First clinical experience using fluorine-19 MRI to track immunotherapeutic dendritic cells in colorectal cancer patients

Eric T Ahrens[1], Anthony Balducci[2], Brooke Helfer[2], Amy Wesa[2], Charles O'Hanlon[2], Claudiu Schirda[3], David Bartlett[4], and Pawel Kalinski[4]

[1]Radiology, University of California at San Diego, La Jolla, CA, United States, [2]Celsense, Inc., Pittsburgh, PA, United States, [3]Radiology, University of Pittsburgh, Pittsburgh, PA, United States, [4]Surgical Oncology, University of Pittsburgh, Pittsburgh, PA, United States

Introduction - We described the first clinical use of a sensitive and non-toxic fluorine-19 based MRI tracer agent specifically designed for MRI cell tracking. Emerging cell therapies, such as those employing immune or stem cells, can benefit from non-invasive imaging to visualize the behavior of cells following transfer into the patient. Imaging of cell trafficking can provide feedback regarding the persistence, motility, optimal routes of delivery and therapeutic doses for individuals [1]. In this study we labeled autologous immunotherapeutic dendritic cell (DCs) with a perfluorocarbon (PFC) tracer agent *ex vivo*. Labeled DCs were inoculated into colorectal cancer patients. Cells were imaged using a conventional 3 T scanner using ^{19}F/^{1}H MRI and ^{19}F MRS. Overall, we show that clinical ^{19}F-based cell tracking is feasible [2] and provides unambiguous information about the cell location, with no background signal, and can be used to quantify cells *in situ*.

Methods - This safety and feasibility study was conducted under protocols approved by the University of Pittsburgh Cancer Institute Institutional Review Board and the Office of Cell, Tissue and Gene Therapy at the US Food and Drug Administration (BB-IND 14,730). A Drug Master File (DMF) covering the commercially available PFC MRI tracer reagent (BB-MF 14,062) was cross referenced in the IND application. This study included adult patients (N=5) with metastatic (Stage 4) colorectal cancer. The autologous live-cell DC vaccine was prepared using a seven day culture protocol, as previously described [3]. On day six, the GMP-grade PFC MRI tracer emulsion (CS1000, Celsense, Inc., Pittsburgh, PA) agent was added to the media for a portion of cells. To assay the ^{19}F content of the DCs post-labeling, sample cell pellets from the batch (~3×10^6 cells) were lysed, spiked with trifluoroacetic acid (TFA) reference compound, and analyzed using high-resolution ^{19}F NMR to calculate the mean ^{19}F/cell [4]. Cells were also assayed for viability and phenotype using fluorescence-activated cell sorting to measure the expression of HLA-DR, CD83, CD86, and CCR7 to compare PFC-labeled versus unlabeled cells. The patient study consisted of three separate intradermal administrations of the DC vaccine, one of the doses being labeled with PFC (1×10^6 or 1×10^7 DCs). The site of administration was the quadriceps near the inguinal crease. At 4 and 24 hours post-injection, subjects had ^{19}F/^{1}H MRI scans using a 3 T Siemens Tim Trio scanner. A custom ^{19}F/^{1}H, 7 cm diameter, surface coil (Stark Contrast, Inc., Germany) for transmit and receive was placed near the injection site. A non-selective 1-pulse ^{19}F MRS sequence was initially used to detect labeled cells (pulse 0.5 ms, TR/TE=1500/0.35 ms, SW=10 kHz, NA=384). Next, ^{19}F images were acquired using a conventional spin-density weighted FLASH sequence with a 9.5 min scan time; the imaging parameters were TR/TE=100/4.15 ms, NA=96, FA=45°, slice thickness 2 cm, NS=3, FOV=$(28.8\text{ cm})^2$, and matrix size 64×64. For anatomical reference, co-registered ^{1}H FLASH images were collected with parameters TR/TE=115/4.92 ms, NA=2, FA=25°, slice thickness 5 mm, NS=12, FOV=$(28.8\text{ cm})^2$, and matrix size 192×192. The ^{19}F/^{1}H images were imported into Voxel Tracker^{TM} software (Celsense) for quantitative analysis.

Results - PFC provides efficient and safe labeling of patient cells. By NMR, the patient DCs had a mean ^{19}F content on the order of 10^{12} to 10^{13} ^{19}F/cell, depending on batch, consistent with previous *in vitro* studies [4]. The cell viability was >95% compared to unlabeled DCs, with no significant changes in expression levels of HLA-DR, CD83, CD86, and CCR7. Fig. 1 shows representative ^{19}F MRS and ^{19}F/^{1}H MRI patient results. The ^{19}F MRS displays (Fig. 1a) two peaks from PFC in labeled cells and from TFA in a reference capillary adjacent the quadriceps in the coil receptive field. Fig. 1b shows a representative composite ^{19}F/^{1}H MRI image in the same patient. The peak voxel SNR of the ^{19}F images ('hot-iron') was >40. *In vivo* quantification of the ^{19}F cell numbers from the MRI data [5] yielded comparable cell numbers as injected. No evidence of cell migration was noted from 4 to 24 hours post-injection, within the uncertainty limits of the experiment (~10^5 cells/voxel).

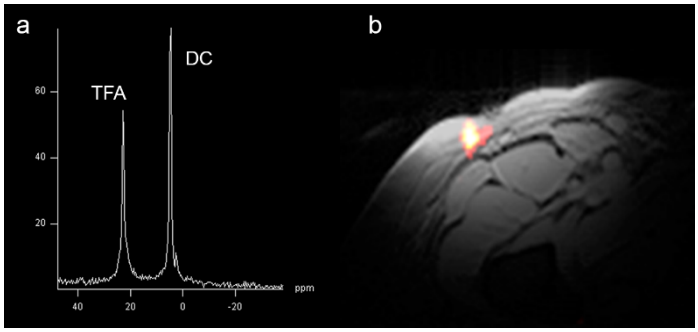

Fig 1. MR data at 3 T in colorectal cancer patient that received PFC labeled DCs (1×10^7) injected intradermal. (**a**) Displays a ^{19}F MRS spectrum of DCs along with external TFA reference. (**b**) Shows a composite ^{19}F/^{1}H image with ^{19}F in hot-iron scale.

Discussion - In the development of new cell therapies, a failure to observe a clinical response raises the question whether a sufficient number of cells were delivered to, and/or persisted at the desired site(s). Conversely, the manifestation of undesired side effects raises the question whether large numbers of cells were delivered off-target. Overall, we show that clinical ^{19}F cell tracking is highly feasible in patients using engineered PFC cell tracking agents. This study used a 'self-delivering' PFC agent, designed and optimized specifically for clinical MRI cell tracking, which can label any cell without transfection agents. An innovative aspect of the regulatory path used to get this PFC agent into the clinic is via the FDA DMF mechanism, which should ease incorporation of this technology into a broad range of cell types and future clinical trials. Future improvements in pulse sequence and coil design will further extend the utility and sensitivity of these technologies.

References – [1] Ahrens & Bulte 2013 *Nat Rev Immunol* 13(10):755-63; [2] Ahrens *et al.* 2005 *Nat Biotechnol* 23(8):983-7; [3] Mailliard *et al.* 2004 *Cancer Res* 64:5934-37; [4] Helfer *et al.* 2010 *Cytotherapy* 12(2):238-50; [5] Srinivas *et al.* 2007 *Magn Reson Med* 58(4):725-34.

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