to the drug of Balb/c 3T3 cells, transfected with H-ras or v-myc. H-ras cells were more resistant than control or v-myc cells. H-ras + v-myc cells were extremely sensitive. Several drug resistance mechanisms were investigated: Intracellular levels of glutathione, methallothioneins and cisplatin accumulation. No single mechanism tested was solely responsible for the pattern of cisplatin resistance. Topoisomerase I amounts and activity was reduced in resistant, H-ras cells, compared to sensitive ras + myc transfected cells. In addition, ras + myc transfected cells, showed unusually high amounts of p53 levels. The pattern of cisplatin sensitivity corresponded directly to the ability of our cells to undergo apoptosis by this drug. We conclude that the oncogenes H-ras and v-myc can modulate drug resistance through apoptosis, in conjunction with changes in p53 and topo I activity.

126 POSTER

INTERACTIONS AND CROSS RESISTANCE PATTERNS BETWEEN VARIOUS SCHEDULES OF 5-FU AND THE NEW, FOLATE-BASED THYMIDILATE SYNTHASE INHIBITOR TOMUDEX (D1694)

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Tomudex (ICI-D1694) is a new, specific inhibitor of thymidilate synthase, based on a folate structure. It has shown promising activity in advanced colorectal carcinoma (response rate 26%). Since it shares the cellular target with 5-FU, the second active drug for colorectal cancer, a detailed evaluation of the interaction of these drugs and of the cross resistance patterns will be important.

Methods: The human colorectal carcinoma cell lines HT29 and HCT8 were used for the interaction studies; the interactions were evaluated by standard isobologram methodology. Four 5-FU resistant sublines, made resistant to either a 1 h application of 5-FU (HT29-1R, M2-1R) or 24 h application of 5-FU (HT29-24R, M2-24R) were used for the cross resistance studies (*AACR* 1995, **1889**). Cytotoxicity was evaluated by the sulforhodamine-B-assay.

Results: Tomudex and 5-FU showed partial cross resistance. Tomudex was active in both cell lines with acquired resistance to a 1 h application of 5-FU whereas both cell lines made resistant to 24 h of 5-FU were highly cross resistant to Tomudex.

When 5-FU and Tomudex were given simultaneously for 24 h, significant synergistic interactions were seen in both colorectal cancer cell lines. However, when 5-FU was given for 1 h prior to a 24 h incubation of Tomudex, a strong antagonism was seen for higher doses of 5-FU combined with low doses of Tomudex, whereas low doses of 5-FU and high doses of Tomudex proved to be synergistic. Reversing the schedule (24 h Tomudex followed by 1 h of 5-FU) resulted in synergistic interactions for all ratios of drugs.

Conclusions: Tomudex exhibits partial cross resistance to 5-FU, especially in cell lines which have been pretreated with protracted schedules of 5-FU. The interactions between Tomudex and 5-FU are schedule dependent. A combination of protracted infusion of 5-FU (e.g. 24 h) and Tomudex appears to be the most active combination. These data might serve as a basis for the design of clinical trials.

127 POSTER

MODULATION OF CIS-DIAMMINEDICHROLO-PLATINUM (II) SENSITIVITY BY A THROMOBOXANE A2 RECEPTOR ANTAGONIST IN NON-SMALL CELL LUNG CANCER CELL LINES

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We evaluated the effect of thromboxane A2 (TXA2) receptor antagonists, calcium 5 (Z)-1R, 2S, 3S, 4S-7-[3-phenylsulfonylamidnobicyclo [2.2.1] hept-2-yl]-5-heptonoate hydrate (S-1452) on cis-diamminedichrolo-platinum (II) (CDDP) sensitivity in PC-9, a non-small cell lung cancer (NSCLC) cell line, and PC-9/CDDP (6.0-fold resistant to CDDP) *in vitro*. In PC-9 cells, treatment with 250 or 500 μM of S-1452 caused 2.1-fold and 4.6-fold increase in IC50 values, respectively. In PC-9/CDDP cells, treatment caused 3.1-fold and 6.0-fold increase in IC50 values. Glutathione contents and glutathione S-transferase activities of these cell lines were not affected by treatment with S-1452. Uptake of CDDP after 2 h drug exposure into PC-9 was 1.3-fold increased by treatment with 500 μM of S-1452 and that into PC-9/CDDP was 1.4-fold increased. These results suggest that TXA2 receptor might be related with sensitivity to CDDP in NSCLC cell lines and increase in CDDP uptake might contribute to the sensitizing effect of S-1452.

