benign or malignant areas of the prostate. Baseline measurements were taken before the first HDR treatment, and then 7 days later before the second fraction. Control pO readings were also obtained within skeletal muscle. Seventeen patients were included in the study.

Results: At baseline, the mean hypoxic fraction was 76.5% in malignant and 63.2% in benign areas of the prostate (p=0.18, paired T test), with mean median pO values of 4.9 mm Hg and 8.7 mm Hg (p=0.40), respectively. The median pO in skeletal muscle was 29.5 mm Hg, with no values in the hypoxic range. One week following treatment, the mean HF was unchanged at 78.6% (p=0.76) in malignant areas and 66% (p=0.75) in benign areas. The mean median pO following treatment was also unchanged at 1.6 mm Hg (p=0.23) and 4.4 mm Hg (p=0.15) in malignant and benign areas, respectively. Of 27 malignant areas measured, 11 had an increase in HF, 14 had a decrease, and 2 were unchanged. Of 33 benign areas, 17 had an increase, 13 a decrease, and 3 no change.

Conclusions: The entire prostates of men with high risk prostate cancer are diffusely hypoxic. The level of hypoxia is not significantly reduced one week after receiving 10 Gy with HDR, indicating that reoxygenation had not occurred.

482 POSTER

Enhancement of radiation response by roscovitine in human breast carcinoma in vitro and in vivo xenograft model

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Frequent deregulation of CDK activation associated with loss of cell cycle control has been found in most of human cancers. Recent development of a new class of antineoplasic agents targeting the cell cycle, has emerged as a small molecule CDK inhibitor, roscovitine which presents potential antiproliferative and antitumoral effects in human tumors. Further studies have reported that roscovitine combined with cytotoxic agents can cooperate with DNA damage to activate p53 protein. However, little is known about the biological effect of roscovitine combined with ionizing radiation in human carcinoma and no studies has been reported so far in p53 mutated carcinoma. In the breast cancer cell line MDA-MB 231 which lack a functional p53 protein, we have found a strong radiosensitization effect of *roscovitine in vitro* by clonogenic survival assay and *in vivo* in MDA-MB 231 xenograft model. Using Pulse field gel electrophoresis (PFGE), a strong impairment in DNA-DSB rejoining was observed following roscovitine + IR treatment as compared to IR alone. Cell cycle analysis has shown a G2 delay and no increase in radiation induced apoptosis in the cells treated with IR or roscovitine+IR. On the other hand, we have found a significant induction in micronuclei frequency following roscovitine +IR treatment as compared to IR alone. In MDA-MB 231 cells, the radiosensitization effect of roscovitine was associated with an inhibition of the DNA-PK activity due to a marked decrease in Ku-DNA binding when we used the electrophoretic mobility shift assay (EMSA). In conclusion, we found a novel effect on DNA repair of the CDK inhibitor roscovitine which acts as a radiosensitizer *in vitro* and *in vivo* in breast cancer cells lacking a functional p53.

483 POSTER

Erythropoietin receptor expression and the in-vitro effect of erythropoietin on the radiation-response of different cancer cell lines.

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Anemia is associated with a poor outcome in patients treated with radiotherapy. Currently, Erythropoietin (EPO) is tested in phase III clinical trials to study its potential role to improve local control in patients treated with ionizing radiation. EPO is a hormone produced by the kidneys. It acts via the EPO receptor (EPOR) to stimulate growth, prevent apoptosis, and induce differentiation of red blood cells precursors. Expression of EPO and EPOR has recently been demonstrated in several nonhematopoietic tissues. This suggests a broader role for EPO in regulating cell growth and survival. It is known that autonomous EPO expression mediates autocrine growth of erythrocytic leukemia cells. This suggests that the expression of EPO and EPOR by tumors of nonhematopoietic tissues may also stimulate cancer cell proliferation.

