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*.