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

776C was calculated from the ratio (F) of FU IC50 without 776C divided by FU IC50 with 776C. 776C was not cytotoxic to any of the cell lines tested. On CAL51 cell line, expressing a high basal DPD activity, FU enhancement by 776C was a saturable phenomenon related to the 776C concentration; the inhibition of DPD increased between 10^{-12} to 10^{-6} M of 776C. For the following studies, 776C was tested at 10^{-6} M. FU IC50 varied from 15 to 7770 μM among cell lines (median 390 μM). Basal DPD activity ranged from not detectable (< 1 pmol/min/mg prot) to 320 pmol/min/mg prot among cell lines (median 53 pmol/min/mg prot). For the 12 cell lines tested, the mean F ranged from 0.7 (no enhancement of FU cytotoxicity by 776C) up to 5.2 and was significantly related to the basal DPD activity: the greater the DPD activity, the greater the FU enhancement factor (Spearman rank correlation, $P = 0.019$). Enhancement of FU cytotoxicity by 776C occurred only in the 6 cell lines expressing the greatest basal DPD activity (> 50 pmol/min/mg prot, F ranging between 1.7 and 5.2), whereas 776C did not modify FU cytotoxicity in the remaining cell lines expressing the lowest DPD activity (< 50 pmol/min/mg prot, F ranging between 0.7 and 1.4); F was significantly different between these 2 groups of cell lines ($P = 0.005$). These results justify clinical trials with DPD inhibitors like 776C.

131 POSTER

CLINICAL RELEVANCE OF P-GLYCOPROTEIN-RELATED RESISTANCE IN PATIENTS WITH ACUTE LEUKEMIA

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Between 1989 and 1994 P-gp expression was prospectively studied in mononuclear bone marrow cells of 304 (221 AML; 83 ALL) acute leukemia patients. In 282 patients P-gp was investigated before and after therapy and in 22 patients only before therapy: 148 AML patients with AML-6 protocol (EORTC), containing daunorubicin, vincristine and conventional-dose cytarabine (ara-C), and 63 AML patients were treated with intermediate-dose ara-C plus amsacrine. Further 71 ALL patients were treated according to a German standard polychemotherapy protocol (BMFT04/1989). For AML patients with P-gp overexpression at primary diagnosis or early relapse/refractoriness, the predictive values for nonresponse to AML-6 protocol were 90% and 94% respectively, while late-relapsed AML patients with P-gp overexpression had a significantly ($P < 0.05$) lower predictive value of 73% for nonresponse. Additionally, in refractory and late-relapsed P-gp-overexpressing AML patients treated with intermediate-dose ara-C plus amsacrine the predictive values for nonresponse were 44% and 38%, respectively, significantly ($P < 0.05$) lower as compared to AML-6 protocol-treated refractory or late-relapsed AML patients. In P-gp-overexpressing treated ALL patients the predictive values of 50% and 55% for nonresponse were calculated at primary diagnosis and late relapse, respectively. P-gp overexpression is a common phenomenon in AML patients and has an inverse influence on AML-6 treatment outcome.

132 POSTER

MRP GENE EXPRESSION IN COLORECTAL CARCINOMAS

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To determine the clinically important mechanisms of multidrug resistance, we studied the expression of the MRP gene in primary colorectal carcinomas (N = 75). MRP RNA was determined by RT-PCR. MRP RNA was detected in 62 (83%) tumor specimens. The expression was independent of size and localization of the primary tumor, lymph node involvement, tumor stage and the survival durations of the patients. However, MRP gene expression correlated with MDR1 gene expression. In conclusion, the frequent expression of the MRP gene suggests its importance as a drug resistance gene in colorectal carcinomas. (Supported by Austrian Science Foundation.)

