Imaging Techniques for Monitoring Cellular Therapy

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Cell Imaging

Approximately $\sim 10^{15}$ cells in 70 kg person

Brain $\sim 10^{11}$

Human Heart 300 grams or $\sim 6 \times 10^9$ cells

IV Injection of $10^5 - 10^8$ cells into blood volume
5L containing $\sim 40 \times 10^{12}$ cells

BMSC 1-5x$10^6$cells/kg iv = 70-350x$10^6$ cells

IM Injection of $10^5$-$10^6$ cells
## Overview of Imaging Modalities

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Resolution</th>
<th>Depth</th>
<th>Time</th>
<th>Imaging Agents</th>
<th>Application</th>
<th>Main Characteristics</th>
<th>Clinical</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRI</td>
<td>10-100µm</td>
<td>No Limit</td>
<td>µsec to Hours</td>
<td>Gadolinium, Manganese, Iron Oxides nanoparticles</td>
<td>Anatomy Physiology Metabolic Molecular</td>
<td>Versatile High soft tissue contrast</td>
<td>Yes</td>
</tr>
<tr>
<td>PET</td>
<td>1-2 mm</td>
<td>No Limit</td>
<td>Min</td>
<td>$^{18}$F, $^{11}$C, $^{15}$O, $^{64}$Cu</td>
<td>Physiology Metabolism Molecular Cellular</td>
<td>Versatile Receptor Studies cyclotron</td>
<td>Yes</td>
</tr>
<tr>
<td>SPECT</td>
<td>1-2 mm</td>
<td>No Limit</td>
<td>Min</td>
<td>$^{99m}$Tc, $^{111}$In, $^{123}$I</td>
<td>Physiology Metabolism Molecular Cellular</td>
<td>Commonly used for MoAb imaging</td>
<td>Yes</td>
</tr>
</tbody>
</table>
Positron Emission Tomography

511 keV nucleus

Positron scatters in tissue losing energy

$e^+ + 511 \text{ keV}$

$e^- + e^+ + e^- + e^+$

Half Life $^{18}\text{F} = 110\text{min}$, $^{64}\text{Cu} 12.7\text{h}$
SPECT cameras are used to determine the **3D** distribution of the radiotracer.

- Position X
- Position Y
- Energy Z

Half Life $^{111}$In = 67hr, $^{99m}$Tc=6hr, $^{123}$I =13hr
Magnetic Resonance Imaging

1. Atoms spin in random directions, like tops, around their individual magnetic fields.

2. In a magnetic field produced by MRI, atoms line up either north or south.

3. About half the atoms go each way, but there are a few unmatched atoms.

4. When the radio frequency pulse is applied, the unmatched atoms spin the other way.

5. The energy sends a signal to a computer. The computer uses a mathematical formula to convert the signal into an image.
Sensitivity of Imaging Technologies

- PET
- SPECT
- Fluorescence
- Bioluminescence
- "Smart"US
- "Smart"MRI
- MRS

Sensitivity (1/[probe])

- $10^{-15}$ moles
- $10^{-12}$ moles
- $10^{-9}$ moles
- $10^{-6}$ moles
- $10^{-3}$ moles

Distance:
- 100µm
- 1mm
- 1cm
- 10cm
- 1m
- 10m

Schematic presentation for non-invasive imaging of cells

Welling MM et al J Cell Physiology 2010;226:1444-52
Cell type analysis of functional fetal dopamine cell suspension transplants in the striatum and substantia nigra of patients with PD

Mendez I and Isacson O et al Brain 2005;128:1498-1510

Post-mortem analysis of 2 patients with PD with received fetal transplant with favorable clinical outcome and positive $^{18}$FDOPA PET
Reporter Gene Based Cell Imaging
A new transgene reporter for *in vivo* magnetic resonance imaging

