinoma in this model and increased apoptosis in the malignant lesions. Efficacy of systemic therapy with the anti-ALK IgG was also observed in a syngeneic model of pancreatic adenocarcinoma that was derived from the Kras mutant mouse tissues.
Conclusions: We propose that the PTN ligand and ALK transmembrane receptor are drivers of pancreatic cancer. Targeting of their interaction by antibody treatment can provide a novel therapeutic approach in cancers that upregulate this pathway including pancreatic adenocarcinoma and other cancers [4].

References

[1] Souttou et al. 1998; J Natl Cancer Inst 90: 1468−73.
[2] Weber et al. 2000; Cancer Res 60: 5284−88.
[3] Stoica et al. 2001; J Biol Chem 276: 16772–779.
[4] Stylianou et al. 2009; Oncogene 28: 3296–3306.

210 POSTER

EML4-ALK is a sensitive client protein of Hsp90, and rearrangements of the ALK locus are associated with clinical response to IPI-504 (retaspimycin hydrochloride), a novel Hsp90 chaperone inhibitor, in patients with non-small cell lung cancer

C. Fritz[1], A. Lim[1], K. Slocum[2], C. Tunkey[1], G. Paez[1], K. West[2], R. Ross[3], E. Normant[1], L. Sequist[4], V. Palombella[5]. *[1]Infinity Pharmaceuticals, Cancer Biology, Cambridge Massachusetts, USA; [2]Infinity Pharmaceuticals, Pharmacology, Cambridge Massachusetts, USA; [3]Infinity Pharmaceuticals, Clinical Oncology, Cambridge Massachusetts, USA; [4]Massachusetts General Hospital, Cancer Center, Boston Massachusetts, USA; [5]Infinity Pharmaceuticals, Drug Discovery, Cambridge Massachusetts, USA*

Hsp90 is an emerging target for cancer therapy due to its important role in maintaining the activity and stability of key oncogenic signaling proteins. Client proteins of Hsp90 include oncoproteins such as HER2, mutant EGFR, KIT and BCR-ABL. Hsp90 is an ATPase and several inhibitors of Hsp90 are currently in clinical development. Infinity Pharmaceuticals is developing two Hsp90 chaperone inhibitors, an oral (IPI-493) as well as an i.v. administered compound (IPI-504).
We show here that the EML4-ALK fusion protein, presumed to be the oncogenic driver in about 5% of patients with NSCLC, is associated with Hsp90 in cells and is rapidly degraded upon exposure of cells to IPI-504. We find EML4-ALK to be more sensitive to Hsp90 inhibition than either HER2 or mutant EGFR with an IC_{50} for protein degradation in the low nM range. This degradation leads to a potent inhibition of downstream signaling pathways and leads to the induction of growth arrest and apoptosis in cells carrying the EML4-ALK fusion. To generate a causative link between the expression of EML4-ALK and sensitivity to IPI-504, we introduced an EML4-ALK cDNA into HEK293 cells and show that the expression of the fusion protein sensitizes cells to IPI-504 both in vitro and in vivo. In a xenograft model of a human NSCLC cell line containing the ALK rearrangement, we observe tumor regression at clinically relevant doses of IPI-504.
Finally, an analysis of a recent phase 2 trial of IPI-504 in NSCLC demonstrated partial responses in patients with ALK rearranged NSCLC.

211 POSTER

Effective therapeutic sensitization of gastrointestinal stromal tumors by a BH3 mimetic

T. Mühlenberg[1], J.A. Fletcher[2], F. Grabellus[3], M. Schuler[1], S. Bauer[1]. *[1]West German Cancer Center, Innere Klinik − Tumorforschung, Essen, Germany; [2]Brigham And Women's Hospital, Dept. Of Pathology, Boston, USA; [3]West German Cancer Center, Dept. Of Pathology, Essen, Germany*

Background: Inhibition of the KIT-kinase by imatinib (IM) represents the standard treatment of gastrointestinal stromal tumors (GIST). However, IM

