

Clinical Cell Tracking Using PET and SPECT

Shahriar Yaghoubi, Ph.D.

Chief Scientific Officer
CellSight Technologies, Inc.

Visiting Professor
UCLA School of Medicine
Dept of Molecular and Medical Pharmacology

Monitoring Kinetics of Cells Transferred into Living Subjects

- Detect whole-body presence and whole-body quantity of cells
- Detect whole-body locations and quantity at every location of cell accumulation
- Monitor changes in cell status through time: proliferation, differentiation, functional change, interaction with other cells, etc ...

Ideal Cell Kinetic Monitoring Technique

- **Can be done at any desired time point after administration into living subjects**
- **Does not involve invasive procedures**
- **Does not perturb the cells while repeatedly imaging them**
- **Is safe for human use**

Applications of Cell Kinetics Monitoring

- **Monitoring pharmacokinetics of therapeutic cells in cell therapy**
- **Monitoring cell trafficking**
 - **Cancer cell metastasis**
 - **Immune cell trafficking and activation**
- **Monitoring pharmacodynamics of therapeutic agents**
 - **Eradication of cancer cells**
 - **Early detection of efficacy & potency**
 - **Effect on cell proliferation**

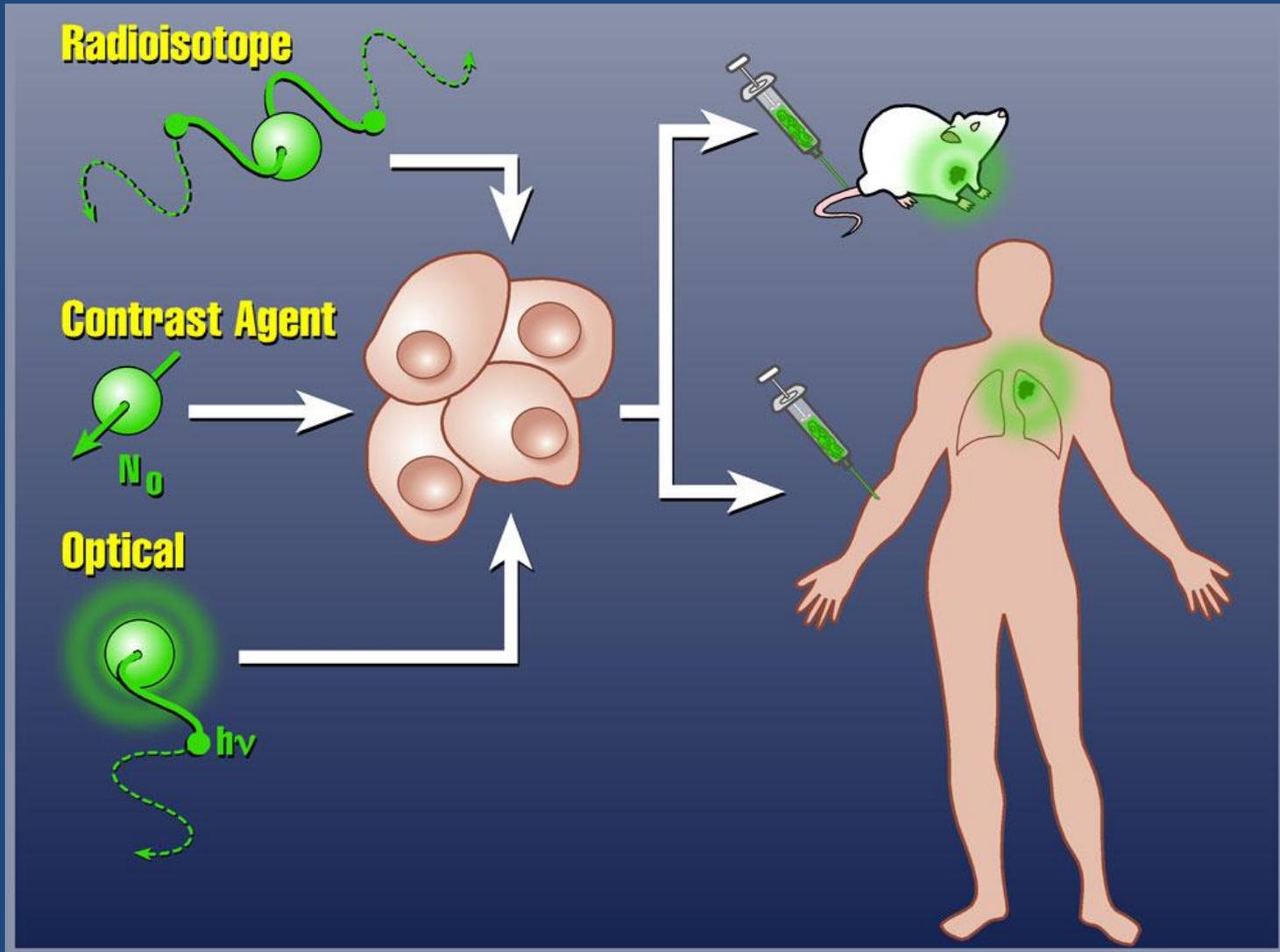
Methods for Non-Invasive Imaging of Cell Kinetics in Living Subjects

Cell Pre-labeling Imaging Probes

Imaging Reporter Genes

Highly Specific Imaging Probes Detecting Specific Cells or a Specific Cell Characteristic

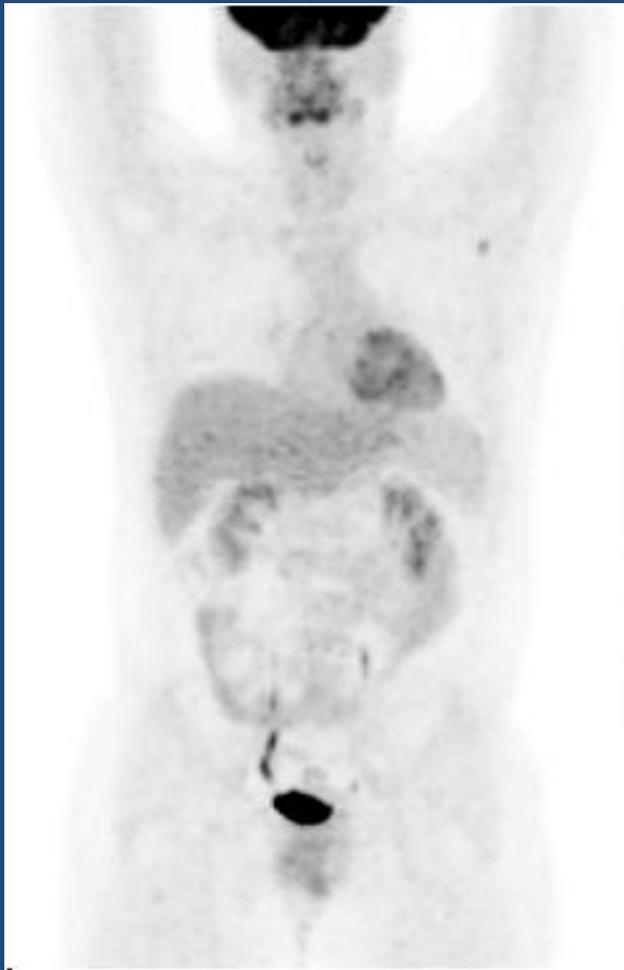
Pre-Labeling Strategies



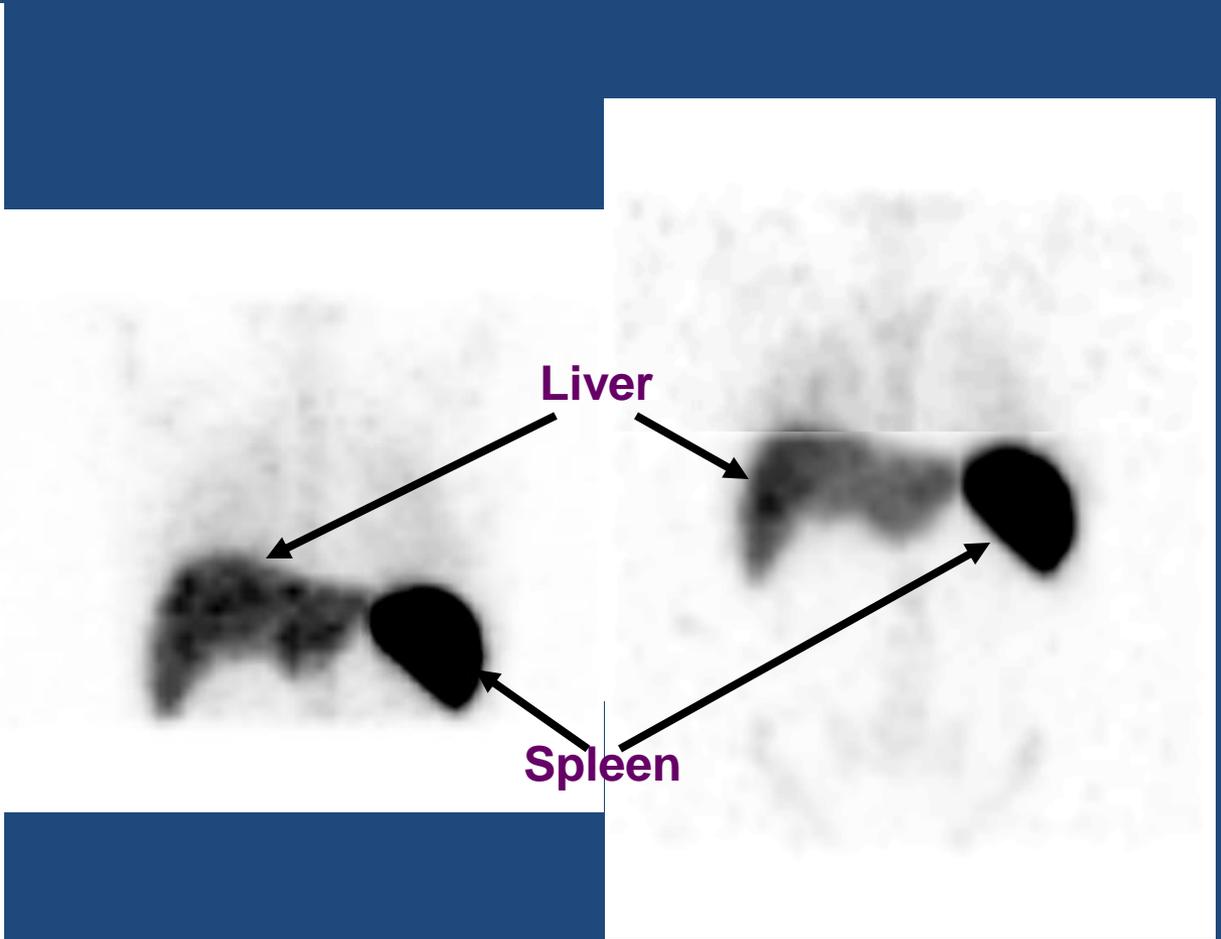
PET/SPECT Pre-labeling Probes

- Indium-111 Oxine and Indium-111 Tropolone
Half-life = 2.8 days
- Technetium-99m Exametazime
Half-life = 6 hours
- [^{18}F]Fluorodeoxyglucose ([^{18}F]FDG)
Half-life = 110 minutes
- ^{64}Cu -pyruvaldehyde-bis(N⁴-methylthiosemicarbazone) ([^{64}Cu]PTSM)
Half-life = 12.7 hours

