Overview:

A challenge in the development and translation of new cellular therapies is effective tracking of cells post-transfer in both animal and human subjects. Cell Sense reagents are engineered to safely and efficiently label cells *ex vivo*, rendering them detectable with fluorine-19 magnetic resonance imaging (^{19}F MRI), a safe, highly specific and quantitative imaging modality. Cell Sense reagents provide positive contrast *independent of the anatomical image and without confounding background*. A variety of therapeutic cell types have been labeled using Cell Sense, enabling applications in regenerative medicine, immunotherapy, and diagnosis of inflammation using *ex vivo* labeled immune cells.

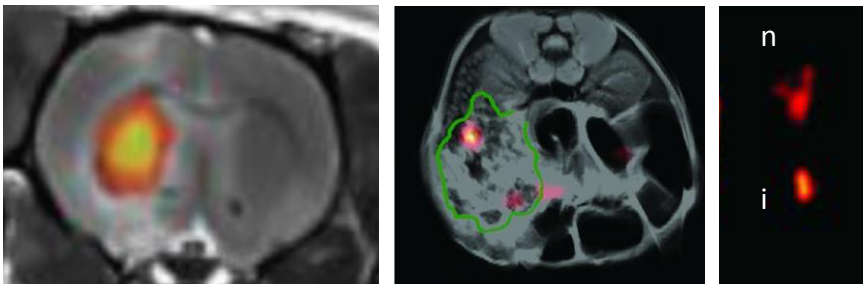


Figure 1. Cell Sense applications, from left: Detection of neuronal stem cells after intracranial delivery; homing of T cells to the pancreas in a transgenic model of inflammatory bowel disease; and visualizing the migration of human dendritic cells to the lymph node (n) from the site of injection (i).

Unmet Need:

The past decade has seen vast investment in development of cell therapies, yet few have shown the safety and efficacy required for FDA approval. This slow technological adoption is partly due to poor knowledge of the persistence and location of therapeutic cells after delivery. While it is well known that the biodistribution of small molecule drugs is essential to their performance, the difficulty in determining the analogous information for cellular therapies has hindered fundamental understanding. For instance, mechanisms of treatment failure, as measured by lack of response or off-target effects, cannot be decoupled from delivery artifacts or lack of cellular persistence without an independent measure of cellular location. Further, the safety aspects provided by knowledge of cellular location may circumvent or delay acquisition of large and expensive datasets. The importance of imaging cellular therapies is underscored by the FDA's Cellular, Tissue and Gene Therapy Advisory Committee, which recommends that sponsors develop labeling and non-invasive imaging methods for tracking cells as an integral element of any patient-monitoring protocol.

Imaging Modalities:

Several technologies exist that are able to non-invasively detect various labels *in vivo*, each with its own advantages and disadvantages in terms of cell tracking. MRI is unique in that three-dimensional, high resolution images can be acquired regardless of tissue depth without the use of ionizing radiation. PET and SPECT modalities are more sensitive; however, these methods rely on the detection of radioactive decay. The probes and detection methods raise important considerations to the patient and possible effects on the cellular therapeutic. Optical methods show great promise in small animal systems; however depth penetration issues convolute quantification efforts and can be insurmountable during clinical translation. Iron oxide and transition metal contrast agents (gadolinium) for proton MRI have also been used in cell tracking. These reagents function by altering the signal obtained in a typical MRI scan, making certain areas appear dimmer or brighter in the anatomical image. These effects create the need for a pre- and post-administration scan to enable determination of changes against background. Also, the anatomical image is adulterated, often in the specific areas affected by the disease state, making monitoring the biological response to the cellular treatment difficult.

"Transplanted cells can be labeled using a ^{19}F-MRI contrast agent prior to transplantation without interfering with T2- or diffusion-weighted imaging, hence affording the visualization of the stroke pathology, tissue regeneration and transplanted cells using serial MRI."

- Bible et al. *Biomaterials,* 2012

^{19}F MRI for cell tracking is a rather novel method which avoids many of the difficulties above. ^{19}F MRI does not involve ionizing radiation and is effective at imaging cells at clinical field strengths, capitalizing on the safety aspects of MRI. Because fluorine and hydrogen resonate at different frequencies, the MRI instrument, once outfitted with an appropriate coil, can be tuned to each independently. Thus, the anatomical image is not altered by the presence of the contrast agent and cell-based information need not be interpreted from changes in anatomical contrast. Also, since there is little to no native fluorine in biological tissues, ^{19}F signal needs only to be separated from noise to determine the presence and quantity of labeled cells (Figure 2). Importantly, ^{19}F MRI is directly clinically applicable. As a result, populations of transplanted cells can be safely, precisely, and quantitatively located in three dimensions, with anatomical reference within a live subject.

