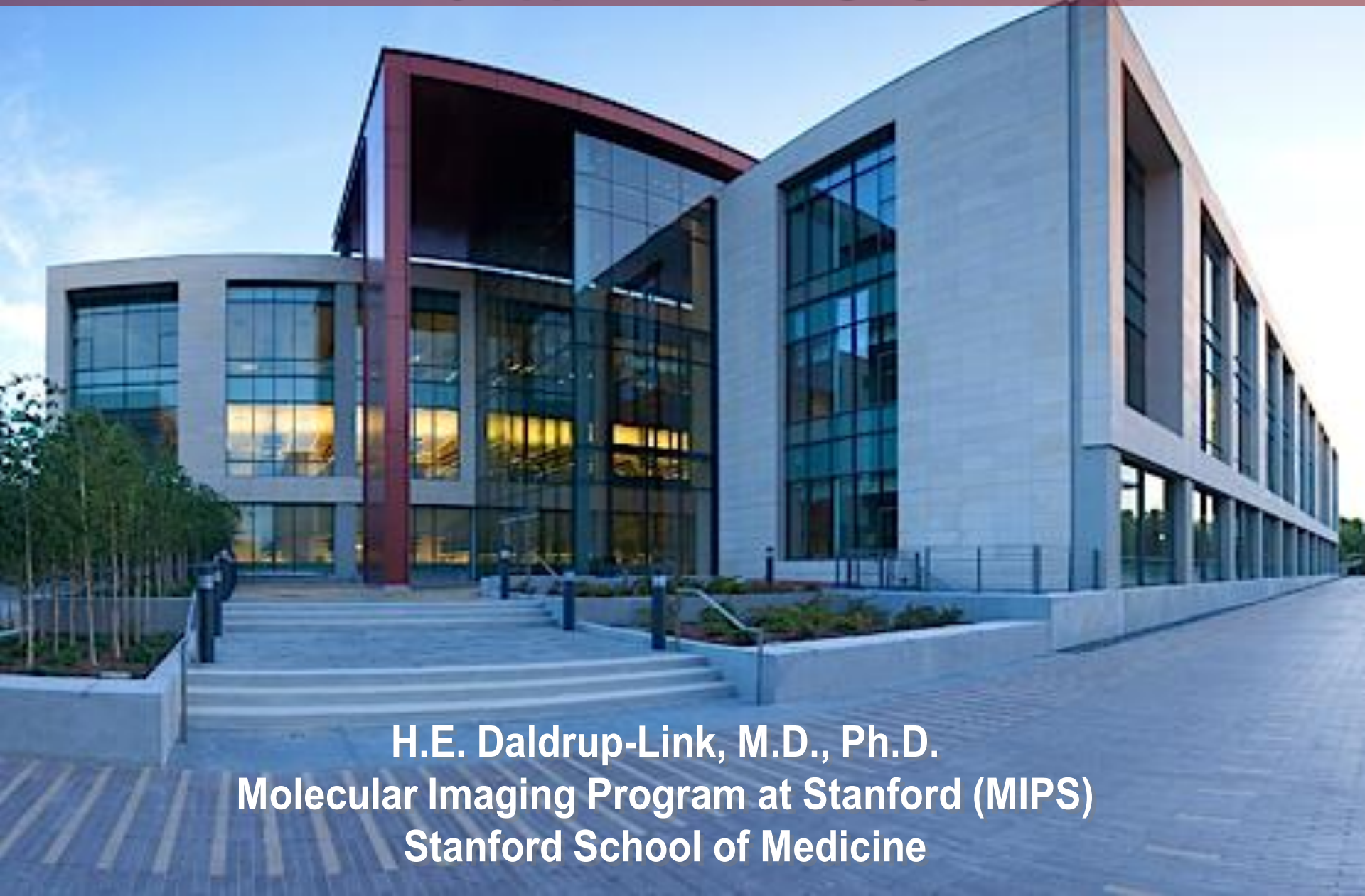


# Assessing Immune Responses of Stem Cell Transplants with clinically applicable Imaging Techniques



**H.E. Daldrup-Link, M.D., Ph.D.  
Molecular Imaging Program at Stanford (MIPS)  
Stanford School of Medicine**

# Imaging Stem Cell Transplants: Assessment of Immune Responses



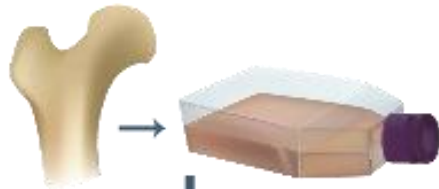
Cell Isolation

Cell Expansion

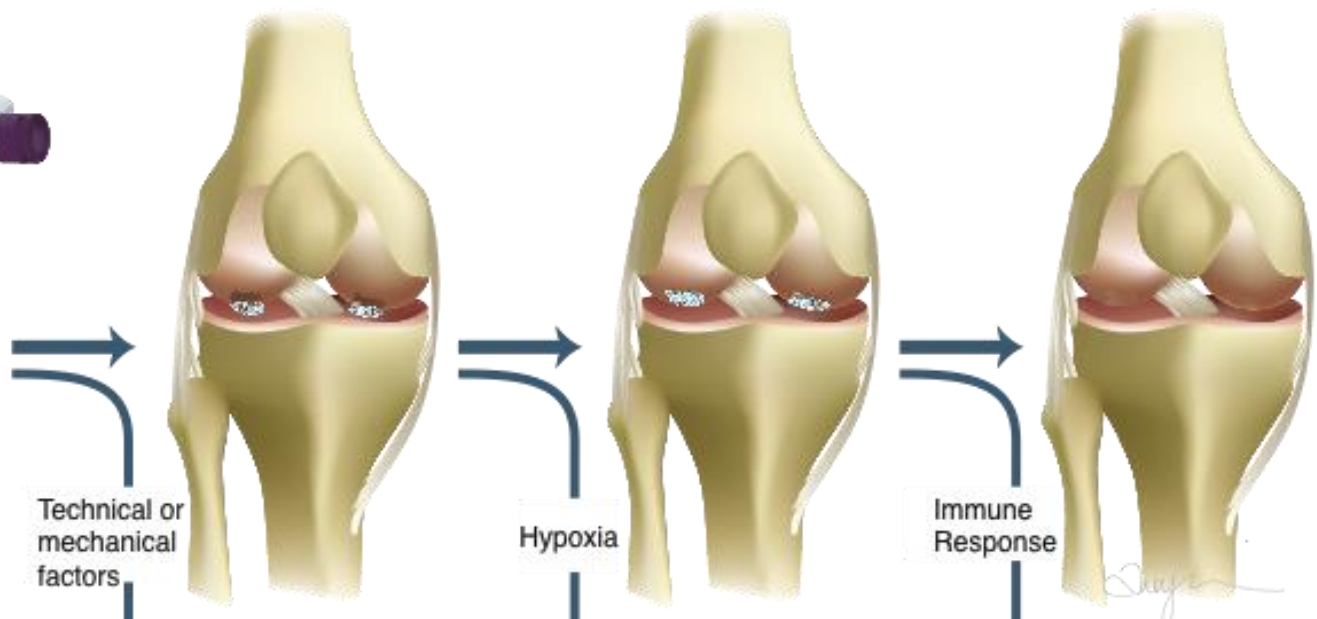
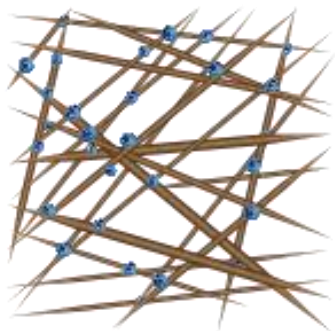
Intra-articular Transplantation

Engraftment

Cartilage Regeneration



Cell Seeded Scaffold



Possible Complications

Cell Loss

Cell Apoptosis

Cell Phagocytosis

Imaging Approaches



Stem Cell Tracking



Detection of  
stem cell apoptosis



Immune Cell Tracking



# Imaging Stem Cell Transplants: Assessment of Immune Responses

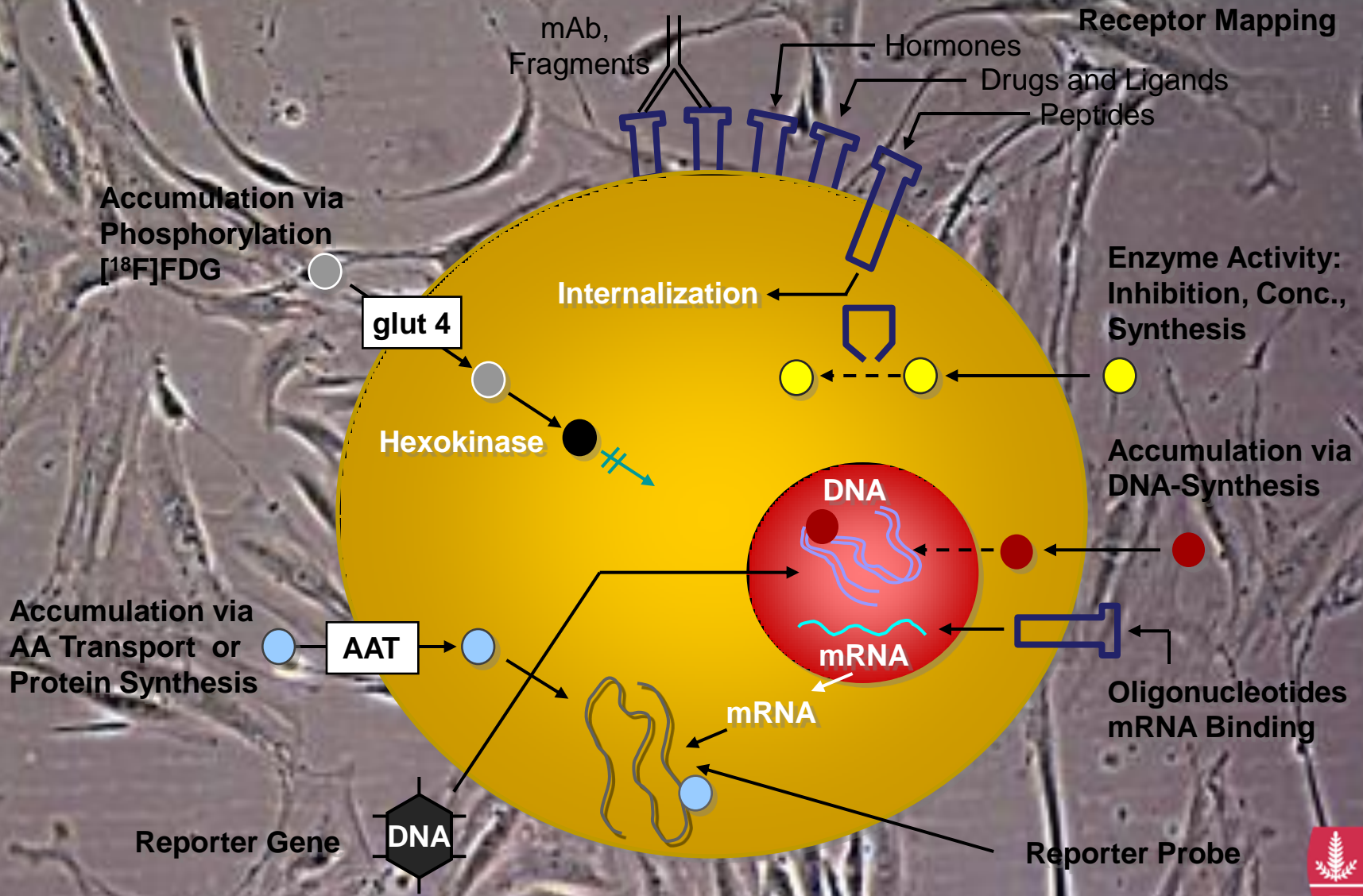


- Repeatedly image the location(s) and numbers of one or more specific population(s) of cells *in vivo*
- Repeatedly image the “status” of stem cells and their progenies *in vivo*, including potential interactions with host cells, such as immune cells
- Do not significantly perturb the cells while repeatedly imaging them
- Allow flexibility to scale across different spatial resolution levels