This prompted us to study the *in-vitro* effect of EPO and the expression of its receptor on the radio-responsiveness of cultured tumor cell lines. The expression of EPO receptor (EPO-R) and its messanger (mRNA) were studied in cell lines including: MCF-7, HeLa, MDA, U87 and Colon 205, as well as a primary carcinoma cell line of the cervix (HT-100) using Reverse Transcriptase and Polymerase Chain Reaction (RT-PCR) and immuno-blot techniques. The radiation cell survival curves of all the cell lines were determined in the absence or in the presence of EPO. In all studied cell lines, there was a consistent and reproducible radiation protection in the presence of EPO. This EPO-induced radiation protection was abolished by the addition of a JAK2-kinase inhibitor, suggesting that the signal transduction pathway of EPO is functional.

Studies are underway to determine whether these *in-vitro* results are reproducible *in-vivo*. If such results are confirmed *in-vivo*, this may have implications on current ongoing clinical trials using EPO as an experimental agent to counteract the effects of hypoxia.

484 POSTER

Biological markers associated with sensitivity of tumour cells to the epidermal growth factor receptor-tyrosine kinase inhibitor ZD1839 and ionizing radiation

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Background: ZD1839 (Iressa ®) , an orally active, selective epidermal growth factor receptor (EGFR)-tyrosine kinase inhibitor, reduces survival and augments radiation response of certain tumour cells through blockage of EGFR signaling. In this study we tested tumour cell lines for EGFR and TGF-alpha mRNA expression, cell cycle distribution and induction of apoptosis in order to identify biological markers that are associated with sensitivity to ZD1839 and that might serve as parameters in a predictive test.

Material and methods: The tumour cell lines A549, H596 (both human non-small cell lung cancer cell lines) and FaDu (human head and neck squamous cell carcinoma cell line) were subjected to ionizing irradiation, treatment with ZD1839 (1 μM, 5 μM) and combined ZD1839 / irradiation treatment. Clonogenic cell survival was determined by colony assays, EGFR and TGF-alpha expression by RT-PCR, cell cycle distribution and apoptosis by flow cytometry.

Results: Whereas in FaDu cells a considerably high amount of EGFR and TGF-alpha transcripts was detected, A549 and H596 cells both expressed moderate amounts of EGFR mRNA and very low levels of TGF-alpha mRNA. Irradiation led to early downregulation of EGFR transcripts in all three cell lines but only FaDu cells which were more radiosensitive than A549 and H596 cells showed a prolonged downregulation of EGFR mRNA expression compared to the expression level of the untreated cell line. Exposure to ZD1839 caused a decrease in EGFR mRNA expression in A549 cells whereas this effect could not be detected in the other two cell lines. Treatment with 1 μM ZD1839 showed marked inhibition of clonogenic growth in FaDu cells whereas it had little effect on clonogenic growth in A549 and H596 cells. Upon treatment with 5 μM ZD1839 survival curves revealed a radiosensitizing effect on A549 cells. A reduction of S phase cells and induction of apoptosis after treatment with 1 μM ZD1839 and combined ZD1839 / radiation treatment was most marked in FaDu cells.

Conclusions: The sensitivity of tumour cells to ZD1839 correlated with the EGFR and TGF-alpha expression level whereas a radiosensitizing effect was associated with downregulation of EGFR mRNA expression. Inhibition of cell proliferation and induction of apoptosis were correlated with a decrease in clonogenic cell survival after treatment with ZD1839.

485 POSTER

Evaluation of the effects of radiotherapy to the chiasm and optic nerve by visual psychophysical-electrophysiological tests

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Purpose: To evaluate the effects of high-dose irradiation to the chiasm and optic nerves in locally-advanced nasopharyngeal carcinoma patients by visual psychophysical- electrophysiological tests.

Materials and Methods: Series of visual tests [Visual evoked potential (VEP) latency, VEP amplitude, contrast sensitivity, visual field and visual acuity tests] were applied to 27 patients with locally-advanced (T4) nasopharyngeal carcinoma who were irradiated to high doses 6 - 74 months

ago. As a control group, the same tests were applied to 40 unirradiated patients who referred to the ophthalmology department for any reason.

