

## MR biomarkers of tyrosine kinase inhibition in Mouse Gliomas

P. A. Schornack<sup>1</sup>, J.-J. Yiin<sup>2</sup>, B. Hu<sup>2</sup>, R. S. Sengar<sup>2</sup>, K.-W. Liu<sup>2</sup>, H. Feng<sup>2</sup>, F. S. Lieberman<sup>2</sup>, J. N. Sarkaria<sup>3</sup>, E. Wiener<sup>2</sup>, H.-I. Ma<sup>4</sup>, and S.-Y. Cheng<sup>2</sup>  
<sup>1</sup>Radiology, University of Pittsburgh, Pittsburgh, PA, United States, <sup>2</sup>University of Pittsburgh, <sup>3</sup>Mayo Clinic, <sup>4</sup>Taiwan National Defense Medical Center

**Purpose:** EGFRvIII is a mutated EGFR that is frequently over expressed in malignant gliomas & implicated in response to receptor tyrosine kinase inhibitors (TKI). We used MR techniques sensitive to T2, T2\*, & ADC in measurements of mouse gliomas with histological correlates to test the hypothesized that ZD6474 (ZACTIMA, vandetanib), a TKI for VEGFR2 & EGFR, exerts better efficacy on inhibition of brain gliomas that express EGFRvIII.

**Experimental Design:** We tested the efficacy of ZD6474 on growth inhibition of intracranial U87MG & short-term cultured primary glioma (GBM) tumors with or without EGFRvIII over expression. The impact of ZD6474 on GBM gliomas was further evaluated using magnetic resonance imaging (MRI) analysis. Treated (+ZD, m=18g) & untreated (-ZD, m=11g) mice with GBM8 glioma tumors at 2 to 3 weeks post-implantation were anesthetized. MR data was acquired @ 9.4T on a Varian scanner. ADC maps were obtained using a spin-echo sequence (te/tr=25/1000ms) with a range of bipolar gradients strengths (up to 10 G/cm) of fixed duration ( $\delta=8$ ms) & separation ( $\Delta=14$ ms) applied simultaneously along X,Y&Z axes (b-values of 16-1261 s/mm<sup>2</sup>). T2 maps were obtained using a spin-echo sequence with 6 TE=10-60 ms & TR=1000ms. Both T2 & ADC map acquisitions, which also yield a range of T2w & ADCw images, were acquired with (16mm)<sup>2</sup> FOV, 64x64 matrix, & 4-5 contiguous 1mm thick image slices. Maps were calculated by single exponential fitting signal intensity vs. b-value (ADC) or TE(T2), respectively. T2\*w 3D Turboflash images were acquired with TE=10, TR=40ms, & (16mm)<sup>2</sup> FOV & (128)<sup>3</sup> & (64)<sup>3</sup> matrix size for the control & treated mouse brain, respectively. Effects of ZD6474 on EGFRvIII signal transducers critical for cell proliferation & survival were also examined.

**Results:** In the brain, ZD6474 inhibited tumor growth, angiogenesis & induced cell apoptosis in various gliomas. Moreover, significant inhibition of EGFRvIII-expressing gliomas in both types of gliomas was observed compared with their isogenic tumors without EGFRvIII. MRI analysis using the apparent diffusion coefficient (ADC) & 3D T2\*w weighted measurements validated a greater inhibition on tumor growth & angiogenesis in GBM8 (EGFRvIII-expressing) compared to GBM14 (No EGFRvIII) tumors. T2\*w images (left) & ADC maps (right) are shown below for untreated(top) & treated(bottom) tumor. (Red & blue ROI from untreated & treated tumor region on H&E stains (not shown), green ROI from the largest in-plane region of hypointensity from 3D T2\*w image). The distribution of tumor ADC values are decreased approximately 8% relative to normal brain in untreated mouse, but 6% higher in treated tumor relative to normal brain in the ZD treated mouse. ADC is negatively correlated with tumor cell density & a shift to higher values has been demonstrated as a quantifiable indicator of treatment efficacy.

We found that untreated tumor exhibits a decreased & broadened distribution of T2\*w signal intensities relative to normal control contralateral brain tissue. T2\*w signal from the entire untreated tumor volume is 10% less & 1.85-fold broader than corresponding contralateral brain; these regions corresponded with H&E stains. Conversely, it was not possible to distinguish between tumor & normal brain in treated mouse on T2\*w images, nor to co-register with the much smaller tumor ROI from treated H&E stained brain sections. Treated tumor & contralateral T2\*w signal was the same @ 0.13 (& same as contralateral untreated signal); the distribution of T2\*w signal intensities was only slightly more heterogeneous as indicated by the FWHM (0.024 vs 0.02). One can also distinguish a region consistent with necrosis on T2w, T2 maps, T2w fast spin echo, T2\*w 3D Turboflash, diffusion weighted, & ADC maps (not all shown). These signal properties are all consistent with regions of reduced cellularity, where water exists in a more uniform environment with minimal structure & macromolecular content. The hypo-intensity of this same ROI on T2\*w 3D images are consistent with a region of increased magnetic susceptibility gradients. This could possibly be due to localized heterogeneously distributed products of hemorrhage in tumor (ie, red blood cells, hemoglobin), which has been noted by others within & adjacent to necrotic regions.<sup>1,2</sup> In vitro, ZD6474 preferentially inhibited cell growth & survival of U87MG/EGFRvIII & GBM8 cells versus non-EGFRvIII expressing cells. ZD6474 also attenuated EGFRvIII-activated phosphorylation of Stat3, Akt & Bcl-XL expression in EGFRvIII-expressing glioma cells compared with cells without EGFRvIII.

**Conclusion:** ZD6474 significantly inhibited growth & angiogenesis of gliomas expressing EGFRvIII by specifically blocking EGFRvIII-activated signaling transducers in brain, which suggests a potential application in treatments for gliomas that overexpress EGFRvIII. Our results indicate that susceptibility/T2\* weighted MR along with ADC and T2 measurements can be used as a means of non-invasively quantifying the efficacy of such treatment protocols.

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**References.** 1. Cha, MRM 49:848-55, 2003. 2. McConville, Clin Cancer Res 13(10):2897-2904, 2007.

