Stem Cell Tracking in Neurologic Applications

Jeff W.M. Bulte, Ph.D.

Professor of Radiology, Biomedical Engineering,
and Chemical & Biomolecular Engineering

Director, Cellular Imaging Section
Institute for Cell Engineering
The Johns Hopkins University School of Medicine
Baltimore, MD, USA

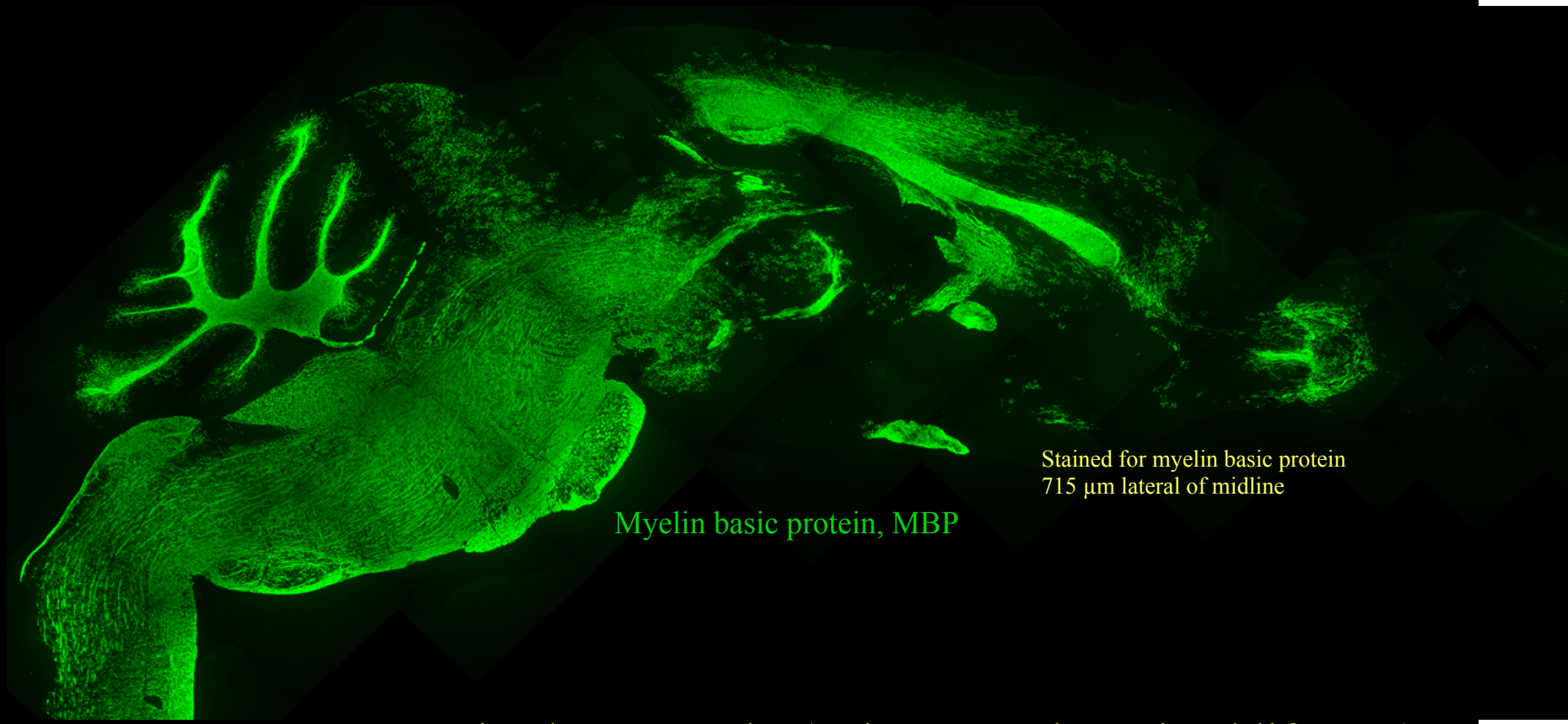
Neural Stem Cell Transplantation:
The Case of the Shiverer Dysmyelinated Mouse Brain

90 days, untreated

300 days, stem cell injection

Windrem & Goldman, Cell Stem Cell 2, 553-565 (2008).

