

Imaging Techniques for Monitoring Cellular Therapy

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Cell Imaging

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

Brain $\sim 10^{11}$

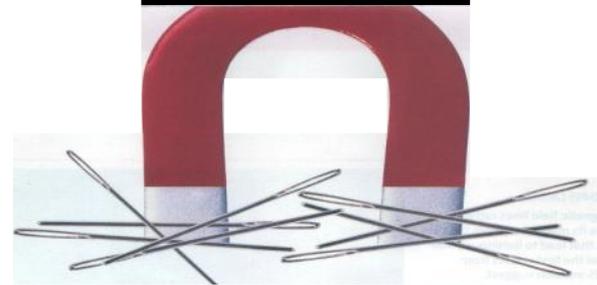


IV Injection of
 $10^5 - 10^8$ cells
into blood volume
5L containing
 $\sim 40 \times 10^{12}$ cells

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



IM Injection of $10^5 - 10^6$ cells



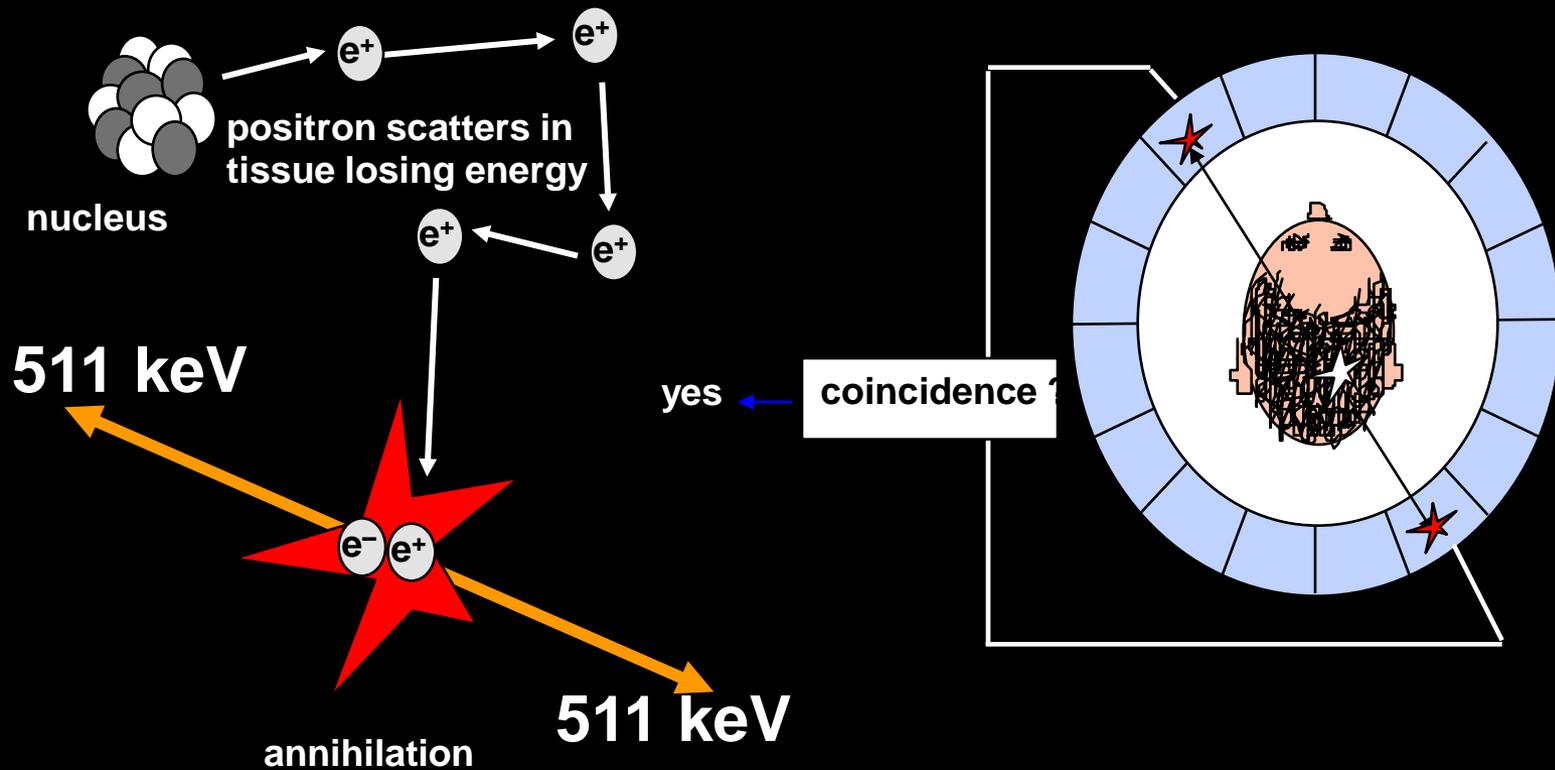
BMSC $1 - 5 \times 10^6$ cells/kg iv = $70 - 350 \times 10^6$ cells



Overview of Imaging Modalities

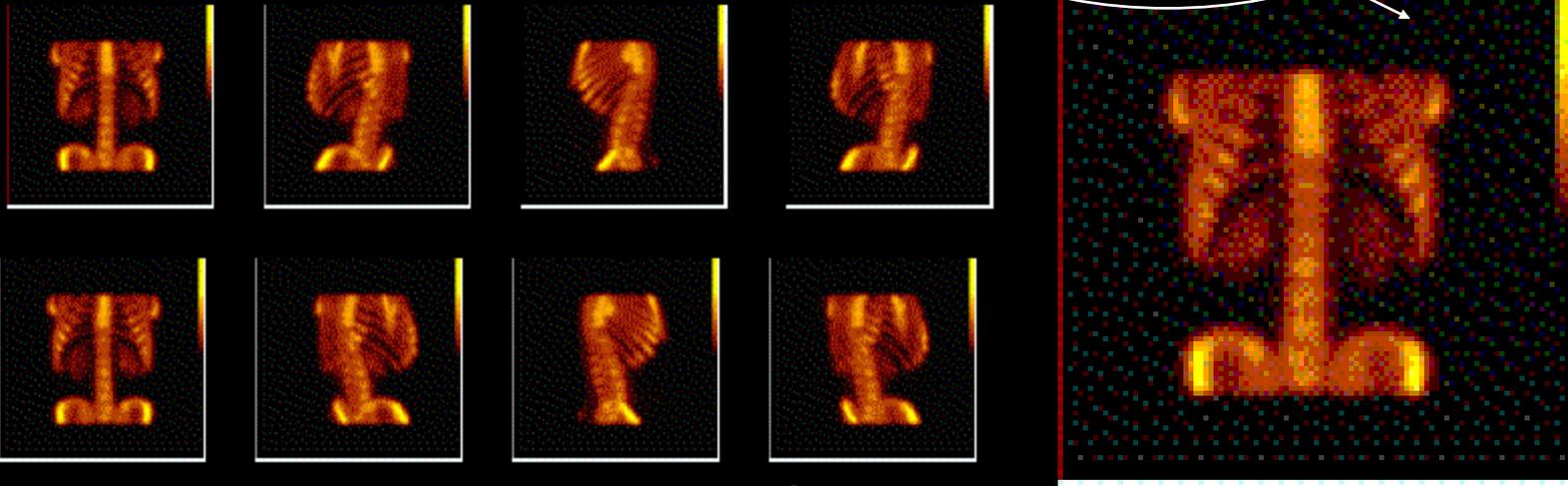
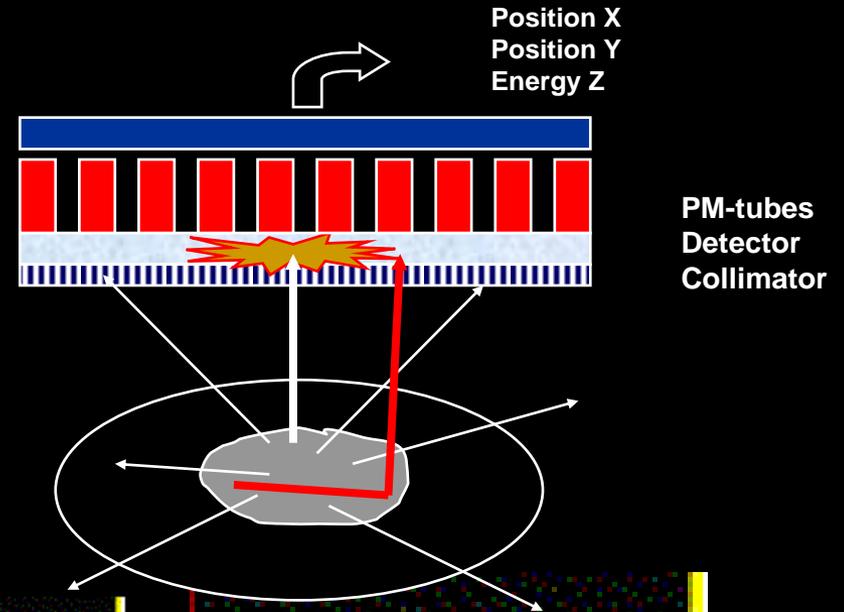
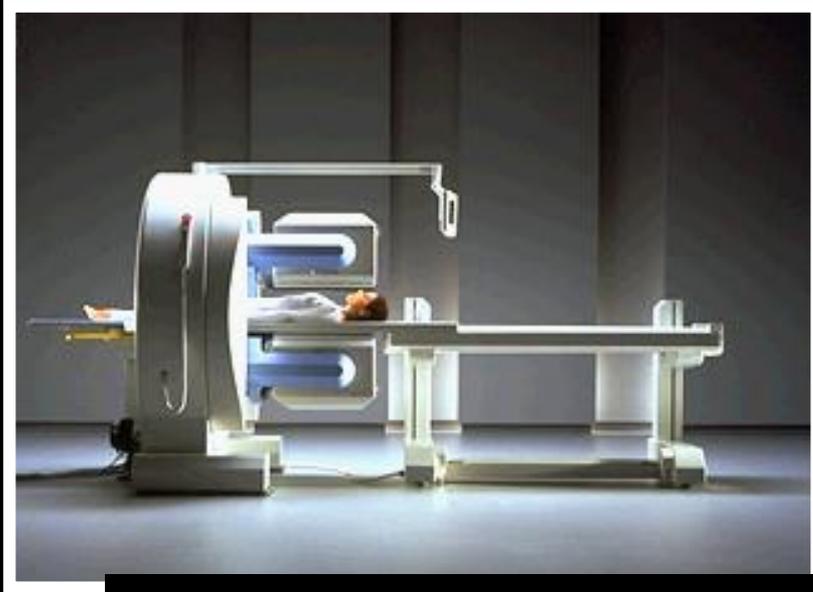
Techniques	Resolution	Depth	Time	Imaging Agents	Application	Main Characteristics	Clinical
MRI	10-100μm	No Limit	μsec to Hours	Gadolinium, Manganese, Iron Oxides nanoparticles	Anatomy Physiology Metabolic <u>Cellular</u> Molecular	Versatile High soft tissue contrast	Yes
PET	1-2 mm	No Limit	Min	^{18}F,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

Positron Emission Tomography



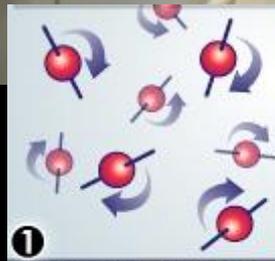
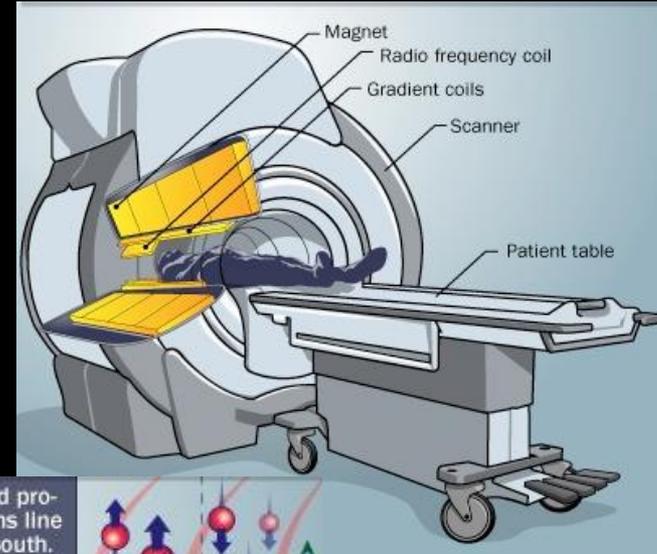
Half Life ^{18}F = 110min, ^{64}Cu 12.7h

