proliferative activity was determined by immunohisto-chemical assessment of the MIB-1 (Ki-67) antigen.

Results: After radiochemotherapy, the apoptotic index increased significantly in nearly every case examined (mean AI [biopsy]: 25.1 ± 11.3 vs. mean AI [resection specimen] 50.3 ± 25.2). When comparing the proliferative activity (MIB-1 index) in biopsies (mean: 47.4 ± 23.5) and corresponding resection specimen (mean 36.6 ± 17.9), a significant decrease was found. Bax immunostaining was detected in 12/31 (39%) biopsies and in 26/31 (84%) resection specimens. However, we did not find a correlation between the (pre- and post-therapeutical) rate of apoptosis or the level of bax expression and the degree of clinical-to pathological downstaging.

Conclusion: Our results indicated, that radiochemotherapy induced an increase in bax expression and also in apoptotic cell death. Further studies are necessary to identify possible regulatory candidates which might be responsible for the observed bax induction and the increase of apoptosis after radiochemotherapy.

85 ORAL

Bcl-2/p53 expression and TP53 mutations: Correlations with *in vitro* radiosensitivity in patients with head and neck squamous cell carcinoma

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Purpose: To determine whether Bcl-2 and p53 status correlate with *in vitro* radiosensitivity, we have examined Bcl-2 expression, p53 expression, and *TP53* mutations in primary tumour specimens of 61 patients with head and neck squamous cell carcinoma.

Methods: Immunohistochemical staining with Clone 124 and DO-7 was used to detect Bcl-2 and p53 expression, respectively, in formalin-fixed paraffin-embedded tissue sections from primary head and neck squamous cell carcinomas. Parallel sections were used for DNA extraction and analysis for gene mutations (exon 5–9) by Denaturing Gradient Gel Electrophoresis (DGGE). *In vitro* radiosensitivity of tumour cells from the primary biopsies was selectively measured using immunocytochemical identification of colonies in the modified Courtenay-Mills soft agar clonogenic assay.

Results: Aberrant Bcl-2 and p53 expression was found in approximately 10% and more than 50% of the tumours, respectively. p53 expression did not correlate with the measured *in vitro* radiosensitivity. However, there was a trend for overexpression of Bcl-2 to be associated with radioresistance.

86 ORAL

DNA damage assays predict normal tissue radiosensitivity

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Introduction: There have been several reports of a correlation between fibroblast radiosensitivity *in vitro* as measured by a clonogenic assay and the severity of late normal tissue reactions. There is increasing interest in more rapid tests such as those that measure DNA damage. There have been few studies examining the relationship between the different methods available. In this work a comparison has been made of fibroblast radiosensitivity measured using a clonogenic assay and three gel electrophoresis techniques: pulsed field, graded voltage and constant voltage gel electrophoresis.

Materials: Eleven fibroblast strains were studied comprising two radiosensitive human strains and nine strains established from vaginal biopsies from patients with carcinomas of the cervix prior to a radical course of radiotherapy.

Methods: Cells were labelled with tritiated thymidnie for 72 h and grown to confluence. After 10 days they were irradiated at high dose rate (1.67 Gy min^{-1}) on a ^{137}Cs source, to doses between 30 and 180 Gy. Residual DNA damage at 24 h was measured as the fraction of activity released (FAR) into the agarose gels.

Results: For all three methods there were highly significant correlations between cell surviving fractions at 2 Gy (SF_2) and the slope of FAR, $r > 0.88$, $p < 0.01$. The correlations among the three gel electrophoresis methods were also highly significant, $r > 0.89$, $p < 0.01$.

Conclusions: The future of DNA damage assays in predicting normal tissue radiosensitivity appears to be promising. This work was supported by the Cancer Research Campaign.

87 ORAL

A new assay to detect *in situ* colony formation in pig epidermis as a possible estimate for radiosensitivity

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Purpose: Radiation-induced moist desquamation in pig epidermis is the result of excessive loss of keratinocytes. Replenishment of cell loss occurs by proliferation of surviving stem cells resulting in colony formation. A new assay is developed to detect these proliferating cells/colonies *in situ* and relate them to dose and the incidence of moist desquamation.

Methods: After enzymatic separation of epidermis and dermis in skin biopsies, 4 mm in diameter, proliferating epidermal cells/colonies are labelled *in vitro* with BrdUrd for 24 hr and visualised by immunohistochemistry.

Results: In unirradiated epidermis 86–87% of proliferating cells were seen as single cells, while 13–14% appeared as pairs which had just passed mitosis. Inter animal variation ranged from 543 ± 59 to 1002 ± 94 proliferating cells per mm^2 (mean ± sem). BrdUrd-positive cells were significantly less in dorsal compared with ventral areas of the flank which might explain observed variations in radiosensitivity with flank position for the epidermal *in vivo* responses. Data on colony formation in relation to dose and *in vivo* responses will be presented.