Results: The median values of VEP latency, VEP amplitude, contrast sensitivity and the rate of visual field defect were significantly worse in the irradiation group ($p=0.06$, $p<0.001$, $p<0.001$ and $p=0.005$ respectively). There was no dose-response relationship in all tests when 50 Gy was the cut-off value. However a positive correlation between time interval after radiotherapy and VEP latency ($r=0.406$, $p=0.003$) and a negative correlation between time interval and contrast sensitivity ($r= -0.499$, $p<0.001$) was noted; no correlation could be established regarding VEP amplitude and time interval.

Conclusion: Radiation-induced injury to the anterior visual pathways could result in an increase in VEP latency and a decrease in VEP amplitude and contrast sensitivity. This injury seems to be a continuous process developing in time.

486 POSTER

Evaluation of the new hypoxic cell radiosensitizer doranidazole in a murine tumour and mouse normal tissue

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Background: Hypoxia is a major factor for tumour resistance to radiotherapy and considerable effort is still being made to find approaches to overcome this problem. The aim of this pre-clinical study was to assess the potential of the new nitroaromatic compound doranidazole (POLA Chemical Industries) as a tumour radiosensitiser.

Material and methods: A C3H mammary carcinoma grown in the right rear foot of female CDF1 mice was used and treated when at 200 mm^3 in size. Doranidazole was dissolved in saline and intravenously injected into mice at a constant volume of 0.02 ml/g. Radiation (240 kV x-rays) was locally administered to the tumours or normal feet of restrained non-anaesthetised animals. Tumour response was assessed by calculating the percentage of animals at each radiation dose showing local tumour control at 90 days and skin damage estimated from the percentage of mice developing moist desquamation in the foot 11-23 days after irradiation. Following logit analysis of the dose response curves the TCD50 (tumour) or MDD50 (skin) doses (radiation doses producing a response in 50% of treated mice) were estimated and from these a sensitizer enhancement ratio (SER; ratio of the TCD50 or MDD50 for radiation alone and radiation with drug) calculated. Statistical analysis was performed using a Chi-squared test ($p<0.05$).

Results: The TCD50 value ($\pm$ 95% confidence interval) for radiation alone was 53 Gy (51-55). Injecting doranidazole (200 mg/kg) at 0, 30 or 60 minutes prior to irradiation significantly enhanced radiation response with the greatest effect seen at the 30-minute interval [TCD50 = 40 Gy (37-44); SER = 1.3]. No enhancement was seen if doranidazole was injected after radiation. Injecting different drug doses 30 minutes prior to irradiation showed a clear linear dose-response relationship, with the SERs going from 1.1 at 50 mg/kg to 1.8 at 500 mg/kg. In skin, using the 200 mg/kg dose and a 30-minute interval, the SER was only 1.1.

Conclusions: Doranidazole at non-toxic doses significantly enhanced radiation response of this tumour in a manner that is consistent with a hypoxic cell sensitizer and did so to a much greater degree than was seen in a normal tissue.

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487 POSTER

Geographical miss of the primary target and nodes in adjuvant breast radiotherapy as assessed by open MRI scanning

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Background: Good radiotherapy requires accurate targeting. Adjuvant breast cancer radiotherapy is planned without benefit of modern three-dimensional imaging in many centres. Even specialised CT scanners that can collect three-dimensional anatomical information in the necessary treatment position fail to demonstrate the primary site without the use of surgical clips. Clips pose problems, both in their placement, and in their final position. Despite this, radiotherapy reduces the chance of local recurrence from about a third of all cases to less than 10%. Improvements in the delivery of radiotherapy may help to reduce long term morbidity and mortality. MRI gives exquisite soft tissue definition in multiple planes, without the use of contrast. The primary site, nodes, surgical cavities and organs at risk are easily identified in three-dimensions.

