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).