128 POSTER

RECOMBINANT DEOXYRIBONUCLEASE I (DNASE I) AND CHIMERAS IN CANCER THERAPY

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DNase I, an endocuclease that degrades double stranded DNA, represents an attractive candidate for tumour targeting since it is normally nontoxic yet highly cytotoxic when redirected to the cell nucleus.

The aim of this study is to explore the cytotoxic potential of mammalian DNase I, recombinantly produced (rDNase) and its chimeras with a tumour-specific single chain antibody (ScFv) directed against human placental alkaline phosphatase.

We studied several bacterial expression systems for the production of rDNase, all cases resulting in no or minimal yields due to enzyme lethality. We identify a tightly controlled T7 promoter-based system, employing M13 phage supply of T7 RNA polymerase, as essential for expression, resulting in overproduction of active rDNase and its chimeric fusions. We describe the construction, expression in E. coli and characterisation of these molecules, showing that they possess DNA degrading and antigen-binding activities when refolded from bacterial inclusion bodies. Metal affinity chromatography was used for protein purification. Direct cytotoxicity of rDNase was tested bycell micro-injections whereas the efficacy of cell killing of chimeras was determined on antigen-positive cells *in vitro* and in xenograft models.

Targeting mammalian enzymes provides a novel therapeutic strategy for selective cell-killing, with less systemic toxicity and immunogenicity than currently used immunotoxins.

129 POSTER

MRP-, MDR1 EXPRESSION AND RHODAMINE-123 EFFLUX IN B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

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Resistance to cancer chemotherapy represents a major problem in the treatment of human neoplasms. We investigated the expression of multidrug resistance-associated protein (MRP) mRNA and of classical multidrug resistance (MDR1) mRNA in 27 patients suffering from B-cell chronic lymphocytic leukemia (B-CLL) by a quantitative polymerase chain reaction (PCR) assay. In addition, efflux of the fluorescent dye rhodamine 123 (Rh123) from the malignant B lymphocytes was measured to evaluate functional activity of the membrane transporter P-glycoprotein. MRP mRNA was detected in all 27 patients analyzed showing low (n = 8), intermediate (n = 9) and high (n = 10) levels of expression. MRP expression was associated with disease progression ($P < 0.005$) in as much as patients with progressive disease had low levels of MRP mRNA. MRP expression was also associated with leukocyte count ($P < 0.01$) but not with Rai stage, duration of disease or prior treatment. Low levels of MDRI mRNA were found in 96% and Rh123 efflux in 89% of B-CLL cases. Rh123 efflux correlated well with MDR1 ($P < 0.0001$) but not with MRP mRNA expression.

130 POSTER

DIHYDROPYRIMIDINE DEHYDROGENASE AS A PIVOTAL TARGET FOR FU BIOMODULATION. ROLE OF 5-ETHYNYLURACIL

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Dihydropyrimidine dehydrogenase (DPD) is the rate-limiting enzyme of fluorouracil (FU) catabolism. Ethynyluracil (776C) is a very potent, mechanism-based irreversible DPD inhibitor that improves the antitumor efficacy and the therapeutic index of FU in laboratory animals. We tested the cytotoxic effects of the FU-776C combination on a panel of 12 human cancer cell lines (4 breast, 4 head and neck, 3 colon, 1 duodenum). Basal DPD activity (radioenzymatic assay) and FU sensitivity (FU IC50, MTT test) were determined. The FU potentiation by