ZD6126 (200 mg/kg ip qwk) plus radiation (2.5 Gy biw) results in enhanced anti-tumor activity over that achieved with single modality therapy.
**Conclusions:** These preclinical experiments strongly suggest that ZD6126 can augment radiation response in H&N and lung cancer model systems. These results complement reports from other researchers [1,2] suggesting the potential value of combining radiation with vascular targeting agents for the treatment of upper aero-digestive tract cancers.

**References**

[1] Siemann DW, Rojiani AM: Enhancement of radiation therapy by the novel vascular targeting agent ZD6126. Int J Radiat Oncol Biol Phys 53:164–71, 2002.
[2] Raben D. Personal communication.

**180** POSTER

**Low-dose metronomic cyclophosphamide induces sustained hypoxia in human tumor xenografts, which can be exploited therapeutically by the combination with the hypoxic cell cytotoxin tirapazamine**

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The frequent administration of low doses of conventional cytotoxic drugs over prolonged periods without breaks, often referred to as low-dose metronomic chemotherapy (LDM), or simply metronomic chemotherapy, targets the tumor vasculature and as such is assumed to increase tumor hypoxia. However, the relationship between antiangiogenic therapies and tumor oxygenation status is both complex and controversial. Moreover, recent evidence suggests that by inducing severe hypoxia, antiangiogenic treatments might select for less oxygen dependent tumor cell populations with potentially more aggressive clinical behavior and resistance to radiotherapy and conventional chemotherapy. We therefore tested a) whether LDM, in our case with cyclophosphamide (CTX), indeed increases tumor hypoxia and b) if combining LDM CTX with the hypoxic cell cytotoxin tirapazamine (TPZ) prevents the outgrowth of less oxygen dependent tumor cells. The hypoxic status of PC-3 human prostate cancer xenografts implanted subcutaneously in male nude mice was assessed by direct oxygen measurements (Eppendorf microelectrode technique) and EF5 staining. Mice with established tumors were treated with 20 mg/kg/d of CTX given in the drinking water, which results in partial tumor regression, followed by prolonged stabilization and eventually regrowth. TPZ was administered intraperitoneally at a weekly dose of 25 mg/kg, the regimen with the best therapeutic index of several schedules tested. In comparison with untreated PC-3 xenografts, LDM CTX treated tumors exhibit more pronounced hypoxia, which was sustained during tumor regrowth. Adding TPZ to LDM CTX did not induce complete tumor eradication. However, the combination resulted in a significant growth delay of more than 6 weeks. LDM CTX combined with TPZ was also beneficial in two other xenograft models using the orthotopically implanted human breast cancer cell line MDA-MB-231, and the subcutaneously growing human colon cancer cell line HT29. We conclude that LDM CTX induces sustained hypoxia, which can be exploited by combination with TPZ. Potential limitations of the combination of antiangiogenic therapies and hypoxic cell cytotoxins will be discussed.

