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POSTER

Pharmacokinetic (PK)-pharmacodynamic correlation, biologic and clinical effects of GW786034 (pazopanib), a VEGF tyrosine kinase inhibitor (TKI), in patients (pts) with solid tumors

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Background: Pazopanib (P) is a potent small molecule TKI of VEGFR-1, -2, -3, PDGF- α , - β , and c-kit. The effects of P on blood pressure (BP), tumor blood flow, circulating biomarkers and wound healing in pts treated on a dose escalation phase I trial are described.

Materials and Methods: 63 pts with a variety of solid tumors were enrolled and continued on study until disease progression (3–107 wks, median 77.5 wks); 5 patients remain on study at the time of the present analysis. P was given orally at doses of 50 mg TIW to 2000 mg qd. Pts had BPs measured at clinic and at home. Dynamic contrast enhanced MRI (DCE-MRI) scans were obtained in duplicate at baseline and on Day 8 and 22 of treatment, in 12 patients at P doses of either 800 mg qd or 300–400 mg bid. Dermal wound vascular scores and phospho-VEGFR2 were evaluated pre- and post-treatment. PK data were collected on Days 1 and 22 of P administration. HTN was defined as an increase in mean arterial pressure from baseline of ≥ 15 mmHg on 3 separate occasions, or the institution/escalation of antihypertensive medication. Logistic regression (LR) was used to examine the relationship between plasma P concentrations and HTN. The effects of age, sex, and prior history (Hx) of HTN on this relationship were examined.

Results: 23/57 pts (40%) developed HTN during the first 3 weeks on study, and 30/57 (53%) developed HTN during the entire study. HTN was responsive to treatment and reversible upon discontinuation/reduction of P dose. LR suggested a relationship between steady-state plasma P concentration at 24 hr (C24) and HTN. The C24 at which the probability of HTN was 50% across all pts was 21 μ g/mL. Hx of HTN had a significant effect on the relationship between C24 and HTN. Mean C24 in patients who received P at 800 mg qd or 300 mg bid were 25 μ g/mL and 31 μ g/mL, respectively. 10 of 12 patients had a $\geq 50\%$ reduction in tumor blood flow as determined by IAUGC₆₀. A transient increase in VEGF was observed during treatment. No change in VCAM or e-Selectin concentrations was observed. No consistent treatment related changes in wound vascular scores were seen in this study; molecular analyses are ongoing.

Conclusions: P administration demonstrated biologic and clinical effects consistent with VEGFR inhibition in cancer patients at doses administered in this study. A concentration-response relationship between HTN and C24 was demonstrated.

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Bevacizumab is taken up by platelets and thereby blocks platelet-VEGF in cancer patients

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VEGF is a key factor to promote angiogenesis and released by tumor cells and host cells. Platelets primarily determine VEGF concentrations in blood. We recently reported that isolated platelets take up immunoglobulins *in vitro* including the monoclonal antibody bevacizumab, a therapeutic antibody against VEGF. This uptake blocked the angiogenic activity of platelets in an endothelial cell proliferation assay. Based on these findings we hypothesized that treatment of cancer patients with bevacizumab results in platelet uptake of the antibody and interferes with platelet function. To study this hypothesis we isolated platelets from patients at different time points after administration of bevacizumab and determined the free VEGF content in these platelets by ELISA (R&D Systems). This ELISA method does not recognize VEGF bound to bevacizumab. Subsequently, we determined the total VEGF content by western blotting according to standard procedures. Furthermore, the angiogenic activity of these isolated platelet samples are being determined in an endothelial cell proliferation assay. In these ongoing studies, we have been able to isolate platelets from 4 patients with advanced cancer during bevacizumab treatment. At 8 hours after bevacizumab administration, free VEGF concentration in platelets was decreased to $<5\%$ compared to pretreatment, while at 2 hours after administration free VEGF concentration was similar

