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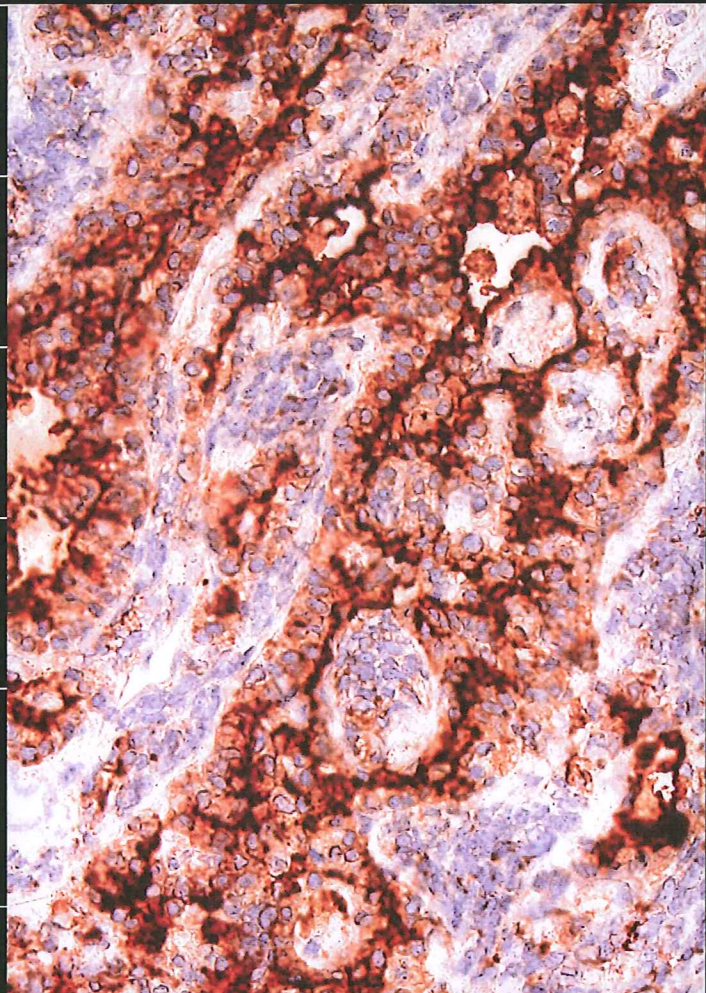
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## EXPERIMENTAL THERAPEUTICS

## ET-01. MOLECULAR IMAGING AND THERAPY PLATFORM: PRE-SELECTING RESPONDING TUMORS TO ANTIANGIOGENIC THERAPY

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The ability to screen patient responders to a chemotherapeutic agent prior to administration of a drug is of significant value. We have developed an integrin-targeted drug delivery system that can target tumor vessels that have upregulated expression of the integrin  $\alpha v \beta 3$ . This platform delivery system can also be used for imaging in many clinical imaging modalities including MRI, PET, and SPECT. We have used this platform to image and deliver genes to the tumor vessels in the rat GBM tumor model. Here we will present the correlation between screening responding tumors using MRI prior to treatment. The difference in therapeutic efficacy will be described in these tumor models. Glioblastoma model U87, was used in nude mice to evaluate temporal changes in T1- and T2-weighted images in MRI using integrin-targeted nanoparticles containing chelated Gadolinium as T1 MRI contrast agent. The treatment group received intravenous injections of integrin-targeted nanoparticles containing a mutated Raf gene that lead to extensive apoptosis of the tumor vessels. Control animals were sham treated with saline. Pixel intensities were measured and positively correlated to therapeutic efficacy. GBM and their tumor models are highly vascular neoplasms that likely respond well to the antiangiogenic therapy like the one we have developed here. Tumors were significantly reduced in size (>95%) and did not recur, even 6 months after treatment completion. Contrary to the GBM model, the SCCVII tumor (model for head and neck cancer) is not very vascular and did not respond well to this anti-angiogenic treatment. molecular imaging (integrin-targeted imaging) using this platform agent as an MRI contrast agent was predictive of therapeutic efficacy. Here we introduce a novel anti-angiogenic agent that can also be used as a molecular imaging probe to predict responding tumors from those that will not respond.

## ET-02. FK506 BINDING PROTEIN (FKBP5) MEDIATES GLIOMA CELL GROWTH AND SENSITIVITY TO RAPAMYCIN TREATMENT THROUGH REGULATING AKT AND NF-KB SIGNAL TRANSDUCTION

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FK506 binding protein 5 (FKBP5 or FKBP51), an immunophilin, belongs to the family of peptidyl prolyl cis/trans isomerases, functions together with chaperones to help protein folding. Using glioma cDNA microarray analysis, we found that FKBP5 was overexpressed in glioma tumors and correlated with overall survival. This finding was further validated by real-time RT-PCR and Western blot analysis. We then employed both RNAi to knock-down FKBP5 expression and cDNA transfection to overexpress FKBP5 to examine the roles of FKBP5 in glioma cells. Cell growth analysis showed that cell proliferation was suppressed after FKBP5 expression was inhibited for 5 days but was enhanced by FKBP5 overexpression. In addition, rapamycin-resistant glioma cells, both PTEN positive and negative, were synergistically sensitive to rapamycin after FKBP5 was knocked down, but the response of glioma cell to rapamycin treatment was suppressed when FKBP5 was overexpressed in glioma cells, suggesting that FKBP5 mediated glioma cell response to mTOR inhibitor treatment and that the effect of FKBP5 on the cell response to mTOR inhibitor was PTEN-independent. To verify the function of FKBP5 in glioma cell signaling, we employed Western blot to analyze the phosphorylation of I $\kappa$ B $\alpha$ , NF- $\kappa$ B and AKT, and EMSA to study the DNA binding ability of NF- $\kappa$ B after FKBP5 expression was regulated by siRNA transfection or overexpression. Western blot analysis showed the expression level of phosphorylated NF- $\kappa$ B and AKT was regulated by FKBP5, and that the expression of I $\kappa$ B $\alpha$  was increased in FKBP5-depleted cells and decreased in FKBP5-overexpressed cells. Moreover, EMSA data showed that overexpression of FKBP5 stimulated DNA-NF- $\kappa$ B binding ability. These results together suggest that FKBP5 involves in AKT and NF- $\kappa$ B pathway activation in glioma cells. In conclusion, our study demonstrates that FKBP5 plays an important role in glioma growth and chemoresistance via regulating signal transduction of AKT and NF- $\kappa$ B pathway.

