Overall cellular radiosensitivity correlates well with the level of radiation-induced G1 arrest (r = 0.856, $P = 0.0067$), with p53 constitutive levels (r = 0.874, $P = 0.0046$) and with p53 protein fold induction (r= -0.882, $P = 0.0038$). The mechanistic basis of these correlations remains to be elucidated in these cells, but the data do suggest that both the constitutive p53 level, and the p53 protein response to radiation, may be good predictive tests for radiosensitivity in some cell types.

819 ORAL

SIGNIFICANCE OF P53 AND BCL2 LEVELS IN THE RADIO-SENSITIVITY OF HEAD AND NECK CANCER

O. Csuka, G. Németh, K. Koronczay, Z. Doleschall, É Remenár
National Institute of Oncology, Budapest, Hungary

Radiosensitivity of tumor cells is thought to be modulated by p53 and bcl2 proteins. High level of wild type p53 is required for radiation induced apoptosis. The aim of the present study is to investigate the significance of gene dosages of p53, bcl2 and c myc in radiation induced apoptosis. The base line and 30 Gy, 60 Gy gamma radiation induced values of p53 and bc12 were estimated by Western blot in 60 biopsies of head and neck cancer. Our results suggest that the radiosensitivity of head and neck cancer depends on the ratio of p53 and bcl2 gene dosages. High level of p53 is counter-balanced by high bcl2 gene dosage resulting in radioresistance. Amplification of c myc can compensate for the low p53 level and suggests radiosensitivity of tumor cells. Our studies indicated that the prediction of radiosensitivity of tumor cells could be based on the simultaneous evaluation of p53, bcl2 and c myc levels.

820 ORAL

IMMUNOHISTOCHEMICAL ANALYSIS OF P34^{CDC2} AND CYCLIN B CELL LOCALIZATION IN RECURRENT HEAD AND NECK SQUAMOUS CELL CARCINOMA AFTER IRRADIATION

E. Cohen-Jonathan[1], C. Toulas[2], P. Rochaix[3], J.F. David[3], N. Daly-Schveitzer[1], G. Favre[2]
[1]Radiotherapy Department
[2]Laboratory of Molecular Oncology
[3]Histopathologic Department-Centre Claudius Regaud, 20-24 rue du pont Saint Pierre, 31052 Toulouse, France

After exposure to ionizing radiation, eukaryotic cells undergo a G2 delay which contributes to the ability of cells to survive irradiation. For some radioresistant cell lines, this delay is prolonged. Entry of cells into mitosis is regulated by a complex of two proteins cyclin B, and the serine-threonine p34^{cdc2} kinase. When this complex is activated, it undergoes a transport from cytoplasm into the nucleus and phosphorylates proteins which lead to mitosis. P34^{cdc2} kinase is activated by binding to cyclin B and by phosphorylation/dephosphorylation of p34^{cdc2}. Since G_2 delay after irradiation has been correlated with a rapid inhibition of p34^{cdc2} activity and an enhanced tyrosine phosphorylation, we hypothesized that radioresistant tumors could have a lack in regulation of p34^{cdc2} kinase activity. In this study, we entered 32 patients treated, from 1983 through 1989 at the Claudius Regaud Center, for head and neck squamous cell carcinoma by surgery and standard post-operative doses of radiotherapy. The paraffin embedded tumor specimens had been sampled before radiotherapy for long term controlled patients (n = 7) and before and after radiotherapy for patients who had developed a recurrence in the irradiation fields (n = 25). Immunohistochemical staining was performed with monoclonal antibodies against p34^{cdc2} (sc-54) and cyclin B (sc-245). A semi-quantitative score was used. For p34^{cdc2} analysis, no difference in intensity of staining was observed between long term controlled patients and those who recurred or, when there was a recurrence before and after radiotherapy. However, there was a highly significant difference ($P < 0.001$) in p34^{cdc2} cell localization with a preferential cytoplasmic localization only for the patients who have a recurrence in the radiotherapy fields. This cytoplasmic localization was present in the primary tumor before radiotherapy and in the recurrence, too. No preferential localization was observed in long term controlled patients. For cyclin B, no difference in intensity of staining was observed anywhere and conversely to p34^{cdc2}, no difference in localization appeared in long term controlled patients nor for patients who have had a recurrence. No correlation existed between localization of p34^{cdc2} and those of cyclin B in patients who recurred. Our results suggest a probably intrinsic abnormality of p34^{cdc2} activity and a lack of association between cyclin B and p34^{cdc2} in head and neck radioresistant squamous cell carcinoma.

