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.

569 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.

570 POSTER

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^{TM}, 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_{50} values against JAK1, JAK2, JAK2 V617F mutant, and TYK2 enzymes are all less than 20 nM, while the JAK3 IC_{50} value is 1650 nM (a 300 fold increase over the Jak2 IC_{50}). 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_{50} 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_{50} 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.

572 POSTER

***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 μg/ml) was evaluated in HT-29 cells using MTT viability assay. For cytokine-induced cells, TNF-α (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.

Results: AST caused growth inhibition in native HT-29 cells, which was exaggerated in TNF-induced cells. These were accompanied by caspase 3 activation, cleavage of poly(ADP-ribose) polymerase and subsequent increase in apoptotic cell numbers. Furthermore, activation of procaspase 8 indicates that the extrinsic apoptotic pathway had been involved, while cleavage of Bid into t-Bid implicates cross-talk with the intrinsic apoptotic pathway (proven to be induced by AST in our previous study). Alternatively, AST caused G2-phase arrest, while in TNF-induced cells S-phase arrest has been observed. On top of our recent suggestion on its correlation with PI3K-Akt signaling, we have now revealed that AST caused overexpression of PTEN and downregulation of mTOR expression, with both effects being more intense in TNF-induced cells. Nevertheless, these events were preceded by continuous ERK 1/2 activation. Our data have also demonstrated that NF-kappa B DNA binding activity was subsequently decreased after AST treatment (with additional reduction in TNF-induced cells), whereas the early nuclear translocation of the NF-kappa B subunit p65 remains to be clarified.
Conclusion: Our findings in this study suggest that AST could induce the extrinsic apoptotic cascade and cause cell cycle arrest in HT-29 cells by modulation of both (PI3K/PTEN/Akt)/mTOR and ERK signaling pathways, of which NF-kappa B has been identified as an imperative molecular target.

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Identification of potent, selective JAK2 inhibitors using a fragment-based screening approach

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Janus Kinase 2 (JAK2) has become a key target in myeloproliferative diseases since the discovery of the activating JAK2V617F mutation in a significant proportion of these patients. This mutation, and others subsequently discovered, induces cytokine-independent proliferation of cells that express erythropoietin receptors. This causes these cells to become hypersensitive to cytokines and to upregulate the phosphorylation of Signal Transducer and Activator of Transcription (STAT) 5 and signalling through this pathway.
We used our fragment-based screening approach, Pyramid™, to identify multiple low molecular weight fragments that bound to the kinase domain of JAK2. These weakly binding hits were then optimised into potent lead compounds against the target using a structure-guided approach.
Lead compounds were identified with sub-10 nM potency against the isolated JAK2 enzyme and were also shown to have sub-micromolar cellular activity in a number of JAK2-based cellular assays. Compounds inhibited proliferation of an engineered JAK2-dependent Ba/F3 cell line. They also inhibited the phosphorylation of the JAK2 substrate STAT5 and other downstream markers in Human erythroleukemia (HEL) cells at similar concentrations, indicating the mechanism of cellular action was through JAK2 inhibition.
These JAK2 inhibitors were less active against a number of other isolated kinases, including other members of the JAK family, JAK1, JAK3 and Tyk2. Optimised lead compounds were 50 and 100-fold selective for JAK2 over JAK3 and JAK1 enzymes respectively. The selectivity for JAK2 over JAK3 seen in the isolated enzyme system translated into cells with the proliferation of a JAK2-driven Ba/F3 cell line being inhibited at least 17 times more potently than that of a JAK3-driven Ba/F3 line by one of our lead compounds.
Overall we have successfully used a fragment-based screening approach to identify potent and selective JAK2 inhibitors, both against isolated enzymes and in cellular systems, which merit further optimisation.

574 POSTER

Identification and preclinical characterization of AZ-23, a novel, selective, and orally bioavailable inhibitor of the Trk kinase pathway