## ET-03. PHENYLACETYLGLUTAMINE (PG) AND PHENYLACETATE (PN) INTERACT ADDITIVELY TO PRODUCE DETACHMENT-INDUCED APOPTOSIS/ANOIKIS IN GLIOBLASTOMA CELLS

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Phenylacetylglutamine (PG) and phenylacetate (PN) are major components of antineoplastons A-10 and AS2-1. These formulations are currently in advanced clinical trials for the treatment of primary brain tumors. Phenylacetate has been examined independently in the past by other researchers as a potential anti-tumor agent in glioblastoma, medulloblastoma and hematological, breast, pancreatic, prostate, and thyroid malignancies. We have investigated the anti-proliferative effects of PG and PN used separately and in combination in U87 human glioblastoma cells. Though this is the cell line we have focused our attention on, we also have briefly examined the effects of these agents on BT20, DAOY, and U373 cells. Our data on anti-proliferative effect of PG and PN show that the two drugs have a constant relative potency, (R) with PN being 5 times more potent than PG. This feature has allowed us to study the effect of fixed ratio combinations of the two drugs in isobolographic analysis. We present here evidence to show that PG and PN when used in combination are additive at lower doses. These combinatorial doses are in the range of therapeutic significance. In median effect analysis, the Combination Index, CI is > 1. We have observed a dose dependant induction of detachment in cells treated with PG or PN. We have been able to demonstrate that PG causes anoikis or detachment-induced apoptosis in glioblastoma cells using TUNEL as well as Annexin V staining. Our studies indicate that PG enters the U87 cells via glutamine channels and also significantly inhibits the uptake of glutamine by cells. This may be an important feature of its mechanism of action since cancer cells are highly dependant on glutamine. Deprivation of intracellular glutamine could be a key factor in growth inhibition caused by PG. Investigation of the cell cycle by propidium iodide staining and flow cytometry shows an increased number of cells in the sub-diploid stage upon treatment with PG and/or PN, indicative of apoptosis. However there does not appear to be any effect on the cell cycle itself. We have conducted a total human gene array screen using the Affymetrix Human Genome plus 2.0 oligonucleotide arrays, for genes regulated by PG and a combination of PG and PN. The gene TXNIP was up-regulated almost 5-fold with PG, and over 120-fold with a combination of PG and PN. Other interesting genes that are significantly up-regulated are CLDN1, ATF3, CASP5, TP53, TRIB3, and UNC5B. Genes that were down-regulated include AKT2, ASPM, and CDCA8. Based on the results of this study we are currently investigating the possible effect of these antineoplastons on the redox system involving thioredoxin, cell division (borealin, ASPM), apoptosis (caspase 5, p53, netrin receptor), and AKT pathway (AKT2, TRB3).

## ET-04. IN VITRO EFFECTS OF CETUXIMAB ON EGFR AND DOWNSTREAM MEDIATORS

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Treatment outcome of Glioblastoma multiforme (GBM) remains poor despite multimodal therapies. Current standard care for newly diagnosed GBM is surgical resection, followed by radiotherapy plus concomitant and adjuvant chemotherapy with temozolomide. Median survival receiving this therapy is 14.6 month. Between 30%–60% of these patients will experience relapse within 6 month of standard therapy. In case of early relapse, treatment options are limited and systematic trials investigating tumor response to new drugs and the mechanisms involved have to be performed. Primary GBMs, are frequently associated with amplification and/or mutations of the epidermal growth factor receptor (EGFR). EGFR is involved in regulation of cell proliferation, growth, survival and motility. Two of the pathways downstream of EGFR are the Ras/Raf/ERK and PI3K/AKT pathways. Inactivating mutations of the tumor suppressor gene PTEN occurs in approximately 15%–60% of all GBM and contributes to an abnormally high activity of the PI3K/AKT pathway. Aim: Investigating the effects of cetuximab on human glioblastoma cell lines: U87MG, U87MGvIII (express the EGFR type III deletion mutant, EGFRvIII), U118MG, U373MG, and SKMG3 in comparison with the human head and neck cell line, HN5. Inhibition of EGFR signaling in vitro with increasing concentrations of cetuximab (Erbix) with or without the addition of EGF showed moderate effects on GBM cell viability. In comparison, HN5 with known EGFR amplification and wild type PTEN is sensitive to increasing concentrations of cetuximab. However, in the presence of EGF, almost no effect on HN5 cell viability was observed. Western blotting experiments showed that EGF is capable of inducing phosphorylation of EGFR in the U87MG, U87vIII and SKMG3 cell lines, all of which have mutated PTEN and that this could be inhibited by increasing concentrations of cetuximab. Both ERK and AKT were phosphorylated in the presence of EGF, however limited or