Guillem Genove³, Ulrike DeMarco¹, Hongyan Xu¹, William F Goins² & Eric T Ahrens³

*Nature Medicine* 2005;11:450

**MRI at 11.7T 3x10⁶ AdV-FT AF 549 cells implanted in mouse**

**Ferritin expression (µg/mg protein)**

- **Ferritin Expression Day 5 after implanting AdV-Ft AF 549 cells**

- **AF549 cells transfected with LacZ**
Tet:EGFP-HA Ferritin transgenic mouse model with Hemagglutinin-ferritin being expressed in hepatocytes and in vascular endothelial cells altering $R_2$ of tissue.
Acute myeloid leukemia is associated with retroviral gene transfer (eGFP) to hematopoietic progenitor cells in a rhesus macaque
Seggewiss R et al Blood 2006;107:3865-67

BM Transplanted 7 Rhesus monkeys in 1999 using MSCV based RD114 pseudotyped retroviral containing eGFP and dihydrofolate reductase gene. 
Insertion analysis revealed eGFP inserted in chromosomes 15 and 9. (Not usually performed)

Stable Hematopoietic cell marking in Bone Marrow 3-5% between 2000-5. One Monkey presented with peripheral blood showed 30% eGFP+ granulocytes.

Myelomonocytic leukemia that was eGFP+ infiltration into Kidney. Animal died 5 days after diagnosis!

How to control insertion sites in chromosomes for a imaging marker gene?
Who can afford to keep animals for 5+yrs to ensure insertion of imaging probe does not result in malignant transformation.

Insertion of eGFP into Chromosomal 15 or 9 resulted in activating BCL2-A1, a gene known to have antiapoptotic properties, dominated multilineage contribution to hematopoiesis after transplantation, became dormant for 4 years, and then re-emerged as the dominant clone contributing to myeloid hematopoiesis and a fatal myeloid sarcoma 5 years after transplantation.

Courtesy of Cindy Dunbar M.D. Ph.D. NHLBI
Agents for Cellular Labeling

- SPECT/PET agents: $^{111}\text{In}$ oxine, $^{99m}\text{Tc}$ HMPAO/Tropoline, $[^{18}\text{F}]$ FDG, $^{64}\text{Cu}$

- Multispectral Imaging: $^{19}$Fluorine

- Paramagnetic Agents: Gadolinium, Manganese, Iron chelates

- Superparamagnetic Agents: Iron, Iron + Mn, Fe + Co, MnO$_2$ in crystal lattice
Magnetic Resonance Tracking of Dendritic Cells in Melanoma patients for monitoring Cellular Therapy

de Vries IJM et al Nature Biotechnology 2005;23:1407-13

Monocytes

Dendritic Cells

Ferumoxides x 2 days

15x10^6

50%

^111In Labeling

50%

 Ultrasound guidance

Scintigraphy

In vivo MRI

Lymph node dissection

Histology

Ex Vivo MRI

50% SPIO Labeling
In vivo imaging platform for tracking immunotherapeutic cells

Perfluoropolyether particle and cationic lipids + Dendritic cell

PFPE-Labeled Dendritic Cell

MRI at 11.7T acquisition time > 3hrs

4x10^6 Injection into Foot Pad 18x10^6 labeled cells iv

Figure 4 FACS analysis of DCs in excised lymph nodes following foot pad injection. BMDCs were labeled with PFPE overnight, and a portion of these were additionally stained with CMFDA. Labeled BMDCs were injected into the foot pad of syngeneic NOD mice. Twenty-four hours later, the popliteal and inguinal lymph nodes were excised and single cell suspensions were generated and the presence of labeled cells was determined by flow cytometry. (a) is PFPE + CMFDA, and (b) is PFPE only. The results shown are representative of two similar experiments.

Bulte JWM Nature Biotech 2005;23:945
$^{19}$F MRI for stem/progenitor cell tracking with multiple perflourocarbon nanobeacons. Partlow KC et al FASEB J 2007;21:1647-54

Acquisition Time ~15 min using dedicated 1H/19F Surface Coil

4x10$^6$ CD34$^+$CD133$^+$ Labeled cells IM
Direct implantation of NC17.2 NSC labeled with Perfluoro-15-crown-5-ether (PFCE) by incubating cells using special coated culture plate and $^19$F MRI imaged at 9.4 T 1mm slice thickness, nex =64 TSE (TR 1080/TE 46), 64x32 in FOV 2.5cm (voxel size 1mm x 0.39mm x 0.78mm)

Scan Acquisition Times approximately 1 hour.

