Using a 19F MRI Tracer Agent for In Vivo Tracking of Human Dendritic Cell Vaccines


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Introduction
The inability to non-invasively track clinically relevant cell types after in vivo administration has been a significant roadblock to the acceptance of cellular therapies. Dendritic cells (DCs), the “professional” antigen presenting cells of the immune system, are increasingly being used as cellular vaccines in the setting of cancer and infectious diseases. Here we ex vivo labeled human DCs using a novel, commercially available fluorine (19F)–based MRI tracer agent to visualize these transferred cells in vivo by 19F MRI. In this study we show for the first time that clinically relevant human DCs can be effectively labeled in vitro with a 19F tracer without impacting cell health, phenotype, or function. Moreover, the 19F-labeled human DCs are capable of being detected by 19F MRI 8 hours post injection into NOD-SCID mice, demonstrating utility and possible clinical application of this method.

Methods
Human monocyte derived immature DCs were generated in the presence of IL-4 (500 IU/ml) and GM-CSF (500 IU/ml) for 5 days, and maturated by exposure to TNF-α, IL-1β, IL-6, and PGE2 for 48 hours as previously described. Two different PFPE tracer reagents (CS1000 or CS1250+; Celsense, Inc., Pittsburgh PA) were used to label the DCs. These reagents were added to day 5 cultures, and the cells were then harvested, washed, and characterized on day 7. The 19F labeling efficiency was determined by NMR of a fraction of the cells. Cell viability and phenotype were determined by microscopic analysis as well as flow cytometry. The IL-12p70 producing capacity of the DCs was tested following CD40L stimulation by measuring protein concentration in culture supernatants by ELISA (Endogen, Woburn, MA). The migratory responsiveness of the DCs to CCL21 (6Ckine; R&D systems, Minneapolis, MN) was examined using 96 well migration chambers (NeuroProbe, Gaithersburg, MD). To determine T cell stimulatory capacity, proliferation assays were performed using allogeneic T cells as responders (allo-MLR). The Cell-Titer Glow Assay (Promega, Madison, WI) was used to determine cell count. Human monocyte derived immature DCs were generated in the presence of IL-4 (500 IU/ml) and GM-CSF (500 IU/ml) for 5 days, and

Results and Discussion
While previous studies have demonstrated the utilization of 19F tracer agents as tools for in vivo imaging of animal cells and immortalized cell lines (3,4), this is the first report demonstrating the utility of 19F tracer agents in labeling clinically relevant human immune cells for in vivo tracking. This study illustrates that the 19F tracer agents tested had no deleterious effect on cell function or viability while providing a high-resolution image of migratory cells following injection into NOD-SCID mice. This study also offers in vivo functional confirmation that transplanted 19F-labeled human DCs can indeed migrate to draining lymph nodes in response to chemokines of a murine host. This novel animal model may now provide researchers with a valuable tool for pre-clinical in vivo human DC studies.

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References
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Fig 1. Migration of 19F-labeled human DCs. (A) In vitro analysis of the migratory responsiveness of immature DCs (iDC) and mature DCs (mDC) to the lymph node associated chemokine CCL21. (B) MRI of a NOD-SCID mouse demonstrating In vivo migration of human mature DC. Images were acquired 8h post-injection of 19F-labeled human DCs (2x106). The DCs were injected into the quadriceps at the location indicated with the asterisks (*). The anatomical 1H image is grayscale and the 19F image is rendered in hot-iron scale showing the accumulation of perfluorocarbon-labeled human mature DCs