accumulation of autophagossomes (using fluorescent dyes) as well as of LC3-II (assessed by western blot), which was also significantly inhibited by SP. These results suggest that UA induction of apoptosis and autophagy is JNK dependent. A decrease in mutated p53 and phospho mTOR, which are associated with an induction of autophagy, were also observed. In conclusion, UA showed anticancer activity by inducing apoptosis and autophagy, which was JNK-dependent in HCT15 cells. In addition, in these resistant cells, UA synergistically cooperate with 5-FU to induce cell death.

Acknowledgements: Cristina Xavier was supported by the Foundation for Science and Technology (FCT), Portugal, through the grant SFRH/BD/27524/2006 and the work was supported by the FCT research grant PTDC/AGR-AAM/70418/2006.

254 Down-regulation of P-gp and drug resistance to csiplatin and VP-16 in human lung cancer cell lines

Q. Wang[1], J. Wang[1], J. Zhang[1], L.O.N.G. Zhao[1], L. Zhang[2]. [1]*The Second affiliated Hospital, Respiratory Medicine, Dalian, China,* [2]*The Zhongshan affiliated Hospital of Dalian University, Respiratory Medicine, Dalian, China*

Background: The aim of this study is to investigate whether the expression of P-glycoprotein is correlated to chemotherapy resistance to cisplatin and VP-16 in four different subtypes of lung cancer cells.

Material and Methods: The expression of P-gp with or without pretreatment of verapamil in four different subtypes of lung cancer cell lines was analyzed with RT-PCR and immunofluorescence. Cell survival to cisplatin and VP-16 was determined by MTT assay.

Results: Our study shows that the expression of P-gp can be inhibited by verapamil in SPCA-1, NCI-H-460 and NCI-H-446 cell line, but not in SK-MES-1 cell line. With the pretreatment of verapamil, NCI-H-446 was more sensitiveto cisplatin (IC_{50}: 67.39±4.3 vs 50.69±2.25).; NCI-H-460, SPCA-1 and NCI-H-446 were more sensitive to VP-16 (IC_{50}: 67.39±4.3 vs 50.69±2.25, 62.37±2.88 vs 45.79±4.47 and 56.35±3.15 vs 43.61±1.64, respectively, $p < 0.05$) as well compared to the control group.

Conclusions: Verapamil can inhibit the expression of P-gp both at mRNA and protein level in NCI-H-460, SPCA-1 and NCI-H-446 lung cancer cell lines. The down-regulation of P-gp is associated with the intrinsic resistance to cisplatin in NCI-H-446 cell line and to VP-16 in NCI-H-460, SPCA-1 and NCI-H-446 cell lines. All these indicated that selecting appropriate mediator to inhibit the expression of P-gp maybe helpful for the reversion of drug resistance in some subtypes of lung cancer cell lines.

255 Withdrawn

256 A dichloromethane fraction of Strobilanthes crispus induces apoptosis and promotes the effect of tamoxifen in MCF-7 and MDA-MB-231 cells

N.S. Yaacob[1], N.N. Nik Mohd Kamal[1], C.S. Lai[2], V. Navaratnam[2], M.N. Norazmi[3]. [1]*University Sains Malaysia, Department of Chemical Pathology School of Medical Sciences, Kubang Kerian, Malaysia,* [2]*University Sains Malaysia, Centre for Drug Research, Pulau Pinang, Malaysia,* [3]*University Sains Malaysia, School of Health Sciences, Kubang Kerian, Malaysia*

Background: The leaves of *Strobilanthes crispus* (*S. crispus*) which is native to the Malay Archipelago, are used in folk medicine for their antidiabetic, diuretic, anticancer and blood pressure lowering properties. Crude extracts of this plant have been found to be cytotoxic to cancer cell lines. In this study, an active fraction of the dichloromethane extract of *S. crispus* (SC/D-F9) was isolated and analysed for its anticancer activities in MCF-7 and MDA-MB-231 breast cancer cell lines.

