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).

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

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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

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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

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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

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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*.

We have assessed the tumouricidal activity of UVI5008, *in vitro* in a panel of cancer cell lines as well as *ex vivo* in leukemia patient's blasts. Our results indicate that UVI5008 inhibits proliferation by inducing apoptosis of these cells. *In vitro* enzymatic assays showed that UVI5008 can inhibit the activity of class I & II HDACs, Sirtuins and DNA methyltransferase.
We could also show that UVI5008 exerts its antitumour effect *in vivo* in xenografted tumours and in mammary tumour model. This activity is p53 independent and selective for cancer cells, without significant toxicity to normal cells. Growth inhibition is achieved by increased acetylation and induction of TNF related apoptosis inducing ligand (TRAIL) and Reactive oxygen species (ROS). We have also observed reduced methylation and re-expression of p16/INK4 and Retinoic acid receptor-beta 2, two tumour suppressor genes usually silenced in tumour cells by promoter hypermethylation.
Taken together, our data strongly suggest that targeting of multiple signaling pathways by a single drug is a feasible and attractive paradigm for new cancer therapies.

267 The effect of bevacizumab on intratumoural angiogenesis of malignant fibrous histiocytoma in animal model

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Background: Vascular endothelial growth factor (VEGF) is considered to be a key mediator among the angiogenic growth factors causing tumour growth and metastasis, hence the development of anticancer drugs targeting angiogenesis and clinical trials have been widely conducted. Bevacizumab is one of the specific inhibitors for angiogenesis and a neutralizing antibody against vascular endothelial growth factor (VEGF), has recently been used as a drug against malignant tumours. In this study, we evaluated an effect of bevacizumab against malignant fibrous histiocytoma (MFH) in the animal model.
Material and Methods: MFH cell line, Nara H, was used. We injected Nara H cells (1.2×10^7) subcutaneously to the dorsal area of nude mice. After implantation, we measured body weight and tumour dimensions twice a week. Tumour volume was calculated according to the formula $V = \pi/6 \times a^2 \times b$, where *a* and *b* represent the shorter and the longer dimension of the tumour.
Effect of bevacizumab on tumour growth: Mice were randomly divided into treatment group (n = 25) and control group (n = 25). We started treatment with bevacizumab (2 mg/kg) or PBS for each group, twice a week, intraperitoneally. We measured body weight and calculated tumour volume and survival rate for 8 weeks.
Immunohistochemical analysis: Nineteen mice received intraperitoneal injection with bevacizumab or PBS twice a week (treatment group (n = 10) and control group (n = 9)). After 18 days, all tumours were removed and immunohistochemical analysis was performed with Factor-VIII and VEGF antibodies to evaluate microvessel density (MVD) and VEGF expression.
Results: *Tumour growth was significantly inhibited by bevacizumab:* We did not find a difference in body weight between two groups. Tumour volume was significantly decreased in treatment group compared with control group after 16 days treatment. At the end of experimental period, the mean tumour volume of treatment group and control group were 2.7×10^{-5} m^3 and 1.4×10^{-5} m^3, respectively. There was no significant difference in survival rate between two groups, however survival rate of treatment group was higher than that of control group (76.6% with treatment group and 59.7% with control group).
Bevacizumab significantly decreased MVD but not VEGF expression: There was no significant difference in VEGF expression between two groups. MVD was significantly decreased in treatment group. The mean MVD value was 4.2 in treatment group and 7.2 in control group (p = 0.005). We also found a significant correlation between tumour volume and MVD in treatment group (p = 0.02, r = 0.53).
Conclusions: In this study, bevacizumab significantly inhibited tumour volume and intratumoural MVD of MFH in vivo, and there was a significant correlation between tumour volume and MVD in treatment group. These results suggest that bevacizumab may suppress tumour growth of MFH via inhibiting intratumoural micro vessel formation and that bevacizumab may be a novel therapeutic agent for MFH.