**181** POSTER

**Suppression of neuroblastoma by targeted delivery of interleukin-12 to tumor vasculature**

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Neuroblastoma (NB) is the third most common cancer of infancy and childhood. The outlook for long-term survival in children diagnosed with NB remains poor. Because NB is considered an immunogenic cancer, new approaches for the treatment of this disease are being explored. Interleukin-12 (IL-12) is a potent stimulator of immune cells, specifically NK and T lymphocytes, and demonstrates both antitumor and antiangiogenic properties. Because its pleiotropic effects may be of benefit to cancer patients with NB, delivery of IL-12 in a manner that avoids the troublesome toxicity associated with its administration would be advantageous. To this end, we engineered a fusion protein, mrIL-12vp (mouse recombinant interleukin-12 linked to vascular homing peptide) composed of the cytokine IL-12 linked to an arginine-glycine-aspartate containing peptide, RGD-4C. Binding of $\alpha v\beta 3$ integrin to its RGD ligands within the extracellular matrix (ECM) triggers signals within endothelial cells (EC) for survival. Disruption of these signals by $\alpha v\beta 3$ antagonists triggers apoptosis of EC. Since $\alpha v\beta 3$ integrin is expressed almost exclusively by dividing EC in developing tumor neovasculature, targeted delivery of IL-12 to $\alpha v\beta 3$ integrin could enhance its immunostimulatory, antiangiogenic, and antitumor activities within the tumor microenvironment and localization may decrease toxicity. Using a corneal neovascular assay, we evaluated the angiogenic potential of NXS2 murine neuroblastoma cells. NXS2 cells loaded onto sponges and implanted into corneal pockets in an avascular area generated a robust angiogenic response. Mice receiving NXS2 corneal implants and given mrIL-12vp by subcutaneous (SC) continuous infusion (CI) showed nearly total inhibition of corneal neovascularization (P=0.02) while inhibition by mrIL-12 was 80%. mrIL-12vp slowed tumor growth when mice were injected SC with 1x106 NXS2 cells and treated with 1 μg/day of mrIL-12vp or mrIL-12 by CI once tumors were palpable. Only mrIL-12vp demonstrated significant inhibition of tumor growth (P=0.03). Thus, targeting IL-12 to developing tumor vasculature may be an effective strategy to suppress angiogenesis and tumor growth with effects superior to those of non-targeted IL-12. This model offers opportunities to extend these results by combining mrIL-12vp with other immunogenic strategies for NB that may prolong survival time for NB patients.

**182** POSTER

**Effect of chemotherapy on human tumor xenografts differently expressing vascular endothelial growth factor (VEGF) and fibroblast growth factor-2 (FGF-2).**

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The aim of this study was to investigate the influence of growth factor-affected vascular morphology and functionality on tumor response to chemotherapy. To this purpose we have investigated transfectants from the human endometrial adenocarcinoma HEC-1-B cell line that differ for VEGF and/or FGF-2 expression. Specifically, Tet-FGF-2/HEC-1-B cells that over express FGF-2 under the control of a tetracycline-responsive promoter and AS-VEGF-Tet-FGF-2/HEC-1-B that show reduced levels of VEGF after further transfection with VEGF antisense cDNA. We have previously shown the expression of FGF-2 and VEGF affect HEC-1-B tumor growth and angiogenesis synergistically; the inhibition of either one of the two growth factors results in a significant reduction in tumor growth and vascularization and the simultaneous down regulation of VEGF and FGF-2 caused additional inhibitory effects (Giavazzi et al., Am. J. Pathol., 2003). Here, nude mice transplanted subcutaneously with Tet-FGF-2/HEC-1-B or AS-VEGF/Tet-FGF-2/HEC-1-B and receiving tetracycline or not in the drinking water were treated with BAY 59–8862 (a paclitaxel derivative, former IDN5109; Nicoletti et al., Cancer Res., 2000) at different doses and schedules. The treatment with BAY 59–8862 induced a significant tumor growth inhibition of Tet-FGF-2/HEC-1-B and AS-VEGF/Tet-FGF-2/HEC-1-B xenograft models; the response was dose and schedule dependent. Although the xenograft variants showed similar tumor growth delay, independently from the expression of the angiogenic growth factors, the best therapeutic response was obtained in tumors with both the growth factors down regulated simultaneously (50% cured mice). The drug distribution into the tumors, was evaluated by HPLC assay. These findings show the alterations of vascularization, associated to growth factor modification, do not impair the response to chemotherapy.
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**183** POSTER

**Gene delivery of Escherichia coli nitroreductase into endothelial cells prolongs the survival of tumor-bearing mice after the treatment with the prodrug CB1954**

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A recent target of cancer gene therapy is tumor vasculature. Here, we present a gene-directed enzyme prodrug therapy (GDEPT) approach to target the tumor angiogenesis *in vivo*, by using the *E. coli* nitroreductase (*ntr*) gene delivery. This gene codes for an enzyme which is able to convert a non-toxic prodrug, such as the CB1954, into a potent cytolytic agent. After transfection of human umbilical vein endothelial cell line (HUV-EC-C) with a plasmid DNA carrying the *ntr* gene, we set up *in vitro* experiments in order to analyse the effects of CB1954 on these *ntr*-transfected cells.

