Iron Labeling of CD 133+ Cells

Joseph A. Frank MS, MD
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MSC Group (n=5)  
Control Group (n=25)

(2) $5 \times 10^7$ MSC IV between 4-5 wks and 7-9 wks following Infarction.

Ann Neurol 2005;57:874
MR Properties of Contrast Agents

- **Paramagnetic**: Dipole-Dipole Interaction (inner sphere effects) with Chelated Metal (Mn, Fe, Gd) resulting in a shortening of NMR (T1>T2) relaxation times and Proton Relaxation Enhancement
  - BBB disruption and GdDTPA

- **Superparamagnetic**: Outer Sphere or dephasing effects due to alterations in local magnetic field gradients (susceptibility effects) Contrast agent (CA). CA may also shorten the T1 and T2 of tissue.
  - Ferumoxides as a Liver Agent

- **Diamagnetic**: Does not alter the relaxation properties of tissues.
Cellular Labeling for *in vivo* MRI

- **Coated Superparamagnetic Iron Oxides Nanoparticles**
  - Dextran Coated: AMI 25, AMI 227, MION-46L, Ferumoxides (Feridex®), Combidex, Endorem, Resovist, Sinerem
  - Cationic and Anionic Coated SPIO: VSOP, WSOP, Citrate, Liposomes, Ferritin
    - **Magnetodendrimer** Bulte et al Nature Biotech 2001
    - Bang Particles (Divinyl Benzene inert microsphere Dragon Green and Iron Oxides) Hinds et al Blood 2003
- **Biochemical Alterations with Proteins or MoAbs**
  - MION-TrF, MION-Tat, CLIO-Tat, MION-OX26, CLIO-NPR, CLIO-HD, Lectins, Sendi Virus Envelopes
- **Complexing Dextran Coated SPIO with Transfection Agents**
Straightforward method for cell labeling

Protamine Sulfate: FDA Approved
(Heparin Antagonist)
No Synthesis Required
No Proprietary Compounds
High Labeling Efficiency

Macropinocytosis of Ferumoxides-Protamine Sulfate Complex into Mesenchymal Stem Cells
• What is the labeling efficiency in stem cells?
• Are ferumoxides complexes toxic to stem cells?
• Does labeling cell alter metabolism or differentiation?
• What happens to the iron in the cells?
• Does iv administration of labeled cells alter biochemical or hematological measures?
• Does the use of ferumoxides labeled cells in experimental models alter morbidity or mortality?
• Can new MRI approaches be developed to improve detection of labeled cells in vivo?
• Can we scale up the process of labeling cells with ferumoxides in a CGMP facility?
What happens to the iron oxide following Labeling with Ferumoxides-PLL complex?

HeLa cells
(cell division 2-3 days)

MSC (growth inhibited)

Day 1 Day 6 Day 21 Day 44

Arbab, AS et al Radiology 2003;229:876
FEPro Labeling is not Toxic nor does it Alter Differentiation or Function of HSCs (CD34) and MSCs

Reactive Oxygen Species FEPro labeled M:

Proliferation (MTT) FEPro labeled M:

FEPro labeled MSC differentiation towards Chondrogenesis

Arbab AS and Frank JA NMR in Biomedicine 2005;18:1447
Ferumoxides-Protamine Sulfate labeled hBMSC CFU formation and immunomodulatory properties

Secondary CFU assay

Mixed lymphocyte reaction

% Control

BMSC  Fe-Pro BMSC

T cell proliferation (CPM)

No MSC  MSC  FE-Pro MSC
FEPro labeling of BMSC does not alter Osteogenic Differentiation or change ability to support hematopoiesis in vivo

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

BMSCs support Hematopoiesis

A cell forms CFU

Transplant

Carrier

Bone

Hematopoietic stroma: supports OC formation

GFP+ osteocytes

PB+ fibroblast-like cells
Efficient In Vitro Labeling of Human Neural Precursor Cells with SPIO Nanoparticles: Relevance for In Vivo Cell Tracking
Neri M et al Stem Cells 2008;26:505-516
Transferrin receptor (TfR-1) and Ferritin Expression in FEPro Labeled cells TaqMan and Western Blot

