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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%;

G3/4, 5.3%) and rash (G1/2, 44.7%; G3/4, 0%). The recommended phase II dose is being explored in expanded cohorts enriched for molecular variations of HER family receptors in multiple tumor types, as well as wild-type KRAS in refractory non-small cell lung cancer (NSCLC). Updated PK/PD results are presented, with preliminary efficacy data for the NCSCL cohort.

Materials and Methods: PK data were collected on day 14 of cycle 1. PD measures included assessments of skin rash, diarrhea, HER-related signaling pathways by immunohistochemistry analyses of serial skin biopsies, and tumor functional (FDG-PET) imaging. PK/PD relationships were assessed by Spearman Correlation analysis. Tumor response was evaluated in patients with NSCLC.

Results: C_{max} and AUC increased with dose and no evidence of dose- or time-dependent PK was seen; the average terminal half-life was ~85 hours. Significant positive correlations were noted between diarrhea severity and PK parameters or dose ($p \leqslant 0.0001$), and between rash severity and dose ($p = 0.0009$). Significant negative associations ($p < 0.05$) were seen between the skin biomarkers, Ki67 and pMAPK, and C_{max} or dose. Ki67 changes also negatively correlated with diarrhea severity ($p = 0.0296$) and positively correlated with changes in pMAPK ($p = 0.0048$). Forty-three patients with NSCLC were enrolled. Four patients achieved a partial response, and disease was controlled in 50% of patients.

Conclusions: At the recommended phase II dose, 45 mg/day, the mean steady-state trough concentration approached the predicted human efficacious concentration. PK/PD analysis in skin suggests that PF-00299804 mechanistically inhibits the EGFR-MAPK signalling pathway, decreases the Ki67 proliferation marker and produces rash/diarrhea in a dose- or exposure-dependent manner. Updated efficacy results and tumor functional imaging data will be presented at the meeting.

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Activity of the anti-IGF-IR antibody CP-751,871 in combination with docetaxel as first-line treatment for castration resistant prostate cancer in a randomized Phase II trial

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Background: CP-751,871 is a fully human IgG2 monoclonal antibody against the insulin like growth factor 1 receptor (IGF-IR). Inhibition of the IGF-IR is a promising novel therapy for prostate cancer. Elevated serum IGF-1 is associated to increased prostate cancer risk; up-regulation of the IGF-IR has been documented in prostate cancer refractory to hormonal therapy (HRPC); and IGF-IR blockade is active in animal models of HRPC.

Methods: We are conducting a phase 2 trial to determine the activity of the combination of docetaxel 75 mg/m^2 q3 weeks (D), prednisone 5 mg p.o. BID (P), and CP-751,871 20 mg/kg q3 weeks (I) in metastatic, chemotherapy-naive HRPC patients (pts) with performance status 0−1. A total of 200 pts will be randomized 1:1 to receive DPI or DP alone. Pts progressing on DP alone are eligible to receive DPI. Pts receiving DPI with response (PR) or stable disease are eligible to receive I or PI upon D discontinuation for up to 12 mos. The primary endpoint is PSA response according to PSAWG criteria.

Results: Ninety seven men with metastatic, HRPC have been enrolled. Median age was 70 yrs; PS 0 (13%), PS 1 (76%), PS 2 (11%). DPI was well tolerated. All causality grade 3, 4 toxicity included (DPI, DP): hyperglycemia (22%, 7%), fatigue (4%, 15%), and neutropenia (41%, 48%). PSA response data are available for 42 patients: 45% of patients responded to DPI and 32% to DP.

Conclusions: DPI is well tolerated and appears active in HRPC. Accrual continues to further assess the clinical activity of this combination treatment.

