Proffered papers

Drug resistance and pre-clinical drug development—new targets

105 ORAL

CORRELATION BETWEEN EXPRESSION OF MDR1, GST-π, K-RAS, MDR3, CEA IN HUMAN TUMOR XENOGRAFTS

C. Weissenberger, S. Heck, D.P. Berger, L. Herbstritt, S. Schulzke, W.A. Dengler, R. Mertelsmann, H.H. Fiebig
University of Freiburg, Department of Internal Medicine I, D-79106 Freiburg, Germany

The drug resistance due to the overexpression of MDR1 is a major obstacle to a successful chemotherapy of human malignancies. In order to survey the mechanisms of regulation of MDR1 the expression of various tumor characteristics were studied in 62 different solid human tumor types growing subcutaneously in nude mice (xenografts). Northern and slot blot analysis revealed a statistical significant correlation ($P < 0.05$) between MDR1 expression and expression of glutathione-S-transferase (GST-π), k-ras, MDR3, but not to the expression of p53. CEA-positive tumors showed a significant higher expression of MDR1 ($P < 0.05$) and GST-π ($P < 0.01$) than CEA-negative tumors. GST-π showed a highly significant correlation ($P < 0.001$) with c-myc, k-ras and a significant correlation with p53 ($P < 0.05$). Additionally, a relationship between mdr3 expression, the expression of the EGF receptor and k-ras was observed ($P < 0.05$) as well as a weak association with the chemosensitivity to doxorubicine ($P < 0.10$). No other significant correlation between MDR1, MDR3 or GST-π expression and the chemosensitivity to 14 tested anticancer drugs could be observed, indicating that drug resistance cannot be explained solely on the basis of the expression of these resistance factors.

In conclusion, the association of MDR1, MDR3 and GST-π with oncogenes (k-ras) as well as with other tumor characteristics (CEA, p53) implies the involvement of the regulation of these resistance factors in complex molecular events during the progression of human cancers.

106 ORAL

CHEMOSENSITISATION OF HUMAN ADENOCARCINOMA CELLS BY ANTISENSE AGAINST THE MULTIDRUG RESISTANCE (MDR1) GENE

P.J. Roberts, J.I. Penny[1], F.C. Campbell[1]
Departments of Surgery, Helsinki University Central Hospital, Finland
[1]Ninewells Hospital, Dundee, U.K.

The MDR1 gene encodes P-glycoprotein (Pgp) which pumps cytotoxic agents out of cells and thus induces chemoresistance. Inhibition of this gene could impair Pgp expression and sensitise the cells to cytotoxic drugs. Pgp expression (by Western blot), sensitivity to doxorubicin (by MTT assay) and efflux of the Pgp mediated substrata rhodamine were investigated in human (Hap 2A) adenocarcinoma cells before and after treatment by antisense (AS) and sense (S) oligonucleotides against MDR1.

Both AS1 (against initiation region) and AS2 (against loop forming site) significantly (approx. 50%) inhibited Pgp expression and gave a 10-fold increase in chemosensitivity to doxorubicin. Both AS1 and AS2 inhibited rhodamine efflux. AS2 appeared more effective in all the tests. PgP expression returned to normal within 5 days of withdrawal of AS. Sense oligonucleotides S1 and S2 were ineffective.

Conclusions: MDR1 antisense inhibits Pgp expression and sensitises human adenocarcinoma cells (Hep 2A) to doxorubicin chemotherapy.

107 ORAL

HETEROGENEITY IN DNA-DAMAGE *IN VIVO* BY FDURD IN MICE BEARING COLON CARCINOMA #26 TUMORS

C. Schöber[1], S. Cao[2], M.B. Yin[2], H.K. Slocum[2], Y.M. Rustum[2]
[1]Hannover Medical University School, 30623 Hannover, Germany
[2]Roswell Park Cancer Center, Buffalo, NY 14263, U.S.A.

