Introduction: Recent studies have indicated that a significant survival advantage is conferred to glioma patients whose lesions harbor mutations in isocitrate dehydrogenase 1 and 2 (IDH1/2). Interestingly, these mutations result in the excessive production of 2-hydroxyglutarate (2HG), which is an onco-metabolite that accumulates in both intracellular and extracellular regions. Although mutations in IDH can be determined by detailed genetic analysis of resected tumor tissue, the ability to detect 2HG levels by using magnetic resonance techniques could have significant implications on patient care. The goal of this study was to determine whether 2HG could be detected in recurrent low-grade glioma tissue samples using proton High-resolution Magic Angle Spinning (1H HR-MAS) spectroscopy, and to correlate the results obtained with IDH1 mutation status as assessed by immunohistochemistry.

Methods: Fifty-three patients with an original pathologically confirmed diagnosis of WHO Grade 2 glioma were included in our IRB-approved study. Patients were recruited immediately prior to resection for suspected recurrence, when progression to higher grade is often observed. They had received prior treatment with surgical resection and/or radiation and chemotherapy.

In vivo MR Scans: Preoperative MR studies were conducted at either 1.5 or 3 Tesla. The scans included 6 directional axial Diffusion Weighted Imaging (DWI) with b=10000/mm²; lactate-edited 3D MRSI with PRESS volume localization; and dynamic Perfusion Weighted Imaging (PWI) with a 5ml/s injection of 0.1nmol/kg body weight Gd-DTPA.

Tissue Acquisition: Tissue sample locations were selected in BrainLab navigation software based on surgically accessible areas with low ADC, elevated Choline/N-Acetylaspartate index (CNI), or elevated PWI peak height and low recovery. After surgical excision tissue samples were immediately bisected: one half was snap frozen in liquid nitrogen and stored at -80°C for 1H HR-MAS spectroscopy; the other half was routinely fixed in 4% formalin, dehydrated by graded ethanols, and embedded in wax using standardized techniques for tissue processing and IDH1 immunohistochemistry using the anti-IDH1R132H antibody. Ex vivo 1H HR-MAS: Tissue samples were placed in a 35µl zirconium rotor custom designed by Varian with 3µl 99.9% atom-D deuterium oxide containing .75 wt% 3(Trimethylsilyl)propionic acid (TSP) from Sigma Aldrich. Samples were scanned at 11.7 Tesla, 1° C, 2250Hz spin rate in a 4mm gHz nanoprobe with a Varian INOVA 500 MHz multi-nuclear spectrometer. A 1D Carr-Purcell-Meiboom-Gill (CPMG) Sequence was run with TR/TE=4s/144ms, 512 scans, 40,000 acquired points, 90° pulse angle, 20000Hz spectral width, with an acquisition time of 35 minutes. To estimate in -vivo Concentrations, the RETIC method was utilized for quantification. 2D Total Correlation Spectroscopy (TOCSY)¹ was performed to further resolve choline- and ethanolamine-containing compounds as well as 2HG. A 110mM 2HG solution was prepared from 2HG stock and scanned independently with the same parameters as our tissue samples. Levels of metabolites were evaluated using a customized High Resolution Quantum Estimation (HR-QUEST) semi-parametric algorithm, with a basis set that included a spectrum of 2HG, as well as standard metabolites known to be present in brain tissue. HR-QUEST Parameter fits with less than 11% Cramer-Rao error estimates were included for analysis. A Wilcoxon rank sum test was performed on mean metabolite values in biopsies for each patient to assess statistical significance (p < 0.05).

Results: The 1D 2HG spectrum gave rise to four multiplets, three of which possessed a complex splitting pattern comprising the alpha, beta, and gamma protons and contained as many as thirteen peaks (see Figure 1). 2HG was detected in 85% of spectra from glioma samples (see Figure 2) of which 62 were 2HG positive, 11 negative, and 37 uncertain due to insufficient signal-to-noise. The third multiplet at 2.28 ppm offered the best method of detection and corresponded to a single multiplet in the other three multiplets. Although other 2HG crosspeaks were detectable between 1.8 and 2.28ppm, this spectral region also included crosspeaks corresponding to GABA, Glu, and Gln. When the presence of 2HG was compared to staining of the paired tissue sample with the IDH1 antibody (n=37 samples), the results were in relatively strong concordance, with a specificity of 0.70, sensitivity of 0.76 and accuracy of 0.73. Note that the anti-IDH1R132H antibody only recognizes mutations at codon 132 of IDH1, and it was expected that samples with alternative mutations in IDH1 (non-histidine) or in IDH2 would exist and were classified as “false positives” for 2HG. A subset of these “false positives” was subsequently confirmed to be IDH1 mutated by full genomic sequencing. Upon stratification among tumor grade at a patient level, relative 2HG levels increased from Grade 2 (0.28 +/- 0.13, n=12) to Grade 3 (0.46 +/- 0.32, n=19) to Grade 4 (0.77 +/- 0.22, n=4). The difference between patients whose lesion remained at Grade 2 versus those who transformed to Grade 4 was significant (p=0.002), while the remaining differences showed a trend but did not reach significance.

Conclusion: The ultimate goal of this work is to improve the clinical management of patients with LGG. Our data demonstrates the utility of HR-MAS in the detection of 2HG as a novel biomarker of IDH1 mutation status in glioma. This work was confirmed by immunohistochemistry and suggests that 2HG is an onco-metabolite of particular interest due to its prognostic value. Furthermore, this study shows differences in 2HG levels between samples from patients whose tumors have transformed to higher grade. While the estimation of in vivo levels of 2HG may be challenging due to significant overlap with neighboring metabolites, this would clearly be desirable for the non-invasive evaluation of changes in 2HG levels associated with transformation of LGG to higher grade.