**P < 0.007, * P < 0.02 when compared to relevant controls, two-tailed Student t test**

Pawelczyk E et al, NMR in Biomedicine 2006;19:581
Summary of Results of Magnetic Cell Labeling

• **Iron Oxide Nanoparticle Labeling of Any Type of Cell.**
  - Functional and Differential Capacity is unaltered by Iron Oxide Labeling.
  - Labeled Cells contain 1.0 - >20 picograms of iron/cell with labeling efficiencies >95% (unlabeled cells < 0.1 pg iron).

• Magnetic Cell Labeling **Does Not Alter** the Physiological or Metabolic properties of the Cells.
  - Iron oxide nanoparticles are digested by lysosomal pH and buffers and stored in cells as ferritin. Trf is decreased and Ferritin is increase following FE labeling.

• 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

Experimental Cellular MRI Studies

- **Cell Types:** Embryonic Stem Cells, Mesenchymal Stem Cells, Hematopoietic Stem Cells, Neural Stem Cells, Skin Precursor Cells, Schwann Cells, Olfactory Ensheathing Cells, Aminotic Fluid Stem Cells, NK Cells, T-cells, Dendritic Cells, and Pancreatic Islets.

- **Brain** (Tumor and Stroke)
- **Spinal Cord** (Demyelination and Trauma)
- **Heart** (Myocardial Infarction)
- **Abdomen:** Liver, Spleen and Kidney
- **Flank Tumors**
Detection of Angiogenesis by MRI in RT2 Glioma in SCID Mouse Following Sublethal Irradiation 5x10^5 Sca1^+ Cells IV 2 Days prior to Tumor Implantation

Prussian Blue + Sca1^+ cells

*In Vivo* 7T MRI 70 x 80 x 500 µm³

SE pre Unlabeled

SE post GdDTPA Labeled

Control pre Unlabeled Labeled

post GdDTPA Labeled

CD31^+ (PECAM-1) and PB +

vWF+ and PB+

Preclinical Data for IND

RT2 500k Glioma Implanted in Mice 8-13 days prior MRI. Mice were Infused IV (Day 2) with FEPro Labeled or Unlabeled Human CD 34+ AC 133+

# Cells

0 3x10^6 3x10^6 3x10^7 3x10^7

Courtesy of Stasia Anderson, Wei Zheng, EJ Read, Howard Fine, Joseph Frank
RT2 500k Glioma Implanted and Infused IV (Day 2) with FePro Labeled or Unlabeled Human CD 34+ AC 133+ (EPCs)

Kaplan-Meier Survival Analysis

<table>
<thead>
<tr>
<th>Days</th>
<th>Percent Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 x 10^5 EPCs</td>
<td></td>
</tr>
<tr>
<td>3 x 10^6 EPCs</td>
<td></td>
</tr>
<tr>
<td>3 x 10^7 EPCs</td>
<td></td>
</tr>
</tbody>
</table>

FePro labeling of CD 34+ AC 133+ cells has no effect on morbidity or mortality

No difference in Hematological or Serum Chemistry or Renal or Liver Function in the SCID mice that received FePro labeled or unlabeled EPCs compared to Sham (saline) infused mice.

Mitochondrial Stain

Prussian blue

Courtesy of Howard Fine, Joseph Frank
Detection of Differential Migration of Endothelial Progenitor Cells Detected by Cellular Magnetic Resonance Imaging with Histological Correlation

IV 3x10^6 FEPro CD34 AC 133

EPCs home to periphery of Tumor

EPCs spread homogeneously throughout Tumor

Tumor cells + CD 34 CD 133 cells in various ratios

MRI at various times after implantation
Tracking Fe-Pro Labeled CD 34 AC 133 (3x10^6 IV) in Xenografted Glioma

Confocal Microscopy of Dual labeled (FEPro and Quantum Dot) CD 34 AC 133 and IV Rhodamine Lectin in Flank Tumor

