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.

573 POSTER

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.

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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,

respectively) appears to hold considerable promise in impairing metastases formation.

576 POSTER

ARRY-768, a highly potent and selective small-molecule PDGFR inhibitor which inhibits cellular and in vivo tumor growth

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Background: PDGFR is an attractive target for treating malignant disease. Constitutive PDGFR kinase activity resulting from point mutations, chromosomal translocations, and autocrine loops has been described for GIST, HES/DFSB/CMML and gliomas, respectively. In addition, the role of PDGFR in maintaining neovasculature through its regulation of pericytes, suggests that the use of PDGFR inhibitors as anti-angiogenic agents could have broad utility against a spectrum of human cancers. Here we describe the characterization of a potent and selective small-molecule PDGFR inhibitor, ARRY-768.
Material and Methods: To evaluate its selectivity against other kinases, ARRY-768 was tested against a panel of purified kinases in addition to its characterization in several cellular kinase assays, including PDGFR, KDR, Kit, and Abl. Cellular potency against PDGFR was evaluated in HS27, C6, and PDGFR fusion-expressing EOL-1 cells under basal or PDGF-induced conditions. *In vivo* activity was evaluated in several models, including the C6 glioblastoma tumor model.
Results: ARRY-768 is a highly potent PDGFR inhibitor with an average cellular IC_{50} of 3 nM. In contrast to many previously described multi-kinase inhibitors which have PDGFR activity, ARRY-768 shows significant selectivity over KDR, Kit and Abl. We show that cellular proliferation of human EOL-1 cells is inhibited by ARRY-768 and that this effect correlates with inhibition of PDGFR phosphorylation. Furthermore, we also show that ARRY-768, at 50 mg/kg, po, inhibits growth of C6 glioblastoma xenograft tumors which express constitutive PDGFR phosphorylation. The EC_{80} for inhibition of tumor PDGFR phosphorylation in this model was determined to be ~250 ng/ml, plasma concentrations that are achievable and tolerated in several pre-clinical species. Additional preclinical data showing ARRY-768 anti-tumor activity will be presented.
Conclusions: ARRY-768 is a potent and selective PDGFR inhibitor that is active in several *in vitro* and *in vivo* models. Its distinctive selectivity profile may provide potent PDGFR inhibition in the absence of off-target kinase toxicities observed with other inhibitors having PDGFR activity. A selective PDGFR inhibitor may allow for therapeutic approaches not achievable with less selective PDGFR inhibitors.

577 POSTER

Selective MEK Inhibitor RDEA119 exhibits efficacy in orthotopic hepatoma models and cytostatic potential in multiple cell based models of cancer

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Introduction: RDEA119, a novel, highly selective MEK1/2 inhibitor currently in clinical trials for the treatment of cancer, is capable of inhibition of MEK1/2 at nanomolar concentrations. This molecule exhibits superb pharmacokinetic properties in man consistent with once per day dosing while maintaining constant drug levels in the pharmacologic range as determined by measurement of pharmacodynamic markers in treated patients.
Results and Methods: The compound exhibits activity in both subcutaneous (melanoma, colon) as well as orthotopic xenograft models including orthotopic hepatoma and orthotopic colon cancer. For these models, the caecum wall or liver of female BALB/c nu/nu mice was inoculated with human HT 29 colorectal adenocarcinoma cells or Hep3B2.1−7 tumor cells, respectively. Beginning 20 days post-inoculation, 21 days of oral dosing with RDEA119 was initiated and tumor number and weights were assessed. Because RDEA119 interacts solely with MEK1/2, as determined by SelectScreen kinase Profiling (Invitrogen) against 205 other kinase targets (>100 fold selectivity), this indicates that these tumors exhibit growth dependence on the MEK pathway. We noted that after withdrawal of compound, certain tumors resumed growth in some of these xenograft models. We therefore tested whether RDEA119 induces a cytostatic response or a cell death response. A375 melanoma cells were treated for 24 hr with RDEA119, washed, permeabilized and stained with propidium iodide and analyzed for cell cycle status. RDEA119 inhibited A375 cell proliferation by inducing cell cycle arrest rather than apoptosis as demonstrated by measuring both cellular membrane integrity (adenylate kinase release) and cell cycle analysis showing a G1 phase cell cycle arrest. We examined the ability of RDEA119 to synergize with multiple anti-tumor agents in vitro and measured cell death response in both BRAF wildtype and mutant cell lines. Significant synergy was observed with several combinations, the magnitude of synergy ranged from 5−80 fold.
Conclusions: Thus, RDEA119 represents a new potential weapon for use as both single agent in selected cancers and in combination with other active agents in a broader array of cancers.

