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

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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

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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

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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

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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