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.

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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.

Results: We determined that TLN-4601 potently inhibited the anchorage-dependent and -independent growth of KRAS-transformed human pancreatic nestin-positive (HPNE) duct-derived cells. We also found that the growth of KRAS mutation-positive pancreatic carcinoma cell lines was inhibited by TLN-4601. We then assessed the ability of TLN-4601 to antagonize RAS signal transduction. Consistent with the ability to directly antagonize RAS, we found that TLN-4601 treatment caused cell context-dependent reduction in RAS and RAF-1 protein expression and an inhibition of p70 S6 kinase and MEK1/2 phosphorylation.
Conclusions: Our results support the use of TLN-4601 for pancreatic cancer treatment and are consistent with a model where the anti-tumor activity of TLN-4601 is mediated, in part, through antagonism of RAS signaling. The mechanism of action of TLN-4601 is cell context-dependent and is associated with antagonism of multiple facets of Ras signal transduction.

580 POSTER

RhoA and RhoB inversly modulate estrogen receptor alpha expression and transcriptional activities in breast cancer cell lines

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Background: About two thirds of mammary tumors express estrogen receptor alpha (ERα) and hormonotherapy is then recommended. Nevertheless, there are systematically resistances to these treatments that impose the search for new pharmacological targets. Estrogens act mainly through the well-known ERα. However, cross-talks have been clearly demonstrated between ER and growth factors signalling pathways. Ras family proteins, such as Rho proteins, are key elements in those cross-talks. RhoA is frequently overexpressed in breast cancers and has been shown to down-regulate ER-mediated transcription. Our purpose was then to decipher the effect of Rho proteins inhibition on ERα expression and transcriptional activities.
Material and Methods: We specifically abolished the expression of either RhoA or RhoB proteins using two independent sequences of interfering RNA for each protein in MCF-7, MELN, T47D, ZR75 cells (hormonodependent breast cancer cell lines) and in LCC2 and LCC9 cells (hormonoresistant breast cancer cell lines). We then studied the impact of RhoA and RhoB inhibition on the one hand on ER target gene expression (by RTq-PCR or by a luciferase assay) and on the other hand, on ERα expression in cell model. Finally, we analyzed ER expression in RhoB knock out mice.
Results: We first showed in MCF-7 cells that RhoA inhibition increases both the expression of a luciferase reporter gene controlled by the vitellogenine Estrogen Responsive Element and the Progesteron Receptor (PR) mRNA. The inhibition of RhoA also increases ERα expression both at the mRNA and proteins levels. On the contrary, the inhibition of RhoB decreases the expression of the luciferase reporter gene controlled by the vitellogenine ERE and PR mRNA in MCF-7. Besides, RhoB inhibition decreases ERα expression in MCF-7, TR47D, ZR75, LCC2 and LCC9 cells. We also confirmed this result in Mouse Embryonnic Fibroblasts (MEFs) from RhoB knock-out mouse.
Conclusion: In brief, our results evidence RhoA and RhoB participation in the balance of expression of ER and in the individual modulation of the expression of various target genes. Further investigations, especially experiments in hormonoresistant cells, are now necessary for a better understanding of hormonoresistance.

581 POSTER

Inhibition of protein kinase C as the molecular basis of the synergism between safingol and irinotecan in colon cancer treatment