Imaging Trafficking of Cytokine Induced Killer Cells with ^{111}In -Oxine



Pre-CIK ^{18}F FDG PET

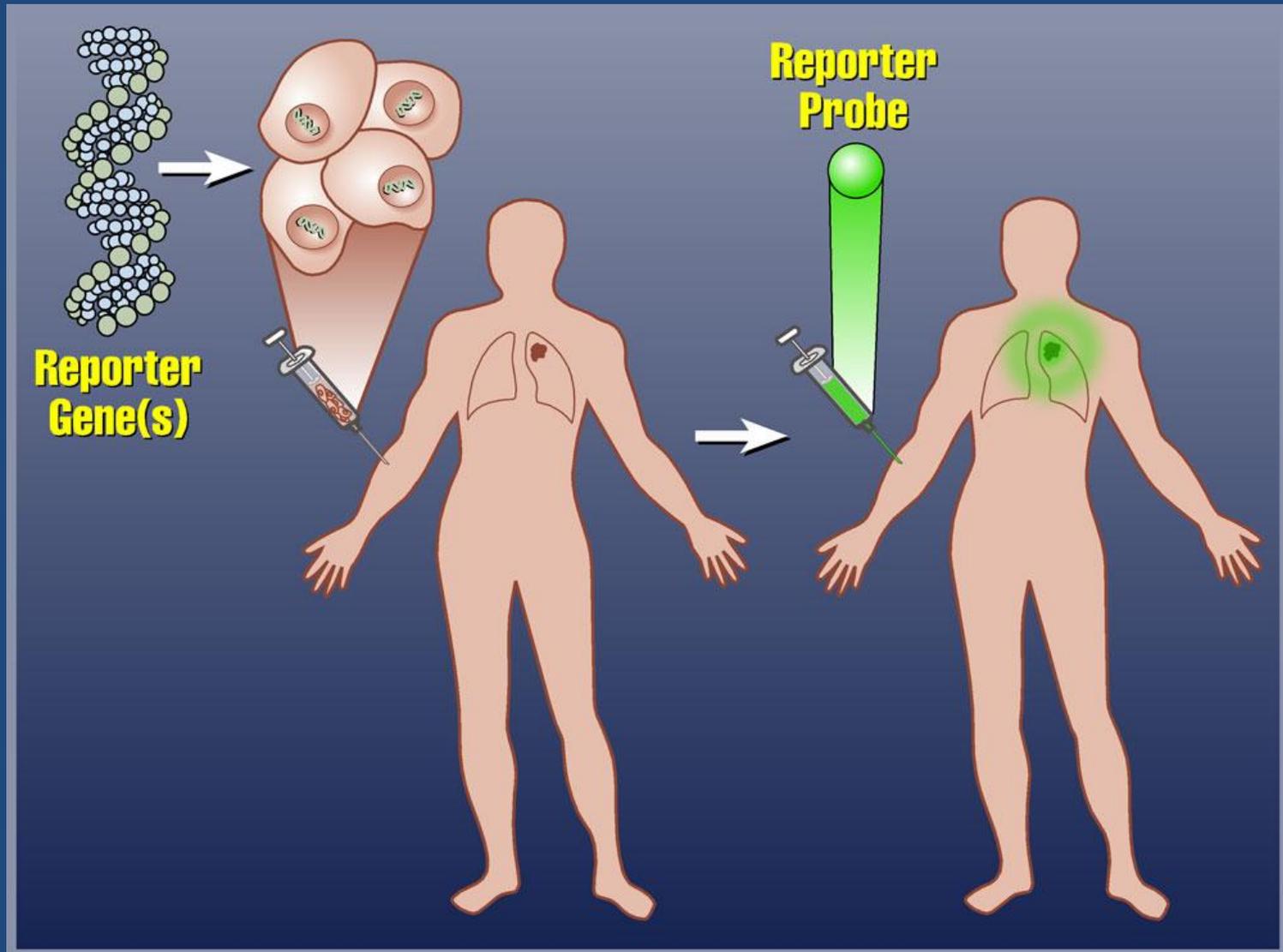


^{111}In CIK SPECT

24 hours

42 hours

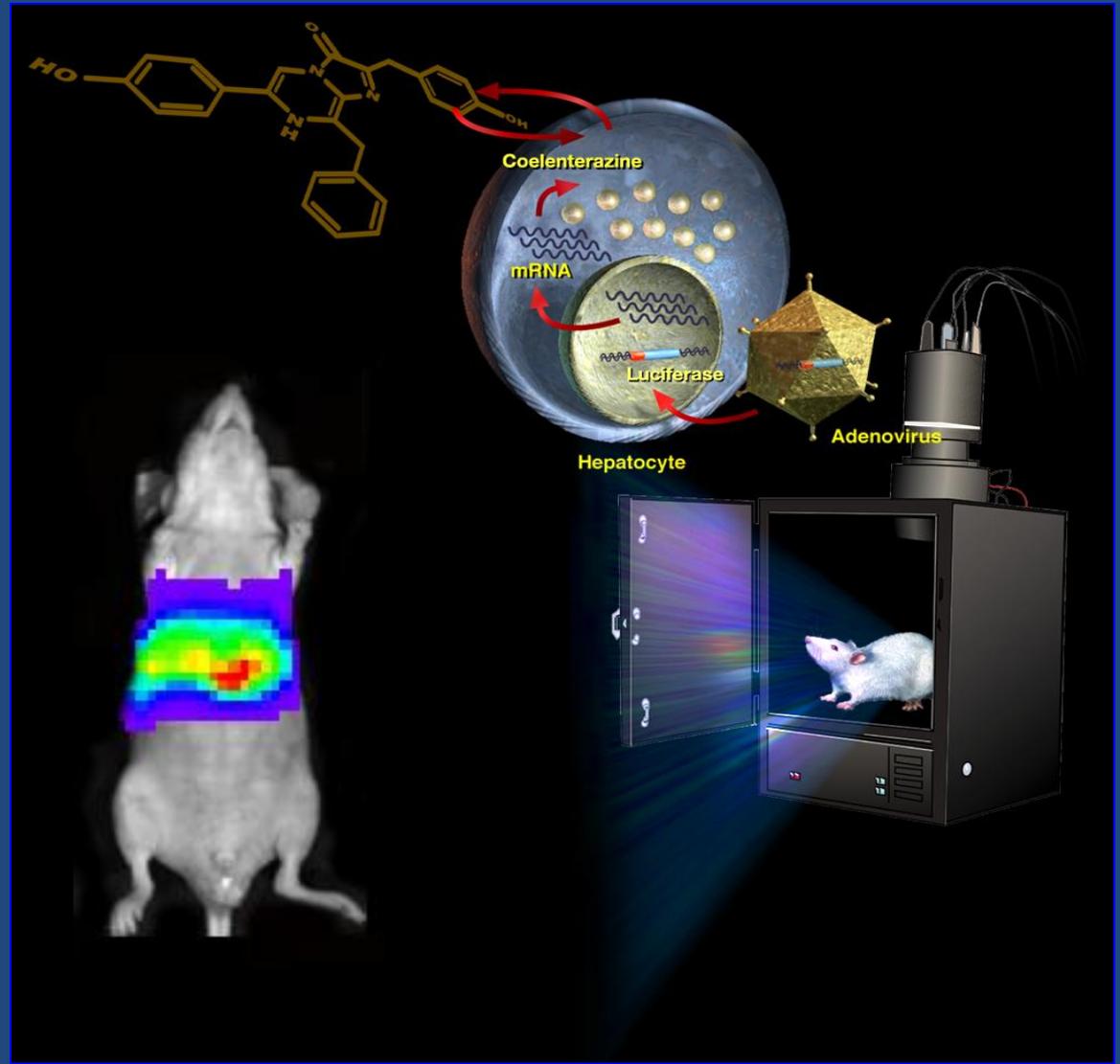
Imaging Reporter Gene Based Cell Kinetics Imaging



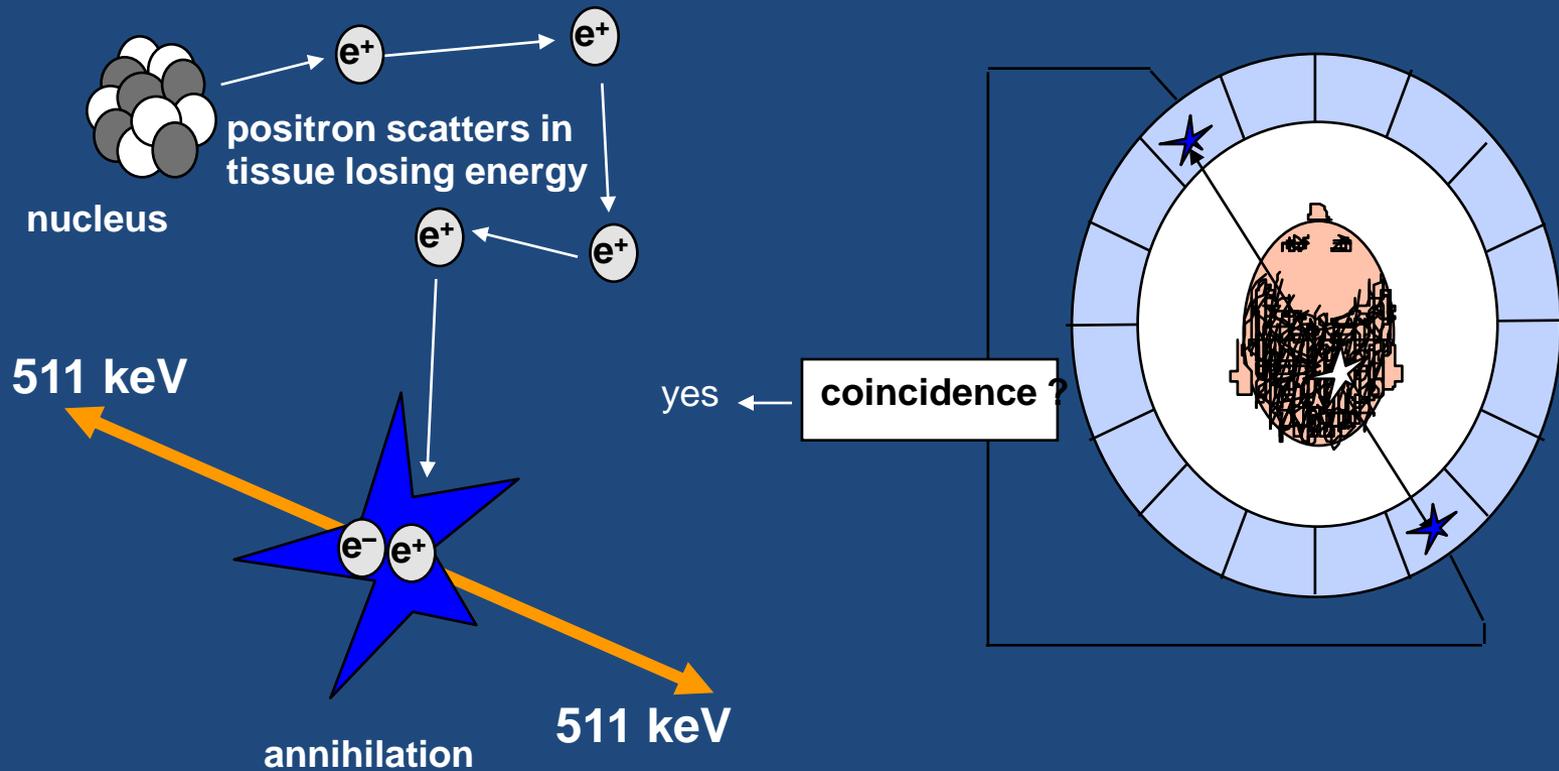
Bioluminescence Reporter Genes/Probes

- **Firefly Luciferase and Optimized Mutants/ D-Luciferin**
- **Renilla Luciferase and Optimized Mutants/ Coelenterazine**
- **Gaussia Luciferase/ Coelenterazine**