SPECT cameras are used to determine the 3D distribution of the radiotracer

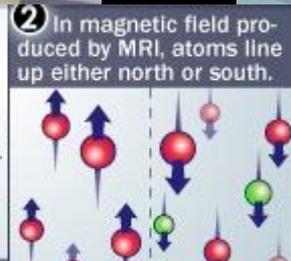


Half Life ^{111}In = 67hr, $^{99\text{m}}\text{Tc}$ = 6hr, ^{123}I = 13hr

Magnetic Resonance Imaging

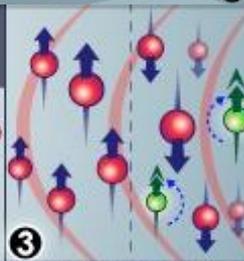


1 Atoms spin in random directions, like tops, around their individual magnetic fields.

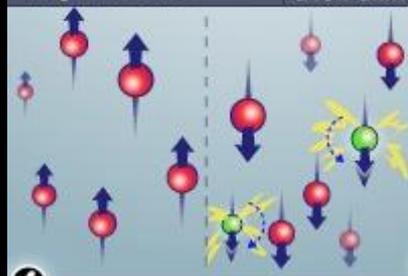


2 In magnetic field produced by MRI, atoms line up either north or south.

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



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

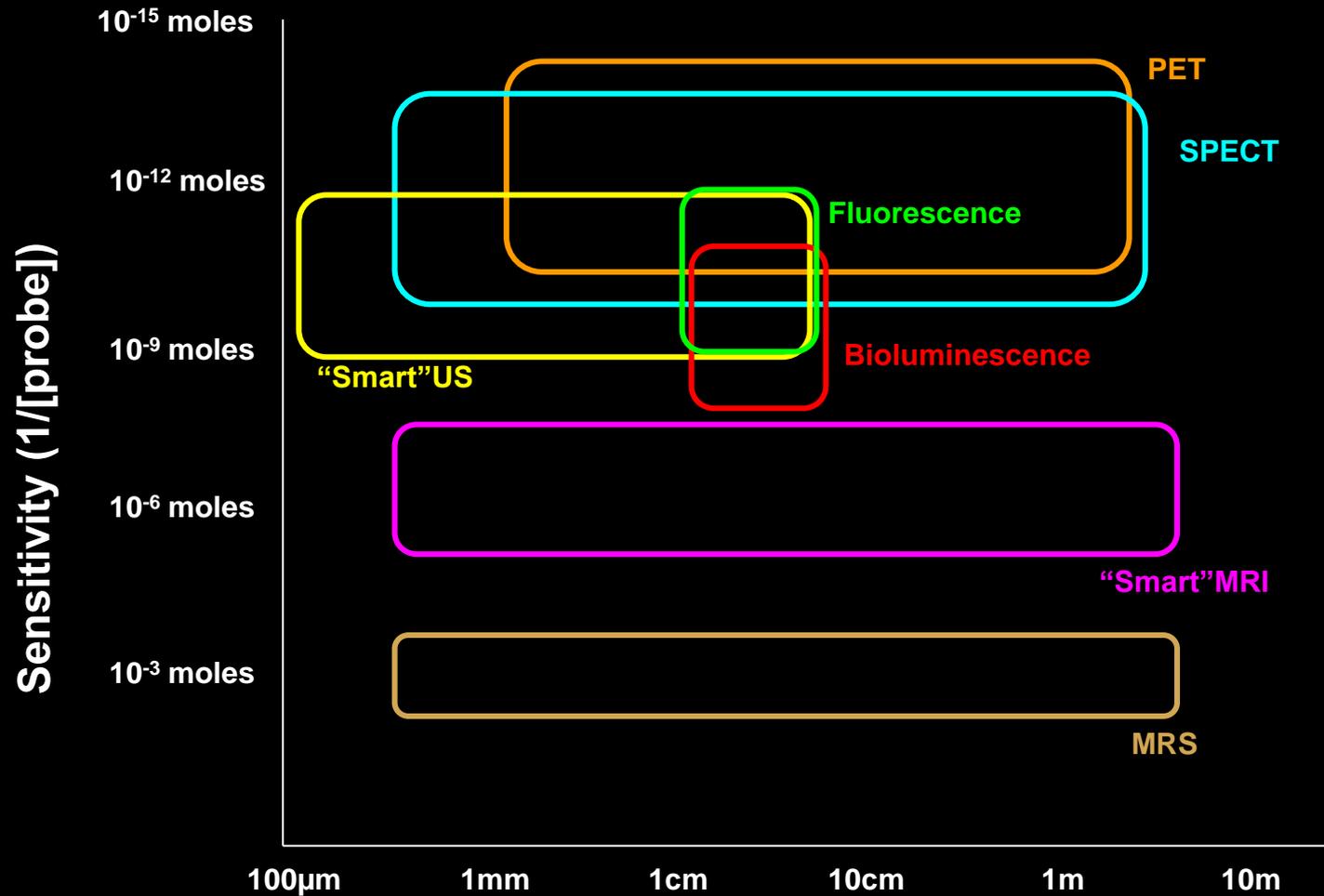


4 When the radio frequency is turned off, the extra atoms return to normal position, emitting energy.

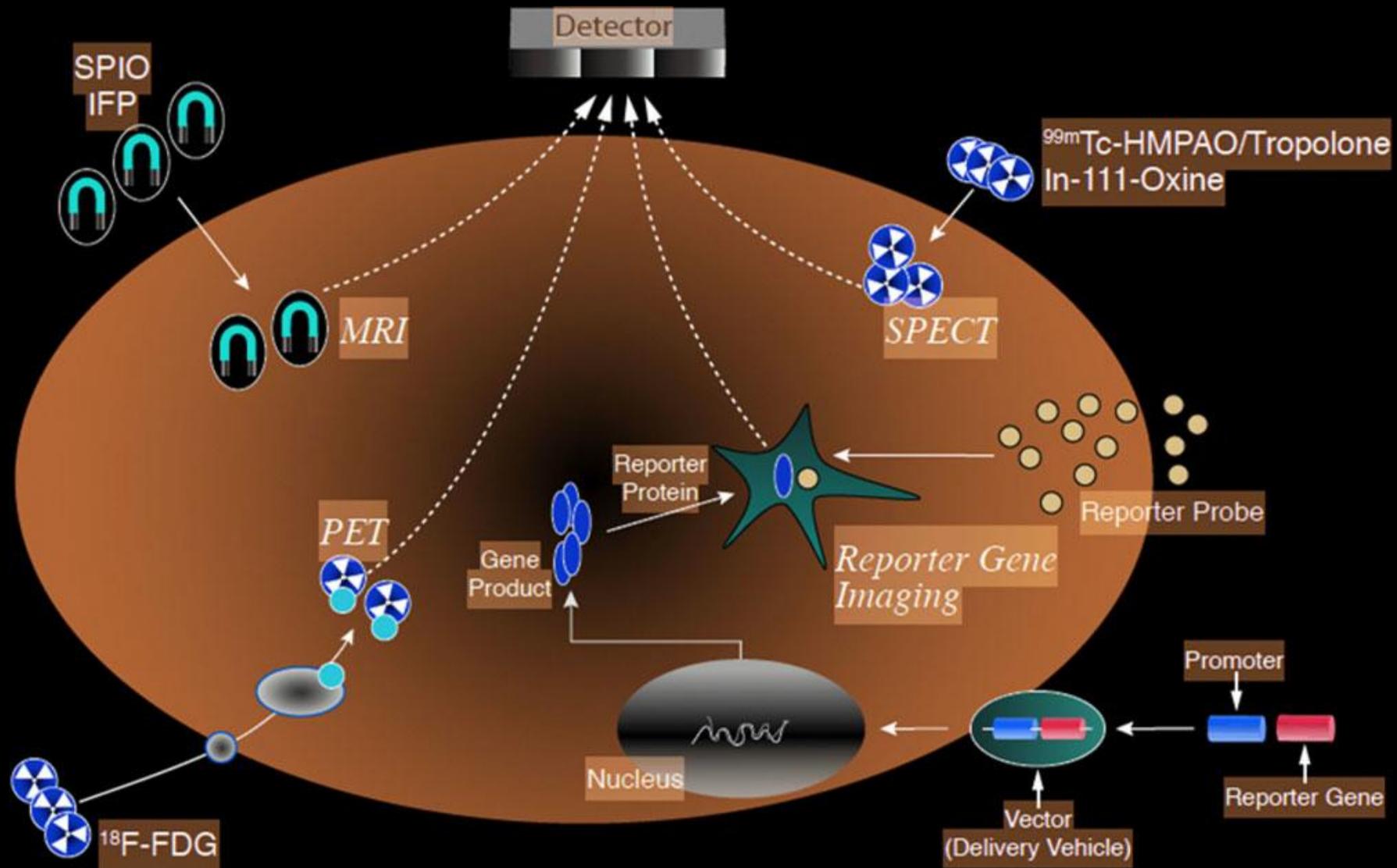


5 The energy sends a signal to a computer. The computer uses a mathematical formula to convert the signal into an image.

Sensitivity of Imaging Technologies

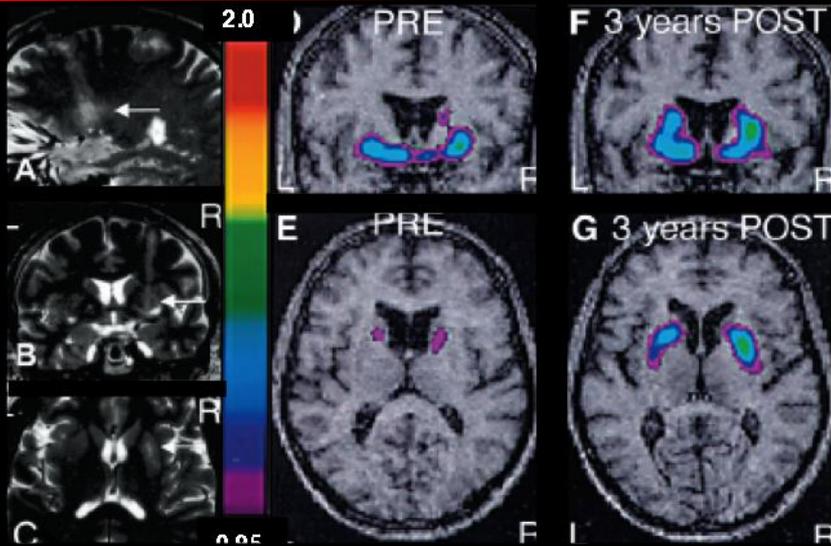


Schematic presentation for non-invasive imaging of cells

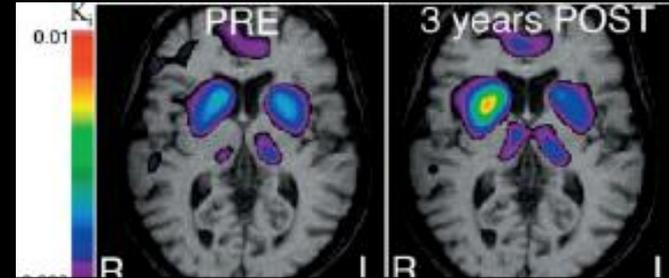


Cell type analysis of functional fetal dopamine cell suspension transplants in the striatum and substantia nigra of patients with PD

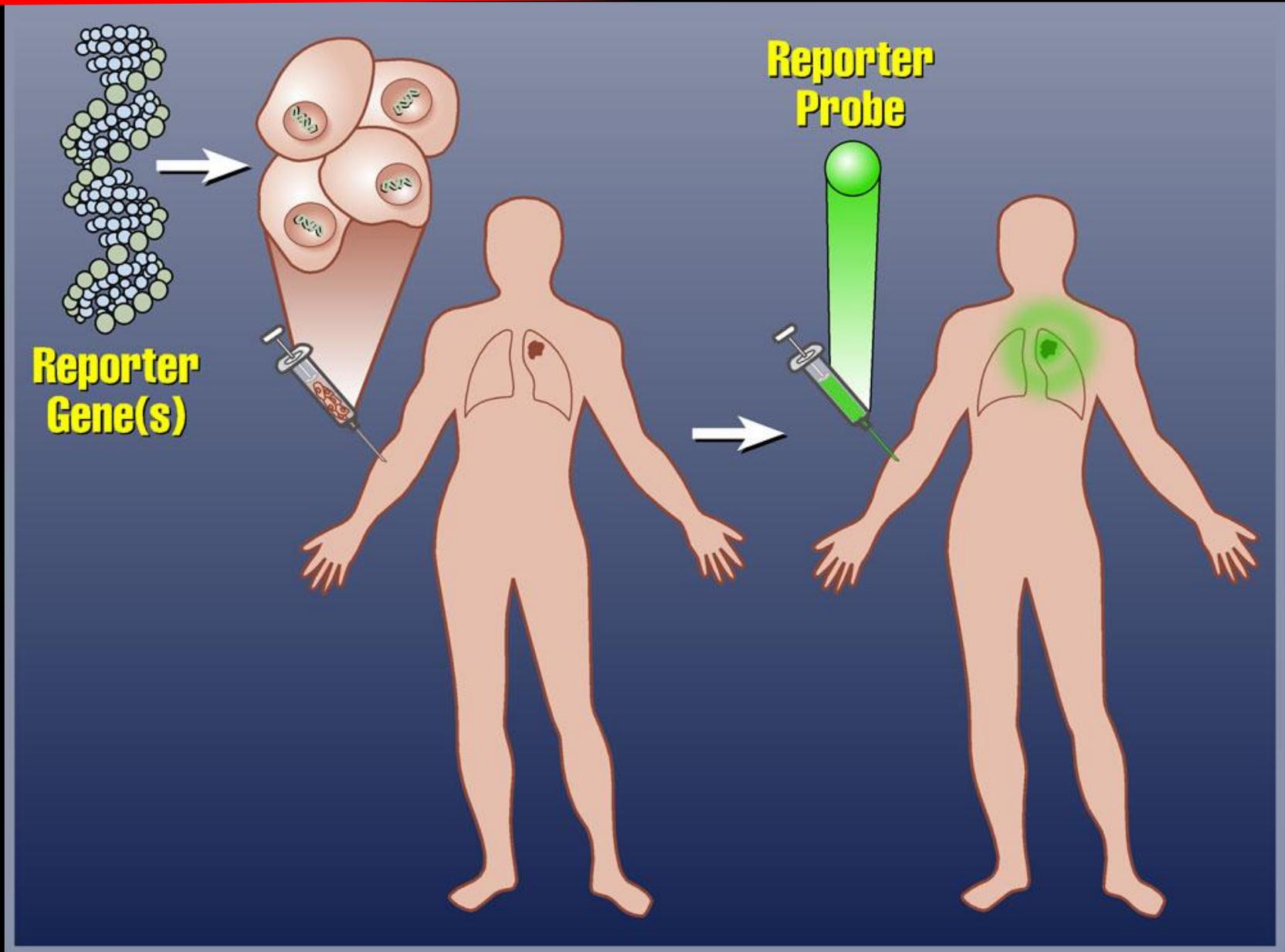
Mendez I and Isacson O et al Brain 2005;128:1498-1510



Post-mortem analysis of 2 patients with PD with received fetal transplant with favorable clinical outcome and positive ^{18}F FDOPA PET



Reporter Gene Based Cell Imaging

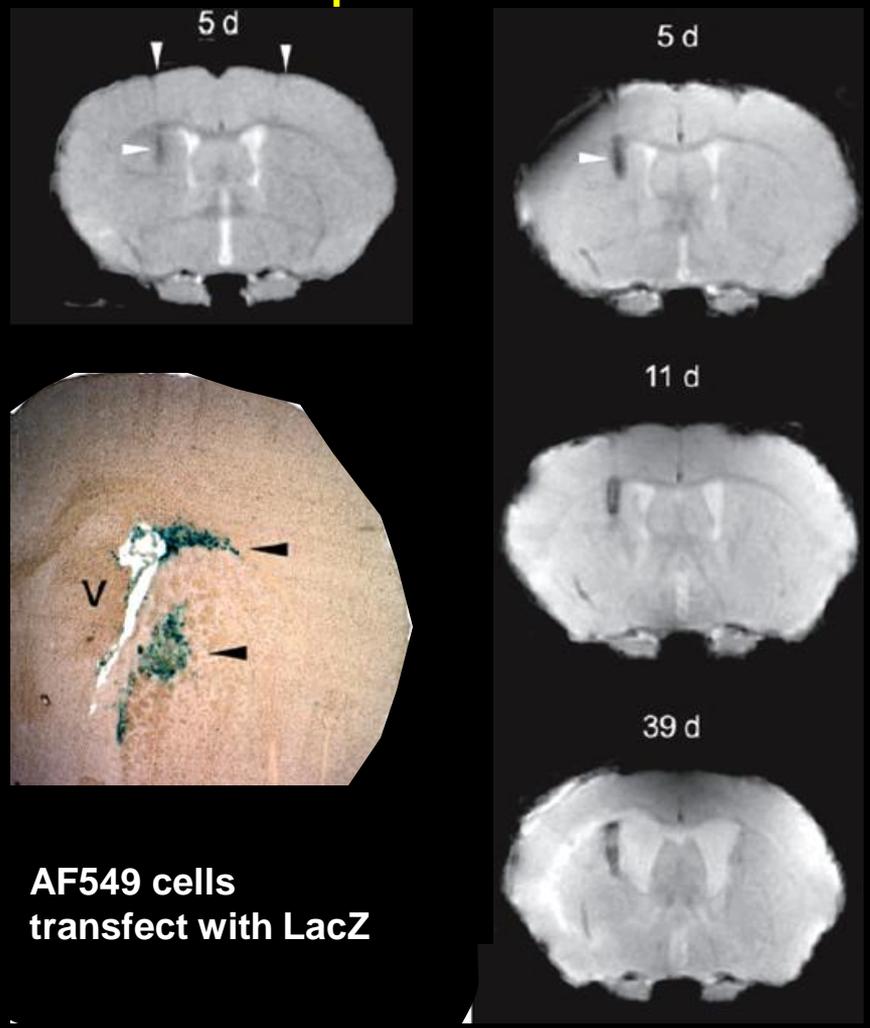


A new transgene reporter for *in vivo* magnetic resonance imaging

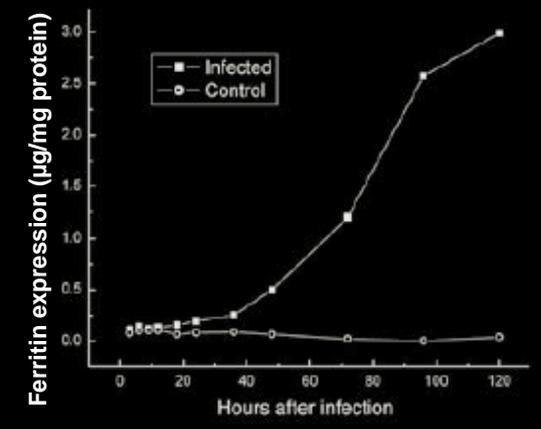
Guillem Genove¹, Ulrike DeMarco¹, Hongyan Xu¹, William F Goins² & Eric T Ahrens¹

