

abundance of receptor and ligand correlates strongly with poor patient outcome in several indications. Hence, inhibition of the kinase activity of Met is an attractive approach to treat cancer.

We have discovered an exquisitely selective, orally bioavailable, Met inhibitor (JNJ-38877605). Under optimal culture conditions, JNJ-38877605 abrogates the proliferation of only Met gene-amplified cell lines, and in mouse xenograft models JNJ-38877605 regresses Met gene-amplified tumors. However, in many other cell lines, when cultured in the presence of HGF, selective Met inhibition impairs migration, invasion, cell scattering and anchorage-independent growth. Moreover, Met inhibition results in the impairment of Akt signaling and sensitizes these cells to apoptosis induced by chemotherapeutic agents, regardless of the presence of PTEN. In clinical specimens, the Met protein is frequently upregulated in metastatic lesions compared to the primary tumor. Consistent with these observations, we find that metastatic cancer cells from three different tissue origins (colon, breast and prostate) have upregulated Met signaling and are more sensitive to Met inhibition in motility and survival assays compared to their non-metastatic counterparts, at least in the presence of HGF.

We conclude that Met inhibition is a promising therapeutic approach, not only as a monotherapy in Met-amplified tumours, but also in metastatic disease characterized by increased Met signaling, particularly as a combination therapy.

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POSTER

#### Preclinical studies and characterization of BMS-777607, a small molecule inhibitor of Met receptor tyrosine kinase

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The Met receptor tyrosine kinase, which is predominantly expressed in epithelial and endothelial cells, is the exclusive high-affinity receptor for the hepatocyte growth factor (HGF) ligand. Met activation and subsequent signaling can occur by ligand binding, receptor overexpression and/or a variety of receptor activating mutations. Receptor activation subsequently elicits important and complex biological responses that include cell motility, migration, proliferation, invasion and survival which underlie tumor growth and metastasis. In human malignancies, activated Met has been identified in a variety of histological tumor types. We have identified and characterized a small molecule inhibitor of Met kinase activity, BMS-777607. This compound, which is currently under clinical evaluation, inhibits both ligand stimulated and constitutive Met phosphorylation. As a result, HGF induced scattering and migration were observed to be inhibited when cells were treated with this compound. BMS-777607 also inhibited tumor cell proliferation in vitro in tumor lines in which Met was constitutively active. In addition, cell cycle analysis demonstrated G1 arrest as a result of drug treatment. In vivo, tumor growth inhibition was observed with BMS-777607 in the GTL-16 human gastric tumor model in which Met is amplified and activated. Using this same model, Met receptor phosphorylation in tumor tissue from mice treated orally with varying doses of BMS-777607 was inhibited in a dose-dependent manner. In vivo activity was also assessed pharmacodynamically in GTL-16 tumor bearing mice using Dynamic Contrast Enhanced-Magnetic Resonance Imaging (DCE-MRI). Consistent with the role Met plays in angiogenesis, DCE-MRI results demonstrated inhibition of contrast agent uptake in a dose-responsive manner.

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#### Activity of IPI-926, a novel inhibitor of the HH pathway, in subcutaneous and orthotopically implanted xenograft tumors that express SHH ligand

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**Background:** IPI-926 is a novel, potent and selective inhibitor of the Hedgehog pathway and functions as a Smoothened (Smo) antagonist. The Hedgehog (Hh) signaling pathway is known to be important in the development of several organ systems, most notably the gastrointestinal tract and lungs. Moreover, Hedgehog signaling is also important for the growth and survival of cancers of these organs. Herein, the in vivo efficacy of IPI-926 was evaluated in pancreatic cancer tumor models.

**Results:** We observed a significant inhibition of xenograft tumor growth which was mediated, at least in part, through inhibition of the Hh pathway in the stroma of tumors that express hedgehog ligand. Thus, daily dose administration of IPI-926 in a subcutaneous (BxPC3) or orthotopic (Panc1) pancreatic cancer model at 40 mg/kg resulted in significant tumor growth inhibition after a 28 day treatment course. When a single dose of IPI-926 was administered in these human tumor models, the result was rapid Hh pathway inhibition, as measured by Gli1 expression, in the murine cells,

but not in the human tumor cells themselves. Consistent with inhibition of Hedgehog signaling by IPI-926, similar results were observed with a single administration of the mAb 5E1, a neutralizing antibody targeted to both SHh and Ihh, strongly implicating a role for ligand produced by tumor cells. These data extend from pancreatic cancer to include a number of other Hh expressing cancers, notably colon cancer in which IPI-926 treatment resulted in a similar pattern of stromal response, presumably driven by tumor derived Hh ligand. Expression of Hh ligand appears to be a common feature of a number of cancer types, including pancreatic, colon, breast and ovarian cancer. Finally, efforts to elucidate the identity of the IPI-926 responsive stromal cells have revealed that these cells reside in a non-CD31 expressing subset of cells, suggesting that the anti-tumor effect of IPI-926 is not directly related to the tumor vasculature.