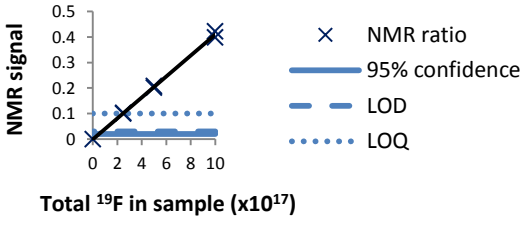


Figure 2. Quantitative measurements. The signal measured by ^{19}F nuclear magnetic resonance (NMR) is directly proportional to the amount of ^{19}F present, which can be quantified by inclusion of a chemically shifted ^{19}F reference. ^{19}F MRI signal is similarly quantitative, and is independent of tissue depth or composition.

The Cell Sense Reagent:

The Cell Sense product has been developed to facilitate the use of ^{19}F MRI for cell tracking purposes. The design of the reagent involved three primary goals: labeling cells without perturbing function, maximizing sensitivity in the MRI, and ease of use.

Cell Sense is optimized for MRI sensitivity on two levels: the molecular design of the imaging agent and the nano-emulsion formulation. The imaging agent is a polymeric perfluorocarbon that contains many chemically-identical fluorine atoms, yielding a single major resonance detectable by MRI. The compound also allows fast image acquisition due to its magnetic relaxation properties (specifically a short T1/T2). The imaging agent is formulated into an emulsion with an engineered droplet surface to enhance cellular uptake. Cells can internalize as many as tens of thousands of emulsion droplets, allowing as few as thousands of cells to be detected by ^{19}F MRI.

Table 1. The unique features of the Cell Sense reagent when combined with ^{19}F MR methods; successfully labeled and detected cell types; and the applications enabled through the use of the technology.

Unique features:	Cell types:	Applications:
Quantitative	Dendritic cells [2-4]	On-/off-target delivery
Non-invasive	T cells [1,5,6]	Biodistribution
High specificity	Hematopoietic stem cells [7]	Homing
Depth independent	Neuronal stem cells [8,9]	Migration
Safe, biologically inert	Mesenchymal stem cells	Diagnostic use
Signal-optimized	Natural killer cells	
Self-delivering	White blood cells	

Extensive testing of labeled cells has revealed little to no change when compared to unlabeled cells (Table 1). Functional tests on a wide variety of cell types have been conducted, including several comprehensive data sets which have been evaluated and published in peer-reviewed journals [4,7,8]. Each study concluded that labeling and tracking cells with Cell Sense occurs without evidence of detrimental effects on cells either *in vitro* or *in vivo*.

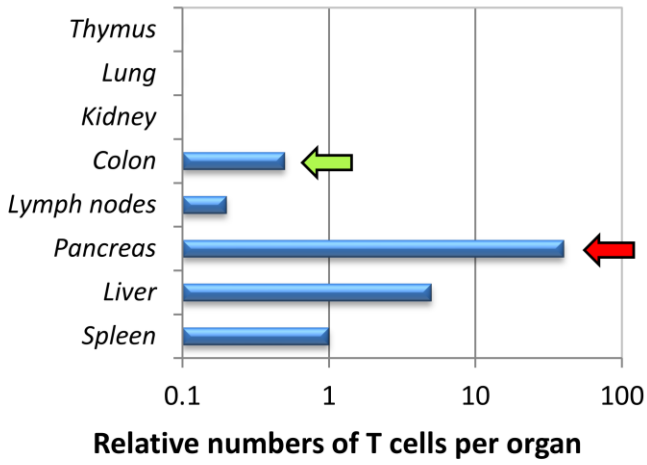
Cell Sense does not require the use of exogenous transfection agents. As such, the cell-labeling process is simply co-incubation of the reagent with the cells of interest and is typically performed at normal culture conditions. The concentration and the duration of the co-incubation are empirically determined to yield optimal cell uptake and cell properties. The labeling duration can be as short as an hour, or as long as two days, permitting integration of Cell Sense into existing cell handling protocols. These properties combine to make the reagent highly user-friendly.

Reagent Detection: ^{19}F nuclear magnetic resonance (NMR)

In vivo detection of the reagent is performed using ^{19}F MRI, however, validation work and initial *in vivo* studies typically use ^{19}F NMR. Based on the same physics, NMR can be used to count the number of fluorine present in a sample (Figure 2, above). Nearly any liquid-state NMR equipped with a fluorine-tunable RF coil is capable of such a measurement.

When a cell pellet of known cell number is measured, a measure of label uptake can be generated. When excised tissue of known mass is measured and the label uptake is known, the number of transplanted cells in a given tissue can be generated. Preliminary biodistribution studies can be performed in this manner, which is a critically important parameter to assess cellular delivery. Further, the coarse level of information available through NMR can lead MRI imaging to gain fine scale details only in areas where cells are known to accumulate.

"Off-target" homing of specific T cells



Antigen-specific T cells may exert their immunological effects at sites where they can (1) migrate and (2) the protein of interest is expressed. Biodistribution evaluation of T cell homing as determined by labeling specific T cells ex-vivo, administration and ^{19}F NMR of excised organs enables the quantification of cells homing to each organ. In this murine study, the antigenic protein was known to be expressed in the colon, and systemically delivered T cells were expected to home to the colon, but not kidney or lungs which did not express the antigen. ^{19}F NMR biodistribution analysis confirmed the presence of T cells in the colon, but not the lung or thymus.