821 ORAL

GLUCOSE UTILIZATION AND *IN VIVO* CELL KINETICS IN RECTAL CANCER, BEFORE AND AFTER PREOPERATIVE RADIOTHERAPY

K. Haustermans, C. Schiepers, F. Penninckx, K. Geboes, J. Nuyts, L. Filez, L. Mortelmans, G. Bormans, M. De Roo, E. van der Schueren
Department of Oncology, Nuclear medicine, Abdominal surgery and Pathology, University Hospital, Leuven, Belgium

Patients with T3/T4 rectal cancers were injected i.v. with 400–555 MBq F-18 FDG. Dynamic imaging was immediately started. Maximum tumor glucose utilization was calculated. Cell kinetics were measured by flow cytometry 6–8 hrs after i.v. injection of IUdR.

Two groups of patients were studied: 1) surgery only (n = 8); and 2) preoperative radiotherapy (30 Gy/10 fractions) followed by surgery (RT, n = 6). At baseline, TuGluc for group 1 was 280 ± 141 (SD) nmol/min/ml, and for group 2:269 ± 161 (P = NS). After RT, TuGluc decreased to 109 ± 61 ($P > 0.05$). Tpot was 3.50 ± 1.21 days for group 1 and 3.23 ± 2.23 days for group 2 before RT (P = NS). A negative correlation was found between TuGluc and Tpot, suggesting increasing glucose utilization for faster dividing cells. After RT, Tpot did not change significantly (4.88 ± 3.80 days), whereas TuGluc fell significantly. *In conclusion:* these results show a direct correlation between tumor cell metabolism and tumor cell proliferation *in vivo*. RT results in a decrease in TuGluc utilization indicating cell loss due to RT, because the proliferating cells don't proliferate more slowly, and so should not have a smaller TuGluc.

822 ORAL

ASSESSMENT OF HUMAN FIBROBLAST RADIOSENSITIVITY BY THE MICRONUCLEUS AND COLONY-FORMING ASSAY

J. Johansen, S.M. Bentzen, J. Overgaard, B. Stausbøl, M. Overgaard, C. Fuhrmann, C. Streffer
Danish Cancer Society and Department of Oncology, University Hospital of Aarhus, Denmark
Institut für Medizinisches Strahlenbiologie, Essen, Germany

The relationship between radiosensitivity of normal human skin fibroblasts determined by the micronucleus (Mn) assay and the colony-forming assay was investigated. Prediction of radiation-induced normal-tissue response in individuals necessitates a rapid and reproducible *in vitro* assay that correlates well with cell survival after irradiation. The Mn-assay is a quick test that was applied to primary skin biopsies from 10 unselected breast cancer patients in whom a significant correlation previously was found between the expression of subcutaneous fibrosis after radiotherapy and the cell surviving fraction at $SF_{3.5}$ (Spearman's rho = −0.81, $P < 0.01$). Early generations of fibroblasts in exponential growth were irradiated with 250 kV X-rays at room temperature. The micronucleus frequency in Cytochalasin B-induced binucleated cells (Mn/BNC) was scored after doses of 0, 1, 2, and 3.5 Gy, and Mn/BNC after irradiation with 3.5 Gy was estimated (range 1.11–1.77). Mn/BNC at 3.5 Gy showed no correlation with $SF_{3.5}$ as determined by the colony-forming assay. A possible reason for lack of correlation include variable numbers of scorable binucleated cells after irradiation with 3.5 Gy (4%–21%).

823 ORAL

IN VIVO AND *IN VITRO* EFFECTS OF TIRAPAZAMINE (SR-4233) ALONE OR COMBINED WITH RADIATION OR CYTOTOXIC DRUGS ON HUMAN CELL LINES

E. Lartigau, M. Guichard
Laboratory of Radiobiology, Institute Gustave-Roussy, 9488 Villejuif, France

Solid human tumours contain areas with low oxygen tension (pO_2). For bioreductive drugs it is important to define the cytotoxic effect according to drug concentration and to clinically relevant pO_2. *In vitro*, the pO_2 dependence of the survival of three human cell lines (HRT 18, Na11 +, and MEWO), exposed to tirapazamine alone or combined with ionizing radiation, was studied at five different oxygen concentrations: air (20.9% O_2), 10, 2, 0.2 and 0.02% O_2 (hypoxia). Tirapazamine below a concentration of 100 μM was not cytotoxic in air or at 10% O_2. At 100 μM tirapazamine was toxic in 2% O_2, and at 50 μM in 0.2% O_2. For $pO_2 < 0.2\%$ O_2, there was a marked increase in cell killing when 10 μM tirapazamine was combined with 2 Gy, compared with either 10 μM or 2 Gy given alone ($P < 0.03$). *In vivo*, tirapazamine was combined with various drugs (5 FU, VP 16, DTIC, CDDP, BLEOMYCINE). Mice lethality, regrowth delay and excision assay were studied. In the latter, the most effective combination was tirapazamine-bleo for HRT 18 and tirapazamine-VP 16 for NA 11+. The activation of tirapazamine at a low concentration and at pO_2 found mainly in tumours could yield a very

