Validation of anti-VEGF Therapy in a Radiation Necrosis Mouse Model

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Purpose: Delayed radiation injury, also known as radiation necrosis (RN), is a serious complication of radiation therapy, seen in up to 23% of patients, that can occur months to years after radiation. We have recently developed a mouse model of focal RN generated via stereotactic radiosurgery with the Leskell Gamma Knife®. Among the newest treatment options for RN is the use of anti-VEGF antibodies. We sought to evaluate the therapeutic potential of a murine anti-VEGF antibody in our RN model, while validating VEGF as a target via immunohistochemistry (IHC).

Methods: All experiments were approved by the Washington University Division of Comparative Medicine and were performed on 8-9 week old female BALB/c mice. A single, 50 Gy radiation dose (50% isodose) from the Leksell Gamma Knife was focused on the cortex of the left hemisphere ~ 3 mm posterior to bregma. Images were acquired with a 4.7-T small-animal Agilent/Varian DirectDrive™ scanner using an actively decoupled volume coil (transmit) and 1.5-cm surface coil (receive). Mice were randomly assigned at week 4 post irradiation (PIR) to one of three treatment groups. The untreated group received no treatment, the B20 group received 10 mg/kg of B20-4.1.1 given intraperitoneally twice weekly, and the GP120 received 10 mg/kg of GP120:9239 given intraperitoneally twice weekly. B20-4.1.1 (Genentech, San Francisco, CA) is a murine antibody capable of recognizing both human and mouse VEGF, while GP120:9239 (Genentech, San Francisco, CA) is a murine antibody of the same isotype that targets the HIV capsid protein. Lesion volumes were quantified in a semi-automated manner on both T1 and T2-weighted images.

Results: IHC staining (Figure 1) demonstrated that VEGF was initially expressed at week 4 PIR with progressive expression over time. This is consistent with our prior observation that permeability does not increase until approximately the same time. Figure 2 shows the quantification of the T2-derived lesion volumes. (T1-derived volumes are not shown but are similar.) Treatment of this mouse model with an anti-VEGF antibody (B20) lead to a reduction in lesion size that could not be accomplished with non-specific antibody treatment (GP120). Figure 2 shows T1 and T2-weighted images after treatment (13 weeks PIR) for the different treatment groups. As would be expected from the volume measurements shown in Figure 2, mice in the B20 treatment group exhibited much smaller areas of enhancement in MRI images. However, some mice in the B20 group eventually exhibited regions of enhancement on both post-contrast T1 and T2-weighted imaging (labeled as B20-resistant in Figure 3). Curiously, the pattern of enhancement seen in both post-contrast T1 and T2-weighted images is different than that seen in either the GP120 or Control groups. Specifically, the B20-resistant mice showed an outside-in pattern, in which enhancement first appeared on the edges of the brain, whereas the GP120 and untreated groups showed an inside-out pattern, in which the initial lesion propagates outward.

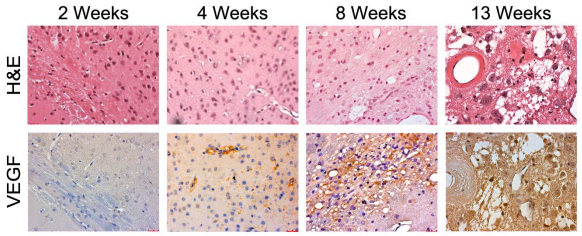

Figure 1. VEGF is elevated as early as week 4 PIR. H&E (top row) and VEGF staining (bottom row) of corresponding regions of irradiated brain are presented. VEGF staining (brown) indicates a progressive increase in VEGF expression.

Discussion and Conclusion: Our mouse model of RN provides a unique tool to investigate late radiation injury, independent of other pathologies. We have shown that our mouse model responds to anti-VEGF therapy similar to clinical RN. IHC for VEGF after therapy was able to detect VEGF expression in all treatment groups (data not shown). In the case of B20 treated mice, remnant VEGF expression could explain why some of the mice become resistant to B20 therapy, though more work is needed to colocalize the MRI signal to VEGF expression as identified by IHC.

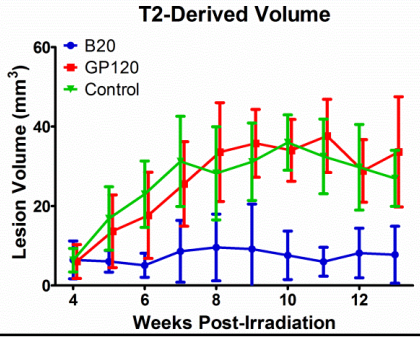

Figure 2. Mice were treated after week 4 PIR with either an anti-VEGF antibody (B20 group), an isotype control antibody (GP120 group), or not at all (Control group). Graphs represent mean ± standard deviation of 5-9 animals. The B20 group is statistically different from both control groups at every time point after week 6 PIR (P<0.05).

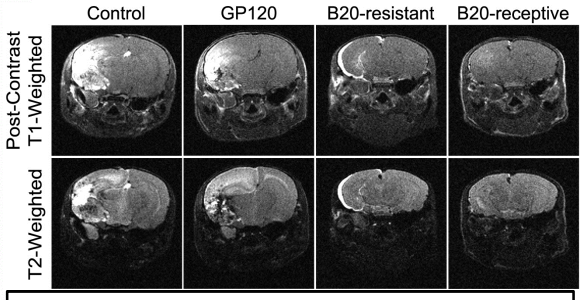

Figure 3. Qualitative analysis of MRI images reveals resistance to B20 treatment. Post-contrast T1-weighted images and T2-weighted images are presented for representative animals from the untreated, GP120, and B20 groups at 13 weeks PIR. The third column shows images of a mouse that appears to develop resistance to B20 treatment, while the fourth column shows images of a mouse that responds to B20 therapy.