excluded. All patients received definitive RT of >60 Gy, predominantly with 6 fractions per week concomitantly with the radiosensitizer nimorazole, but without chemotherapy. Weight loss was expressed by the relative weight change per week as fitted by linear regression from observations during week 0−7 of RT. For the statistical analysis, critical weight loss was defined as weight loss of >1.0% per week. Multivariate linear regression analysis was applied.

Results: The average weight loss during RT for the whole group was 5.65 kg corresponding to an average absolute weight loss of 7.3% over $7\frac{1}{2}$ weeks. 245 patients (50%) experienced a critical weight loss of more than 1% per week which on multivariate analysis was significantly associated with accelerated RT (OR=2.6; CI 1.1−5.7), BMI (OR=2.5; CI 1.2−4.8), non-glottic tumour sites (OR=3.6; CI 2.2−5.7), and disease stage (OR=1.9; CI 1.2−2.9). Tube feeding was prescribed for 24% (119/490) which was significantly related to non-glottic tumour sites (OR=2.6; CI 1.2−5.5) and disease stage (OR=3.6; CI 2.0−6.7), as well as to lower age (OR=0.6; CI 0.5−0.8) and poor performance status (OR=1.8; CI 1.1−3.0), but not to BMI.

Conclusion: Accelerated RT, BMI, disease stage, and non-glottic tumour sites predicted critical weight loss during RT. Of these factors, only disease stage and non-glottic tumour sites were linked to the prescription of tube feeding besides age and performance status; the latter indicating clinicians' preferences.

8517 POSTER DISCUSSION

Comparison of Clinical Outcome Between Proton and Carbon-ion Radiotherapy in the Same Treatment Protocols

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Purpose: To compare retrospectively our treatment results after proton radiotherapy (PRT) or carbon-ion radiotherapy (CiRT) in patients with malignant tumours originated in the H&N, the lung and the liver.

Methods: From June 2005 to December 2010, 699 patients, aged from 26 to 98 (median 71), with H&N (n = 122), lung (208) and liver cancer (369) were treated by PRT (330) or CiRT (369) with radical intent. All patients except for liver cancer were fresh cases. The RBE values for protons and carbon-ions were determined as 1.1 and 2.0−3.7, respectively, by in vivo and in vitro studies. Three protocols consisting of 70.2 GyE/26Fr (BED_{10}=89.2), 66 GyE/10Fr (109.6) and 52.8 GyE/4Fr (122.5) were employed for either proton or carbon-ion therapy (Table). The selection of protons or carbons was made for all patients based on the DVH analysis (D95 of CTV and PTV, V20−60 of OAR). Overall survival (OS) and local control (LC) rates were calculated by Kaplan–Meier and Log-rank test.

Results: The median follow-up periods were 22.2 months. As for LC and OS rates, there were no significant differences between PRT and CiRT in the same treatment protocols (the same total dose and the same fractionation) in patients with H&N, lung and liver cancer (Table).

Discussions: Our clinical experiences suggested that GyE calculated by the above described RBE values was equivalent for tumours with different histological types.

Conclusions: There were no significant differences of LC and OS rates between PRT and CiRT in the same treatment protocols.

		H&N			Lung			Liver		
		n	2 year OS (%)	p value	n	[1] 2 year OS (%)	p value	n	2[3] year OS (%)	p value
52.8 GyE/4Fr	proton	0	–	–	18	94.4	0.669	26	83.9[74.6]	0.61
	carbon	0	–		55	86.1		82	80.3[65.5]	
66.0 GyE/10Fr	proton	0	–	–	57	74	0.34	154	63.0[60.3]	0.194
	carbon	0	–		54	78.5		107	80.2	
70.2 GyE/26Fr	proton	66	61	0.389	9	72.9	0.399	0	–	–
	carbon	56	83.1		15	[92.3]		0	–	
			2 year LC (%)	p value		[1]2 year LC (%)	p value		2[3] year LC (%)	p value
52.8 GyE/4Fr	proton	0	–	–	18	87.4	0.908	26	95.0[95.0]	0.819
	carbon	0	–		55	89.6		82	93.0[89.2]	
66.0 GyE/10Fr	proton	0	–	–	57	76.9	0.186	154	94.3[94.3]	0.253
	carbon	0	–		54	100		107	83.5	
70.2 GyE/26Fr	proton	66	66.9	0.425	9	[65.6]	0.225	0	–	–
	carbon	56	81.9		15	100		0	–	