Reporter Gene Imaging with Bioluminescence Optical Imaging



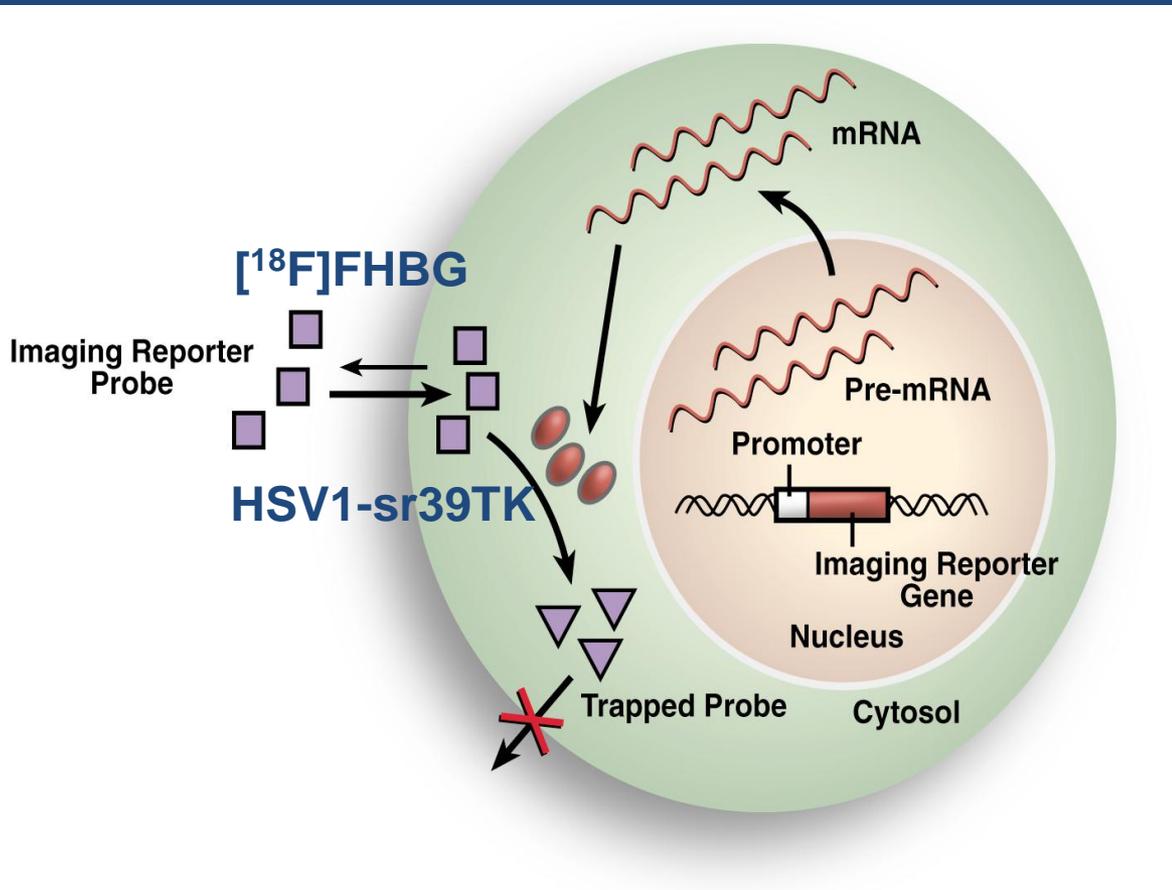
Positron Emission Tomography (PET)



PET/SPECT Imaging Reporter Genes

- Herpes Simplex Virus Type 1 Thymidine Kinase and multiple optimized mutants, such as HSV1-sr39tk
- Dopamine Type 2 Receptor
- Sodium-Iodide Symporter
- Somatostatin type 2 Receptor
- Human Norepinephrine Transporter
- Human Estrogen Receptor α Ligand Binding Domain
- Mutants of Human Mitochondrial Thymidine Kinase 2
- Mutants of Human Deoxycytidine Kinase
- Recombinant Carcinoembryonic Antigen
- Engineered Antibody Fragments
- Anti-Polyethylene Glycol

PET Reporter Gene/Probe System (Enzyme Based)

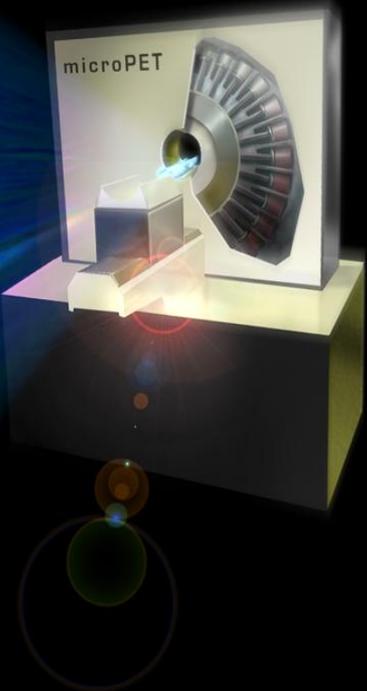
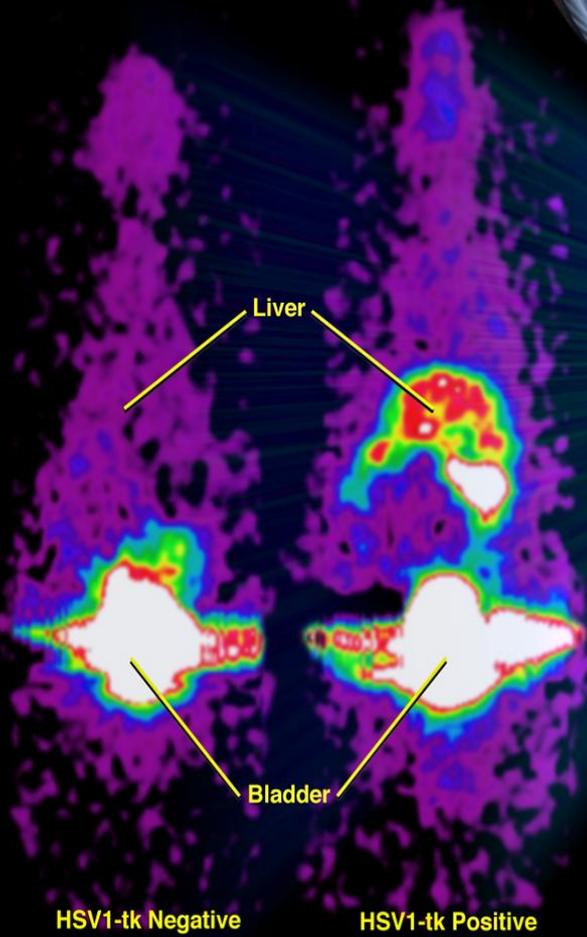
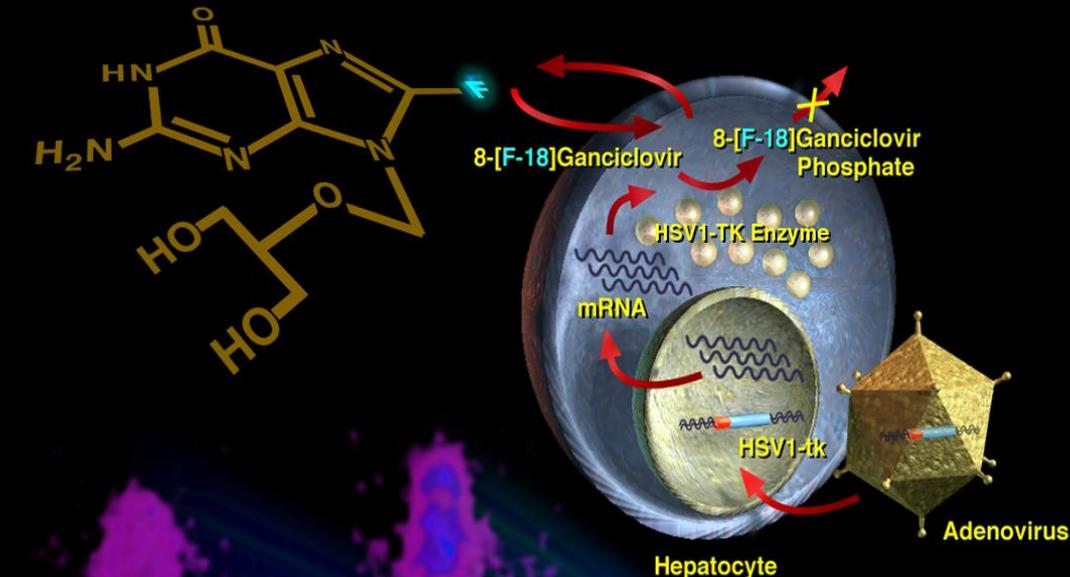


