26 ORAL

Single nucleotide polymorphisms in candidate genes correlate with clinical normal tissue radiosensitivity

C.N. Andreassen[1], J. Alsner[1], M. Overgaard[2], J. Overgaard[1]. [1]*Aarhus University Hospital, Dept. of Experimental Clinical Oncology, Aarhus, Denmark;* [2]*Aarhus University Hospital, Dept. of Oncology, Aarhus, Denmark*

Background: Several observations indicate that normal tissue radiosensitivity may have a genetic basis. However, we are still far from having a complete understanding of the genetic variation that may underlie differences in normal tissue reactions when unselected cancer patients undergo radiotherapy. Single nucleotide polymorphisms (SNPs) make up approximately 90% of naturally occurring sequence variation. Previously, we have hypothesized that SNPs in genes related to the biological response to radiation injury may account for some of the variability in normal tissue radiosensitivity (Radiotherapy and Oncology, 64 (2002) 131-140). In this study we investigate whether 7 selected SNPs in 5 candidate genes affect the risk of three different radiotherapy complications.

Material and methods: The 41 patients included in this study were given post-mastectomy radiotherapy between 1978 and 1982. All patients have been evaluated in three individual treatment fields with regard to subcutaneous fibrosis, telangiectasia and acute skin erythema after radiotherapy. SNPs in *XRCC1* (codon 399), *XRCC3* (codon 241), *APEX* (codon 148), *SOD2* (codon 16) and *TGF-B1* (position -509, codon 10 and codon 25) were analyzed by PCR and single nucleotide primer extension. Dose-response curves were established for subcutaneous fibrosis and telangiectasia in patients with different genotypes. Differences in radiosensitivity were quantified in terms of ED50 values and enhancement ratios. For acute skin erythema, the incidence was directly compared between patients with different genotypes.

Results: The *XRCC1* codon 399 genotype and the *SOD2* codon 16 genotype correlated with risk of radiation-induced subcutaneous fibrosis. The *XRCC3* codon 241 genotype was associated with risk of subcutaneous fibrosis as well as telangiectasia. For *TGFB1*, the codon 10 genotype correlated with risk of subcutaneous fibrosis whereas the codon 25 genotype was associated with risk of telangiectasia. Heterozygotes exhibited intermediate radiosensitivity compared to patients being homozygous for the alleles causing increased or decreased radiosensitivity. Our study was unable to demonstrate any correlation between the assessed SNPs and risk of acute skin erythema.

Conclusion: Our data demonstrate that SNPs account for some of the variability in the occurrence of normal tissue reactions. This observation supports the assumption that clinical normal tissue radiosensitivity should be regarded as a complex trait dependent on the cumulative effect of variation in several genes. Such insight could become useful in the attempts to establish gene-based predictive assays and will enter into the ESTRO GENEPI project.

27 ORAL

Prospective assessment of cd4 and cd8 T-lymphocyte apoptosis in the prediction of radiation-induced late toxicity in 399 individual consenting patients

M. Ozsahin[1], L. Li[1], N.E.A. Crompton[2], Y. Shi[2], A. Zouhair[1], P. Coucke[1], R.O. Mirimanoff[1], D. Azria[1]. [1]*Centre Hospitalier Universitaire Vaudois (CHUV), Radiation Oncology, Lausanne, Switzerland;* [2]*Paul Scherrer Institute, Department of Health Sciences, Villigen-PSI, Switzerland*