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Background: Tropomysin-related kinases (TrkA, B and C) are high affinity growth factor receptors for the neurotrophin family of soluble ligands that include nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF). Trk receptors are associated with neuronal maintenance and survival during development but also have been shown to be potent oncogenes with roles in several human cancers including neuroblastoma, AML, thyroid, breast and lung. Here we report the identification and characterization of AZ-23, a potent, selective, ATP-competitive Trk kinase inhibitor with potential for utility as a cancer therapeutic.
Methods: A high throughput screen of AstraZeneca's compound collection yielded potent inhibitors of in vitro Trk kinase activity, culminating in the identification of AZ-23. The cellular potency of AZ-23 against both ligand driven and ligand independent Trk phosphorylation was determined using phospho-Trk ELISAs and immunoblotting in a range of cancer cell lines. The selectivity of AZ-23 was assessed through use of large commercial kinase panels as well as through extensive cellular profiling. In vivo, short-term pharmacodynamic (PD) studies were performed with oral dosing of AZ-23 in a TrkA-3T3 allograft model and anti-tumor efficacy was investigated following a 4-day dosing regimen of AZ-23 given either once or twice daily at both 10 and 50 mg/kg.
Results: AZ-23 was found to potently inhibit ligand-driven and constitutive Trk kinase activity with cellular EC50's in the 1−2 nM range. AZ-23 inhibited all three Trk receptor isoforms (A/B/C) equivalently but showed significant selectivity versus a wide range of over 170 other kinases. At the cellular level, AZ-23 demonstrated at least a 35-fold window of selectivity against multiple kinases using specific phospho-endpoint assays. In 3-day growth assays, AZ-23 potently inhibited the Trk/NGF-driven proliferation of MCF10 and TF-1 cell lines (EC50's ~1 nM) but had little if any effect on proliferation of these same cell lines when driven through other growth factors. The in vivo PD-PK relationship for AZ-23 was determined using a mouse tumor model that expresses constitutive, human TrkA kinase activity and it was found that approximately 180 ng/ml of plasma-resident AZ-23 was required to inhibit greater than 80% of the phospho-TrkA activity in tumor lysates. Repeat dosing of AZ-23 to tumor bearing mice resulted in potent, dose-dependent anti-tumor efficacy with tumor regressions noted with all dosing regimens. AZ-23 was well tolerated with no weight loss or overt toxicity observed even at doses of 100 mg/kg twice daily for up to 14 days.
Conclusions: AZ-23 represents a potent, selective, and novel Trk inhibitor of the pyrazole-pyrimidine chemistry class with potential for further development in clinical settings including the treatment of cancer.

575 POSTER

Targeting metastatic tumor cell functions by inhibition of new blood vessel formation with the selective VEGF signal inhibitor, cediranib (RECENTIN™, AZD2171) or Src signaling interference using the small molecule Src inhibitor, AZD0530

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Introduction: The metastatic spread of cancer cells remains the principal cause of treatment failure and indicator of poor prognosis in patients with cancer. Molecular strategies to target tumor metastases have focused on interrupting key steps in the metastatic cascade. Two critical functional aspects of this process are the tumor cell's ability to invade and initiate new blood vessel networks. VEGF is considered the important pro-angiogenic factor associated with tumor cell-induced angiogenesis. Src tyrosine kinase plays an important role in tumor cell survival, proliferation, migration and invasion. The present studies evaluated the effects of cediranib (RECENTIN™, AZD2171), an oral, highly potent and selective VEGF signaling inhibitor of VEGFR-1, -2, and -3, and AZD0530, a potent, oral Src inhibitor that modulates multiple key signaling pathways, in the highly metastatic rodent KHT sarcoma cell line.
Methods: The effect of cediranib and AZD0530 on tumor cell functions, including viability, cell cycle, migration and invasion, were examined by trypan blue exclusion, flow cytometry, and migration/invasion assays, respectively. *In situ* effects were assessed by measuring the number of vessels growing to intradermal tumor cell inoculates. Tumor cells were injected and then treated daily with cediranib and AZD0530 alone or in combination for 3 weeks at which time the number of lung metastases was determined.
Results: *In vitro* cediranib (5–10 nM) significantly impaired migration and tube formation of endothelial cells. AZD0530 doses >2.5 μM significantly reduced Src phosphorylation and functionally reduced the ability of KHT sarcoma cells to migrate or invade through a transwell membrane (0.5–2.5 μM). *In vivo,* both agents affected the ability of KHT tumor cells to induce angiogenesis. Daily doses of cediranib (6 mg/kg) or AZD0530 (10 mg/kg) also reduced the number of metastases formed by a factor of ~2. Combination treatment led to a greater reduction in metastatic foci than that achieved with either agent alone.
Conclusions: The present study explored a molecular-targeting strategy against different aspects of the metastatic cascade. Since both the initiation of new vessel formation and the ability of the cancer cells to invade are critical to a tumor's growth and survival at a secondary site, a double assault on VEGF and Src signal inhibition (cediranib and AZD0530 treatment,