[¹⁸F]FHBG

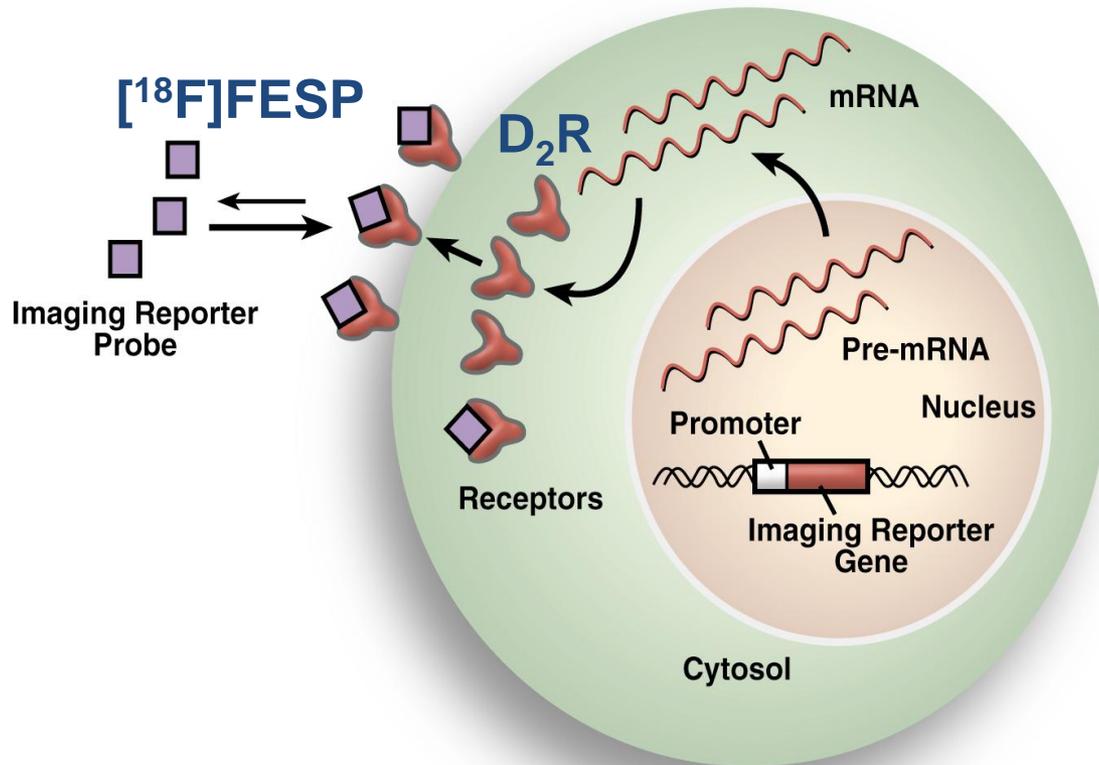


9-[4-[¹⁸F]Fluoro-3-(hydroxymethyl)butyl]guanine

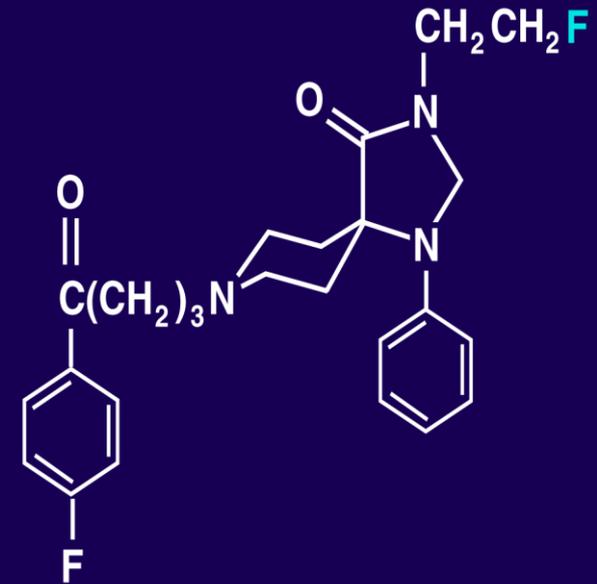
Reporter Gene Imaging with microPET



PET Reporter Gene/Probe System (Receptor Based)

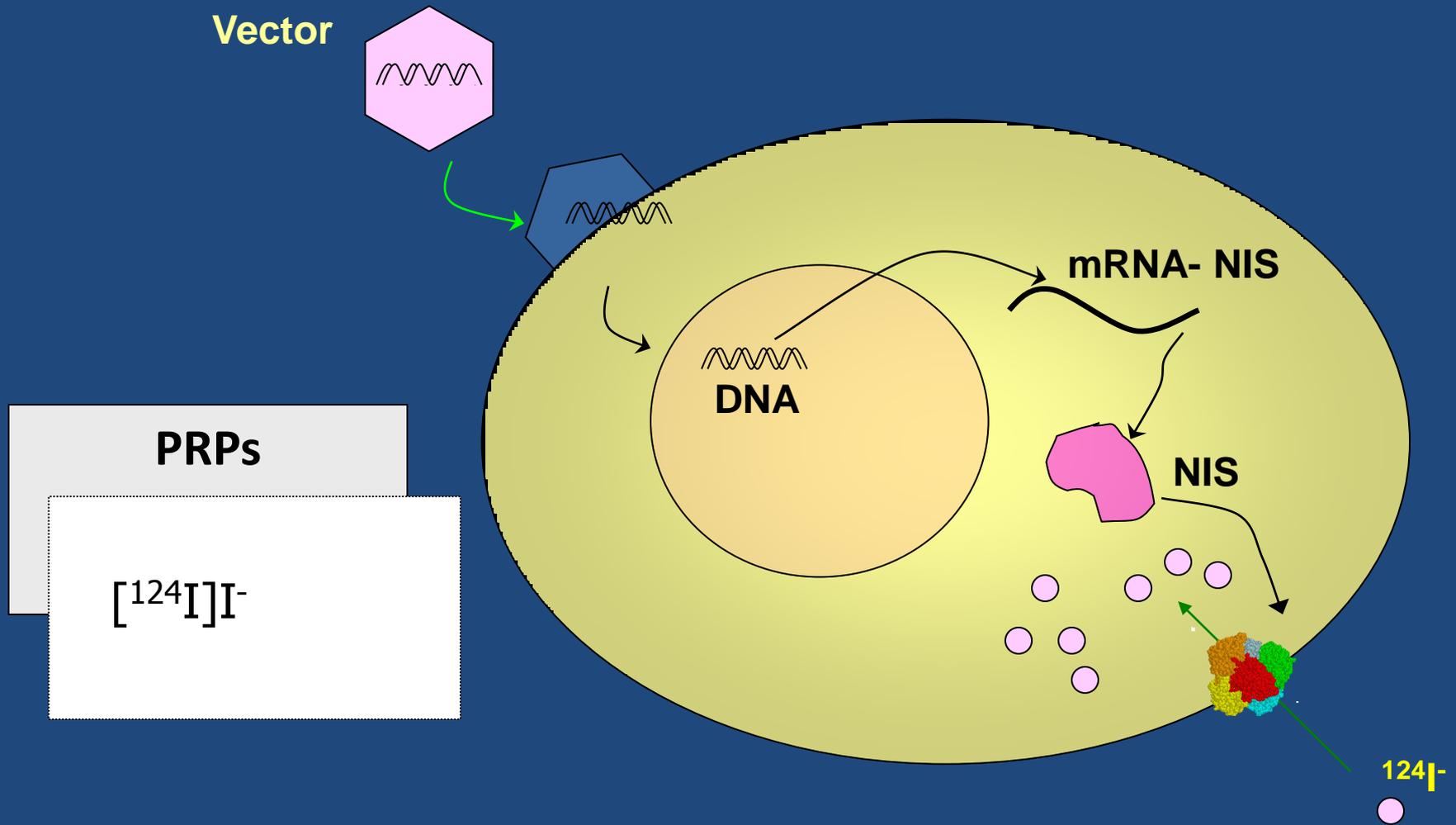


[¹⁸F]FESP

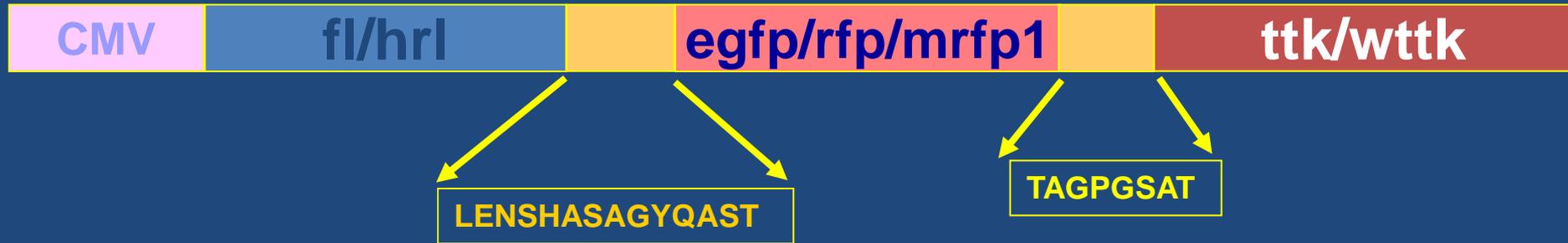


**3-(2' [¹⁸F]Fluoroethyl)
spiperone**

Transporter Based PET Reporter Gene/Probe System



Tri-fusion Multimodality Reporters



Reporter Genes:

Bioluminescence:

- ❖ Firefly luciferase (fl)
- ❖ Synthetic renilla luciferase (hrf)

Fluorescence:

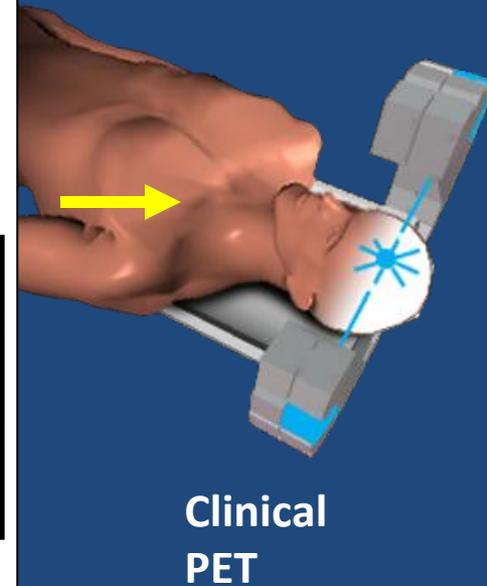
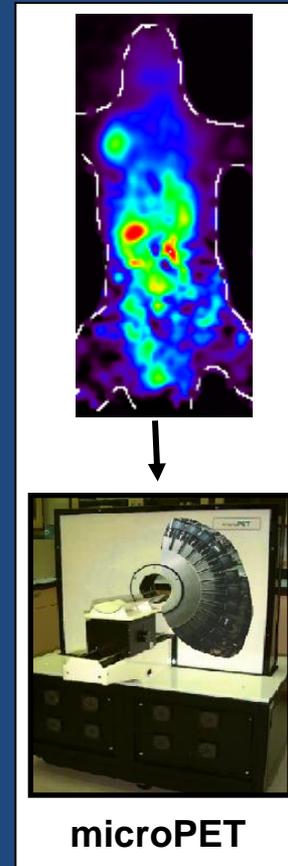
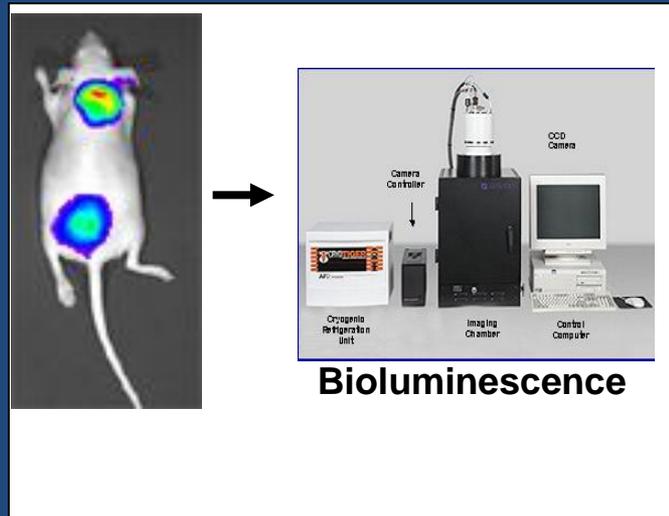
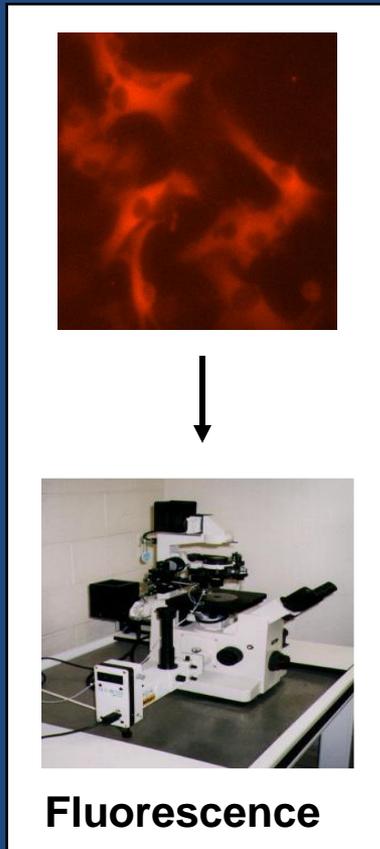
- ❖ Enhanced Green Fluorescence protein (egfp)
- ❖ Red Fluorescence Protein (rfp)
- ❖ Monomeric Red Fluorescence Protein (mrfp1)

