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

carried out in 0.1 M ammonium acetate at pH 5.5, 37ºC and 15 min. The IC_{50} was determined on HT-29 cell membranes. Cell uptake and internalization was studied in HT-29 and PC3 cells. The biodistribution of the radiotracer was investigated in HT-29 tumour bearing NMRI nu/nu mice (5 min, 60 min p.i.; 4 animals per time point) and imaged by small animal PET (8 animals). The metabolic stability was analyzed in Wistar rats.

Results: The binding affinity of the radiotracer towards NTR1 was 7 nM (4−12 nM, 95% confidence interval). The radiochemical purity after one step radiolabeling was greater than 92%. After single intravenous administration the activity concentration increased fast in the tumour (0.8±0.1 SUV, 5 min p.i.) and decreased to 0.3±0.1 SUV (60 min). At 60 min p.i. the tumour to organ ratios were 2.8±0.7 (blood), 5.2±0.9 (muscle), 4.2±0.6 (pancreas), 0.6±0.5 (liver), and 0.4±0.4 (kidneys). The radiotracer was fast accumulated in the kidneys (3.7±0.6 SUV, 5 min p.i.; 0.8±0.1 SUV, 60 min p.i.) and eliminated in the urine (60±6% injected dose, 60 min p.i.). The tumours were clearly delineated in the PET images. The tumour uptake of the radiotracer was competitively inhibited by 73% by simultaneous injection of the neurotensin derivative 8−13. In rat plasma 33% of the radioactivity accounted for the original compound at 60 min p.i.

Conclusions: The novel ^{64}Cu-neurotensin analog with good stability and high receptor affinity allows for the in vivo imaging and functional characterization of NTR1 receptor overexpressing tumours. These findings are a prerequisite for other imaging applications, e.g., using SPECT radionuclides (^{111}In), and potentially also for targeted radionuclide therapy (^{67}Cu, ^{90}Y or ^{177}Lu).

Acknowledgement: This project was supported in part by the EC (Grant agreement no. 223057, GIPIO).

263 Participation of the immune system in glioma lysis initiated by parvovirus H1

I. Kiprianova[1], N. Thomas[1], M. Fischer[1], J. Rommealere[1], J. Schlehofer[1].
[1]*DKFZ, Tumourvirologie F010, Heidelberg, Germany*

Malignant gliomas represent the largest number of malignant brain tumours in humans. To date, treatment of gliomas includes neurosurgery, radiation, and chemotherapy but still with a very limited prolongation of survival of patients. Therefore, an alternative therapeutic concept is urgently needed, e.g. oncolytic virotherapy. The rodent parvovirus H-1 (H-1PV) may be an appropriate candidate virus, since it kills selectively malignant cells and is innocuous for normal (non-transformed) cells.

Recently, we have reported complete, stable remission of advanced intracerebral gliomas (RG-2 cell-derived) in a rat model after infection with H-1PV *(Geletneky et al., NeuroOncology, 2010)*. However, in experiments with human glioma xenografts implanted in immunodeficient animals, we observed only a partial regression of the tumour mass. This indicated a role of T-cells in the oncolytic activity of H-1PV *in vivo*.

Indeed, after depletion of T-cells in immunocompetent animals, H-1PV-mediated regression of gliomas was impaired.

To further analyze immune mechanisms in H-1PV-mediated virotherapy, we investigated the potential contribution of IFNγ, a major trigger of immune response produced by T cells.

In vitro, treatment of glioma cell lines (RG2 [rat] and U87 [human]) with IFNγ was not cytotoxic, and did not interfere with H-1PV-mediated cell killing. Therefore, we tested the role of IFNγ in an *in vivo* model. Tumours established from U87 cells implanted stereo-tactically into the brain of immunodeficient (RNU) rats were treated with intratumoural injection of H-1PV alone or combined with intravenous injection of recombinant INFγ. Under these conditions, treatment was as successful as in immunocompetent animals.

The data suggest that INFγ contributes to the efficiency of H-1PV-mediated anti-cancer effect *in vivo*. This involvement seems to be indirect, since *in vitro,* IFNγ application had no impact on the oncolytic activity of H-1PV against glioma cells. The presented results lead us to hypothesize that H-1PV-mediated oncosuppression may – in addition to virus-mediated oncolysis – require an immune component, modulated by IFNγ.

264 Sensitization of melanoma cells to TRAIL-R2 agonist antibody by low-dose anisomycin

A. Slipicevic[1], G.F. Øy[2], B. Engesæter[2], G.M. Mælandsmo[2], V.A. Flørenes[1].
[1]*Oslo University Hospital The Norwegian Radium Hospital, Deparment of Pathology, Oslo, Norway*, [2]*Oslo University Hospital The Norwegian Radium Hospital, Deparment of Tumour Biology, Oslo, Norway*

Background: Tumour necrosis factor related apoptosis-inducing ligand (TRAIL) has been shown to induce apoptosis in malignant cells while leaving most normal cells unharmed, making it a potential anticancer drug. In the present study, the human melanoma cell lines FEMX-1 and WM239 were treated with the TRAIL-R2 agonistic antibody lexatumumab (HGS-ETR2) alone or in combination with subtoxic concentrations of the protein translation inhibitor anisomycin.