Nature Medicine 2005;11:450

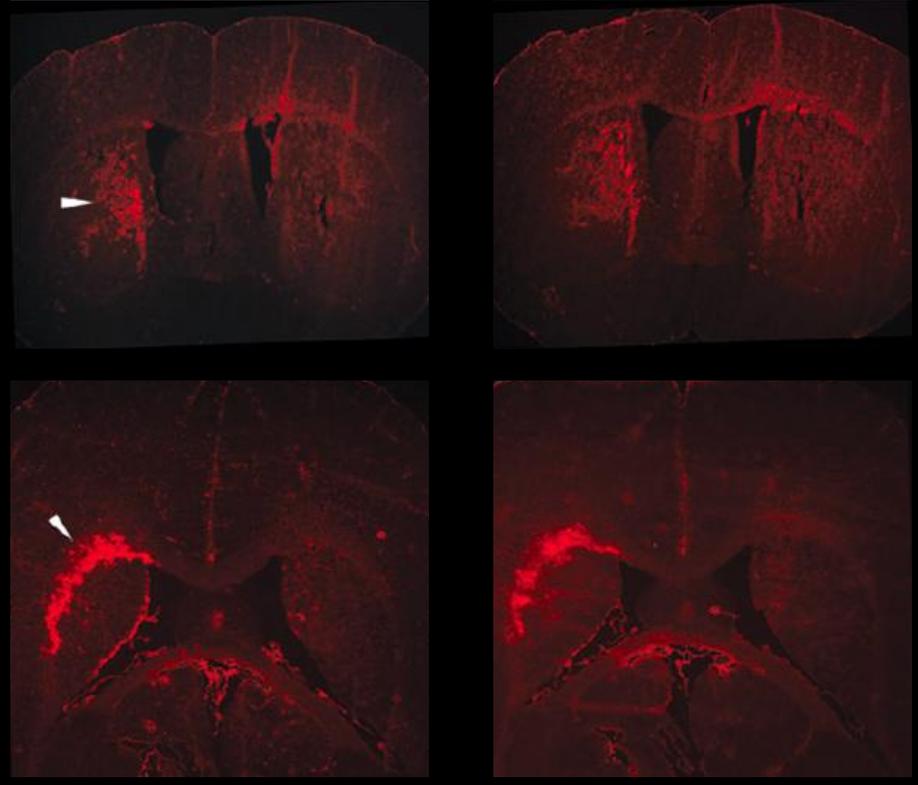
MRI at 11.7T 3×10^6 AdV-Ft AF 549 cells implanted in mouse



AdV-Ft transfected AF 549 cells



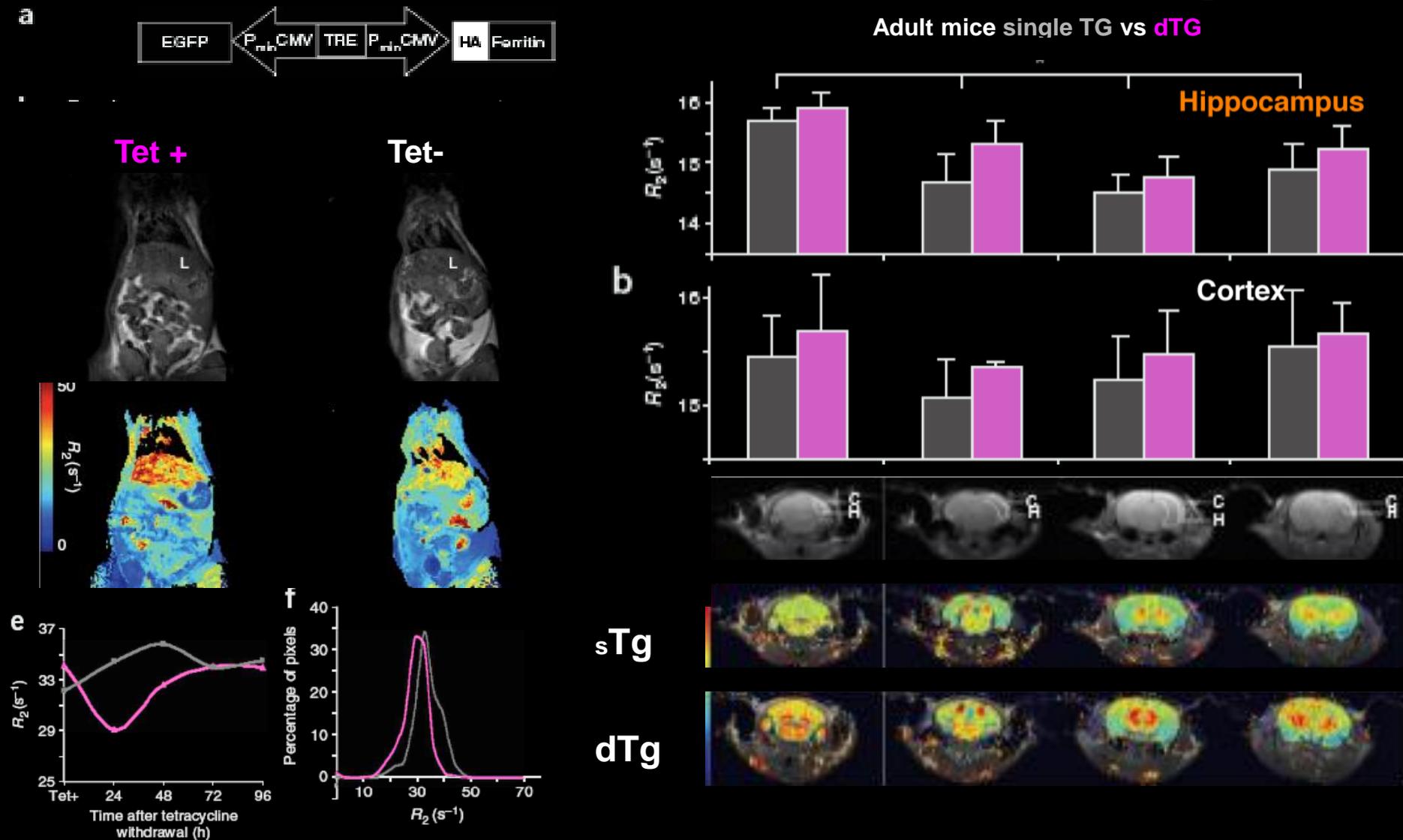
Ferritin Expression Day 5 after implanting AdV-Ft AF 549 cells



MRI detection of transcriptional regulation of gene expression in transgenic mice

Cohen B et al Nature Medicine 2007;13:498-0503

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

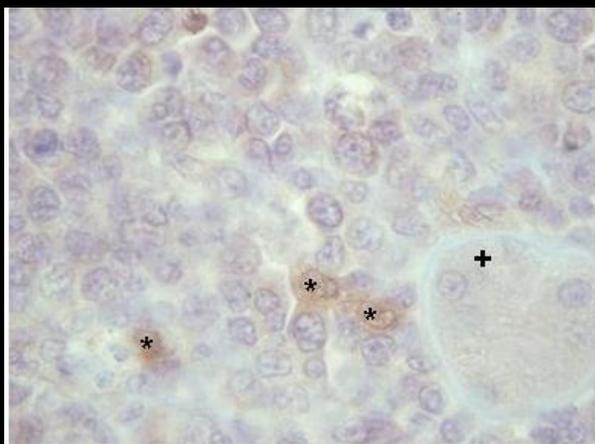


BM Transplanted 7 Rhesus monkeys in 1999 using MSCV based RD114 pseudotyped retroviral containing eGFP and dihydrofolate reductase gene.

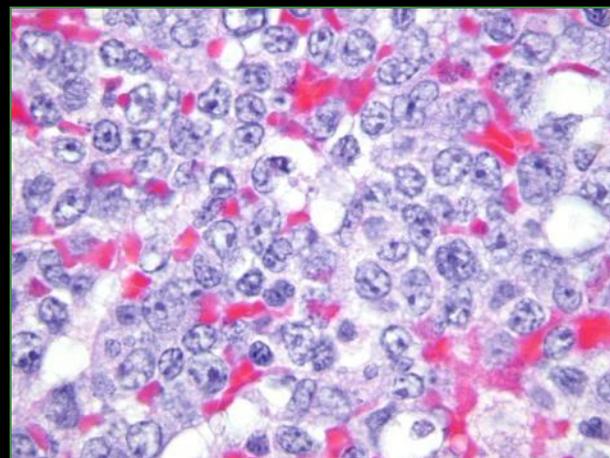
Insertion analysis revealed eGFP inserted in chromosomes 15 and 9. (Not usually performed)

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!



eGFP staining of the kidney infiltrates (monoclonal GFP antibody), weakly positive blast cells (*), negative renal tubulus (+).

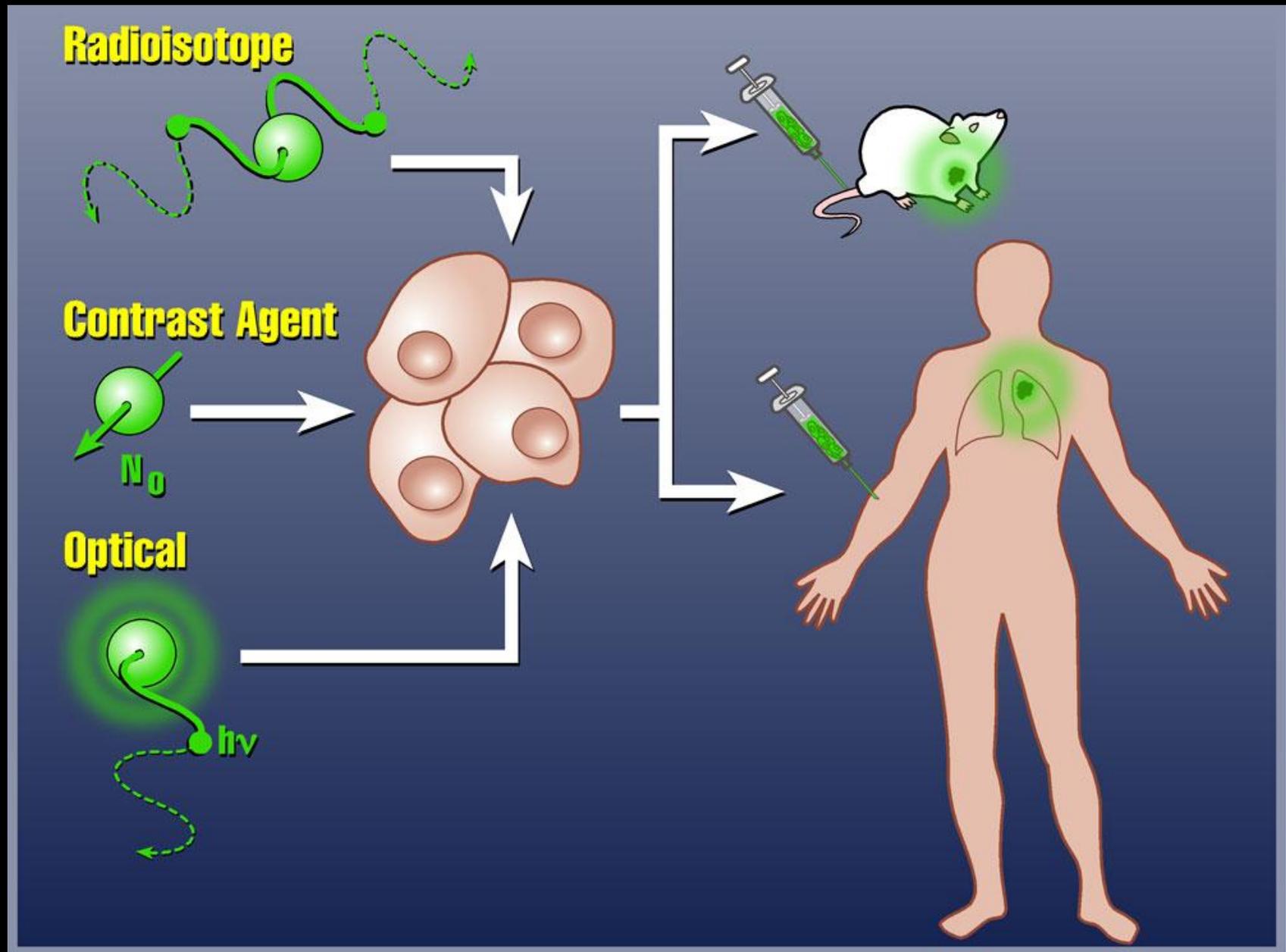


Insertion of eGFP into Chromosomal 15 or 9 resulted in activating *BCL2-A1*, a gene known to have antiapoptotic properties, dominated multilineage contribution to hematopoiesis after transplantation, became dormant for 4 years, and then re-emerged as the dominant clone contributing to myeloid hematopoiesis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

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.