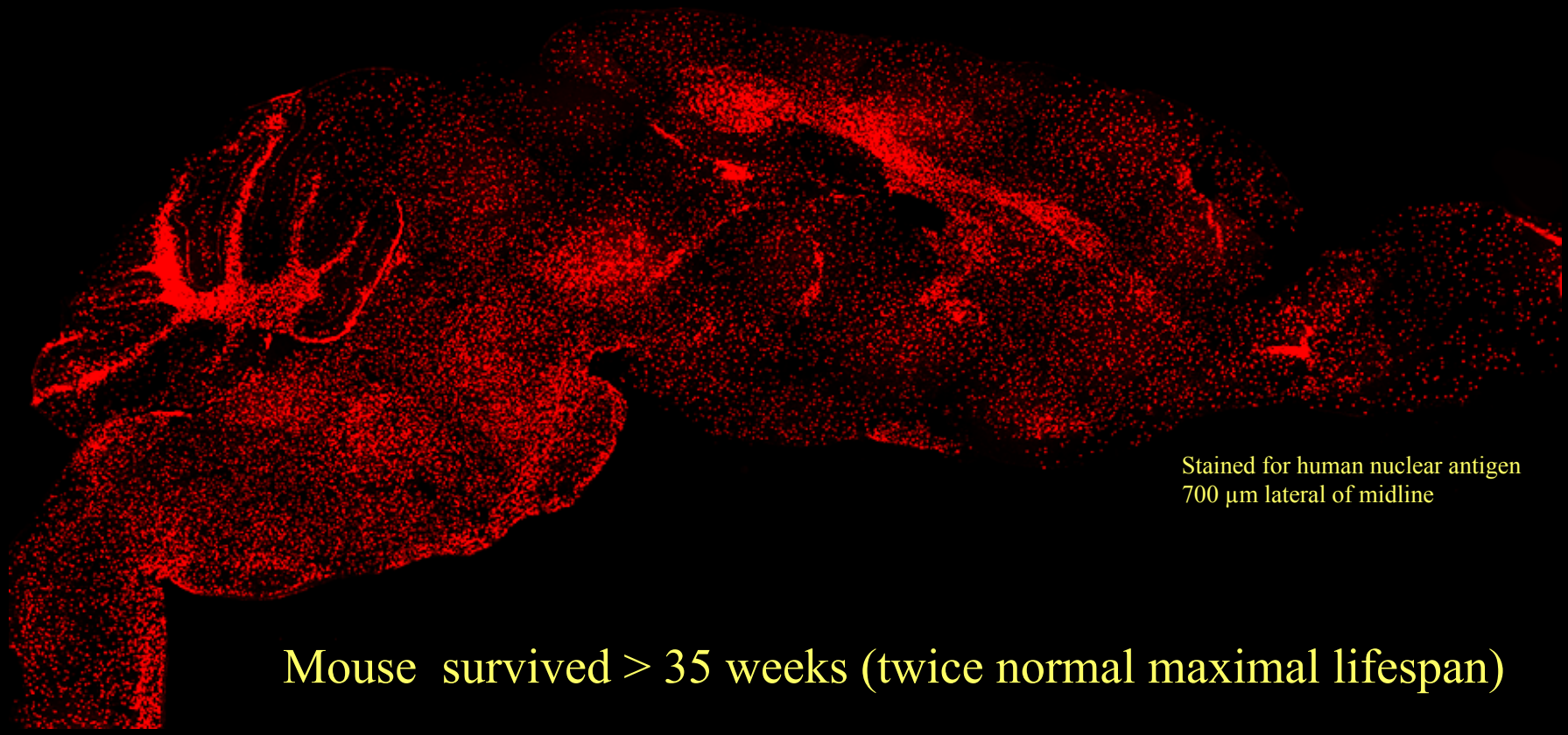
Human fetal glial progenitor cells engrafted at birth into shiverer mice



