to other known high affinity VEGF binders. sFLT01 binds to both human and murine VEGF.
Methods: sFLT01 was evaluated in vitro in VEGF binding assays quantified by ELISA and endothelial cell-based proliferation assays. sFLT01 was evaluated for anti-tumor activity in vivo in the A673 sarcoma xenograft model as well as the syngeneic B16F10 melanoma and RENCA renal cell carcinoma models. sFLT01 was delivered by intraperitoneal injection twice a week at doses ranging from 5−25 mg/kg. Microvessel density (MVD) and vessel integrity was analyzed by immunohistochemical methods with antibodies against CD31 for endothelial cells and NG2 or smooth muscle actin for pericytes.
Results: sFLT01 bound to human VEGF with great affinity and inhibited HUVEC proliferation with an IC90 of approximately 1.0 nM. Subcutaneous A673 tumors were inhibited reproducibly at doses of 5 mg/kg with a doubling in survival time. Importantly, sFLT01 was also efficacious when treatment began when tumors were larger, 400–500 mm3 in size. MVD was reduced by 10-fold in the A673 model after three doses of sFLT01 compared to control. The blood vessels of these tumors were smaller with less pericyte involvement. B16F10 tumor growth was significantly reduced following a treatment regimen of sFLT01 at 25 mg/kg.
Conclusions: sFLT01 is effective at inhibiting blood vessel growth in tumors by binding VEGF and preventing the development of new vasculature. sFLT01 reduces intratumoral blood vessel count, prolongs survival, and delays tumor progression in mice without any apparent side effects.

49 POSTER

Analyses of pharmacodynamic (PD) assessments collected during expanded cohorts (EC) of a phase I trial with OSI-930, a multi-targeted oral tyrosine kinase inhibitor (TKI)

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Background: OSI-930 is an oral TKI with potent activity against Kit, VEGFR2, and PDGFR. Preclinical studies demonstrate tumor stasis and regression with long-term remissions across multiple xenograft models.
Material and Methods: A multicenter, phase I study of continuous oral OSI-930 administered QD or BID was performed in patients (pts) with advanced solid tumors. Upon determination of the maximum tolerated dose (MTD) and recommended phase 2 dose (RP2D), two expansion cohorts of pts with GIST (n = 10) and/or other advanced solid tumors (n = 10) were enrolled. Detailed PD assessments taken pre- and post-drug administration in the EC included PET imaging in GIST pts and either paired tumor biopsies or DCE-MRI in pts with solid tumors. Levels of soluble VEGFR2 (sVEGFR2) in plasma were measured. Additional pharmacokinetic analyses were also performed.
Results: Thirty-five pts were enrolled in the escalation phase where the MTD was 500 mg BID with no MTD determined for QD dosing. Dose limiting toxicities were rash (2 pts), elevated GGT (1 pt) and myalgia (1 pt). Common grade 1/2 toxicities included diarrhea, fatigue, nausea, rash and anorexia. Median duration of therapy in the BID cohorts was 13 wks. An additional 21 pts with a median age of 50 (range 20−81) were enrolled in the EC including 10 non-GIST and 11 GIST pts. Treatment was initiated at 500 mg BID. Two grade 3 events have occurred in the EC to date: fatigue (1 pt) and asymptomatic elevated lipase (1 pt). Other grade 1/2 toxicities were limited to fatigue, nausea, vomiting and flatulence. Of the non-GIST pts, 5 had DCE-MRI and 4 had biopsy studies. All GIST pts had PET studies. Initial PD data available at submission demonstrate significant changes in tumor vascularity by DCE-MRI between pre (day −1) and post OSI-930 (days 2 and 22) with good correlations to tumor shrinkage on CT evaluation. Decreases in plasma sVEGFR2 levels were also observed.
Conclusions: The single agent MTD and RP2D of OSI-930 is 500 mg BID. OSI-930 has been well tolerated with promising anti-tumor activity. Findings from paired tumor biopsies, PET, or DCE-MRI will be presented.