Arbab AS et al Stem Cells 2006;24:671-8
Detection of Differential Migration of Endothelial Progenitor Cells Detected by Cellular Magnetic Resonance Imaging with Histological Correlation

Fluorescent microscopic images show incorporation of locally implanted Dil labeled EPCs (E, arrows) into the tumor vasculatures. Arrows indicate lectin positive endothelial lining and Dil positive EPCs. (F) Superimposed image.
Expression of different angiogenic and chemoattractant factors at the sites of migrated labeled EPCs in rat glioma at various tumor sizes

<table>
<thead>
<tr>
<th></th>
<th>PB +DAB</th>
<th>MMP-2</th>
<th>PDGF</th>
<th>VEGF</th>
<th>HIF-1α</th>
<th>SDF-1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>0.5 cm</strong></td>
<td><img src="A" alt="Image" /></td>
<td><img src="B" alt="Image" /></td>
<td><img src="C" alt="Image" /></td>
<td><img src="D" alt="Image" /></td>
<td><img src="E" alt="Image" /></td>
<td><img src="F" alt="Image" /></td>
</tr>
<tr>
<td><strong>1.0 cm</strong></td>
<td><img src="A" alt="Image" /></td>
<td><img src="B" alt="Image" /></td>
<td><img src="C" alt="Image" /></td>
<td><img src="D" alt="Image" /></td>
<td><img src="E" alt="Image" /></td>
<td><img src="F" alt="Image" /></td>
</tr>
<tr>
<td><strong>1.5 cm</strong></td>
<td><img src="A" alt="Image" /></td>
<td><img src="B" alt="Image" /></td>
<td><img src="C" alt="Image" /></td>
<td><img src="D" alt="Image" /></td>
<td><img src="E" alt="Image" /></td>
<td><img src="F" alt="Image" /></td>
</tr>
</tbody>
</table>


- HIF-1α and SDF-1 were strongly expressed in tumors at periphery.
- MMP-2 and PDGF were expressed in tumors and surrounding tissues.
- VEGF was not essential for the migration of EPC to periphery of tumor.
- Will co-implantation of EPCs with tumor cells result in an increase in metastasis? (Weinberg RA Nature 2007;449:557)
Dual-Modality Monitoring of Targeted Intraarterial Delivery of Mesenchymal Stem Cells After Transient Ischemia


MR images of intracerebral MSC ($10^6$) cell engraftment after Intracarotid artery (A-F, H) and IV (G) injection
Homing Induced by Injection SDF-1+AVD into Anterior Wall Myocardium

IV injection FEPro labeled 10x10^6 AC133 cells. In-vivo MRI Day 6.

Prussian blue and HE staining

6 wks post MI

In Collaboration with Aarif Khakoo and Toren Finkel
Cardiac repair with intramyocardial injection of allogeneic MSC after myocardial infarction

Engraftment of allogeneic porcine FEPro labeled MSCs assessed with MRI

A Day 2 Week 1 Week 4 Week 8

Iron-labeled Retention (%)

B Non-Injected Region MSC injected Region

H&E 64x H&E 160x H&E 160x PB 160x

Subendocardial rim thickness (mm)

Amado, LC et al PNAS 2005;102:11474-11479
What Percentage of Activated Macrophages will take up SPIO label from implanted cells?

- 30% to >80% of directly transplanted cells into tissues are Dead or are undergoing Apoptosis soon after implantation*.
- How often does endogenous label from directly implanted Stem Cells get endocytosed by inflammatory cells?

Macrophage Uptake of Intracellular Exogenous Cell Label from Stem Cells. Implication for Cell Transplantation and Cellular Imaging