Materials and Methods: The dichloromethane extract of *S. crispus* was chromatographed on a silica gel and SC/D-F9 was isolated by gradient step elution using a combination of hexane, DCM and MeOH. Cytotoxicity was measured using the LDH assay and apoptosis was determined using Annexin V antibody and analysed by flow cytometry and fluorescence microscopy. Alterations in the mitochondrial membrane potential were also determined by flow cytometry. Modulation of specific gene expression was determined using PCR array and analysed by real-time PCR.

Results: SC/D-F9 is relatively more cytotoxic to the MCF-7 and MDA-MB-231 cells compared to tamoxifen, paclitaxel and doxorubicin, while is non-cytotoxic to the normal breast epithelial cell line, MCF-10A. Cell death occurs by apoptosis via depolarization of the mitochondrial membrane potential and transcriptional modulation of specific signaling molecules. In addition, SC/D-F9 promotes the apoptotic effects of low dose tamoxifen on both breast cancer cell lines.

Conclusion: These findings suggest the potential of SC/D-F9 as a cancer therapeutic agent.

257 Effects of Drug-X on cisplatin-resistant and cisplatin-sensitive ovarian cancer cells

J. Nordquist[1], S.C. Chauhan[1], D. Maher[1], M. Ebeling[1], M. Bell[2], M. Jaggi[1]. [1]*Sanford Research/USD, Cancer Biology Research Center, Sioux Falls SD, USA,* [2]*Sanford School of Medicine, Department of OB/GYN, Sioux Falls SD, USA*

Background: With 204,000 new cases and 125,000 deaths annually worldwide, ovarian cancer is the most lethal gynecological malignancy. Using current treatment options, recurrence is very common. The median survival rate for relapsing women is two to three years with only 30% surviving five years from diagnosis. Resistance to existing treatments (such as cisplatin) attributes to this poor survival rate. Consequently, our laboratory has identified a potentially new drug for treating ovarian cancer, Drug-X (name of the drug can not be disclosed at this point due to proprietary reasons). For decades, Drug-X has been widely used for other clinical indications. Herein, we investigated the effects of Drug-X on cisplatin-sensitive and cisplatin-resistant ovarian cancer cells.

Material and Methods: Three ovarian cancer cell lines (SKOV-3 [cisplatin-resistant], A2780-CP [cisplatin-resistant], and A2780 [cisplatin-sensitive]) were cultured and treated with Drug-X. To determine colony forming effects, 1000 cells were plated overnight, treated for 14 days, stained, and colonies were counted. To test mitochondrial effects, cells were treated (10−20 μM) at three different time points (6, 12, and 18 hours), incubated in 100 nM tetramethylrhodamine (TMRE) for 20 minutes, and analyzed by flow cytometry. In addition, after 48-hour treatment (1−27 μM), cells were analyzed using these methods: (1) Cell proliferation data was gathered by counting cells with an automated cell counter. (2) Apoptotic cells were identified using Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL) and propidium iodide (PI) staining assays. (3) For immuno-blotting, cell lysates were prepared using RIPA buffer, electrophoretically resolved on SDS-PAGE, transferred to PVDF membrane, and probed with various apoptosis-related antibodies. Experiments were performed in triplicate, and statistical significance was determined using a two-tailed student t-test with equal variance.

Results: In all three cell lines, Drug-X inhibited cell proliferation and reduced colonogenic potential in a dose-dependent manner. Further analysis using TUNEL and PI staining revealed a dose-dependent increase in percent of apoptotic cells. Immuno-blotting assays showed a dose-dependent decrease in full-length caspase-9 and caspase-3 (indicating an increase in activity), and a marked increase in cleaved Poly (ADP-ribose) polymerase (PARP). The TMRE assay also revealed a dose-and time-dependent decrease in mitochondrial membrane potential ($\Delta\Psi m$) which is an early sign of the intrinsic apoptotic pathway.

Conclusions: Drug-X effectively inhibits growth of ovarian cancer cells *via* induction of caspase-mediated apoptosis. Our data suggest that Drug-X induces an intrinsic apoptotic pathway by altering mitochondrial membrane potential, triggering caspase-9 activity, and subsequently increasing caspase-3 activity and PARP cleavage. Therefore, Drug-X may be a potential treatment modality for cisplatin-resistant and cisplatin-sensitive ovarian cancer.