8518 POSTER DISCUSSION

Genetic Factors and Late Adverse Effects of Tissue After Radiotherapy in Breast Cancer Patients − Results From the German MARIE(RAD) Study

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Background: After breast conserving surgery (BCS), breast cancer patients are routinely treated with radiotherapy (RT) to reduce the rate of local recurrences. However, late adverse effects such as telangiectasia and fibrosis can occur as a consequence of RT. The risk of these events can be modified by individual genetic susceptibility. As RT leads to increased levels of oxidative stress, we assessed the association of polymorphisms in genes related to oxidative stress and RT-induced late adverse effects.

Methods: For this analysis, breast cancer patients from the study region Rhein-Neckar-Karlsruhe of the German MARIE study were eligible if they received RT after BCS (2002–2005) and had no chemotherapy, no metastases at diagnosis or any previous cancer(s). 414 patients participated (participation rate: 84%). Late adverse effects were evaluated by physical examination and classified according to standardized EORTC/RTOG scoring (0=none to 4=severe adverse effects) by an experienced study physician. 109 common single nucleotide polymorphisms (SNPs) were genotyped using Illumina Golden Gate and 22 SNPs for replication using iPlex application. Associations of genotype with skin alterations (e.g. telangiectasia) and with fibrosis, respectively, were assessed in up to 363 patients, excluding individuals who received intraoperative or interstitial boost to achieve a homogeneously exposed population. Multivariate logistic regression was used to adjust for potential confounding factors. A dominant model was assumed, comparing carriers of the variant allele to non-carriers. An independent study of 390 breast cancer patients (RT after BCS in 1998–2001, same study region) was used for replication.

Results: After a median follow-up time of 67 months, 46 of 414 patients (11%) developed skin alterations of grade 2 or 3. A total of 43 patients developed fibrosis, of whom 23 also experienced telangiectasia. None of the patients presented with grade 4 toxicities. Two SNPs in *NQO1* in high linkage disequilibrium were associated with a significant risk reduction for skin alterations (OR 0.3, 95% CI 0.1−0.9) that was replicated in the independent study (OR 0.4, 95% CI 0.2−0.8). For fibrosis, SNPs in *TXN, TNF* and *NQO1* showed significant associations in one study.

Conclusion: Polymorphisms in oxidative stress-related genes might influence the occurrence of RT-induced late adverse effects. Our findings need further replication by larger studies and support from functional studies.

8519 POSTER DISCUSSION

Setup Margins in a Thermoplastic Shell Can Be Reduced to Those of a Stereotactic Frame Using Daily Online Correction − a Prospective Comparison Between Shell and Frame

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Background: Patients undergoing fractionated stereotactic cranial radiotherapy (SCRT) who cannot tolerate a relocatable frame may be immobilised in a thermoplastic shell but larger CTV-PTV margins are applied to account for the reduced relocation accuracy. This prospective study compares the setup accuracy and intrafraction motion achieved using daily online correction with the ExacTrac (ET) system for frame and shell based treatment. The primary endpoint is to evaluate whether margin reduction to 3 mm (as used in a frame) is safe in shell patients.

Methods: Approval was granted by the Committee for Clinical Research, Royal Marsden Hospital. Margin reduction will be considered safe in the shell if $\geqslant$168 of 179 fractions are accurate (defined as maximum error <2 mm on post correction *and* post treatment imaging).

All patients undergoing SCRT for benign brain tumours were included. For each fraction, stereoscopic kV image pairs were acquired using the ET system:

- pre-correction (at initial setup)
- post-correction pre-treatment
- post-treatment
- additional image pair acquired after floor twist to 90° for the first 5 fractions

Population systematic & random errors were calculated for each image sets.