We isolated two HUV-EC-C/ntr$^+$ clones presenting a sensitivity to CB1954 that was 15- to 30-folds higher with respect to untransfected HUV-EC-C cells (HUV-EC-C/ntr$^-$). These clones were injected subcutaneously, in association with the murine melanoma cell line B16-F10, into nude mice that were then treated with CB1954. Animal survival, as well as histological analysis of tumors, lung, spleen and liver were evaluated.
After the treatment of the animals with CB1954, we observed a prolonged survival of animals carrying the HUV-EC-C/ntr$^+$ clones with respect to control animals injected with HUV-EC-C/ntr$^-$ cells, but no significant differences in tumor growth. However, histological analysis of tumors showed large areas of necrosis, likely due to tumor ischemia, in the presence of HUV-EC-C/ntr$^+$ clones with respect to control animals. Histological analysis of lung and spleen did not show the presence of tumor metastasis, as well as histological analysis of liver showed neither tumor metastasis nor animal toxicity.
To our knowledge, this is the first report showing the efficacy of the GDEPT-based approach to prolong tumor-bearing animal survival after the delivery of the *ntr* gene to tumor vasculature and the treatment with CB1954, without inducing animal toxicity. Altogether our data indicate that the targeting of the tumor vasculature by this GDEPT strategy may be an effective approach for cancer treatment *in vivo*, relied on one possible bystander mechanism based on tumor ischemia.

**184** POSTER

**Pharmacokinetics of CNTO 328 in a three month intravenous dose toxicity study in cynomolgus monkeys with concomitant IL-2 therapy**

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**Background:** CNTO 328 is a chimeric (murine-human) monoclonal antibody (mAb) specific for IL-6, which is in involved in tumor growth, invasion and metastasis as well as tumor angiogenesis. IL-6 is also considered to be a causative factor in cancer-related morbidity from afflictions such as asthenia/cachexia and bone resorption. The goal of this study was to determine the pharmacokinetics of CNTO 328 when administered once weekly via an intravenous (IV) infusion to cynomolgus monkeys for 13 weeks, in combination with IL-2, followed by a one-month recovery period.
**Material and Methods:** Cynomolgus monkeys, 32 male and 32 female, were randomly assigned to 4 groups (16 monkeys per group) and were infused either with saline and IL-2 therapy alone, or a combination of weekly 10 and 50 mg/kg CNTO 328 with IL-2 therapy. Pharmacokinetic calculations were conducted using WinNonlin. These data were then compared with the PK parameter estimates from another study where CNTO 328 was administered alone.
**Results:** Animals treated with CNTO 328, in combination with IL-2 therapy, received extensive exposure to CNTO 328 over the three-month dosing and one month recovery periods. A five-fold increase in dose from 10 to 50 mg/kg resulted in an approximately 3.49 and 3.37 fold increase in Cmax and AUC (0–96hr), respectively. Steady state serum CNTO 328 serum concentrations were achieved by Week 11 (Day 71) with mean steady state trough concentrations of approximately 446.39 μg/mL and 3162.88 μg/mL (on Day 85) for the 10 and 50 mg/kg dose groups, respectively. The mean apparent terminal half-life was approximately 19.89 days.
**Conclusion:** CNTO 328 PK estimates in this combinatory study was comparable with the results obtained in previous study in which CNTO 328 was administered alone. This indicated that the intravenous infusions of CNTO 328 with or without combination of IL-2 do not appear to influence CNTO 328 exposure.