Direct Labeling Strategies

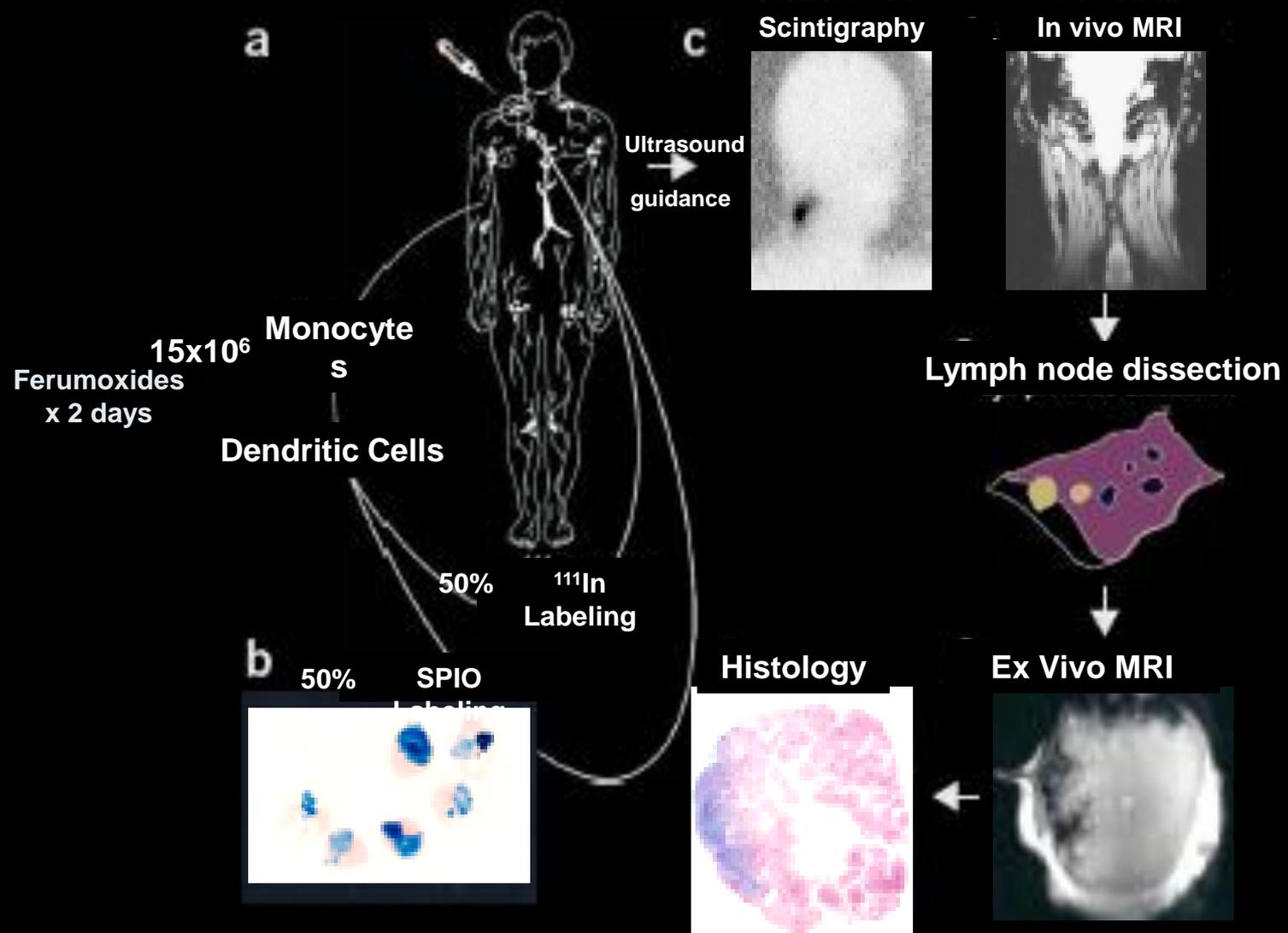


Agents for Cellular Labeling

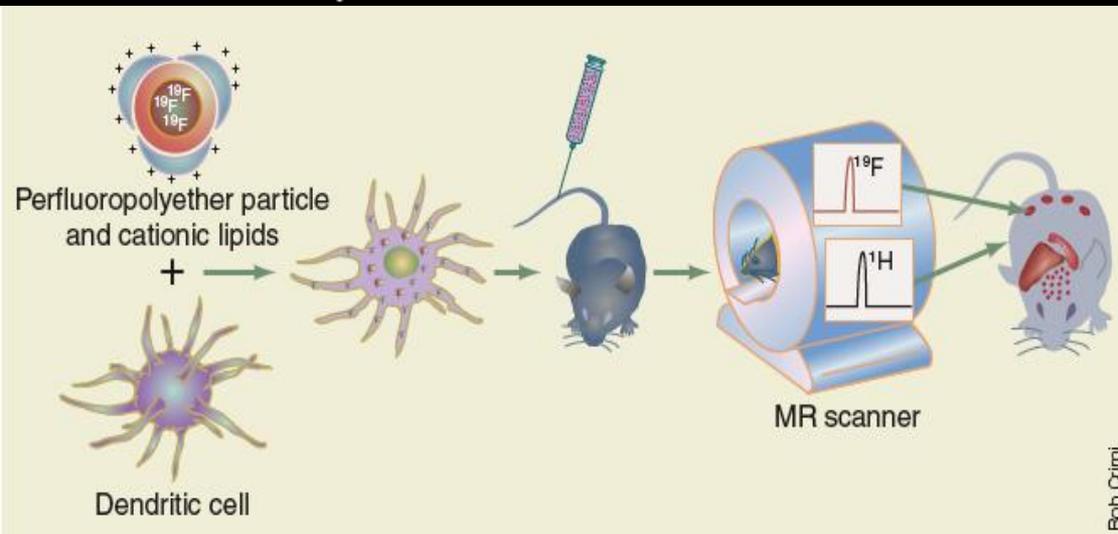
- **SPECT/PET agents: ^{111}In oxine, $^{99\text{M}}\text{Tc}$ HMPAO/Troponine, ^{18}F FDG, ^{64}Cu**
- **Multispectral Imaging: ^{19}F Fluorine**
- **Paramagnetic Agents: Gadolinium, Manganese, Iron chelates**
- **Superparamagnetic Agents: Iron, Iron + Mn, Fe + Co, MnO_2 in crystal lattice**

Magnetic Resonance Tracking of Dendritic Cells in Melanoma patients for monitoring Cellular Therapy

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



In vivo imaging platform for tracking immunotherapeutic cells



PFPE-Labeled Dendritic Cell

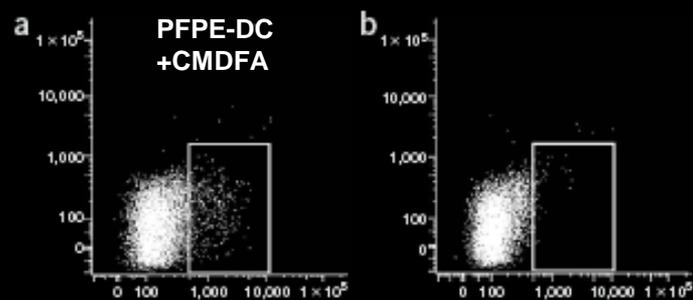
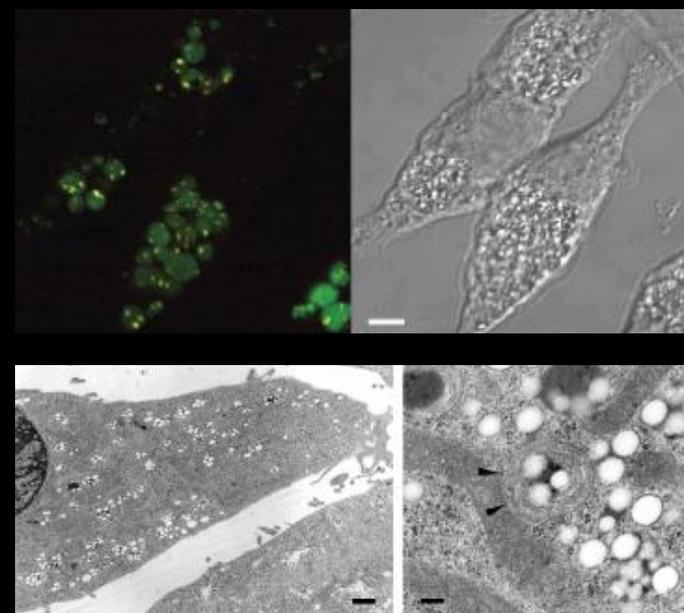
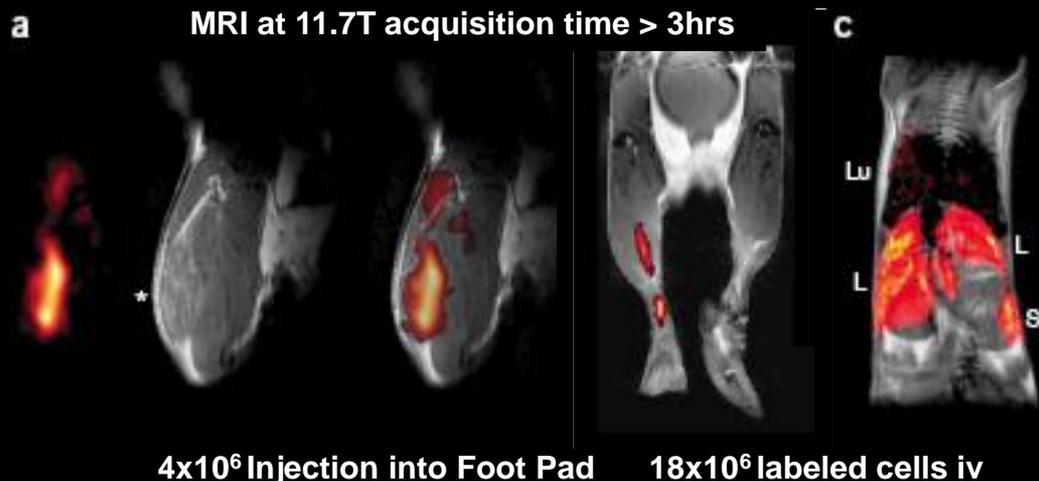


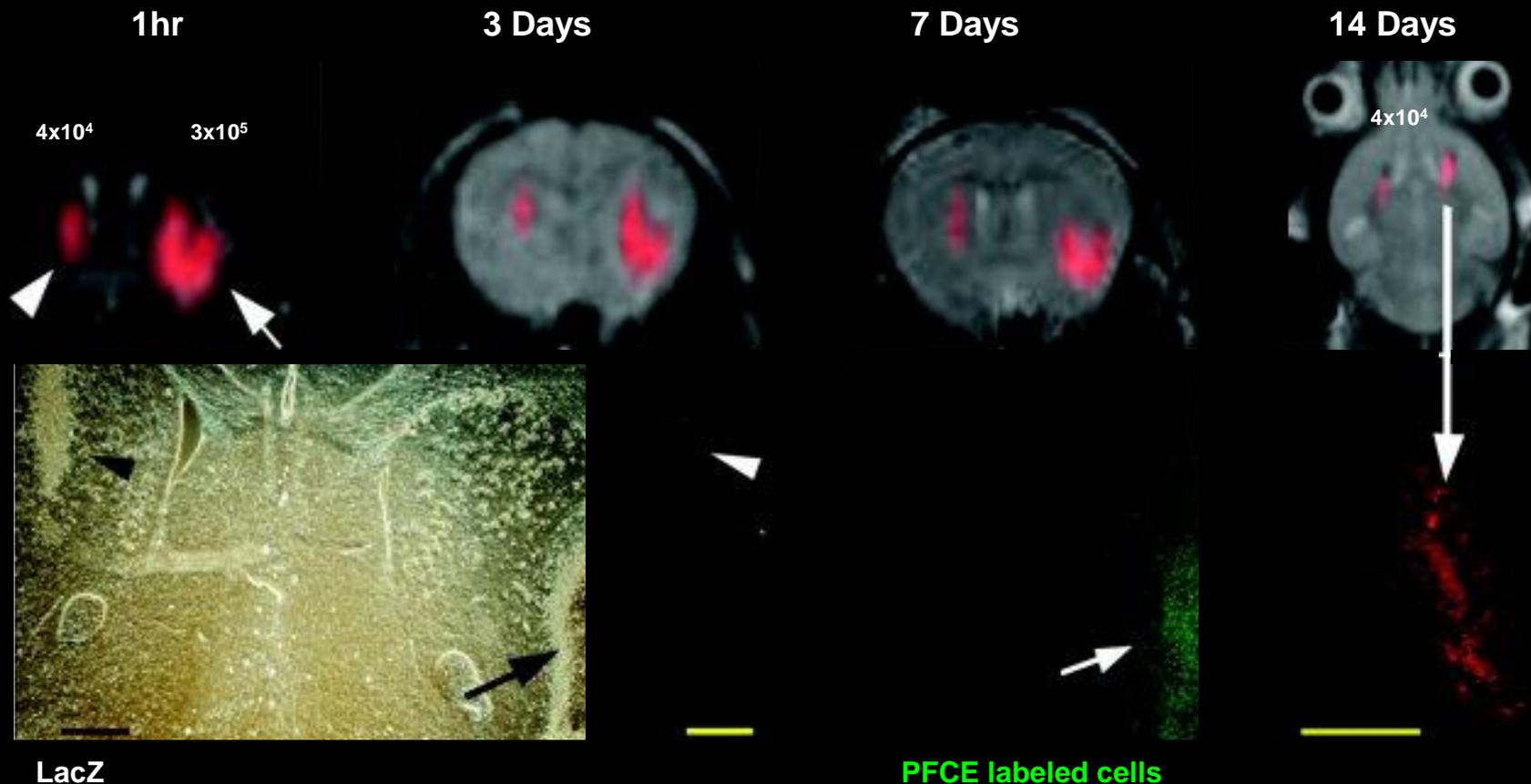
Figure 4 FACS analysis of DCs in excised lymph nodes following foot pad injection. BMDCs were labeled with PFPE overnight, and a portion of these were additionally stained with CMDFA. Labeled BMDCs were injected into the foot pad of syngeneic NOD mice. Twenty-four hours later, the popliteal and inguinal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



In vivo “hot spot” MR imaging of neural stem cells using fluorinated nanoparticles

Ruiz-Cabello J et al MRM 2009;60:1506-11

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)

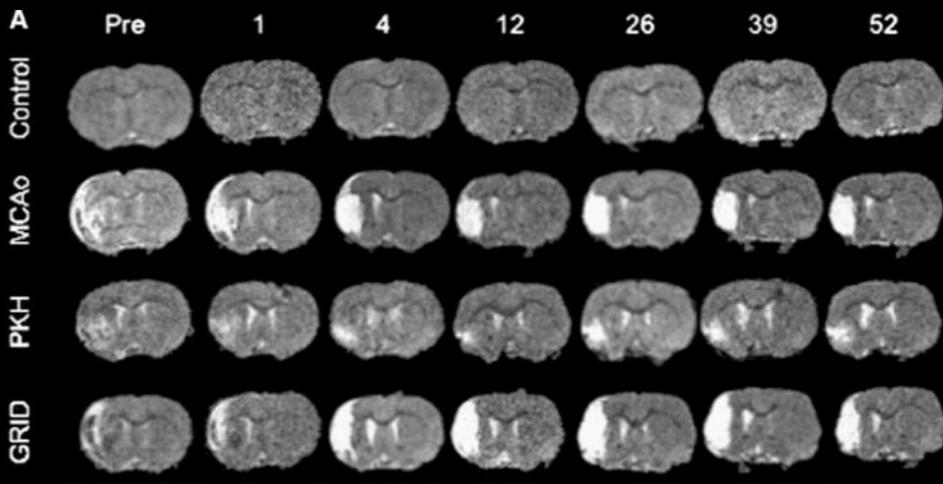


Scan Acquisition Times approximately 1 hour.
Will this technique be translated to the clinic?
Cost for surface coil for 3T \$20-40k.

