F MRS and Diffusion MRI Analysis of Cytosine Deaminase-Uracil Phosphoribosyltransferase Fusion Protein Results in Enhanced Deamination of 5-Fluorouracil and Increased Therapeutic Efficacy

J. Stojanovska[1], D. A. Hamstra[2], J. M. Tychewicz[3], K. C. Lee[4], V. D. Shepkin[5], B. A. Moffat[5], M. Chen[2], K. J. Dornfeld[2], J. G. Tjuvajev[2], T. L. Chenevert[5], B. D. Ross[5], A. Rehemtulla[5]

[1]Center for Molecular Imaging, Departmet of radiology, University of Michigan, Ann Arbor, MI, United States, [2]Department of Radiation Oncology, University of Michigan Medical Center, Ann Arbor, MI, United States, [3]Department of Biochemistry, University of Michigan, Ann Arbor, MI, United States, [4]Department of Biochemistry, University of Michigan Medical Center, Ann Arbor, MI, United States, [5]Department of Radiology, University of Michigan Medical Center, Ann Arbor, MI, United States

Synopsis: To evaluate a novel Gene Dependent Enzyme Prodrug Therapy (GDEPT) for glioma, genetically engineered rat glioma cells were developed which expressed either a fusion protein between *Saccharomyces cerevisiae* cytosine deaminase (CD) and *Haemophilus influenzae* Uracil phosphorobosyltransferase (UPRT) or just CD alone. The activity of these proteins and their therapeutic efficacies were then followed *in vitro* and *in vivo* using both ^{19}F MRS and diffusion weighted MRI.

Introduction: Cancer gene therapy using the conversion of pharmacologically non-toxic compounds or prodrugs to chemotherapeutic agents has been studied using a number of different gene and drug combination. One of the most promising GDEPT utilizes the combination of the yeast Cytosine Deaminase (CD) which deaminates the non-toxic anti-fungal agent 5-flurocytosine (5FC) to the commonly use chemotherapeutic 5-fluoruracil (5FU) (1). Uracil Phosphoribosyltransferase (UPRT) directly converts 5FU to 5-fluorouridine-5'-monophospate (FUMP), thus by-passing degradation pathway for 5FU and yielding increased cytotoxicity from 5FU. The combination of both CD and UPRT using a dual GDEPT strategy has also been demonstrated either using transduction of both genes on separate adenoviral vectors, or through a fusion protein between CD and UPRT(2). Little, however, is known about the *in vivo* pharmacokinetics of this system. We, therefore, chose to evaluate this system using ^{19}F MRS and MRI in a preclinical model of high-grade glioma.

Methods: 9L tumors were induced as flank tumors in CD-1 *nu/nu* mice or intracerebrally in Fischer 344 rats as previously described (3). Briefly, 9L cells that expressed either CD or CD-UPRT were injected subcutaneously in nude mice and once tumors had developed were followed using ^{19}F MRS on a 9.4 Tesla Varian Unity Inova Imaging system with a custom designed surface coil. Spectra were then acquired at 376 MHz and averaged over 20 min for a minimum of 3 hrs using a single pulse with a TR of 4 sec and a spectral width of 10 MHz (4). For intracerebral tumors the 9L cell lines were implanted in the right forebrain at a depth of 3 mm, and T2-weighted and diffusion weighted MRI were obtained both prior to and during treatment (5). Briefly, an isotropic, diffusion weighted sequence was employed with two interleaved *b*-factors (Δb= 1148 sec/mm^2) and the following acquisition parameters: TR/TE 3500/60 msec, 128x128 matrix, and a 3-cm FOV. The low *b*-factor images were essentially T2-weighted to allow tumor volume measurements, as previously described. Isotropic ADC maps were calculated for each image.

Results: *In vitro* MRS demonstrated increased deaminase activity for the fusion protein relative to native CD which was demonstrated to be secondary to enhanced thermal stability. MRS demonstrated that in animals bearing CD expressing tumors there was limited conversion of 5FC to 5FU with no accumulation of cytotoxic fluorinated nucleotides (F-nucs). In contrast, CD-UPRT expressing tumors had similar peak 5FC concentrations, but 3-fold higher intratumoral accumulation of 5FU and significant generation of F-nucs. Finally, the enhanced production of 5FU and F-nucs from CD-UPRT gave increased efficacy in an orthotopic animal model of high-grade glioma. More importantly, early changes in diffusion weighted MRI were predictive of both durable response and increased survival. These results demonstrate the increased efficacy for the CD-UPRT GDEPT compared to CD alone both biochemically and in a preclinical model, and validate MRI as a tool to assess gene function and early therapeutic efficacy.

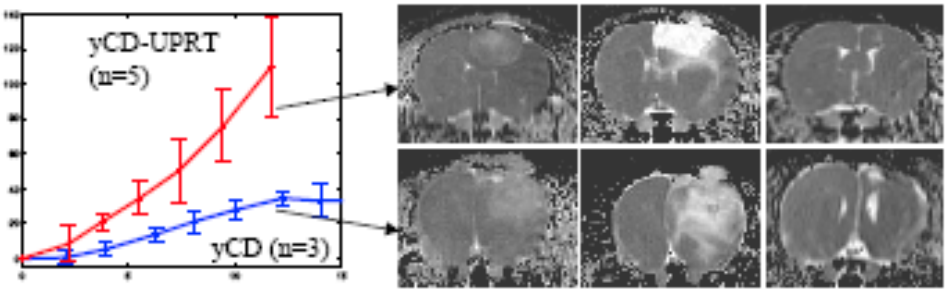

Figure 1: Diffusion MRI results for efficacy of CD and CD-UPRT gene therapies of 9L gliomas. The ADC maps on the right and the percentage change in mean ADC on the left show that the more stable CD-UPRT enzyme is significantly faster and more effective in killing the 9L tumors than the CD enzyme.

Conclusions: CD-UPRT is more thermal stable and has greater cytosine deaminase activity as witnessed by *in vitro* and *in vivo* MRS, and this translated into enhanced changes in diffusion weighted MRI and tumor regression *in vivo*.

References.

1)Hamstra, D. A., et al *Human Gene Therapy., 10:* 1993-2003, 1999.
2)Erbs, P.et al *Cancer Research., 60:* 3813-3822, 2000.
3)Ross, B. D., et al. *PNAS., 95:* 7012-7017, 1998
4)Chenevert, T. L., et al *Clinical Cancer Research., 3:* 1457-1466, 1997
5 Stegman, L. D., et al *PNAS 96:* 9821-9826, 1999

Acknowledgements: This work was supported in part by the following NIH/NCI grants:P01CA85878, R24CA83099, P50CA093990.