MR tracking of magnetically-labeled dendritic cells migrating to lymph nodes in melanoma patients: First clinical experience

I. de Vries[1,2], W. Lesterhuis[3], J. Barentsz[4], G. Gambarota[4], T. Scheenen[4], N. Scharenborg[1], A. Veltien[4], J. Rijntjes[5], O. Boerman[6], W. Oyen[6], G. Adema[1], J. van Krieken[5], J. Bulte[7], C. Punt[3], A. Heerschap[4], C. Figdor[1]

[1]Tumor Immunology, NCMLS, UMC Nijmegen, Nijmegen, Netherlands, [2]Pediatric Oncology, UMC Nijmegen, Nijmegen, Netherlands, [3]Medical Oncology, UMC Nijmegen, Nijmegen, Netherlands, [4]Radiology, UMC Nijmegen, Nijmegen, Netherlands, [5]Pathology, UMC Nijmegen, Nijmegen, Netherlands, [6]Nuclear Medicine, UMC Nijmegen, Nijmegen, Netherlands, [7]Radiology and Institute for Cell Engineering, Johns Hopkins University School of Medicine, Baltimore, MD, United States

Introduction Dendritic cells (DC) are the professional antigen presenting cells of the immune system[1]. Recent clinical studies indicate that mature DC are effective cancer vaccines when loaded with tumor antigens[2]. To induce an effective immune response, these cells should not only express high levels of antigen presenting and co-stimulatory molecules but also migrate to the lymph nodes (LN) to interact with naïve T cells. Monitoring cellular migration in vivo by MRI is feasible[3] and is important to improve the efficacy of DC-based therapies[4]. Here we demonstrate that DCs can be efficiently labeled with Endorem, a clinical iron oxide formulation, without the need for transfection agents. This allowed in vivo MR tracking of intranodally injected DC in melanoma patients and ex-vivo MRI of DC distribution in LN.

Patients and Methods Mature monocyte-derived DC were cultured from peripheral blood of melanoma patients planned for surgical LN resection[5]. DC were cocultured in the presence of Endorem super paramagnetic iron oxide nanoparticles (SPIO) which were spontaneously taken up by DC. Forty-eight hours before surgery, ^{111}Indium oxinate-labeled and SPIO-labeled DC (15×10^6) were mixed and patients received a single injection directly into a tumor draining LN under ultrasound guidance. Migration of the labeled DC from the LN was recorded using scintigraphy of radioactively labeled cells and MRI of SPIO-labeled cells. This protocol was approved by our institutional review board. Before and 48 hours after intranodal injection, patients were imaged with a gamma camera and by MRI. MRI was performed on a 3T Siemens MR system with a body phase array coil. A pulse sequence was used which combined the signals of three gradient echoes into one R2* weighted image with an average echo time of 15 ms. (flip angle 36 degrees, TR 1060 ms, total time ~9 minutes, resolution 0.50 x 0.50 x 3.50 mm). Apart from the R2*-weighted images, which are very sensitive to the presence of iron-oxide, we also acquired Turbo Spin Echo images with short echo time (18 ms) (0.83 x 0.50 x 3.50 mm, exact same 30 slice locations, TR 2.5s, hyperechoes, total time ~6.5 minutes) as an additional reference to make sure that the decreased signal intensity originates from magnetic field inhomogeneities caused by SPIO. After the last imaging session, the LN basin was resected and radioactive LN were embedded. Sections were dipped in photographic emulsion (to detect radioactive indium labeled cells) and after exposure developed and counterstained with H&E. Alternatively, sections were stained with Prussian blue for iron to detect SPIO-labeled cells. Ex-vivo MR imaging of LN was performed on a 7T MR-spectrometer using a 20 mm diameter RF coil. The LN were placed in a plastic tube filled with Fomblin, to reduce susceptibility artifacts at the tissue–air interface. Multislice gradient-echo imaging was performed at two different echo times (TR = 1000 and TE = 6 and 9 ms, voxel-size = 136x136x700 mm). Multislice spin-echo imaging was then performed. (TR = 2000 and TE = 11 and 28 ms, same voxel-size as in the gradient-echo imaging).