Results: To date:
- 14 patients have completed treatment in a frame, 6 in a shell (2 more now on treatment)
- 494 fractions delivered, 478 (97%) are adequate for inclusion, corresponding to 3136 kV images.

Data is available from 158 fractions in shell patients.
- Post correction pre-treatment, translational errors are ⩾2 mm in 0/158 fractions
- Post treatment, errors are ⩾2 mm in 9/158 fractions.

Table: Systematic & Random errors

	Systematic errors						Random errors					
	Translational (mm)			Rotational (°)			Translational (mm)			Rotational (°)		
	Vert	Long	Lat	Yaw	Roll	Pitch	Vert	Long	Lat	Yaw	Roll	Pitch
Shell												
Pre-correction	0.23	1.71	1.30	1.53	0.38	1.08	0.85	1.05	0.72	0.63	0.85	0.90
Post-correction	0.08	0.25	0.21	0.28	0.13	0.16	0.21	0.21	0.26	0.28	0.21	0.24
Post-treatment	0.29	0.39	0.28	0.23	0.23	0.34	0.43	0.45	0.35	0.43	0.27	0.32
Post floor twist (1st 5 fractions)	0.45	0.47	0.35	0.37	0.16	0.45	0.38	0.23	0.28	0.34	0.22	0.22
Frame												
Pre-correction	0.42	0.60	0.30	0.24	0.34	0.37	0.40	0.54	0.42	0.29	0.21	0.22
Post-correction	0.07	0.13	0.07	0.08	0.05	0.09	0.22	0.18	0.17	0.16	0.11	0.20
Post-treatment	0.19	0.26	0.24	0.16	0.09	0.16	0.42	0.29	0.35	0.19	0.16	0.24
Post floor twist (1st 5 fractions)	0.17	0.22	0.25	0.14	0.11	0.19	0.31	0.25	0.20	0.19	0.14	0.19

Conclusion: Data is awaited from the final 2 patients but margin reduction is likely to be safe in shell-immobilised patients when using ExacTrac for daily online correction.

Poster Presentations (Sun, 25 Sep, 09:30–12:00)
Head and Neck Cancer

8520 POSTER

Podoplanin Regulates the Proliferation of Oral Squamous Cell Carcinoma Cells via Its Binding to Extracellular Matrix

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Background: We have already reported that podoplanin (PDPN), one of the representative immunohistochemical markers for lymphatic endothelial cells, is enhancedly expressed in carcinoma in-situ and squamous cell carcinoma (SCC) of the oral mucosa, though its pathophysiological function remains largely unknown. Our aim was to determine its molecular role in oral SCC cells, based on a hypothesis that PDPN plays an important role in SCC cell activities such as cell proliferation and migration.
Material and Methods: Surgical specimens of oral SCC were used for immunohistochemistry for PDPN and Ki-67. ZK-1 and the other two SCC cell systems as well as three ACC cell systems of salivary adenoid cystic carcinoma origin were used for in-vitro experiments. PDPN expression levels were determined by quantitative real-time PCR, western blotting, and immunofluorescence. Transient PDPN knockdown was performed with siRNA. Cell growth curves were compared between ZK-1 cells with or without PDPN knockdown or in the absence or presence of anti-PDPN monoclonal antibodies capable of selectively recognizing its cell surface domain. The apoptotic effect of PDPN knockdown in ZK-1 was analyzed by flow cytometry. Their cell attachment, migration, and invasion assays were also conducted by conventional methods using microplates, scratch wounding, transwells, and matrigel.
Results: Immunohistochemically, PDPN was characteristically localized in the peripheral cells of SCC foci where Ki-67 positive cells were simultaneously localized. PDPN was specifically expressed in SCC cells, especially in ZK-1, but not in ACC cells at mRNA as well as protein levels. The cell growth was significantly suppressed in PDPN-knockdowned ZK-1 cells, which was not always due to apoptosis (less than 5%) but rather due to the inhibition of cellular attachment in initial stages after plating in plastic dishes. ZK-1 cell growth was also significantly repressed in the anti-PDPN antibodies in the culture medium. In contrast, migration and invasion activities were not affected at all in ZK-1 cells knockdowned by siRNA for PDPN.
Conclusion: The findings indicated that PDPN functions in cell proliferation but not in migration or invasion of oral squamous cell carcinoma cells by binding with extracellular matrix molecules via its extracellular domain.

