Conclusions: The subtraction method described here validates the x-ray markers as an accurate surrogate for the HDR brachytherapy source position. These markers were then appropriate references for validation of the algorithm used to determine source position in a two dimensional plane parallel to the imaging panel, as derived using an EPID based delivery validation system described elsewhere at this meeting. EPID measurements of source position were confirmed to be accurate within ± 1 mm at source-detector distances up to 200 mm.

POSTER DISCUSSION: 5: PREVENT: EFFECTS OF RADIOTherAPY ON NORMAL TISSUE

PD-0184
Protective effect of leuprorelin on radiation-induced intestinal toxicity
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Purpose/Objective: Prostate cancer patients treated with neoadjuvant androgen ablation experience less radiation-induced intestinal toxicity. This can be due to the size reduction of bulky prostatic tumours and optimization of the target volume. The aim of this study was to evaluate if leuprorelin exerts itself a protective effect on irradiated bowel.

Materials and Methods: C57BL/6J mice underwent 12Gy total body irradiation (TBI), with or without a monthly i.p. injection of leuprorelin (enantone®, 0.054 mg/Kg), started three months before TBI. Mice were sacrificed 24 or 72h after TBI and small bowel (jejunum, ileum) segments were collected for histological and molecular analysis. Microcolony survival assay was also performed, to evaluate radiation-induced damage on crypts and the potential protective effect of leuprorelin. Radioprotection was correlated with crypt regeneration; crypts containing at least 10 viable cells were considered regenerating crypts.

Results: At hematoxylin eosi staining, untreated controls showed normal jejunal wall features, with long villi and well developed tubular glands. Irradiation caused a time-dependent reduction of the tubular glands and occurrence of a marked inflammatory infiltrate. Uroplakin pre-treatment appeared to blunt the radiation-induced changes: the columnar epithelium mostly showed normal features, with continuous striated border and intercalated goblet cells; the inflammatory infiltrate in the mucosal/submucosal stroma was markedly reduced in comparison with the irradiated mice.

Real-Time RT-PCR analysis showed that leuprorelin significantly decreased radiation-induced collagen type I and type III, TGFbeta, Mmp2, Mmp13 and p53 gene expression in the jejunum of treated mice.

Leuprorelin pre-treatment was also able to inhibit TGFbeta and p-NFkB protein expression induced by irradiation in the jejunum, as revealed by western blot analysis.

Pre-irradiation administration of leuprorelin significantly increased the number of regenerating crypts in the jejunum, as compared to mice undergone irradiation alone.

Conclusions: Pretreatment with leuprorelin exerts a protective effect against radiation-induced intestinal injury in mice. These findings confirm clinical observations in irradiated prostate cancer patients and suggest that prostate size reduction is not the only mechanism at the basis of better intestinal tolerance after neoadjuvant androgen ablation. More investigations are needed to establish a possible way of action for the reported effect of leuprorelin on irradiated intestinal mucosa.

PD-0185
Effect of high dose rate and flattening filter free beams on DNA repair deficient, normal and cancer cell survival
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Purpose/Objective: The introduction of flattening filter free (FFF) photon beams to deliver higher instantaneous dose rates and shortened overall treatment times, re-introduced controversy about the biological consequences of these high dose rates. We hypothesize that high dose rate irradiation will decrease cell survival in normal and cancer cell lines and even more in cancer cells deficient for homologous recombination. Additionally, we hypothesize that removal of the flattening filter will result in an enhanced relative biological effectiveness (RBE), independently of the dose rate.

Materials and Methods: Human breast adenocarcinoma (MCF7), colorectal adenocarcinoma (HCT116 and DLD1), glioblastoma (U87 and U373) and non small cell lung adenocarcinoma (A549) cells were irradiated in the range 0 - 10 Gy. Additionally, DNA repair deficient HCT116 (DNA-PKcs-/-, NHEJ deficient) or DLD1 (BRCA2-/-, HR deficient) and normal breast (MCF10A) and lung bronchial (NL20) epithelial cell lines were exposed to similar dose schedules. To investigate the effect of high dose rate irradiation, clonogenic survival was assessed after exposing cells to 4 or 24 Gy/min with or without flattening filter. RBE estimations were performed comparing the different photon spectra of FF and FFF beams.

Results: Cell survival in tumor or normal tissue cell lines was not influenced by high dose rate irradiation. The survival curves for A549 non small cell lung cancer cells are shown as an example. Although DNA repair deficient cell lines showed an increased radiosensitivity, high dose rate irradiation did not influence survival. Furthermore, RBE was not significantly different from unity in any of the cell lines between FFF and conventional flattened beams.

Conclusions: High dose rate irradiation did not affect long term cell survival and DNA repair for human cancer and normal cell lines of different origin. These results suggest that high dose rate does not...
influence treatment outcome or treatment toxicity. Furthermore, no enhanced RBE was found for FFF compared to FF photon beams.

**PD-0185**

**Carotid intima-media thickness as a marker of radiation-induced atherosclerosis**

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**Purpose/Objective:** Radiotherapy to collateral structures such as the carotid artery leads to atherosclerosis and increased stroke risk. Arterial thickening is a precursor to atherosclerosis. Carotid intima-media thickness (CIMT), a measure of arterial thickening, is a validated surrogate for prediction of cardiovascular events. Its application in irradiated arteries as a measure of accelerated atherosclerosis has shown variable results. This study investigates CIMT as an early marker of radiation-induced damage in head and neck cancer patients.

