dose distribution, dose statistics, and dose volume histogram (DVH) of PTVs were used to evaluate differences between respiratory gated conventional 2-D plans and respiratory non gated 3-D conformal treatment plans. In addition, the risk of radiation exposure of surrounding normal liver and organs are evaluated by means of DVH and normal tissue complication probabilities (NTCPs).

Results: The vertical movement of liver ranged 2–3 cm in all patients. We found no difference between respiratory gated 2-D plans and 3-D conformal treatment plans with the patients breathing freely. Treatment planning using DVH analysis of PTV and the normal liver was used for all patients. DVH and calculated NTCP showed no difference in respiratory gated 2-D plans and respiratory non gated 3-D conformal treatment plans.

Conclusion: Respiratory gated radiation therapy was very important in hepatic tumors because radiation induced hepatitis was dependent on remaining normal liver volume. Further investigational studies for respiratory gated radiation treatment combined with 3-D conformal treatment are required.

352 PUBLICATION

Measurement of patient entry and exit doses during total body irradiation

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Purpose: There is no standard algorithm to calculate the dose distribution for TBI under translation conditions with commercial treatment planning systems. Therefore it is necessary to observe the applied dose which has to be calculated by hand for several points with in vivo measurements.

Methods: The doses at relevant points of the body are calculated using the beam-zone method and data from systematical phantom measurements. To verify these calculations we use a set of at least 6 up to 12 semiconductor probes which are attached to the patients skin to measure the entrance and exit doses. The dose at body-center can be calculated from these values. The results are corrected for varying patient geometries.

Results: Dose measurements for actual 35 patients show a correspondence between calculated and applied doses in the range of ±10% for the center of the lung and in the range of ±5% for a reference point in the middle of the body.

Conclusion: In the clinical routine the use of semiconductor probes provides an easy and fast way to measure point doses during TBI. This method allows an online verification of calculated doses.

353 PUBLICATION

Intraoperative high dose rate interstitial irradiation of hepatic metastases

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Resection of hepatic metastases offers long-term survival (25–30% 5-year), and possibly cure for selected patients. Thirty per cent of patients undergoing laparotomy for possible resection have unresectable metastatic disease confined only to the liver. Irradiation might provide benefit in median survival if a tumoricidal dose could be administered to the hepatic metastases.

Eligibility criteria included only liver disease with the primary tumor controlled and no extrahepatic involment, no cirrotic liver, acceptable surgical risk, patient life expectancy greater than 3 months and a total volume of potentially unirradiated liver of 30–40% (defined by 3D reconstruction with MRI). All the patients must have been treated at least with a first line of chemotherapy. From July 1998 to February 1999, 7 procedures have been done in our Unit. Patients underwent laparotomy in the operative suite of the Radiotherapy Unit, adjacent to the radiotherapy bunker where the afterloaded high intensity (10 Ci) iridium 192 source is kept. Careful abdominal exploration define both, resectability or implant of hepatic metastases. Liver was mobilized by disecction of the falciform ligament. Patients with resectable lesions underwent lobectomy or wedge resection. Unresectable disease and the scar of resectable metastases are encompassed within a volume implant or implants. Intraoperative ultrasound is employed to confirm diagnosis and for guiding the insertion of the implant covering the area wanted to be irradiated. The dosimetry is done in real time with optimization of the dose to the required volume. After irradiation, the patient is carried back to the operating room, the needles are removed, hemostasis is achieved and abdominal closure is completed. There are not acute or chronic complication referable to irradiation. Hospitalizations were from 8 to 15 days (median 10 days). We will present this early experience and the technical aspects of the Intraoperative radiation therapy technique

354 PUBLICATION

The impact a multileaf collimator (MLC) may have on a departments confidence and quality assurance

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Purpose: To investigate the effect of introducing an MLC into a radiotherapy department. Is confidence affected by the possible 80 variables involved in making a conformal field, compared to none with customised blocks. Can confidence be restored?

Methods: A strict efficient method of Quality Assurance (QA) was employed involving all types and levels of operational staff. Strict protocols and test tools that graphically represented the correct function of the device were used. Methods for physically setting up the MLC during a service or repair that minimised downtime and Physics acceptance were employed.

Results: The MLC specification once proved during acceptance was the base for designing QA tools. Experience using the MLC quickly showed where time was lost and which items or problems most frequently occurred.

Conclusions: The final regimes allow a speedy daily QA and if a problem is detected, is followed by various levels of protocols, and tools, all of which may be carried out efficiently by any trained member of staff. An MLC expert is not required.

355 PUBLICATION

Concurrent radiotherapy and chemotherapy with topotecan and stealth liposomal doxorubicin for the treatment of locally advanced pelvic tumours

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Purpose: Topoisomerases may have an important role in the repair of DNA damages induced by radiotherapy. The feasibility of radiotherapy combination with a topo-I inhibitor (topotecan, Hycamtin®) and a topo-II inhibitor (stealth liposomal formulation of doxorubicin, Caelyx®) was assessed in a pilot study.

Methods: Twelve patients (pts) with locally advanced pelvic tumours were recruited (3 sarcomas, 4 bladder carcinomas, 2 adenocarcinomas of unknown origin and 3 pelvic masses metastatic from cholangiocarcinoma or pancreatic carcinoma). Caelyx was given once every 2 weeks (d1) at a dose of 20 mg/m^2 and, Hycamtin was given 3 times every 2 weeks (d4, 8, 11) at a dose of 1 mg/m^2. Four cycles were given during the course of radiotherapy (64–70 Gy). The regimen was supported with G-CSF 480 μg sc. every Saturday and Sunday. Six received prophylactic use of HuEPO (20.000IU every Saturday and Sunday) and 6 did not.

Results: Complete symptomatic relief was obtained in all pts. Complete response was noted in 6/12 (50%) cases. No severe "in-field" radiation toxicity was observed. None of the pts developed neutropenia. Hemoglobin (Hb) levels were substantially reduced in 6 patients that did not receive HuEPO (mean Hb decrease 1.8 g/dL), while no Hb reduction was noted in pts supported with HuEPO.

Conclusion: Combination of radiotherapy with topotecan and stealth liposomal doxorubicin is feasible, well tolerated and a promising approach for locally advanced inoperable pelvic tumours.

356 PUBLICATION

Subcutaneous administration of Amifostine in patients treated with radiotherapy. A preliminary report

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Purpose: To demonstrate the efficacy of Amifostine (AF) in patients treated with External Beam Radiotherapy (EBRT) plus chemotherapy.

Material and Methods: From January to August 1998 we enrolled in our study 6 patients affected by non small cell lung cancer (NSCLC) at stage IIIb; 6 patients affected by rinoipo-oropharyngeal cancer; 4 affected by cervix cancer. EBRT was delivered with a 6–23 Mv linear accelerator. In all patient treatment doses were >50 Gy. AF was delivered subcutaneously 10 minutes before EBRT with the dosage of 500 mg.

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.

Cancer cell biology & angiogenesis

357 ORAL

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.

358 ORAL

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.

359 ORAL

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.

360 ORAL

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