**Conclusion:** These data suggest that tumor-stromal interactions, mediated by Hh ligand, are an important attribute for the growth of pancreatic cancer, and may be important for other cancers as well.

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#### Modulation of JAK2 signaling pathways in vitro and in vivo by SGI-1252, a potent small molecule JAK2 inhibitor

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JAK2 is an intracellular protein tyrosine kinase whose dysregulation has been implicated in myeloproliferative disorders (MPD) and hematological and solid tumor malignancies. Increased kinase activity of JAK2 has been shown to be caused by point mutation of the JH2 autoinhibitory region, formation of JAK2 fusion proteins, and down-regulation of JAK2 regulatory proteins. Due to the dysregulation of the kinase activity, increased activation of downstream signaling pathways affecting cell differentiation, proliferation, migration, and apoptosis can occur. Through the use of CLIMB<sup>TM</sup>, our proprietary drug discovery process, we identified a subset of leads from a large, virtual library. From these lead compounds we designed, optimized, and synthesized less than 30 inhibitors of JAK2. SGI-1252 was selected from those optimized inhibitors as our lead candidate. SGI-1252 exhibits potent low nanomolar activity against all members of the Janus kinase family, with the exception of the JAK3 kinase. IC<sub>50</sub> values against JAK1, JAK2, JAK2 V617F mutant, and TYK2 enzymes are all less than 20 nM, while the JAK3 IC<sub>50</sub> value is 1650 nM (a 300 fold increase over the Jak2 IC<sub>50</sub>). Consistent with the inhibition of the JAK2 enzyme, activity of downstream signaling partners are severely decreased. The phosphorylation level of STAT5, a downstream effector of JAK2 signaling, in treated HEL cell lysates was analyzed by western blot. These results showed that SGI-1252 caused an inhibition of STAT5 phosphorylation at an EC<sub>50</sub> of 76.2 nM. Another downstream target of JAK2, Bcl-XL, was evaluated for gene expression levels via RT-PCR. In the presence of SGI-1252, Bcl-XL levels were reduced with an EC<sub>50</sub> value of 778 nM. In mouse xenograft tumor models treatment with SGI-1252 was efficacious in decreasing tumor growth rates by as much as 80%. Pharmacokinetic analysis of SGI-1252 in rats has shown the oral bioavailability to be ~65%. Current work is focused on determining modulation of pharmacodynamic markers in mouse in vivo models. SuperGen's lead selective JAK2 inhibitor, SGI-1252, is a potent inhibitor of the JAK2 enzyme leading to inhibition of cellular signaling pathways and cancer cell proliferation in in vitro and in vivo models.

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#### Astragalus saponins (AST) modulate mTOR and ERK signaling with NF-kappa B as target in native and cytokine-induced HT-29 colon cancer cells

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**Background:** The total saponins of *Astragalus membranaceus* (AST) possess potential anti-tumorigenic effects in human colon cancer cells and tumor xenograft (Carcinogenesis 28:1347–1355, 2007). In the present study, the proapoptotic effects of AST were investigated in native or TNF- $\alpha$  treated HT-29 cells to further unveil its mechanism of action.

**Materials and Methods:** The growth-inhibitory action of AST (60  $\mu$ g/ml) was evaluated in HT-29 cells using MTT viability assay. For cytokine-induced cells, TNF- $\alpha$  (5 ng/ml) was added 1 h following AST treatment. Western immunoblotting had been used to assess the protein expression of apoptotic and transcription factors. Electrophoretic mobility shift assay was conducted to reveal NF-kappa B DNA binding activity. Modulation of cell proliferation by phase-specific cycle arrest was tested by flow cytometry. Apoptotic analysis and detection of NF-kappa B subunit translocation were determined by immunofluorescence nuclear staining.