Will this technique be translated to the clinic?

Cost for surface coil for 3T $20-40k.$
A chronic 1 year assessment of MRI contrast agent-labeled neural stem cell transplants in stroke

Modo M et al Neuroimage 2009;47:T133-142

Injected $2 \times 10^5$ GRID labeled NSC 2 weeks MCAO in rat MRI at 4.7T SE 4000/64 234x234x350 μm

Brain Volume

Lesion Volume

MRI over 1 year indicated that GRID-labeled transplants resulted in a slight increase in lesion size compared to MCAo-only animals, whereas PKH26-labeled cells significantly decreased lesion size by 35%.
Prussian Blue + MSCs
DAB Prussian blue
CD34 CD133 Cells

Protamine Sulfate: FDA Approved
(Heparin Antagonist)
No Synthesis Required
No Proprietary Compounds
Optimization and Validation of FEPro Cell Labeling Method
Magnetic Labeling of Stem Cells: How to get from Bench-To-Bedside

- What is the labeling efficiency of the agent?
- Is the label toxic to cells?
- Does the label alter cellular metabolism or differentiation?
- What happens to the iron in cells?
- Does iv administration of labeled cells alter biochemical or hematological measures?
- Do labeled cells alter morbidity or mortality?
- Can we scale up cell labeling in a CGMP facility?
- Does the labeling alter stemness or potency of cell?
- Can new MRI approaches be developed to improve detection of labeled cells in vivo?
- Which Agency should review the IND application? CBER vs CDER

FEPro Labeling is not Toxic nor does it Alter Differentiation or Function of HSCs (CD34) and MSCs

**CFU-GM**
- Labeled
- Control

**BFU-E**
- Labeled
- Mixed
- Control

**Number of CFU/100 cells**
- FE-Pro
- Control

**% Cell Viability**
- FE-Pro
- Control

**Reactive Oxygen Species FEPro labeled MSC**
- FE-Pro
- Control

**Proliferation (MTT) FEPro labeled MSC**
- FE-Pro
- Control

**AC133 Migration to SDF: Effect of Iron Labeling**

**Glycosaminoglycan**
- Unlabeled
- FEPro labeled

**Collagen X**
- Unlabeled
- FEPro labeled

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Arbab AS et al NMR in Biomedicine 2005;18:1447
FEPro labeling of BMSC does not alter In Vivo Differentiation or change ability to support hematopoiesis

Pawelczyk, E, Kuznetzov SA, Frank JA, Robey PG, Balakumaran A Blood submitted

How do you determine stemness+ and potency?

a single cell forms CFU

carrier

Transplant

Bone

Fat

hematopoietic stroma: supports OC formation

BMSCs support Hematopoiesis

GFP+osteocytes

Adipocytes are PB+

PB+ fibroblast-like cells

Superparamagnetic iron oxide nanoparticles (FEPro) labeling of bone marrow stromal (mesenchymal) cells does not affect their “stemness”

Pawelczyk, E, Kuznetzov SA, Chaudhry A, Frank JA, Robey PG, Balakumaran A Blood submitted

Greater inter-individual differences than between subject’s BMSCs than due to labeling cells with FEPro or Au nanoparticles compared to unlabeled cells.

36,000 probes in the array only those genes that were expressed by BMSCs intensity> 2 ( \( P<0.01 \)) were analyzed. **No distinct clustering associated with labeling methods was found.**

FEPro- labeled BMSCs or Au nanoparticle labeled BMSC compared individually to unlabeled BMSCs were related by ion binding, ion or vesicle transport, genes related to cytoskeleton or signal transduction pathways. Ferritin was up-regulated in FEPro-labeled BMSCs and transferrin receptor was not changed*

No change in FEPro-labeled BMSCs in genes critical for “stemness” such WNT pathway genes, OCT 4 or NANOG when compared to unlabeled BMSCs.