Mouse survived > 35 weeks (twice normal maximal lifespan)

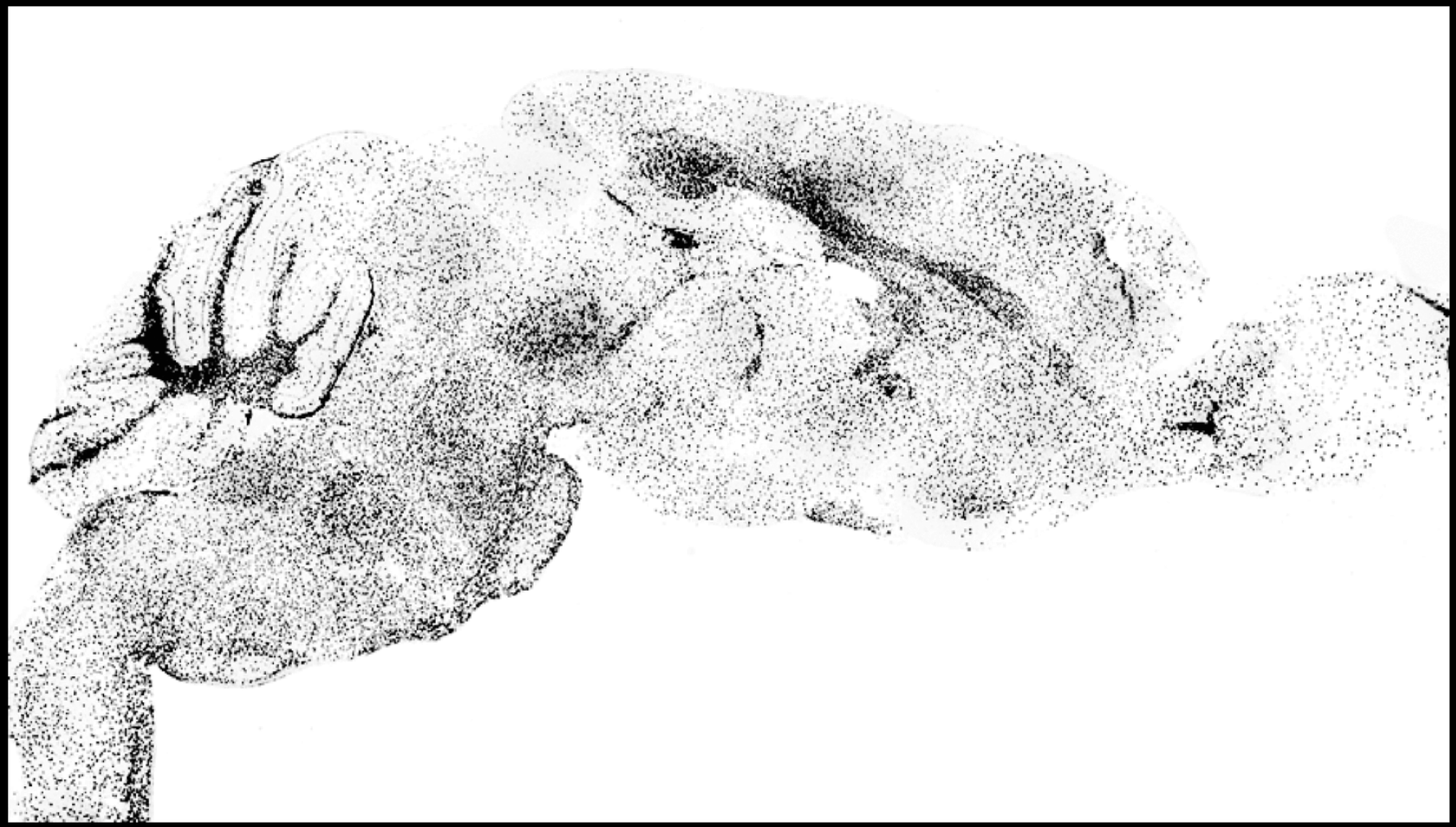
Windrem & Goldman, Cell Stem Cell 2, 553-565 (2008).