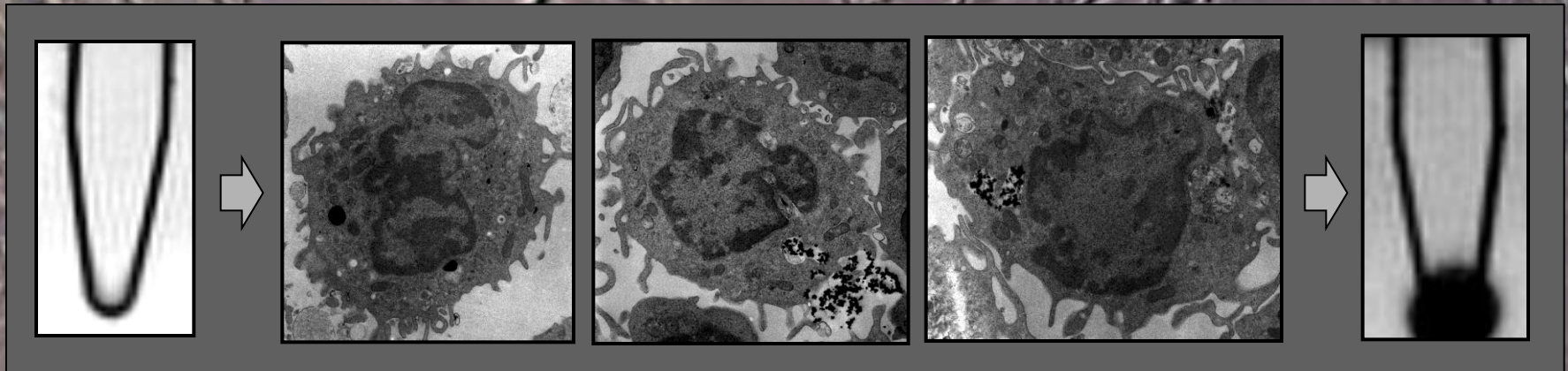


# Cellular Imaging Targets / Probes





# Imaging Stem Cell Transplants: Assessment of Immune Responses



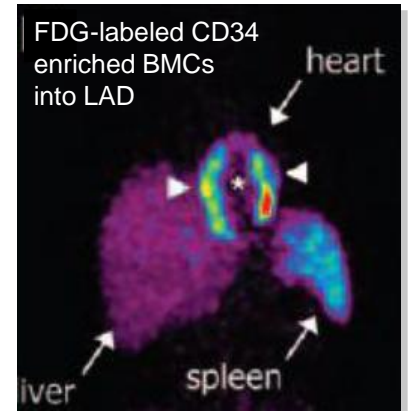
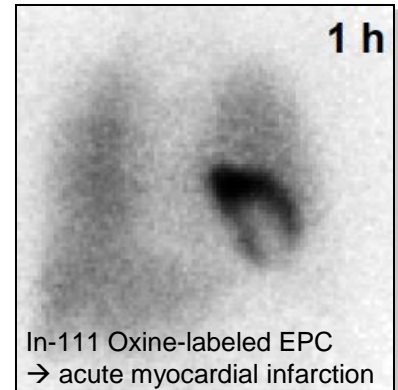
1. *In vivo* tracking of transplanted stem cells
2. *In vivo* tracking of immune cells
3. *In vivo* imaging of tissue regeneration

# Clinically applicable labels for *in vivo* tracking of stem cells



## SPECT and PET Imaging

- **In-111 Oxine (half-life 2.8 days)**
- **Tc99m HMPAO (half life 6 h)**
- **[18F]-FDG (half life 2 h)**
- **[18F]-Fluoro-3-(hydroxymethyl)butyl]guanine**



# Clinically applicable labels for *in vivo* tracking of stem cells

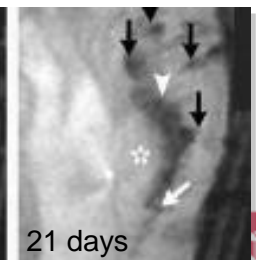
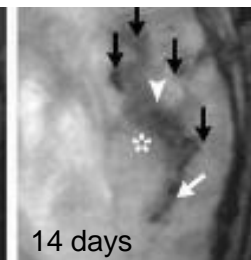
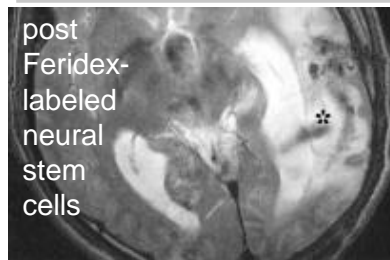
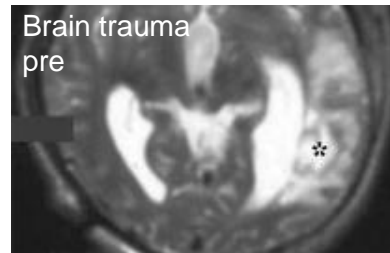


## SPECT and PET Imaging

- In-111 Oxine (half-life 2.8 days)
- Tc99m HMPAO (half life 6 h)
- [18F]-FDG (half life 2 h)
- [18F]-Fluoro-3-(hydroxymethyl)butyl]guanine

## MR Imaging

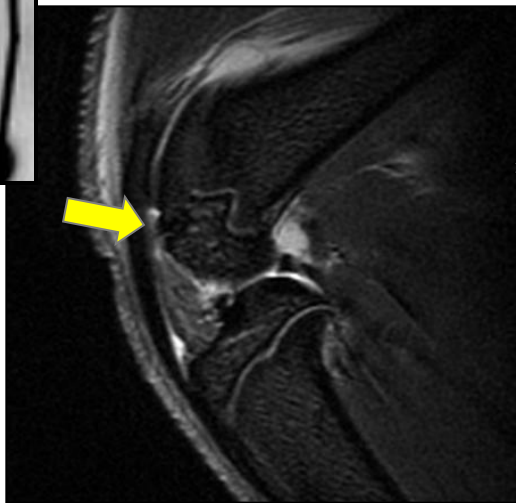
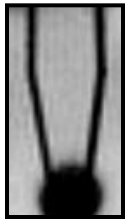
- Gd-Chelates
- Iron Oxide Nanoparticles



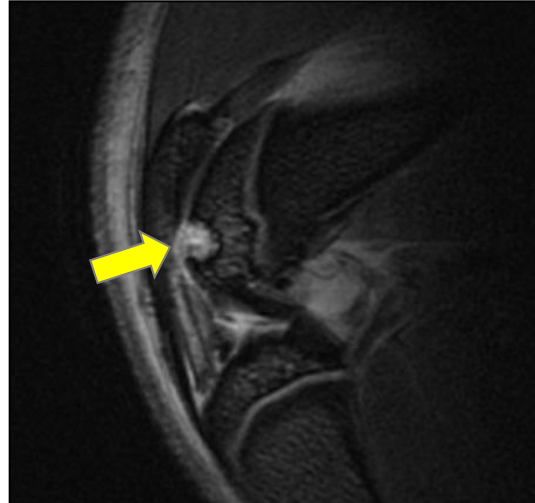
# Ferumoxytol (Feraheme™)



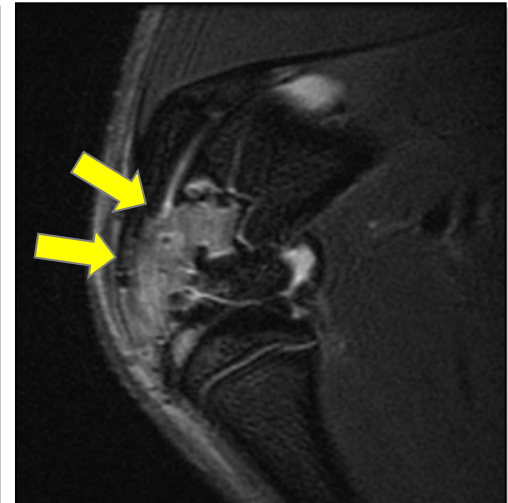
- FDA approved iron supplement
- Mean hydrodynamic diameter: 30nm
- Non-ionic carboxhydrate coating of polyglucose sorbital carboxymethylether
- $r_1$ :  $15 \text{ mM}^{-1} \text{ sec}^{-1}$
- $r_2$ :  $89 \text{ mM}^{-1} \text{ sec}^{-1}$  at 1.5 Tesla and  $37^\circ\text{C}^2$



Labeled Stem Cells



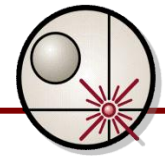
Stem Cell Loss



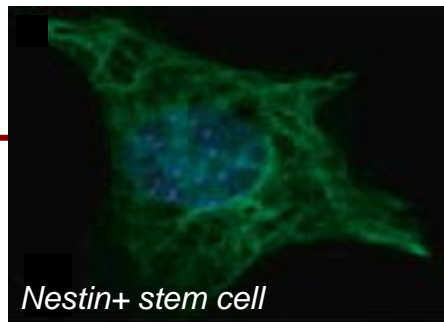
Stem Cell Hypertrophy



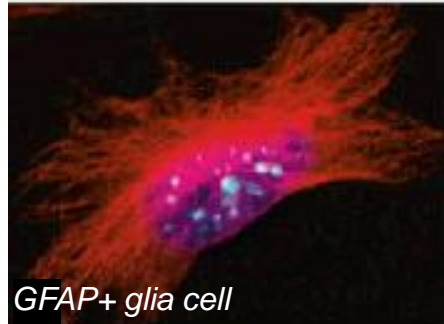




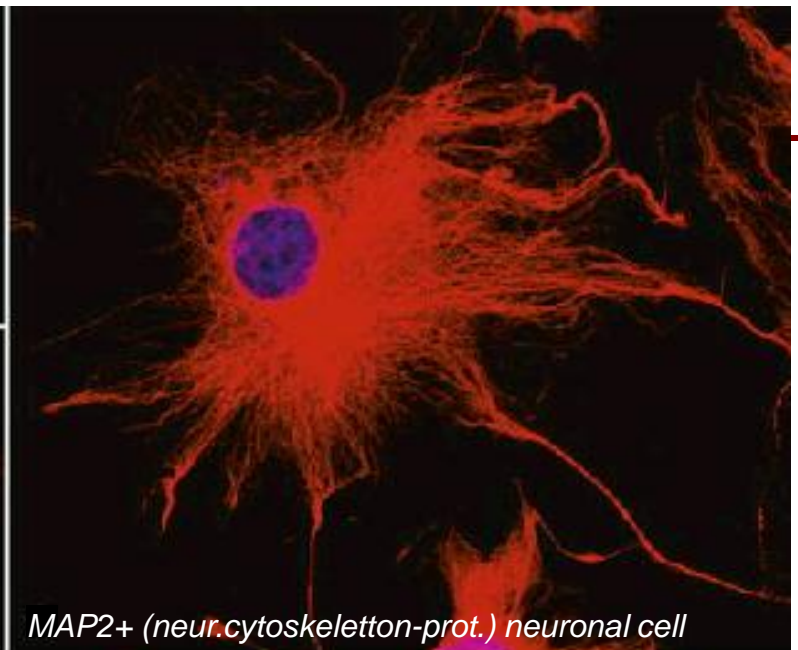
*Normal  
differentiation  
of CM-labeled  
stem cells*



*Nestin+ stem cell*

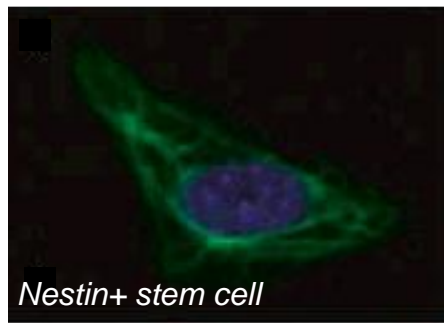


*GFAP+ glia cell*

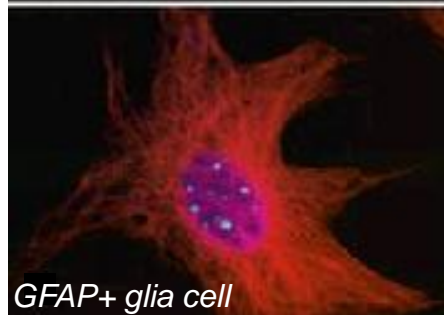


*MAP2+ (neur.cytoskeleton-prot.) neuronal cell*

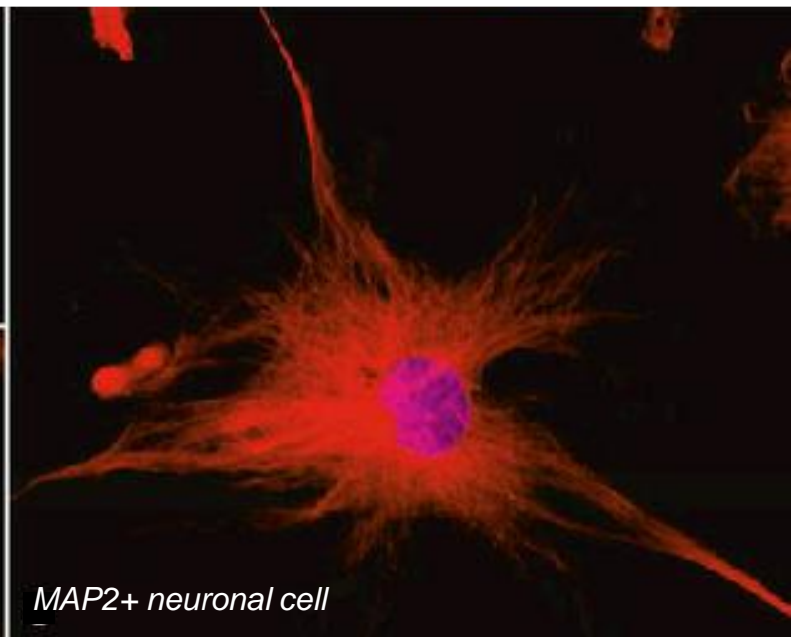
*Normal  
differentiation  
of unlabeled  
controls*