Pawelczyk E et al, NMR in Biomedicine 2006;19:581
Matrigel Plug Model of Angiogenesis* and Inflammation† in 129/SvImJ mouse
Uptake by Macrophages of BrdU, GFP or FEPro from Labeled BMSC

Pawelczyk E et al PLoS ONE 2009;4:e6712

No difference between mouse or human BMSC were used in uptake of label by AM

BrdU uptake from BMSC by Macrophages

P=0.0214
P=0.0055

GFP uptake from BMSC by Macrophages

P=0.0214
P=0.0211

FEPro uptake from BMSC by Macrophages

P=0.0055
P=0.0221

* Bertolini F et al Nature Reviews Cancer 2006 6 835-4
Summary of Results of Magnetic Cell Labeling

- **Iron Oxide Nanoparticle Labeling of Any Type of Cell.**
  - Functional and Differential Capacity is unaltered by SPIO Labeling.
  - Labeled Cells contain 1.0 - >20 picograms of iron/cell (unlabeled cells <0.1 pg iron).

- **Magnetic Cell Labeling Does Not Alter** the Physiological or Metabolic or Stemness properties of Cells.
  - Iron oxide nanoparticles are stored in cells as ferritin.

- **No Short or Long Term Toxicity** was observed as a result of labeling compared to unlabeled cells.

- **MRI detection of Ferumoxides Labeled Cells in vivo.**
  - Can detect approximately <50 labeled cells/voxel in mice and an estimated 500 cells/voxel in humans
  - Transfer of SPIO to local activated macrophages in vivo occurs about 10-20% and represents small fraction of total iron injected in transplanted labeled cells.
Clinical Trials with SPIO Labeled Cells
Ferumoxides Labeled Dendritic Cells missed Lymph Node

MRI and Indium$^{111}$oxine SPECT of labeled (1.5x10$^6$) Dendritic Cells

MRI can visualize about 500 labeled cells/ voxel

Approximately 150,000 labeled cells

Area became isointense with fat in 30 days
34 y.o. Male with open brain surgery, extracraniofacial cultured for NSCs were Effectene cultured for points around MRI guidance.

Ferumoxides labeled

10 weeks after implant T2* effect from labeled NSC could not be detected at 3T.
MRI of Pancreatic Islets Transplanted Into the Liver in Humans
Saudek, F et al Transplantation 2010;90:1602

35-60x10^4 Islets Labeled with Ferucarbotran infused in portal vein

Day -5  Day 1  Day 7

Day 30  Day 168

<table>
<thead>
<tr>
<th>TABLE 2: Decline in regional signal loss (mean±SD in patients 3–6), relative to day 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Days after the first transplant</td>
</tr>
</tbody>
</table>
| No. spots (%)                  | 100   | 55.8±3.9
d | 45.8±5.8 | 32.8±7.8 |
| Area of spots (%)              | 100   | 54.3±7.7
d | 33.2±5.8 | 16.0±4.3 |
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<tbody>
<tr>
<td>Note: A significant decrease in both number of spots and their area, compared with the initial values, occurred at 7 d after transplantation.</td>
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</tbody>
</table>

^ P<0.01, d 1 vs d 7; Wilcoxon paired test.
SPIO Nanoparticles for Cell Labeling

• **USP Grade Agents**
  - MRI contrast agents Ferumoxides (Feridex or Endorem) and Ferucarbotran- Resovist (Taken off the Market 2009-10)
  - **Ferumoxytol- (FeraHeme®) Introduced in July 2009**
  - Treatment for Iron Deficiency Anemia for CKD
  - Miltenyi Biotech - Iron Dextran Beads for cell separation that are administered clinically (CD34+ cells cord blood Tx)
  - Dyna Beads (Invitrogen magnetic cell isolation)

• **Experimental Agents**
  - [www.biopal.com](http://www.biopal.com)
  - [www.genovis.com](http://www.genovis.com)
  - [www.micromed.com](http://www.micromed.com)
  - [www.miltenyi.com](http://www.miltenyi.com)
  - [cmir.mgh.harvard.edu/chem/chem_probes](http://cmir.mgh.harvard.edu/chem/chem_probes)
  - [www.bangslab.com](http://www.bangslab.com)
Ferumoxytol ZP = -49mv, ~33nm
LD50 420mg/kg in rat
Heparin ZP = -60mv, 18Kd
LD50 5000mg/ml in mouse
Protamine ZP = 7.2mv, 4.6Kd
LD 50 100mg/kg in mouse

