Methods: HPMVEC proliferation assay was performed as previously described. HPMVEC (5 * 103 cells/well) were incubated with serum-free media containing various agonists (100 nM MS, DAMGO or VEGF) for 24 h at 37ºC. The in vitro cell proliferation was analyzed by measuring increases in cell number using the CellTiter96TM MTS assay. 24 transwell units with 8 μM pore size were used for monitoring in vitro cell migration. HPMVEC (~1×10^4 cells/well) were plated with various treatments to the upper chamber and various agonists were added to the lower chamber. Cells were allowed to migrate for 18 hrs. Cells from the upper and lower chamber were counted by the same assay.
Results: S1P, VEGF, PDGF, MS and DAMGO induced P and M of EC which was inhibited by pretreatment with MNTX (0.1 μM, 1 hr). Silencing mu opioid receptor expression (siRNA) blocks MS and DAMGO-induced EC P and M while also inhibiting S1P, VEGF and PDGF-induced EC P and M. Immunoprecipitation followed by immunoblot indicate that S1P, VEGF and PDGF treatment of EC induced serine/threonine phosphorylation of the mu opioid receptor (indicating receptor transactivation) and activation of the G-protein, RhoA. MS and DAMGO treatment of EC induced tyrosine phosphorylation of the VEGF receptor, PDGF receptor and S1P3 receptor along with RhoA activation. MNTX pretreatment of EC attenuated MS, DAMGO, S1P, VEGF and PDGF-induced receptor phosphorylation events and RhoA activation. Finally, silencing RhoA expression blocked agonist-induced EC proliferation and migration.
Conclusion: These results indicate that MNTX inhibits agonist-induced EC P and M via inhibition of receptor phosphorylation/transactivation and subsequent inhibition of RhoA activation. MNTX inhibition of angiogenesis may be a useful therapeutic intervention for cancer treatment.
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Modulation of the radiation response of zebrafish embryos by targeting the VEGFR2 tyrosine kinase using ZD6474

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Background: Preclinical studies have suggested that combining ionizing radiation with antiangiogenic agents enhances the therapeutic effect of ionizing radiation. Here, we addressed this issue *in vivo* using a novel vertebrate model, zebrafish embryos. Zebrafish are rapidly and prolifically bred and easily maintained, and embryos are optically transparent, facilitating direct observation of internal organs. Previously, we established zebrafish embryos as a model for the genotoxic stress response and pharmacologic modulation thereof (McAleer et al., *Int J Radiat Oncol Biol Phys* 61(1):10−13). The present study was designed to assess whether ZD6474 (AstraZeneca, Manchester UK), an inhibitor of VEGF receptor-2 (KDR) tyrosine kinase, modulated radiation sensitivity of zebrafish embryos.
Materials and Methods: Zebrafish were mated in embryo collection tanks. Viable embryos were washed and sorted at the one-cell developmental stage, and maintained under normoxic conditions at 28.5ºC for normal development. Morphology and survival was assessed visually using a light transmission microscope at 24-h intervals up to 144 hours post fertilization (hpf). The criterion for embryonic survival was the presence of cardiac contractility. Inhibition of angiogenesis was determined by monitoring the development of the main dorsal artery and intersegmental vessels.
Results: Treatment of live fish embryos with 10 μM ZD6474 at 24 hpf completely blocked formation of all blood vessels including the aorta as assessed at 48 hpf. At 3.3 μM ZD6474 approximately half (53%) of the embryos completely lacked vessel formation and none had developed intersegmental vessels, while at 1 μM only the development of the intersegmental vessels were perturbed (43%). When ZD6474 was administered within 30 min prior to ionizing radiation (0−20 Gy) at 24 hpf overall survival was markedly reduced. At 120 hours after irradiation only a fraction of the ZD6474-treated embryos (3.3 μM; 1.0 μM) were alive (10±5.8% and 34.8±14.7%, respectively) compared to 61.4±15.5% of control embryos receiving vehicle. Radiation-induced defects in midline development were significantly ($p < 0.05$) increased in ZD6474-treated irradiated embryos (93.3±5.8% and 82.8±13.4%, 3.3 μM; 1.0 μM respectively) vs. radiation alone (59.4±8.3%).
Conclusions: ZD6474 (10 μM) alone severely disturbed vascular development in zebrafish embryos. Concurrent administration of lower concentrations of ZD6474 and ionizing markedly reduced survival of zebrafish embryos, and sensitized them to radiation-induced morphological malformations. This model may help facilitate the evaluation of radiation modifiers.