133 POSTER

EFFECT OF PACLITAXEL ON THE UPTAKE OF CIPROFLOXACIN AND OFLOXACIN BY HUMAN NEUTROPHILS

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Ciprofloxacin (CPLX) and ofloxacin (OFLX) are antimicrobial agents which concentrate and remain active within phagocytic cells. We have evaluated by a fluorometric assay the effect of paclitaxel in comparison with methotrexate, doxorubicin, Cis-platinum and etopoxide on the intracellular penetration of CPLX and OFLX in human neutrophils. The preincubation of cells for 30 min at 35°C with therapeutical concentrations of these antineoplastic agents yielded the following cellular to extracellular concentration ratio values (C/E) for CPLX and OFLX (at 20 min; 35°C; extracellular concentration: 5 mg/l).

		C/E	
Antineoplastic	**mg/l**	**CPLX**	**OFLX**
None		4.7 ± 1.2	4.6 ± 0.9
Paclitaxel	5	4.8 ± 1.0	4.3 ± 0.4
Methotrexate	10	5.1 ± 1.2	4.4 ± 0.7
Doxorubicin	1	14.3 ± 1.4	4.6 ± 1.1
Cis-Platinum	10	4.7 ± 1.6	3.7 ± 0.5
Etopoxide	10	5.5 ± 1.0	4.8 ± 1.3

Similar results were obtained when other extracellular concentrations of the antineoplastic agents were used. It is concluded that paclitaxel and the other drugs evaluated did not affect the intracellular penetration of quinolone antimicrobial agents.

134 POSTER

ENHANCED TUMOR RADIOIMMUNOTARGETING OF CHIMERIC ^{125}I-BR96-BIOTIN IN A SYNGENEIC RAT TUMOR MODEL USING WHOLE BLOOD EXTRACORPOREAL IMMUNOADSORPTION (ECIA)

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Chimeric BR 96 is a human IgG1 isotype with a high tumor selectivity for most human carcinomas of breast, lung, ovary, and gastrointestinal tract. The rapid internalization into tumor cells is another important feature for BR96. The aim of the study was to investigate if whole blood ECIA has an influence on tumor and normal tissue radioimmunotargeting.

Material and methods.: 30 BN-male rats inoculated intramuscularly (IM) and beneath liver- or kidney capsule (SR) with syngeneic rat colon carcinomas, expressing Ly Ag, were investigated. The rats were injected i.v. with 3.5–4.5 MBq of ^{125}I-BR96-biotin. ECIA of whole blood, using avidin-gel adsorption column, was performed 12 h after injection of Mab. *Results:* After completion of ECIA, whole body radioactivity was reduced by 48–62%, and plasma activity (%/g) by 85%. After finish of ECIA, the uptake in the liver-, SR-, and IM-tumors decreased by only 11, 23 and 13%, respectively, whereas the uptake in normal tissues was considerably diminished. T(umor)/bone marrow, T/liver, T/kidney and T/lung uptake ratios were enhanced in all 3 tumor models by a factor varying from 2.2 to 4.2. The uptake of Mab in Liver and IM-tumor was enhanced by increasing amount of Mab injected. *Conclusion:* ^{125}I-BR96-biotin proved high tumor-to-normal tissue ratios, which were even more enhanced by ECIA of whole blood.

135 POSTER

PHARMACOKINETICS AND PHARMACODYNAMICS OF TENIPOSIDE (VM26) COADMINISTERED WITH CYCLOSPORIN A (CSA) IN PATIENTS WITH METASTATIC RENAL CELL CANCER (RCC)

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Background: Chemosensitizers could alter the pharmacokinetics (PK) and pharmacodynamics (PD) of antineoplastic drugs. It was previously demonstrated that CsA modifies PK and PD of etoposide, an analog of VM26, but the effect of CsA on VM26 has not been clarified yet.

Methods: Thirteen patients with RCC in progression after standard therapy were accrued. Demographics: median age 61 years (range 44–75), male:female 9/4, median WHO P.S. 2 (range 1–3). The patients