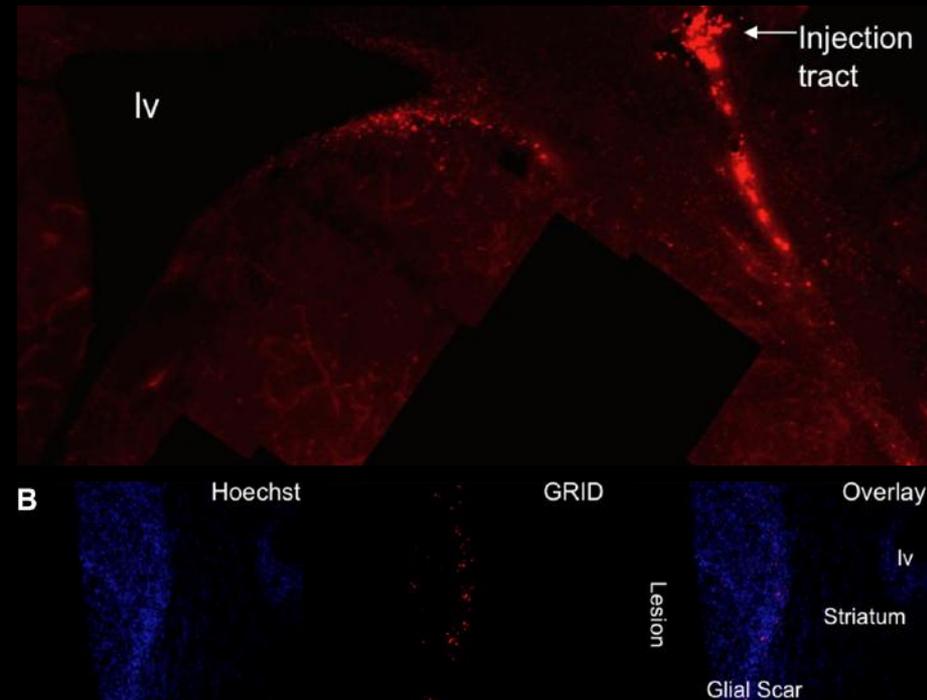
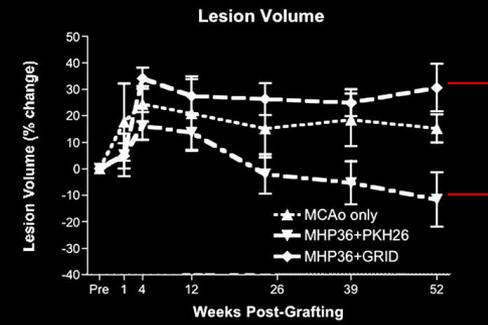
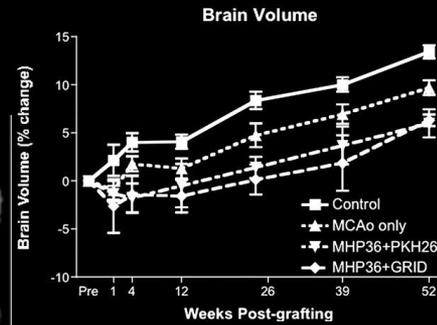
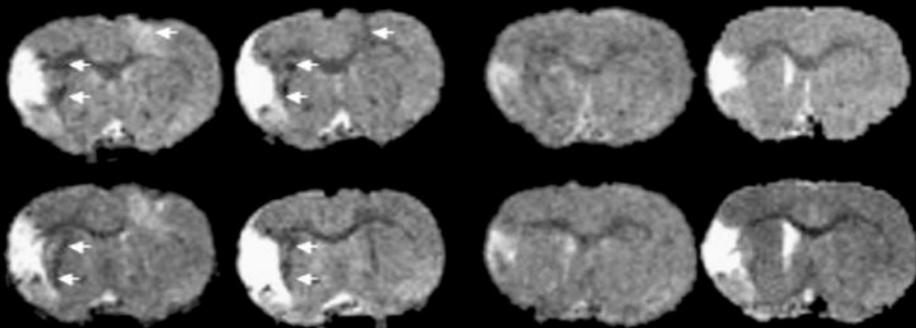
A chronic 1 year assessment of MRI contrast agent-labeled neural stem cell transplants in stroke

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

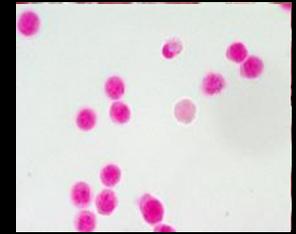
Injected 2×10^5 GRID labeled NSC 2 weeks MCAO in rat MRI at 4.7T SE 4000/64 234x234x350 μm



GRID **PKH26**
1week 1 month 1 week month



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%.



Incubate
2-6 hrs in Serum
Free Media

50% FE-PRO + 50% Media



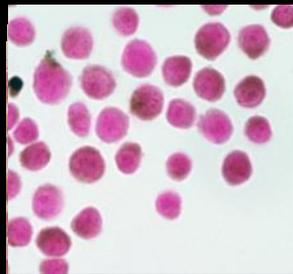
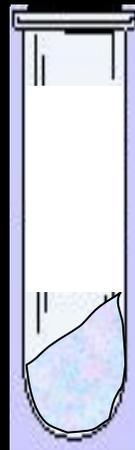
Incubate
2-12 hrs
Incubate
2-4 hrs

Wash x 3



Collect

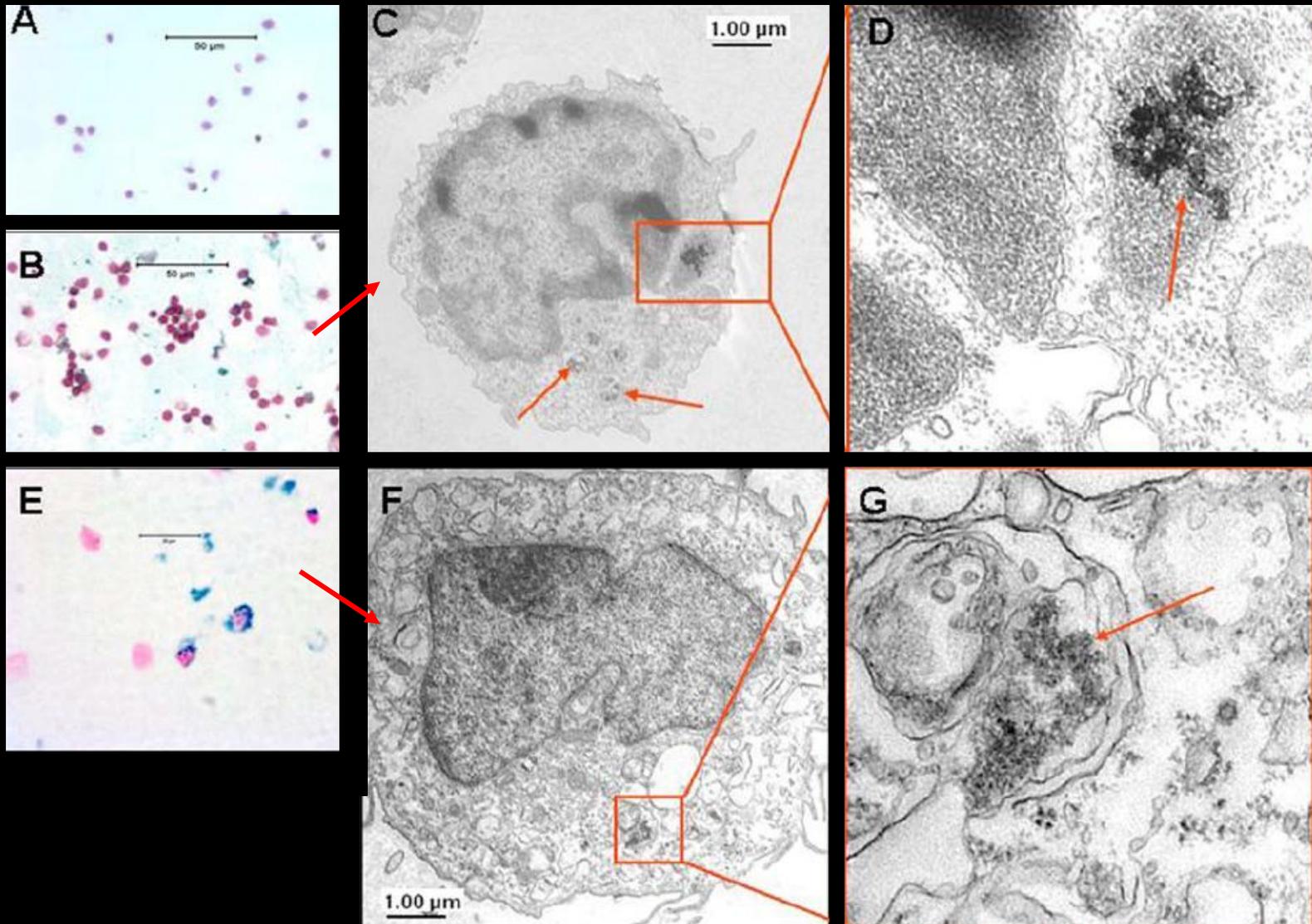
Collect



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

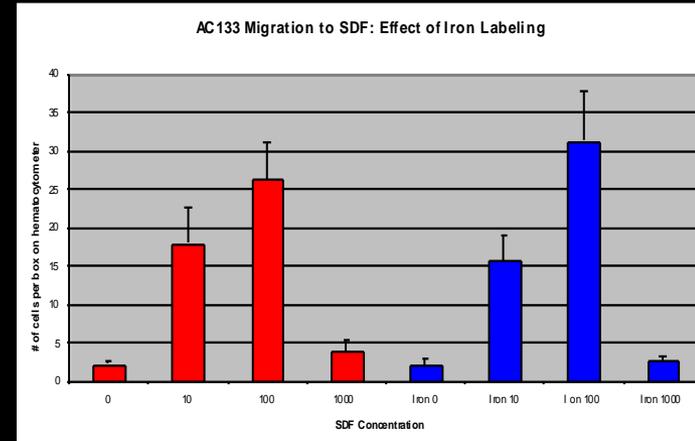
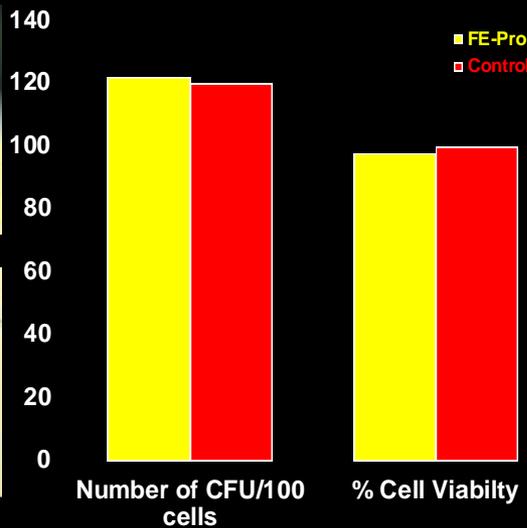
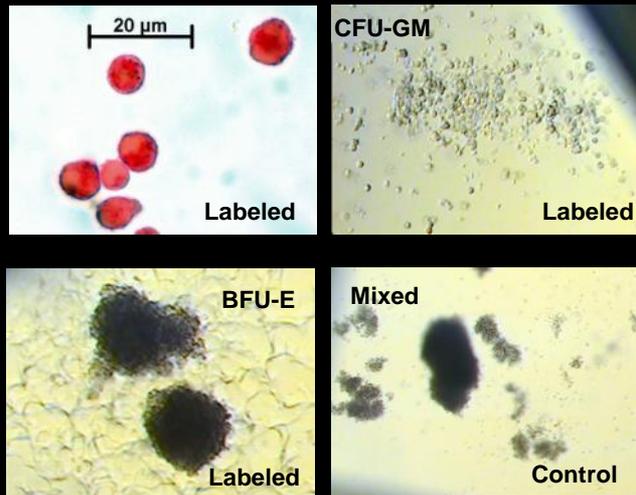


Magnetic Labeling of Stem Cells: How to get from Bench-To-Bedside

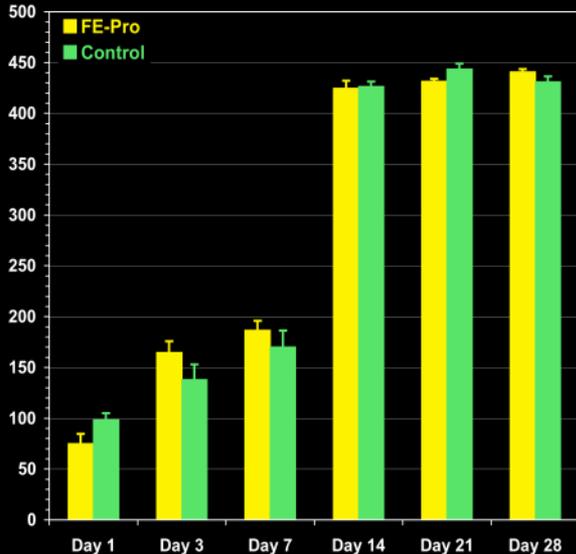
- What is the labeling efficiency of the agent?
- Is the label toxic to cells?
- Does the label alter cellular metabolism or differentiation?
- What happens to the iron in cells?
- Does iv administration of labeled cells alter biochemical or hematological measures?
- Do labeled cells alter morbidity 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

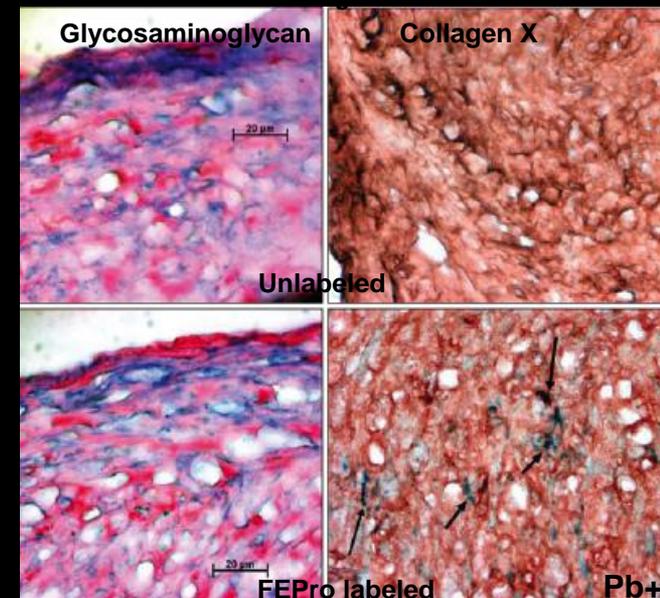
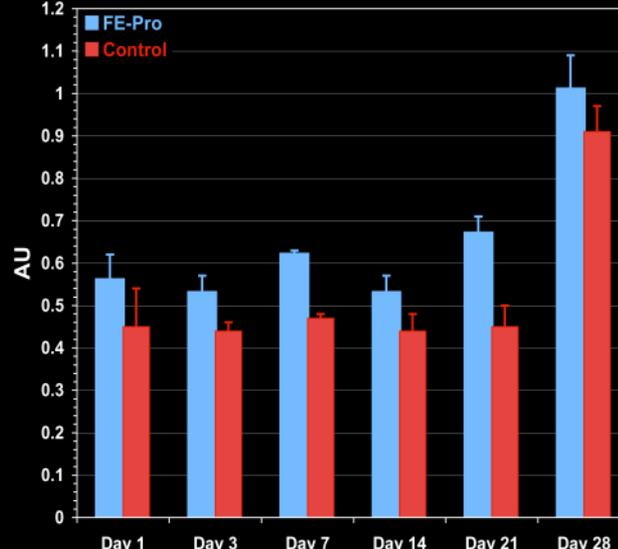
FEPro Labeling is not Toxic nor does it Alter Differentiation or Function of HSCs (CD34) and MSCs



