# Dynamic Contrast-Enhanced MR Imaging Detected Early Changes in Vascular Permeability following Anti-DR5 Antibody Therapy in Breast Tumor Xenografts

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**Purpose:** Dynamic contrast-enhanced MR imaging (DCE-MRI) was applied to measure the early therapeutic response of a novel anti-DR5 antibody (TRA-8), bevacizumab, and TRA-8 combined with bevacizumab in breast tumor xenografts.

**Methods:** Four groups (n=5/group) of nude mice bearing subcutaneous breast tumors (2LMP) were used. Groups 1-4 were intraperitoneally injected with saline, bevacizumab (0.1 mg), TRA-8 (0.2 mg), and TRA-8+bevacizumab on day 0, respectively. DCE-MRI and anatomical MRI were performed on days 0 (before dosing), 1, 2, and 3. Four days prior to imaging, a vascular access port was implanted in each mouse to facilitate repeated intravenous Gd-DTPA injections. Two animals were imaged simultaneously, reducing the total image-acquisition time by half (fig. 1A). Prior to Gd-DTPA injection, a $T_1$ map was acquired with a gradient-echo multiflip-angle approach (10°, 20°, 30°, 40°, 50°, 60°, and 70°), and then the DCE-MRI was performed with the fixed flip angle of 30°. A total of 3~5 1-mm thick slices (0.2-mm gap) were used to cover tumor regions of interest. Five baseline images were acquired before Gd-DTPA injection, and then 20 images were collected after Gd-DTPA injection (0.2 mmol/kg BW, 150 μl (10 μl/sec)) with time resolution of 58 seconds. Reference Region (RR) model (1) was employed to calculate vascular permeability ($K^{trans}$). The averaged $K^{trans}$ values in both the entire tumor region (fig. 1C) and 0.5-mm thick peripheral tumor region (fig. 1D) were calculated, and compared with changes in tumor volume. Repeated measure analysis of variance (RM ANOVA) (2) was used to analyze the difference between groups over all 3 days.

**Results:** Figure 1 shows representative DCE MR images at (A) 1 minute before and (B) 5 minutes after Gd-DTPA injection, with $K^{trans}$ maps in (C) the entire tumor region and (D) the 0.5-mm thick peripheral region. Figure 2 presents the $K^{trans}$ changes of groups 1-4 over 3 days after therapy initiation in (A) the entire tumor region and (B) the peripheral tumor region. In both the regions, the $K^{trans}$ values of groups 3 and 4 were significantly lower than that of control (p < 0.05). The $K^{trans}$ values were significantly reduced by TRA-8 monotherapy in the peripheral tumor region (p=0.0181), but not in entire tumor region (p=0.503). For tumor-volume analyses, no significant difference between any treated group and control group was detected over 3 days (p > 0.05). Figure 3 demonstrates that the $K^{trans}$ changes over time for all treated groups followed a 2nd order polynomial.

**Discussion:** Sequential DCE-MRI in the same mice was successfully performed every 24 hours over 3 days, detecting significantly lower $K^{trans}$ levels in breast tumors responsive to therapy, as compared to controls, within 3 days after therapy initiation. Sequential DCE-MRI may provide a sufficient number of data points to enable non-linear mathematical modeling for intratumoral vascular permeability changes; the non-linear characteristics could be considered as a time-independent imaging biomarker (3). Peripheral-region analyses markedly increased the accuracy of the vascular permeability measurements (fig. 2), which can be translated for clinical trials immediately. The proposed techniques may be utilized to improve "Personalized Medicine" during breast-tumor preoperative therapy.
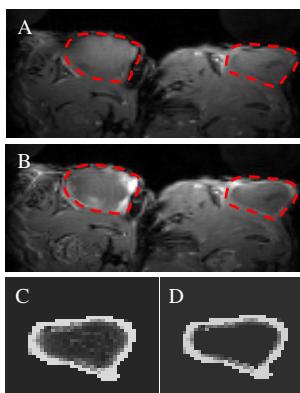


**Figure 1:** Representative dynamic contrast MR images at (A) 1 minute before contrast agent (Gd-DTPA) injection and (B) 5 minutes after contrast agent injection (two mice were imaged simultaneously. The tumor regions are indicated with red dotted circles. $K^{trans}$ maps of the right-side tumor for (C) the entire tumor region and (D) the 0.5-mm thick peripheral tumor region.
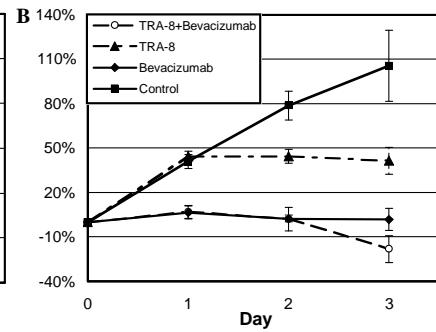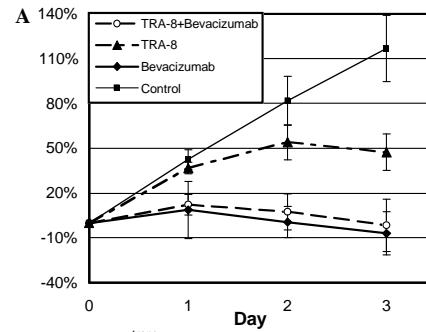


**Figure 2:** $K^{trans}$ changes (mean±SE) of (A) the entire tumor region and (B) the 0.5-mm thick peripheral tumor region at 1, 2, and 3 d, after mice were treated with saline (control), bevacizumab, TRA-8, and TRA-8+bevacizumab (groups 1, 2, 3, and 4, respectively: n=5 per group) on day 0.
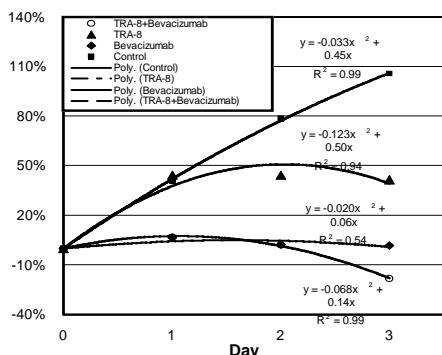


**Figure 3:** Vascular permeability ($K^{trans}$) changes in 0.5-mm thick peripheral region fit a 2nd order polynomial for all treated groups: averaged $K^{trans}$ changes of groups 1, 2, 3, and 4 over 3 days with the best fitting 2nd order polynomial curves, equations, and R-squared values. The characteristics of non-linear $K^{trans}$ changes can be utilized to measure early anti-angiogenesis independently of time.

**References:**
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