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Phase I study of ABT869, a multiple receptor tyrosine kinase inhibitor, in patients with refractory solid malignancies.

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ABT869 is an orally administered, potent and specific inhibitor of VEGF and PDGF family tyrosine kinases, including FLT-3, and c-kit receptors. A phase I study of ABT869 in patients with refractory solid malignancies was initiated to determine the maximum tolerable dose (MTD) of ABT869 given by continuous once-daily oral dosing in escalating doses; evaluate ABT869 pharmacokinetics; assess pharmacodynamic effects on plasma VEGF, flt-1 receptor, circulating endothelial cells and other potential biomarkers; and to evaluate tumor response, including an assessment of microcirculatory parameters (blood flow, F and capillary permeability, PS) with DCE-MRI. Dose escalation was planned in cohorts of 3 patients each. ABT869 was administered before bedtime except on days 1 and 15. Treatment periods (TP) were defined as 21 days and tumor assessments were performed using CT scans after every 6 weeks. DCE-MRI was done at baseline, day 3 and 14 of the first cycle. Cohort expansion to 6 patients was planned if dose limiting toxicity (DLT) occurred in the first cycle of treatment, and MTD was defined as the dose at which $\geqslant$2/6 patients experienced DLT. 4 male, 5 female patients (median age 55; range 29−73) have received a total of 34 TP; 6 at 10 mg per day and 3 at 0.25 mg/kg/day. Weight adjusted dosing was implemented to minimize interpatient variability. Cycle 1 toxicities included fatigue (grade 3 DLT in 1 patient at 10 mg), asthenia, myalgia (grade 2 in 4/9), skin rash (maculopapular, vasculitic in 1 patient), hand foot syndrome, hypertension, proteinuria and mouth irritation. Hypertension and proteinuria were reversible on dose interruption. Pharmacokinetics of ABT869 demonstrated plasma clearance of 2.8±1.3 L/h, with a corresponding mean half-life of 16±5 h. Drug accumulation was not significant with continuous dosing (day 15/day 1 accumulation ratio 1.16). The target AUC (4.9 μg h/mL) for activity based on preclinical models has been reached with daily dosing of 10 mg (mean 4.1±2.2 μg h/mL). A carboxylate-derivative was identified as a major metabolite, suggesting cytochrome p-450 enzymes play a role in ABT-869 metabolism. 5/6 patients at 10 mg achieved stable disease, with CT scan evidence of tumor necrosis and DCE-MRI evidence of reduced Ktrans, ve, F and PS. In conclusion, continuous dosing of ABT869 is tolerable and achieves target exposure at doses studied and demonstrates early DCE MRI evidence of reducing tumor flow and capillary permeability.

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Metronomic oral vinorelbine: dose escalation study, pharmacokinetics and assessment of predictive biomarkers

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Background: Metronomic chemotherapy, the rhythmical dense administration of low doses of cytotoxics is believed to exert antitumor activity though damaging the activated endothelial cells in tumor vasculature. We investigated vinorelbine, an orally bioavailabe antimitotic drug, at a metronomic schedule of administration.
Material and Methods: patients with resistant metastatic tumors were treated with, escalated doses of oral vinorelbine, three times a week (TIW) without break until disease progression or unacceptable toxicity (UT) defined as any grade 2 toxicity according to CTC version 3. Patients were initially followed biweekly and later every month for assessing disease status, toxicity and blood sampling for pharmacokinetics and quantification of circulating angiogenesis related factors [VEGF, VEGFr2, TSP1, IL-8, FGFb and p53]. The study should close if UT occurred in two patients treated at minimum 3 months. The dose below this would be the highest metronomic dose (HMD).
Results: Eighty patients [39 women, median age 58, median PS 1] enrolled between June 2004 and December 2005 and treated at 7 dose levels: 20 mg (16 pts), 30 mg (17(, 40 mg (26), 50 mg (13), 60 mg (6) and 70 mg (2 pts). Median duration of treatment was 19 weeks (range 4 to 85+). Unacceptable toxicity occurred in 2 patients at the 60 mg dose level (leucopenia of grade 4 on 14th week of treatment and epistaxis on 9th week