irradiance 100 mW/cm2, wavelength=652 nm). Response was determined after 12 weeks. Follow-up for 2-;5 years is on-going.

Results: 85% of patients achieved CR (95% CI: 76-90%) through application of PDT alone, and a further 6% of patients who initially had a partial response achieved a CR with other adjunctive therapy, giving a total CR rate for PDT alone and PDT with adjunctive therapy of 91%. The majority of CRs (59%) were biopsy-confirmed (26 patients with clinical CR did not undergo biopsy). Mean duration of response was 621 days; one and two-year CR rates were 89% and 86% respectively, survival rates were 90% and 81% respectively. Censored mean patient survival time was at least 650 days (follow-up continues). The most common adverse event was local pain at treatment site. Mild to moderate photosensitivity reactions occurred in 13% of patients. Twelve patients died during the first year (none considered related to Foscan PDT). There were 23 non-fatal serious adverse events, of which 5 were considered related to treatment: two burns, one photosensitivity reaction, one excessive tissue necrosis, one increase in pain and dysphagia.

Conclusion: Foscan PDT is an effective treatment for small primary tumours of the oral cavity, yielding CR rates comparable to those published for surgery or radiotherapy. It is without major toxicity, preserves form and function and does not compromise future treatment options for recurrent, residual or second primary disease.

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"High" (60 mCi) vs. "low" (30 mCi) activities of 131I as adjuvant treatment for papillary thyroid cancer: the results of a prospective randomized trial

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Purpose: To compare the effectiveness of 30 mCi vs. 60 mCi for ablation of thyroid remnants in papillary thyroid cancer (PTC) treated with total thyroidectomy.

Matherial/Methods: Since 1998 188 patients with PTC in a clinical stage T1-T3N0M0 have been randomly assigned to receive "high" or "low" ablative dose of 131I: 95 (50,5%) received 60 mCi, and 93 (49,5%) received 30 mCi. Post-therapeutic whole body scintygraphy (WBS), the uptake over the neck (UON), and serum thyreoglobin level (Tg) was obtained 6-12 months after ablation. Other routine imagining procedures included ultrasonography of the neck, and chest X-ray. Ablation has been considered as: very good if there was no uptake in WBS, and UON <0.09%, and Tg ≤ 4 ng/ml; good - if there was only scattered uptake over the neck on WBS, and UON =0.1-0,39%, or Tg =4.1-10 ng/ml.; doubtful - if there was residual uptake over the neck, and UON =0.4-0,9%, or Tg =10.1-30 ng/ml.; insufficient - if there was a distinct uptake over the neck, or UON => 1%, or Tg <100 ng/ml.; local recurrence or dissemination of disease - if WBS and/or other imagining procedures revealed a recurrent tumor or metastases, or Tg ≥100 ng/ml.

Results: In a group of patients who received 60 mCi ablation was considered very good in 82 (44%) patients, good in 12 (6%), there were no doubtful or insufficient results, and one (0.5%) early nodal recurrence. In a 30 mCi group ablation was considered very good in 62 (33%) patients, good in 21 (11%), doubtful in 9 (5%), insufficient in one (0.5%), and there were no local recurrences or dissemination. This difference is highly significant (p< 0,005) in favour of 60 mCi, as shown by non-parametrical distribution tests.

Conclusion: The results show a significantly higher effectiveness of 60 mCi for ablation of thyroid remnants in PTC treated with total thyroidectomy. Although long-term effectiveness of adjuvant radioiodine therapy has not been assessed in this study it can be postulated that "low"activities of 131I should not be routinely used, particularly for patients with high risk of locoregional relapse and/or distant dissemination.

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Locally advanced laryngeal cancer: surgery and radiotherapy vs. radiotherapy alone. A multivariate locoregional control analysis in 2220 patients

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Purpose: To compare locoregional tumors control rate (TCP) of laryngeal cancer after radiotherapy alone (RTA) or after postoperative radiotherapy (PRT).

Material/methods: For the purpose of the analysis the data on RTA (1493 patients) and PRT (727 patients) were combined. A logistic analysis of the dose-response relationship, and multivariate Cox proportional hazard regression model of recurrence-free survival has been used. The presence of surgery has been considered as one of the variables included in the analysis.

Results: The presence of surgery, T, N stage, Hb concentration at the end of radiation course, total radiation dose and overall radiation treatment time (OTT) have significantly influenced the recurrence-free survival. It is predicted from the logistic model, that for a tumor in a clinical stage T3N0 to achieve the same cure rate as PRT, RTA would require an increase in total dose of as much as 32 Gy given in 2 Gy fractions (TCD50% 36 Gy vs. 68 Gy). It is, however, predicted that an increment in radiation dose of only 12 Gy, with simultaneous shortening of OTT by 14 days and increase in Hb of 1.5 g% may, in the same case, provide an isoeffect of dose escalation alone.

Conclusion: A large difference between TCD50% for PRT and RTA may indicate that surgery and PRT will so-far remain a treatment of choice for a considerable proportion of patients with advanced head and neck tumors. The acceptable cure rates in organ-sparing therapy may likely be achieved by enhancing dose intensity of local treatment with concurrent increase (or prevention of decrease) of Hb concentration during radiotherapy.

Cell biology/Genetics I

132 ORAL

Growth patterns of pulmonary metastases of renal cell carcinoma and colorectal adenocarcinoma are distinct in angiogenesis

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Objective: Metastatic disease needs angiogenesis to grow, but other vascularisation patterns which are not based on endothelial cell proliferation are described. The hypothesis of this study was that lung metastases (LM), present in a highly vascularised organ with a reduced hypoxic drive for angiogenesis, can demonstrate distinct growth patterns.

Methods: Tissue sections of 59 LM were analysed (13 pts with renal cell carcinoma (RCC) and 6 with colorectal carcinoma (CA). A hematoxylin-eosin and reticulin stain of all LM was done to evaluate growth patterns. Immunohistochemical staining with CD34 antibodies was done to count new formed blood vessels. Microvessel density across the LM was assessed.

Results: 2 growth patterns were found. A nodular growth pattern (NG) did not respect the lung parenchyma architecture, in contrast with the alveolar growth pattern (AG) in which tumor cells filled the alveoli. In each patient, all LM expressed the same growth pattern. The AG was present in 38% and 100% of the patients with RCC and CA respectively (p=0.07). The ratio of the microvessel density of the marginal zone over the central zone was at least 2 in 56% of the LM with an AG compared to 9% with a NG (p=0.02). The number of CD34-positive single endothelial cells was higher in NG than AG, indicating more angiogenesis in NG.

Conclusion: By co-opting existing alveolar wall capillaries, the alveolar growth pattern of pulmonary carcinoma metastases is less angiogenesis-dependent than the nodular growth pattern. Clinical implications are important since several angiogenesis-inhibitors are tested as anticancer agents, and lung metastases with a low angiogenesis intensity will probably not benefit from such treatment.

133 ORAL

Effects of adriamycin and interferon, applied in metronomic doses, on tumor volume, metastases and vessel density in murine renal cell carcinoma

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Introduction: Chemotherapy is effecting tumor cells as well as endothelial cells. The application of low dose continuous (metronomic) chemotherapy may optimize the effect on the tumor endothelial cells, acting like an unspecific antiangiogenic regimen. Therefore, we evaluated clinically relevant

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.

134 ORAL

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

135 ORAL

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.