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.

79 POSTER

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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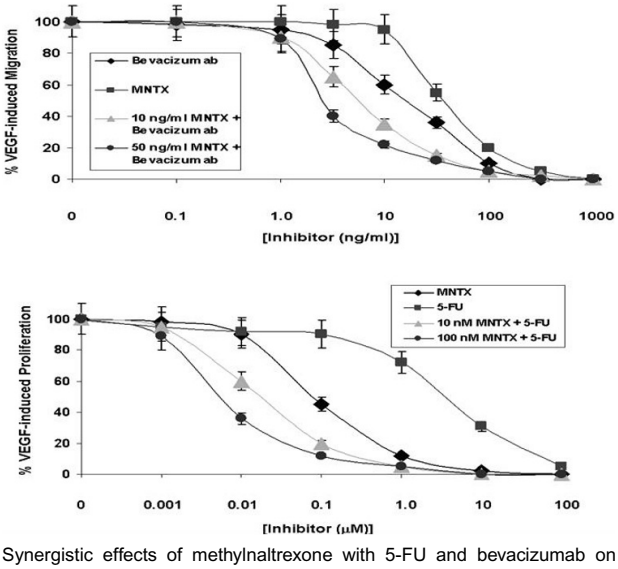
Background: Many patients with cancer receive combinations of drug treatments which include 5-fluorouracil (5-FU) and bevacizumab. Therapeutic doses of 5-FU are often associated with unwanted side effects and bevacizumab is costly. Therefore, agents that can reduce the therapeutic concentration of these 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.