*Nestin+ stem cell*



*GFAP+ glia cell*



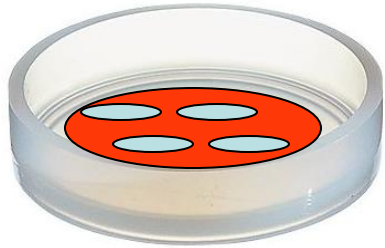
*MAP2+ neuronal cell*



# Differentiation of hESCs into cardiomyocytes



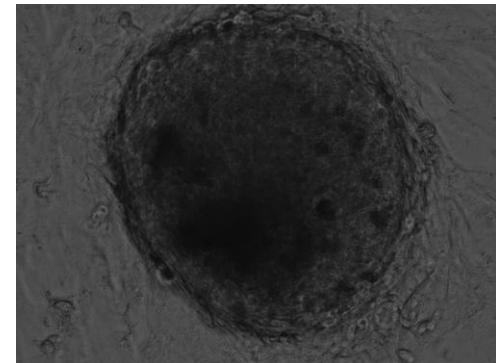
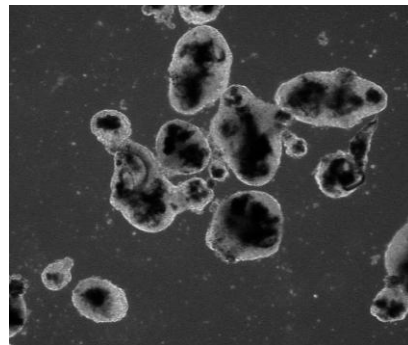
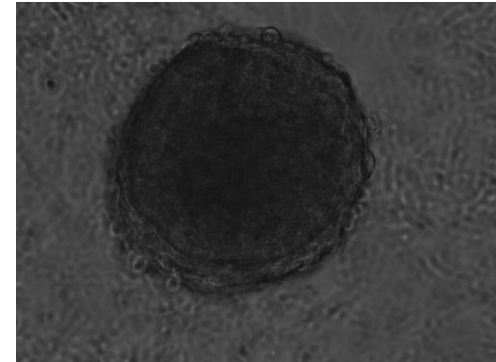
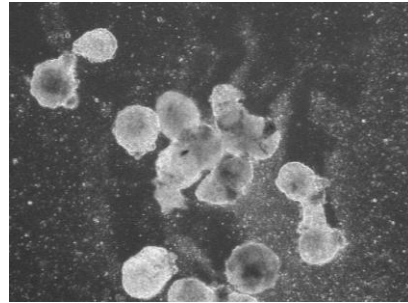
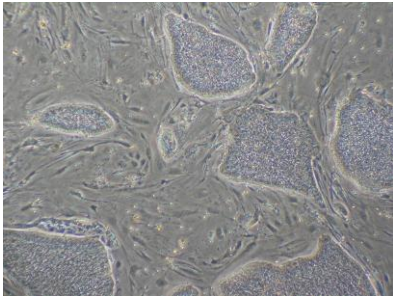
hESCs



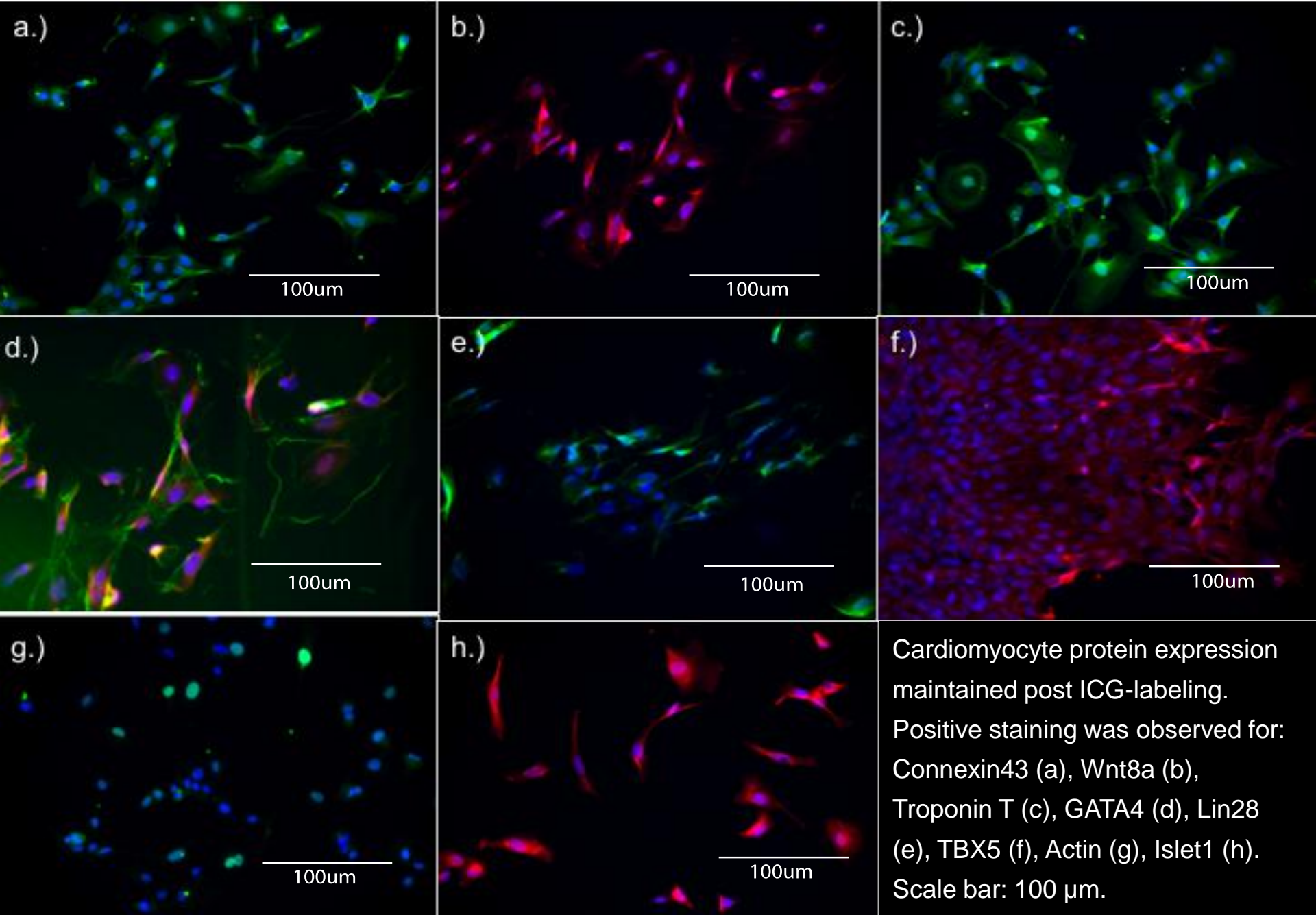
Embryoid Bodies



Differentiation into cardiomyocytes

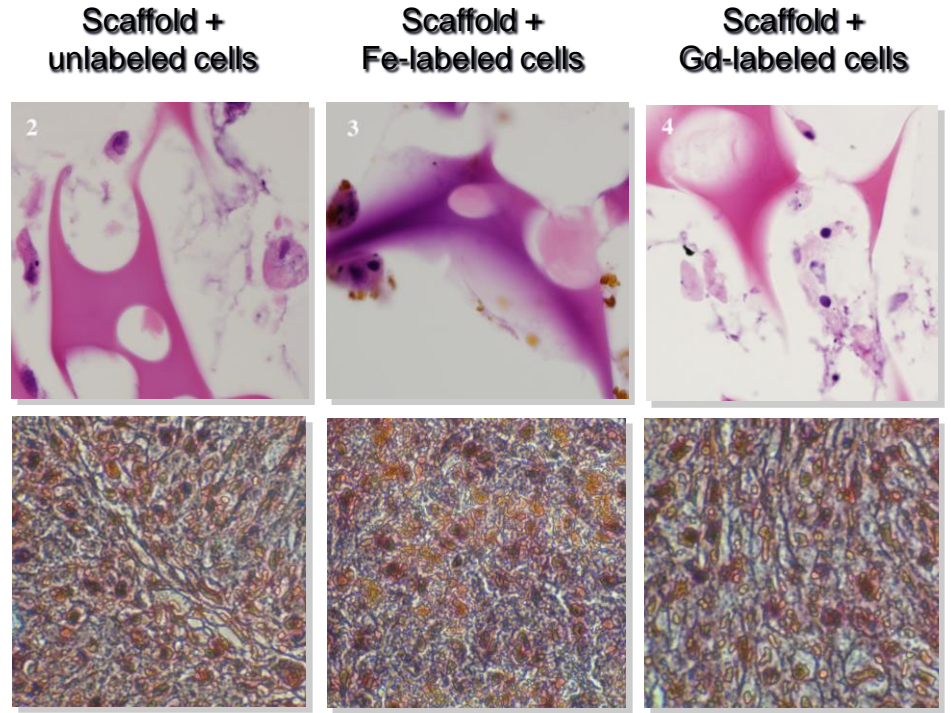
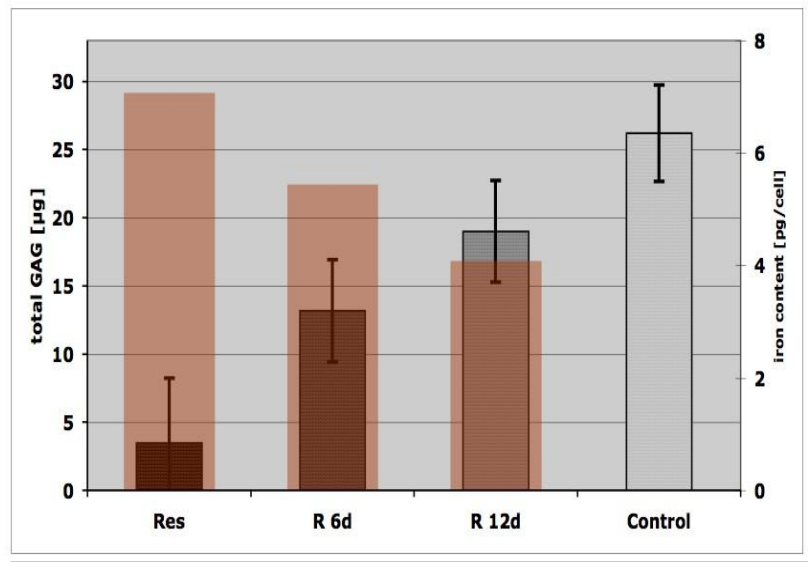








