Multi-Parametric Imaging of Tumor Treatment Response to VEGF Blockade

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Introduction: Anti-angiogenic therapies, aimed at inhibiting vascular growth in tumors, are currently employed for treatment of cancer patients. Aflibercept (VEGF Trap), a compound designed to neutralize endogenous VEGF that is composed of the Ig2 domain of VEGFR1, the Ig3 domain of VEGFR2 fused to the Fc domain of IgG1, is one such agent that has shown promise. The aim of this study was to monitor effects of VEGF signaling blockade on a glioma rat model by quantitative permeability and diffusion-weighted MRI.

Methods and Materials: *Animal Model:* Male Fischer 344 rats (14) were implanted intracranially with a suspension of 9L rat glioma cells. Animals were imaged and then separated into control (n=5) and treated (n=9) groups. The treated group was injected subcutaneously with 25 mg/kg Aflibercept (Regeneron Pharmaceuticals Inc., Tarrytown, NY) or control (human Fc protein), at a dose of 12.5 mg/kg, twice weekly for two weeks.

MRI Experiment: Each animal was imaged the day before the first treatment and the day after every treatment using a 9.4T Varian *Direct Drive* system and a quadrature rat head RF coil (Doty Scientific, Inc.). Anatomical images were acquired using a fast spin-echo sequence with the following parameters: TR/TE = 4000/60 ms, field of view (FOV) = 30 mm, matrix size = 256x128, slice thickness = 1 mm, 2 averages. Diffusion-weighted images were acquired using a spin-echo sequence, with a navigator echo and gradient waveforms sensitive to isotropic diffusion, with the following parameters: TR/TE = 4000/47 ms, field of view (FOV) = 30 mm, matrix size = 128x64, slice thickness = 1 mm, and b-values of 120 to 1200 s/mm^2. Permeability imaging was done using a T_1-weighted gradient-echo sequence with the following parameters: TR/TE = 85/3.2 ms, FOV = 30 mm, matrix size = 128x64, slice thickness = 1 mm, and 13 slices. The TR was repeated 83 times, with ~11s for each image set, resulting in a 15 minute scan time. After about 1 minute (6 images), a bolus dose of Gd-DTPA (0.15 mmol/kg, diluted in 0.9% saline solution to 0.06 mmol/mL) was administered via tail-vein catheter at a rate of 4 mL/min.

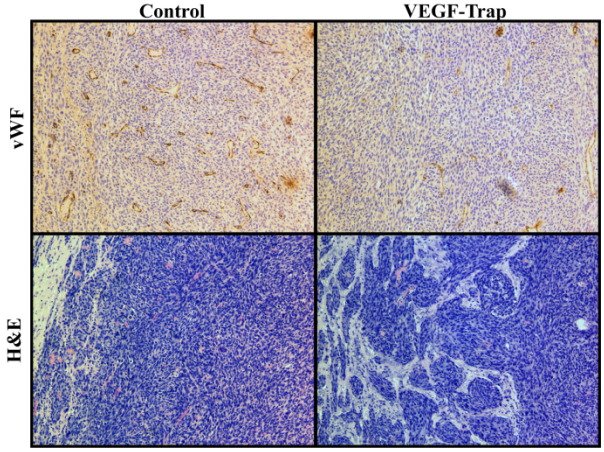

Figure 1: Representative histological images for vWF and H&E stains for both groups.

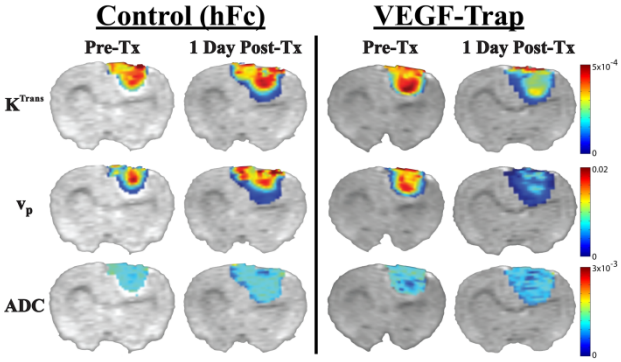

Figure 2: Representative images with color overlays of parametric maps are shown from the day before and the day after the initial treatment. (Units: ADC in mm^2/s, K^{Trans} in s^{-1})