50 POSTER

Unique enzymatic profile of a potent and selective VEGFR/PDGFR tyrosine kinase inhibitor, TAK-593: potent pseudo-irreversibility against VEGFR and PDGFR

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TAK-593 is a novel small molecule that potently and selectively inhibits tyrosine kinases in the VEGFR and PDGFR families. TAK-593 inhibited VEGFR1, VEGFR2, VEGFR3, PDGFRα and PDGFRβ kinases with an IC50 value of 3.2, 0.95, 1.0, 4.3 and 13 nmol/L, respectively. TAK-593 also showed moderate inhibition of c-Kit and FGFR1 kinases with a IC50 value of 100 and 350 nmol/L, respectively. In addition, the IC50 values against 14 other protein kinases were in the range from 5900 nmol/L over 10000 nmol/L. TAK-593 dose-dependently inhibited the VEGF-induced proliferation of HUVECs (human umbilical vein endothelial cells) with an IC50 value of 0.30 nmol/L and PDGF-BB-induced proliferation of CASMC (coronary artery smooth muscle cell) with an IC50 value of 3.5 nmol/L. TAK-593 also inhibited the VEGF-induced VEGFR2 phosphorylation of HUVECs (IC50 = 0.34 nmol/L) and PDGF-BB-induced PDGFRβ phosphorylation of CASMCs (IC50 = 2.1 nmol/L). As expected, TAK-593 showed much less effect on the proliferation of various cancer and normal cells including lung carcinoma A549 (IC50 = 30 μmol/L), pancreas carcinoma CFPAC-1 (IC50 = 13 μmol/L), prostate carcinoma DU145 (IC50 = 18 μmol/L), colon carcinoma HT-29 (IC50 = 14 μmol/L), breast carcinoma MDA-MB-231 (IC50 = 8.6 μmol/L), and fetal lung fibroblast MRC-5 (IC50 = 24 μmol/L). These data indicated a high level of selectivity and potency of TAK-593 against VEGFR and PDGFR kinases.
In order to clarify the mode of inhibitory activity of TAK-593, the recovery of enzymatic activity after a large dilution of the enzyme-inhibitor complex was measured for VEGFR2 kinase. The progress curve for the control reaction (no inhibitor) was essentially linear for 25 min after dilution. In contrast, the activity of VEGFR2 kinase pre-incubated with TAK-593 was very low even at 90 min after dilution to a concentration of 1/10 the IC50 value of TAK-593 (95 pmol/L). This result indicates a unique mode of TAK-593 to inhibit VEGFR2 kinase with reversible, but very slow kinetics, i.e., pseudo-irreversibility. In addition to VEGFR2 kinase, we also demonstrated that TAK-593 inhibited PDGFRβ kinase in a pseudo-irreversible manner. These results suggest that TAK-593 might have prolonged action against VEGFR2 and PDGFRβ kinases in both clinical and preclinical settings.

51 POSTER

TAK-593, a potent and selective VEGFR/PDGFR tyrosine kinase inhibitor, effects the tumor vascularity and vascular permeability

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TAK-593 is a novel small molecule compound that potently and selectively inhibits VEGFR and PDGFR tyrosine kinases. TAK-593 uniquely shows potent pseudo-irreversibility against VEGFR2 and PDGFRβ that leads to potent antitumor activities against various types of cancers with good tolerability.
Effects of TAK-593 on tumor angiogenesis were investigated in this study. HUVECs (human umbilical vein endothelial cells) are known to give rise to tube-like formation by coculture of fibroblasts in the presence of VEGF in vitro. TAK-593 inhibited the VEGF-induced tube formation in a dose-dependent manner (IC50 = 0.32 nmol/L). Angiogenesis inhibition of TAK-593 in A549 lung carcinoma xenograft was examined by measuring blood vessel density. Vascular endothelial cells were detected by immunostaining with an anti-CD31 (PECAM) antibody. TAK-593 significantly decreased blood vessel density of human lung carcinoma A549 xenografts at 3 and 7 days after initial treatment at doses of 0.25 and 1 mg/kg. Along with the angiogenesis inhibition, the suppression of cancer cell proliferation and the apoptosis induction were shown by Ki-67 immuno-staining and TUNEL method, respectively. These results show a significant activity of TAK-593 to inhibit tumor angiogenesis contributes to the *in vivo* antitumor activity.
Next, the effect of TAK-593 on tumor vascular permeability was investigated by DCE-MRI (dynamic contrast-enhanced magnetic resonance imaging). Nude mice with HT-29 colon carcinoma xenograft were treated with TAK-593 at doses of 0.25 and 1.5 mg/kg, twice-daily for 4 days. DCE-MRI was carried out followed by administration of gadolinium diethylene triamine pentaacetate. Ktrans value (index of vascular permeability) in the tumor decreased significantly in 0.25 and 1.5 mg/kg treatment groups, although clear antitumor activity was still not observed at the time point. These results suggest that TAK-593 has a suppressive effect on vascular permeability of the blood vessels in tumor xenograft at an early stage of