# Chondrogenic differentiation of Fe-labeled *hMSC*

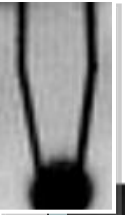






# In vivo diagnosis of stem cell proliferation vs apoptosis

## Matrix associated stem cell implants in osteochondral defects

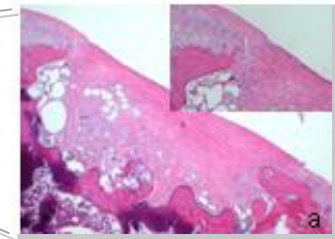
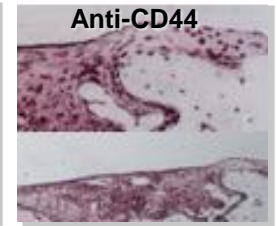
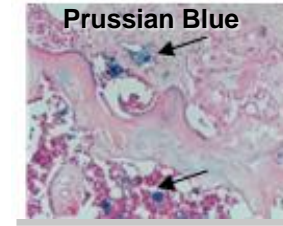
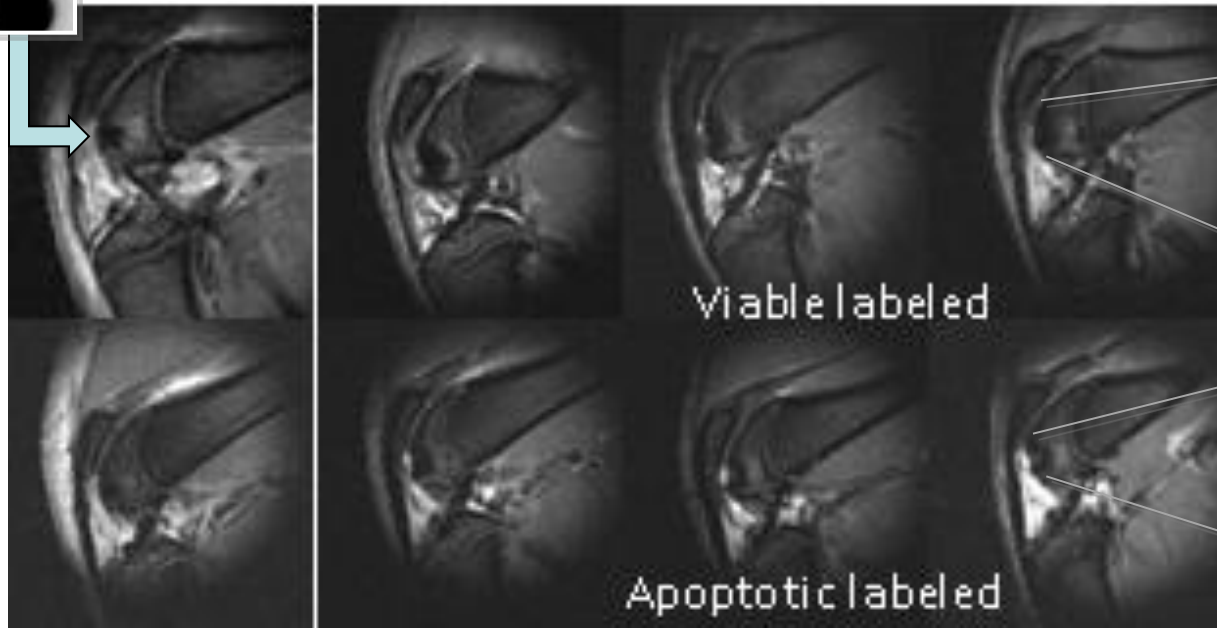


Day 0

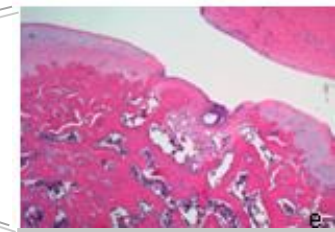
Week 2

Week 4

Week 8



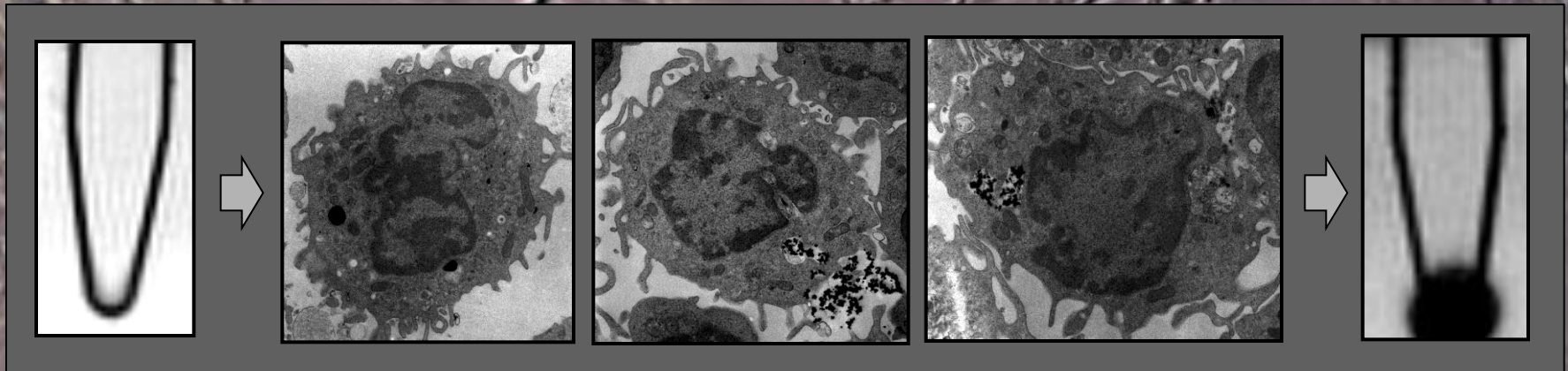
VIABLE



APOPTOTIC



# Imaging Stem Cell Transplants: Assessment of Immune Responses



1. *In vivo* tracking of transplanted stem cells
2. *In vivo* tracking of immune cells
3. *In vivo* imaging of tissue regeneration



# Clinically applicable labels for *in vivo* tracking of leukocytes

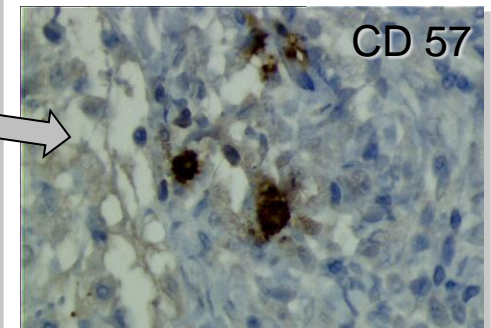
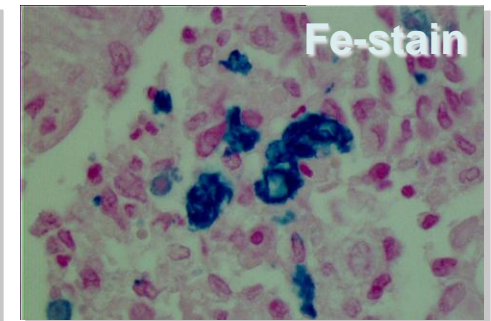
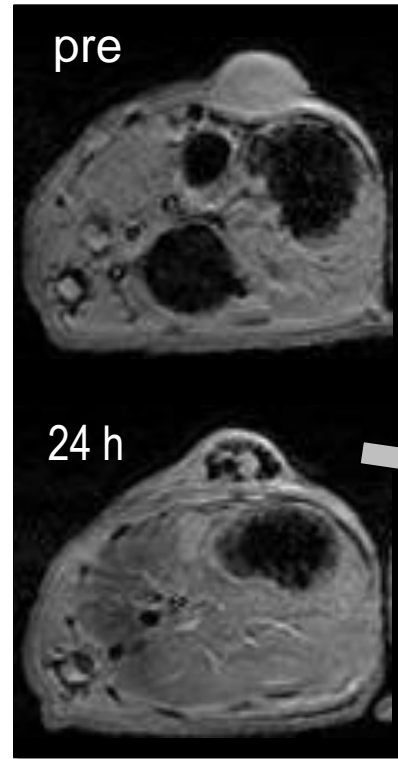
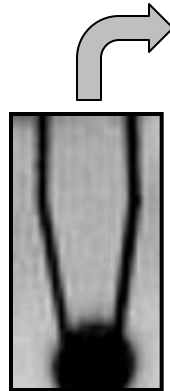


## SPECT and PET Imaging

- In-111 Oxine (half-life 2.8 days)
- Tc99m HMPAO (half life 6 h)
- [18F]-FDG (half life 2 h)
- [18F]-FHBG