Reactive Oxygen Species FEPro labeled MSC



Proliferation (MTT) FEPro labeled MSC

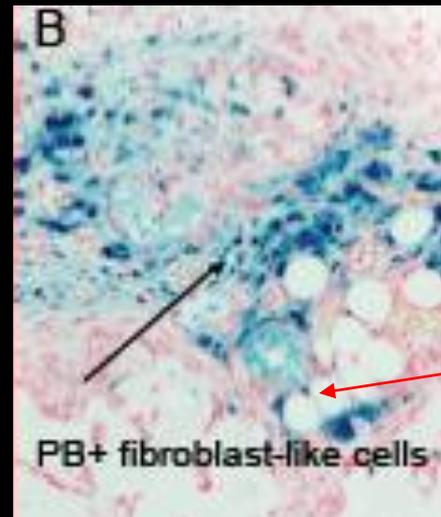
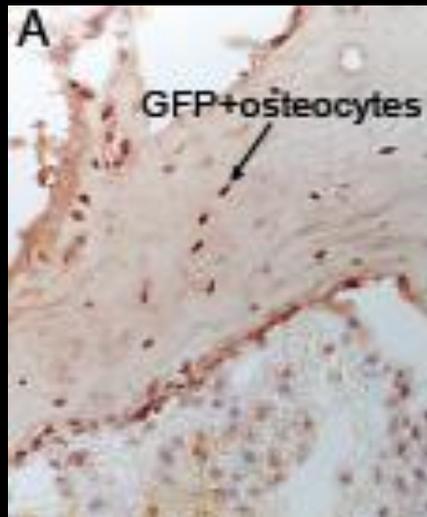
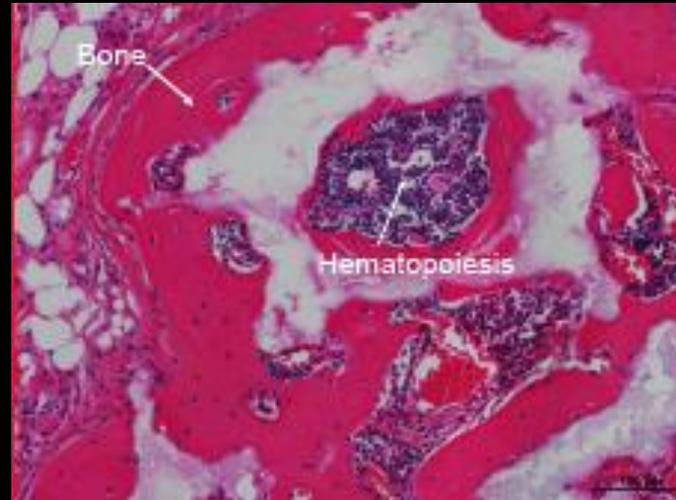
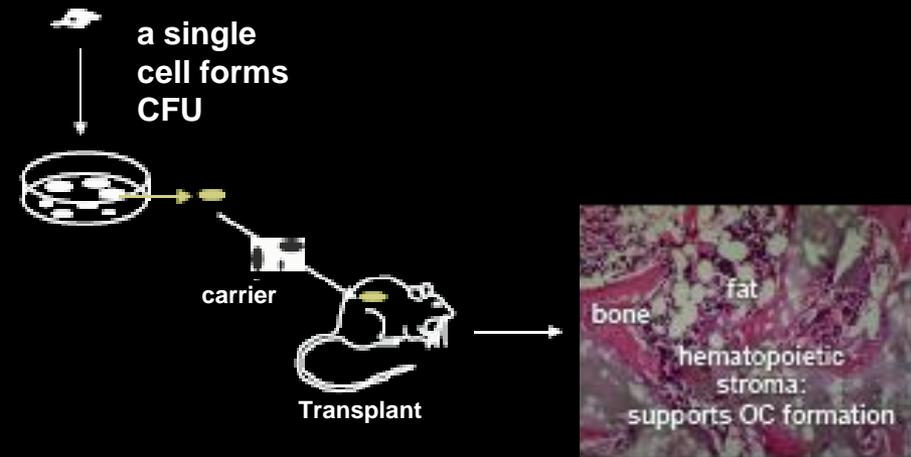


FEPro labeling of BMSC does not alter In Vivo Differentiation or change ability to support hematopoiesis

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

How do you determine stemness+ and potency?

BMSCs support Hematopoiesis

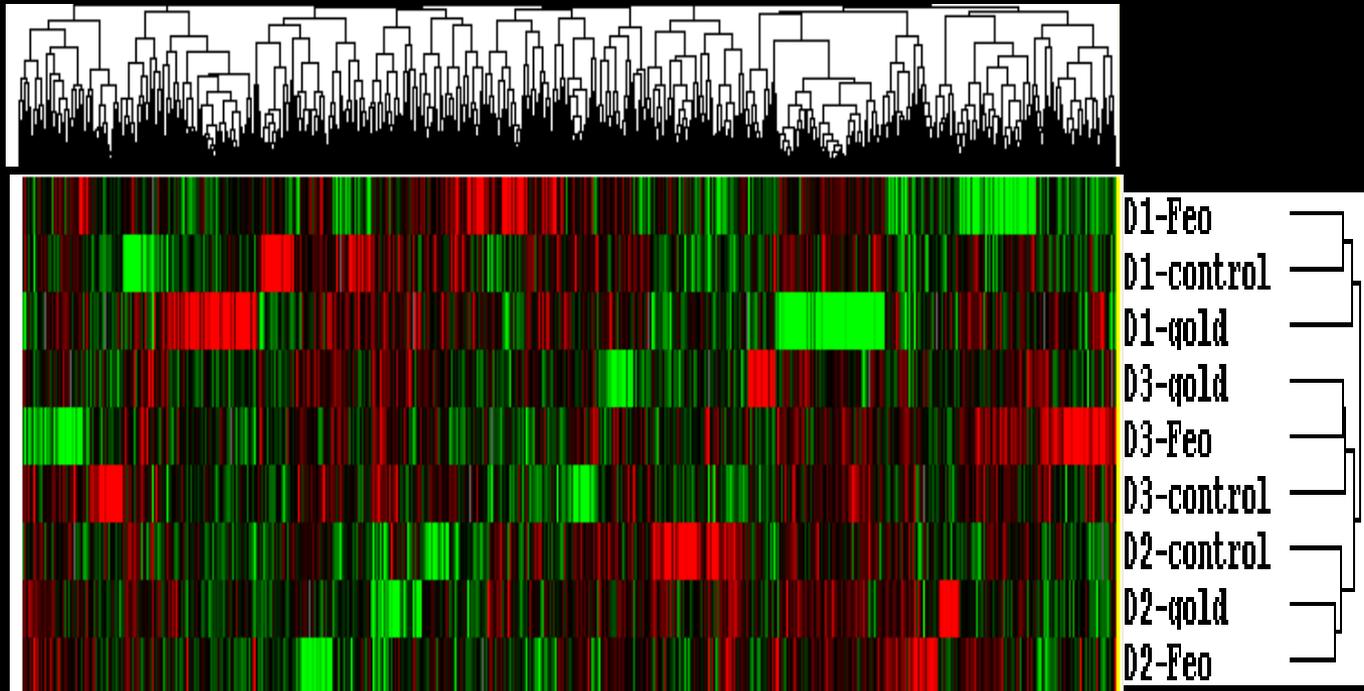


Adipocytes are PB+

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

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

Greater inter-individuals 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*

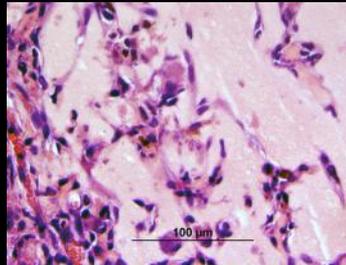
No change in FEPro-labeled BMSCs in genes critical for “stemness” such WNT pathway genes, OCT 4 or NANOG when compared to unlabeled BMSCs.

Matrigel Plug Model of Angiogenesis⁺ and Inflammation[!] in 129/SvImJ mouse

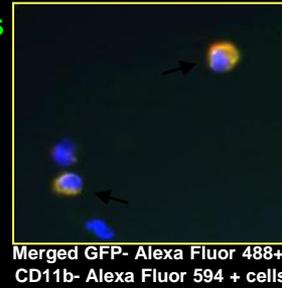
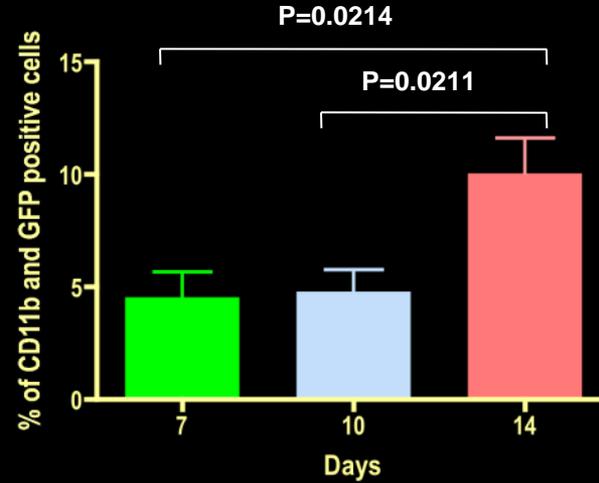
Uptake by Macrophages of BrdU, GFP or FEPro from Labeled BMSC

Pawelczyk E et al PLoS ONE 2009;4:e6712

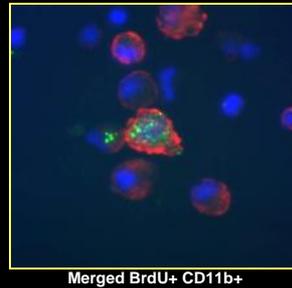
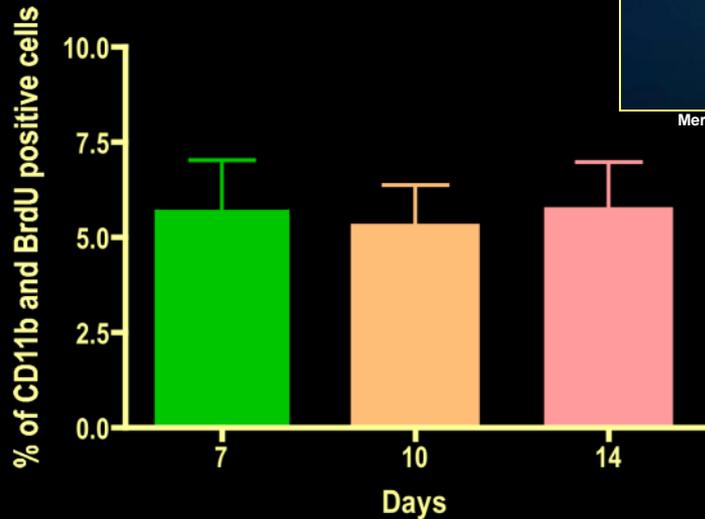
No difference between mouse or human BMSC were used in uptake of label by AM



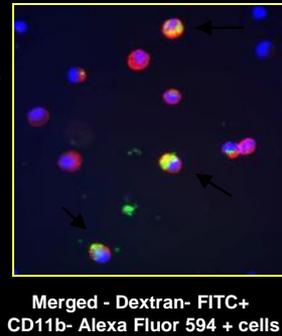
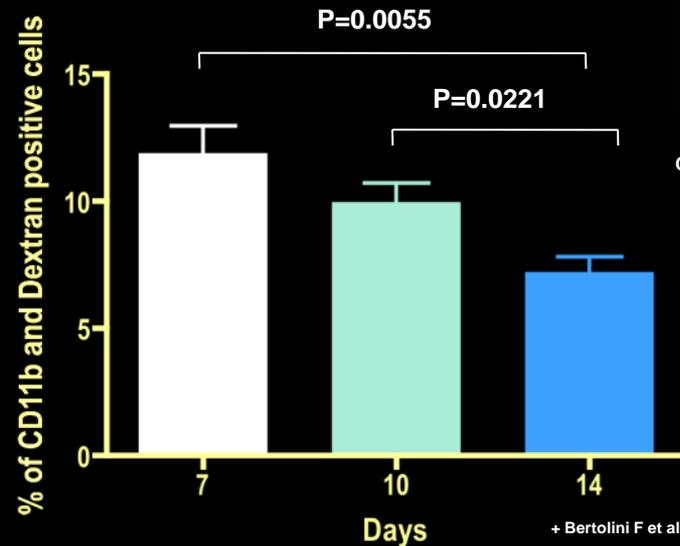
GFP uptake from BMSC by Macrophages



BrdU uptake from BMSC by Macrophages



FEPro uptake from BMSC by Macrophages



Summary of Results of Magnetic Cell Labeling

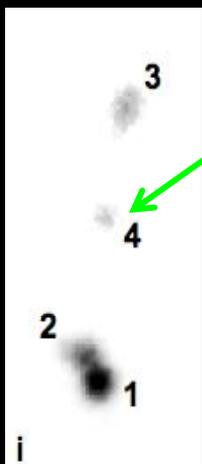
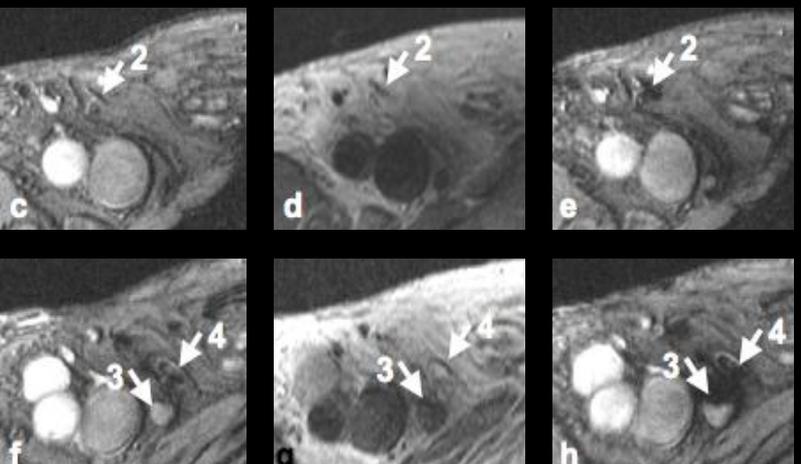
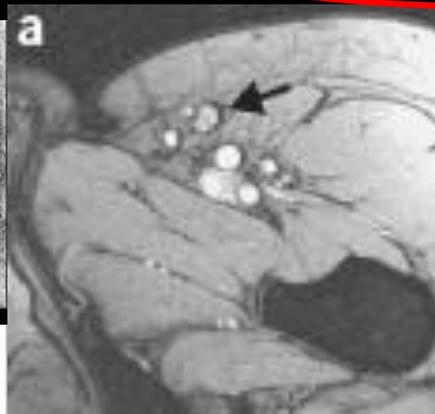
- Iron Oxide Nanoparticle Labeling of Any Type of Cell.
 - **Functional and Differential Capacity is unaltered by SPIO Labeling.**
 - **Labeled Cells contain 1.0 - ≥ 20 picograms of iron/cell (unlabeled cells ≤ 0.1 pg iron).**
- Magnetic Cell Labeling Does Not Alter the Physiological or Metabolic or 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

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

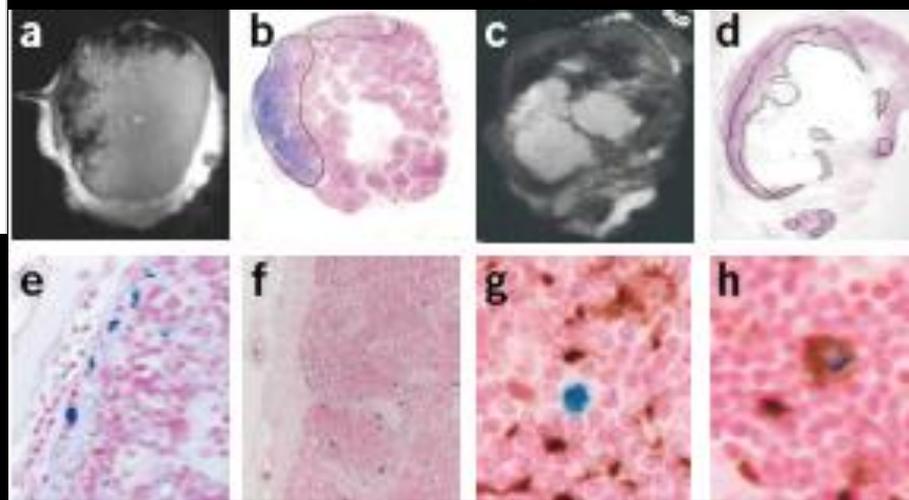
Ferumoxides Labeled Dendritic Cells missed Lymph Node