We wanted to assess prospectively the usefulness of radiation-induced CD4 and CD8 T-lymphocyte apoptosis in the prediction of individual intrinsic radiosensitivity. Between 1998 and 2000, 399 consenting patients with miscellaneous cancers treated by curative radiation therapy (> 50 Gy) were, therefore, included in the KFS 00539-9-1997/SKL 00778-2-1999 study. All patients were tested using a rapid (48 h) leukocyte apoptosis assay, where fresh blood samples were irradiated with 8-Gy X rays. Following irradiation, lymphocytes were collected and prepared for flow cytometric analysis. Cytotoxicity was assessed by gradual degradation of internucleosomal DNA which resulted in a sub-G1 peak on the DNA histogram. The majority of the patients had breast (n = 149), head and neck (n = 75), or prostate (n = 36) cancers. Male to female ratio was 165/228, and median age was 60 years (range: 18-85). Acute (WHO and CTC 2.0 scales) and late (RTOG/EORTC scale) toxicities were graded in all patients. Any grade > 2 toxicity was considered as an event in terms of statistical evaluation. Six patients refused radiation therapy following blood collection and were, therefore, excluded from analyses. Receiver operating characteristic (RoC) curves (sensitivity vs. 1-specificity) and corresponding areas under the curve (AuC) were used to assess the prediction power of the assay. Median follow-up period for all treated patients (n = 393) was 29 months (range: 1-73). Following 8-Gy irradiation, median CD4 and CD8 apoptosis was 12.46% (range: -3.63–45.48) and 20.68% (range: 3.38–70.41), respectively. No correlation was found between early toxicity and T-lymphocyte apoptosis. However, among the 393 patients, grade 2 and 3 late toxicity was observed in 94 (24%) and 25 (6.4%) patients, respectively. Radiation-induced CD4 and CD8 T-lymphocyte apoptosis significantly predicted grade 2 and 3 late effects. Among the patients who showed CD4 radiation-induced apoptosis below the median (n = 197), 92 presented grade > 2 toxicity compared to 27 above the median (n = 196; $p < 0.0001$). The same relation was observed for CD8 apoptosis as well: 105 grade > 2 late effects out of 197 vs. 14 out of 196 ($p < 0.0001$). Considering only grade 3 late toxicity, patients with late effects (25 out of 197) all showed CD4 or CD8 radiation-induced apoptosis below the median ($p < 0.0001$ for both CD4 and CD8). The areas under the curve of the receiver operating characteristic curves of CD4 and CD8 apoptosis separately, or CD4 and CD8 analyzed together were 0.84, 0.89, and 0.92; respectively. When analyzing major grade 3 toxicities separately, we observed significant relations between radiation-induced lymphocyte apoptosis and skin ($p < 0.0001$ for both CD4 and CD8), subcutaneous ($p < 0.0001$ for both), salivary glands ($p = 0.0009$ and < 0.0001 for CD4 and CD8, respectively), bladder (p = NS for CD4 but 0.02 for CD8), and intestinal (p = NS for CD4 but 0.002 for CD8) late toxicities. To our knowledge, this is the first rapid assay of intrinsic radiosensitivity confirmed prospectively. It can predict significantly the differences in radiation-induced late toxicity between individuals, and could be used as a rapid screen for genetically hypersensitive patients. Patients in future dose escalation studies could be stratified using the apoptosis assay.

28 ORAL

Radiotherapy and concomittant mitomycin c (MMC) for locally advanced head and neck cancer. Final report of the IAEA multicentre randomized trial.

C. Grau[1,2], J.P. Agarwal[3], K. Jabeen[4], A.R. Khan[5], S. Abeyakoon[6], T. Hadjieva[7], I. Wahid[8], S. Turkan[9], H. Tatsuzaki[10], J. Overgaard[1]. [1]*Aarhus University Hospital, Experimental Clinical Oncology, Aarhus, Denmark;* [2]*Aarhus University Hospital, Oncology, Aarhus, Denmark;* [3]*Tata Memorial Hospital, Radiotherapy, Mumbai, India;* [4]*NORI, Radiotherapy, Islamabad, Pakistan;* [5]*Liaquat Medical College and Hospital, Radiotherapy, Jamshoro, Pakistan;* [6]*National Cancer Institute, Radiotherapy, Colombo, Sri Lanka;* [7]*University Hospital "Queen Giovanna", Radiotherapy, Sofia, Bulgary;* [8]*Medical Faculty, University of Malaya, Department of Medicine, Kuala Lumpur, Malaysia;* [9]*Cerrahpasa Medical Faculty, Department of Radiation Oncology, Istanbul, Turkey;* [10]*International Atomic Energy Agency, Applied Radiation Biology and Radiotherapy Section, Vienna, Austria*

Mitomycin c (MMC) is the prototype hypoxic cytotoxin. Administration of the drug during radiotherapy has been shown to improve the outcome in single institution trials with head and neck cancer. In order to confirm these findings in a broader worldwide setting, the International Atomic Energy Agency (IAEA) initiated a multicentre trial randomising between radiotherapy alone versus radiotherapy plus MMC. Patients with advanced head and neck cancer were treated with primary curative radiotherapy (66 Gy in 33 fractions with 5 fractions per week) ± a single injection (15 mg/sqm) of MMC at the end of the first week of radiotherapy. Stratification parameters were tumour localization, T-stage, N- stage, and institution. A total of 558 patients were recruited in the trial from Feb 1996 to Dec 1999. Insufficient accrual and reporting led to the exclusion of three centres. The final study population consisted of 478 patients from seven centres. Patients had stage III (n=223) or stage IV (n=255) squamous cell carcinoma of the oral cavity (n=230), oropharynx (n=140), hypopharynx (n=65) or larynx (n=43). Prognostic factors like age, gender, site, size, differentiation and stage were well balanced between the two arms. The results showed that the haematological side effects of MMC were very modest (<5% grade 3-4) and did not require any specific interventions. Furthermore, MMC did not enhance the incidence or severity of acute and late radiation side effects. Confluent mucositis and dry skin desquamation was common, occurring in 56% and 62% of patients, respectively. The overall 3-year primary locoregional tumour control, disease-specific and overall survival rates were 19%, 36% and 30%, respectively. Gender, haemoglobin drop, tumour site, tumour and nodal stage were significant parameters for loco-regional tumour control. There was no significant effect of MMC on locoregional control or survival, except for the 161 N0 patients, where MMC resulted in a better loco-regional control (3-year estimate 16% vs. 29%, p=0.01). In conclusion, the study did not show any major influence of MMC on loco-regional tumour control, survival or morbidity after primary radiotherapy in