Human fetal glial progenitor cells engrafted at birth into shiverer mice



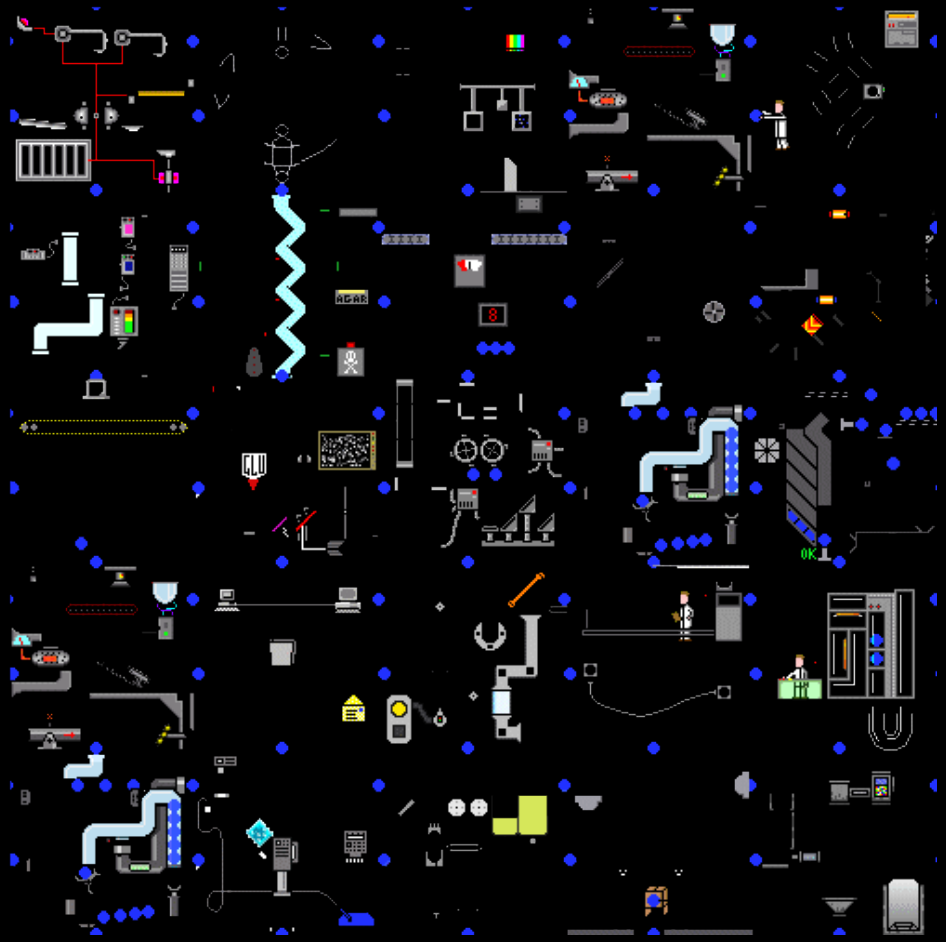
Mouse survived > 35 weeks (twice normal maximal lifespan)

Windrem & Goldman, Cell Stem Cell 2, 553-565 (2008).



