pelvic (IWP), and low pelvic (LP) fields. We recorded the grade of diarrhea according to the common toxicity criteria (CTC) until 39.6 Gy. The overall and ∗Grade 2 diarrhea rates were compared among groups.

Results: The diarrhea rate was 86%, 78%, and 63% (p=0.0058) in patients with WP, IWP, and LP fields, respectively. The corresponding rate of ∗Grade 2 diarrhea was 27%, 16% and 17% (p=0.0914). The distribution of full dose volume was 18% (NVWT), 62% (SVWT), and 20% (LVWT). The diarrhea rate of WP fields was 63%, 95%, and 92% in the NVWT, SVWT, and LVWT group (p=0.0088), respectively. The corresponding rate of ∗Grade 2 diarrhea was 6%, 22%, and 46% (p=0.0154). For patients with WP irradiation, small bowel within target (S VWT+LVWT) was the factor predictive for risk of overall diarrhea in both univariate (p=0.0021) and multivariate (p=0.0016) analysis. More amount of small bowel within target was the only factor predictive for risk of ∗Grade 2 diarrhea in both univariate and multivariate (p=0.0154) analysis.

Conclusion: Whole pelvis irradiation resulted in higher incidence of overall diarrhea. Overall incidence of diarrhea is always higher while small bowel is within the whole pelvic target. There is a positive correlation of small bowel amount within whole pelvic target and incidence of ∗Grade 2 diarrhea. The scoring method may be used to evaluate risk of diarrhea before whole pelvic irradiation.

502 POSTER

Repopulation of the moderately well differentiated GL human squamous cell carcinoma growing in nude mice

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Purpose: Several clinical trials and experimental investigations showed important influence of overall treatment time on results of fractionated radiotherapy (RT). This so-called time factor has consistently been observed in human squamous cell carcinoma (hSCC) and is considered to be caused by accelerated repopulation of clonogenic tumour cells. It has been suggested that, reminiscent of the regulated proliferative response of normal squamous epithelium, SCC which have preserved characteristics of differentiation have a greater repopulation capacity during fractionated RT than undifferentiated tumours. The aim of the present study was to investigate repopulation in moderately well differntiated GL hSCC in nude mice.

Methods and Material: GL hSCC were transplanted subcutaneously into the right hindleg of nude mice. Tumours were irradiated every, every 2nd or 3rd day with 6, 12 or 18 fractions (fx) of 5.4 Gy (clamp) or 2.0 Gy (ambient), assumed OER = 2.7. Graded top-up doses were applied under clamp hypoxia, to determine the tumour control dose 50% (TCD50).

Results: With increasing number of daily 5.4 Gy fx under clamp hypoxia the top-up TCD50 values decreased significantly from 50.9 Gy [95% CI 47, 54] after single doses to 0 Gy after 18 fx. For the same number of fx the top-up TCD50 increased with increasing overall treatment time. The results are consistent with a constant repopulation rate with a clonogenic doubling time (Tclon) of 12.7 days [8.6, 16.8]. Under ambient blood flow the top-up TCD50s for daily 2 Gy fx decreased significantly but less pronounced than for 5.4 Gy under clamp hypoxia. For a given number of fx under ambient conditions the top-up TCD50s did not increase significantly with overall treatment time except for RT with 12 fx in 36 days compared to 12 and 24 days. The Tclon value from these data was 27.7 days [11.6, 43.8] Conclusion: Our data demonstrate significant capacity for repopulation of clonogenic tumour cells during fractionated RT of GL hSCC under clamp hypoxia without indication of a change of the repopulation rate during treatment. Less pronounced repopulation was observed for RT under ambient conditions, which might be explained by preferential survival of hypoxic and therefore nonproliferating cells. Taken together with our previous studies on poorly differentiated FaDu tumours the results support important heterogeneity of kinetics and mechanisms of repopulation in different experimental SCC.

503 POSTER

Quantification of a differentially expressed gene, RTP801, in irradiated HeLa cells using real time PCR

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Purpose: RTP801, a hypoxia-inducible factor-I-responsive gene, was cloned and characterized in MCF7 human epithelial breast carcinoma cells in 2002 and was strongly up-regulated with hypoxia. We tried to quantify the scarce RTP801 mRNA accurately and to compare the gene expression patterns of irradiated HeLa cells and non-irradiated controls.

Materials and Methods: Cells were harvested and total RNA was extracted 4 h after exposure to 0.1, 0.5, 1, or 2 Gy. We performed real-time PCR using CYBR green I dye with the iCYCLER IQ system from BIO-RAD.

Results: A 200-fold decrease was observed at 0.1 Gy, while the response subsequently declined at 0.5, 1, and 2 Gy, by 11, 6, and 2.5 times, respectively.

Conclusion: We observed that doses in the range 0.1-2.0 Gy reduced the amount of RTP801 mRNA at a given time. Interestingly, the lowest dose, 0.1 Gy, clearly decreased transcripts more than the higher doses. These results demonstrate that it is possible to identify and quantify differential gene expression using sparse mRNA with real time PCR. Further studies of down-regulation in *RTP801* gene expression and the implications of the strong response to low doses could be useful for elucidating the biological response of HeLa cells to radiation and developing novel therapeutic targets.

504 POSTER

Quality aspects and time gain of an automated procedure for generating an optimized plan in the routine treatment of breast cancer with external tangential beam irradiation.

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Background: We use two tangential, single isocentre, photon half beams as standard technique for breast irradation in our center. On average 275 patients are treated yearly for breast cancer using this setup. Every patient has a planning CT in treatment position of at least the treated region (average 15 cm cranio caudally) and the neighbouring tissues with a margin of 4 cm. At 1 cm interval this leads up to a total of about 25 CT slices per patient. The routine and reproducible drawing of the CTV and the skin on all these CT slices takes considerable time. We investigated if this time could be reduced by automating part of this work and automating the planning optimization procedure.

Material and methods: To delineate the breast contour we use a lead collar around the conserved breast or chest wall. A maximum of 3 cm of projected lung tissue is accepted by setting the gantry, collimator angle, field aperture and isocentre. A third beam (called collar beam) uses the same isocentre and is used only for the contouring software. Its gantry angle is orthogonal to the axis of the tangential beams and the aperture includes the complete palpable breast contour delineated by the lead collar. From the combined use of the CT data, the location of the isocenter and this collar beam a fully automated procedure was developed for the delineation of the planning optimization volume and PTV and the computer optimized planning procedure.

Results: In a feasibility study 43 consecutive and unselected patients were included. Three different plans were considered: (1) a 2D plan using only one slice, on screen manual contouring and dosimetrist-guided

Partial volumes of relevant organs at risk for right and left-sided breast tumors with the three planning procedures

	Partial volume in cc					
	Right-sided tumors			Left-sided tumors		
	2D	3D	CMI	2D	3D	CMI
Lung						
Volume receiving more than 20 Gy	132.1	162.7	127.4	120.6	116.4	108.7
Volume receiving more than 40 Gy	71.2	71.6	72.3	64.3	65.9	53.4
Heart						
Volume receiving more than 20 Gy	0.01	0	0	8.2	5.9	6.1
Volume receiving more than 40 Gy	0	0	0	5.4	3.8	4.1