8521 POSTER

Novel Candidate Genetic Polymorphisms Identified in Genome-wide Association Study for Base of Tongue Squamous Cell Carcinoma Susceptibility

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Background: In head and neck, we have traditionally combined squamous cell carcinomas (SCC) of the oral cavity, oropharynx, larynx and hypopharynx in clinical treatment. However, with the advances in our understanding of molecular biology of cancer, the tumours that where once considered to be relatively homogenous diseases are now being recognized as comprising distinct subtypes. Inherited genetic alterations, such as single nucleotide polymorphisms (SNPs), were described in association with oropharyngeal cancer risk in only few reports. Base of tongue (BT) SCC are common tumours of oropharynx; however, the association of SNPs and BTSCC risk is still not clarified and, therefore, this was the aim of the present study.
Methods: DNA of 49 BTSCC patients and 49 controls was extracted using the Qiagen QIamp kit (Qiagen®). Each sample was genotyped individually using DNA high-resolution microarrays containing 500.568 SNPs (SNP array 5.0, Affymetrix®). Further sample processing, including digestion, adaptor ligation, amplification, fragmentation, labelling, hybridization, washing and scanning was assayed according to the standard protocol. Genotype data were acquired by genotyping calling of samples using the corrected robust linear model with maximum likelihood classification algorithm provided by Bioconductor software, as per the recommended guidelines. The differences between groups were analysed by the logistic regression model.
Results: We observed 6.609 SNPs with distinct frequencies between BTSCC patients and controls. 52 SNPs (0.8%) were located in coding sequence (CDS) of amino acids, 51 (0.8%) in 3′ and 5′ untranslated regions (UTR), 3.461 (52.4%) in up or downstream regions and 3.045 (46.0%) in introns. The SNPs were clustered to their main function, evidencing those localized in CDS, 3′- and 5′-UTR, related to cell cycle (*CHFR*, *COSMC*, *ERP29*, *IQCE*, *IRS2*, *KANK4*, *MAU2* and *USP2*), apoptosis (*ARHGEF18*, *CTSB*, *GFRAL*, *JMJD6*, *PIHID1*, *RAB6C* and *SPATA4*), DNA repair (*CCDC6* and *GEN1*), transcriptional process (*GCN2*, *MLL3*, *RPAD1*, and *ZNF415*), cell adhesion (*COL6A3*, *COL22A1*, *IKAP* and *KIND1*) and metastasis (*GCNT1*, *LARS* and *SYNJ2*).
Conclusions: Our preliminary results suggest that SNPs in genes involved in tumour origin and development may predispose individuals to BTSCC. However, these results should be confirmed by functional studies of coded proteins and validated in larger epidemiological studies.
Financial support: FAPESP and FINEP.

8522 POSTER

Human Papilloma Virus in Head and Neck Squamous Cell Cancer

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Background: Epidemiologic and molecular evidence have established the strong link between high risk types of human Papilloma virus (HPV) and a subgroup of head and neck squamous cell carcinoma (HNSCC). We evaluated the frequency of HPV positivity in HNSCC and its relationship to demographic and some risk factor variables in an open case-control study.
Materials and Methods: Fourteen newly diagnosed patients of squamous cell cancer of oropharynx, hypopharynx and larynx aged between 18–50 years were examined from 2008–2010 in Tabriz/Iran. HPV DNA was extracted from paraffin-embedded block of each patient for evaluating by PCR. Saliva samples of 94 control cancer-free subjects were collected for DNA analysis. Multivariable logistic regression method was used to calculate odds ratio for case-control comparisons.
Results: High risk HPV was detected in 6 (42.8%) of patients and 6 (5.3%) of control subjects, which statistically was significant ($p < 0.0001$). HPV-18 was the most frequent type both in the case and control group. HPV-16