**185** POSTER

**Phage display-derived peptides specific to the galectin-3 carbohydrate recognition domain inhibit metastasis-mediated cancer cell adhesion**

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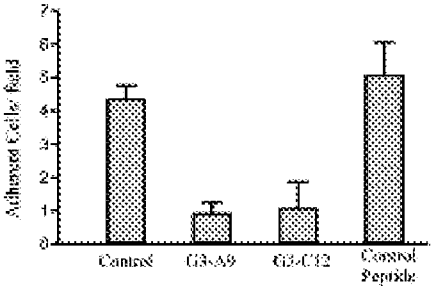
**Background:** Interactions between circulating cancer cells and the endothelial cells of blood vessels have a significant influence on metastasis. Studies indicate that galectin-3 (gal-3), a member of the galectin family of carbohydrate-binding lectins, is involved in carbohydrate-mediated metastatic events, and interacts with the tumor specific Thomsen-Friedenreich glycoantigen (TFA). Our laboratory is actively pursuing TFA and gal-3 as potential targets for breast, prostate, and colon cancer detection and therapy (Glinsky, Can. Res. 60:2584 and 61:4851). Because increased carcinoma cell adhesion is associated with increased metastatic potential, we hypothesized that inhibition of the galectin-3-TFA interaction would reduce homotypic (between carcinoma cells) and heterotypic (between carcinoma cells and endothelium) adhesion. To test this, we identified peptide antagonists of gal-3 using combinatorial bacteriophage (phage) display.
**Methods:** Two random phage display libraries, f88/15 and f88/Cys6 encoding for random 15- and 6-amino acid peptides respectively, were used for affinity selections against purified recombinant gal-3. After 4 rounds of selection, eighty individual phage clones were analyzed. ELISA, immunoblot, and fluorescence quenching were employed to analyze peptide affinity and specificity. The ability of the peptides to functionally modulate adhesion was tested using MDA-435 human breast carcinoma cell homotypic adhesion, and heterotypic adhesion to human bone marrow endothelium in a parallel plate laminar flow chamber system, which reconstructs *in vitro, in vivo* microvascular blood flow.
**Results:** Peptides bound to purified gal-3 protein with high affinity ($K_d$ ≅17–80 nM), but did not bind other galectins or lectins tested. Experiments using truncated gal-3 proteins indicated that the selected peptides bound to the carbohydrate-recognition domain (CRD) of gal-3. Two such peptides, G3-A9 and G3-C12, blocked the interaction between gal-3 and TFA, and inhibited MDA-MB-435 breast carcinoma cell homotypic and heterotypic adhesion by greater than 60% (Figure 1).
**Conclusions:** Our results have physiological importance because they demonstrate that carbohydrate-mediated metastatic cell adhesion can be inhibited efficiently with peptides that bind to the CRD of gal-3. Small galectin-3-specific peptides, offer advantages over antibody or carbohydrate gal-3 modulators, and have the potential to affect the entire metastatic process and control hematogenous cancer spread.




MDA-435 cells were infused across human endothelial cell monolayers in the presence or absence of 25 μM G3-A9, G3-C12, control peptide or no peptide (control). After stabilization, the number of rolling and adherent tumor cells was recorded. Gal3 peptides inhibit human breast cancer cell adhesion to endothelium under flow conditions.

**186** POSTER

**Potent analogues of the antiangiogenic agent NM-3 have enhanced antiproliferative activity in vitro**

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NM-3 (8-hydroxy-6methoxy-3-(2-methylcarboxymethyl)-isocoumrarin) is an orally active antiangiogenic agent that is being evaluated in clinical trials. In preclinical studies, NM-3 inhibits the proliferation of human umbilical vein endothelial cells (HUVEC) as well as enhances anti-tumor effects of standard chemotherapy and radiotherapy in mice xenografts without noticeable toxicity. NM-3 has also been shown to induce lethality in some human carcinoma cell lines in vitro, however, at concentrations above 100 μM. In this project, NM-3 analogues have been synthesized which displayed enhanced antiproliferative activity on HUVEC (200 times NM-3), as well as on different tumor cell lines that are not sensitive to NM-3. We investigated the mechanism whereby these compounds, in comparison with NM-3, exert their activity on HUVEC and on tumor cell lines. Immunoblotting analysis of different cell cycle-associated proteins in cells treated with these novel compounds indicated that they are at least 5 fold more potent in increasing p53 and p21/WAF levels than NM-3 and that cells are blocked in G2.
In summary, NM-3 analogues have been synthesized and shown to have significantly more potent antiangiogenic and antiproliferative activities which might contribute to their antitumor activity in vivo.