cytotoxic agents and interferon for their IC-50 on endothelial cells compared to different tumor cell lines. Additionally, the most promising compounds were studied in metronomic doses in a murine renal cell carcinoma model to investigate their antitumor and antiangiogenic activity in vivo.

**Methods:** For in vitro studies, influence of different cytotoxic agents and interferon-alpha 2a on proliferation (BRdU assay) and apoptosis (FACS-analysis) were studied on tumor cell lines as well as on endothelial cell lines to determine IC-50 and mechanism of action.

For in vivo studies, intrarenal application of RENCA cells in syngenic Balb/c mice was used. 21 days after application, mice developed a primary tumor and metastases to the lung and abdominal lymph nodes. Mice received either, 12 mg adriamycin i.v. on day 10 and 17 or 1,2 mg adriamycin i.v. on day 10 and 17 or 0,24 mg adriamycin i.v. on day 10-19 or vehicle. The interferon groups received either 10.000 or 100.000 IU interferon-alpha 2a i.p. on day 10-17. All mice were sacrificed on day 21 and tumor weight and volume, lung weight and lung metastases as well as vessel density in primary tumors (immunohistochemical staining against CD-31) were detected.

**Results:** The IC-50 of cytotoxic drugs (adriamycin, idarubicin, 5-FU, paclitaxel) is decreased by 3 orders of magnitude or more for the endothelial cells compared to different tumor cell lines. Adriamycin, applied at cyclic MTD to RENCA mice resulted in a partial remission in tumor volume but showed increased vessel density in primary tumors compared to the control group. Metronomic application of adriamycin resulted in a partial remission of tumor volume but showed a significant decrease in vessel density of primary tumors. The reduction in dose of interferon-2-alpha therapy in RENCA mice did not lead to changes in anti-angiogenic activity.

**Conclusions:** The application of cytotoxic drugs in the metronomic way shows clear antiangiogenic efficacy. It therefore may become a future alternative to conventional applied chemotherapy. Additionally, interferon-2-alpha still shows antiangiogenic activity at 10 fold reduced dose.

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## Plasma levels of vascular endothelial growth factor (VEGF) in patients with cervical cancers: prognostic significance and impact of platelet count and hemoglobin level

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**Objective:** VEGF (Vascular Endothelial Growth Factor) is a protein with high biological activity. This study investigates the role of the pretreatment VEGF-levels in sera (sVEGF) and plasma (pVEGF) in patients with unresectable cervical cancers and its relationship to the clinical course of the disease. Furthermore we analyzed the impact of hemoglobin and platelet count on the VEGF-expression.

**Patients, Material and Methods:** 41 patients with locally advanced cervical cancer (FIGO IB-IVA), who were treated with primary radiotherapy, were analyzed. VEGF-concentrations were measured with a quantitative immunoassay (Quantikine, R&D Europe). The VEGF-levels were compared with the clinical outcome. The statistical analysis were performed using SPSS 9.0 for windows.

**Results:** The VEGF-concentration did not correlate with tumor stage. The sVEGF-level was 258 ± 54 pg/ml in stage II (n = 6), 450 ± 61 pg/ml in stage III (n = 24) and 815 ± 304 pg/ml in stage IV (n = 8) (n.s.). Patients with complete tumor response (CR; n = 22) showed significantly lower sVEGF-levels (320 ± 44 pg/ml) than patients with progressive tumor (PD; n = 19; sVEGF 674 ± 138 pg/ml; p = 0.025). The 3-year-survival of patients with sVEGF<600pg/ml was 63 ± 9%. All 9 patients with sVEGF>600pg/ml died within 3 years. The release of VEGF from platelets during serum preparation was demonstrated by a correlation between serum-VEGF and the platelet counts (r = 0.518; p < 0.01). In the cases with tumor response, the platelet counts were also lower (224 ± 17 Gpt/l) than in the cases with progressive disease (309 ± 28 Gpt/l; p = 0.012). The 3-year-survival, dependent on the median of the platelet-count (250Gpt/l), was 68 ± 11% for patients <250Gpt/l (N = 20) vs. 36 ± 11% for >250Gpt/l (N = 21); p<0.01. The VEGF in blood-plasma (without the platelet-released VEGF) was in anemic patients higher(51 ± 6 pg/ml) than in non -anemic patients (29 ± 3pg/ml; p <0.01).

