Imaging oncolytic virotherapy delivery using a CEST reporter gene

Christian T. Farrar[1], Jason S. Buhrman[2], Guanshu Liu[3,4], Assaf A. Gilad[3,4], Michael T. McMahon[3,4], and Giulia Fulci[2]

[1]*Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, United States,* [2]*Department of Neurosurgery, Massachusetts General Hospital and Harvard Medical School, Charlestown, MA, United States,* [3]*F. M. Kirby Center for Functional Brain Imaging, Kennedy Krieger Institute, Baltimore, MD, United States,* [4]*Department of Radiology, The Johns Hopkins University School of Medicine, Baltimore, MD, United States*

Target Audience: Researchers and clinicians who are interested in molecular and cellular MRI, particularly in non-invasive monitoring of gene expression, cell therapy, and transplantation, as well as pre-clinical drug screening with advanced MRI-based techniques.

Purpose: One of the major challenges in treating glioblastoma (GBM) is the poor efficiency of drug delivery. Oncolytic viruses (OVs) that can selectively replicate in tumor cells and target the infiltrating margins of the tumor represent a promising new tool in cancer therapy. However, to date oncolytic virotherapy has not proven effective in treating GBM due to multiple physiological barriers, such as host anti-viral immunity and the extracellular matrix, that prevent viral delivery to the cancer and viral intratumoral spread. Several attempts are ongoing to combine oncolytic virotherapy with chemotherapeutics that suppress anti-viral defense systems in pre-clinical research and clinical trials [1,2]. However, the lack of a means to detect the OV in a non-invasive fashion limits the evaluation of such treatments. Here we demonstrate the use of a lysine-rich protein (LRP) reporter for imaging oncolytic viral delivery using chemical exchange saturation transfer (CEST) MRI that may for the first time provide a tool for the in vivo monitoring of oncolytic virotherapy.

Methods: D74 rat glioma cells were implanted into the brains of Fischer 344 rats and imaged 7 days following tumor cell implantation. Baseline T2-weighted and CEST MR images were acquired on a 9.4T Bruker MRI scanner. CEST images were acquired with a saturating field of 3.6 μT and 43 field offsets from -2100 to +2100 Hz. The LRP reporter gene [3] was incorporated into the oncolytic Herpes Simplex Virus (HSV) G47Δ (G47Δ-LRP) through the flip-flop HSV recombination method [4]. G47Δ-LRP or G47Δ-empty, containing no LRP reporter gene, was injected into the tumors following baseline imaging. Tumors were then re-imaged 6-10 hours after virus injection.

Results and Discussion: An increase in CEST contrast was clearly visible in the tumor following G47Δ-LRP viral therapy, as shown in the representative images in Figure 1. LacZ staining of the *ex vivo* brain tissue revealed extensive viral distribution in the tumor. The average CEST contrast following G47Δ-LRP virus injection (3.9%) was significantly greater ($p<0.05$, $n=3$) than the baseline tumor CEST contrast (1.8%). Preliminary studies of rats injected with G47Δ-empty virus ($n=3$) observed only a 20% increase in MTR_{asym} compared to a 101% increase for the G47Δ-LRP virus. The slight increase in CEST contrast observed with the empty virus is attributed to inflammation and slight hemorrhage due to the virus injection.

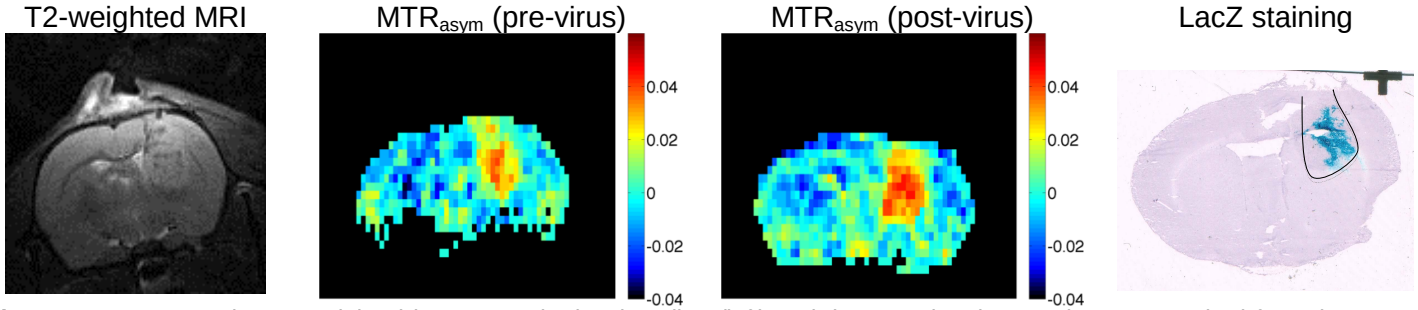

Figure 1: Representative T2-weighted image acquired at baseline (left) and the associated CEST images acquired from the same image slice at baseline and 8 hours following injection of the G47Δ-LRP oncolytic virus. Representative histology slice showing extensive LacZ staining of tumor (right). LacZ staining indicated that 60% of the tumor was infected with virus.

Conclusions This preliminary study suggests that CEST imaging can be effectively used for the *in vivo* detection of OV intratumoral delivery and to quantify the spatiotemporal changes in tumor viral distribution.

References: [1] Diop-Frimpong B, Chauhan VP, Krane S, Boucher Y, Jain RK. Proc Natl Acad Sci USA 2011;108:2909-14. [2] Fulci G, Breymann L, Gianni D, *et al.* Proc Natl Acad Sci USA 2006;103:12873-8. [3] Gilad AA, McMahon MT, Walczak P, Winnard PT, Raman V, van Laarhoven HWM, Skoglund CM, Multe JWM, van Zijl PCM. Nature Biotech 2007;25(2):217-219. [4] Kuroda T, Martuza RL, Todo T, Rabkin SD. BMC Biotechnol 2006;6:40.