Materials and Methods: Patients were scanned in the conventional treatment position in an open MRI scanner, using positioning lasers. A Siemens Open 0.2 Tesla scanner was used, with distortion corrected automatically using data derived from in-house phantom studies. In addition to axial scans, sagittal and coronal views can be constructed. A series of 528 patients with early operable breast cancer following conservative surgery were set up in the conventional treatment position, using standard positioning lasers, starting in January 1997. The field margins marked were the midline and the mid-axillary line. A Clinical Target Volume (CTV) of 1.5 cm surrounding the post-surgical tumour cavity was chosen, and the degree of geographical miss was calculated.

Results: It was found that 57% of all cases were receiving half or less of the prescribed dose to at least part of this CTV. Visible axillary nodes received less than half the dose in 47% case: this rises to a total of 80% patients if the post-surgical axillary cavity is also taken into account. Excluding peri-cardial fat, 73% patients had more than half the prescribed dose to the myocardium. Some lung received 50% or more of the dose in 95% cases. The radiotherapy plans were subsequently amended. All boosts were targeted using MRI.

Conclusion: The apparent effectiveness of poorly targeted radiotherapy raises interesting speculation. Improving treatment related morbidity and mortality might improve survival. Recurrence rates observed in 542 patients treated over the last seven years with MR modified two-dimensional radiotherapy will be presented.

488 POSTER

Selective conformal post-operative radiotherapy in patients with head and neck squamous cell carcinoma (HNSCC). Could the "bath of X-rays" be avoided?

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Background: Indications for post-operative radiotherapy in patients with HNSCC have been well established over the years. For both technical and conceptual reasons, target volumes typically included the entire neck and the site of tumor resection. This study presents data on post-operative irradiation (PORT) where the target volumes have been tailored based on the surgical procedure and the pathologic report.

Materials and Methods: Between January 2000 and June 2002, 47 patients (35 males and 12 females) with HNSCC (22 oral cavity, 10 larynx, 8 oropharynx, 7 hypopharynx) were treated with curative surgery on the primary tumor and on the neck using recommended procedures (Robbins, Arch Otolaryngol Head Neck Surg. 1991). There were 26 pT1-pT2 tumors and 21 pT3-pT4 tumors. Thirty-four patients were pN+ and 12 pN0. Indications for PORT followed published recommendations (Peters, Int J Radiat Oncol Biol Phys. 1993). Moderate and high risk patients received 60 Gy (range 59.2-60.8 Gy) and 64 Gy (range 63.2-65.7 Gy), respectively. Selection of targets volumes was tailored on the pathological finding, e.g. unilateral neck irradiation if pN0 on one side of the neck, or no neck irradiation in case of pN0 neck on both sides. Target volumes were delineated on a 3D basis using published guidelines (Grégoire, Radiother. Oncology, 2000). Planning was performed using forward planning IMRT. Typically, minimal dose to 95% of the PTV was 95% of the prescribed dose, and not more than 7% of the PTV received more than 107% of the prescribed dose. Maximum dose to the spinal cord was set at 50 Gy on the envelope. During the follow-up (FU) patients with loco-regional recurrence were imaged with CT, MR or FDG-PET and the relapse site was matched with the dose distribution of the planning CT scan.

Results: With a median FU of 16.6 months (5.1-34.9), 2 year actuarial loco-regional control and overall survival reached 80% and 68%, respectively. Presence of pathological lymph node infiltration, extracapsular spread and R1 resection were adverse prognostic factors, although they did not reach the level of significance due to the small number of patients. Two patients experienced a progressive disease in the target volume during PORT. Seven patients experienced a loco-regional relapse (1 local and 6 regional) within 1 year following surgery. In 3 of these patients, the relapse occurred outside the target volume (dose < 25 Gy). In 2 of these patients, relapses occurred at the same time outside the target volume (dose <25 Gy) and inside the target volume. In 46 patients, the mean dose to the controlateral parotid was below 30 Gy (range 1.7-33.8 Gy).