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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 $\geqslant$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 μg/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 μg/mL and 31 μg/mL, respectively. 10 of 12 patients had a $\geqslant$50% reduction in tumor blood flow as determined by $IAUGC_{60}$. 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 colocarcinoma. 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.

The present studies sought in particular to determine whether sunitinib disrupts pericytes as well as endothelial cells of tumor vessels. We also sought to distinguish effects of VEGFR inhibition from those of PDGFR inhibition. For this purpose, we compared the actions of sunitinib to those of two more selective RTK inhibitors: AG-028262, a selective inhibitor of VEGFR, and CP-673,451, a selective inhibitor of PDGFR. Pancreatic islet tumors in RIP-Tag2 transgenic mice were used as a model. Experiments focused on changes on endothelial cells (CD31 immunoreactivity) and pericytes (alpha-smooth muscle actin immunoreactivity) after 7 days of oral administration. Changes in tumor vascularity were inferred from changes in the endothelial cell population. Treatment with sunitinib for 7 days reduced both the endothelial cell population (−75%) and pericyte coverage (−63%) of tumor vessels. After sunitinib treatment, some tumor vessels lost patency within 1 day and regressed within 2 days. AG-028262 reduced the tumor endothelial cell population (−61%) but not pericyte coverage (+0.6%). CP-673,451 had little effect on endothelial cells (−5%) but significantly reduced pericyte coverage (−50%). The combination of AG-028262 and CP-673,451 produced large reductions in both endothelial cells (−71%) and pericytes (−70%) in RIP-Tag2 tumors, comparable to those caused by sunitinib alone. These findings show that sunitinib caused regression of both endothelial cells and pericytes of tumor blood vessels. The rapid reduction in tumor vascularity appeared to result largely from inhibition of VEGFR signaling, and the accompanying pericytes loss from inhibition of PDGFR signaling.

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Correlative results of in-vivo monitoring of platelet-derived growth factor receptor (PDGFR) activation status in a randomized placebo-controlled trial of docetaxel +/− imatinib in castration-resistant prostate cancer (CRPC) and bone metastases (BM)

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Background: To further examine preclinical (Uehara, *JNCI* 2003) and clinical (Mathew, *JCO* 2004) evidence that the PDGFR inhibitor, imatinib, may favorably modulate taxane activity in CRPC with BM, a randomized placebo-controlled study of weekly docetaxel +/− daily oral imatinib was conducted (Mathew, *ASCO* 2006). In order to a) assess whether differential p-PDGFR inhibition during therapy could be detected and b) whether this predicted clinical outcomes, in-vivo monitoring of p-PDGFR status was explored using surrogate tissue.
Methods: Among 115 men randomized, there were 88 [(41 docetaxel + imatinib (DI), 47 docetaxel + placebo (DP)] sample pairs for study of p-PDGFR status. Peripheral blood leucocyte p-PDGFR status was determined by immunofluorescent antibody staining; p-PDGFR fluorescent intensities of 2000 individual leucocytes at baseline and after 1 cycle of therapy (6 weeks) were captured by laser scanning microscope. Within-patient estimators (WPEs) of the probability that p-PDGFR decreased [(Pr(Decr)] were computed using a Wilcoxon-Mann-Whitney statistic. Weighted averages of WPEs were computed to obtain overall group level estimates (GLEs) for all patients, each treatment arm, patients with/without 50% decline in PSA, dose-limiting toxicity and change in bone markers respectively. Time-to-progression (TTP) for two groups of patients having WPEs of Pr(Decr) above or below median was assessed.
Results: The magnitude Pr(Decr) in DI (median, 0.487) was higher than DP (median, 0.447), $p < 0.0001$. Pr(Decr) was higher among patients *without* PSA response (median, 0.505) than patients with PSA response (median, 0.406), $p < 0.0001$. Logistic regression suggested a lower Pr(Decr) marginally predicted a higher probability of PSA response ($p = 0.052$). By contrast, Pr(Decr) did not correlate with dose-limiting toxicity ($p = 0.43$), decrease in bone specific alkaline phosphatase ($p = 0.236$) or urine N-telopeptide ($p = 0.065$). TTP for WPEs of Pr(Decr) below median of 0.46 was 5.7 months compared to 4.1 months for above median, $p = 0.032$.
Conclusions: In-vivo evidence of differential p-PDGFR inhibition in the DI arm was obtained. Additionally and unexpectedly, the data suggest that systemic p-PDGFR inhibition associated with inferior response and TTP outcomes with docetaxel therapy. These observations qualify the appraisal of p-PDGFR as a therapeutic target for combination therapy with docetaxel in CRPC with BM.

