cycles). In order to estimate the entity of dose reduction, we compared for every patient INRT dose distribution profiles in different organs with dose profiles of classic involved fields, as if patients were treated outside the trial, and with mantle field as "historical" example of extended fields approach. All patients had mediastinal involvement in at least one nodal station at diagnosis, 4/5 patients had supraclavicular involvement and 3 middle-upper neck involvement. No axillary localizations were present. INRT fields were contoured following EORTC guidelines specifically designed for H10 trial (Girinsky et al, 2007–2008). A classic AP-PA parallel opposing fields technique with personalized shaped blocks was employed. Organs at risk were: breast (bilateral, as a whole organ), lung (bilateral, as a whole organ), thyroid gland, coronary arteries (origin). Breasts were considered at risk only in female patients (4/5). Mean dose and dose received by 50% of the volume (D50) were selected as parameters for comparison for every organ and calculated separately, then average values were taken into account for final comparison.
Results: Average dose reductions (expressed in percentage for mean dose and for D50) for breast, lung, thyroid gland and coronary arteries are shown in table I.

Table I

	Dose reduction	
	INRT vs. IFRT	INRT vs. MF
Breast:		
Mean dose	57.1%	57.3%
D50	44.1%	73.5%
Lung:		
Mean dose	38.8%	58.2%
D50	65.5%	88%
Thyroid gland:		
Mean dose	25.6%	41.5%
D50	9.1%	30%
Coronary artery:		
Mean dose	2.2%	22.2%
D50	1%	21%

Conclusions: As preliminary findings, our data suggest that for breast and lung a clear advantage in terms of global dose reduction is evident with INRT if compared with IFRT (and intuitively greater if compared with a traditional mantle field approach). The potential benefit for thyroid gland and coronary arteries sparing is not so evident, and has to be evaluated prospectively in a larger series. In order to spare these central structures, probably a different technical approach including IMRT and various IGRT options for thoracic radiotherapy is needed.

2026 POSTER

Response of melanocytes to low doses of fractionated radiotherapy

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Background: Low doses of ionizing radiation will inhibit cell division of the basal kreatinocytes of human epidermis, and reduce the ability to maintain the normal amount of cells in the germinal cell layer. Previously a hypersensitivity to doses below 0.3 Gy was determined by our group for DNA double-strand breaks, growth arrest and apoptosis throughout a treatment course of 7 weeks. The aim of this study is to determine the melanocyte response by molecular markers to daily low doses of radiation and to establish whether hyper-radiosensitivity also occurs for this cell type.
Material and Methods: Skin punch biopsies from 33 patients treated for prostate cancer with radiotherapy were used. Sampling of biopsies for each patient was performed before treatment and after 1 or 6.5 weeks into the radiotherapy course. The daily doses per fraction were about 0.1, 0.2, 0.45 and 1.1 Gy at the different areas of the exposed skin where biopsies were taken. The number of melanocytes per mm of the basal membrane was determined using immunohistochemical staining with eosin-PAS, ΔNP63-negative, MITF and Bcl-2. Three paraffined sections from each biopsy were assessed for every marker. The dose-response relationships were determined from the mean values of each staining versus fraction size.
Results: Both after 1 week and 6.5 weeks of radiotherapy an increase in the numbers of eosin-PAS, MITF and Bcl-2 stained melanocytes were observed. Small fraction doses of 0.04 Gy had a trigger effect, causing the melanocytes to reveal a more distinct morphology in eosin-PAS and express higher levels of MITF and Bcl-2. The number of ΔNP63-negative cells was constant, and independent of fraction size.
Conclusions: Melanocytes are radioresistant to low doses of radiotherapy over 7 weeks. Several molecular markers indicate an induced radioresistance. An effective DNA damage response of melanocytes preserves their cell number intact.

2027 POSTER

4D FDG-PET/CT combined with diffusion weighted MRI for planning of stereotactic radiation therapy of liver metastasis

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Background: Stereotactic radiotherapy is a highly effective method for treatment of liver metastasis in not operable patients. Although MRI and CT are standard imaging modalities for therapy planning, it is frequently difficult to define an exact target volume based on these methods. PET and diffusion weighted MRI (DWMRI) may help to improve the accuracy of target volumes. Aim of this study was to investigate feasibility and value of coregistration of respiratory gated PET-CT in treatment position and DWMRI.
Methods: 11 patients assigned to stereotactic body radiation therapy of liver metastases were examined by standard planning contrast enhanced CT and MRI scans, 4D FDG-PET/CT using respiratory gated PET and CT in treatment position and by diffusion weighted MR sequences. DWMRI data was acquired in breath hold (end-expiration). Immobilization for image acquisition (CT, PET-CT) and treatment was carried out in a vacuum couch with a low-pressure foil as used for regular SBRT. The different imaging studies were fused on a BrainLab workstation (iPlan net). The quality of the fusion was rated on a scale from 1 (very good) to 5 (bad). Gross tumor volumes were defined using conventional morphological imaging (CT, T1 and T2 weighted MRI) (Vcon), visual PET information (Vpet) and all modalities combined (Vcom). A composite volume from all different GTV was then created.
Results: 15 lesions were identified, in 2 patients the diagnosis of liver metastases was not confirmed in FDG-PET. Optimal fusion could only be achieved in 20% of the patients. The tumor volumes differed significantly when contoured in contrast-enhanced CT or MR compared to FDG-PET. The mean Vcon was 40 ccm while the mean Vpet and Vcomb were 59 and 89 ccm respectively. Difference of the volumes were up to a factor of 3.5 between Vpet and V con (mean 0.9) and up to a factor of 4.8 between Vcon and V comb (mean 1.8).
Conclusions: Coregistration of imaging modalities due to anatomic colocalisation was most feasible when planning CT and PET-CT in treatment position was used. MRI acquired in end-expiration was difficult to fuse with the other imaging modalities. Using visual information of FDG-PET for GTV-delineation the GTV was significantly enlarged. Prior to decide about target volume adaption (expansion) due to additional information provided by functional MRI or PET quantitative analysis should be performed.