268 Expression profile of genes influencing the efficiency of taxanes in breast cancer therapy

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Background: Taxanes have been successfully used in the therapy of various cancers, mainly breast and ovarian cancers. However, multidrug resistance (MDR) of tumour cells to anticancer drugs (i.e. taxanes) represents a problem in the cancer chemotherapy. MDR is a significant cause of the failure of chemotherapy in tumours with inherent or acquired resistance due to enhanced expression of ABC transporters, especially P-glycoprotein (encoded by *ABCB1*). Together with the transporter-mediated resistance, alterations in apoptosis induction by taxanes may be related to tumour resistance, but molecular mechanism(s) is not fully understood. One of causes of the resistance to apoptosis can be the role of caspase-2, mainly different expression of caspase-2S (antiapoptotic) and caspase-2L (proapoptotic) isoforms.
Material and Methods: Expression profile of ABC transporter genes (*ABCB1, ABCC1* and *ABCC2*) was evaluated in 33 breast cancer patients treated by neoadjuvant chemotherapy. Gene expression was quantified in paired tumour and non-tumour breast tissue samples using real-time PCR method with absolute quantification and normalization to cyclophillin A as a housekeeping gene. In addition, the particular isoforms of caspase-2 gene were detected using RT-PCR method.
Results: Caspase-2S/L isoforms as well as ABC transporter genes were identified in examined subjects. ABC transporters were expressed in majority of cases with inter-individual variability in their expression. The levels of expression were as follows; *ABCC1* > *ABCB1* > *ABCC2. ABCC1* was up-regulated in 60% of all tumours, while opposite was observed for *ABCC2. ABCB1* was up-regulated in about half of tumour samples (51.5%).
Conclusions: High expression of *ABCB1* gene in particular tumour samples seems to be important prognostic factor, because patients with high *ABCB1* expression treated with P-gp substrates anthracycline- or taxane-containing regimens had significantly shorter disease-free survival than those treated by other regimens (P = 0.031). In addition, our findings indicate that gene *ABCC1* is, due to its high expression in breast tumour tissue, another potential candidate gene for breast cancer resistance. The presence of antiapoptotic isoform caspase-2 (caspase-2S) seems relevant for additional decrease of efficiency of taxane-based regimen due to the inhibition of the apoptosis in cancer cells caused by taxanes.
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269 Intermittent treatment schedules with rapamycin against malignant glioma xenograft model

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The main aim of this study was to establish a drug dose and treatment schedule to enhance the efficacy in the treatment of malignant glioma xenograft model with rapamycin, improving the survival time of animals bearing brain tumours.
In vitro toxicity experiments were carried out with established human glioblastoma multiforme cell lines U87-MG and U251-MG. The drug activity at different concentrations and times of incubation was studied. Survival studies were performed using the well-established intracranial glioblastoma tumour xenograft model U87MG. Animals were treated intraperitoneally using different schedules and doses in order to compare the efficacy and the benefits of each system. In vitro and in vivo studies of the mammalian target of rapamycin, mTOR, were also accomplished in order to know the activity of rapamycin in each case and apply this knowledge to improve the treatment.
The efficacy of rapamycin in the in vitro and in vivo experiments was found to be no dose-dependant. Animals in the control group had a median survival (MS) of 14 days. Animals treated with rapamycin at 10 mg/kg once a week had a MS of 45.2 days. An important improvement in the survival was not observed when the dose increased to 25 mg/kg, MS = 48.2 days, (P = 0.0271 pair wise comparison). Comparing restricted treatment of rapamycin (days 7, 8, 9, 15, 16 and 17 after tumour implantation) with intermittent treatment (every 5 days) using the same dose (10 mg/kg) we observed a significant improvement in survival. Restricted treatment had a MS = 32.5 days and intermittent treatment showed a MS = 55 days, (P = 0.00313 pair wise comparison).
Our results suggest that a treatment with intermittent intraperitoneal injection is more effective than a daily injection for a restricted period. Intermittent injections allow to keep mTOR pathway inhibited for a longer time. When rapamycin starts to be cleared due to the activity of cytochrome P450 and the stability of the FKBP-Rapamycin complex starts to become weak, a new administration of rapamycin prolongs mTOR pathway inhibition. We demonstrated also, with this study that is more important to keep the activity of rapamycin in the brain than to provide a higher concentration. The response to every 5 days treatment (10 mg/kg) was better than every 7 days treatment (25 mg/kg) even at lower dose, because the administration was provided when mTOR pathway seems to recover the activity. The efficacy of intermittent treatment schedules suggests a therapeutic window reducing the toxicity due to the drug, decreasing the dose to the minimum effective dose that is able to inhibit the mTOR pathway. We conclude that intermittent doses of rapamycin may be an effective treatment option for malignant gliomas.