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

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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.

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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.

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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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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.