Material and Methods: Cell viability was measured by the MTS-assay and synergistic or additive effects of the treatments was determined using CalcuSyn software package. DNA-fragmentation, depolarization of mitochondria membranes and expression of TRAIL-R2 was measured by Flow Cytometry. Proteins of interest were analyzed by Western Blot.

Results: Administration of lexatumumab at doses ranging from 0.75−3.0 μg/ml reduced cell viability by 20−30%. However, when combined with subtoxic doses of anisomycin (20−80 nM), a 60−75% synergistic decrease in cell viability was obtained for both cell lines. Strong activation of the pro-apoptotic proteins caspase 3 and 8 was found in both cell lines after combination treatment. Surprisingly, DNA fragmentation was present only in the WM239 cell line, where the combination treatment showed a two fold increase in TUNEL-positive cells compared to single agent treatment with lexatumumab. No effect on the mitochondrial membrane potential was observed in either the cell line, suggesting increased activation of the extrinsic apoptotic pathway may be responsible for the enhanced cell death. However, increased cell death could not be attributed to increased cell surface expression of TRAIL-R2. Interesting, a rapid activation of MAPK/p38 and enhanced cleavage of the anti-apoptotic protein Livin were observed both in FEMX-1 and WM239 cells.

Conclusion: Use of subtoxic doses of anisomycin sensitize melanoma cells to lexatumumab-induced cell death and suggest that such combination treatment may have a significant efficacy in the treatment of melanoma.

265 Differential effects of EGFR inhibitors in pancreatic carcinoma cell lines

M. Saceda[1], N. López-Riquelme[1], T. Mata-Balaguer[1], P. Garcia-Morales[1], E. Carrasco-García[1], L. Rocamora-Reverte[2], S. Grasso[2], A. Gomez-Martinez[2], J.A. Ferragut[2], I. Martinez-Lacaci[2]. [1]*Elche University General Hospital, Unidad de Investigación, Elche, Spain,* [2]*Universidad Miguel Hernández, Instituto de Biología Molecular y Celular, Elche, Spain*

Erlotinib, an Epidermal growth factor receptor (EGFR) inhibitor is used as therapy in pancreatic carcinoma. We have determined the effects of the EGFR inhibitors AG1478, erlotinib, geficitinib and cetuximab in pancreatic carcinoma cell lines (IMIM-PC-1, IMIM-PC-2, RWP-1 and PANC-1), founding that all four cell lines were resistant to the antiproliferative effect of cetuximab as determined by MTT analysis as well as by cell cycle analysis using flow cytometry. Meanwhile, all cell lines were sensitive at least to one of the EGFR tyrosin-kinase activity inhibitors (AG-1478, erlotinib and geficitinib). We have found that IMIM-PC-2 cell line was sensitive to all the EGFR-TK inhibitors, RWP-1 cells were sensitive to geficitib and erlotinib but they were quite resistant to AG-1478, IMIM-PC-1 cells were sensitive to geficitinib and to a lesser extent to erlotinib and, finally PANC-1 cells were only moderately sensitive to geficitinib. The discrepancies found between the differential effects of cetuximab versus EGFR-TK inhibitors as well as the differences observed in the effects of the different TK-inhibitors upon the same cell lines, suggest that the EGFR inhibitors act in these pancreatic carcinoma cell lines not only inhibiting EGFR but also having differential effects on secondary targets.

To determine the putative secondary targets, we have first discard alternative explanations, such as differential expression of EGFR (EGFR was determined by western blot and we have shown that the levels of EGFR are unrelated to EGFR inhibitor's effects). We have also discarded the presence of mutated EGFR that could account for differential effects of EGFR inhibitors. We have also have studied the effects of these inhibitors on other members of the HER receptor family (HER2, HER3 and HER4), founding that EGFR-TK inhibitors are able to abrogate HER-3 and HER-4 phosphorylation in our cell lines, suggesting that these protein could be also putative targets of the EGFR-TK inhibitors, Finally, we have determined the level of expression of several tirosin-kinases in the four pancreatic cell lines. Interestingly, in PANC-1 cells there are several TKs that are expressed in quite high levels. Taking in consideration that PANC-1 were almost resistant to all the EGFR inhibitor that we have tested, we have studied the putative role that those TKs may play in the response of PANC-1 cell line to EGFR inhibitors. Our results will be presented at the meeting.

266 UVI5008, a novel epigenetic enzyme inhibitor

H. Khanwalkar[1], A.R. De Lera[2], L. Altucci[3], H. Stunnenberg[4], H. Gronemeyer[1]. [1]*IGBMC, Department of Cancer Biology, Strasbourg, France,* [2]*University Vigo, Departamento de Quimica Organica, Vigo, Spain,* [3]*Seconda Università degli Studi di Napoli, Dipartimento di Patologia Generale, Napoli, Italy,* [4]*Nijmegen Centre for Molecular Life Sciences, Department of Molecular Biology, Nijmegen, The Netherlands*

It is becoming increasingly clear that cancer is a consequence not only from genetic but also from epigenetic alterations. Results from recent studies have brought epigenetic effectors into the focus of the search for new anti-cancer therapies. Chromatin remodeling enzymes, in particular histone deacetylases (HDACs) and DNA methyltransferases (DNMTs), have recently emerged as new promising targets of the so-called "epigenetic drugs" for the treatment of cancer. We have synthesized a derivative of the natural compound Psammaplin A, UVI5008 that targets several epigenetic effector enzymes and displays anti tumour activity *in vitro* and *in vivo*.