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

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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- β 1* (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 *TGF- β 1*, 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.

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

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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.

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

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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) \pm 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

stage III-IV head and neck cancer except in N0 patients where loco-regional control was significantly improved.

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Erythropoietin improves the oxygen carrying capacity of mouse blood without changing hypoxia in a murine tumour model

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Background: It has been suggested that by increasing the haemoglobin content of blood one should be able to increase its oxygen carrying capacity and thereby decrease tumour hypoxia. The aim of this pre-clinical study was to investigate the ability of erythropoietin (EPO) to achieve this.

Material and methods: A C3H mammary carcinoma grown in the right rear foot of female CDF1 mice was used when at 200 mm³ in size (the radiobiological hypoxic fraction is 10-15% at this size). EPO (2000 U/ml; Janssen-Cilag) was dissolved in saline and different concentrations injected intraperitoneally 3 times/week at 0.02 ml/g body weight. Single blood samples (80 µl) were taken from the suborbital sinus of mice and analysed using an ABL300 Acid-Base Laboratory and OSM Hemoximeter, and haematocrit levels determined on an Adams haematocrit reader. Tumour oxygen partial pressure distributions were obtained using an Eppendorf oxygen electrode. Radiation (240 kV x-rays) was locally administered to tumours using a fractionated schedule of 10 fractions in 2 weeks (4 Gy/day) followed by graded top-up doses and response assessed by calculating the percentage of animals showing local tumour control 90 days later. Following logit analysis the TCD50 value (radiation dose producing tumour control in 50% of treated animals) was calculated. When combined with EPO the radiation and EPO treatments were started at the same time.

Results: In untreated animals the mean (\pm 1 S.E.) haematocrit level and oxygen carrying capacity of mouse blood was 49% (\pm 0.5) and 9.0 mmol/L (\pm 0.2), respectively. Injecting EPO caused a gradual increase in haematocrit reaching a plateau within 2-3 weeks. The haematocrit concentration and oxygen carrying capacity at the time of this plateau were EPO dose dependent, being 56% (\pm 0.3), 62% (\pm 0.5) and 67% (\pm 0.3), and 10.6 mmol/L (\pm 0.2), 11.7 mmol/L (\pm 0.1) and 12.3 mmol/L (\pm 0.1), with EPO doses of 200, 400 and 800 U/ml, respectively. However, there was no change in tumour oxygenation with any of these EPO doses. Nor was the radiation response altered; the TCD50 values (\pm 95% confidence limits) were 85 Gy (81-89) for animals given radiation alone and 84 Gy (80-87) for those that received concomitant EPO (600 U/ml) and radiation.

Conclusions: EPO improved haematocrit and the oxygen carrying capacity of mouse blood in a dose dependent fashion, but since it had no influence on tumour oxygenation status or radiation response, EPO may not be the answer to the hypoxia problem.

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Recurrences after parotid-sparing radiotherapy

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Purpose: Evaluation of loco-regional failure patterns and survival after parotid-sparing three-dimensional conformal and intensity modulated radiotherapy (IMRT) for head and neck cancer.

Methods and materials: From June 1999 until July 2002, seventy-two selected patients with head and neck cancer were irradiated using a parotid-sparing technique. Three-dimensional conformal planning was used in sixty-eight patients, four patients were treated by IMRT. In all patients the junctional (or high level II) nodes contra-lateral to the tumor were omitted from the clinical target volume to spare the adjacent parotid. All patients presenting with persistent or recurrent loco-regional disease had the radiological image of the failure transposed to the pre-treatment computed tomography used for treatment planning. Minimum, mean and maximal doses administered at the level of the failure were calculated, dose-volume histograms were computed for each failure. The failures were defined to be in-field, marginal misses or out-field depending on the percentage of their volume respectively \geq 95%, between 20% - 95% and < 20%- receiving 95% of the prescribed dose. The rates of 2-year loco-regional control and overall survival were calculated using the Kaplan-Meier method.

Results: The median follow-up was 19 months (range 10-30 months). Both loco-regional control and overall survival rates at 2 year were 67.4%. At the time of analysis, twenty of the seventy-two patients developed a loco-regional failure. Two of these patients (2/20) presented with distant metastasis in combination with a loco-regional relapse. Fifteen of the twenty failures (15/20) occurred within the high dose region, five patients (5/20) developed marginal misses. When reviewing these marginal misses by means of beams-eye-views, in all cases the center of the recurrence was located in the high dose region. No relapses were seen out field and no patients relapsed in the spared junctional area contra-lateral to the tumor.

Conclusion: Parotid sparing radiotherapy, by omitting from irradiation the junctional nodes contra-lateral to the tumor, proved to be safe in our selected patients, since no recurrences developed in that area. As this parotid sparing technique reduces significantly the dose to the parotid contralateral to the tumor and is easy to perform, it should be considered for all such patients.

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Final results of a randomized fractionation trial on radiotherapy for neuropathic bone pain (Trans-Tasman Radiation Oncology Group, TROG 96.05)

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Background: Although there is little evidence for a radiotherapy (RT) dose-response above a single 8 Gy fraction for uncomplicated metastatic bone pain, there have been no prospective studies on bone pain where there is a neuropathic component. The Trans-Tasman Radiation Oncology Group therefore undertook a randomized trial comparing a single 8 Gy (arm 1) with 20 Gy in 5 fractions (arm 2) for this type of pain.

Materials and Methods: Eligible patients had plain x-ray or bone scan evidence of skeletal metastases from a known malignancy with no other metastases along the distribution of the neuropathic pain, and no change in systemic treatment within 6 weeks before RT or anticipated within 4 weeks after RT. This was an "equivalence" trial, the purpose being to determine whether arm 1 was as effective as arm 2. The primary end points were response within 2 months of commencing RT and time to treatment failure. The accrual target was 270 patients (pts).

Results: Between February 1996 and December 2003, 272 pts were randomised from 15 centres (11 Australian, 3 New Zealand, 1 UK). 253 pts satisfied the eligibility criteria (arm 1:2 = 128:125). The commonest primary sites were lung (32%), prostate (28%) and breast (8%); 73% were male; median age was 67 (range 29-89); index sites were spine (88%), rib (10%), other (2%).

The only grade 3 acute RTOG toxicities were (arm 1:arm 2) upper GI tract 1:2 and lung 1:1. In addition, severe but temporary pain "flare" was observed in 7:2 pts. No grade 4 toxicities were reported. There were no statistically significant differences between the treatment arms in development of cord/cauda equina compression or pathological fracture at the index site. On an intention-to-treat basis, the overall response rate was 58% (95% CI = 51-64%) with 26% achieving a complete response. Estimated median time to treatment failure was 3.1 months (95% CI 2.6-3.7 months), and estimated median survival 4.8 months (95% CI 4.0-5.8 months).

Conclusion: Overall, about 3 in 5 patients with neuropathic bone pain responded to RT for a significant proportion of their remaining life span. The results by treatment arm will be presented at the meeting.