## Advantages of Micron-Sized Magnetic Particles for Tracking Dendritic Cells in Preclinical Cancer Vaccine Studies

**R. Rohani[1], G. Dekaban[2], C. Willert[2], S. De Chickera[2], and P. Foster[1]**

[1]Imaging, Robarts Research Institute, London, Ontario, Canada, [2]Biotherapeutic, Robarts Research Institute, London, Ontario, Canada

**Introduction:** Dendritic cell (DC) immunotherapy is under investigation for the treatment of cancer. However, the poor migration of DC *in vivo* has been a significant barrier to the clinical implementation of DC immunotherapy. At present most studies rely on node biopsy to evaluate the migration of DC. A number of labs have begun to explore the use of cellular MRI to track DC migration *in vivo*, in mice and humans, after labeling DC with superparamagnetic nanoparticles (SPIO) and protamine sulfate[1,2]. The aim of this study is to investigate the use of micron-sized superparamagnetic iron particles (MPIO) for DC tracking. For preclinical work MPIO has some advantages. Cells can be labeled with higher iron loadings using MPIO, enabling smaller numbers of cells to be detected. In addition, MPIO are inert so the label will persist longer *in vivo* than SPIO. Here we show that the migration of MPIO-labeled DC can be tracked *in vivo* in mice and that the signal loss due to DC in the nodes can be measured.

**Methods:** Bone marrow-derived DC were isolated from EGFP+ transgenic mice and labeled with MPIO (0.9 μm, Bangs Labs) by overnight incubation. $1x10^6$ MPIO labeled DC were injected into the left footpad of recipient C57Bl/6 mice for delivery to the popliteal lymph node. $1x10^6$ unlabeled DC were injected into the right footpad. Images were acquired on a 3T GE whole body scanner equipped with a custom-built high-performance gradient coil and a mouse body solenoid RF coil. Anesthetized mice were scanned using a 3D balanced steady state free precession (bSSFP) pulse sequence. Imaging parameters were: TR/TE=4.2/2.1, 200x200x200 micron spatial resolution, FA=20, eight phase-cycles and the acquisition time was 21 minutes. Scanning was performed pre-injection and at 2, 3 and 7 days post-injection.

**Results:** Signal loss in the popliteal node could be easily detected and the volume of signal loss could be measured and related to the presence of DC that have migrated from the footpad to the node. Figure 1 shows a representative coronal image slice of the mouse body at the level of the popliteal nodes. Figure 2 shows representative images (cropped and enlarged) of the popliteal lymph node at days 2, 3 and 7 post DC injection. Measurements of the volume of the popliteal node and the volume of the region of signal loss within the node are shown in Figure 3. The node volume increases (on both sides) after the DC injection, due to the infiltration of DC and subsequent T cell proliferation. The volume of the region of signal loss within the right popliteal node also increased between 2 and 7 days post DC injection, suggesting that DC continue to infiltrate the node over this time period.

**Discussion:** The bSSFP sequence is very sensitive to susceptibility effects and has a very high SNR efficiency allowing the acquisition of 3D cellular MR images of the whole mouse body in less than 30 minutes. bSSFP relating banding artifact at 3T was minimized using RF phase cycling. The images allow the clear visualization and quantification of the mouse lymph nodes and the iron-labeled cells within them. This approach can now be used to test different strategies for enhancing cell migration, a key obstacle to the realization of DC for cancer therapy. Although MPIO are not suitable for clinical use they provide an excellent option for imaging small numbers of cells *in vivo* in mice for important preclinical investigations of disease.

**References:** [1] Baumjohann et al. Eur J Immunol 2006, [2] de Vries et al. Nat Biotechnol 2005.

Figure 1.

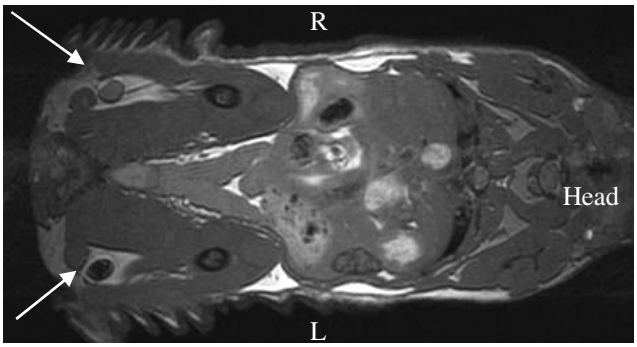



Figure 2.

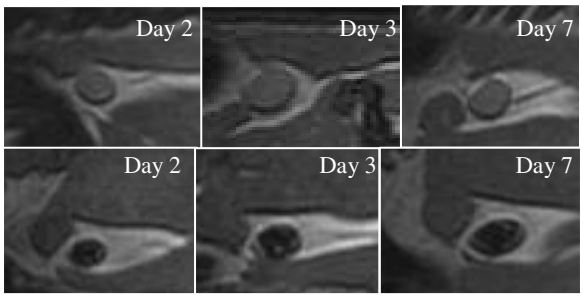
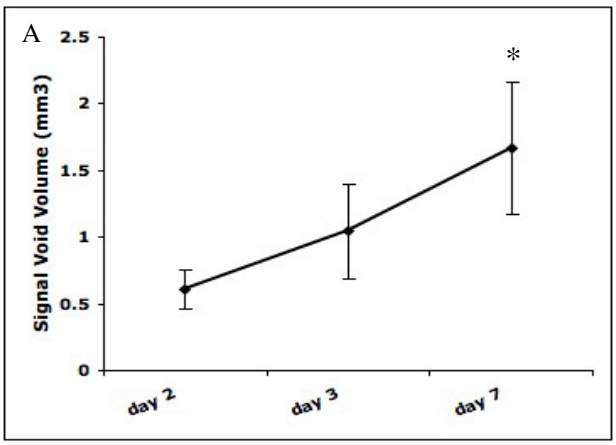
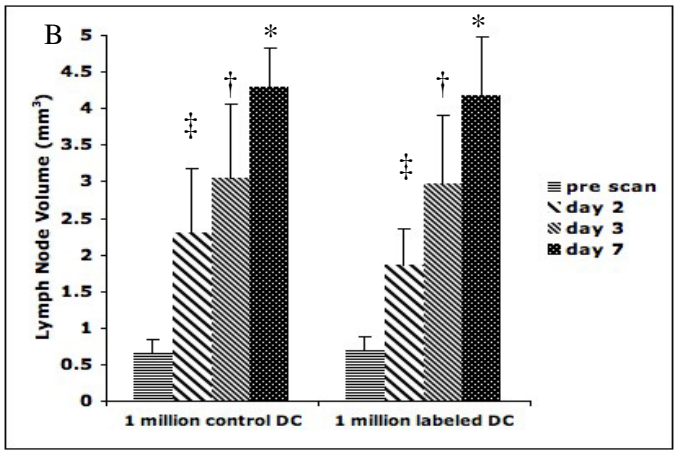



Figure 3.




*P<0.01 for Day 3 vs. Day 7




‡P<0.05 for Prescan vs. Day 2, †P<0.01 for Prescan vs. Day 3
*P<0.01 for Prescan vs. Day 7