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.

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

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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.

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

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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].

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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

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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.

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Effective therapeutic sensitization of gastrointestinal stromal tumors by a BH3 mimetic

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