2028 POSTER

Second Cancer after Total Body Irradiation: a retrospective analysis of 773 patients

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Background: To retrospectively evaluate second cancer in a group of children and adults treated with TBI. The database includes late toxicities; this analysis focused on second cancers.
Materials/Methods: Between October 1984 and June 2002, 773 patients received TBI in their conditioning regimens prior to autologous or allogeneic stem cell transplantation (SCT). TBI was performed at the Léon Bérard Cancer Center -France. The median follow-up from TBI was 4 years (range 0–27.4 yrs). The study registers 347 deaths (45%). Among 773 patients, 259 survived longer than 4 years with regular follow-up. Median age of patients at the time of the TBI was 32.4 years (range 0.3−95). Median TBI dose was 1090 cGy (8−12 Gy)/3 fraction (fx)/3 days with 6 MV linear accelerator.
Results: A total of 39 second cancers were recorded with a 2.9 years median time-to-TBI (range 0.5−17.2 yrs). Thirty-three second cancers occurred among the 259 more than 4 years survivors. Second cancer distribution listed 5 haematological malignancies, 3 cutaneous malignancies, 2 brain cancers, 6 gastro-intestinal cancers, 3 head-and-neck

cancer, 2 prostatic cancers, 5 breast cancers, 2 parotids cancers and 7 thyroid cancers. Thyroid and parotids cancer occurred mostly in children (respectively 2/2 and 6/7).
Conclusions: This retrospective study confirms the need for long-term follow-up in patients undergoing TBI, principally in children. Nevertheless, young age seems to be a risk factor after TBI regimen irradiation, in particular for parotid and thyroid second cancers).

2029 POSTER

Noninvasive detection of tumour's oxygen status using diffuse optical tomography

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Hypoxia is a key factor of tumor progression and resistance to therapy due to its effects on various metabolic processes. Growing comprehension of its importance in cancer progression and therapy, gave an essential impetus to develop imaging methods to detect and assess tumor oxygen status. Diffuse optical tomography (DOT) is an imaging modality with potential to provide information related to tissue oxygenation. The study objective was to test this approach for tumor's hypoxia identifying and to check its validity by immunohistochemical analysis.
Experiments were performed using white outbreed male rats. Rat's breast cancer-1 and Pliss's lymph sarcoma (6 animals for every tumor model) were transplanted subcutaneously into the right hind-leg of the rats. The tumor sizes at the start of monitoring were about 12 mm. DOT was carried out on the experimental setup with three laser fibers coupled in a single bundle. They scan the studied volume at 684 nm, 794 nm, and 850 nm and provided information about oxygenated hemoglobin and deoxygenated hemoglobin concentrations. Distribution of HbO_2, HHb, total Hb and oxygen saturation was reconstructed numerically. Immunohistochemical analysis with Hypoxyprobe™-1 kit was carried out under Natural Pharmacia International recommendations. Histological material was collected from the centre and periphery of the tumors. The cross-sections were scanned for the FITC (green) fluorescence signal using LSM 510 META. The determined by DOT distribution of the hypoxic areas within the tumor was compared with allocation of pimonidazole positive zones.
Pliss's lymph sarcoma is characterized by rapid growth and early occurrence of necrotic areas. DOT images of this tumor demonstrated the increased concentration of deoxygenated hemoglobin in the centre and the increased concentration of oxygenated hemoglobin at the periphery. The substantial decrease of oxygen saturation was observed in the centre of Plisse's lymph sarcoma as compared with periphery. On the contrary, DOT images of rat's breast cancer-1, which is noted for the rather slow growth and satisfactory oxygenation, showed relatively uniform and high oxygen saturation of the tumor tissue. Immunohistochemial analysis confirmed the distribution of hypoxic and oxygenated areas in both tumor models.
Diffuse optical tomography represents a useful tool for detection and monitoring of oxygen status of the tumors. Its validity was confirmed by the distribution of pimonidazole-positive zones on two different tumor models.