## MR Imaging

- Gd-Chelates
- Iron Oxide Nanoparticles

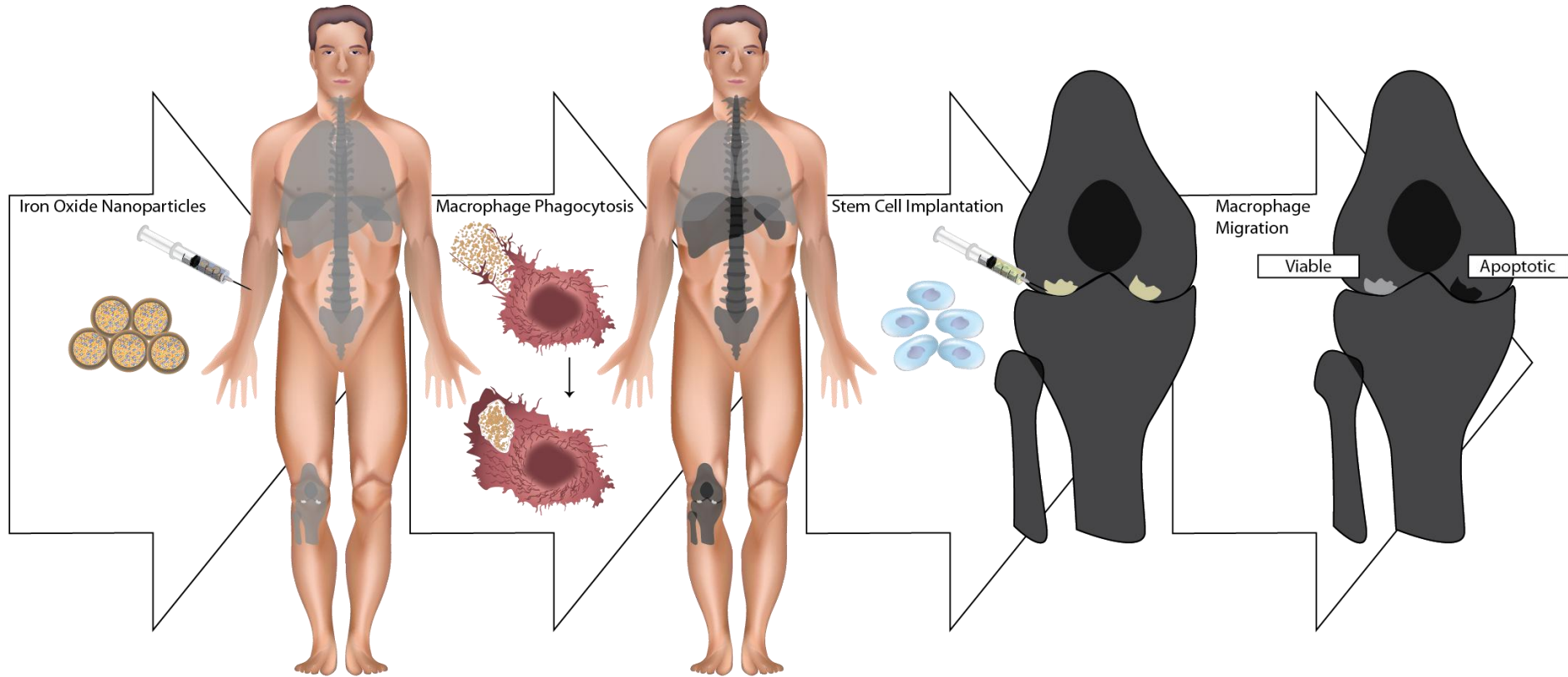


## Cancer Immunotherapy

Tracking EpCAM-targeted NK cells  
-> EpCAM-positive cancer



# MR Imaging of Macrophage Migration into MASI

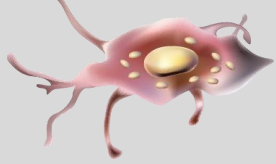


# Macrophage Imaging

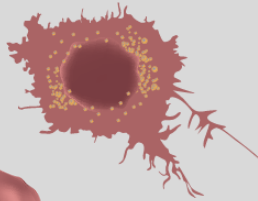
Bone Marrow

Reticuloendothelial System

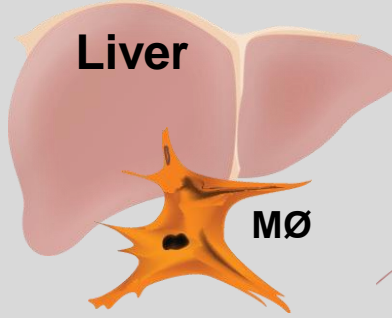
Progenitor cell



MØ



Liver



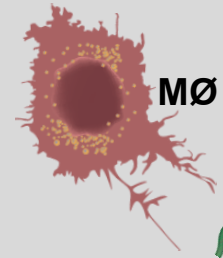
MØ

Spleen

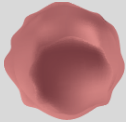


MØ

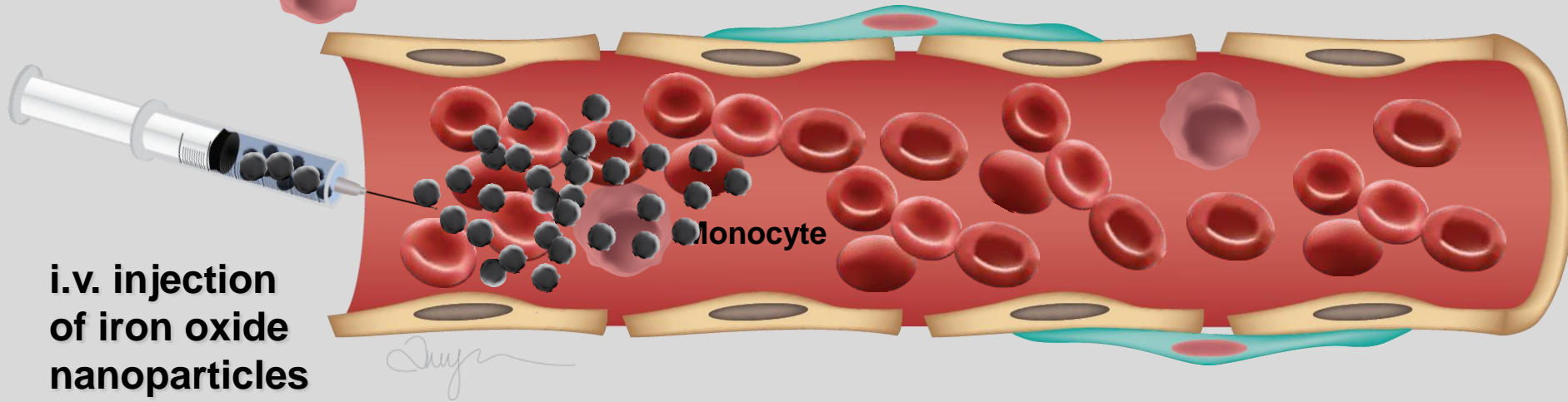
Lymph Nodes



Monocyte



i.v. injection  
of iron oxide  
nanoparticles



*Dujze*

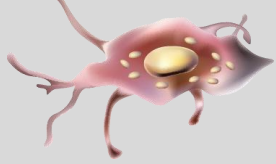


# Macrophage Imaging

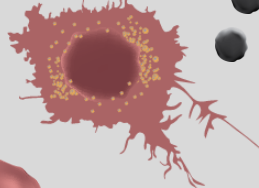
Bone Marrow

Reticuloendothelial System

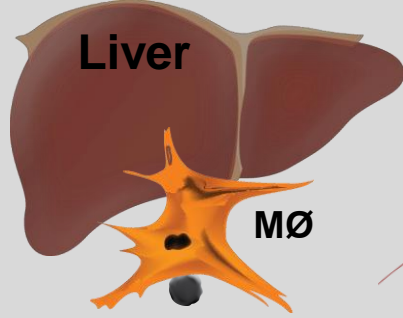
Progenitor cell



MØ

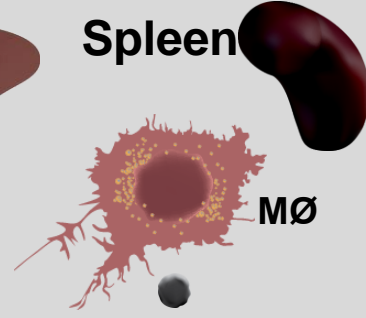


Liver



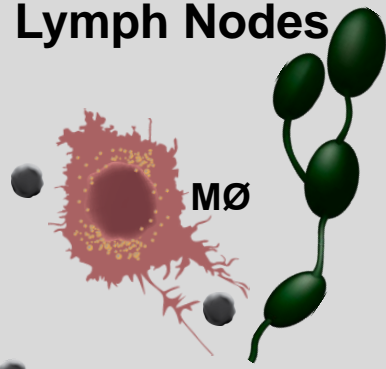
MØ

Spleen



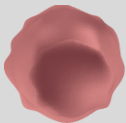
MØ

Lymph Nodes

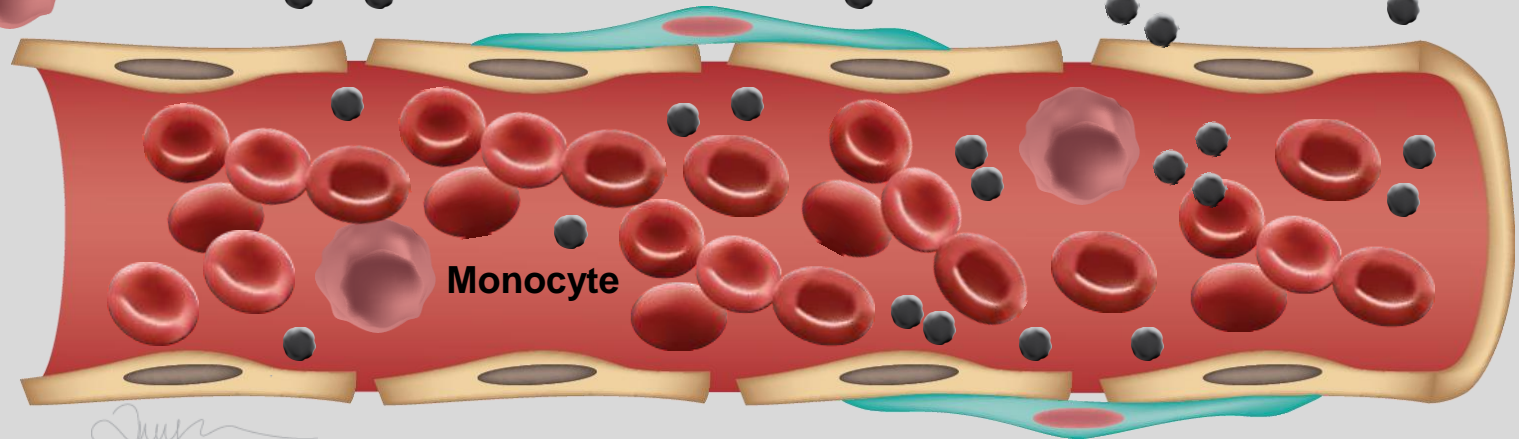


MØ

Monocyte



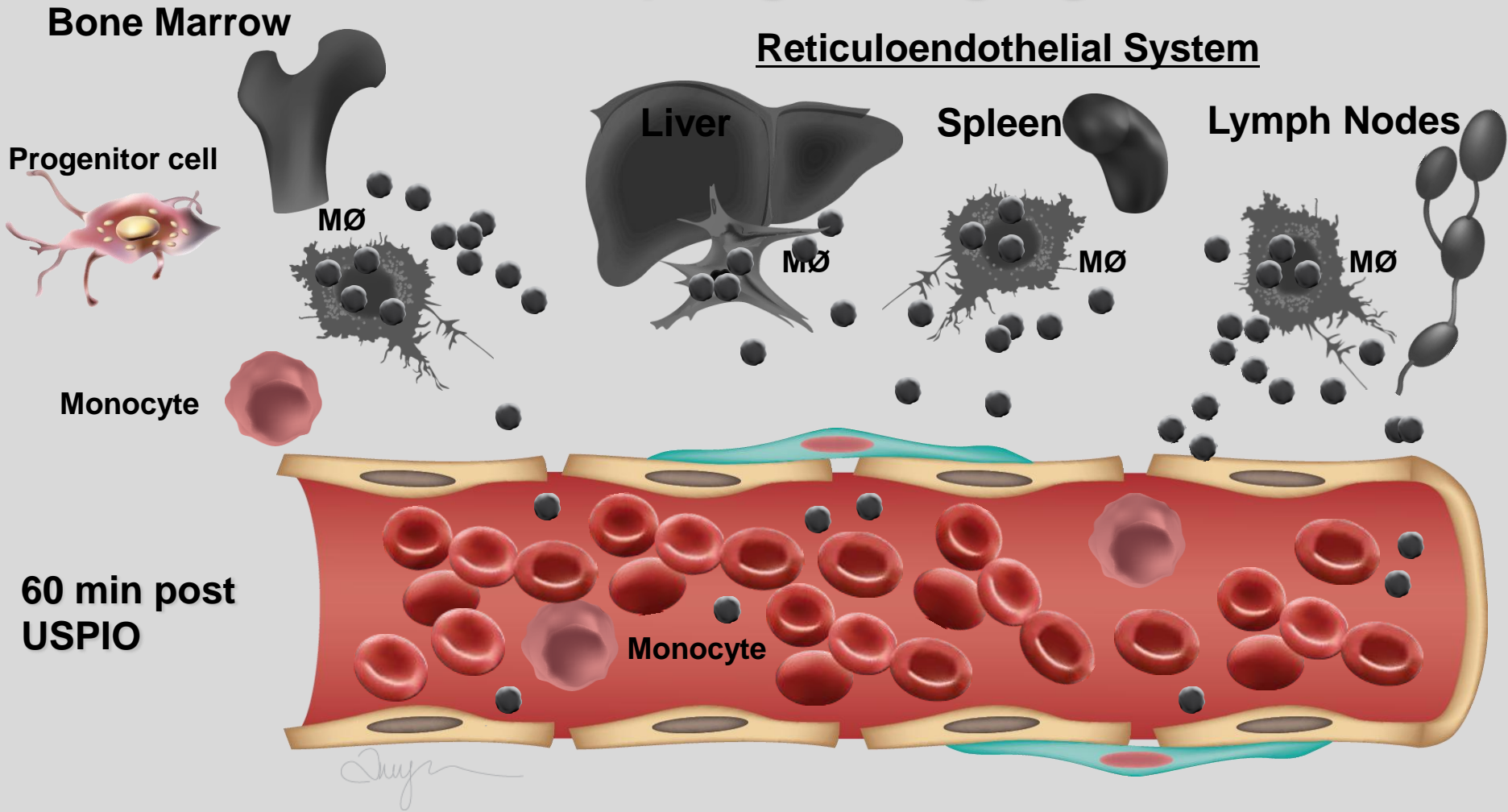
15-60 min  
post USPIO



Monocyte

*Dujze*

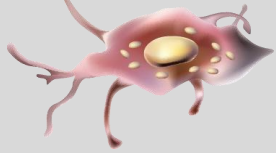
# Macrophage Imaging



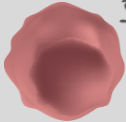
# Macrophage Imaging

Bone Marrow

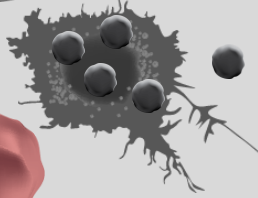
Progenitor cell



Monocyte

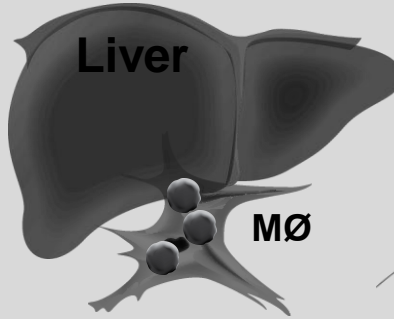


MØ



Reticuloendothelial System

Liver



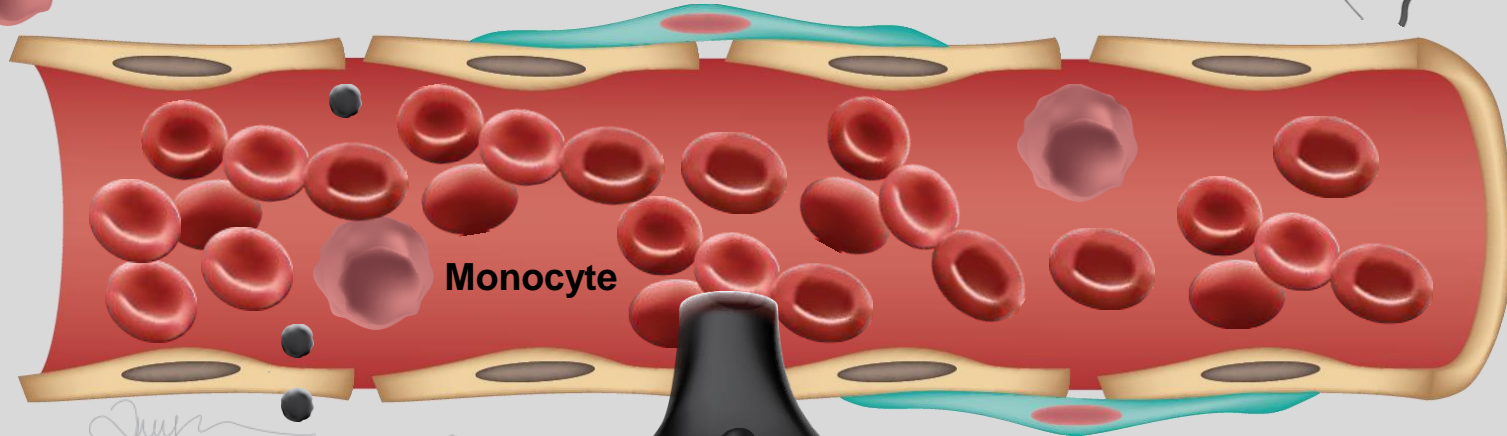
MØ

Spleen



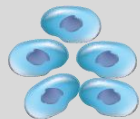
MØ

Lymph Nodes



*Dujze*

Stem Cell Transplant



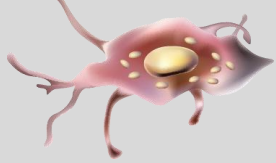


# Macrophage Imaging

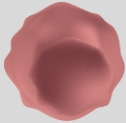
Bone Marrow

Reticuloendothelial System

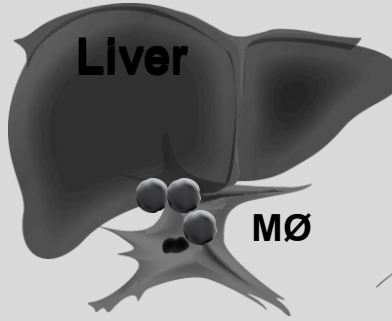
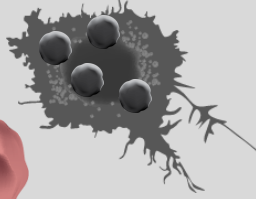
Progenitor cell



