	New Pts (24)	Previous treatment (49)	P value
Response			
CR	13 (55%)	11 (23%)	0.007
PR	9 (37%)	20 (40%)	NS
NR	2 (8%)	15 (31%)	0.03
Died	0 (0%)	3 (6%)	
Engraftment			
Pits 25 × 10^9/l	14 days	17 days	0.004
Neuts 1000/L	24 days	34 days	0.002
Inf start (Med)	55 days	68 days	0.017

The overall survival by the Kaplan Meier estimate at 2 years is 79.3% with a median follow up of 7.75 months and the progression free survival is 58% in the whole group of 73 patients. Interferon (inf) maintenance was started at a median of 61 days in 58/73 patients. We therefore conclude that the previously untreated group is a better risk group with respect to achieving remission as well as rapid engraftment. Inf was also started earlier in the untreated group. Longer follow up will be required however to comment on the efficacy of PBSCT. A CR rate of 55% in previously untreated patients is lower than our previously reported CR rate with ABMT in a comparable group of patients and the possibility of contamination of the stem cell grafts with myeloma should be borne in mind.

815 PUBLICATION

CA-125 SERUM LEVELS SIGNIFICANTLY CORRELATE WITH PROBABILITY OF RESPONSE IN MALIGNANT LYMPHOMAS

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Aim: to determine whether CA-125 serum levels correlate with disease extension and prognosis in patients with malignant lymphoma.

Methods: Ca-125 serum levels were assessed by using the OC 125 monoclonal antibody before treatment in 53 consecutive patients with malignant lymphoma (41 NHL/12 HD) and no sign of concomitant chronic hepatic disease.

Results: Increased serum levels (> 30 IU/ML) were observed in 27/53 (51%) pts. A significant difference between NHL (22/41) and HD (5/12) was not found. To date, 42 pts. are evaluable for response after the third cycle of chemotherapy. Basal abnormal CA-125 serum levels were associated with a significantly lower major response rate (14/22 vs 19/20; $P = 0.015$). At multivariate logistic analysis, presence of extranodal involvement was the only independent variable predictive of response. A further statistical estimation was made by considering a different cut-off for CA-125 (> 100 vs ⩽ 100). Only 2 of the 8 pts. with > 100 IU/ml CA-125 serum levels showed major responses, as compared to 31/34 responses in the other group ($P = 0.0004$). At logistic analysis CA-125 serum level > 100 IU/ml was the only parameter which significantly correlated with a lower response-rate ($P = 0.03$).

Conclusions: abnormal CA-125 serum levels are present in a significant rate of patients with malignant lymphoma. Our preliminary results show that very high serum levels (>100 IU/ml) correlate with a lower probability of response.

816 PUBLICATION

DEVELOPMENT OF AN ACTIVE CHOP-MODIFIED REGIMEN WHICH ALLOWS MORE CONTINUOUS AND BETTER TOLERATED TREATMENT FOR DIFFUSE AGGRESSIVE NON-HODGKIN'S LYMPHOMAS (NHL)

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A CHOP-variant regimen was developed in order to allow administration of full-doses therapy with reduced gastrointestinal and neurologic toxicity. The traditional CHOP regimen was modified as follows. Adriamycin 25 mg/m^2 iv day 1 and day 8, Cyclophosphamide 500 mg/m^2 iv day 1 and day 8, Vincristine 1.2 mg/m^2 iv day 1 and day 8, Prednison 50 mg/m^2 po days 1–8. Vincristine doses did not exceed 2.0 mg. The regimen was repeated every 21 days for 6–8 cycles. This schedule, allowing a more continuous treatment, would also adopt some of the basic concepts of the 2nd and 3rd generation regimens. Between March 1989 and March 1995 42 patients (age 25–84 yr) with stage II–IV diffuse aggressive NHL (Working Formulation F, G, H) were treated with acceptable toxicity. Most patients had grade 2 leukopenia and/or thrombocytopenia. No platelets transfusions were needed and the use of growth factors (G or GM-CSF) was not required. No life-threatening infections and no toxic deaths were observed. The regimen was safely administered also in elderly patients (17 patients had > 70 years). The large majority of patients experienced only mild nausea and vomiting. All patients had grade 3 alopecia.

The overall response rate was 85% (56% CR and 29% PR). Actuarial 3-years failure free survival is approx. 45%. These results appear to superimpose those achieved in the SWOG/ECOG trial (Fisher *et al.* NEJM, 1993).

This regimen therefore represents a CHOP variant that retains efficacy and appears easier to be administered especially in elderly patients.

Radiobiology

817 ORAL

TIME COURSE OF RADIATION-INDUCED APOPTOSIS IN THE ADULT RAT SPINAL CORD

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Radiation-induced apoptosis has been reported in thymic, lymphoid and hematopoietic cells but is infrequently documented in other adult mammalian cell types. In this study, we examined the time course of radiation-induced apoptosis in the adult cervical rat spinal cord following a single dose of 8 or 25 Gy. Apoptosis was assessed by morphological criteria under light and electron microscopy, and immunohistochemically in-situ using ApopTag to detect 3'-OH ends of DNA fragments. Little evidence of apoptosis (0.3 ± 0.3 apoptotic nuclei/spinal cord section) was observed in control un-irradiated spinal cord. A significant increase in the number of apoptotic cells was seen at 4 hr, the number peaked at 8 hr (53.7 ± 3.5 per spinal cord section after 8 Gy, and 60.7 ± 8.7 after 22 Gy) and returned to the baseline level by 24 h. A dose of 22 Gy induced apoptosis than 8 Gy at 4, 6, 10 and 12 h ($P < 0.006$), but not at 8 h. More apoptosis was observed in white matter (79 ± 3%) than in gray matter (21 ± 3%). All the apoptotic cells were observed in GFAP negative glial cells and none in vascular endothelial cells and neurons. We conclude that apoptosis in glial cells may represent a biologically relevant mechanism of radiation induced cell kill in the central nervous system. (Supported by NCIC.)

818 ORAL

RELATIONSHIP BETWEEN P53 CONSTITUTIVE/INDUCED LEVELS AND CELLULAR RESPONSE TO RADIATION

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The inhibition of replicative DNA synthesis, in which p53 has a pivotal role, is an important component of the cellular response to radiation-induced DNA damage. In this study we have examined the relationship between p53 levels before and after irradiation, radiation-induced cell cycle delays and radiosensitivity in a panel of 8 human tumour cell lines.

The cell lines differed widely in their clonogenic survival after radiation, (SF2 = 0.18–0.82). Constitutive p53 protein levels varied from 2.2 ± 0.4 to 6.3 ± 0.3 OD units per 10^6 cells. p53 after irradiation (6 Gy) also varied among the cell lines, ranging from no induction to a 1.6 fold increase in p53 levels 4 hours after treatment.

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

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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

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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

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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

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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

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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