ZP = zeta potential 37°C in water
pH = 7

3 FDA approved agents
Heparin, Protamine & Ferumoxytol
Self Assemble to form complex that can be used to magnetically label cells
<table>
<thead>
<tr>
<th>Unlabeled</th>
<th>Labeled</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>NSC</strong></td>
<td>![NSC Unlabeled] ![NSC Labeled]</td>
</tr>
<tr>
<td><strong>BMSC</strong></td>
<td>![BMSC Unlabeled] ![BMSC Labeled]</td>
</tr>
<tr>
<td><strong>Monocytes</strong></td>
<td>![Monocytes Unlabeled] ![Monocytes Labeled]</td>
</tr>
<tr>
<td><strong>HSC</strong></td>
<td>![HSC Unlabeled] ![HSC Labeled]</td>
</tr>
</tbody>
</table>

**Prussian blue (DAB) stains HPF labeled or unlabeled Cells**

- **OSTEOGENESIS**
  - Unlabeled
  - HPF-labeled

- **ADIPOGENESIS**
  - Unlabeled
  - HPF-labeled

- **T-cell**
  - Unlabeled
  - Labeled

**NSC** stands for Neural Stem Cells, **BMSC** for Bone Marrow Stem Cells, and **HSC** for Hematopoietic Stem Cells.
T2*w MRI at 3T of Rat with implanted HPF labeled BMSC

10³ UL BMSC

10⁴ HPF BMSC

5x10³ HPF BMSC

10⁴ HPF BMSC

PB

huMit

huMito
Monitoring Cellular Therapy: The Role of Imaging

- **Patient Selection**
  - Evaluation and Characterization of Pathology
    - Location, Extent of Pathology or Abnormality
  - Delivery Routes
    - Direct Implantation versus Vascular Routes

- **Cell Selection (Stem Cells or Combination of Cells)**

- **Safety of Therapy**
  - Damage to Target Organ, Malignant Transformation, GVH

- **Cell Survival, Migration and Differentiation**
  - Mechanism and Microenvironment

- **Physiologic, Metabolic and/or Morphologic Improvement**
  - Direct Effect to Host or Bystander Effect

- **Optimization of Cell Based Therapy**
  - How Many, How Often and When to Give Cells

- **Evaluation of New Drug or Cytokine Therapies on Cells**

- **What Combination of Imaging Modalities should be used to Assess Cellular Therapy?**
Encapsulation of HPF complexes in Cells

EM of HPF labeled cells

- NSC
- T-cell
- BMSC
- Mono
MRI for Quantitative in vivo cell tracking

Sensitivity
~10^4
1-5x10^2
1-5x10^5
The MR Tracking of transplanted ATDC5 cells using fluorinated poly-l-lysine-CF$_3$

Poly-L-Lysine derivatives

3x10$^7$ PLLCF$_3$ ATDC5 cells implanted in skull

Dilution of $^{19}$F signal over time
MR Tracking of Transplanted Cells with “Positive Contrast using Manganese Oxide Nanoparticles
Gilad AA et al MRM 2008;60:1-7

2x10^5 9L Glioma Cells 9.4T MRI performed 24hr after injection

MnO FeO MnO FeO Ctrl FeO

R1

R2

Merged

Convertible Manganese Contrast for Molecular and Cellular MRI
Shapiro EM and Koretsky A MRM 2008;60:265-9

Mn\textsubscript{3}O\textsubscript{4} nanoparticles in PBS and Na citrate pH =5
PBS or culture media

T1 maps rat brains

Aoki I et al NMR Biomed 2006 noted cell toxicity at >0.5mM Mn

MnCO\textsubscript{3}

Day 0

Day 10

Mn\textsubscript{3}O\textsubscript{4} nanoparticles

Thalamus