Monocyte



MØ



Liver

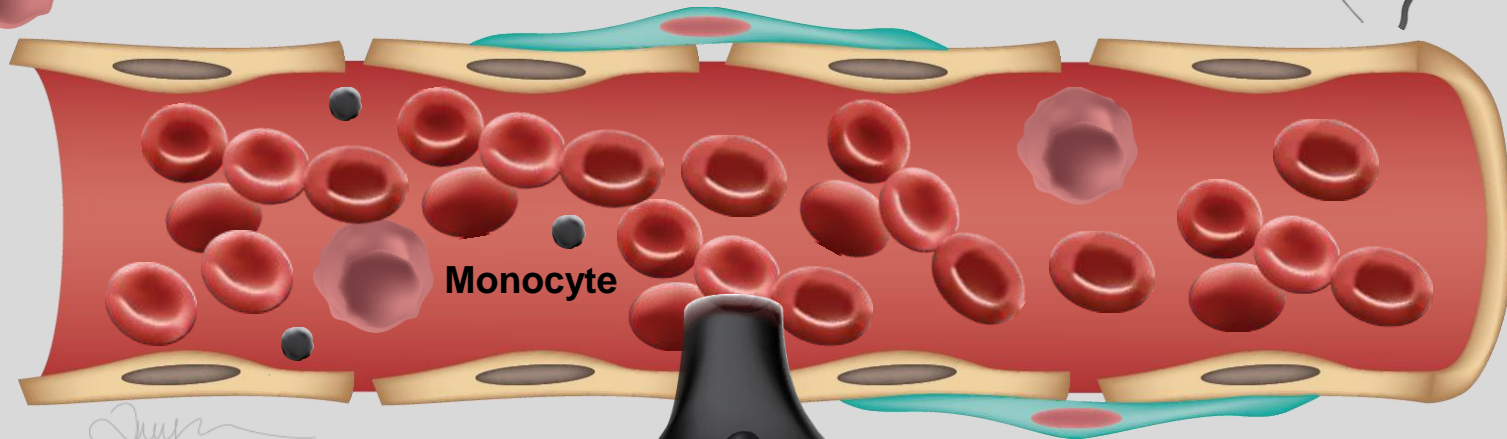
MØ

Spleen



MØ

Lymph Nodes



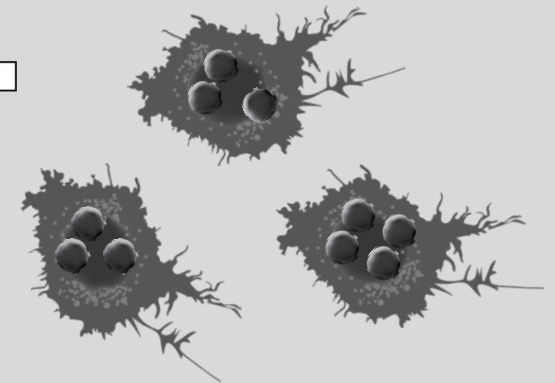
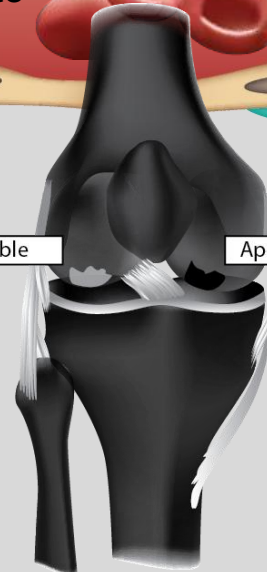
Monocyte

*Duys*

Viable

Apoptotic

Stem Cell Transplant

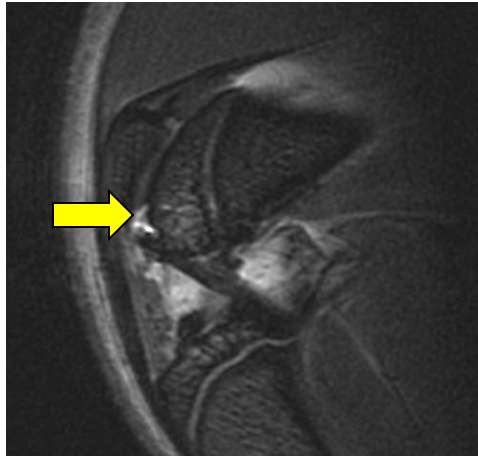




# In vivo tracking of macrophages

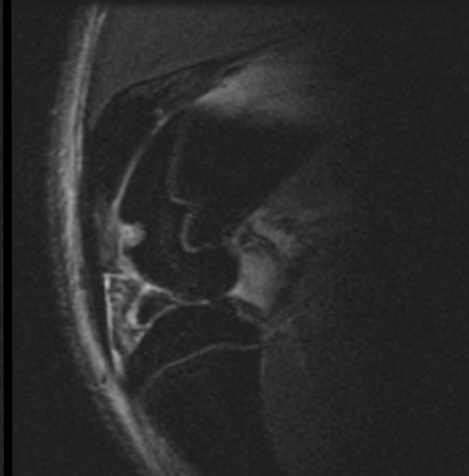
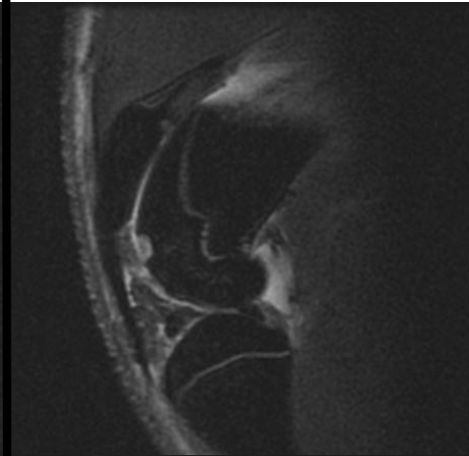
No Fe

Stem Cell Implant

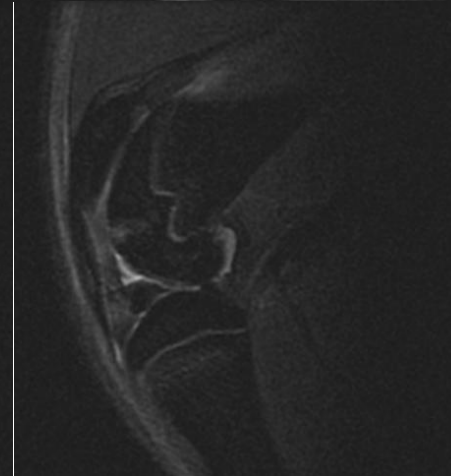
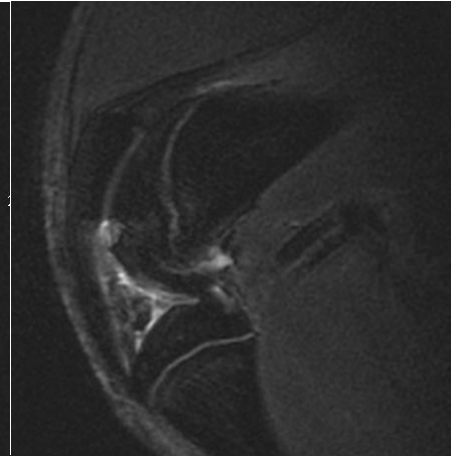


IV ferumoxytol → labeled bone marrow macrophages

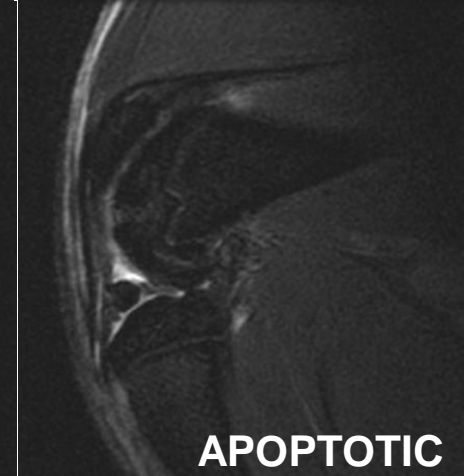
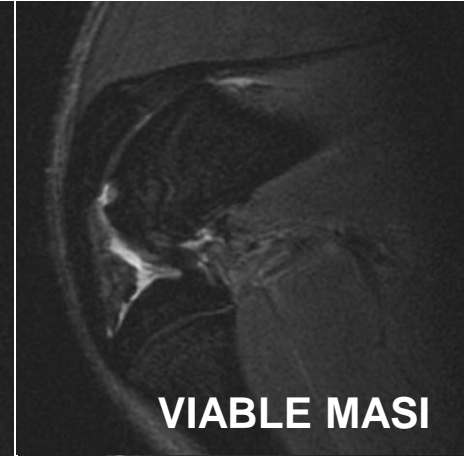
Stem Cell Implant



Week 2

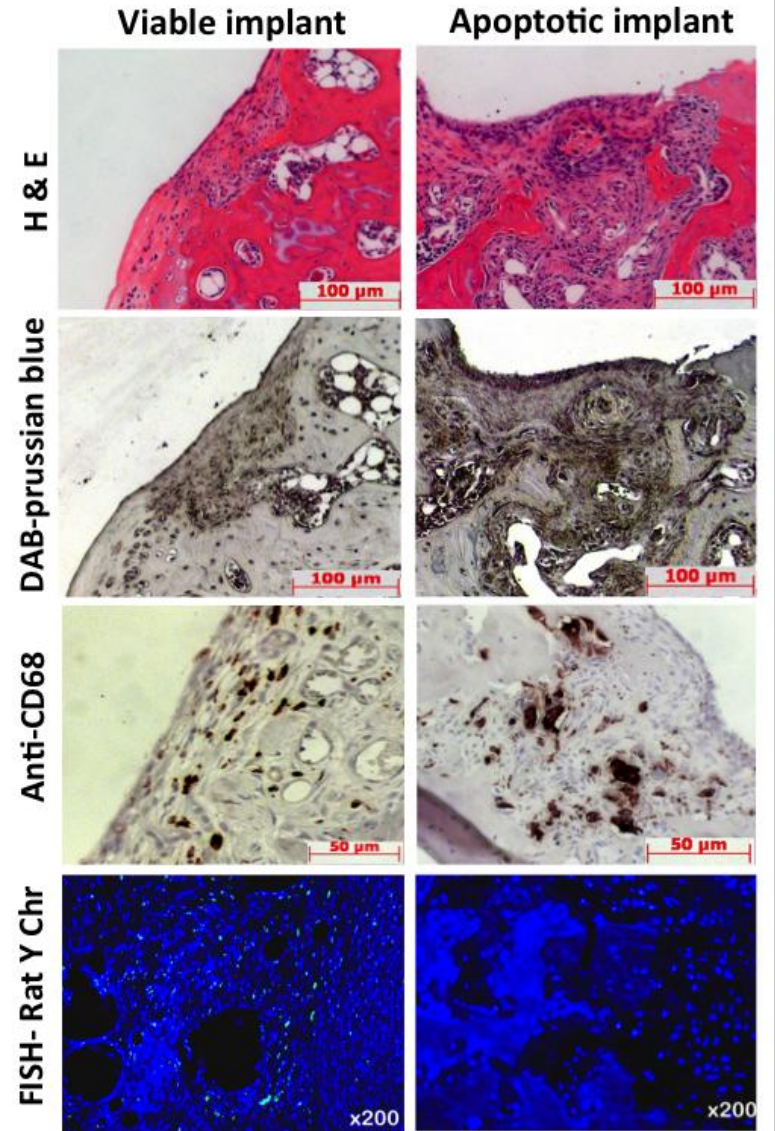
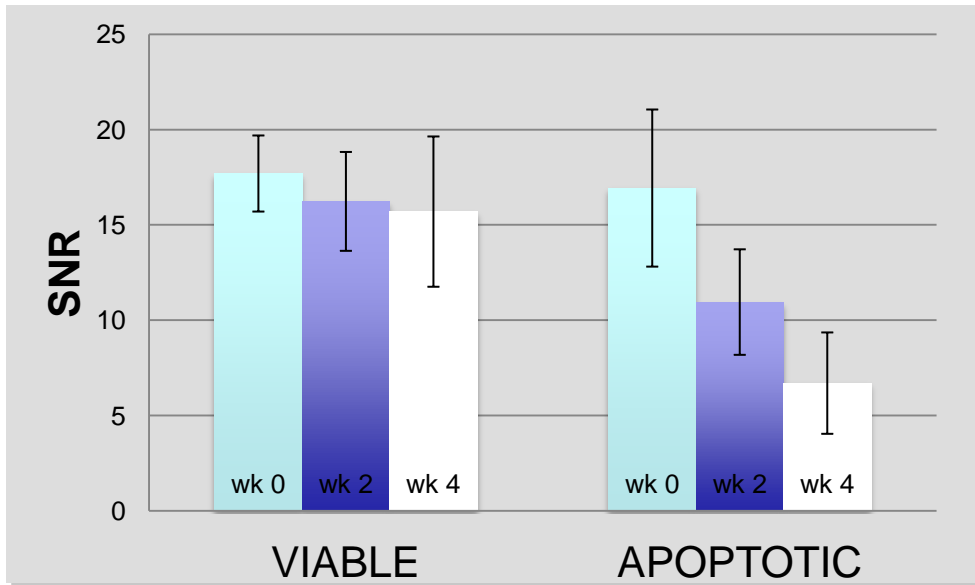


