# Improved Molecular Imaging of Sparse Neovascular Biomarkers with a Novel Lipophilic Gd-DOTA chelate on Targeted Nanoparticles

**P. M. Winter[1], S. D. Caruthers[1,2], A. H. Schmieder[1], T. A. Williams[1], D. Riley[3], W. McGhee[3], S. A. Wickline[1], and G. M. Lanza[1]**

[1]Washington University, St. Louis, MO, United States, [2]Philips Medical Systems, [3]Kereos, Inc., St. Louis, MO

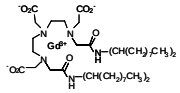
**Introduction:** MRI contrast agents with higher relaxivity can induce larger changes in image intensity, and therefore have a lower minimum detection level. Increasing the relaxivity of molecular imaging agents may provide higher sensitivity to the targeted biomarker and allow earlier detection of disease. The expression of $\alpha_v\beta_3$-integrin, a heterodimeric transmembrane glycoprotein, is upregulated in proliferating versus quiescent endothelial cells and is considered a neovascular biomarker for molecular imaging of tumor growth. The purpose of this study was to compare the signal enhancement from $\alpha_v\beta_3$-targeted nanoparticles formulated with either Gd-DTPA-BOA or Gd-DOTA-Amide-PE (Figure 1) targeted to angiogenic vasculature in a tumor model.

**Methods:** Nanoparticles consisted of a perfluorooctylbromide core encapsulated in a surfactant mixture. The surfactant included a peptidomimetic vitronectin antagonist for targeting to the $\alpha_v\beta_3$-integrin and either Gd-DTPA-BOA or Gd-DOTA-amide-PE as the paramagnetic chelate. The relaxivity of each nanoparticle formulation was measured at 3.0T with a mixed spin-echo, inversion recovery imaging sequence. The "mixed" sequence generates a series of images with different amounts of $T_1$ and $T_2$ weighting. Pixel-by-pixel fitting with RLSQ algorithms yields values of $T_1$, $T_2$ and proton density.

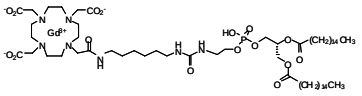
Rabbits bearing 14 day old Vx-2 carcinoma tumors in the popliteal fossa were imaged at 3.0T with an 8 element coil array. 3D gradient echo, fat suppressed, black blood, $T_1$-weighted images were collected (TR/TE = 42/5.7 ms, flip angle = 45°, in-plane resolution = 250 μm by 250 μm, slice thickness = 500 μm, 67 slices, 2 signal averages, total scan time = 25 minutes) before and 3 hours after IV injection (1 ml/kg) of either Gd-DTPA-BOA (n=6) or Gd-DOTA-amide-PE (n=6) nanoparticles. Blood samples were collected 3 hours post-injection and the $Gd^{3+}$ content was measured by neutron activation analysis. Post-injection enhancement was defined as pixels with a signal intensity that exceeded the mean tumor signal at baseline by more than two standard deviations. The tumor periphery was identified as pixels in the outer 1/6th of the tumor radius. The average signal increase in enhancing pixels was multiplied by the percent area of enhancement to calculate the contrast index, a composite measure of both the extent and the intensity of tumor enhancement.

**Results:** Gd-DOTA-Amide-PE nanoparticles had a 50% higher relaxivity at 3.0T compared to Gd-DTPA-BOA nanoparticles (15.4 $(s*mM)^{-1}$ vs. 10.4 $(s*mM)^{-1}$, respectively). In the Vx2 tumor, nanoparticles formulated with Gd-DOTA-Amide-PE produced 74% more signal enhancement in the tumor periphery (Figure 2), contrast index = 607 ± 72, compared to Gd-DTPA-BOA particles, contrast index = 349 ± 49 ($p < 0.05$). This was mostly due to a 69% increase in the number of pixels enhancing above the detection threshold, 11.3 ± 1.2% vs. 6.67 ± 0.61% ($p < 0.05$), while the average signal enhancement increased only 9%, 53.2 ± 2.0 vs. 48.9 ± 3.0 ($p$ = NS). Blood levels of $Gd^{3+}$ at the time of MRI were identical, 24.1 ± 3.9 μM vs. 31.4 ± 6.7 μM ($p$ = NS), indicating that the increased enhancement was not due to differences in nanoparticle clearance rates. Three dimensional reconstruction of the tumor enhancement with targeted Gd-DOTA-Amide-PE nanoparticles shows some small concentrated areas of angiogenesis along with fine dispersed neovessels feeding the growing tumor (Figure 3). The lower relaxivity agent was capable of detecting the larger areas, but often could not distinguish the finer details.

**Conclusions:** This study shows that nanoparticles formulated with Gd-DOTA-Amide-PE have higher relaxivity than Gd-DTPA-BOA particles. The improved relaxivity caused increased in vivo enhancement, which could provide more sensitive detection of tumor angiogenesis with molecular imaging.



Gd-DTPA-BOA



Gd-DOTA-Amide-PE

**Figure 1:** Chemical structures of Gd-DTPA-BOA (Top) and Gd-DOTA-Amide-PE (Bottom). The DOTA agent displays increased relaxivity compared to the linear chelate.

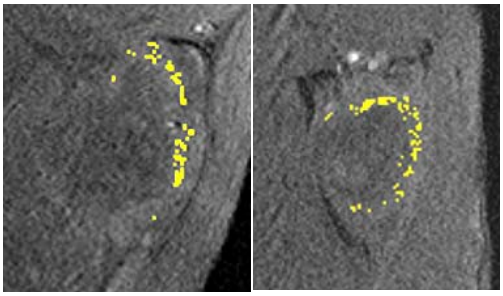


**Figure 2:** Molecular imaging of tumor angiogenesis with Gd-DTPA-BOA (Left) or Gd-DOTA-Amide-PE (Right) nanoparticles. The area of enhancement (colorcoded in yellow) is larger with the Gd-DOTA-Amide-PE agent.

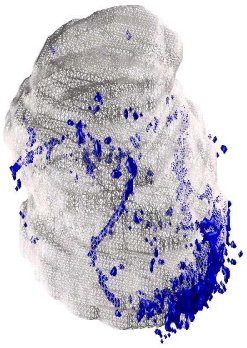


**Figure 3:** 3-D reconstruction of signal enhancement with Gd-DOTA-Amide-PE nanoparticles (colorcoded in blue) shows the fine structures of neovessels feeding the tumor.