to pretreatment concentrations. In all other samples (n=14) during treatment with bevacizumab, free VEGF concentration decreased to $<5\%$ of pretreatment concentrations. In contrast, total VEGF contents of these isolated platelets (free and bound VEGF) as determined by western blotting remained unchanged during treatment. Taken together, these results reveal that bevacizumab binds to VEGF inside of the platelets. Assessment of the angiogenic activity of these platelets is ongoing and the results will be presented during the meeting. In conclusion, we report that treatment with bevacizumab results in platelet uptake of this antibody in cancer patients. Based on these findings, we propose that platelets may deliver therapeutic antibodies at prothrombotic sites such as wounds and tumors. In addition, platelet uptake of bevacizumab may explain side effects of bevacizumab such as impaired woundhealing.

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VLA-4 antagonism inhibits angiogenesis-dependent hepatic colon carcinoma metastasis via endothelium-myofibroblast interaction blockade

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Previously, we have developed a novel family of synthetic non-peptidyl small molecule antagonists to very late antigen-4 (VLA-4) integrin that inhibit the experimental hepatic melanoma metastasis via melanoma cell adhesion blockade to cytokine-activated endothelial cells. Transient expression of VLA-4 on endothelial cells promotes their motility, proliferation and adhesion to vascular cell adhesion molecule-1 (VCAM-1) expressing-pericytes during blood vessel maturation. Therefore, anti-tumoral effects of these VLA-4 antagonists were evaluated *in vivo* through an experimental hepatic colonization model of non-VLA-4-expressing cancer cells using CT26 colocal carcinoma. In addition, the potential antiangiogenic activity of these VLA-4 antagonists was also studied through cell migration and adhesion assays between hepatic stellate cells (HSC) and hepatic sinusoidal endothelial (HSE) cells isolated from vascular endothelial growth factor (VEGF)-injected mice. Mice were given one intraperitoneal injection of either vehicle or VLA-4 antagonist (2.5 mg/kg) 1 hour prior to CT26 cell intrasplenic injection and the same dose was repeated on days 2, 3, 8, 9, 10 and 11 after cancer cell injection. As histologically assessed, the hepatic volume fraction occupied by metastatic tissue decreased by 50% in VLA-4 antagonist-treated mice as compared to vehicle-treated mice.

However, not statistically significant variations in the number of metastatic foci were observed between both treated groups. Interestingly, VLA-4 antagonist treatment inhibited endothelial cell (as CD31+ cells) and myofibroblast (as smooth muscle alpha-actin expressing cells) recruitment into hepatic metastases that correlated with decreased angiogenesis. *In vitro*, VLA-4 antagonist inhibited tumor-induced HSE migration through type I collagen. VLA-4 antagonist also completely abrogated HSC adhesion to HSE cells isolated from VEGF-intraperitoneal injected mice having increased VLA-4 expression. This enhanced adherence was also inhibited when HSC were preincubated with anti-VCAM-1 monoclonal antibody before their addition to HSE cells confirming that adhesion between both cell lineages was VLA-4/VCAM-1-interaction dependent. In summary, these results demonstrate for the first time antiangiogenic effects of VLA-4 antagonists during cancer metastasis development and suggest that antitumoral effects of VCAM-1/VLA-4 interaction blockade may occur in part by targeting tumor-activated endothelial-myofibroblast interactions.

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Combined anti-VEGFR and anti-PDGFR actions of sunitinib on blood vessels in preclinical tumor models

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Studies using mouse tumor models have shown that inhibitors of VEGF signaling can reduce the vascularity of tumors as much as 80%, but most pericytes (mural cells) in tumors survive the treatment. Surviving pericytes and basement membrane sleeves contribute to the rapid regrowth of tumor vessels after the treatment ends. Preclinical studies also suggest that therapies that target both VEGFR and PDGFR signaling pathways are more efficacious than those targeting individual pathways, but the mechanism is unclear. Sunitinib malate (SU11248) is a multitargeted receptor tyrosine kinase (RTK) inhibitor that blocks VEGFR and PDGFR at roughly equivalent concentrations. This dual action was used to explore the combined effects of VEGFR and PDGFR inhibition on tumor blood vessels.