Week 4





# In vivo tracking of macrophages





# Clinically applicable labels for *in vivo* tracking of leukocytes

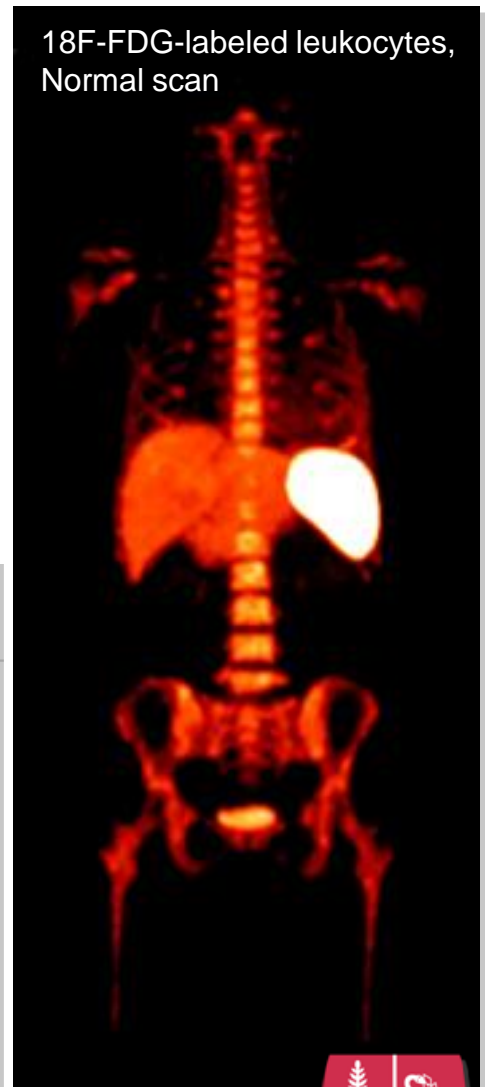
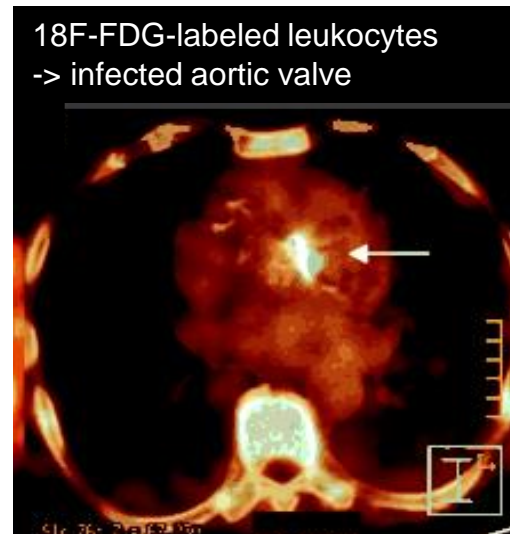


## SPECT and PET Imaging

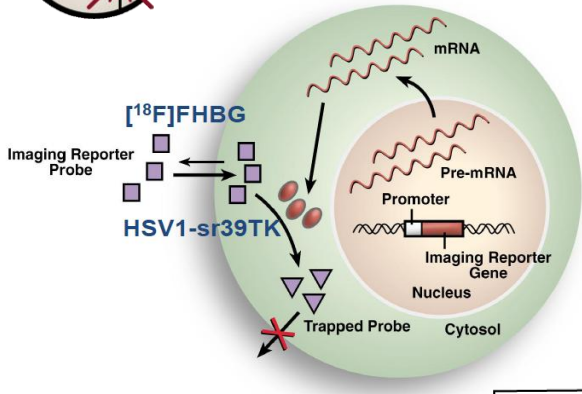
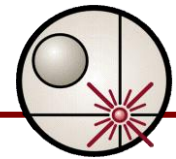
- In-111 Oxine (half-life 2.8 days)
- Tc99m HMPAO (half life 6 h)
- [18F]-FDG (half life 2 h)
- [18F]-Fluoro-3-(hydroxymethyl)butyl]guanine

## MR Imaging

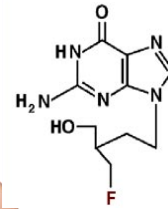
- Gd-Chelates
- Iron Oxide Nanoparticles



# PET Reporter Gene Imaging for Tracking CTL

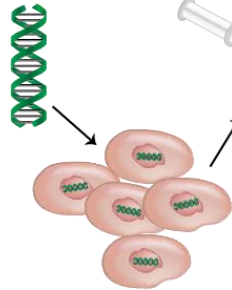


9-[4-[<sup>18</sup>F]-Fluoro-3-(hydroxymethyl)butyl]guanine

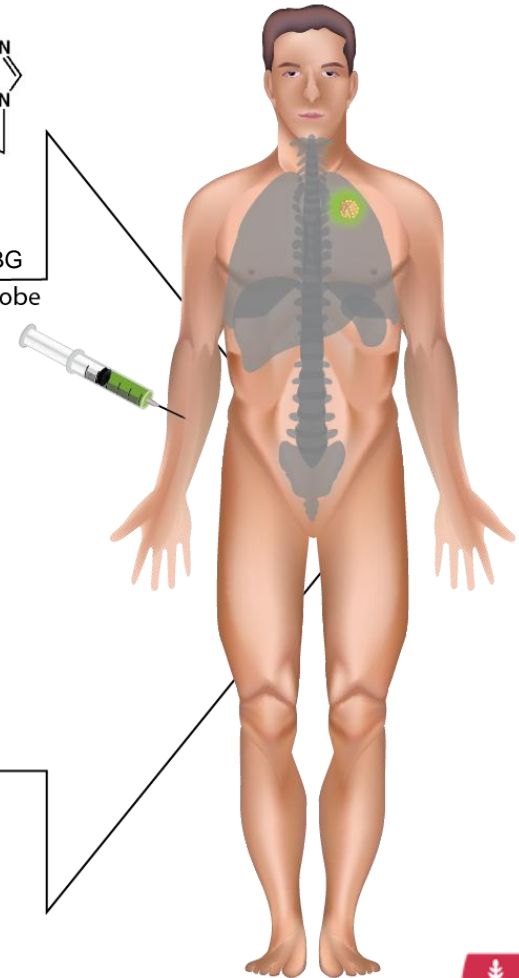
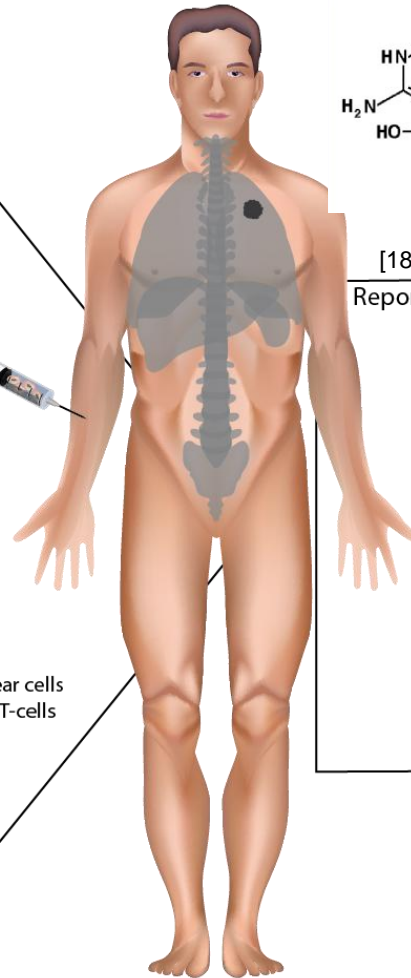


[<sup>18</sup>F]FHBG  
Reporter Probe

Reporter Gene(s)

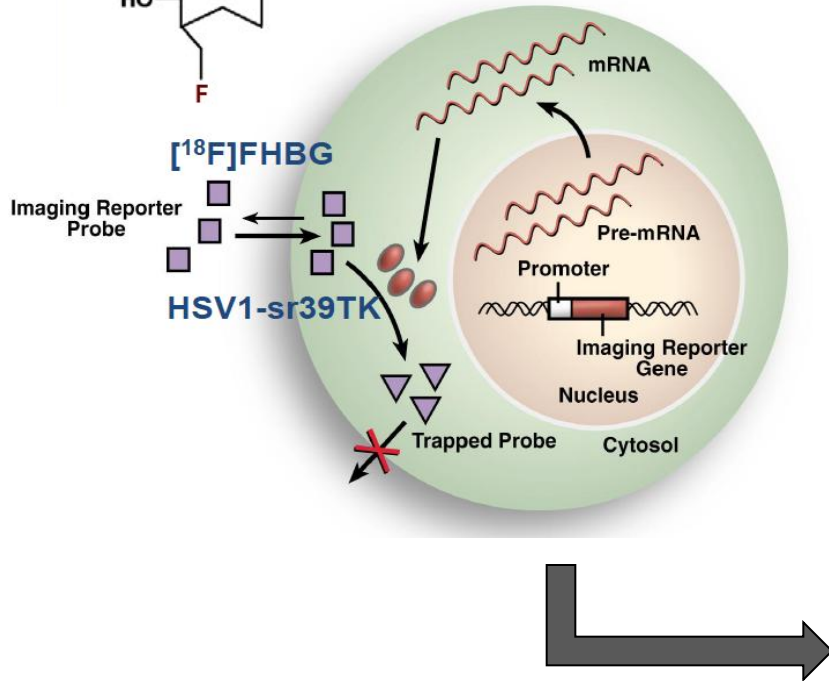
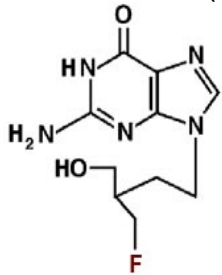


- 1) Leukapheresis,
- 2) Separation of mononuclear cells
- 3) Activation of autologous T-cells
- 4) Expansion with IL-2