MRI and Indium¹¹¹oxine SPECT of labeled (1.5x10⁶) Dendritic Cells



MRI can visualize about 500 labeled cells/ voxel

MRI and Prussian blue stain of Lymph Node

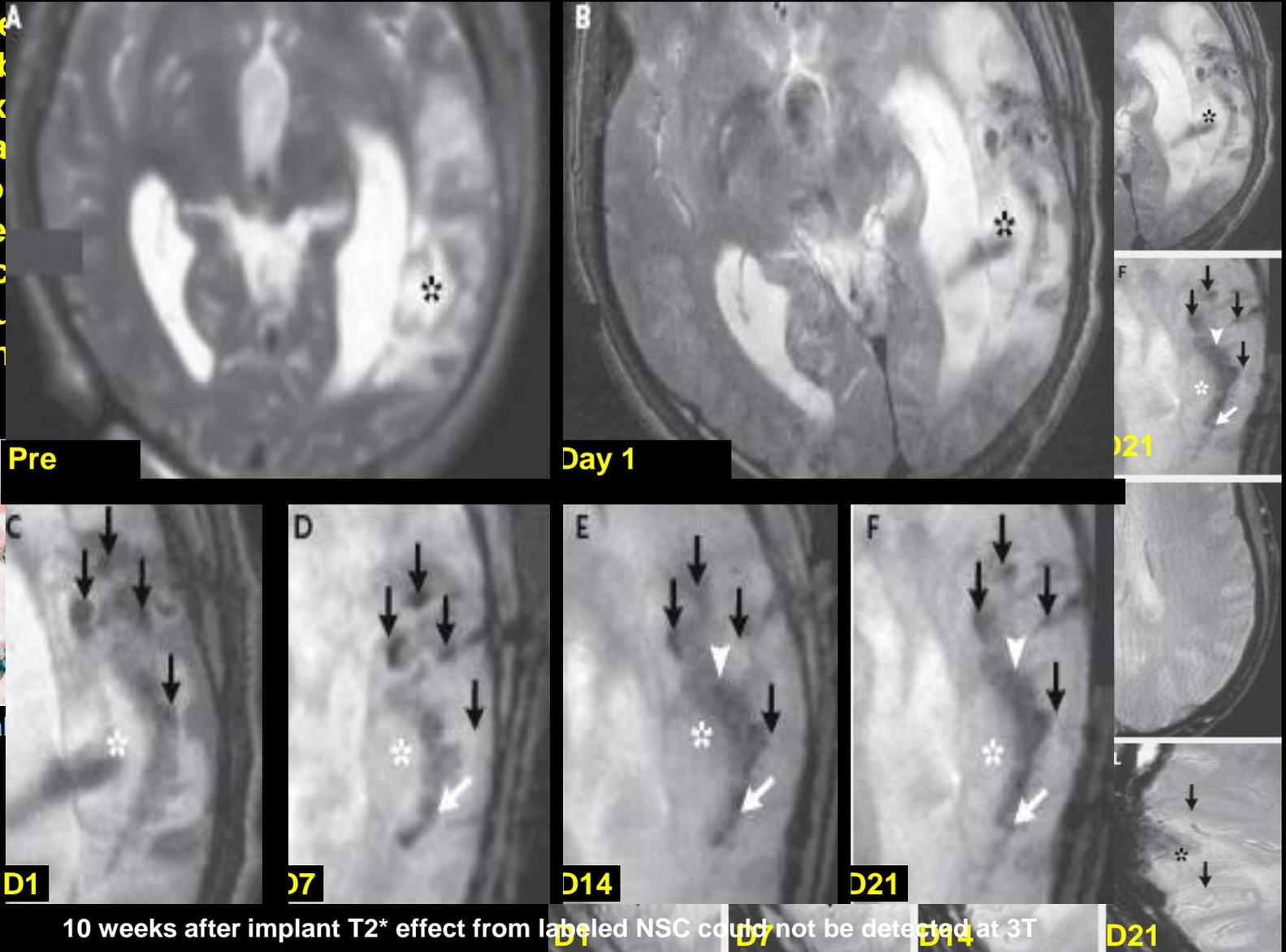
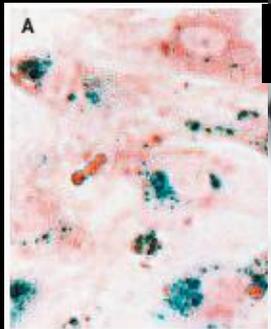


Approximately 150,000 labeled cells

Tracking Neural Stem Cells in Patients with Brain Trauma

Zhu, J et. Al., NEJMED 2006;355:2376-78

34 y.o. Male with open brain surgery, extracranial cultured NSCs were effectively tracked around MRI guidance



MRI of Pancreatic Islets Transplanted Into the Liver in Humans

Saudek, F et al Transplantation 2010;90:1602

35-60x10⁴ Islets Labeled with Ferucarbotran infused in portal vein

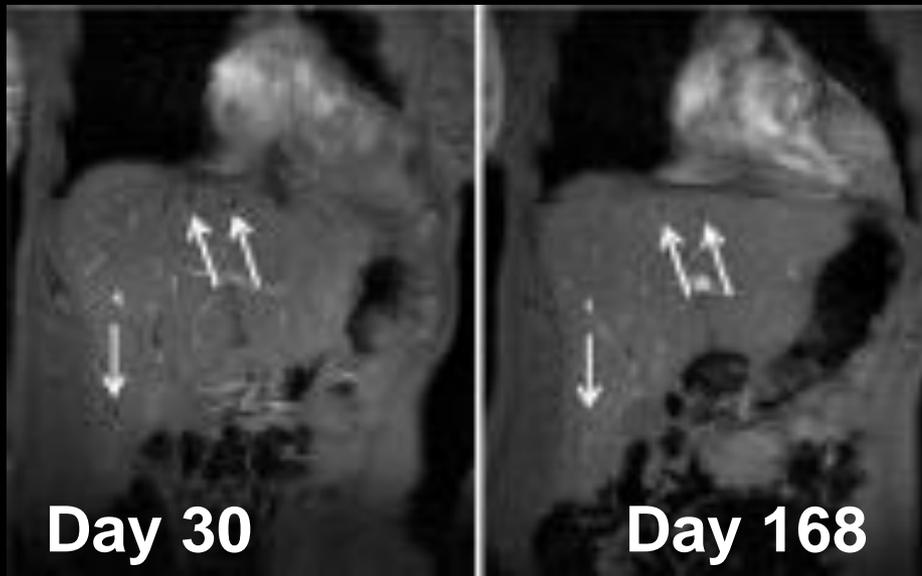
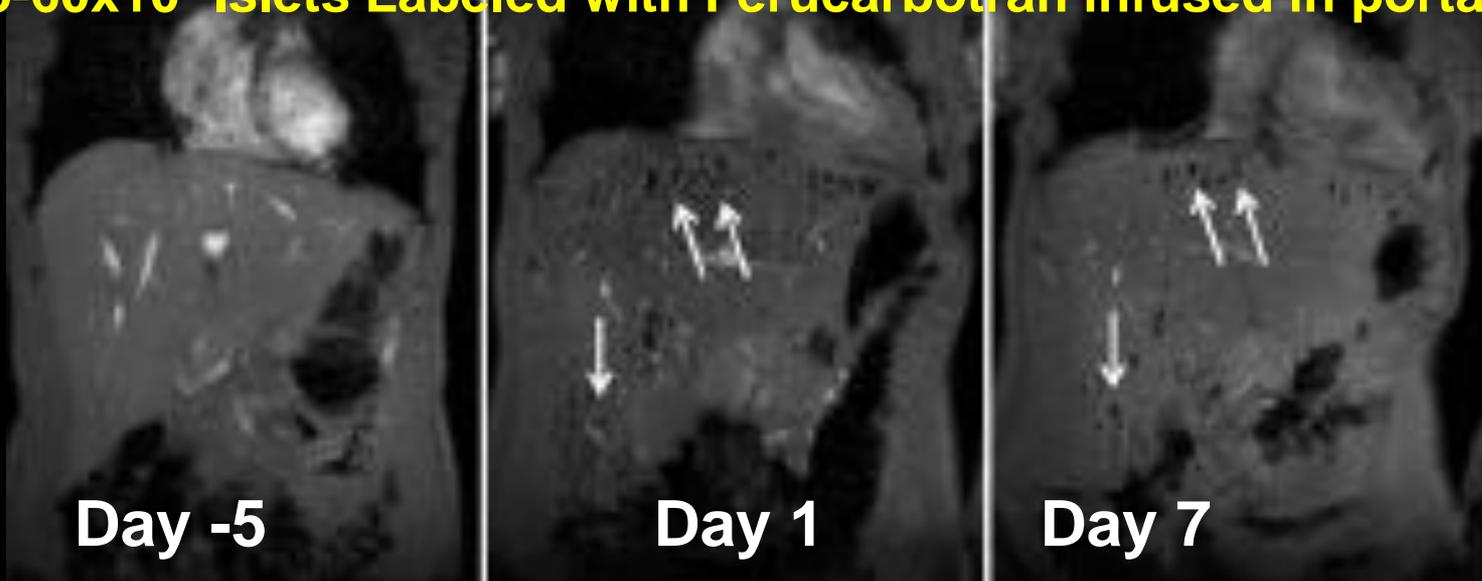


TABLE 2. Decline in regional signal loss (mean±SD in patients 3–6), relative to day 1

Days after the first transplant	1	7	30	168
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

A significant decrease in both number of spots and their area, compared with the initial values, occurred at 7 d after transplantation.

^d $P < 0.01$, d 1 vs. d 7; Wilcoxon paired test.

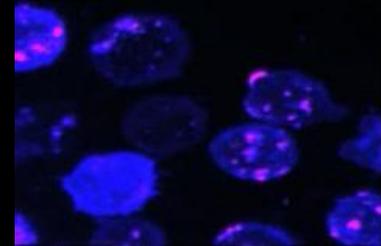
SPIO Nanoparticles for Cell Labeling

- **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
- www.genovis.com
- www.micromed.com
- www.miltenyi.com
- cmir.mgh.harvard.edu/chem/chem_probes
- www.bangslab.com

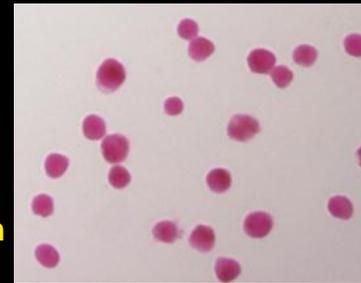




Ferumoxytol ZP = -49mv, ~ 33nm
LD50 420mg/kg in rat
Heparin ZP = -60mv, 18Kd
LD50 5000mg/ml in mouse
Protamine ZP = 7.2mv, 4.6Kd
LD 50 100mg/kg in mouse
ZP = zeta potential 37°C in water
pH = 7



Incubate
2-4 hrs in Serum
Free Media



50% HeProFera + 50% Media



Incubate
4-12 hrs

Wash x 3

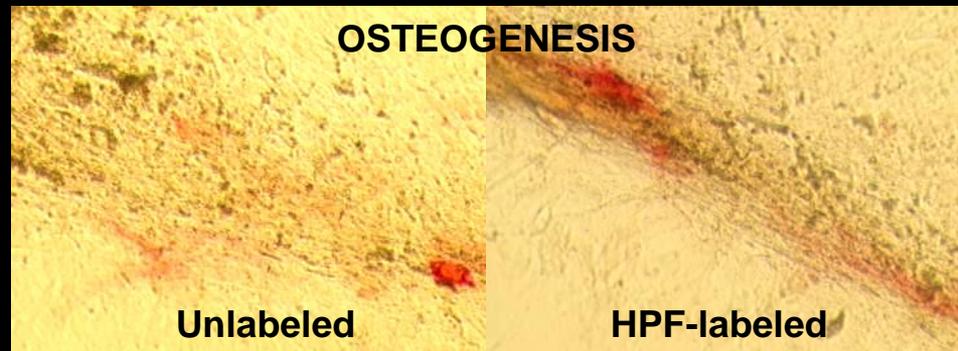
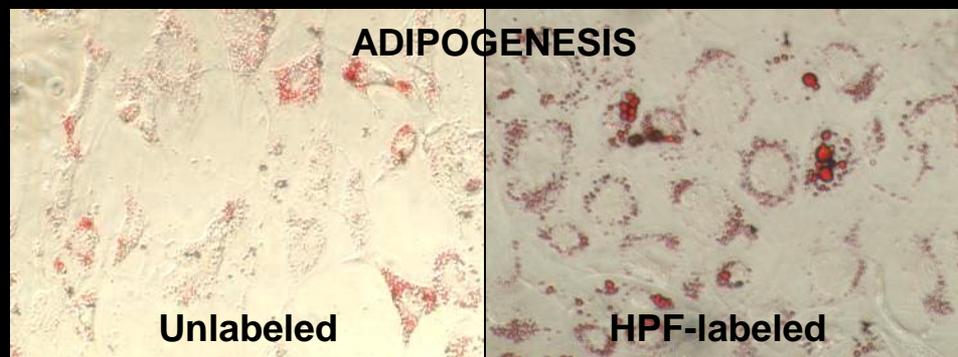
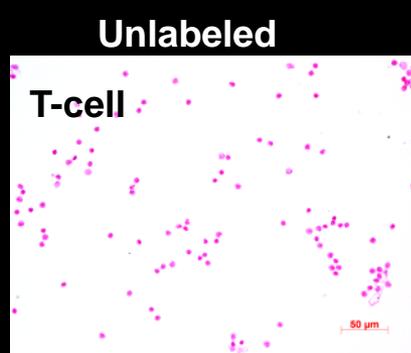
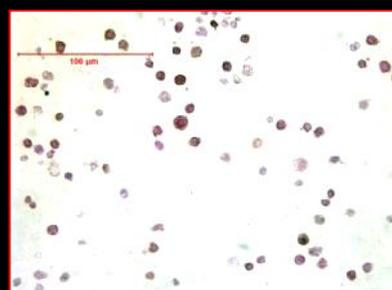
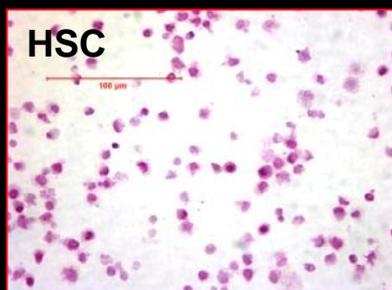
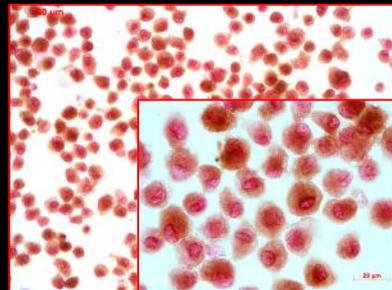
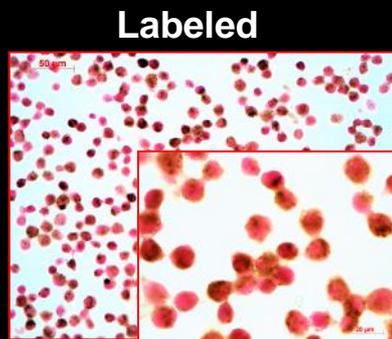
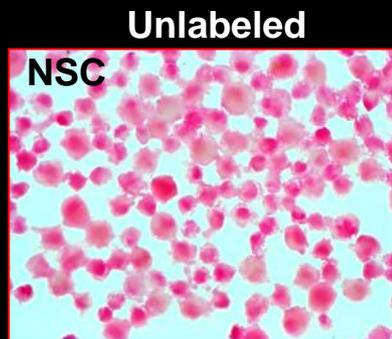
PBS &
Heparin