JOHNS HOPKINS
MEDICINE

Neurorepair According to an Imaging Scientist



JOHNS HOPKINS MEDICINE

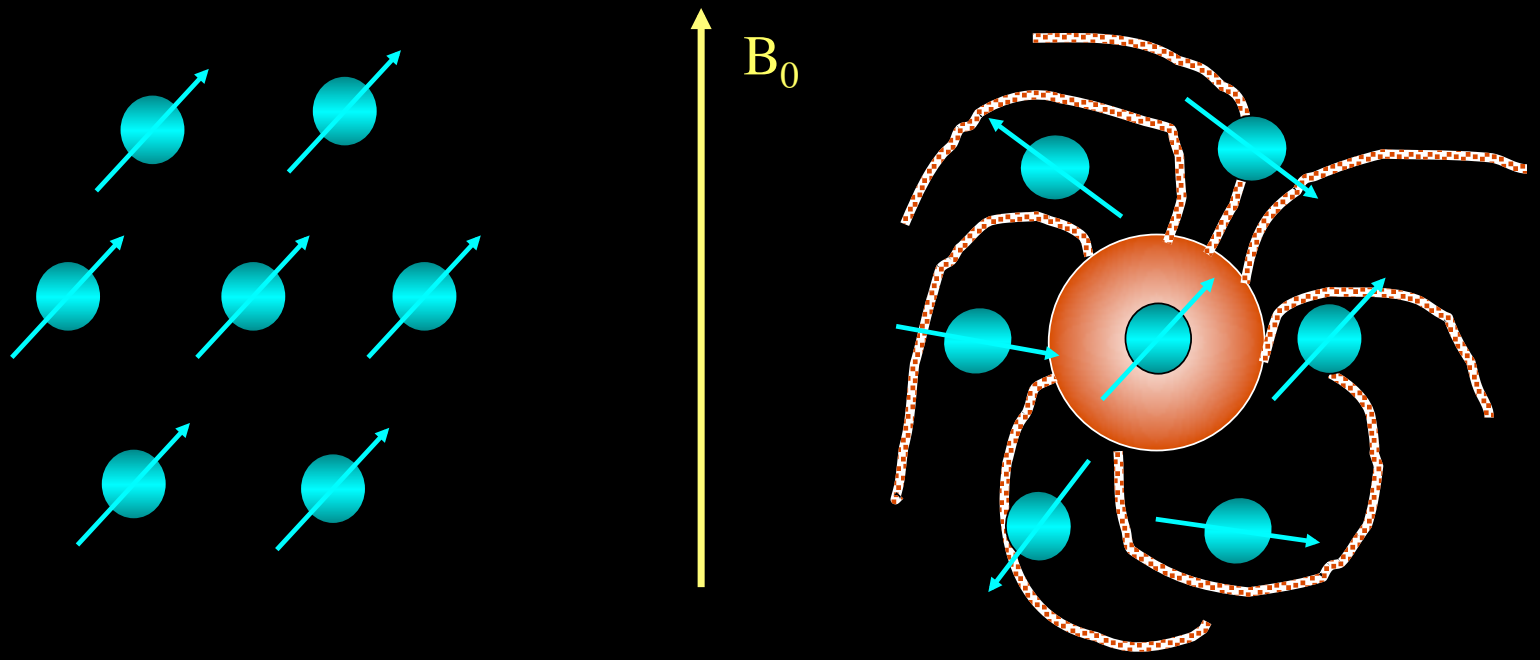
Molecular and Cellular Imaging Modalities

	Clinical Gen/Cells		Reso lution	Sensit. n cells	Anatomy	Long evity	Reporter gene	Quant.
Optical	endo	no	+++	?	no	+++	yes	no
NIR	no	no	+	?	no	?	no	no
BLI	no	no	+	10,000	no	+++	yes	~yes
PET	yes	yes	+	50,000	no	+/+/+	yes	~yes
ϒ-rad	yes	yes	++	?	no	+	~~	YES
^{1}H MRI	yes	yes	++++	1-1000	yes	++	yes	~no
^{19}F MRI	no	no	++	7,500	no	++	no	YES
CEST MRI	yes	no	+++	25,000	yes	+++	yes	~yes
X-ray	yes	no	++++	high n	yes	++	no	~yes
US	yes	no	+	1	yes	++	no	~yes