PET:

- ❖ Deletion mutant of HSV1-sr39tk (ttk)
- ❖ Deletion mutant of Wild type HSV1-tk (wttk)

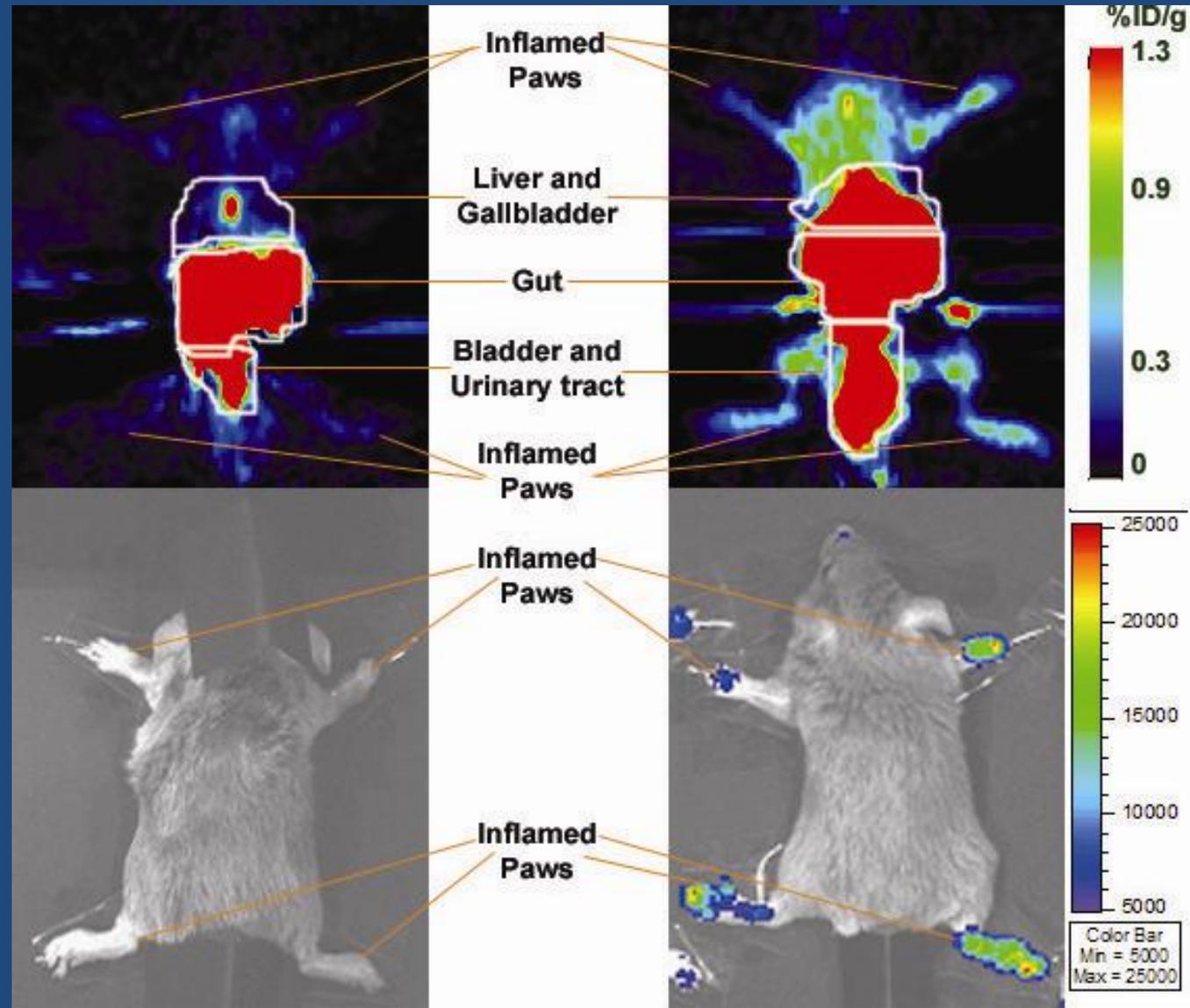
Ray et. al., Cancer Research (March 2004)

Translational Cell Imaging

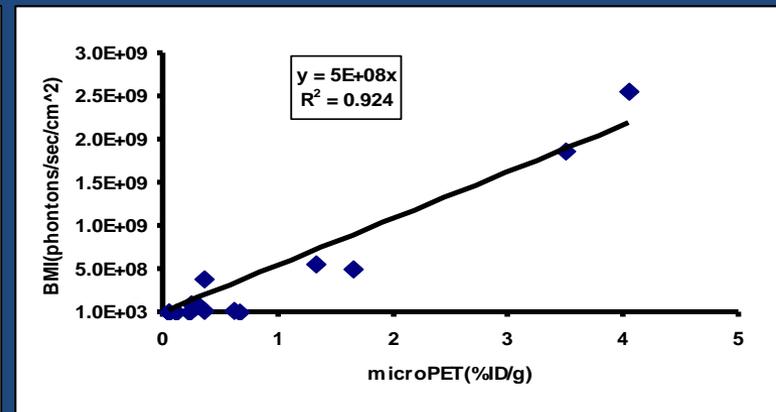
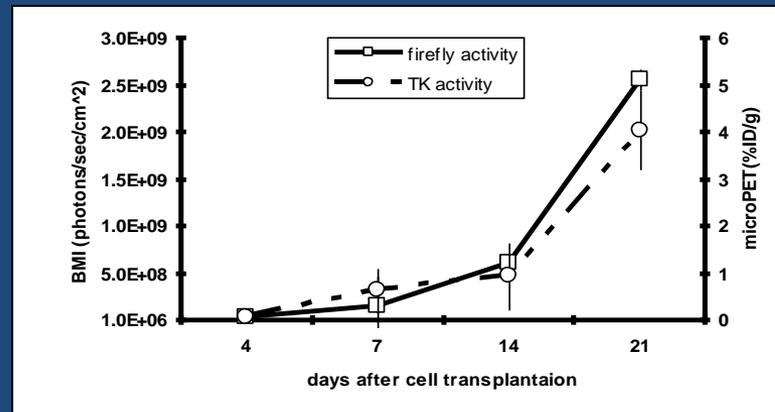
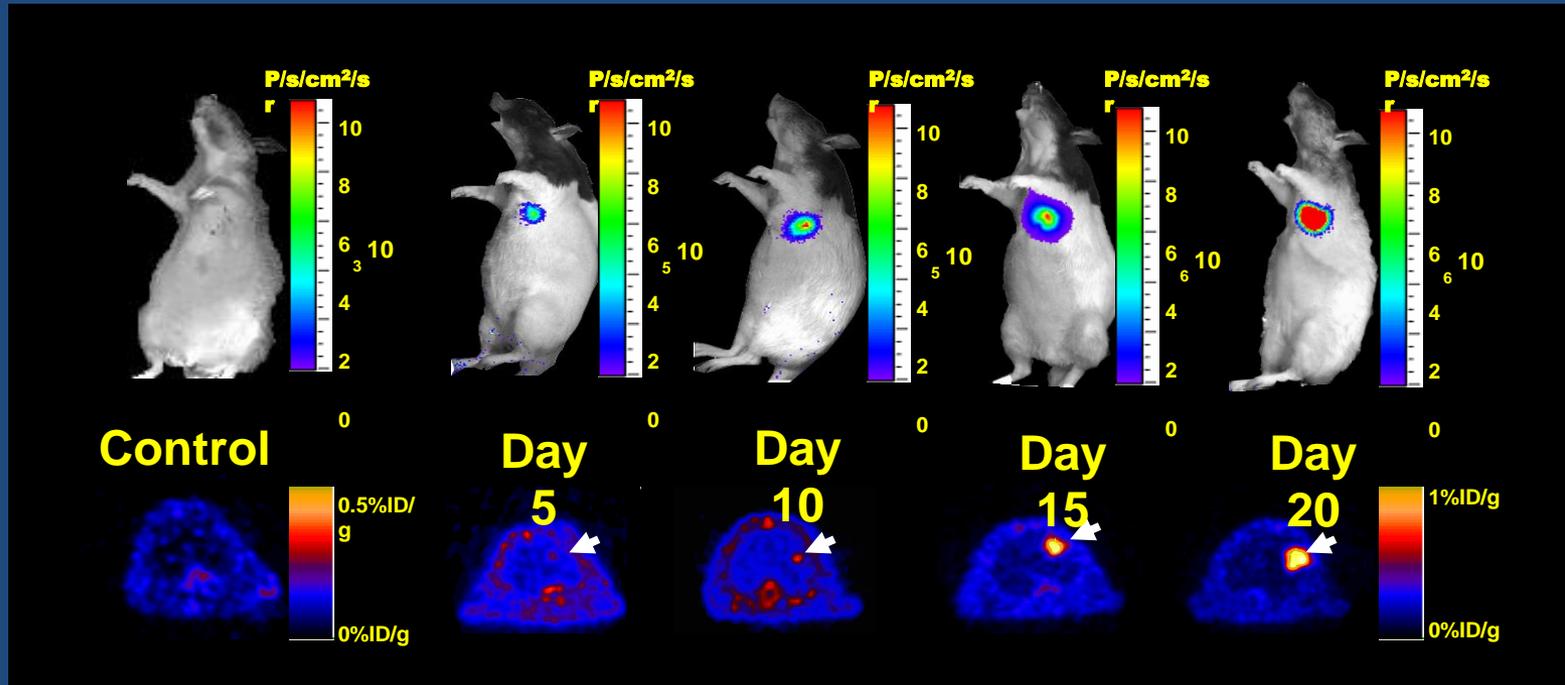


