Results: The use of AF was associated with a significantly less mucositis in the patients with tumours of the pharyngeal tract (30% reduction if compared with the group of patients treated with the same doses without AF). In the group of patients with NSCLC the use of subcutaneous AF was associated with a dramatic decrease of Grade III-IV side effects and of nausea and vomiting. Moreover AF allowed the treatment with higher doses of chemotherapy. In the cervix cancer group at the moment is not showed an improvement in patients conditions during EBRT.

Conclusions: The subcutaneous administration of AF during EBRT is an extremely simple procedure that reduces, in selected patients, the incidence of severe side-effects linked to the radio-chemotherapy association and probably allows the administration of higher doses of chemotherapy, without increasing the side effects. We need further studies to identify the subgroups of patients that can obtain better results.

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Antiangiogenic and antitumor effect of VEGF antisense oligonucleotide in combination with anti-EGFR C225 monoclonal antibody in human colon cancer

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Angiogenesis plays a key role in tumor growth and metastasis. Vascular endothelial growth factor (VEGF) is secreted by cancer cells to stimulate endothelial cell growth through paracrine mechanisms. The transforming growth factor alpha (TGFalpha)-epidermal growth factor receptor (EGFR) autocrine pathway controls the production of VEGF and other angiogenic factors by cancer cells. We evaluated the antiangiogenic and antitumor activity of HYB 676, a novel human VEGF antisense 21-mer oligonucleotide with modified backbone structure, alone and in combination with MAb C225, an anti-EGFR chimeric human-mouse monoclonal antibody, in a human colon cancer xenograft model. The effect of HYB 676 on VEGF production by GEO cells was evaluated in vitro and in vivo by Western blotting and immunocytochemistry. The in vivo antitumor activity of HYB 676 and/or MAb C225 was determined in athymic mice bearing established human GEO colon cancer xenografts. The Student's t test and the Mantel-Cox logrank test were used for statistical evaluation. HYB 676 determined a dose-dependent inhibition of VEGF production by GEO cells in vitro. Treatment of mice bearing established GEO xenografts for three weeks with HYB 676 or with MAb C225 determined a reversible inhibition of tumor growth. In contrast, a prolonged inhibition of tumor growth was observed in all mice treated with the two agents in combination with a significant improvement in mice survival compared to controls ($P < 0.001$), to MAb C225 group ($P < 0.001$), or to HYB 676 group ($P < 0.001$). All mice died within 5, 7 and 10 weeks following tumor cell injection in the control, HYB 676 and MAb C225 groups, respectively. In contrast, 50% of mice treated with the combination of HYB 676 and MAb C225 were alive at 15 weeks. Immunohistochemical analysis of GEO xenografts demonstrated a significant reduction of VEGF expression after treatment with HYB 676 with a parallel reduction in microvessel count. A potentiation in VEGF inhibition in GEO tumors with little or no microvessels was observed following the combined treatment with the two agents. These results demonstrate that anti-EGFR MAbs and VEGF antisense oligonucleotides have a cooperative antiangiogenic and antitumor activity.

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Free circulating vascular endothelial growth factor (VEGF) is detectable only in small tumours of the WAG/Rij rat rhabdomyosarcoma tumour model

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Background: VEGF is a glycoprotein with potent angiogenic, mitogenic and vascular permeability enhancing activities specific for endothelial cells, synthesised and secreted by a variety of tumours and seems to be the major inducer of angiogenesis. We investigated the detection of free circulating VEGF in 11 normal WAG/Rij rats and in 19 WAG/Rij rats bearing a syngeneic rhabdomyosarcoma subcutaneously implanted and randomly selected for these measurements (tumour volume: 0–50 cm3).

Methods: A blood sample of 1 ml was obtained by intracardial puncture under a short general anaesthesia with ether. A plasma separator gel and a 1:10 anticoagulant mix of sodium citrate, theophylin, adenosine and dipyridamole to achieve maximal platelet stabilisation were used. Samples were immediately placed on ice and centrifuged at 2500 G at 4°C and were measured with a human VEGF ELISA (Citymmune®, Maryland, US) with cross reactivity against rat VEGF (detection range: 0.195–50 ng/ml).

Results: The following VEGF plasma concentrations (ng/ml; range; median; SEM) were measured: controls (n = 11): 0–0.420; 0; 0.046 // tumours (n = 19): 0–2.823; 0; 0.209 // tumours < 4.5 cm3 (n = 8): 0–2.823; 1.178; 0.353 // tumours > 4.5 cm3 (n = 11): 0–0.180; 0; 0.016.

Overview: In this rat rhabdomyosarcoma tumour model, free circulating VEGF levels could only be detected in small tumours < 4.5 cm3, not in larger tumours or in control rats. Apparently, in this pilot study, an excess of VEGF is measurable in the plasma until a certain tumour volume is established. This in vivo observation emphasises the role of VEGF as a major inducer of angiogenesis and can be of potential importance for the preclinical studies investigating angiogenesis inhibitors.

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Plasma D-dimer levels, serum VEGF, b-FGF and IL-6 in metastatic breast cancer (MBC): Correlation with tumour load and response to therapy

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Purpose: Plasma levels of D-dimer reflect both coagulation and fibrinolysis. This activation might be associated with progression and angiogenesis. Plasma indices of activated coagulation, serum levels of angiogenic cytokines were determined in a group of patients with MBC.

Methods: Plasma D-dimer levels, routine coagulation tests, serum levels of VEGF, IL-6 and b-FGF were measured and analyzed for any relationship with a series of clinicopathological parameters in two groups Group A consist of 30 patients considered to be free of disease after locoregional treatment for BC, group B of 100 patients with progressive MBC.

Results: In group A D-dimer levels were elevated in 4/30 with a median level of 330. In group B plasma D-dimer levels were elevated in 94/100 pts ($p = 0.001$) with a median level of 1820. D-dimer levels in group B did not correlate with age, tumour type, disease-free interval, ER or PR status, number of sites of disease, fibrinogen level, aPTT or PTT. In group B serum VEGF was increased in 43%, and b-FGF levels were increased in 40%. A positive correlation between platelet count and sVEGF ($r = 0.44$; $p < 0.005$), and fibrinogen level and s IL-6 ($r = 0.78$; $p < 0.0001$) was shown.

Conclusion: Plasma D-dimer levels are nearly always elevated in patients with MBC. Both progression kinetics and volume of disease seem to determine the level of D-dimers. Further analysis of the angiokine levels and D-dimers and progression kinetics will be presented.

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In vitro differentiation of endothelial cells (EC) from AC133-positive progenitor cells

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The formation of new blood vessels can be due to two different processes. The first is vasculogenesis, which implies the primary differentiation of endothelial progenitor cells from haemangioblasts, and their subsequent organization into a primary capillary plexus. The second is angiogenesis, which is the formation of new vessels by a process of sprouting from pre-existing vessels. It is currently assumed that vasculogenesis is limited to early embryogenesis, while angiogenesis occurs both during development and post-natal life. However, the possibility that endothelial stem cells or haemangioblasts persist into adult life where they may differentiate and contribute to the formation of new blood vessels, e.g. in malignant tumours, through circulating EC, remains to be explored. In this study we investigated the ability of various growth factor combinations to generate EC. Human AC133-positive (+) cells that represent a subset of CD34+ stem and progenitor cells with haematopoietic potential were isolated from