multiple myeloma, breast, renal, and liver tumor cell lines as indicated by combination indices below 0.7. In the colon cancer cell line SW620 cell cycle analysis revealed G2M arrest as mechanisms of action for Perifosine, whereas two representative antimetabolites, i.e. 5-Fluorouracil and 6-Thioguanine, induced S-phase arrest as expected. In combination, synergistic effects were observed in terms of apoptosis, e.g. caspase activation.
In summary, these results demonstrate potent synergistic activity of Perifosine with various antimetabolites in human colon, multiple myeloma, breast, renal, and liver tumor cell lines. Synergism seems to be based on combining G2M arrest by Perifosine and S-phase arrest by the antimetabolite resulting in synergistic induction of cellular apoptosis. Further experiments addressing Perifosine's mechanism of action in combination with antimetabolites are ongoing. Currently, Perifosine is in a phase III clinical trial in combination with Capecitabine in patients with refractory advanced colorectal cancer.

204 POSTER

Effects of EGFR inhibition with tyrosine kinase inhibitors on invasive properties of EGFR mutant and wild type lung cancer cells

I.A. Umelo[1], O. De Wever[2], E. Teugels[1], M. Bracke[2], J. De Grève[1]. *[1]Universitair Ziekenhuis Brussel, Laboratory of Molecular Oncology and Department of Medical Oncology, Brussel, Belgium; [2]Universitair Ziekenhuis Ghent, Laboratory of Experimental Cancer Research and Department of Radiotherapy, Ghent, Belgium*

Background: The epidermal growth factor receptor (EGFR) pathway is known to be involved in the invasive and metastatic process. Furthermore, it is also known that activating mutations of EGFR confer increased sensitivity to small molecule tyrosine kinase inhibitors (TKI's) in the treatment of non-small cell lung cancer (NSCLC). The effects of various TKI's on lung cancer cell proliferation and survival have been previously investigated. However, the effects on invasion (and metastasis) have been studied less. Our present study investigates the effects of EGFR TKI's on the invasion of lung cancer cells with a wild-type or a mutant EGFR gene in an *in vitro* invasion assay.
Materials and Methods: The model used is based on the preparation of native collagen type I, the main interstitial matrix component of solid tumors. Three NSCLC cell lines − NCI-H358 (EGFR-WT), NCI-H1650 (EGFR-ΔE746-A750) and NCI-H1975 (EGFR-L858R/T790M) − were evaluated with several inhibitors of EGFR (erlotinib, lapatinib, BIBW2992, and cetuximab) for the effects on their invasive properties. Invasion- induced changes in cellular structure and F-actin organization were analyzed with phase contrast and confocal microscopy techniques. Invasive index, and factor shape were measured via image processing.
Results: Qualitative and quantitative analysis show that while lapatinib and cetuximab have a moderate effect on the attenuation of epidermal growth factor (EGF) stimulated invasion of mutant NCI-H1650, erlotinib and BIBW 2992 significantly abrogate cellular invasion ($P < 0.0001$). Similarly, BIBW 2992 abrogates invasion in the T790M mutant NCI-H1975 cell line ($P < 0.01$), whereas no effects are observed with any of the first-generation inhibitors. Interestingly, erlotinib significantly promoted EGF stimulated invasion of wild-type NCI-H358 ($P < 0.001$), while BIBW2992 did not.
Conclusions: These findings show that, as assessed in the pre-clinical *in vitro* collagen type I assay, erlotinib has differential effects on the invasive phenotype depending on the genomic status of the EGFR gene, promoting invasion in wild type cells. Our study also supports the use of BIBW 2992 as a therapeutic option in tumors bearing the EGFR-T790M resistance-conferring mutation.

205 POSTER

Distinct inhibitory properties of MEK inhibitors on pathway feedback translate into differential potency in BRAF and RAS mutant cancer cells

M. Belvin[1], L. Berry[1], J. Chan[1], K. Hoeflich[1], B. Liu[1], S. Malek[2], M. Merchant[1], C. Orr[1], K. Song[1], G. Hatzivassiliou[1]. *[1]Genentech Inc., Cancer Signaling/Trans Onc, South San Francisco, USA; [2]Genentech Inc., Biochemical Pharmacology, South San Francisco, USA*