258 Anticancer activity of a novel kaempferol glucoside, Tac, is mediated by a mechanism involving RSK inhibition

C. Tsimplouli[1], L. Mahaira[1], G. Rassidakis[2], K. Dimas[1]. [1]*Biomedical Research Founation Academy of Athens (BRFAA), Pharmacology-Pharmacotechnology, Athens, Greece,* [2]*National and Kapodistrian University of Athens, First Department of Pathology, Athens, Greece*

Background: The Ras/mitogen-activated protein kinase (MAPK) pathway regulates diverse cellular processes such as proliferation, survival, growth and motility. p90 ribosomal S6 kinase (RSK) constitutes a family of protein kinases downstream of the MAP kinase cascade and has been shown to regulate cancer progression by controlling cell proliferation. Recent studies on RSK's involvement in cancer pathways have shown that some flavonoids can act as specific inhibitors against members of the RSK family. In this regard, we have further investigated the mechanism of action of the semisynthetic kaempferol glusoside, named Tac, and whether this compound acts downstream to MAPK pathway via RSK inhibition. Finally, we have evaluated the antitumour activity of *Tac* in the human tumour xenograft/immunodeficient mouse model.

Materials and Methods: The antiproliferative effect of *Tac* was examined using the SRB assay. COMPARE algorithm was further employed for an initial evaluation of the mechanism of action. *Tac*-induced cell death perturbations on cell cycle were further examined on the HCT116 human colon cancer cell line using FACS analysis. Western blot was utilized for the detection of caspase activation, PARP cleavage and changes in the levels of MARCKS, p-MARCKS, ERK2, p-ERK1/2, RSK, p-RSK, total EF2, eEf2 and p-eEf2. The *in vivo* antitumour activity of *Tac* was also evaluated against HCT116 xenografts.

Results: COMPARE analysis revealed great similarities with DNA damaging agents. FACS analysis with PI stain indicated that *Tac* treatment resulted in

a delay in the transition from G_2- to M-phase, subsequent blockage of the transition from G_1- to S-phase, and apoptosis through caspase activation. Under these specific experimental conditions *Tac* did not affect MARKCS or ERK1/2 protein levels and phosphorylation state but did alter RSK's activity as this was depicted by the eEF2 phosphorylation levels. Intraperitoneal (ip) administration of *Tac* at the maximum tolerated dose (MTD), following a [(Q1D5)×2] schedule significantly suppressed growth of HCT116 tumours in xenografts.

Conclusions: The results indicate that *Tac* induced apoptotic cell death to colon cancer cells by a mechanism involving MAPK pathway and more specifically RSK. COMPARE analysis further revealed similarities of the mechanism of action of *Tac* to that of DNA damaging agents thus linking RSK to DNA damage. In conclusion, the *in vitro* and *in vivo* results taken together suggest RSK may be an important novel target for the development of new anticancer therapies.

259 Epstein-Barr Virus-Encoded BILF1 receptor and its porcine homologs: signalling mechanism and tumour formation

V. Kubale[1], T. Kledal[2], M. Vrecl[3], R. Lyngaa[2], M. Rosenkilde[4]. *[1]Veterinary Faculty, Institute of Anatomy Histology and Embryology, Ljubljana, Slovenia, [2]Laboratory for Cell Biology and Virology Department of Micro and Nano-technology, Danish Technical University DTU-Nanotech, Roskilde, Denmark, [3]Institute of Anatomy Histology and Embryology, Veterinary Faculty, Ljubljana, Slovenia, [4]The Laboratory for Molecular Pharmacology, Faculty of Health Sciences Copenhagen University, Copenhagen, Denmark*

Backgroud: The Epstein-Barr virus (EBV) open reading frame BILF1 encodes a seven-transmembrane (7TM) G protein-coupled receptor that was recently shown to signal with high constitutive activity through $G\alpha_i$. The main aim of the presented research is to understand the action of EBV, connection to BILF-1 and determine connection between human and porcine homologs, through characterization of these receptors in the aspect of their cell surface expression, determination of their constitutive activation, mechanism of activation of different reporter genes, as well as their other signaling and internalization pathways.