**Isotype**

**CD68 vs Anti-Dextran**

14.6%

**CD68 vs BrdU**

5.4%

**Iron Uptake by CD 68+ cells**

96 Hours

Mean CD 68 (AM) Dextran+ cells

<table>
<thead>
<tr>
<th>Ratios</th>
<th>AM</th>
<th>MSC</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:1:1</td>
<td>6x10^5</td>
<td>3x10^5</td>
<td>3x10^5</td>
</tr>
<tr>
<td>6:1:1</td>
<td>1.8x10^6</td>
<td>3x10^6</td>
<td>3x10^6</td>
</tr>
<tr>
<td>10:1:1</td>
<td>1x10^6</td>
<td>1x10^6</td>
<td>1x10^6</td>
</tr>
<tr>
<td>1:2:1</td>
<td>1.8x10^5</td>
<td>1.8x10^5</td>
<td>1.8x10^5</td>
</tr>
<tr>
<td>6:1:0</td>
<td>6x10^5</td>
<td>6x10^5</td>
<td>6x10^5</td>
</tr>
<tr>
<td>1:2:0</td>
<td>6x10^5</td>
<td>6x10^5</td>
<td>6x10^5</td>
</tr>
<tr>
<td>6:1:1</td>
<td>1.8x10^6</td>
<td>1.8x10^6</td>
<td>1.8x10^6</td>
</tr>
<tr>
<td>3.3:Fe:1</td>
<td>1.8x10^6</td>
<td>1.2x10^6</td>
<td>1.2x10^6</td>
</tr>
</tbody>
</table>

**P < 0.0001**

**P < 0.004**

**P < 0.0001**
In vivo MRI of Bone Marrow Cells Trafficking to Atherosclerotic Plaques
Qui B et al. JMRI 2007;26:339

Possible Biomarker for Atherosclerosis Therapy
FDA’s CBERs Recommendations:

- Develop a Master File for all Human Cells to be Labeled with **Ferumoxides-Protamine Sulfate** (HSC, MSC, T-cells, Monocytes, Dendritic Cells)
  - Ex vivo Evaluation of Toxicity and Sterility,
  - Scale up Protocols for Labeling Cells

- **Proof of Concept: Preclinical Models of Disease. Can we Detect Labeled Cells by MRI in Cell Escalation studies?**

- Apply for Disease Specific INDs and IRB Protocols
Translating Magnetic Cell Labeling Techniques from *Bench to Bedside*

- **Proof of Concept: Preclinical Models of Disease with Cell Escalation studies.** Cell labeling must be performed according to CGMP and procedures that will be used for Clinical Studies.

- **Example of Cell Escalation Study (e.g. GBM into SCID mice IV infusion of cells at a specific time point).**

<table>
<thead>
<tr>
<th>#Cells/Mouse</th>
<th>Labeled CD 34</th>
<th>Unlabeled CD 34</th>
<th>Control (no cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3x10^5</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>3x10^6</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>3x10^7</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

- 3 mice from each group at each cell level will undergo in vivo MRI.
- KM statistics to determine survival differences among groups.

- **CBC with Diff, LFTs, Renal Function and Electrolytes at time of euthanasia.** Mean Iron content per cell will be measured.

- **Histopathology (H&E, Prussian Blue and Human markers, 1-4 sections per organ):** Pathology, Brain, Lungs, Heart, Liver, Spleen, Kidneys, Bone Marrow.

- **All Data to be submitted as part of IND.**
Pipeline for Labeling Cells with Ferumoxides-Protamine Sulfate in Cell Processing Section, DTM, CC.

Quality Control and Testing for IND Submission to FDA on Magnetic Cell Labeling for CD 34.

Apheresis (MNC) 5x10^8 PL146 bag

Wash Cells, add FE-Pro add media

MNC + FE-Pro (FEP bag)

37°C CO₂ incubator 2-8 hrs

Labeled MNC (FEP bag)

Wash Cells, concentrate and re-suspend in plasmalyte A & 2% HSA

Labeled MNC washed new FEP bag

Filter (170µm)

CBC, Volume, Sterility

CBC, flow cytometry, Endotoxin, Sterility, Mycoplasma Prussian Blue, Trypan Blue Iron Content, MTT

Labeled MNC FEP bag

Infusion into Patient over 30-60 minutes
Procedures of Labeling with FePro

Important points:

1. Wash cells x3 with serum free media prior to labeling
2. Cells in Suspension cell numbers per ml of media = 4x10^6 cells
3. Optimize cell numbers per cm^2 of growth plate (1x10^6/cm^2)
4. Ferumoxides - 100 µg/ml
5. Protamine sulfate – 3 (or 4) µg/ml of cell suspension (Adherent)
6. Ferumoxides first and mix for 30-60 sec
7. Protamine sulfate and mix for 30-60 sec
8. Incubate 15 minutes-2 hrs minutes at 37°C
9. Add equal volume of complete media and incubate further up to 4 hours to overnight at 37°C
10. Wash Cells with Heprinized 10-20u/ml Serum Free Media x 3
## Evaluating labeling efficiency, toxicity, viability and function

<table>
<thead>
<tr>
<th><strong>Labeling efficiency:</strong></th>
<th><strong>Cellular viability:</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Prussian blue staining for iron</td>
<td>Trypan blue dye exclusion test</td>
</tr>
<tr>
<td>Measurement of iron concentration</td>
<td>Incorporation of PI (propidium iodide)</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td></td>
</tr>
<tr>
<td>Magnetopheresis</td>
<td></td>
</tr>
<tr>
<td>Flow cytometer – Antidextran Ab</td>
<td></td>
</tr>
</tbody>
</table>

| **Cellular toxicity:** | |
|------------------------| |
| MTT or similar analysis for metabolic or proliferative activity | |
| Apoptosis, such as Annexin-V by FACS or fluorescent reader | |
| Determination of Reactive Oxygen Species (ROS) | |
| Cell activation (especially for T-cells and macrophages) | |
| FACS of cell surface markers | |
| Changes in RNA and protein expression | |
| Gene analysis | |

| **Cellular functions:** | |
|------------------------| |
| Chemotaxic | |
| Differential capacity of stem cells | |
| Release of cytokines following stimulation | |
Release Criteria for Labeled Cells

- **CD34**
- **CD133**
- Isotype
- **CD34**
- **CD133**
- FEProlabeled

**Anti-Dextran Ab for FACS**

**BMSC Isotype**

**BMSC FEProlabeled**

- Gram Stain Negative
- >90% viability
- PB + cells cytospin
- >70% dex+ FACS
- <3% CD3/4 +cells
Aims and Outcome Measures

- **Safety and Toxicity** of transfusing autologous CD 133\(^+\) cells labeled with FEPro complex in patients with GBM.
- To determine if IV infused FEPro labeled CD 133\(^+\) cells can be Detected in the tumor by MRI.
  - Do CD 133 cells contribute to GBM neovasculature?

Entry Criteria **Patients with GBMs**

- PET/CT FDG + and increased rCBV on DCE-MRI at baseline

Protocol: Cell Escalation (Ferumoxides labeled cells per Kg)

- 6 pts = 1x10\(^6\)/kg, 3-6pts = 2x10\(^6\)/kg, 3-6pts = 3x10\(^6\)/kg and 6 pts at 4x10\(^6\)/kg obtained from a single apheresis and 5 day expansion.
- MRI at Baseline, Days 1, 3, 7, 14 and 28
- Clinical Follow-up
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% SPIO Labeling

Ultrasound guidance

Lymph node dissection

Scintigraphy

In vivo MRI

Histology

Ex Vivo MRI
Magnetic Resonance Imaging at 3T Tracking of Ferumoxides labeled Dendritic Cells

MRI and Indium\(^{111}\)oxine SPECT of labeled \((1.5 \times 10^6)\) Dendritic Cells

MRI can visualize about 500 labeled cells/voxel

Ferumoxides Labeled Dendritic Cells missed Lymph Node

Approximately 150,000 labeled cells

MRI and Prussian blue stain of Lymph Node

de Vries IJM et al Nature Biotechnology 2005;23:1407-13
34 y.o. Male patient admitted to ER with open brain trauma. During surgery, exposed brain mixed with craniofacial debris was collected and cultured for Neural Stem Cells (NSC). NSCs were labeled with Ferumoxides-Effectene complex and implanted at 4 points around damaged area under MRI guidance.
MR Tracking of magnetically labeled autologous bone marrow CD 34+ cells transplanted into the spinal cord via LP technique in patients with chronic spinal cord injury:

