Vessel Size Index MRI for Evaluating the Effects of Multiple Anti-Angiogenic Therapies in Sequence

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Introduction. *In vivo* imaging of tumors treated with anti-angiogenic agents can reveal information about the therapeutic effects on tumor vasculature beyond simple tumor volume measurements. Vessel size index (VSI) MRI determines the fractional blood volume, mean vessel size, and Q (a dimensionless parameter related to vessel density) on a voxel-by-voxel basis, using changes in T_2 and T_2* caused by the injection of an iron oxide contrast agent [1,2]. In this study, we use VSI MRI to examine the effects of two potential therapies in sequence: B20-4.1, an antibody to vascular endothelial growth factor (anti-VEGF) [3], and anti-neuropilin (anti-NRP1^B), an anti-angiogenic agent which is believed to inhibit vessel maturation [4]. To account for tumor heterogeneity, we use viable tumor segmentation, restricting the VSI parameter analysis to the viable tumor tissue as determined by multispectral k-means clustering [5]. This technique has been previously shown to correlate well with *ex vivo* micro-CT angiography and histology [6]. In this study, the viable tumor VSI technique detected effects on the vasculature that showed a significant advantage to dosing with anti-NRP1^B before anti-VEGF, rather than vice versa; this may indicate that anti-NRP1^B treatment leaves vessels in an immature, VEGF-dependent state which then responds strongly to anti-VEGF treatment.

Methods. The institutional AAALAC-accredited review board approved all animal procedures. MRI scans were performed on a 4.7 T Varian Direct Drive MRI system with a 20 mm two-loop surface coil (Varian Inc, Palo Alto, CA), for 25 mice with Calu6 human lung cancer tumors grown subcutaneously on the leg. A multi-slice FSE sequence with diffusion weighting was used to calculate an apparent diffusion coefficient (ADC) map (6 b-values ranging 300-1100 s/mm^2, TR = 3 s, ETL = 4). A multi-slice spin echo sequence was used to calculate T_2 maps (TR = 3s, TE = 5,26,47,68 ms), and a multi-echo multi-slice gradient echo sequence was used to calculate T_2* maps (TR = 345 ms, TE = 5,10,15,20,25,30,35,40 ms). Data were collected as 8 coronal 1-mm-thick slices (FOV = 25.6×25.6mm, 64×64 matrix for ADC and T_2 maps, 128×128 for T_2* maps). A USPIO contrast agent (Molday ION, BioPAL) was then injected via a tail-vein catheter (200 µmol/kg), and the SE and GE sequences were repeated to calculate the post-contrast T_2 and T_2* maps. Mice were randomized into three groups based on tumor volume caliper measurements: Group 1 (N=8) was treated with appropriate control antibodies twice a week for 3 weeks, Group 2 (N=8) was treated first with B20 twice a week for 1.5 weeks and then with anti-NRP1^B twice a week for 1.5 weeks, and Group 3 (N=9) was treated first with anti-NRP1^B twice a week for 1.5 weeks and then with B20 twice a week for 1.5 weeks. All mice were imaged after the first 10 days of treatment and again after the second 10 days of treatment.

Vessel size index (VSI) maps, blood volume (BV) maps, and Q maps were calculated voxel-by-voxel [1,2]. Multispectral analysis for tissue segmentation was performed using a k-means clustering algorithm [5]. The tumor was segmented into four classes: viable tumor tissue, subcutaneous fat, and two necrosis classes. The viable tissue class was used as a mask to calculate mean VSI, BV, and Q parameters only within the viable tissue.

Results & Discussion. After 3 weeks, both treated groups had significantly reduced viable tumor volume relative to the control group. There was no difference in mean VSI between the three groups. However, Group 3 had significantly reduced blood volume and Q relative to both Groups 1 & 2. This may indicate that initial treatment with anti-NRP1^B makes tumor vasculature more susceptible to later treatment with anti-VEGF, while the reverse is not the case. In conclusion, we have demonstrated that VSI MRI can detect different changes in the microvasculature of viable tumor tissue in response to the order of treatment with two anti-angiogenic therapies.

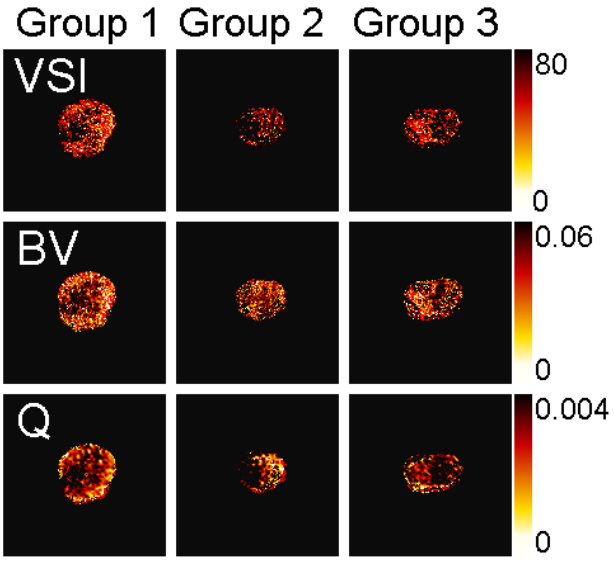

Figure 1. Representative images from each group at Day 20. VSI maps show reduction in vessel size in all groups. BV maps and Q maps both show significant reduction for Group 3 relative to Groups 1 & 2.

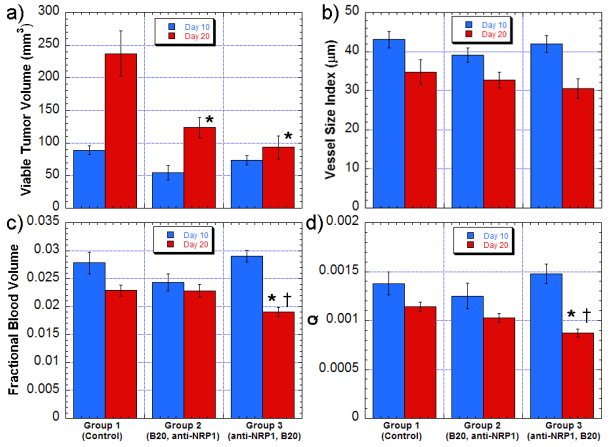

Figure 2. Effect of sequential anti-angiogenic therapies on (a) viable tumor volume, (b) vessel size index, (c) blood volume, and (d) Q (vessel density). * indicates $p < 0.05$ relative to Group 1; † indicates $p < 0.05$ relative to Group 2.

[1] Tropres *et al.*, **MRM** 2001, p. 397.
[2] Jensen *et al.*, **MRM** 2000, p. 224.
[3] Liang *et al.*, **J Biol Chem** 2006, 951.
[4] Pan *et al.*, **Cancer Cell** 2007, p. 53.
[5] Carano *et al.*,**MRM** 2004, p. 542.
[6] Ungersma *et al.*, **ISMRM** 2008, 449.