Collect

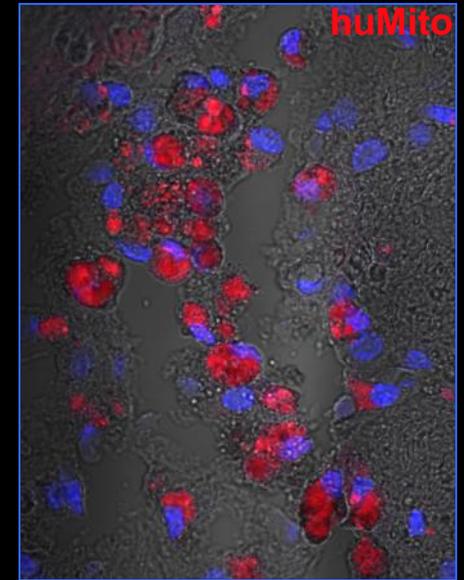
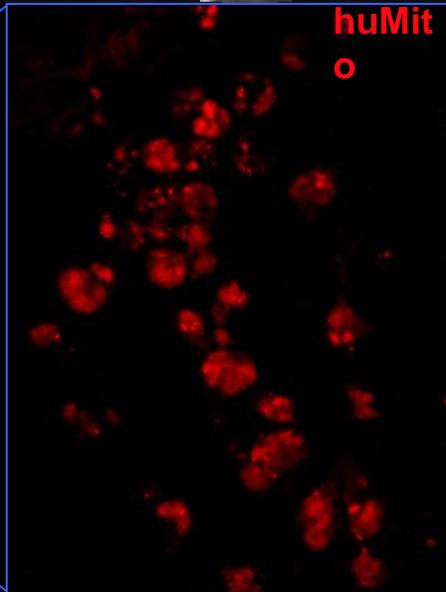
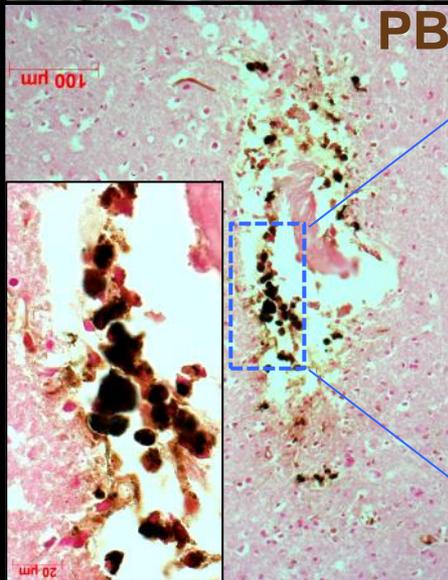
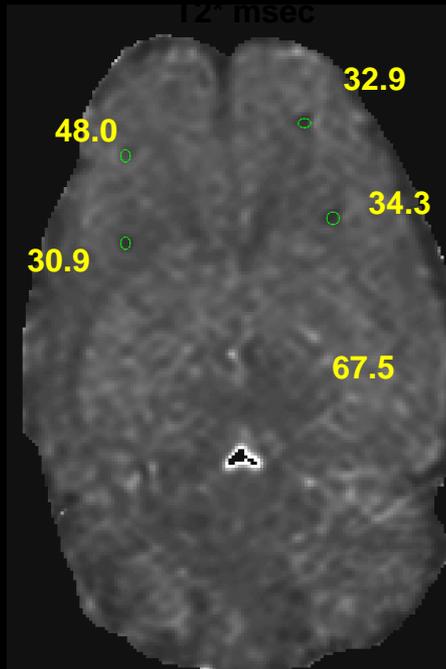
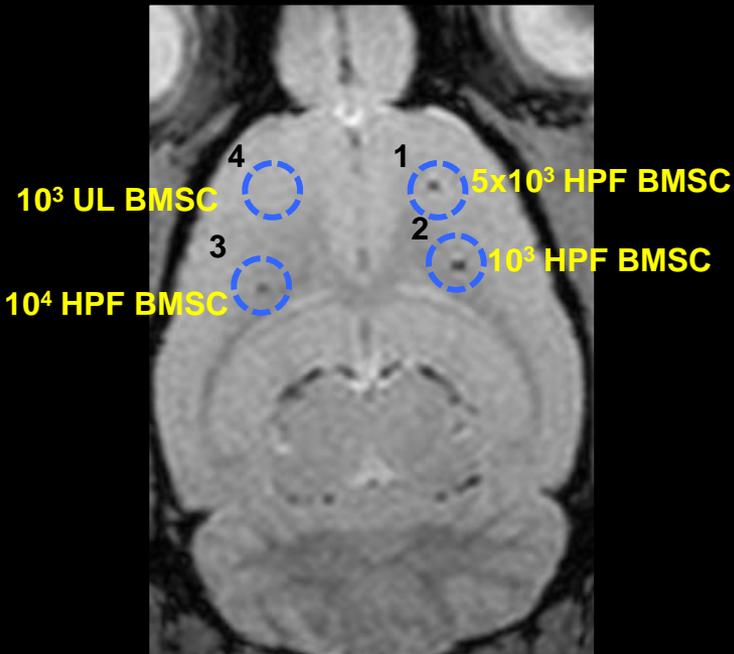


3 FDA approved agents
Heparin, Protamine &
Ferumoxytol
Self Assemble to
form complex that can
be used to magnetically
label cells

Prussian blue (DAB) stains HPF labeled or unlabeled Cells



T2*w MRI at 3T of Rat with implanted HPF labeled BMSC

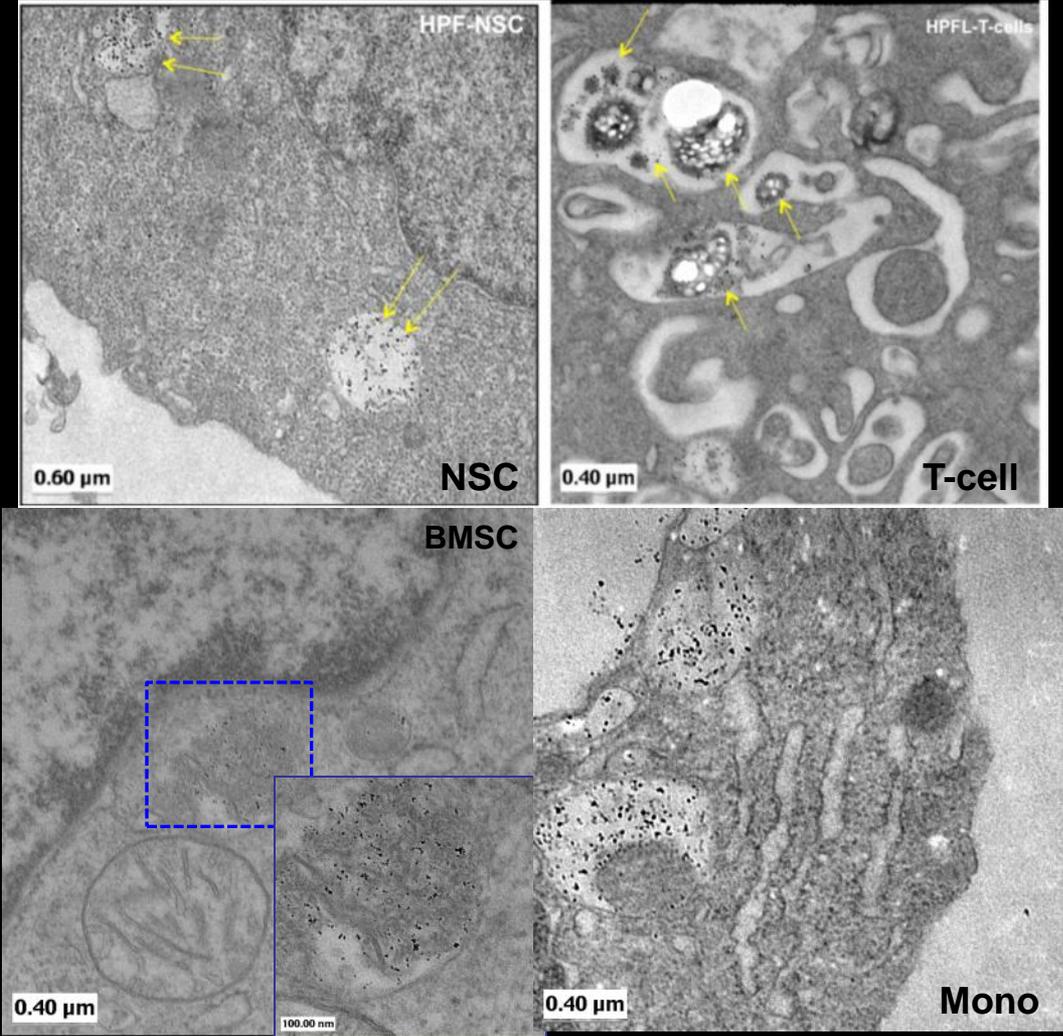


Monitoring Cellular Therapy: The Role of Imaging

- **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

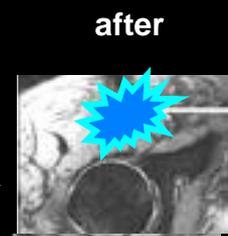
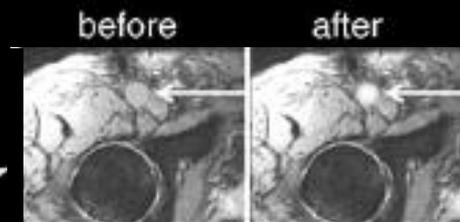
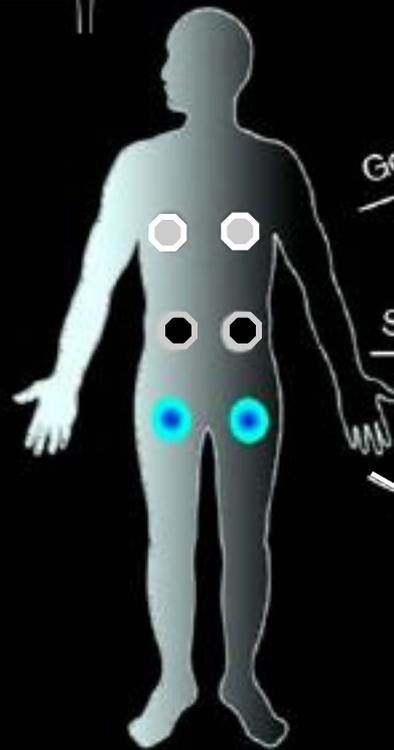


MRI for Quantitative in vivo cell tracking

Srinivas M et al, Trends Biotechnol. 2010 Jul;28(7):363-70

Labeled therapeutic cells

In vivo MRI



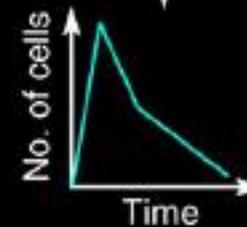
Sensitivity

$\sim 10^4$

$1-5 \times 10^2$

$1-5 \times 10^5$

^1H – anatomy
 ^1H – cells (Gd)
 ^1H – cells (SPIO)
 ^{19}F – cells

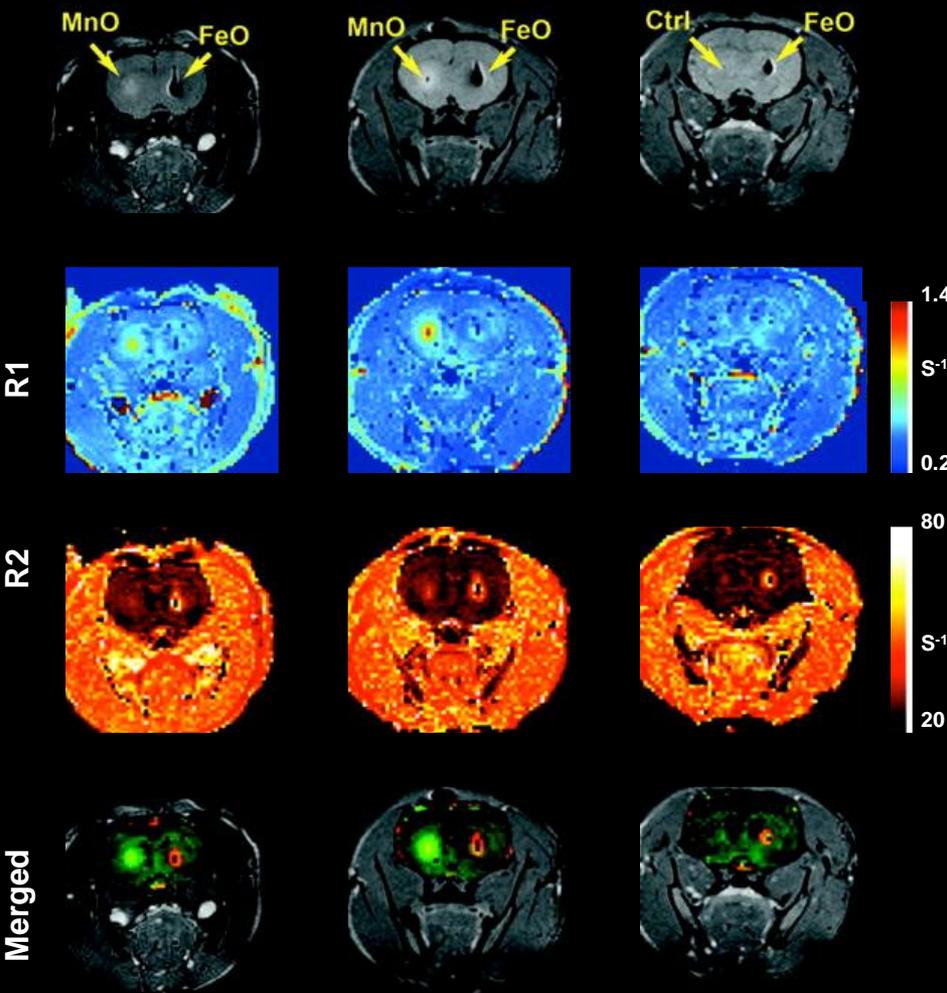


Magnitude Dilution of mask
 Interactive image
 Masked Image select ROI (>99% CI)

MR Tracking of Transplanted Cells with "Positive Contrast using Manganese Oxide Nanoparticles

Gilad AA et al MRM 2008;60:1-7

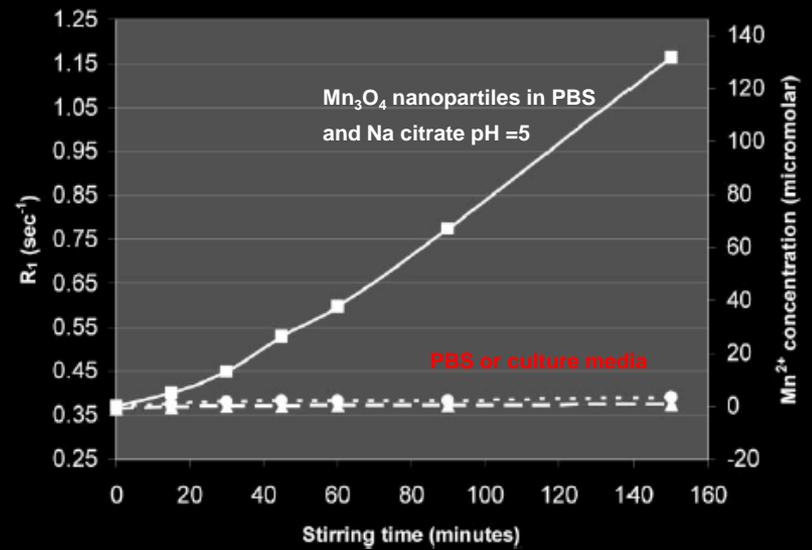
2x10⁵ 9L Glioma Cells 9.4T MRI performed 24hr after injection



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

Convertible Manganese Contrast for Molecular and Cellular MRI

Shapiro EM and Koretsky A MRM 2008;60:265-9



T1 maps rat brains