CD 34+ cells’ migration into the injured site

Callera F et al. Stem Cells and Development 2007;16:461-466

Table 2. Labeled CD34+ Group and Parameters Associated with Resonance Tracking

<table>
<thead>
<tr>
<th>Patients</th>
<th>Labeled CD34+ viability before transplantation (%)</th>
<th>Labeled CD34+ injected (× 10⁶)</th>
<th>Labeled CD34+ resonance tracking</th>
<th>Migration distance (from L4-L5 level to)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>81</td>
<td>0.7</td>
<td>Yes</td>
<td>C7-T1</td>
</tr>
<tr>
<td>2</td>
<td>83</td>
<td>0.45</td>
<td>No</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>82</td>
<td>0.90</td>
<td>Yes</td>
<td>T4-T5</td>
</tr>
<tr>
<td>4</td>
<td>81</td>
<td>1.10</td>
<td>Yes</td>
<td>T1-T2</td>
</tr>
<tr>
<td>5</td>
<td>85</td>
<td>1.22</td>
<td>No</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>85</td>
<td>0.53</td>
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<td>—</td>
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<td>7</td>
<td>82</td>
<td>0.61</td>
<td>No</td>
<td>—</td>
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<tr>
<td>8</td>
<td>85</td>
<td>0.83</td>
<td>No</td>
<td>—</td>
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<tr>
<td>9</td>
<td>84</td>
<td>0.65</td>
<td>No</td>
<td>—</td>
</tr>
<tr>
<td>10</td>
<td>84</td>
<td>0.65</td>
<td>No</td>
<td>—</td>
</tr>
<tr>
<td>Median</td>
<td>83</td>
<td>0.70</td>
<td>Yes 5</td>
<td>—</td>
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<tr>
<td>(range)</td>
<td>(81–86)</td>
<td>(0.45–1.22)</td>
<td>No 5</td>
<td>—</td>
</tr>
</tbody>
</table>

Dyna bead labeled cells delivered via LP
The Role of Imaging in Monitoring Cellular Therapy

- **Subject Selection**
  - Evaluation 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 Cell Therapy (Tumors, GVH)**

- **Cell Survival, Migration, Differentiation and Function**

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

- **What Combination of Imaging Modalities should be used to Assess Cellular Therapy?**

- **To optimize methods that will accelerate the translation of Cellular Therapy from Bench-to-Bedside.**
Experimental Neuroimaging

E. Kay Jordan D.V.M.
Jodi Haller Ph.D.
Matthew Budde Ph.D.
Eddy Pawelczyk Ph.D
HoTaek Song M.D. Ph.D.
Bobbi K. Lewis B.A.
Eric Gold B.S.
Wei Liu Ph.D.
Aneeka Chaudhry B.S.
Melissa Smith B.S.

CC
Charlie Carter B.S.
Elizabeth Read M.D.
Hanh Khuu M.D.
Vicki Fellowes B.S.
David Stroncek M.D.

NHLBI
Stasia Anderson Ph.D.
Toren Finkel M.D.

NICDR
Pamela Robey Ph.D.
Arun Balakumaran M.D.

NCI
Howard Fine M.D.

Henry Ford Hospital Systems
Ali S. Arbab M.D. Ph.D.
End of Slides
Outstanding Questions in Cellular Therapies

- **Environment**
  - Will transplanted cells be subject to the same pathological processes that lead to the initial development of pathology?
  - How can we enhance and direct migration of transplanted cells to target?
  - Can the biochemical effects of astrogliosis and mechanical effects of astroglial scarring be overcome?
  - Are the appropriate signals for repair within damaged regions of the CNS?
  - What is the influence of inflammation on the survival, differentiation, repair or replacement potential of transplanted cells?
  - How can we overcome rejection of transplanted cells without compromising the health of the patient?

- **Cells**
  - What is the ideal stem cell source or type of stem cell?
  - Are the stem cells free of undesired retroviral sequences or rodent molecules?
  - Can the cells be amplified with genetic stability to sufficient numbers for clinical viability?
  - What level of purity is required for clinical use?
  - Has the tumorigenic potential been eradicated in the transplant population?