Materials and Methods: Main methods used in this study were different signaling assays (CREB, NFAT, SRE, NFkB) and proliferation assay *in vitro* were employed and nude mice for *in vivo* assay.

Results: Data suggest that BILF1, when expressed during EBV infection, could indeed be involved in the pathogenesis of EBV associated malignancies. Furthermore, the correlation between the receptor activity and the ability to mediate cell transformation *in vitro* and tumour formation *in vivo* supports the idea that inverse agonists for BILF1 would inhibit cell transformation and could be relevant therapeutic candidates. Herpesvirus homologs of porcine EBV – receptors for porcine lymhotropic herpesviruses (PLHV) 1−3, are important for post-transplantation-associated lymhoproliferative diseases (PTLD). Signal transduction properties are determined for PLHV1, 2 and 3.

Conclusions: Obtained results are important especially in the relation to homeostasis in the organism and in relation to develop specific treatment for EBV cancers in the state of organism immunodeficiency. Similarity of human and porcine homologs is extremely important in the view of xenotransplantations.

260 Tumour vascular occlusion by vascular targeted photodynamic therapy

N. Madar-Balakirski[1], C. Tempel-Brami[2], V. Kalchenko[3], O. Brenner[3], D. Varon[4], A. Scherz[5], Y. Salomon[1]. *[1]Weizmann Institute of Science, Biological Regulation, Rehovot, Israel, [2]Tel Aviv University, MRI Unit, Tel Aviv, Israel, [3]Weizmann Institute of Science, Veterinary Resources, Rehovot, Israel, [4]Hadassah-Hebrew University Medical Center, Hematology, Jerusalem, Israel, [5]Weizmann Institute of Science, Plant Sciences, Rehovot, Israel*

Background: Antiangiogenic and anti-vascular therapies present intriguing alternatives to other anti-cancer approaches. However, the clinical benefit of the currently applied approaches is marginal and, for the most part, in combination with chemo or radiotherapies. This deficiency reflects the inability of these methods to obliterate the entire tumour vasculature and, subsequently, ablate the entire tumour tissue. Of particular significance, is the sparring of the vasculature in the tumour rim where tumour relapse usually occurs shortly after treatment. In this study, novel bacteriochlorophyll based photosensitizers, Tookad (WST09) and Tookad-soluble® are i.v injected and locally activated by light on the target tumour. Activation of the circulating photosensitizer promotes an instantaneous and irreversible occlusion of the tumour feeding arteries and draining veins. The vascular-confined sensitizer generates therapeutic levels of superoxid and hydroxyl radicals that induce the occlusion of the supporting vasculature and microcirculation; this is followed by necrosis of the tumour and its rim, eradication and, subsequently healing in a few weeks. This vascular-targeted photodynamic-therapy (VTP) with vascular occluding agents (VOA) has shown significant clinical efficacy in first and second line treatments of patients with localized prostate cancer in several medical centers in Europe, and North America.

Material and Methods: We used a mouse earlobe tumour model and three complementary, non-invasive online imaging techniques: Fluorescent intra-vital microscopy, Dynamic Light Scattering Imaging and Photosensitized MRI.

Results: VTP induced a prompt vasodilatation of tumour feeding arteries, along with a significant transient increase of blood-flow rate, followed by rapid vasoconstriction, blood clotting, vessel permeabilization, and flow arrest within 63.2 sec ±1.5 SEM. Blood-flow in draining veins slowed down, with a slight delay, and was accompanied by frequent changes in the flow direction before reaching a standstill. Tumour necrosis ensued within 24−48 h of vessel occlusion. Neighboring normal tissue vessels of similar size remained functional.

Conclusion: The proposed VTP approach appears to rapidly target the feeding and draining tumour vessels. To the best of our knowledge, this is the first antivascular modality primarily aimed at the larger tumour vessels, depicting high cure rates in both the preclinical and clinical arenas.