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Background: Safingol is a synthetic sphinganine which has been developed as a protein kinase C (PKC) inhibitor, and is currently evaluated in Phase I clinical trials. As PKC has been found in elevated levels in colon cancer cells, the aim of this study was to investigate the effects of safingol on colon cancer cell viability and its potential to enhance the cytotoxic effect of irinotecan for colon cancer therapy.
Materials and Methods: The anti-cancer effects of safingol as single agent or in combination with irinotecan in HT-29 and LS-174T colon cancer cells were determined using MTT assay. The combination index (C.I.), based on the median effect principle by Chou and Talalay, was computed to determine drug synergism. Treated cells were stained with annexin-V/7AAD to determine the extent of apoptosis. The expression levels of phosphorylated PKC and its downstream substrate, MARCKS, were determined using Western blot.
Results: As a single agent, safingol reduced colon cancer cell viability in a concentration-dependent manner, with IC_{50} values of $2.5\pm1.1\,\mu M$ and $3.4\pm1.0\,\mu M$ in HT-29 and LS-174T, respectively. Over 50% of treated HT-29 cells underwent apoptosis after a 48-h exposure to $10\,\mu M$ safingol. Interestingly, 24.9% of treated cells were annexin-V^- but $7AAD^+$, suggesting the possibility of necrosis or other death mechanisms. Further studies with the pan-caspase inhibitor, Z-VAD-FMK, indicated that cell death was not prevented in safingol-treated cells, indicating that safingol exerted its cytotoxicity via a caspase-independent mechanism. A 1:1 (mol/mol) combination of safingol/irinotecan was synergistic in both HT-29 and LS-174T, with C.I. values <1.0. This combination enabled significant dose reduction of irinotecan, with 4-fold and 250-fold reduction in HT-29 and LS-174T, respectively. Although safingol was developed as a PKC inhibitor, no decrease was observed in the expression of p-PKC or the downstream substrate p-MARCKS with $10\,\mu M$ safingol. However, treatment with safingol/irinotecan combination was associated with decreased expression of p-PKC and p-MARCKS, suggesting a possible molecular basis for the observed synergistic effect.
Conclusions: Our results show that inhibition of PKC by safingol/irinotecan combination could be a potentially effective strategy for colon cancer treatment. Future *in vivo* studies are warranted to further explore the therapeutic potential of this drug combination.

582 POSTER

An acquired point mutation in MEK2 causes resistance to allosteric MEK inhibitors

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Mutations in the ATP binding site are emerging as a common acquired resistance mechanism for ATP competitive kinase inhibitors, however potential resistance mechanisms for allosteric kinase inhibitors are poorly understood. We studied drug resistance mechanisms for an allosteric MEK inhibitor by generating a drug-resistant cell line in vitro. Exposure of the k-ras mutant colon cell line HCT116 (gIC50=2 nM) to increasing concentrations of the MEK inhibitor GSK1120212 led to isolation of a drug resistant population capable of growing under high concentration ($1\,\mu M$) of drug (gIC50 > $7\,\mu M$). The drug resistant population was also resistant to other allosteric MEK inhibitors while remaining sensitive to inhibitors of other targets (KSP and PI3K). Clones were isolated and several MAPK pathway related genes were sequenced. A single point mutation in MEK2 resulting in the amino acid change L119P was identified. MEK2-L119 is located within the allosteric binding site for GSK1120212 as well as other reported MEK inhibitors (PD0325901 and AZD6244). siRNA to MEK2 reduced levels of MEK2-L119P but not MEK1 and re-sensitized these cells to GSK1120212. The homologous MEK1 L115P mutant construct was engineered to test whether it could similarly confer resistance to GSK1120212. Exogenous expression of the MEK1-L115P mutant but not MEK1-wt was demonstrated to confer drug-resistance to tumor cell lines sensitive to GSK1120212. Finally, HCT116 (MEK2-L119P) formed tumors in mice that were relatively resistant to GSK1120212 compared to wild type HCT116 tumors. These data demonstrate that resistance to MEK inhibitors including GSK1120212 can be caused by a mutation of MEK2-L119P or MEK1-L115P permitting phosphorylation of ERK in presence of drug. To date, these mutations or polymorphisms have not been identified in clinical tumor samples.

583 POSTER

Analysis of MAP kinase signalling pathway in KIT & PDGFRA wild-type GISTs

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Background: Gastrointestinal stromal tumours (GISTs) are commonly driven by oncogenic mutations in KIT and PDGFRA genes, which are important molecular targets to specific kinase inhibitors, such as imatinib mesylate. However, 10−40% of GISTs patients are wild-type for KIT and PDGFRA genes. The prognostic significance of wild-type GISTs is controversial, and they rarely respond to imatinib mesylate. MAPK pathway is implicated in some tumor types through alterations in RAS, RAF or RKIP (Raf Kinase Inhibitor protein) molecules. Few studies have investigated the