Conclusion: A reliable immunohistochemical assay has been developed for the detection of proliferating cells/colonies in pig epidermis.

88 ORAL

Describing patients' normal tissue reactions: A necessity for development of predictive testing of normal tissue radiosensitivity

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The recent demonstration of a relationship between in vitro cellular sensitivity and normal tissue response, both of which exhibit normal distributions, suggests that predictive testing to individualise radiotherapy dose prescriptions is a real possibility. This requires collaboration between clinicians and scientists in different groups, but is hampered by the lack of clear definition of what comprises the range of reactions regarded as 'normal', and what constitutes an excessive reaction. Established scoring systems (eg RTOG/EORTC) are excellent at quantitative scoring of early and late reactions, but they do not yet allow the severity of the individual's reaction to be described in relation to the normal range.

We propose a terminology for describing normal tissue reactions which is relative, and should facilitate comparison between centres using different radiotherapy techniques. A numerical description is suggested, dividing the normal range into five categories, from highly radioresistant (category 1), through average (category 3), to highly radiosensitive, or 'HR' (category 5). A definition is proposed to separate those individuals who have severe or extreme reactions, known as "Over-Reactors" from the normal range. It is hoped that this will aid communication between groups working in the field of predictive testing.

89 POSTER

The role of gemcitabine-induced cell cycle synchronization in radiosensitization of A SCC61 human head & neck squamous carcinoma cell (HNSCC) line

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Purpose: Gemcitabine (dFdC) has been shown to radiosensitize human HNSCC lines on a drug incubation time-dependent manner. To understand the basis of radiosensitization, the effect of dFdC on SCC61 cell cycle synchronization was examined by flow cytometry (FACS).

Methods: Confluent cells were incubated with dFdC for 1, 3, 6, 18 or 24 h, washed, pulse-labeled with BrdUrd (10 μM), fixed and then processed for FACS analysis. Alternatively, after drug incubation, cells were irradiated with 4 Gy photons and plated for a colony assay.

Results: For incubation times of 1 to 6 h, dFdC induced a quasi complete inhibition of DNA synthesis with accumulation of cells at the G1-S boundary. From a 6 h incubation time, cells started to reinitiate DNA synthesis, and at 24 h, a significant cell fraction was accumulated in early S-phase. Radiosensitization increased up to a 3 h drug incubation time, thereafter decreased up to 24 h where no more radiosensitization was observed.

Conclusions: In SCC61 cells, dFdC induced a transient block at the G1-S phase boundary. Subsequent accumulation of cells in early S-phase was associated with a loss of radiosensitization. Our data suggest a possible role for dFdC-induced cell cycle synchronization in the kinetics of dFdC's radiosensitization.

90 POSTER

Intravital lectin perfusion of tumours: Visualisation of efficiently and inefficiently perfused microvessels

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Purpose: Histological correlates to high-field rapid acquisition MR images of tumours aid explanation of regional hereogeneities in CM uptake and washout. Protocols for intravital lectin perfusion were developed and assessed for use in localising regions of inefficient perfusion.

Methods: C3H mice with subcutaneous passaged AT17 adenocarcinomas were perfused intravenously with fluorochrome-labelled lectins, given as boluses applied in various protocols, such as sequential injection at different intervals. The tumours and other organs were snapfrozen, and cryostat sections evaluated by fluorescence microscopy to visualise lectin-binding microvessels. Wide-area composite images were correlated with MR data.

Results: Brilliant contrasty images of the tumour microvasculature were obtained up to 4 hours post-bolus. Lectin clearance from the blood was largely complete within 15 minutes, so an intravenous bolus preserved a snapshot view of the microvessels accessible to perfusion near the time of injection. Sequential application of lectins visualised colocalisation in large micro-vessels and many narrow-caliber ones, but also frequent mismatch labelling: evidence of intermittent perfusion in many narrow microvessels.

Conclusion: Over a timescale of a few minutes to a few hours intravital lectin perfusion visualises intermittent perfusion and permits correlation with MR data obtained from the same animals in dynamic studies.

91 POSTER*

Influence of fractionation on the response of pulmonary micrometastases of the R1H-tumour to fractionated irradiation

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The aim of the study was the examination of the influence of the dose per fraction and overall treatment time on tumour control rate of micrometastases.

Lung metastases were induced by i.v. injection of viable tumour cells. Treatment was started 14 days later, when metastases reached an average size of 4 cells. Total doses of 16 to 28 Gy were administered within an overall treatment time of 11 or 25 days, using doses per fraction of 1, 2, or 4 Gy. Tumour response was quantified by local control and number of lung metastases. TCD37% and corresponding 95% C.I. were calculated applying the maximum likelihood method.