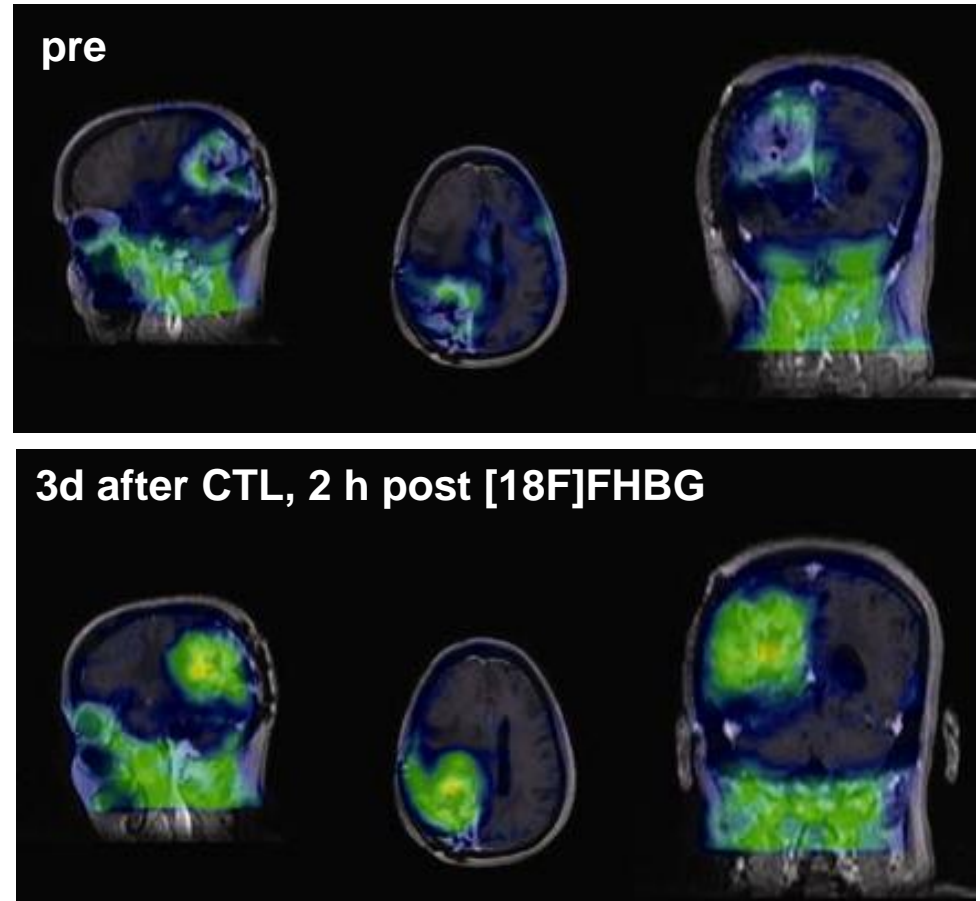


# PET Reporter Gene Imaging for Tracking CTL

9-[4-[<sup>18</sup>F]-Fluoro-3-(hydroxymethyl)butyl]guanine



## Tumor accumulation of CTL in recurrent Glioma

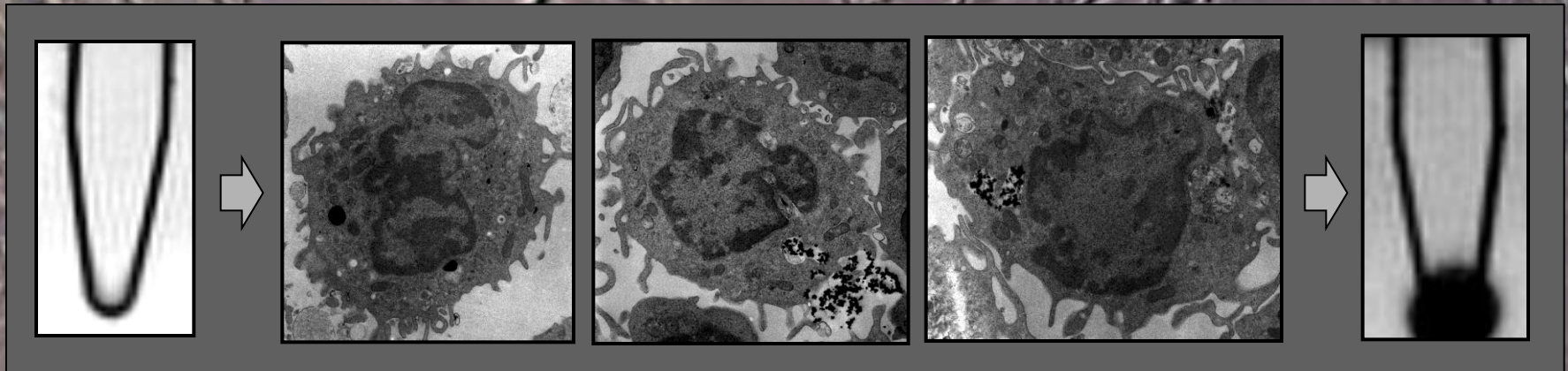


courtesy of S. Gambhir, Stanford University





# Imaging Stem Cell Transplants: Assessment of Immune Responses



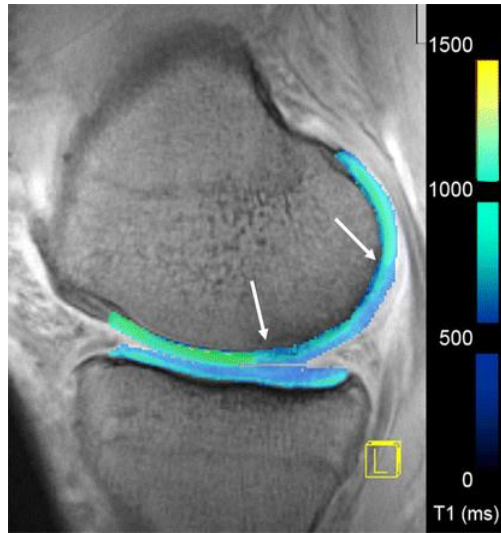
1. *In vivo* tracking of transplanted stem cells
2. *In vivo* tracking of immune cells
3. *In vivo* imaging of tissue regeneration

# Imaging Cartilage Regeneration

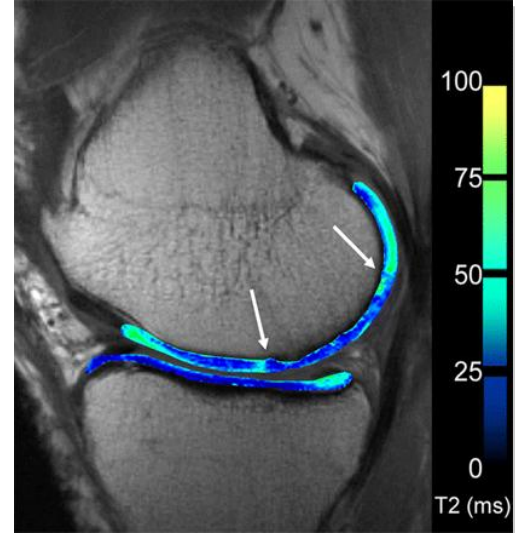
4 years after matrix associated chondrocyte transplant:  
**Successful cartilage repair**



**PDW 3200/30**  
→ Anatomy



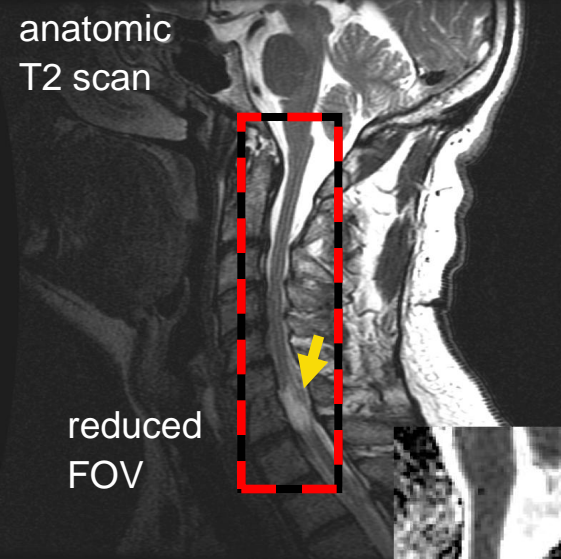
**T1 map**  
**dGEMRIC 490/13**  
→ GAG content



**T2 map,**  
**SE 2700/10-20**  
→ proton content

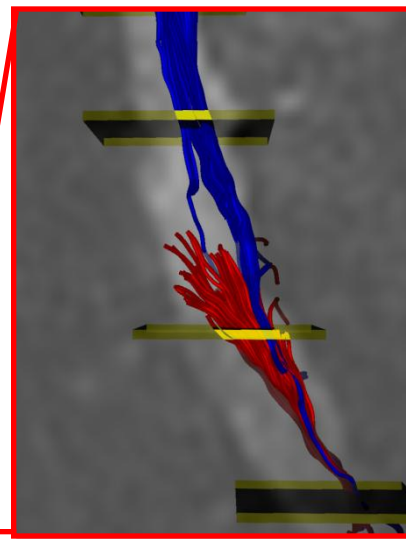
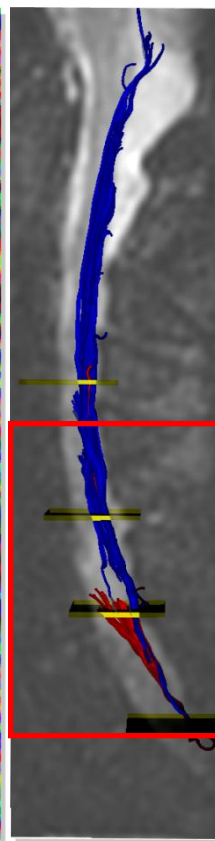
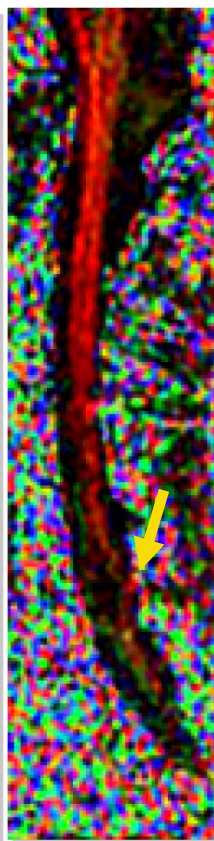
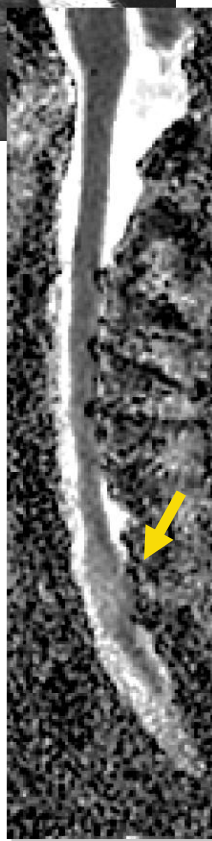




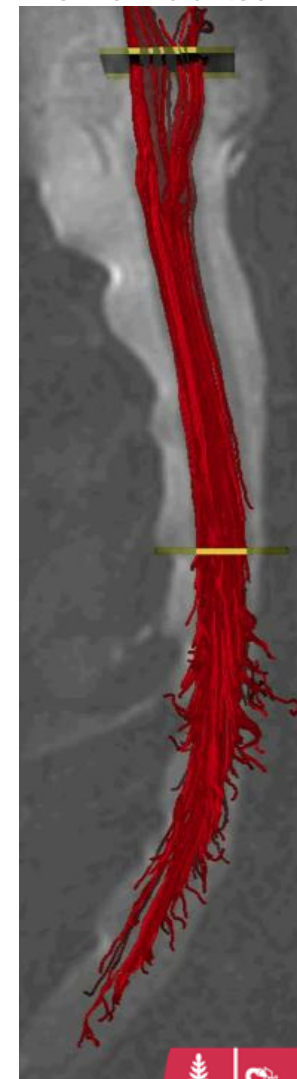


# Imaging Tissue Regeneration

## Traumatic Cord Injury



Normal Volunteer







***What comes next along the path?***





SPECT  
PET

Optical Imaging

Fusion  
Scans

MR Imaging

Radiography

# TAKE HOME MESSAGE

**Clinically applicable techniques  
for imaging stem cell transplants  
& related host immune responses  
are readily available**





# Translational Tumor and Stem Cell Imaging Lab, Lorry Lokey Stem Cell Research Building @ Stanford



## **Stanford Radiology**

**Aman Khurana**  
**Hossein Nejadnik**  
**Fanny Chapelin**  
**Olga Lenkov**  
**Su Hyun Hong**  
**Jessica Donig**  
**Rakhee Gawande**  
**Qiaoyun Shi**  
**Celina Anasari**  
**Christopher Klenk**  
**Beverly Newman**  
**Richard Barth**  
**Amy Morris**  
**Jennifer Vancil**

**Jianghong Rao**  
**Deju Ye**  
**Laura Pisani**  
**Brian Rutt**  
**R. Paulmurugan**  
**Nikita Derugin**  
**Greg Zaharchuk**  
**Deqiang Qiu**  
**Michael Moseley**  
**Andrew Quon**  
**John Ronald**  
**Sanjiv Sam Gambhir**

## **Institute for Stem Cell & Regenerative Medicine**

**Charles Chan**  
**Jeong Hyung**  
**Michael Longaker**

## **Electrical Engineering**

**Sungmin Lee**  
**Dwight Nishimura**

## **Northwestern University**

**Thomas Meade**

## **Grant Support:**

**NIH/NIAMS 2R01AR054458-05, R21AR059861**  
**Stanford Cancer Institute, (CIRM 2007-09)**

<http://daldrup-link-lab.stanford.edu/>





# Translational Tumor and Stem Cell Imaging Lab, Lorry Lokey Stem Cell Research Building @ Stanford



Thank you!



*Daldrup-Link Lab, Molecular Imaging Program @ Stanford*

<http://daldrup-link-lab.stanford.edu/>