2030 POSTER

Experimental study of a new enzyme-targeting radiosensitizer containing hydrogen peroxide & sodium hyaluronate for intra-tumoral injection using mice transplanted with SCCVII tumor

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Background: In radiation therapy (RT), it is known that low oxygen environment decreases effect of RT. We have developed a new enzyme-targeted radiosensitization treatment named KORTUC I using a hydrogen peroxide (H_2O_2) solution (Oxydol) for superficially exposed & unresectable neoplasms (Ogawa Y et al. Oncol Rep 19: 1389–1394, 2008), based on our experimental results demonstrating H_2O_2 as a strong radiosensitizer (Ogawa Y et al. Int J Mol Med 12: 453–458, 845–850, 2003, 14: 845–850, 2004). When H_2O_2 is injected into tumor tissue, anti-oxidative enzymes such as peroxidase/catalase is inactivated. Moreover, oxygen is generated from degradation of H_2O_2 by peroxidase/catalase, and hypoxic tumors can be reoxygenated. In our previous study, it was concluded that the most suitable combination of drugs for preserving high intra-tumoral oxygen concentration is sodium hyaluronate & H_2O_2 (Tokuhiro S et al. Radiother Oncol 90: S84, 2009). In this study, the effect of radiosensitization treatment using H_2O_2 & sodium hyaluronate was studied on the transplanted SCCVII tumor of female C3H/He mice following RT.
Materials and Methods: For the experiment, PBS alone (control), PBS containing 0.5 w/v% H_2O_2 (PBS-H_2O_2), and 0.8 w/v% sodium hyaluronate containing 0.5 w/v% H_2O_2 (hyaluronate-H_2O_2), were prepared just prior to the injection, respectively. First of all, approximately 10^5 cells of SCCVII tumor were inoculated into right hind thigh of each of C3H/He mice. And when each tumor grew up to approximately 10 mm in diameter, RT of 30 Gy of 6 MeV electron beam following injection of 0.25 ml of the each combination of drugs mentioned above were performed. For irradiation, mice were anesthetized, and were fixed on an apparatus specially developed for local irradiation of mice (Ogawa Y et al. Int J Raidat Oncol Biol Phys 9: 533–537, 1983).
Results & Conclusions: The growth of the tumors in the group that had been given RT combined with intratumoral injection of hyaluronate-H_2O_2 was remarkably inhibited, and there was a statistically significant difference between the group and another groups receiving RT alone or RT combined with intratumoral injection of PBS-H_2O_2. Moreover, in the group receiving RT combined with intratumoral injection of hyaluronate-H_2O_2, the tumors in three mice disappeared macroscopically. In this experimental study, it was concluded that radiosensitizing effect was achieved by adding sodium hyaluronate to H_2O_2 as has already been shown clinically in our recent paper (Ogawa Y et al. Int J Oncol 34: 609–618, 2009, Radiother Oncol 90: S73, 2009).

2031 POSTER

Potential biomarkers of a complete response and local control for definitive chemoradiotherapy in resectable esophageal squamous cell carcinoma

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Background: Definitive chemoradiotherapy (CRT) has curative potential for patients with esophageal squamous cell carcinoma (ESCC), especially at a resectable stage. However, there is considerable locally persistent/recurrent disease after definitive CRT and salvage esophagectomy may increase morbidity and mortality. The ability to predict CRT outcomes in individual patients would greatly aid therapeutic planning. This study sought to identify molecular markers that predict the response to CRT for resectable ESCC.
Materials and Methods: Tumor biopsy specimens were taken from 38 patients with ESCC who had received definitive CRT between October 2001 and January 2005. All tumors were considered resectable, but the patients chose CRT as the initial treatment and were recruited in a phase II clinical trial at our hospital that consisted of two cycles of cisplatin and fluorouracil with split-course concurrent radiotherapy of 60 Gy in 30 fractions. The patient characteristics were as follows: median age 63.5 (range 45–79) years, 35 males and 5 females, and Stage I, IIA, IIB, and III in 8, 7, 8, and 15, respectively. The expression of cyclin D1, cyclin A, p21, vascular endothelial growth factor (VEGF), epidermal growth factor receptor (EGFR), urokinase-type plasminogen activator (uPA), and plasminogen activator inhibitor type-1 (PAI-1), the microvascular density (CD34), and cell proliferation activity (Ki-67 labeling index) were evaluated immunohistochemically, and the complete response and local control rates of the patients were analyzed.
Results: At a median follow-up of 47.8 months, the 3- and 5-year overall survival was 65.8 and 63.2%, respectively. Eleven patients underwent salvage esophagectomy. A complete clinical response at the conclusion of CRT was achieved in 29 patients (76.3%) and local control in 17 (44.7%). Univariate analysis showed that the Ki-67 labeling index ($p = 0.038$) was significantly higher in the patients who achieved a CR, and that over-expression of uPA ($p = 0.030$) and VEGF ($p = 0.114$) were unfavorable prognostic factors for local control. Logistic regression analysis showed that uPA and VEGF were independent predictors of local control.
Conclusions: We found that the Ki-67 labeling index and the expression of uPA and VEGF were potential predictive biomarkers of the CRT outcome in ESCC, and we are now investigating these factors in another population as part of the same clinical trial to confirm the results.