The relation between antitumor response and variation of DNA-damage of individual cells *in vivo* following FdUrd i.v.-bolus treatment was investigated in colon carcinoma 26 bearing BALB/c mice using the single cell gel alkaline electrophoresis (SCG-assay) according to SINGH *et al.* Ten mice received a single i.v. bolus of 1500 mg/kg bodyweight FdUrd, a group of 10 mice served as control. Immediately before and 24 h later, tumor cells were collected by fine needle aspiration and processed with the SCG-assay. In control animals, more than 90% of cells had damage with a migration pattern of less than 20 μm which was considered to represent little to moderate damage. In treated animals, about 60% of cells showed considerable DNA-damage in excess of 20 μm with only 40% of cells showing DNA-fragmentation comparable to controls. All tumors showed a regression in tumor size but only 4 out of 10 were partial remissions. The extent of regression correlated to the amount of bulk DNA ($P = 0.046$). The SCG assay is able to define subsets of tumor cells uneffected by the action of FdUrd treatment *in vivo*. The high proportion of undamaged calls in treated animals may explain the relative resistance of this tumor.

108 ORAL

ANTIPROLIFERATIVE ACTIVITY AND MODE OF ACTION OF NOVEL COMPOUNDS OF MARINE ORIGIN

G. Balconi[1], E. Erba[1], M. Bonfanti[1], S. Filippeschi[1], T. Garcia de Quesada[2], J.M. Jimeno[2], M. D'Incalci[1]
[1]Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy
[2]PHARMA MAR, Tres Cantos, Madrid, Spain

LoVo and mdr-1 overexpressing LoVo/DX cells were exposed for 1 or 24 h to 14 compounds with novel structures (mycaperoxide B, Kahalide, isobengazole, crambescidin, thiocoraline, isohomoalichondrin B, epidioximanadic acid A, sesbanimida, lamellarin, MB-2, LL-15, Palauamine, compound 21 and dehydrodidemnin). The cytotoxic potency ranged from <1 pM to >1 μM. Most drugs were less active against LoVo/DX than against LoVo cells with few exceptions (e.g. isohomoalichondrin B). Flow cytometry studies showed that some compounds caused an accumulation of cells in G_2M phases. Detailed studies on the mode of action of these drugs are in progress. Preliminary data indicate that thiocoraline inhibit the decatenation reactions catalyzed by DNA-topoisomerase II, suggesting that a novel topoisomerase II inhibitor has been identified.

109 ORAL

ECTEINASCIDIN (ET) 743: DEVELOPMENTAL STATUS OF A MARINE (M) DERIVED ANTICANCER COMPOUND (AC)

G. Faircloth, J. Avila[1], J.L. Fernández Puentes, K. Rinehart[2], M. García-Rocha[1], D. García Grávalos, T. García de Quesada, J. Jimeno
PharmaMar R+D, Boston–U.S.–Madrid/León–Spain
[1]CBM-UAM, Madrid, Spain
[2]CBM-UAM, U. Illinois, U.S.A.

ET743 belongs to a new class of MAC isolated from a Caribbean tunicate. ETs are isoquinolines related compounds sharing antiviral, immunosuppresive and antitumour activity (ANT). ET-743 has *in vitro* specificity in melanoma (MEL) and NSCLC cell lines ($IC50_s$ <10 nM); ET743 inhibits DNA, RNA and protein synthesis at ET[] 0.03, 0.008 and 0.1 ng/ml, respectively. ET743 has a novel effect on the organisation of the microtubule network in COS1 and HELA cells at 40 nM (being reversible and not competitive with colchicine). Moreover ET-743 binds

non-specifically to DNA and NCI-COMPARE correlates ET743 with doxo, daunorubicin and morfholino-ADR. *In vivo*, ET-743 shows significant ANT in IP implanted B16 MEL and P388 leukaemia; moreover ET-743 is curative in nude mice bearing sc implanted early (up to 100% tumour free at 60 μg/kg/d/iv-qd × 4) and advanced (up to 40% tumour free animals at day 58 after implant) MX-1 breast ca xenografts respectively. The MTDs in mice, rat and dog have been determined. A clinical oriented formulation has been achieved and the identification of assay method is ongoing. Large scale supply of ET-743 is feasible by recollection and/or industrial culture of the tunicate (life cycle achieved). ET-743 will start phase I ecaluation in the U.S. and in Europe.

110 ORAL

AMIFOSTINE PROTECTS AGAINST CYCLOPHOSPHAMIDE AND CISPLATIN-INDUCED MUTAGENESIS WITHOUT AFFECTING THERAPEUTIC EFFECTIVENESS

Y. Kataoka[1], J. Perrin[2], N. Hunter[3], D. Grdina[1,2]
[1]U of Chicago, Chicago, IL
[2]Argonne Natl Lab, Argonne, IL
[3]MD Anderson Hosp & Tumor Inst, Houston, TX, U.S.A.

