analysis. Kruskal-Wallis one-way nonparametric AOV was used to estimate whether the LRP expression was related to: tumor differentiation (well, moderately, poorly); TNM classification and histology. IH of the tumor sections showed different expression of LRP in the different histological subtypes of lung cancer (squamous cell carcinoma 83%; adenocarcinoma 59%; large cell/undifferentiated carcinoma 36% and SCLC 5%). LRP expression was significantly higher in squamous cell carcinoma than in the other subtypes ($P < 0.05$). Furthermore adenocarcinomas showed a significant ($P < 0.05$) higher LRP expression than the SCLC. No significant difference in expression levels was found between patients with different TNM-classification or tumor differentiation. In this relatively small group of patients, there was no relation between LRP expression and survival. Prospective research is being performed in patients undergoing chemotherapy.

121 POSTER

IDOXIFENE DELAYS ACQUIRED ANTI-OESTROGEN RESISTANCE IN MCF-7 HUMAN BREAST CANCER XENOGRAFTS

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Idoxifene (IDOX) is a new anti-oestrogen with less agonist activity than tamoxifen (TAM). We (i) compared the inhibition by IDOX and TAM of the growth of MCF-7 breast cancer xonografts, (ii) determined whether IDOX delayed acquired resistance, and (iii) assessed whether IDOX inhibited the growth of TAM resistant tumours. Forty tumours were established with oestradiol (E2) support in ovariectomised athymic mice and continued with E2, no support, TAM or IDOX (mean serum TAM 35 ng/ml, IDOX 28 ng/ml). The reductions in tumour volume (mean percentage baseline ± SEM) after 2 and 6 m were as follows: TAM 71.8% (± 10.5) and 81.1% (± 14.8); IDOX 47.2% (± 9.3) and 51.0% (± 14.3); E2 withdrawal 30.7% (± 5.2) and 13.1% (± 4.3), respectively. IDOX appeared to give greater tumour regression compared with TAM. Furthermore, after 6 months 3/10 TAM-treated but 0/10 IDOX-treated tumours developed acquired resistance and started to re-grow. In separate studies significantly fewer TAM-resistant tumours were supported by IDOX than by TAM (3/12 vs 8/12; $P = 0.04$ Chi-Squared). These data indicate that IDOX shows reduced growth support of MCF-7 xenografts compared with TAM and appears to delay the development of acquired resistance. Furthermore IDOX may share only partial cross-resistance with TAM. The reduced agonist activity of IDOX may, in part, explain these observations.

122 POSTER

MARINE (MA) DEPSIPEPTIDES (DEP) WITH ACTIVITY (A) AGAINST SOLID TUMOURS (ST) MODELS

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Two MADEP from our R + D program are presented. KahalalideF (KF) is a MADEP isolated from a Hawaiian mollusc; it displays selective A *in vitro* (IVT) in Prostatic cancer cell lines (PROca) (TG1 at 4.22E−07 MOLAR), COMPARE analysts (COa) fails to match KF with any standard anticancer drug and initial data indicates KF promotes an extensive vaculorization in cultures COS1 and HELA cells and might interact with specific component(s) of the cell surface. *In vivo*, KF lacks A in P388 but has A in A549 lung ca xenograft (X) (37% T/C at KF dose = 2 mg/kg/Q4D × 3). Thiocoraline (THIO), MADEP isolated from a MA micro-organism from Mozambique; THIO has IVT A in melanoma, colon and lung ca cell lines (TGIs = 4.09E-09, 2.50E-09 and 2.50E-09 respectively). THIO binds to DNA (> luM); kinetic studies suggests THIO inhibits cell cycle progression at G1, S, G2 and M phases (reversible after washing) and COs matches THIO with doxo and daunorubicin. THIO lacks *in vivo* A in P388 but has A in A549 X (31%T/C; 6 mg/Kg/Q4Dx3). THIO assessment in NSCLC and colon Xs is ongoing. Large scale supply is feasible by fermentation.