261 Clinical importance of GGH −401C>T and the RFC1 A(80)G polymorphism in children with osteosarcoma

M.Z. Hegyi[1], A.F. Semsei[2], E. Csagoly[2], D. Erdelyi[2], C.S. Szalai[2], G. Kovács[1]. *[1]Semmelweis University, II. Department of Peadiatrics, Budapest, Hungary, [2]Semmelweis University, Department of Genetics Cell- and Immunobiology, Budapest, Hungary*

Background: The human gamma-glutamyl hydrolase (GGH) plays an important role in the antifolate-resistance in the tumour cells. Presence of the −401T allele in the promoter of the GGH gene causes increased gene expression in leukemic cell lines. G(80)A polymorphism has been described in the reduced folate carrier(RFC1)gene which encodes the major methotrexate transporter. Children with acute lymphoblastic leukemia homozygous for A(80) had worse prognoses and higher levels of MTX than the other genotype groups. We examined the association of the GGH promoter polymorphism and the RFC1 G(80)A polymorphism with respect to toxicity and pharmacokinetics of methorexate treatment in children with osteosarcoma.

Materials and Methods: We analysed the data of 571 methotrexate blocks administered to 72 patients treated with COSS 86 or 96 protocol between 1987 and 2004. From medical records we examined serum drug levels 6, 24, 36, 48 hours after methorexate infusion; the highest serum GPT, GGT, bilirubin values and the lowest number of granulocyte and serum protein levels in the first two weeks after methotrexate treatment. The polymorphisms were determined by a PCR-RFLP method using DNA extracted from peripheral blood.

Results: The incidence of grade IV acute hepatotoxicity was less frequent ($p = 0.0033$) and drug serum levels were significantly lower in the cellular elimination phase ($p = 0.0003$ at 48 hours) in patients homozygous for the GGH −401T allele than in the group with −401CC or CT genotypes. There was no significant differences between patients with RFC1 80GG or AG genotype and patients homozygous for the A allele, however, in the group with RFC1 80AA and GGH-401CC+CT genotypes, the drug serum levels at 48 hours were significantly higher than in the others. The frequency of grade IV acute hepatotoxicity was significantly higher ($p = 0.001$) in patients with RFC1 80AA genotype than in those who carried the G allele. This difference was even higher between patients with RFC1 80AA plus GGH-401CC+CT genotypes and patients with other genotypes ($p = 0.00005$).

Conclusions: Patients homozygous for the GGH −401T allele had less hepatotoxicity and faster methotrexate elimination compared to those with −401CC or CT genotype. The hepatotoxicity was more frequent in patients homozygous for the RFC1 80A allele than in those who carried the G allele and the difference was intensified without the protective effect of GGH −401TT genotype. Our results indicate that certain gene polymorphisms might be considered for treatment dose individualization in the future.

262 Imaging of neurotensin receptors in tumours by a novel stabilized Cu-64-DOTA-neurotensin analog

R. Bergmann[1], L. Brans[2], D. Tourwe[2], K. Schlottig[1], J. Pietzsch[1]. *[1]Institute of Radiopharmacy Forschungszentrum Dresden-Rossendorf, Radiopharmaceutical Biology, Dresden, Germany, [2]Vrije University Brussels, Department of Organic Chemistry, Brussels, Belgium*

Background: Neurotensin (NT) and its receptors (NTR) are overexpressed in various tumours (breast, prostate, lung, ductal pancreas, pituitary) and play a crucial role in tumour progression and malignancy. For tumour diagnosis and optimized targeted, individualized therapy it is important to image and quantify functional expression of these receptors. The development and radiopharmacological characterization of a novel stable neurotensin analog radiolabeled with ^{64}Cu is described.

Material and Methods: The peptide ($Arg\Psi(CH_2NH)ArgProdmTyrtLeuLeu$-OH) was synthesized by manual solid phase synthesis on a Merrifield-resin and conjugated with DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid). Radiolabeling of the peptide (3 nmol) with $^{64}CuCl_2$ was