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Pyrazolo[3,4-d]pyrimidines as dual kinase inhibitors of both insulin-like growth factor receptor (IGF-IR) and members of the epidermal growth factor receptor family (EGFR and ErbB-2)

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As kinase targeted therapies for cancer have reached the clinic, selective agents have generally failed to yield durable clinical responses. However, the use of these agents in combination with other classes of therapeutics (antibodies, receptor tyrosine kinase inhibitors, cytotoxics) has yielded improved clinical results. For some combination therapies, the rationale is to not only target the desired oncogenic protein (kinase), but also to target known resistance mechanisms.

For the epidermal growth factor (ErbB) family of receptor tyrosine kinases (RTKs), the clinical effectiveness of trastuzumab is significantly diminished by overexpression of the insulin-like growth factor receptor (IGF-IR) and its corresponding ligands. Additionally, cellular systems expressing both RTKs have shown decreased sensitivity to not only trastuzumab, but also, gefitinib. In vitro, we have previously demonstrated that inhibition of both the insulin-like growth factor receptor-I (IGF-IR) and the ErbB-family of RTKs results in a synergistic reduction in cancer cell proliferation, and increased induction of apoptosis.

A therapeutic strategy that simultaneously targeted inhibition of both members of the ErbB-family and the IGF-family would be potentially superior to either selective approach. High throughput screening of Abbott's compound collection indicated that pyrazolo[3,4-d]pyrimidines possess activity versus either IGF-IR or ErbB-1 (EGFR). Therefore, appropriate functionalization of the pyrazolo[3,4-d]pyrimidine scaffold might afford analogs with dual IGF-IR and ErbB-family in vitro and in vivo activity. The structure–activity relationships that were discovered during our lead optimization program will be presented. The result of these efforts led to the synthesis and characterization of A-947864, a pyrazolo[3,4-d]pyrimidine with dual IGF-IR and ErbB-family enzymatic and cellular activity.

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GSK1120212 is a novel Mek inhibitor demonstrating sustained inhibition of ERK phosphorylation and selective inhibition of B-Raf and *RAS* mutant cells in preclinical models

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GSK1120212 is an orally available, potent and selective allosteric inhibitor of the Mek1/2 enzymes. In biochemical assays it inhibits Mek1 activation by B-Raf ($IC50 = 0.4 \pm 0.1$ nM) and the phospho-Mek1 kinase activity ($IC50 = 10 \pm 2$ nM). Consistent with an allosteric mode of inhibition, GSK1120212 is highly selective with $IC50 > 10\ \mu M$ against more than 200 different kinases tested. Antiproliferative activity of GSK1120212 was measured in tumor cell lines and demonstrated potent inhibition of growth ($gIC50 < 50$ nM) in cell lines harboring an activating *RAS* or *BRAF* mutation, but was less active against tumor cell lines having wild-type *RAS* and *BRAF*. GSK1120212 demonstrated minimal activity against human normal non proliferating cells. In vivo studies using daily dosing for 14 days at 3 mg/kg demonstrated a sustained inhibition of phospho-Erk1/2 in A375PF11 (melanoma cell line; B-Raf V600E) xenograft with reduction of KI67 and increase of p27Kip1 levels correlating with inhibition of tumor growth. In a Colo205 (CRC cell line; B-Raf V600E) xenograft tumor model we demonstrated that efficacy of GSK1120212 increased with BID versus QD treatment at 1 mg/kg over a 14 day experiment. In this same model we demonstrated that long term efficacy with improved tolerability was observed with alternating weekly drug treatment at 1 mg/kg QD. Additional in vivo efficacy with GSK1120212 was also demonstrated in *RAS* mutant (HCT116; CRC cell line) xenograft models. The favorable properties of this compound make it a suitable candidate for further development for the treatment of cancer.

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Selective inhibition of Met kinase activity impairs metastatic cancer cell motility and survival

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The Met receptor tyrosine kinase is highly expressed in cancer cells in a significant fraction of solid tumors, whereas the Met ligand, HGF, is produced by stromal cells in the tumor microenvironment. The combined