Fractionation had a significant influence on local control ($p = 0.05$). After application of 1, 2, or 4 Gy and an overall treatment time of 11 days the TCD37% was 25.4 Gy (21.5–32.0), 20.7 (17.0–24.0), and 18.5 Gy (14.9–21.6), respectively.

When overall treatment time was prolonged to 25 days the TCD37% increased to 25.5 Gy (21.3–33.5) after application of 2 Gy per fraction ($p = 0.056$). Comparison with results obtained in vitro allows calculation of a formal doubling time of 8 days, whereas the doubling time of untreated mictometastases of this size is about 4 days.

The results show for well oxygenated micrometastases a strong influence of fractionation on treatment outcome, which is in contrast to findings on macroscopic subcutaneous R1H-tumours. Furthermore repopulation of microscopic lung metastases seems to be decelerated during fractionated radiotherapy.

92 POSTER

In vitro intraction of radiation and paclitaxel compared to the solvent cremophor EL/ethanol

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Purpose: Cremophor EL/ethanol (Cr/e), the diluent of paclitaxel (P), has shown to be active in tumor samples. The aim of our study was to evaluate the interaction of radiation and paclitaxel compared to the effect of cremophor EL/etahnol.

Methods: Single cells derived from the two common human carcinoma cell lines U-138 MG (glioblastoma) and SK-LU-1 (lungcarcinoma) were used. Cells were treated with P and with Cr/e. Cr/e concentration was equivalent to that found in P. As controls, cells were exposed to a phosphate buffered salt solution (PBS). Drug exposure was investigated alone and in combination with irradiation. Cytotoxicity of P and Cr/e was examined at concentrations varied from 2–50 μM with 3 hours incubation time. Radiation doses ranged from 0–10 Gy. In combination treatment irradiation followed 9 hours after the end of drug or PBS incubation. Cell survival was determined applying the clonogenic assay.

Results: In the lungcancer cell line single P exposure with 0/10/30/50 μM resulted in a clonogenic survival of 100/54/31/10% compared to the glioblastoma cell line with 100/54/43/16%. In both cell lines Cre/e exposure resulted in a survival of 100/100/90/92%. Radiation with 0–10 Gy of both cell lines led to 100–0.05% survival. 10 μM P and 10Gy irradiation resulted in 0.03% (lung cancer) and 0.1% (glioblastoma) survival. 10 μM Cre/e and 10 Gy irradiation led to 0.06% (lung cancer) and 1.1% (glioblastoma) clonogenic survival.

Conclusion: Paclitaxel and radiation used concomitantly produced an additive effect in both cell lines with an enhancement ratio of 1.4 (lungcarcinoma) and 2.3 (glioblastoma). Cr/e was without significant cytotoxic or radiosensitizing effect.

93 POSTER

Response of two different human squamous cell carcinoma xenografts to irradiation at varying sizes: Relationship between tumor volume and TCD_{50}

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Purpose: Clinical experience shows that higher doses are needed for larger tumors to achieve local control (LC). Under ideal conditions the single-dose needed for LC should be dependant only on tumor volume and radiosensitivity assuming cell killing to be an exponential function of dose as well as sensitivity and number of clonogenic cells per volume being constant. This relationship was investigated in two human SCC-xenografts.

Methods: FaDu (poorly differentiated) and GL tumors (moderately well differentiated) were transplanted s.c. into nude mice. Local single-dose irradiation was performed when tumors reached sizes of 36, 80, 180 and 470 mm^3. To avoid the influence of varying oxygen concentrations all irradiations were performed under clamp hypoxia. Experimental endpoint was LC at day 120 (FaDu) or 180 (GL).

Results: The radiation dose required to control 50% of the tumors (TCD_{50}) of sizes between 30 and 470 mm^3 increased from 36.6 to 44.1Gy (FaDu) resp. 29.9 to 41.7 Gy (GL) showing very similar responses with overlapping confidence intervals. Calculations of the D_0 from the slopes of the regression lines resulted in values of 1.0 for FaDu and 1.4 for GL (both corrected for an OER of 3.0). The results of both tumors are also well described by a common regression line with a D_0 of 1.2.

Conclusion: The results indicate that the intrinsic radiosensitivity to single-dose irradiation and the number of clonogenic cells per unit of tumor volume are similar in both tumors. In contrast other experiments from our laboratory have shown that the response to fractionated irradiation with 30 f/6 w under ambient conditions shows a marked difference (TCD_{50} FaDu $\approx$60 Gy, GL$\approx$44 Gy) suggesting that other factors e.g. repopulation and reoxygenation importantly influence the outcome of fractionated radiotherapy.