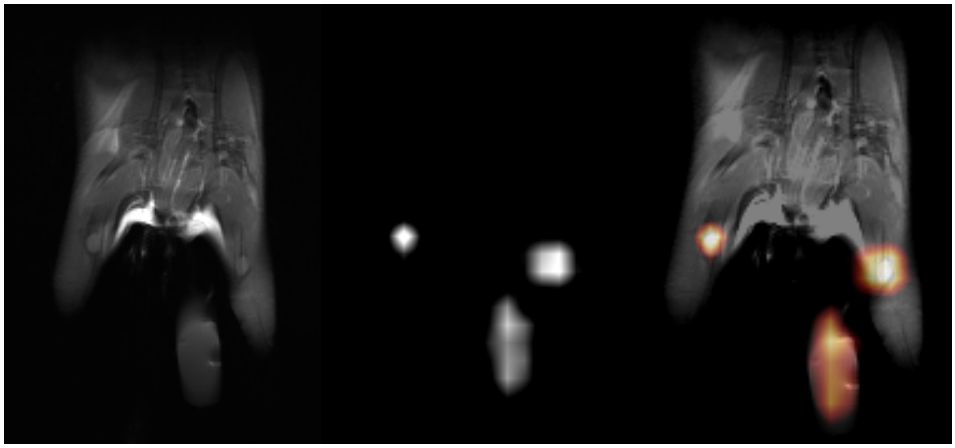
Unexpectedly, extremely high numbers of T cells were found in the pancreas in this model. Follow up studies confirmed the previously unknown expression of the protein in the pancreas as well as infiltration of the transferred T cells in the pancreas by histological methods. For a therapeutic product, so called "off-target" homing could lead to a devastating effect. (Adapted from [1])

Reagent Detection: ^{19}F MRI

Cell Sense can be visualized *in vivo* using MRI with ^{1}H/^{19}F tunable RF coils and traditional fast imaging pulse sequences. The small T1/T2 ratio of the imaging agent allows rapid acquisition of multiple averages, increasing the attainable signal to noise. Like ^{19}F NMR, MRI produces a quantitative measure of the amount of fluorine present in a given volume. An external reference calibrates the signal and an NMR measure of the cellular ^{19}F content can be used to yield a quantitative measure of the number of cells in a given location.

Monitoring a dendritic cell (DC) vaccine

Left to right: ^{1}H anatomical MRI of murine torso, ^{19}F MRI of the same location, and the ^{1}H/^{19}F fused image.



Dendritic cells have been called "nature's adjuvant" due to their role in inciting a general immune response, and are being developed clinically by many researchers and clinicians as a cellular vaccine for a variety of human diseases, from HIV to cancer. As the migration of DC to the lymph node is thought to be essential to their immune-stimulating capacity, evaluating their migratory capacity *in vivo* can provide insight into their effectiveness. Here, MRI cell tracking was employed to evaluate the migration of murine DC injected into the footpad. Two days post-delivery, ~5% of injected DC were found in the left and right popliteal lymph nodes. (Image courtesy of Dr. E. Ahrens, Carnegie Mellon University, and Drs. J. Urban and P. Kalinski, University of Pittsburgh Cancer Institute)

Cell Sense in the Clinic:

A clinical grade of Cell Sense is manufactured under cGMP conditions. Designated a drug by the FDA, Cell Sense is regulated primarily by CBER. An open Drug Master File is filed with the FDA for cross-referencing by customers sponsoring clinical trials of cellular products which incorporate the Cell Sense MRI tracer. The DMF compiles not only the manufacturing details, but includes safety data such as GLP toxicology studies (including acute toxicity, genotoxicity) as well as detailed reports of labeling and safety data for a variety of human therapeutic cells. Investigators are encouraged to contact Celsense about cross-referencing the DMF in their pending regulatory filings. In 2011, Cell Sense was authorized by the FDA for use in a Phase I clinical trial to image a dendritic-dell based vaccine.

Related Products:

Cell Sense Dual-Mode: A fluorescent dye (green or red fluorescence) has been incorporated into the perfluorocarbon phase of the nanoemulsion, allowing optical detection of the reagent inside cells and a qualitative measure of cell uptake via fluorescent microscopy, flow cytometry or with a fluorescent plate reader.

Voxel Tracker: A web-based software package allowing facile superposition of the ^{19}F and ^{1}H MRI data, and which provides cell quantification tools. Enables study management and password protected data sharing through cloud-computing.

Product Support: Celsense, Inc. provides detailed protocols for cell labeling and live support through a team of PhD scientists. Understanding that each cellular product is unique, our expertise in labeling and implementing cell tracking for a wide variety of cellular therapeutic approaches enables researchers to focus on answering their hypotheses rather than technicalities. Written summaries of data on labeling and safety studies in particular cell types are available for incorporation into grants and other project proposals. Celsense also invites proposals for contracted research on labeling cells, biodistribution studies and imaging.

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