**Materials and Methods:** Patients with head and neck cancer treated with a wedged-pair and matched neck technique or hemi-neck radiotherapy (unirradiated (unirr) side as control) at least 2 years previously were included. Patients had been prescribed a dose of at least 50 Gy to the neck. CIMT was measured on B-mode ultrasound using semi-automated detection software. Measurements were taken from the far wall in 4 arterial segments: proximal- (prox), mid-, distal (dist) common carotid artery (CCA), and bifurcation and were compared to corresponding segments in the unirradiated artery. CIMT measurements >75th percentile of a reference normal population were considered abnormal and at increased cardiovascular risk.

**Results:** 24 patients (16 males) with a median age of 58 yrs (interquartile range (IQR) 49.2 - 64.2) were included. The mean maximum dose to the irradiated (irr) artery was 61.2 Gy (IQR 52.6 - 61.8) and 1.1 Gy (IQR 1.0 - 1.8 Gy) to the unirradiated artery. Mean CIMT was significantly greater in irr carotid arteries compared to unirr arteries: mid-CCA (0.75mm ± 0.2 (irr) vs 0.64mm ± 0.12 (unirr) (P = 0.0057), distal CCA (0.79mm ± 0.24 (irr) vs 0.64mm ± 0.14 (unirr) (P = 0.005), and bifurcation (0.85mm ± 0.28 (irr) vs 0.77mm ± 0.17 (unirr) (P = 0.0231)). For the irr prox CCA, 23/24 (95.8%) had a CIMT >75th percentile vs 11/24 (45.8%) for unirr bifurcation.

**Conclusions:** CIMT is increased in irr carotid arteries, suggesting this may be a useful marker of radiation-induced carotid atherosclerosis. The proximal CCA appears to be less sensitive to radiation-induced damage.

**PD-0187**

**Hyperbaric oxygen therapy for late adverse events after particle radiotherapy**

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**Purpose/Objective:** This is the first report on the application and outcomes of hyperbaric oxygen therapy (HBO) for late adverse events which developed after proton or carbon-ionbeam radiation therapy.

**Materials and Methods:** Between April 2008 and May 2012, 40 patients underwent HBO for late adverse events (105 episodes of 8 events) which developed more than 5 months after particle radiotherapy. There were 24 male and 16 female patients aged 15-83 years (median: 67 years), and the performance state (PS) at the initiation of HBO was: PS0:PS1:PS2:PS3= 0:24:13:3. The primary diseases treated with particle radiotherapy were head-and-neck tumor in 23 patients, prostate cancer in 8, bone soft tissue tumor in 5, liver cancer in 4, and lung metastasis in 1. The late adverse events treated with HBO were classified into 8 events using CTC-AEver. 4.0. HBO was performed 3 times or more weekly, as a rule. The HBO chamber was compressed with 100% oxygen to 2.0 ATA inside, and the duration of treatment (pressure-keeping time) was 60 minutes. Responder of HBO was defined as a case of improvement in CTC-AE score.

**Results:** HBO was initiated 5-64 months (median: 19 months) after particle radiotherapy. The total number of HBO was 4-120 (median: 29). Table shows grading score of late adverse events before and after HBO. Response rate (number of responder/total cases) of 8 events was: hematuria, 100% (4/4); rectal bleeding, 67% (4/6); pain, 56% (18/32); central nervous necrosis, 50% (2/4); mucosal ulcer or fistula, 36% (8/22); mandibular bone necrosis, 28% (5/18); trismus, 15% (2/13); and skin ulcer, 0% (0/9). Total response rate was 40% (43/105). The average number of HBO sessions significantly higher in responder (69±48.9) than in non-responder (44±27.9) groups (p<0.001). Adverse events due to HBO were minimal. Otitis media (non-infectious) occurred in 14 patients (35%) (Grade1 in 12 (30%), Grade2 in 1 (2.5%) and Grade3 in 1 (2.5%). TIA, diarreha, bronchitis, cerebral infarction and sinusitis occurred in each one patient, however these events were transient and HBO did not be discontinued.

**Conclusions:** HBO was effective for late radiation disorders after particle radiotherapy especially in hematuria, rectal bleeding, pain, and central nervous necrosis. Total response rate was 40% (43/105). It was suggested that many times (69±48.9) applications of HBO are necessary to obtain an effect.

**PD-0188**

**Assessing the uncertainty in clinical dose-response outcomes with a bootstrap analysis**

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**Purpose/Objective:** Numerous studies investigate the normal tissue dose-response relation. However, limited numbers of patients per study and the low incidence of toxicities render the relation uncertain. The aim of this study is to apply a statistical bootstrap analysis to evaluate the uncertainty in the predicted dose-response due to sampling variability.

**Materials and Methods:** Two clinical endpoints were considered: myelopathy of the cervical spinal cord and pneumonitis. Data was taken from the recently published QUANTEC review. In order to evaluate the uncertainty in the clinical data, a Monte Carlo-based bootstrap analysis was applied. Ten thousand bootstrap replicates of the original dataset were produced by random sampling with replacement. This simulates alternative outcomes at each dose in a different sample of patients of the same size from the same population. The analysis requires only the dose, the number of patients, and the number of occurrences of the studied endpoint. The dose reported in the QUANTEC review was used: the equivalent dose given in 2-Gy fractions (EQD2) for the spinal cord, and the mean dose for the lung. Two-dose-response models, a Poisson-based model and the Lyman model, were fitted to each bootstrap replicate using maximum likelihood.

**Results:** The bootstrap analysis generates a family of curves representing the range of plausible dose-response relations. The 95% confidence intervals of the curve families for the two models overlap for doses included in the clinical study, but diverge beyond that. For higher doses, the Lyman model indicates a steeper slope than does the Poisson-based model. The bootstrap distributions of the model parameters (Do and y) indicate negative (positive) correlation. For both data sets, the likelihood of the observed data was higher for the Lyman model. This result was robust over the bootstrap analysis with higher likelihood of the Lyman model for over 90% of the bootstrap replicates, in both data sets.