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Discovery and preclinical characterization of a series of novel JAK2 small molecule inhibitors for the treatment of myeloproliferative diseases

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Janus Kinase 2 (JAK2) is a cytoplasmic tyrosine kinase that, in normal physiology, plays a role in cytokine signaling. JAK2 kinase is constitutively activated as a consequence of a point mutation in a regulatory domain that converts valine at position 617 to phenylalanine ($JAK2^{V617F}$). The $JAK2^{V617F}$ mutation is found in a large fraction of all myeloproliferative diseases; in almost all cases of polycythemia vera (PV), and in about half of all cases of both essential thrombocythemia (ET) and myelofibrosis (MF). Here we describe the identification and preclinical characterization of a series of potent, selective, and orally bioavailable inhibitors of $JAK2^{V617F}$.
We have identified a series of potent, ATP-competitive inhibitors of both JAK2 kinase (representative $IC_{50} = 0.3$ nM) and the $JAK2^{V617F}$ mutant (representative $IC_{50} = 1$ nM) without significant activity against 40 other protein kinases tested including other JAK family members. Compounds from this series selectively inhibit STAT5 phosphorylation (representative $IC_{50} = 3$ nM) in HEL92.1.7 erythroleukemia cells (HEL) and cellular proliferation in three human hematological cell lines: HEL, TF-1 (erythroleukemia), and SET-2 (essential thrombocythemia). TF-1 cells are homozygous for wildtype JAK2 ($JAK2^{WT}$), SET-2 cells express both $JAK2^{WT}$ and $JAK2^{V617F}$, while HEL cells are homozygous for the $JAK2^{V617F}$ mutation. The biochemical selectivity of the series within the JAK kinase family translated well in the cellular context when additional murine Ba/F3 engineered cell lines containing TEL fusions with the kinase domains of JAK1, JAK2, and JAK3 were evaluated.
In vivo, compounds from this series are orally bioavailable with good overall pharmacokinetic properties. Compounds from the series demonstrated potent anti-tumor activity when dosed orally in human xenograft models and were well-tolerated. The efficacy seen in the xenograft models correlates well with the pharmacokinetics and the pharmacodynamic biomarker, phospho-STAT5.

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The anti-insulin-like growth factor I receptor antibody EM164 (murine AVE1642) enhances anti-tumor activity of temozolomide against neuroblastoma cell lines and xenografts

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Background: Inhibition of insulin-like growth factor type I receptor (IGF-1R) pathway has been suggested as a promising new targeted approach for cancer treatment. We showed significant anti-tumor activity of the IGF-1R antagonistic monoclonal antibody EM164 against neuroblastoma cell lines and xenografts [Geoerger, AACR 2006]. The present study evaluated EM164, for cell growth inhibition in vitro and in vivo, in combination with the alkylating agent temozolomide (Tmz).
Materials and Methods: In vitro anti-proliferative activity against SK-N-AS and IGR-N91 cell lines was measured by MTT and 3H thymidine incorporation assays in 3 independent experiments using EM164 at 0.7 μg/ml administered simultaneously with, depending on IC50 doses, Tmz at 400 and 800 μM for IGR-N91 and 200 and 400 μM for SK-N-AS cells in 10% FCS conditioned medium during 48 hours.
Results: EM164 treatment reduced IGR-N91 cell proliferation to 68% compared to controls, Tmz at 800 μM to 55% and EM164-Tmz combination to 28%. In SK-N-AS cells, EM164 reduced proliferation to 76%, Tmz at 200 μM to 61% and the combination to 42%, suggesting an additive effect of both agents in both cell lines, which was also observed with other doses of Tmz. Anti-tumor activity in vivo was evaluated in athymic mice bearing subcutaneous SK-N-AS tumors of 58–224 mm3 (median: 136 mm3). EM164 40 mg/kg injected intravenously twice weekly during 5 weeks yielded significant tumor growth delay in median time to reach 5 times initial tumor volume (TGD) of 18.1 days compared to controls ($p < 0.05$; Kruskall-Wallis test), a log cell kill (LCK = (T-C (median times to reach 750 mm3) in days)/(3.32 x Td) of 1.6, and one complete tumor regression below the palpation limit (CR < 63 mm3). Temozolomide given orally at the MTD of 100 mg/kg for 5 consecutive days resulted in no tumor regression, a median TGD of 11.4 days ($p = ns$) and a LCK of 1.4. Combined treatment with temozolomide and EM164 starting after the second Tmz dosing showed significant TGD of 29.5 days ($p < 0.001$), a LCK of 2.6 and two CR.
Conclusion: Enhanced anti-tumor effects of EM164 in combination with Tmz hold promise for the treatment of high grade neuroblastoma. A humanized version of this antibody, AVE1642, is now in clinical testing.

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SGX126: a novel, potent and highly selective small molecule inhibitor of the c-Met receptor tyrosine kinase

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Background: MET (c-Met) the receptor for hepatocyte growth factor (HGF) is well established as an important target in the development and progression of cancer and has recently been identified as a possible culprit in some cases of acquired resistance to EGFR inhibitors. We previously described a highly selective small molecule inhibitor of MET, SGX523. We present here a second generation MET inhibitor, SGX126.
Methods: Crystal structures of SGX523, and related compounds, bound to MET inspired the design and characterization of a series of compounds with improved potency and efficacy. One compound, SGX126, was tested in a variety of in vitro and in vivo assays to evaluate its potential as a drug development candidate.
Results: SGX126 is a low molecular weight, ATP-competitive inhibitor of MET. In purified enzyme assays and various cell-based assays SGX126 inhibits MET at low nM concentrations. Proliferation of a gastric cancer cell line with amplified MET is inhibited with an IC_{50} of 0.022 μM and proliferation of Ba/F3 cells engineered to express the activated fusion protein TPR-MET is inhibited with an IC_{50} of 0.011 μM. At comparable concentrations, SGX126 inhibits MET autophosphorylation and HGF-driven signaling in cells. Of 42 human kinases tested at 1 μM, SGX126 only inhibits MET by greater than 90%. *In vivo* SGX126 is orally bioavailable with pharmacokinetics and pharmacodynamics consistent with once or twice daily dosing. SGX126 demonstrates potent anti-tumor activity when dosed orally in human tumor xenograft models with no overt toxicity. Pharmacodynamic studies show a close correspondence between *in vivo* antitumor activity and inhibition of target autophosphorylation.
Conclusions: Our results demonstrate that SGX126 is a potent, orally bioavailable, and remarkably selective MET kinase inhibitor. SGX126 is currently undergoing IND-enabling studies.

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First-in-human (FIH) study of PF-00299804 in advanced cancer patients: correlation between pharmacokinetics (PK) and pharmacodynamics (PD)

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Background: PF-00299804 is an orally bioavailable, potent and irreversible small molecule inhibitor of the HER tyrosine kinases, HER1, HER2 and HER4. Preliminary results from an ongoing, FIH, phase I, dose-escalation study (PF-00299804 0.5–60 mg/day as a continuous regimen) in 79 patients with advanced refractory solid tumors have been reported previously. The most common treatment-related adverse events at the maximum tolerated dose (45 mg; n = 38) included diarrhea (G1/2, 71%;