578 POSTER

RGB-286638 is a novel multitargeted protein kinase inhibitor with activity in chronic myelogenous leukemia (CML) models

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Background: RGB-286638 is an indenopyrazole compound that is a low nanomolar inhibitor of a wide panel of tumor cells in vitro. Its activity against cell cycle and non-cell cycle cyclin-dependent kinases (Cdks), and ability to induce cell cycle arrest and apoptosis have been demonstrated previously. Here, we sought to determine the activity of RGB-286638 against additional protein kinases and in cell and animal models of CML.
Materials and Methods: The biochemical selectivity of RGB-286638 was tested on a panel of 201 protein kinases outside the Cdk family. The IC50 of RGB-286638 in BaF3 cells transformed with wild-type or mutant Bcr-Abl was determined and compared with that of imatinib and dasatinib. Further, the survival of BaF3 mice with wild-type or mutant Bcr-Abl-dependent disease was assessed in animals treated with RGB-286638, imatinib and dasatinib.
Results: RGB-286638 was active against several non-receptor (eg, Abl, Jak, c-Src family members) and receptor (eg, Flt1, Flt3, Flt4, Fms, TrkA) tyrosine kinases and inhibited the serine/threonine kinases AMPK, GSK3, PIM1, HIPK1−3 and MAPK. RGB-286638 inhibited BaF3 cells transformed with either wild-type (IC50: 0.020 uM) or T315I mutant (IC50: 0.051uM) Bcr-Abl. In contrast, imatinib and dasatinib did not inhibit BaF3 T315I cells (IC50: 7.56 uM and 6.12 uM). In BaF3 cells transformed with several other Abl mutant alleles, RGB-286638 showed activity similar to that observed in BaF3 wild-type and T315I mutant cells. RGB-286638 was also active against non-transformed BaF3 cells cultured in the presence of IL-3 (IC50: 0.024 uM), showing that molecular targets other than Bcr-Abl contribute to its activity in this model. Treatment of Bcr-Abl wild type-driven BaF3 mice with RGB-286638 resulted in a dose-dependent survival benefit comparable to that observed with imatinib or dasatinib treatment. In the T315I mutant-driven BaF3 mouse model, which is resistant to imatinib and dasatinib, a survival benefit of 16.5 to >50 days was observed with RGB-286638.
Conclusions: RGB-286638 is a novel multitargeted protein kinase inhibitor with activity against Cdks, receptor and non-receptor tyrosine kinases and several serine/threonine kinases. RGB-286638 showed potent anti-proliferative activity in BaF3 models of CML driven by wild-type Bcr-Abl as well as mutants resistant to imatinib and dasatinib. These findings suggest the potential for RGB-286638 as a treatment for a broad array of solid and hematologic tumors.

579 POSTER

Biological and biochemical activity of TLN-4601 in pancreatic cancer

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Background: TLN-4601 (Formely ECO-4601) is a structurally novel farnesylated dibenzodiazepinone discovered though Thallion's DECIPHER® technology platform. The compound has demonstrated broad anti-tumor activity *in vitro* and *in vivo* against various tumor models. One proposed mechanism of action of TLN-4601 involves its ability to disrupt the activity of RAS signaling by interacting at the level of RAS and RAF-1.
Material and Methods: Since mutational activation of *KRAS* is associated with 90% of pancreatic cancer, we have assessed the activity of TLN-4601 in two cell models for KRAS-driven pancreatic cells by MTT viability and soft agar colony formation assays. To determine the ability of TLN-4601 to modulate Ras function, Western blot analysis was used to evaluate the steady-state levels of total K-Ras and RAF-1 and of phosphorylated p70 S6 kinase and MEK1 and MEK2 protein kinases, activators of the ERK MAPKs.