Data Analysis: Image analysis was done using in-house software developed in MATLAB (The MathWorks, Inc., Natick, MA). VOIs were drawn around the tumors on the T_2w images for volume, mean permeability and diffusion measurements. Permeability images were analyzed by performing a three-parameter fit (K^{Trans}, kep, and v_p) to the acquired time-resolved data using a tri-exponential arterial input function (AIF) along with a two-compartment Patlak kinetic model (1). Mean whole-tumor parameter values were analyzed for each time point.

Histology: On the final day of treatment, three representative animals were selected randomly from each group for histology. Slices were processed using standard protocols and H&E, Von Willebrand Factor (vWF), TUNEL, and Ki-67 staining were done for each animal. Vessels were counted by positive staining in the vWF-stained slides, and the percent of cells showing positive stains for Ki-67 were used to quantify cell proliferation.

Statistics: Student t-tests were used to compare control and treated groups at each time point. Significance was assessed at p-values<0.05.

Results: H&E staining (Fig 1) verified similar tumor cellularity between groups, whereas vascular disruption was evident in the treated group (vWF stains). Representative images of K^{Trans}, v_p, and ADC are presented as color overlays on T2-weighted images (Fig2) pre treatment and 1 day after start of treatment. As presented in Fig3, Aflibercept treated tumors showed a significant decrease in K^{Trans} by the first day post-therapy, followed by no detectable recovery to baseline levels before end of study. The blood plasma volume fraction (v_p) showed a similar trend to that of K^{Trans}, with a steep drop by the first day post-therapy. A drop in kep was seen as well (data not shown), though not as steep. Diffusion was seen to gradually decrease in both groups, with the Aflibercept-treated group dropping significantly more. When compared to controls, treated animals had significantly smaller tumor volumes at days 8 and 11 post-treatment.

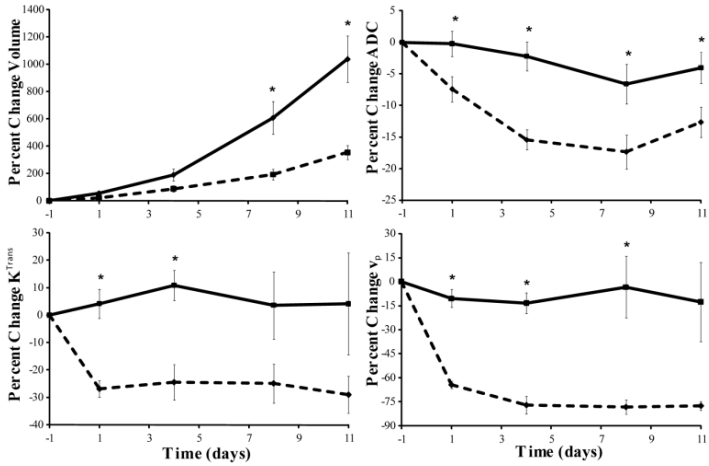

Figure 3: Plots of relative change in volume, diffusion, and perfusion parameters for the treated group (dotted) shown together with the control (solid). Error bars shown represent the standard error of the mean. Significant difference between groups is indicated by (*).

Discussion: Aflibercept had a significant effect on tumor permeability, with a 29% and 64% drop in K^{Trans} and vp, respectively, as early as the first day post-treatment. No increase in water diffusion was observed in the treated group suggesting that tumor cellularity remained unchanged, which was supported by histology. Permeability imaging did show the expected drop in vascular permeability following Aflibercept, which may account for the some of the slowing of tumor growth, since diffusion measurements suggest no appreciable cell death in the tumor.

References:

1. Tofts, Paul S. Modeling Tracer Kinetics in Dynamic Gd-DTPA MR Imaging. JMRI 1997; 7:91-101.
2. Rudge, John S., et al. VEGF Trap complex formation measures production rates of VEGF, providing a biomarker for predicting efficacious angiogenic blockade. PNAS 2007; 104(47):18363-18370.