123 POSTER

ACTIVITY OF N4-OCTADECYL-ARA-C IN HUMAN SOLID TUMOR XENOGRAFTS AND LEUKEMIAS

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A series of new Ara-C derivatives with alkyl chains at N4 has been tested in-vivo in subcutaneously growing human xenografts. The liposomal preparation of N4-Octadecyl-Ara-C (NOAC) was studied in 2 human leukemias and in 6 solid cancers. At the MTD of 150 mg/kg/day injected ip on day 1, 4, 7 and 10 NOAC showed a higher antitumor activity than the equitoxic dose of Ara-C in the promyelocytic leukemia HL-60, tumor volumes being 3% and 52% of the controls, respectively. In the acute T lymphoblastic leukemia CCRF-CEM both compounds were highly active. An impressive *in vivo* activity could be demonstrated in solid tumors. In the mammary cancer MAXF 401 NOAC effected a T/C of 18% versus 42% obtained with Ara-C, in the small cell lung cancer LXFS 605 T/C values were 27% versus 56%. The new derivative showed activity in a large cell cancer of the lung and in the prostate cancer PC3M with a T/C of 17%. The activity in PC3M was higher than obtained with 7 standard agents. In conclusion the new Ara-C derivative NOAC is a promising new compound which should be developed in solid tumors (mammary and prostate cancers) as well as in leukemias.

124 POSTER

ADDITION OF OXALIPLATIN (L-OHP®) TO CHRONOMODULATED (CM) 5-FLUOROURACIL (5-FU) AND FOLINIC ACID (FA) FOR REVERSAL OF ACQUIRED CHEMORESISTANCE IN PATIENTS WITH ADVANCED COLORECTAL CANCER (ACC)

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L-OHP®/5-FU/FA CM combination partially circumvented 5-FU resistance in patients (pts) with ACC (*Cancer* 1992, **69**, 893). L-OHP® delivered as q 3 wks CM or bolus showed a 10% (14/138) overall response rate (ORR) in ACC pts with proven progressive disease (PD) while getting 5-FU/FA treatment. The non-cross resistance and/or synergy of L-OHP® with 5FU/FA was assessed in 25 pts with acquired resistance (20 with CT scan-proven [PD]—and 5 with median 5 months [2.5–12] disease stabilisation [ST]) while on 5-day (d) CM 5-FU (700–1000 mg/sqm/d)/FA (300 mg/sqm/d) (peak delivery at 4.00 h) (FF). L-OHP® (20 to 25 mg/sqm/d, peak at 16.00 h) was added to this schedule in 2nd (12 pts) or 3rd line (13 pts) (FFL) according to 2 different schedules: 5d q 3 wks (14 pts) or 4 d q 2 wks (11 pts). *Selection criteria:* Pretreated ACC, no other intercurrent chemotherapy between the 2 schedules, measurable lesion. *PT Characteristics:* M/F = 10/15, median age = 59, colon/rectum = 14/11, PS 0–1 vs 2–3 = 22/3, nb of sites <2 vs ⩾ 2 = 8/17, liver involvement = 21 (94%). Previous 5-FU time exposition = median 7.2 months (1.5–25.2), FF (CM) median dose intensity (DI) = 1050 mg/sqm/wk. *FFL (CM) Treatment:* 171 cycles, median = 7 (1–15), time to exposition = 5 mo (1–9), 5-FU DI = median 1072 mg (831–1580), L-OHP® DI = median 35.7 mg (24–43). *Toxicity (per pt):* grade 3–4: nausea-vomiting = 25%, diarrhea = 16%, mucositis = 8%. No gr 3–4 hematotoxicity. No renal or auditive toxicity was observed. No toxic death. *Efficacy:* 7 PR (29.2%), 5 minor reponses (21%), 4 SD (17%) and 8 PD (33%). One pt too early. Duration of response was 8.5 mo, disease-free progression 5.8 mo and median survival 12 mo. *Conclusion:* Addition of L-OHP® can reversed FF resistance to CM 5-FU/FA in one third of pts. This further suggests a synergistic and/or modulatory effect between 5-FU and L-OHP®.

125 POSTER

DRUG RESISTANCE MECHANISMS TO CISPLATIN IN H-HAS AND V-MYC TRANSFECTED FIBROBLASTS

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Cisplatin is a widely used antitumor agent. To investigate the role of oncogenes in drug resistance to cisplatin we determined the sensitivity

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