Results DC can efficiently be labeled with ^{111}Indium oxinate[5] and with SPIO (Figure 1A)[5]. In vitro, no effect of the labeling was observed on viability and function (e.g. migration of cells in vitro).DC migrated from the injected LN to subsequent LN as could be visualized with both scintigraphy imaging and MRI. Furthermore, migrated DC could not only be detected very efficiently by MRI in vivo (Figure 1D) but also the intranodal distribution pattern of the injected cells in the LN could be analyzed by high resolution MRI of resected LN (Figure 1C). The localization of DC in the total LN was confirmed by iron staining of LN sections (Figure 1B). Injected DC entered the LN via their natural route, the lymphatic vessels, and subsequently enter the T cell areas.

Conclusions We show for the first time that migration of magnetically labeled cells can be efficiently tracked in humans by non-invasive MRI, providing excellent anatomical detail on the internodal and intranodal DC migration patterns. High resolution MRI using SPIO-labeled DC allowed the detection of small numbers of these cells in LN. The presence of the DC could be confirmed by the detection of radioactive-labeled DC that were co-injected and detected by scintigraphy. Furthermore, the distribution of intact iron-labeled DC was validated by immunohistological stainings of LN sections.

A major advantage of MRI over imaging using radioactive labels is the superior anatomical information obtained. Combination of both techniques facilitates in vivo double label imaging of differently labeled cells (DC subsets, DC in different activation or maturation stages) injected in one and the same site. This is particularly valuable to further optimize cell-based anti-cancer therapies for cancer patients.

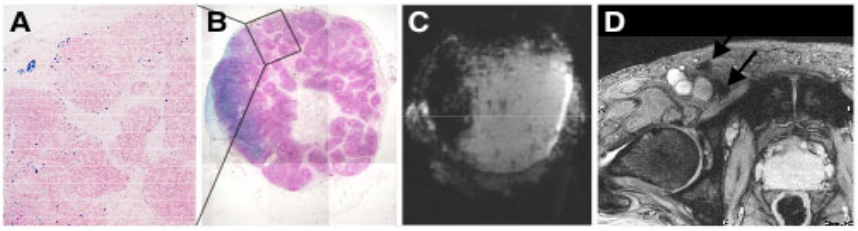

***Figure 1*:** Immunohistological and MR images of SPIO-labeled DC. A. Prussian blue staining for iron (individual cells) of the indicated part of the LN section. B. Overview of a LN, blue indicates the presence of in vitro-labeled DC that have migrated to this LN. C. MRI at 7T of the intact resected LN imaged at 7T MR. The black areas closely match the blue iron staining in B. Panel D shows a transverse slice of an MRI examination of a melanoma patient; the dark spots (arrows) indicate the presence of iron in LN, representing migrated DC.

References

1. Banchereau J, Steinman RM: Dendritic cells and the control of immunity. Nature 392:245-52, 1998
2. Schuler G, Schuler-Thurner B, Steinman RM: The use of dendritic cells in cancer immunotherapy. Curr Opin Immunol 15:138-47, 2003
3. Bulte JW, Douglas T, Witwer B, et al: Magnetodendrimers allow endosomal magnetic labeling and in vivo tracking of stem cells. Nat Biotechnol 19:1141-7, 2001
4. Figdor CG, de Vries IJ, Lesterhuis WJ, et al: Dendritic cell immunotherapy: mapping the way. Nat Med 10:475-80, 2004
5. De Vries IJ, Krooshoop DJ, Scharenborg NM, et al: Effective migration of antigen-pulsed dendritic cells to lymph nodes in melanoma patients is determined by their maturation state. Cancer Res 63:12-7, 2003