Keirstead HS Trends in Neurosciences 2005;28:677
Contrast Agents as Cellular Markers

• Why Label Cells (Stem or Others) for non-invasive Imaging?
  – To Monitor Cell Trafficking.
  – To further the understanding of Normal and Disease Processes.
  – To Develop Innovative Experimental and Clinical Trials using Stem Cells or Genetically Engineered Cells that can be used for Repair, Replacement or Treatment.
Hematologic Aspects of Myeloablative Therapy and Bone Marrow Transplantation

Multiple Stem Cell Doses and Schedules
CG-4 oligodendrocyte progenitors

Add magnetic label

Collect magnetically tagged cells

Migration

10-14 Days

Myelination

Histopathological correlation

3D MR microscopy

**In Vivo Tracking of Magnetodendrimers (MD 100) Labeled Progenitor Cells**

Approx. $5 \times 10^4$ MD-100 LacZ CG-4 cells transplanted into the IC region of neonatal Long Evans Shaker Rats

1.5 Tesla, 3D SPGR, 1.2 mm slices

1.5 Tesla, 3D SPGR, 1.2 mm slices

Day 18

Day 25

Day 44

Non Specific Labeling MD 100 at 25 μg Fe/ml

CO 4 Progenitor Oligodendrocytes

Fe (II) oxidant

γ-Fe$_2$O$_3$

Strable E et al., Chem Mater 2001;13:2001


Strable E et al., Chem Mater 2001;13:2001

4.7T 3D MPGR

Anti-MBP

Electrostatic Interaction between Dextran Coated Superparamagnetic Iron Oxide Nanoparticles and Transfection Agents (i.e., Dendrimer)

Zeta Potential for SPIO
Ferumoxides  -32.7 ± 0.98 mv

Zeta Potential for TA
Superfect  1.0 ± 2.5 mv
Polyfect  19.1 ± 1.01 mv
Poly-L-Lysine  30.1 ± 17.8 mv
Lipofectamine  65.3 ± 5.22 mv
Protamine Sulfate  7.2 ± 2.7 mv

Kalish H and Frank JA et al MRM 2003;50:275
Monitoring of implanted stem cell migration *in vivo*: A highly resolved *in vivo* magnetic resonance imaging investigation of experimental stroke in rat

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PNAS 2002;99:16267.

Migration of Sinerem-Fugene labeled ES cells (3x10⁴) along CC and lining of ventricular wall implanted 2 weeks after stroke
GFP+/Magnetically Labeled Stem Cells Day 30 following Implantation and Stroke
FE-PLL labeled MSC in rat liver: MRI at 1.5T Day 15

Unlabeled MSC

Labeled MSC

PB, Labeled MSC

PB, control liver

Unlabeled MSC

Labeled MSC

GRE 300/20/20°

PB, Labeled MSC

PB, control liver

Anti-HLA-1 + FITC and DAPI

Dil labeled MSC + DAPI

10^6 Labeled cells were administered intravenously

Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi Sarcoma


4x10^6 FePro and Dil labeled MSC injected on Day 0 simultaneously with KS cells implanted in flanks of mice. Serial MRI at 7 Tesla

DiI positive MSC in KS tumor

Necrosis
Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi Sarcoma


4x10^6 FePro and Dil labeled MSC injected on Day 0 simultaneously with KS cells implanted in flanks of mice. MRI Day 3 Labeled MSC found in multiple organs.

KS

KS + MSC

KS

KS + MSC

Bone Marrow

Lung

Spleen

Liver
Targeted cell delivery by Magnetic Field Gradient (MFG)
Can MFG slow the momentum of FE-PLL Labeled Cell?

![Graph showing normalized signal intensity and percent iron content over time with and without a magnet.](image)

DAB PB+

Clinical MRI of Pancreatic Islet Grafts After Iron Nanoparticle (Resovist) Labeling
Toso C et al American J Transplantation 2008;8:701-6

Table 1: Islet transplant characteristics
Average age 52yo

1.5T MRI with T2+ TE 18 ms TR 220ms Slice 1.3x1.3x5mm