Cyclophosphamide and cisplatin, while extremely effective in killing tumor cells, are both highly mutagenic and carcinogenic to normal cells. When administered at doses ranging from 50 to 200 mg/kg to C3H mice, cyclophosphamide induced mutations at the *hprt* locus in splenocytes in a linear dose response manner, e.g., mutant frequency increased from 1.5 × 10^{-6} to 4 × 10^{-5}. Under similar conditions, pretreatment with amifostine protected against cyclophosphamide-induced mutagenesis. Mutant frequency was reduced from 1.6 × 10^{-5} to 1.8 × 10^{-6} with no reduction in the therapeutic response of cyclophosphamide on fibrosarcoma cells. Cisplatin at a dose of 4 mg/kg increased mutant frequency from 1.5 × 10^{-6} to 3 × 10^{-6}. Amifostine reduced this frequency back to control levels (i.e., 1.5 × 10^{-6}). Amifostine's anti-mutagenic effects are being monitored in a clinical trial of amifostine and high-dose cyclophosphamide. Data from this trial will be presented. Amifostine has a potential clinical role for antimutagenesis and anticarcinogenesis in radiation and/or chemotherapy protocols. Supported by Chicago Center for Radiation Therapy, DOE CONT W-31-109-ENG-38 and NIH CA-37435.

111 ORAL

PHASE I CLINICAL AND PHARMACOKINETIC TRIAL OF THE PODOPHYLLOTOXIN DERIVATIVE NK 611 USING AN ORAL DAILY ADMINISTRATION SCHEDULE

I. Raßmann, T. Schilling[1], H. Schrödel[1], A. Kaeser-Fröhlich[2], K. Burk[2], J. Rastetter[1], A.-R. Hanauske[1]
[1]Klinikum r. d. Isar der TU München, Munich, Germany
[2]Asta Medica AG, Frankfurt, Germany

We have performed a clinical and pharmacokinetic trial of the new podophyllotoxin derivative NK 611 administered orally for 21 days repeated every 35 days. Eighteen patients (pts) [9 female, 9 male; median age 60 years (37–73)] with histological proven solid tumors were enrolled in this study. Dose levels were 5 mg/day (105 mg absolute (abs)), 10 mg/day (210 mg abs), 12.5 mg/day 1 (262.5 mg abs) and 15 mg/day (315 mg abs) with a total of 39 courses administered. Toxicity has been evaluated by NCI-CTC criteria. At dose level 5 mg/day, no hematological toxicities (HT) were observed. At dose level 10 mg/day 2/6 pts developed °3 leukopenia (WBC), 1/6 pts °3 granulocytopenia (ANC), 1/6 pts °4 hemoglobin (Hb) and one pt thrombocytopenia (ptl) °2. At dose level 12.5 mg/day 1/8 pts developed °4 WBC, 4/8 pts °4 ANC, 2/8 pts °3 Hb and 1/8 °3 ptl. One pt at 15 mg/day showed °3 WBC, °4 ANC and °2 Hb and °2 ptl. Non-HT at dose level 10 mg/m^2 were 1/6 °2 pain, 1/6 °3 anorexia and 1/6 °4 dysphagia. Non-HT at dose level 12.5 mg/m^2 included 5/8 °2 alopecia, 1/8 °3 dyspnoea, 1/8 °2 fever, 1/8 °2 anorexia, 1/8 °2 nausea and 1/8 °2 vomiting. Non-HT at dose level 15 mg/m^2 (1. pt) consisted of neutropenic fever and °2 alopecia. Pharmacokinetic analysis of 6 pts treated with 12.5 mg qd are available. Using a 2-compartment model, $t_{1/2\alpha}$ ranged from 0.47 to 1.54 h, $t_{1/2\beta}$ ranged from 2.0–11.6 h. Mean Cmax was 1.477 ± 0.331 μg/ml. Mean AUC at 12.5 mg/m^2 was 13.666 ± 3.81 μg/ml·h. No objective tumor response was observed. The Maximum Tolerated Dose was 12.5 mg/d. Dose Limiting Toxicity was ANC °4. The recommended dose for clinical Phase II studies is 10 mg/m^2.- Supported by a grant from ASTA Medica AG