Ray et. al., Cancer Research (March 2004)

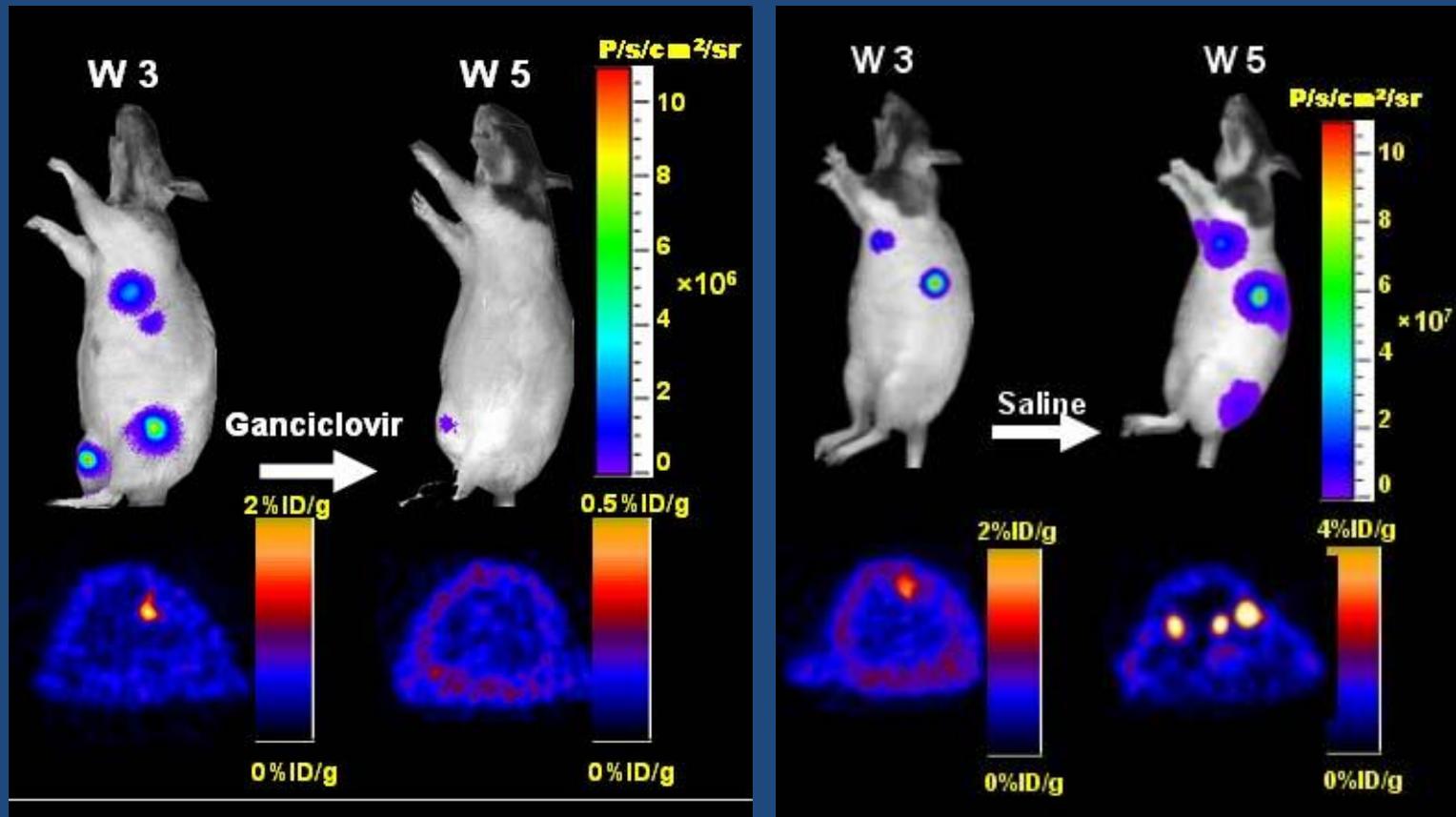
Imaging Trafficking of T Cell Hybridomas Stably Expressing a Triple Fusion Reporter Gene in Collagen Induced Arthritic Mice



Imaging ES Cell Survival & Proliferation

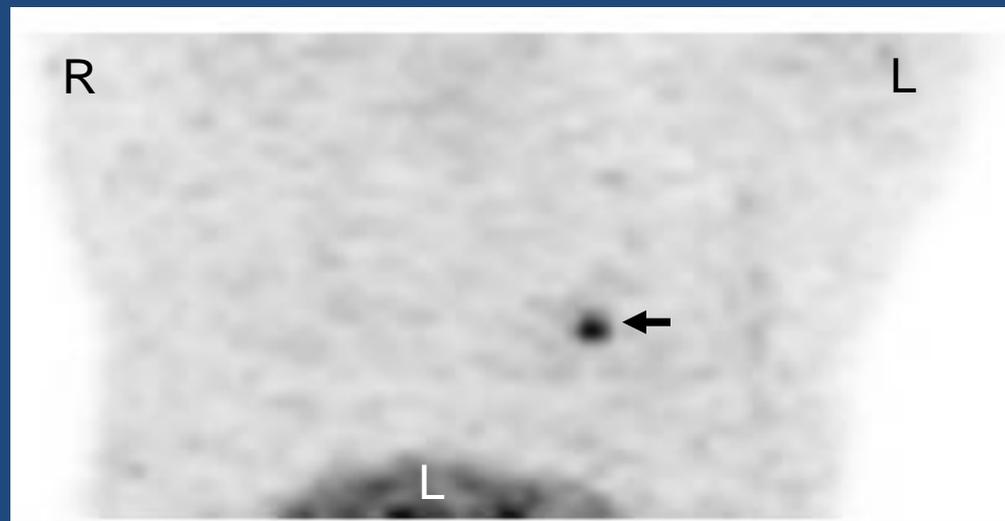
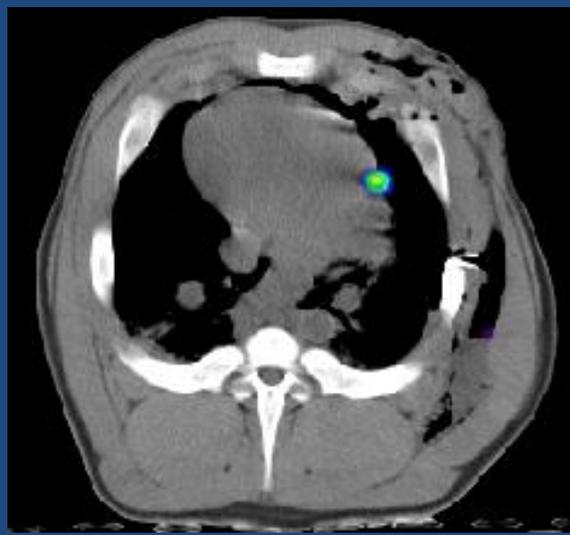
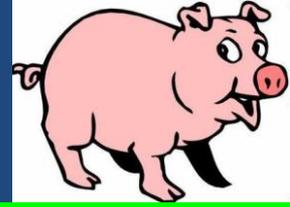


Ablation of Cellular "Misbehavior" with TK Suicide Gene Therapy



Thymidine kinase serves as a PET reporter gene when [^{18}F]-FHBG PET reporter probe is used in pico-nanomolar concentration. Thymidine kinase serves as suicide gene when ganciclovir is administered in milligram dosages.

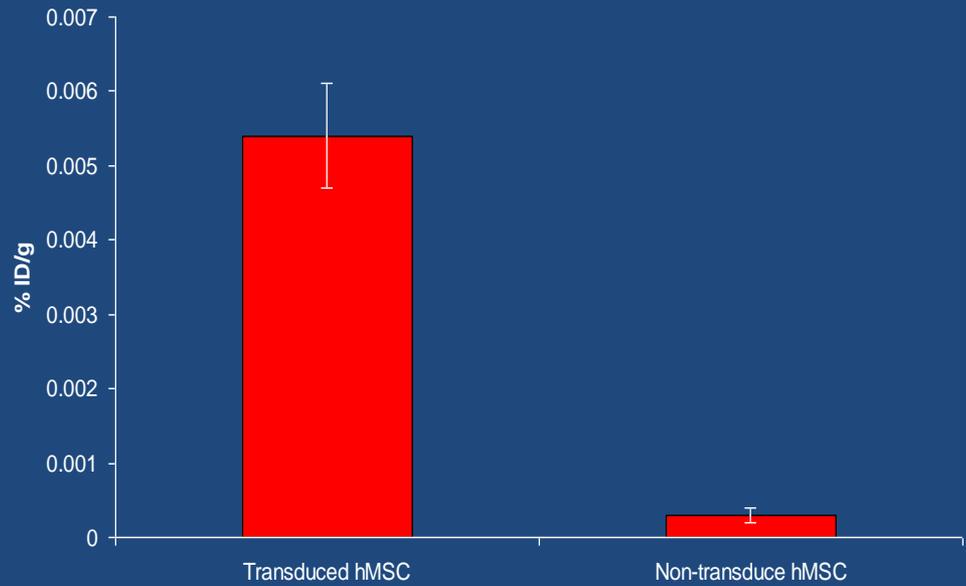
Imaging hMSC Transduced with Adenoviral Vectors Carrying HSV1-sr39tk