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Methylnaltrexone potentiates the effects of 5-fluorouracil (5-FU) and Bevacizumab on inhibition of VEGF-induced endothelial cell proliferation and migration

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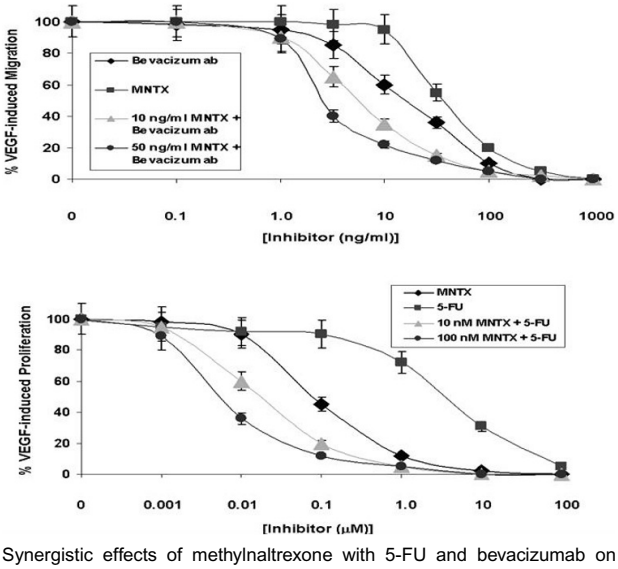
Background: Many patients with cancer receive combinations of drug treatments which include 5-fluorouracil (5-FU) and bevacizumab. Therapeutic doses of 5-FU are often associated with unwanted side effects and bevacizumab is costly. Therefore, agents that can reduce the therapeutic concentration of these drugs can have significant clinical utility. We have previously shown that methylnaltrexone, a peripheral mu opiate antagonist inhibits VEGF and opioid-induced endothelial cell (EC) proliferation and migration, two key components in tumor-associated angiogenesis. In this study, we therefore examined whether methylnaltrexone had a synergistic effect with 5-FU and bevacizumab on angiogenic events.
Methods: Human pulmonary microvascular EC (HPMVEC) proliferation assay was performed as previously described. For measuring cell growth, HPMVEC [5×10^3 cells/well] were incubated with 0.2 ml of serum-free media containing various concentrations of 5-FU, methylnaltrexone or both (1 nM to 100 μM) with or without 100 nM VEGF for 24 h at 37ºC in 5% CO_2/95% air in 96-well culture plates. The *in vitro* cell proliferation assay was analyzed by measuring increases in cell number using the CellTiter96™ MTS assay. For EC migration assays, twenty-four transwell units with 8 μM pore size were used. HPMVEC (~1×10^4 cells/well) were plated in serum-free media containing various concentrations of 5-FU, methylnaltrexone or both (1 nM to 100 μM) to the upper chamber and 100 nM VEGF was added to the lower chamber. Cells were allowed to migrate for 18 hours. Cells from the upper and lower chamber were quantitated using the CellTiter96™ MTS assay and read at 492 nm. Percent migration was defined as the number of cells in the lower chamber divided by the number of cells in both the upper and lower chamber.
Results: We observed that methylnaltrexone inhibited EC proliferation with an IC50 of ~100 nM. Adding 100 nM methylnaltrexone to EC shifted the IC50 of 5-FU from ~5 μM to ~7 nM. Further, adding ~50 ng/ml methylnaltrexone shifted the IC50 of bevacizumab on inhibition of EC migration from ~25 ng/ml to ~6 ng/ml.



Synergistic effects of methylnaltrexone with 5-FU and bevacizumab on inhibition of VEGF-induced EC proliferation and migration.

Conclusion: Taken together, these results indicate that methylnaltrexone is synergistic with 5-FU on EC proliferation and with bevacizumab on EC migration. Therefore, addition of methylnaltrexone could potentially lower the therapeutic doses of 5-FU and bevacizumab.