Background: The RAS/RAF/MEK pathway is active in over 30% of human tumors, often due to mutation in BRAF or RAS family members. Several MEK inhibitors, aimed at treating tumors with RAS/RAF pathway alterations, are in various stages of clinical development. Despite their similarities, MEK inhibitors from distinct chemical series differ in their ability to modulate and inhibit signaling in BRAF and KRAS mutant cell lines and tumors. Here we explore the biochemical nature of this differential potency.
Results: GDC-0973 is a potent, selective, allosteric MEK1/2 inhibitor that is currently being tested in early stage clinical trials. G-573 is an allosteric MEK1/2 inhibitor from a distinct structural class with similar biochemical potency and selectivity as GDC-0973. While GDC-0973 and G-573 have similar cellular potencies in $BRAF^{V600E}$ mutant cells, G-573 displays up to 10 fold higher potency in KRAS mutant cell lines, and shows greater efficacy in vivo in KRAS mutant xenograft tumors. In vivo, GDC-0973 shows stronger maximal efficacy than G-573 in $BRAF^{V600E}$ mutant xenograft models whereas G-573 shows stronger efficacy than GDC-0973 in KRAS mutant models. To investigate the basis for the different activities of these two MEK inhibitors, we analyzed their effects on components of the RAF/MEK/ERK pathway in $BRAF^{V600E}$ vs. RAS mutant cells. We found that GDC-0973, but not G-573, increases levels of phosphorylated MEK (pMEK) and displays a potency shift in blocking pERK in KRAS vs, $BRAF^{V600E}$ mutant cells. This pMEK increase is mediated by RAF family members which are activated due to the release of negative feedback in the MAPK pathway. Although G-573 leads to a similar negative feedback release and RAF activation, it blocks MEK phosphorylation by activated RAF and is more effective at blocking downstream ERK activation in KRAS mutant cells. This effect translates into distinct cellular potencies for the two inhibitors in RAS mutant models, where the negative feedback is present, but not in $BRAF^{V600E}$ models, where it is absent.
Conclusions: These findings provide an explanation for the potency differences of MEK inhibitors in RAS vs. $BRAF^{V600E}$ mutant xenograft tumors and support a model in which potency in RAS mutant tumors correlates with the ability of MEK inhibitors to effectively block MEK activation by RAF. As a consequence, different classes of MEK inhibitors may show distinct efficacy profiles in the clinic.

206 POSTER

The cis/trans effect of the T790M drug resistant mutation in non-small cell lung cancer

I. Umelo[1], G. Chen[1], E. Teugels[1], J. De Grève[1]. *[1]Universitair Ziekenhuis Brussel, Laboratory of Molecular Oncology and Department of Medical Oncology, Brussel, Belgium*

Background: Activating mutations of the epidermal growth factor receptor (EGFR) in non-small cell lung cancer (NSCLC) confer increased sensitivity to small molecule tyrosine kinase inhibitors (TKIs). However, despite initial response, tumors often develop the resistance conferring T790M mutation. Although resistance mechanisms of T790M have been studied, the impact of T790M arising in either *cis* or *trans* to the activated allele remains to be established. The aim of our study was to compare the effects of EGFR primary activating mutations associated with TKI sensitivity to the TKI insensitive EGFR-T790M arising in *cis* or *trans*.
Materials and Methods: The model used is based on the interleukin-3 (IL-3) dependent Ba/F3 system. We transformed the Ba/F3 cells to an epidermal growth factor (EGF) dependent system by the exogenous introduction of wild-type and mutant forms of the EGFR gene through stable transfection (wild-type EGFR, mutant EGFR and *cis* constructs) and stable co-transfection (*trans* configurations). We assessed the functionality of our constructs with two EGFR tyrosine kinase inhibitors, erlotinib and a novel irreversible inhibitor, BIBW 2992 through ^{3}[H]thymidine incorporation, MTS, Annexin V/7-AAD and western blot analysis.
Results: Our results show that T790M arising in *trans* to a primary activating EGFR mutation exhibits increased activation of AKT, ERK1/2 and STAT5 when compared to its *cis* counterpart. We also found that BIBW 2992 overcomes resistance in all erlotinib resistant T790M conformations by decreasing proliferation, increasing apoptosis and promoting G_1 cell cycle arrest.
Conclusions: The T790M mutation activates the EGFR signal transduction pathway more effectively in the *trans* than in the *cis* conformation relative to primary activating mutations. The covalent EGFR/HER2 inhibitor BIBW 2992 has activity in both conformations.

207 POSTER

A novel selective MET inhibitor combined with erlotinib overcomes erlotinib facilitated resistance in patient derived NSCLC xenografts in vivo

M. Kissel[1], T. Müller[2], I. Fichtner[3], H. Hess-Stumpp[1]. *[1]Global Drug Discovery Bayer Schering Pharma Wuppertal Berlin Germany, GDD, Wuppertal, Germany; [2]Global Drug Discovery Bayer Schering Pharma Wuppertal Berlin Germany, Statistics Department, Berlin, Germany; [3]EPO, GmbH, Berlin, Germany*

Most advanced non-small-cell lung cancers (NSCLCs) and especially the fraction with activating epidermal growth factor receptor (EGFR) mutations initially respond to the EGFR tyrosine kinase inhibitors (TKIs) gefitinib and erlotinib. However, most tumors develop acquired resistance to EGFR TKIs via secondary resistance mutations. The amplification of the MET oncogene is present in 20% of TKI-resistant tumors, and in half of the cases

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