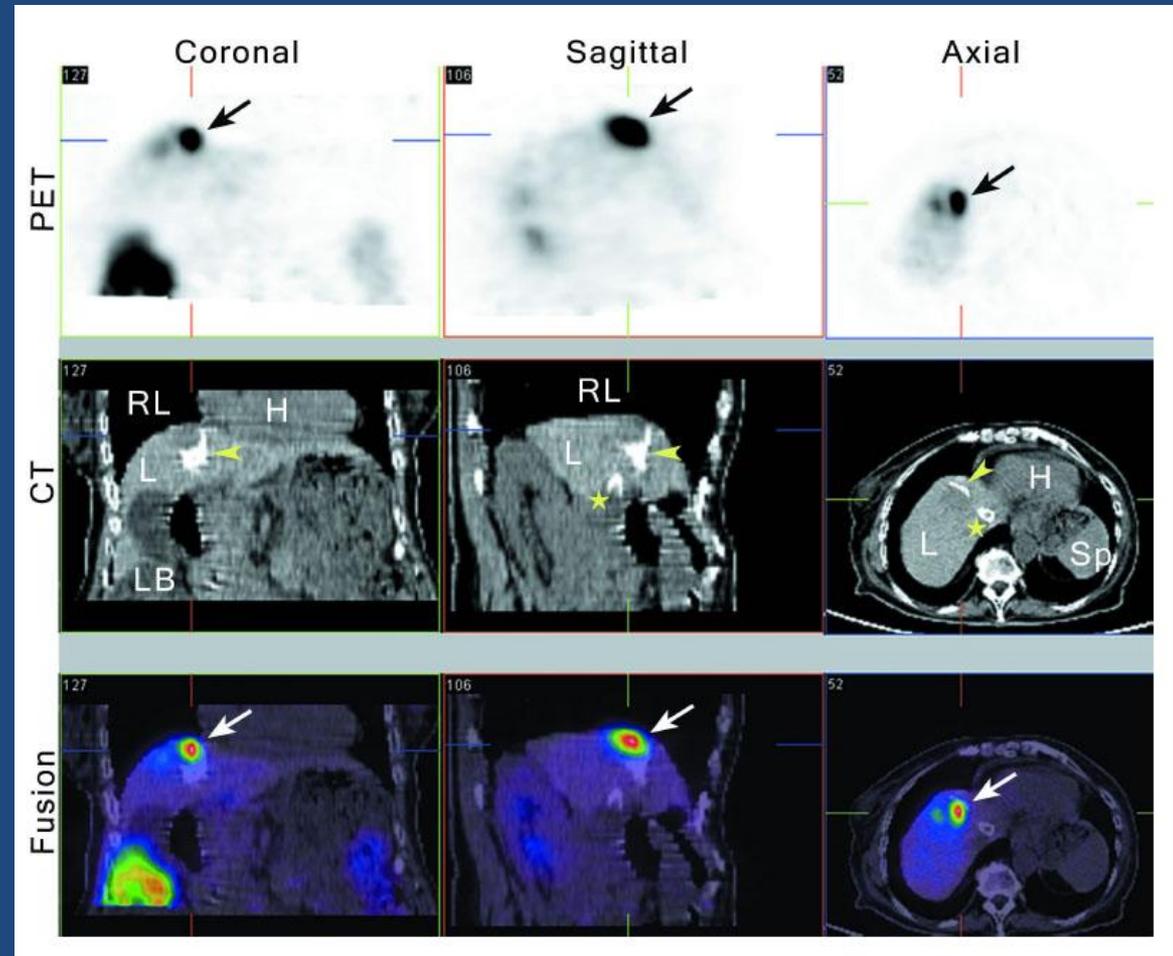
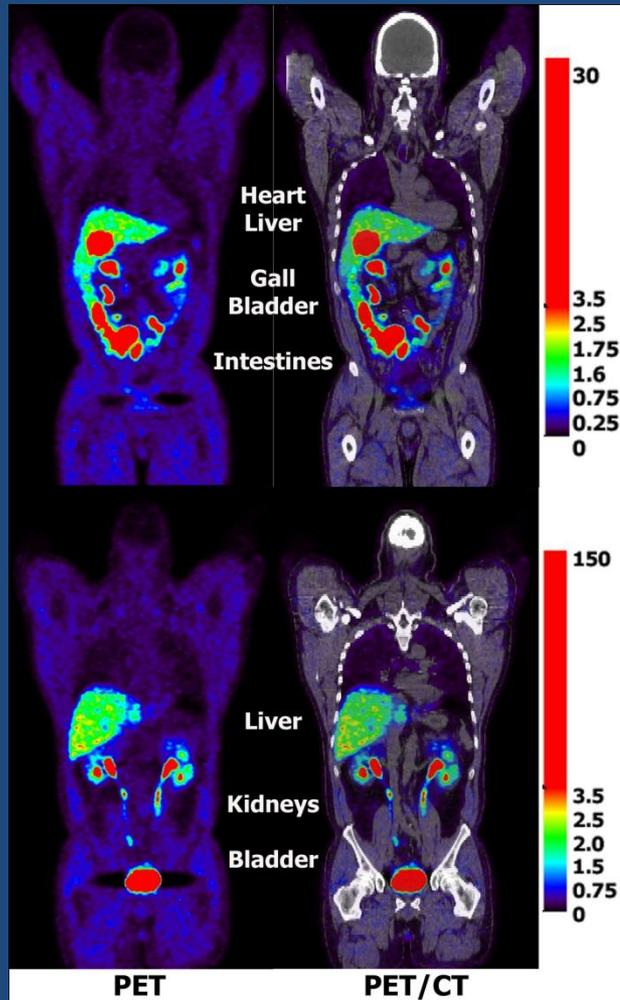
[¹⁸F]FHBG PET/CT [¹⁸F]FHBG



[¹⁸F]FDG

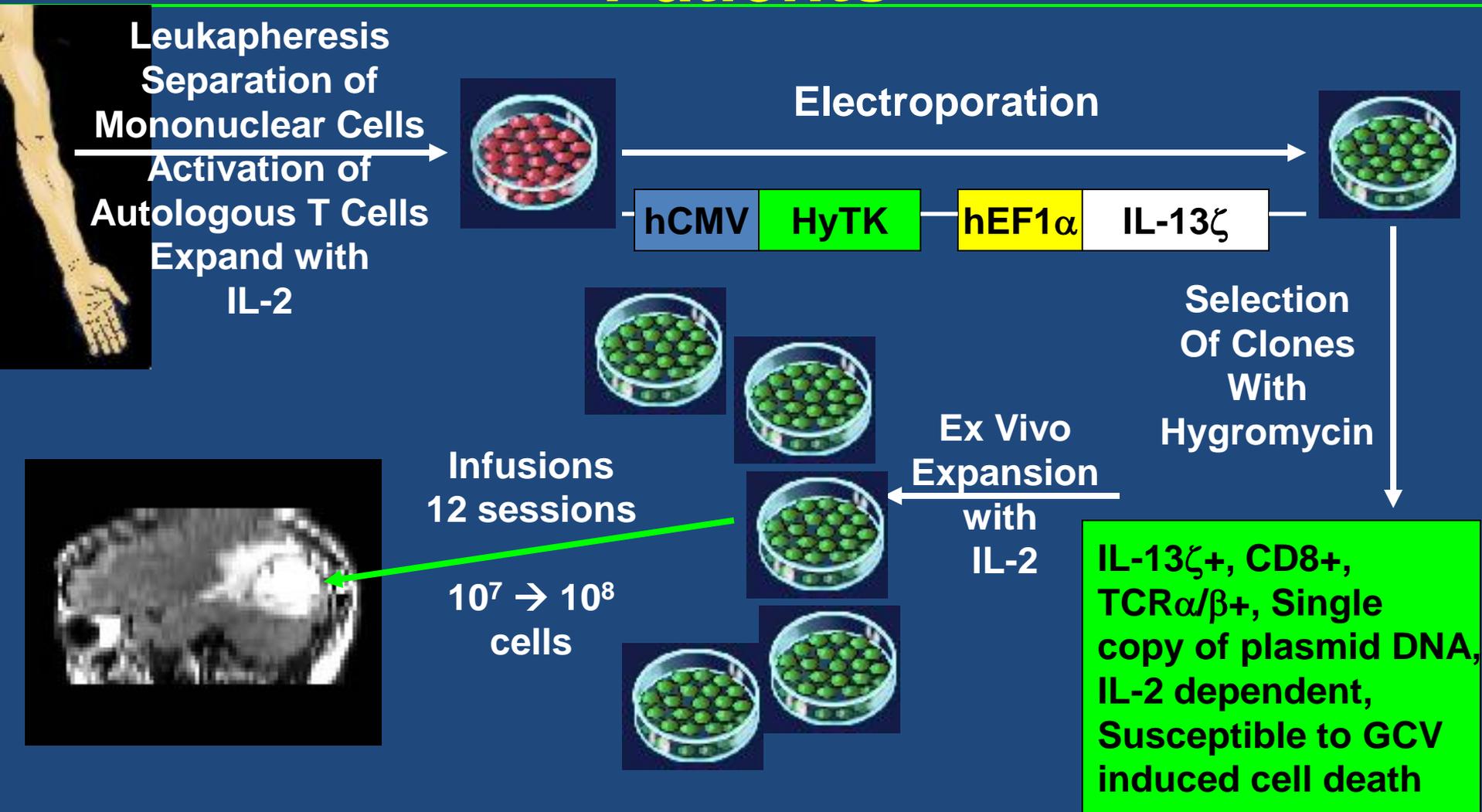


[¹⁸F]FHBG PET Imaging in Humans

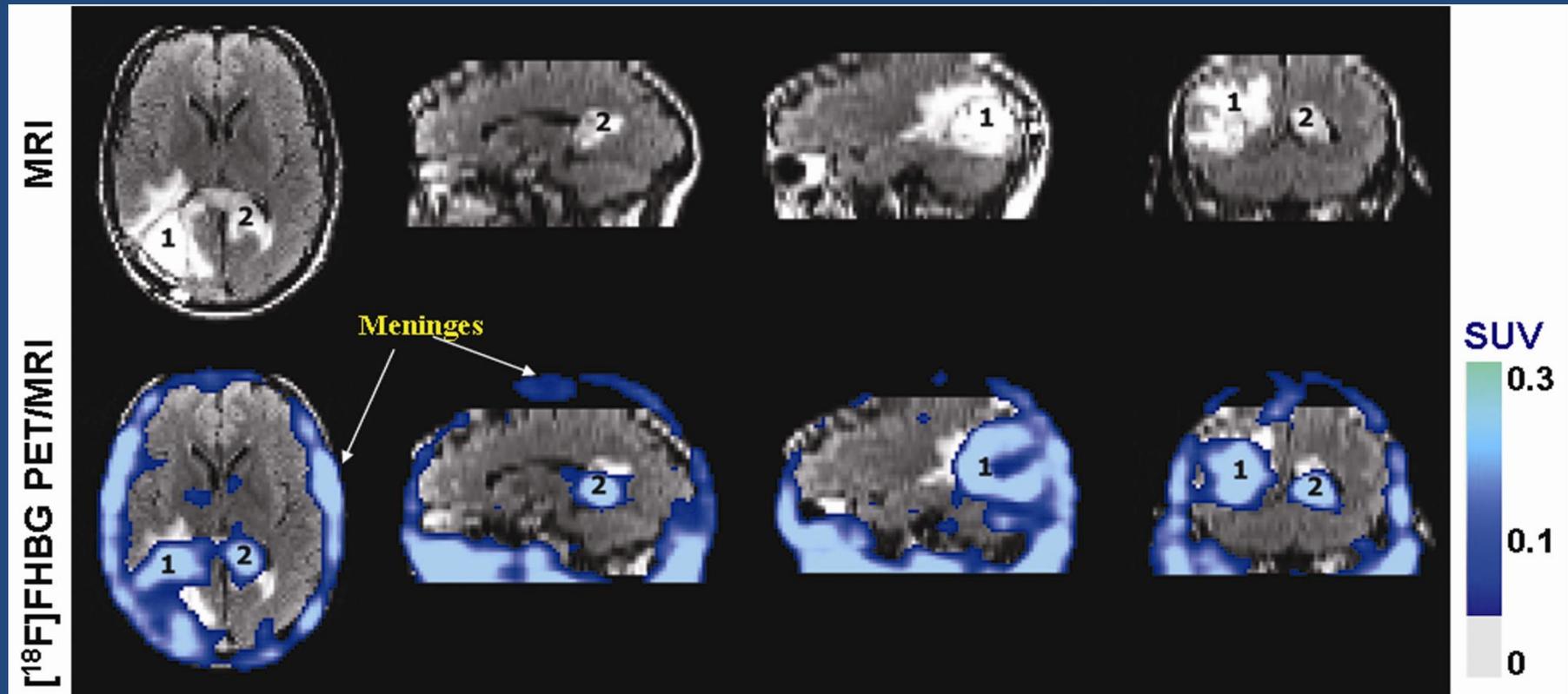


Yaghoubi et al. Nature Clinical Practice Oncology 6(1): 53-58 (2009)
Penuelas . et. al. Gastroenterology 128:1787-95 (2005)