treatment results only in low pCR rates, which is reflected by a moderate induction of apoptosis. IM-induced apoptosis is executed via the intrinsic (mitochondrial) pathway of caspase activation, which is regulated by the BCL-2 protein family. Overexpression of antiapoptotic BCL-2 proteins is frequently observed in a wide range of tumor entities and is associated with resistance. Here we have studied the therapeutic potential of a prototypic BH3 mimetic in GIST in vitro.
Methods: The BH-3 mimetic ABT-263, an inhibitor of BCL-2, BCL-XL and BCL-W, was used alone and in combination with anticancer agents(IM, 17-AAG, nutlin-3 and doxorubicin) in GIST models. Cell viability was measured using SRB assays; induction of apoptosis was analysed by annexin-V/7AAG staining and measurement of activated caspase 3/7. Expression of BCL-2 proteins and caspase-dependent protein cleavage was detected by immunoblotting (IB).
Results: Expression of pro- and antiapoptotic BCL-2 family members varied in the examined cell lines with high levels of BCL-2 in GIST882, GIST430 and GIST48B. ABT-263 given alone only moderately reduced viability with IC50s ranging between 500 nM (GIST48B) and 10 μM (GIST48). In IM-sensitive GIST882 combination of IM 1μM with of ABT-263 100 nM led to a significant increase of PARP cleavage (15-fold, compared to 9- and 1.5-fold for IM and ABT-263 alone) as measured by IB. Synergistic effects were also seen in cytometric assays (42% apoptotic cells for combinational treatment compared to 16% and 1% for respective single treatment). Combining ABT-263 with the MDM2 inhibitor nutlin-3 in IM-resistant cell lines GIST48, GIST430 and GIST48B led to an 11-, 15- and 19-fold activation of caspases 3 and 7, respectively, as compared to monotherapy with nutlin-3 (2-, 1- and 4-fold, respectively) or ABT-263 (each 2-fold). Combining ABT-263 with doxorubicin or 17-AAG failed to reveal a positive or negative interaction.
Conclusion: Antagonizing BCL-2 proteins with pharmacologic BH3 mimetics in GIST is a promising strategy to enhance apoptosis induced by several active agents, including IM, 17-AAG and nutlin-3. This provides a first rationale for clinical application of this strategy. Individual profiling of GIST-specific expression of BCL-2 family proteins may guide the selection of patients with the most benefit.

212 POSTER

Potential therapeutic effect of oxidative stress modulators in hepatocellular carcinoma

A.L. Ferreira[1], S. Sousa Neves[2], A.M. Araújo[1], J.D. Branco[1], A.B. Sarmento Ribeiro[2], J.M. Nascimento Costa[3]. *[1]University of Coimbra, Faculty of Medicine, Coimbra, Portugal; [2]Faculty of Medicine University of Coimbra, Center of Investigation on Environment Genetic and Oncobiology – CIMAGO, Coimbra, Portugal; [3]Faculty of Medicine/CIMAGO and Medicine Service and Hepatology Unity, Faculty of Medicine and University Hospital of Coimbra, Coimbra, Portugal*

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, often diagnosed at an advanced stage when the most potentially curative strategies are no longer effective. Several known environmental risk factors for HCC development lead to generation of reactive oxygen species (ROS), promoting oxidative stress. On the other hand, taking in account that mitochondria are the main site for ROS production, it is conceivable that it may have a relevant role in carcinogenesis. Besides, neoplastic cells have a higher mitochondrial membrane potential than normal cells, which may also be explored in the development of new approaches to treat HCC.
The aim of this study was the evaluation of the therapeutic efficacy of new compounds targeting the mitochondria, such as dequalinium (DQ), a lypophilic cation, and the natural bioactive compounds, vitamin C (ascorbic acid, AA, and dehydroascorbic acid, DHA) and epigallocatechin-3-gallate (EGCG), a green tea polyphenol, as in monotherapy and/or in association with conventional antitumoral therapies.
For this purpose, we used the well-established HCC cell line, HUH-7, maintained in culture in absence and presence of increasing concentrations of the DQ, EGCG, AA and DHA, either in monotherapy or in combination with the conventional anticarcinogenic drugs, 5-Fluorouracil and doxorubicin. The antiproliferative effect was assessed by the Alamar Blue assay and cell death analysis performed by morphological studies and flow cytometry. In order to evaluate the involvement of oxidative stress and mitochondria in the observed cytotoxicity, the intracellular ROS accumulation was studied using the fluorescent probes DCFH2-DA and DHE; the mitochondrial membrane potential was determined using the fluorescent probe JC1.
The results obtained suggested that DQ and EGCG as single agents had an antiproliferative and cytotoxic effect in a dose and time dependent manner, while AA and DHA, when used alone, only showed a modest effect under the mentioned test conditions. However, when they were used in combination, a synergistic antiproliferative and pro-apoptotic effect could be observed. These compounds induced cell dead preferentially by apoptosis which may be related with the higher mitochondrial membrane potential depolarization and mediated by the observed increase in ROS production (especially superoxide).
This study suggested that DQ and natural bioactive compounds may constitute a new therapeutic option for HCC. However, new drugs associations, as well as new administration schemes, should be tested in order to improve the therapeutic efficacy in HCC.