beneficial therapeutic ratio. Such a bioreductive drug will be preferably used in combination with ionizing radiations and/or cytotoxic drugs.

824 ORAL

HIGH DOSE RATE BRACHYTHERAPY: DOSE ESCALATION IN THREE-DIMENSIONAL MINIORGANS OF THE HUMAN BRONCHUS

M. Souvatzoglou, A. Leberig, F. Gamarra, R.M. Huber
Pneumology, Klinikum Innenstadt, University of Munich, Ziemssenstr. 1, D-80336 München, Germany

Aim of the study was to establish an ex-vivo three-dimensional cell culture system (minorgans) for evaluation of high dose rate brachytherapy in the human bronchus. *Methods:* Non-malignant bronchial tissue was obtained by bronchoscopy. The biopsies were cultured as described (Gamarra F *et al.* Eur. Respir. J. *6* (1993), Suppl. 17, 182s). After 3 weeks the acellular stroma is surrounded by multilayer respiratory epithelium. The miniorgans were then exposed to different dosages of Iridium 192 (30, 45, 60 and 75 Gy). After irradiation the miniorgans were brought back to culture or were incubated in trypsin to obtain a single cell suspension. To measure vitality the cells were incubated for I rain with acridinorange and ethidiumbromid. The percentage of nonvital cells was counted under microscopic view with uv light. *Results:* 10 miniorgans were irradiated. There was no significant difference in vitality between the control group and the group with 30 Gy (10% nonvital cells). The percentage of nonvital cells increased significantly after 45 Gy but remained constant after 60 Gy. The maximum was after 75 Gy (25%). The reincubated miniorgans (45 Gy) were examined after 2 weeks. There were significant more nonvital cells compared to those examined three hours after irradiation (24%). *Conclusion:* Human bronchial epithelium may tolerate higher radiation dosages. Further experiments will focus on time-depending aspects of irradiation induced cell death. This may be of importance for brachytherapy of lung cancer.

825 POSTER

CELL CYCLE EFFECTS OF TAXOL IN A MURINE TUMOUR AND ITS IMPLICATIONS IN RADIOSENSITIZATION

A. Cividalli, E. Cordelli, E. Livdi, D. Persiani, D. Tirindelli Danesi
AMB Bio Med, Enea CR Casaccia, Rome, Italy

The new chemotherapeutic agent taxol (TX) was tested as single agent and combined with an X-ray treatment in a murine mammary carcinoma. Furthermore we performed DNA flow cytometric analysis of tumour cells after *in vivo* TX treatment, to assess the proliferative perturbation of cell cycle. Female hybrid (C3H/RixDBA/2J) mice were used. TX (Paclitaxel, Bristol Myers Squibb P.R.I.) was administered i.p. in single doses of 30, 45, 60, 75 mg/kg b.w.. Irradiation was delivered with an X-ray machine (operating at 15 mA, 250 kV, 0.5 mm Cu filter). TGT4 (the time needed to reach 4 times the initial treatment volume) was evaluated as end-point. In the tested range there was a linear dose-response between tumour growth delay and TX dose. In the combined protocol TX was administered 30 min before a 10 Gy X-ray treatment. Our results in the combined treatments show a linear regression line almost parallel to that resulting in TX alone, with a growth delay of about 6 days. The effect seems to be additive. Flow cytometric analysis demonstrated a G2/M block of tumour cell, induced by both the tested TX concentrations (30 and 45 mg/kg). An increase of G2/M fraction was evident 8 h after treatment, and about 60% of cells were in G2/M within 24 h. After that, cells began again to divide, and after 48 h the percentage of G2/M cells decreased; furthermore, a depletion of cells in S phase was obtained suggesting that TX also avoids the starting of DNA synthesis. Considering these results, combined treatment with an interval of 24 h between TX administration (45 mg/kg) and X-ray treatment (10 Gy) was performed. Although the result obtained with the last schedule was better than the previous one, no significant difference between the two protocols was observed.