Imaging Cytolytic T Cells in Glioma Patients

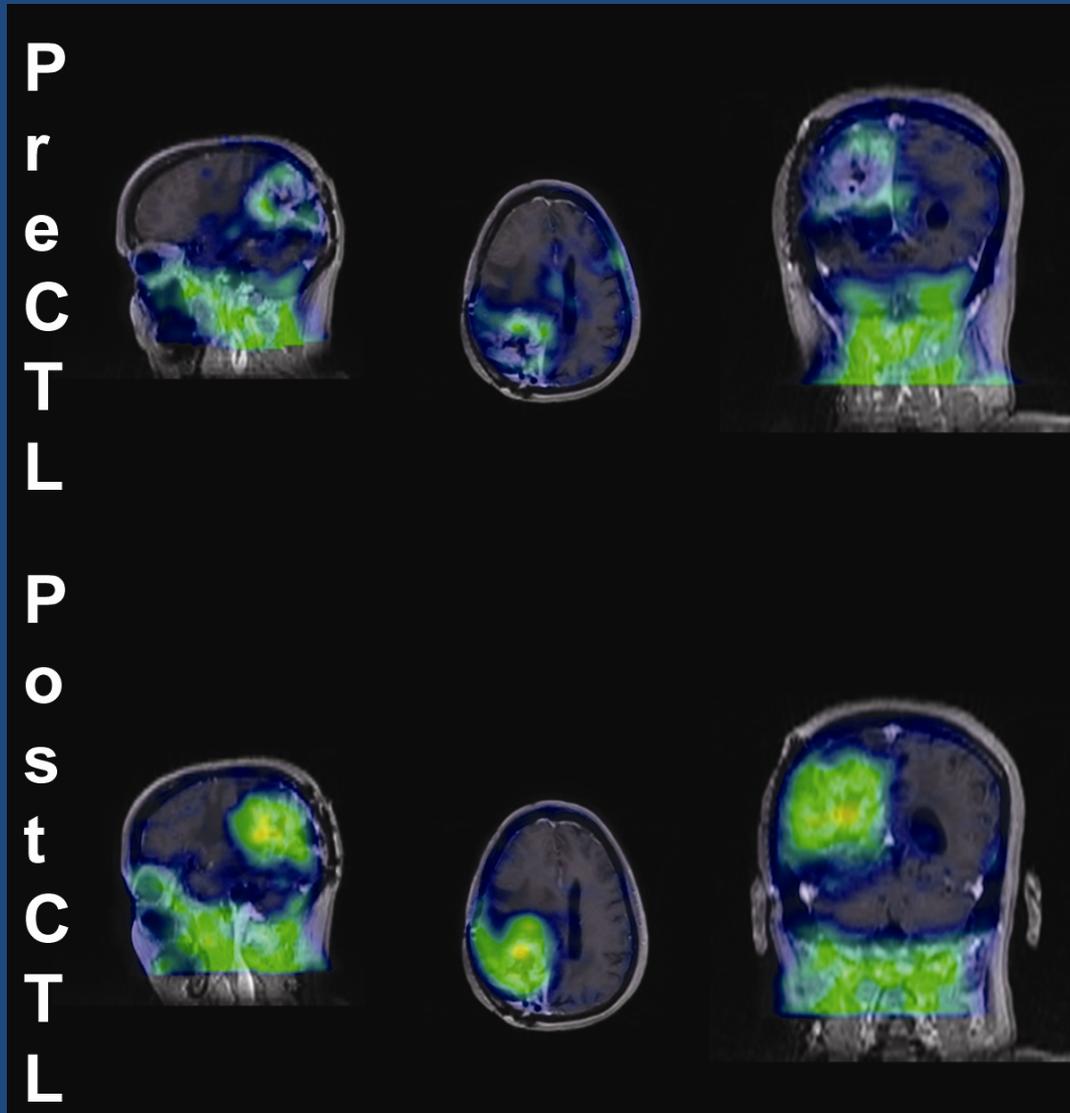


Reporter Gene Based Imaging of Therapeutic Cells (First Clinical Demonstration)

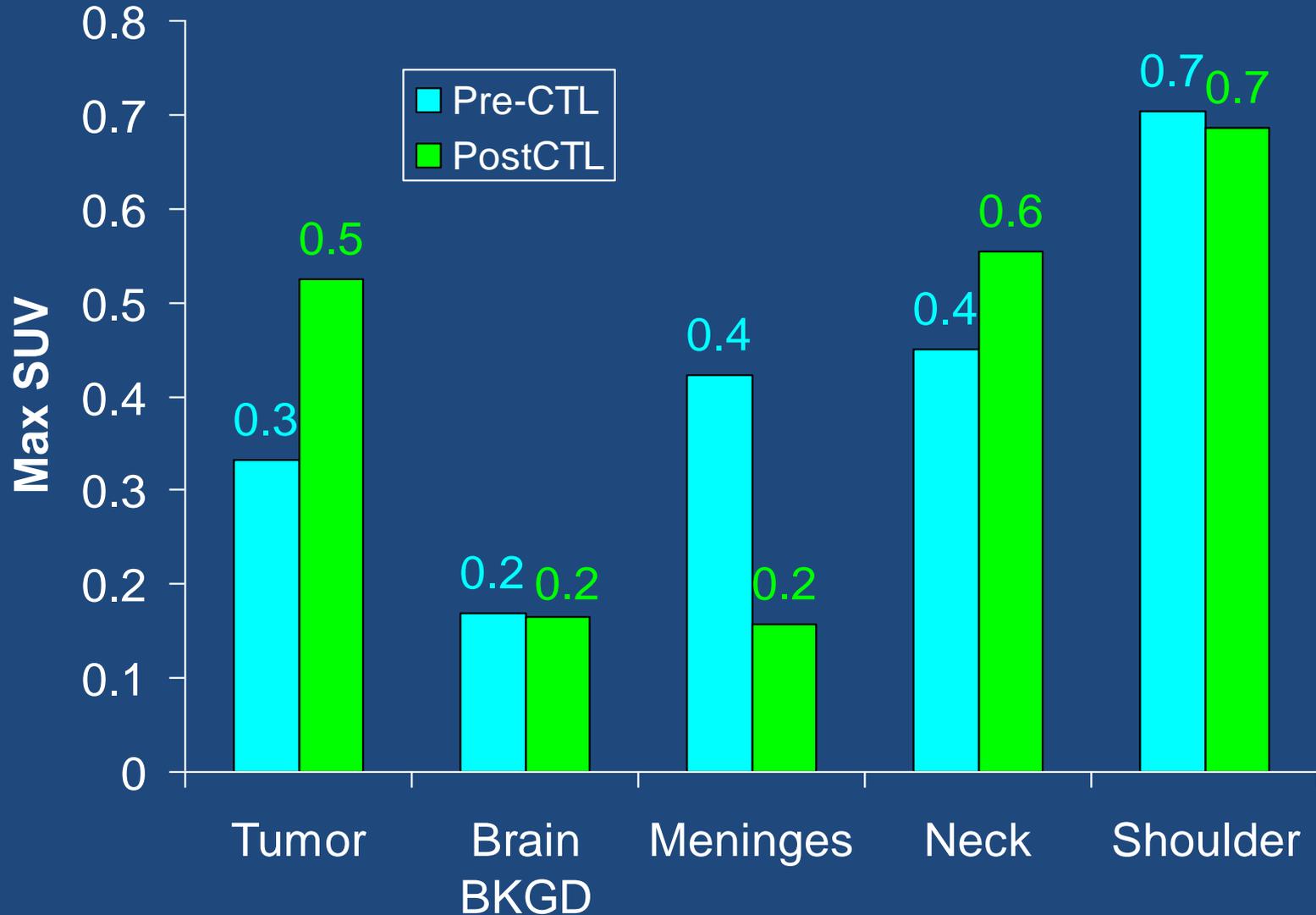


Yaghoubi et al. [Nature Clinical Practice Oncology](#) 6(1):53-58 (2009)

[¹⁸F]FHBG Brain PET Superimposed Over Brain MRI



Standard Uptake Values (SUV) Max Intensity in Regions of Interest (ROI)



Percent Change in [¹⁸F]FHBG Accumulation and Tumor/BKGD [¹⁸F]FHBG Post CTL Infusion

FHBG Accumulation:

Tumor/Tissue Ratios:

Tumor **58%**

Brain BKGD -2%

Meninges -63%

Neck 23%

Shoulder -3%

Tumor/Brain BKGD **61%**

Tumor/Meninges 423%

Tumor/Neck 28%

Tumor/Shoulder 62%

PET/SPECT Probes for Non-Invasive Cell Kinetics Imaging

- ^{64}Cu radiolabeled DOTA conjugated to Anti-Thy1.2 antibodies for pre-clinical T cell trafficking monitoring. [Matsui et al. 2004.](#)
- $^{99\text{m}}\text{Tc}$ radiolabeled humanized Anti-CD3 monoclonal antibody for T cell trafficking monitoring. [Malviya et al. 2009.](#)
- $^{99\text{m}}\text{Tc}$ radiolabeled HYNIC-IL12 for imaging Th1 T lymphocytes. [Annovazzi et al. 2006.](#)
- $[^{18}\text{F}]\text{FAC}$ and $[^{18}\text{F}]\text{F-AraG}$ for imaging T cell activation with PET. [Radu et al. 2008 and Namavari et al. 2010.](#)

Summary & Future Directions

- **Imaging Reporter Genes/Probes are General Tools for Non-Invasively Monitoring All Aspects of the Kinetics of Cells in Living Mammals, Including Humans**
- **Direct Labeling with PET/SPECT Cell Tracking Probes is Also a General Tool, Allowing Non-Invasive Imaging of Cell Biodistribution for a Limited Period of Time Following Administration Into Living Subjects**
- **Specific PET and SPECT Probes are Available for Imaging T cells and T cell Activation**
- **Incorporating Non-Invasive Imaging Into Cell Therapy Trials Should Help Optimize Protocols and Predict Efficacy or Potential Adverse Effects**

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Patient Volunteers