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

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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.

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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.

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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.