826 POSTER

CISPLATIN, CARBOPLATIN AND IRRADIATION IN HUMAN OVARIAN CANCER CELLS

C.H. De Pooter, P. Scalliet, A.T. Van Oosterom
Lab. Ca. Res. & Clin. Onc, Antwerp University, 2610, Belgium

Cisplatin (CDDP) and carboplatin (CBDCA) have a similar spectrum of antitumor activity, but a different toxicity. The combination with radiotherapy has been introduced in order to increase therapeutic efficacy. Therefore, we examined whether CDDP and CBDCA interact in the same way with irradiation.,

Human ovarian cancer cells were continuously exposed to CDDP (0.5, 1, 2.5, 5 μM) or CBDCA (2.5, 5 μM) 16 h and 4 h before and after irradiation with cobalt 60 γ-rays. Survival was determined with the clonogenic assay. Interaction was evaluated by determining the dose-enhancement-factors (DEF) and by constructing the isobolograms at different survival levels.

The combination of CDDP and irradiation resulted in an independent cell kill. There was no modification of the survival curve. The DEF = 0.89–1.08 and in the isoeffect-plot the interaction was additive.

Five μM CBDCA before or 4 h after irradiation resulted in supra-additivity. There was an abolition of the shoulder of the dose survival curve. The DEF >1 (1.69–2.68 at 10% survival) and higher at higher survival levels. In the isoeffect-plot the interactions were supra-additive. The combination with CBDCA 16 h after irradiation (DEF = 0.85–0.96) or at 2.5 μM (DEF = 1) was additive.

CBDCA induced enhancement of cell kill for irradiation in certain sequences and from a certain dose on. The combination with CDDP was purely additive. In our study, CBDCA was much better than CDDP as far as interaction with irradiation was concerned.

827 POSTER

RADIOTHERAPY-ENHANCING EFFECT OF IFOSFAMIDE IN HUMAN NSC LUNG CANCER XENOGRAFTS

W. Hinkelbein, T. Ruhnau, R. Schlag, H.-H. Fiebig
Department of Radiotherapy, University Hospital Benjamin Franklin, Free University 12200 Berlin, Germany

The "radiosensitizing" effect of ifosfamide (IFO) was investigated in 6 different human NSCL and 1SCL cancer xenograft, growing subcutaneously in nude mice. Given alone and combined with IFO radiotherapy (RT) was administered in daily fractions of 2 Gy for 10 days. IFO alone was administered at 130 mg/kg/day intraperitoneally on day 0–2. In combined treatments IFO was injected at 100 mg/kg/day 2 hours before RT. Irradiation in combined treatments was given for 7 and 10 days, respectively. Therapy started with average tumor volumes of 200 to 500 m^3. IFO increased the effect of RT in 4 out of 7 xenografts. An increase in tumor inhibition and also an additional growth delay was observed in 3 squamous cell carcinomas (LXFE 397, 409, 937) and 1SCLC (LXFS 650). In 1 squamous cell carcinoma (LXFE 839) and the adenocarcinoma LXFA 629 there was no synergistic effect. In the large cell model LXFL 529 IFO alone induced complete tumor remission. Our study demonstrates radiopotentiating effect of IFO in NSCLC and also in the SCLC model, suggesting clinical studies.

828 POSTER

EFFECT OF EPIDERMAL GROWTH FACTOR (EGF) ON ACUTE RADIATION DAMAGE TO MOUSE INTESTINE AND EPIDERMIS *IN VIVO*

J.C. Lindegaard, L. Vinter-Jensen, J. Overgaard
Department of Experimental Clinical Oncology, Danish Cancer Society
Department of Oncology
Department of Clinical Biochemistry, Aarhus University Hospital, DK-8000 Aarhus, Denmark

Epidermal growth factor (EGF) has been shown to play an important role in growth and maintenance of intestinal mucosa and epidermis. The aim of the present study was to investigate if extent and time course of acute radiation damage to epidermis and intestine could be moderated by EGF. Twelve-to-sixteen weeks old female CDF1 mice were treated either by single dose total body irradiation (TBI) or local irradiation to the right hind leg. EGF was given s.c. at a dose of 5 μg/day for 4 weeks alter radiation. Control animals were treated with isotonic NaCl. During the first week after local radiation weight increased by 10% and 0% in EGF and NaCl treated animals, respectively. However, median skin score was marginal but not significantly smaller in EGF treated animals. Lethality by day 7 alter TBI has previously been shown to be caused by intestinal damage. EGF did not influence day 7 lethality after TBI.