**Conclusions:** A high pretreatment serum-VEGF was associated with poor response to radiotherapy and decreased overall survival in locally advanced cervical cancer. The role of platelet-count on survival needs to be further investigated. Anemia showed an impact on VEGF-expression

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## Overexpression of lymphanglogenic growth factor VEGF-C in human pancreatic cancer

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Vascular endothelial growth factor C (VEGF-C) is an lymphangiogenic polypeptide that has been implicated in cancer growth. In the present study we characterized VEGF-C expression in cultured human pancreatic cancer cell lines and determined whether the presence of VEGF-C in human pancreatic cancers is associated with clinicopathological characteristics. VEGF-C mRNA transcripts were present in all 5 tested cell lines (Capan-1, MIA-PaCa-2, PANC-1, COLO-357 and T3M4). Immunoblotting with a highly specific anti-VEGF-C antibody revealed the presence of VEGF-C protein in all the cell lines. Northern blot analysis of total RNA revealed about 2.2-fold increase in VEGF-C mRNA transcript in the cancer samples by comparison with the normal pancreas. Immunohistochemical analysis confirmed the expression of VEGF-C and its receptor flt-4 in the cancer cells within the tumor mass. Immunohistochemical analysis of 51 pancreatic cancer tissues revealed the presence of strong VEGF-C immunoreactivity in the cancer cells in 80.4% of the cancer tissues. The presence of VEGF-C in these cells was associated with increased lymphatic vessels invasion (ly) and lymph node metastasis (n), but not with decreased patient survival. These findings indicate that VEGF-C and its receptor is commonly overexpressed in human pancreatic cancers and that this factor may contribute to the lymphangiogenic process and metastasis in this disorder.

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## Inhibition of angiogenesis using rofecoxib (Vioxx) and ionizing radiation

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Two specific COX-2 inhibitors, rofecoxib (VioxxTM) and celecoxib (CelebrexTM), are FDA approved. To date, there is no published data regarding the ability of rofecoxib to impact on endothelial cell function. The purpose of this study was to examine the effects rofecoxib on endothelial cell processes involved in angiogenesis, at clinically relevant doses, at, or below, the steady-state concentrations achieved at for use in the treatment of arthritis in combination with ionizing radiation. These include the effect of rofecoxib on the proliferation, attachment, and differentiation of cultured human umbilical vein endothelial cells (HUVEC) in-vitro, capillary sprouting of rat aortic ring explants embedded in collagen (ex-vivo) and Matrigel-induced angiogenesis (in-vivo) and COX-2 expression (Immunohistochemical).

Single-donor human umbilical vein endothelial cells (HUVECs) were used at passage 3-5. Cells were incubated at a subconfluent density with different concentrations of rofecoxib (0.5-2.0 μM/ml) and the effect of the drug +/- radiation (2 Gy) on cell proliferation, migration (modified Boyden chamber assay), tube differentiation (3D matrigel tube formation assay). In addition, a rat aortic ring explant embedded in Matrigel (ex-vivo) assay of Nicosia was used. The aorta capillary sprouts represent all phases of angiogenesis (invasion, proliferation, migration and tube formation). An in-vivo Matrigel plug assay was used to evaluate the effect in nu/nu mice. Tunnel assay was used for apoptosis.

Proliferating HUVECs had a high baseline expression of COX-2. Rofecoxib inhibited endothelial cell proliferation, migration, and tube formation (differentiation) in a dose dependent manner. The IC50 dose was 0.5 μM (p <0.001). In combination with low dose radiation, inhibition of sprouting and tube formation increased by 50-100%. Inhibition of angiogenic processes suggests at least an additive effect at low doses of both drug and radiation. Combination therapy increased the percentage of cells undergoing apoptosis. In-vivo, rofecoxib inhibited Matrigel induced angiogenesis.

In-vitro, clinically relevant concentrations of rofecoxib inhibited endothelial cell proliferation, migration and tube formation in response to chemotactic and mitogenic growth factors. The addition of low dose ionizing radiation potentiated these antiangiogenic effects in a dose dependent manner. Phase II trials using a COX-2 inhibitor and radiation therapy are planned by the RTOG.