213 POSTER

The role of MNK kinase inhibition in combination with mTOR inhibition in the proliferation of renal cancer cells

M. Wheater[1], G. Broadley[1], E. Alveyn[1], P.W.M. Johnson[1], J.P. Blaydes[1]. *[1]University of Southampton, Cancer Sciences Division, Southampton, United Kingdom*

Mitogen-activating protein kinase signal-integrating kinases (MNKs) are the sole kinases of eukaryotic initiation factor 4E (eIF4E) at serine 209. MNK1 is activated through MAPK signaling. eIF4E is an oncogene over-expressed in several cancers. It regulates the synthesis of proteins involved in progression to the malignant phenotype, including cyclin D1, VEGF and MDM2. The oncogenic activity of eIF4E can be dependent on its phosphorylation at serine 209. eIF4E is additionally regulated by binding to 4E-BP under the control of mTOR and it is already a therapeutic target in renal cancer via the mTOR inhibitors temsirolimus and everolimus. Inhibition of mTOR can result in an increase in eIF4E phosphorylation in some model systems. We have investigated the anti-proliferative effects of MNK inhibition in renal cancer cell lines both alone and in combination with mTOR inhibition.
Renal cancer cell lines were treated with CGP57380 (a MNK kinase inhibitor) or rapamycin or both. Western blotting was used to assess eIF4E and 4E-BP phosphorylation and downstream targets of eIF4E phosphorylation such as cyclin D1. The effects of treatment on proliferation and survival were assessed and assays of cell cycle analysis and apoptosis performed.
The proliferation of renal cancer cell lines is sensitive to inhibition of MNK kinases. This is predominantly due to cell cycle arrest and consistent with a reduction in cyclin D1 at both the protein and RNA level. Cells which showed sensitivity to mTOR inhibition showed an additive effect to the addition of MNK kinase inhibition.
Inhibition of eIF4E phosphorylation results in a reduction in proliferation in both VHL mutant and VHL wild-type renal cancer cell lines. There was an additive effect of the addition of rapamycin. The MNK kinases are worthy of further investigation as a therapeutic target in renal cancer.

214 POSTER

Human ependymoma tumor-initiating cells (TICs) as a model for preclinical studies on EGFR-kinase inhibitors (EGFR-KIs)

D. Meco[1], T. Servidei[1], N. Trivieri[2], G.F. Zannoni[3], R. Pallini[4], G. Maira[4], R. Riccardi[1]. *[1]Catholic University of the Sacred Heart, Department of Pediatric Oncology, Rome, Italy; [2]University of Milano-Bicocca, Department of Biotechnology and Biosciences, Milano, Italy; [3]Catholic University of the Sacred Heart, Department of Pathology, Rome, Italy; [4]Catholic University of the Sacred Heart, Department of Neurosurgery, Rome, Italy*

Background: Ependymomas represent 10% of pediatric brain tumors, with a dismal prognosis in 50% of patients. Increasing evidence shows that ependymomas arise from TICs, which are believed to be responsible for tumor development, progression, and recurrence. Given the high dependence of TICs on EGF, we explored the effects of the EGFR-KIs gefitinib and AEE788 on ependymoma TICs.
Material and Methods: We used neural stem cell permissive conditions to isolate TICs from pediatric ependymomas. Cells were characterized for the expression of stemness markers (CD133, nestin, and brain lipid binding protein), neurosphere-renewal ability, multipotency and tumorigenicity. The effects of gefitinib (which targets EGFR), and AEE788 (which simultaneously targets EGFR, HER2, and VEGFR1/2), were evaluated on cell proliferation and EGF-induced signaling *in vitro* and in TIC-driven xenografts.
Results: We established two TIC-lines that fulfilled all TIC criteria. When orthotopically implanted into nude mice, cells gave rise within 2–3 months to tumors that phenocopied parental tumors. Both lines exhibited a high pEGFR/EGFR ratio. EGFR-KIs reduced dose-dependently the proliferation and survival of TIC-lines, and effectively blocked EGF-induced and basal activation of EGFR, HER2, Akt and ERK. Treatment reduced CD133 expression dose- and time-dependently, suggesting selective effects of EGFR-KIs on the TIC subpopulation. On removal of growth factors, lines showed morphological changes towards neuronal- and astrocytic-like cells, and strongly up-regulated GFAP, while down-regulating the expression of CD133 and activated HER2. Differentiated TICs were less sensitive