112 ORAL

CYCLIN D1 EXPRESSION IN OVARIAN CANCER: A POTENTIAL THERAPEUTIC APPROACH BY ANTISENSE OLIGOMERS

A. Alama, F. Pedullà[1], F. Barbieri, M. Cagnoli, G. Foglia[1], N. Ragni[1]
Department Exp. Pharmacology, Istituto Nazionale Ricerca Cancro
[1]Clinic of Obstetric and Gynecology University of Genova, 16132 Italy

Cyclin D1 (CD1) is a key regulator of the G1 phase of the cell cycle and is required for the progression to S-phase. CD1 has been found to be overexpressed in a variety of human tumors functioning like an oncogene. In the current study, the expression of CD1 in samples from patients bearing benign or malignant ovarian tumors has been investigated. Preliminary data, in 15 patients, indicate that the majority of carcinomas express higher levels of CD1 protein compared to benign neoplasms. Furthermore, 3 ovarian cancer cell lines have been used to study the effects induced by antisense oligonucleotides to CD1 gene, "*in vitro*". An 18 mer oligomer, complementary to the translation start site of the CD1 cDNA, has been synthesized and administered to the OVCAR-3 ovarian cancer cells at increasing concentrations. Significant inhibition of cell growth (55%) was reported at 401 μM after 3 days of culture. Equivalent effects were obtained with the SW626 and IGROV-1 cells resulting in 48% inhibition as compared to controls. In addition, a marked reduction of the mRNA and protein contents was reported. These preliminary results show the potential role of CD1 as a target for the control of ovarian cancer-growth by antisense oligomers.

Supported by AIRC and PF ACRO CNR grants.

113 POSTER

MICROFILAMENT ACTIVITY (CELL DIVISION BLOCK) OF CYTOCHALASIN D AS A SENSITIVE PARAMETER FOR FUNCTIONAL P-GLYCOPROTEIN DETECTION

L. Elbling, M. Micksche, R.M. Weiss, D. Prinz, G. Fritsch, W. Berger
Institute of Tumorbiology/Cancer Research, 1090 Vienna, Austria

Classical multidrug resistance (cMDR) and its reversal by MDR-modulating agents are of increasing clinical importance. However, the accurate detection of functional activity of the *mdr1* gene product P-glycoprotein (P-gp) is critical. We report here on the sensitivity and specificity of the cell division blocking activity of cytochalasin D (CD) as a parameter for actual P-gp transporting capacity by documenting that: (1) CD is a specific P-gp substrate (detected by drug-accumulation, drug-cytotoxicity, photaffinity-labelling) in a large panel of tumor cell lines (parental n = 9, resistant sublines n = 17) of different origin (men and animal, solid and hematopoietic) and degree of resistance (2.1- to 800-fold); (2) that the CD cell division blocking activity (determined by actin staining, microscopical evaluation of bi-multinucleated cells) is a sensitive parameter for P-gp activity as well as for its modulation evaluated by a large panel of chemically unrelated chemosensitizers (n = 26). For making the assay easily manageable it was automatized using FACS ploidy analysis. Correlations with common cMDR detection methods demonstrate the high sensitivity and specificity of the presented functional cMDR assay.

114 POSTER

IDARUBICIN AND DAUNORUBICIN BINDING TO DNA IN SENSITIVE AND RESISTANT LEUKEMIA K562 CELLS

T.A. Boqush[1], J. Robert[2]
[1]Cancer Research Center, Moscow, Russia
[2]Universite de Bordeaux II, Foundation Bergonie, France

Idarubicin (IDA) is a most active anthracycline in treatment of acute myelogenous leukemias including those resistant to daunorubicin (DAU). To study this phenomenon we have compared IDA and DAU intracellular accumulation and binding to DNA in sensitive (sens) and resistant (res) human leukemia K562 cells and verapamil (Ver) influence on these parameters. It was shown: (1) binding to DNA of IDA in sens and res cells is higher than that of DAU; (2) both drugs' binding to DNA is about two times higher in sens than in res cells; (3) after Ver preincubation IDA (but not DAU) binding to DNA in res cells achieves about the same value as in sens cells. The results can explain the higher clinical efficacy of IDA than DAU in treatment of sens and res leukemias and allow supposition that IDA efficacy can be further elevated by its combination with Ver. Supported by UICC, International Soros Foundation and Russian Foundation of Basic Researches.