JOHNS HOPKINS
MEDICINE

MAGNETIC "DYE"

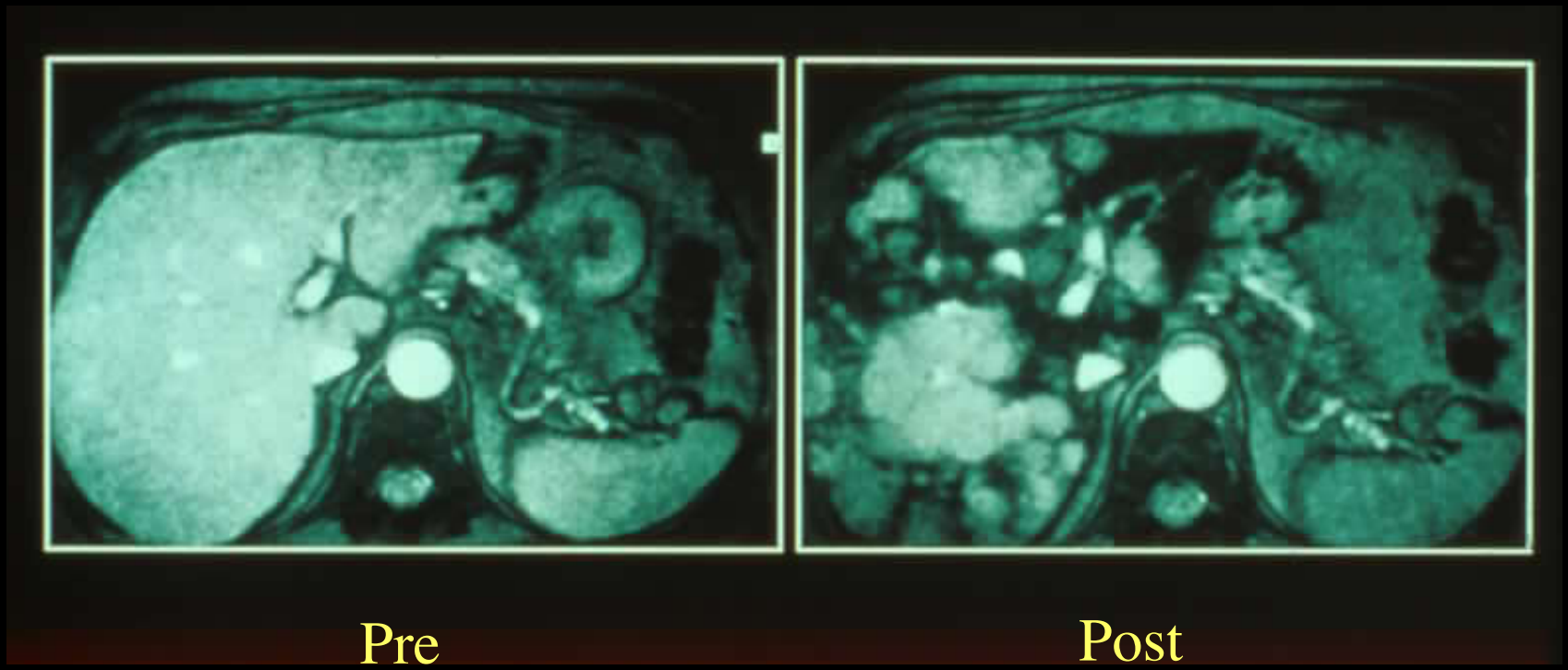
Dextran-Coated Superparamagnetic Iron Oxide Particles (SPIO):
Microscopic Magnetic Field Inhomogeneities
Induce Rapid Dephasing of Proton Spins
and Loss of Imaging Signal



LLS size: ~80 nm

JOHNS HOPKINS
MEDICINE

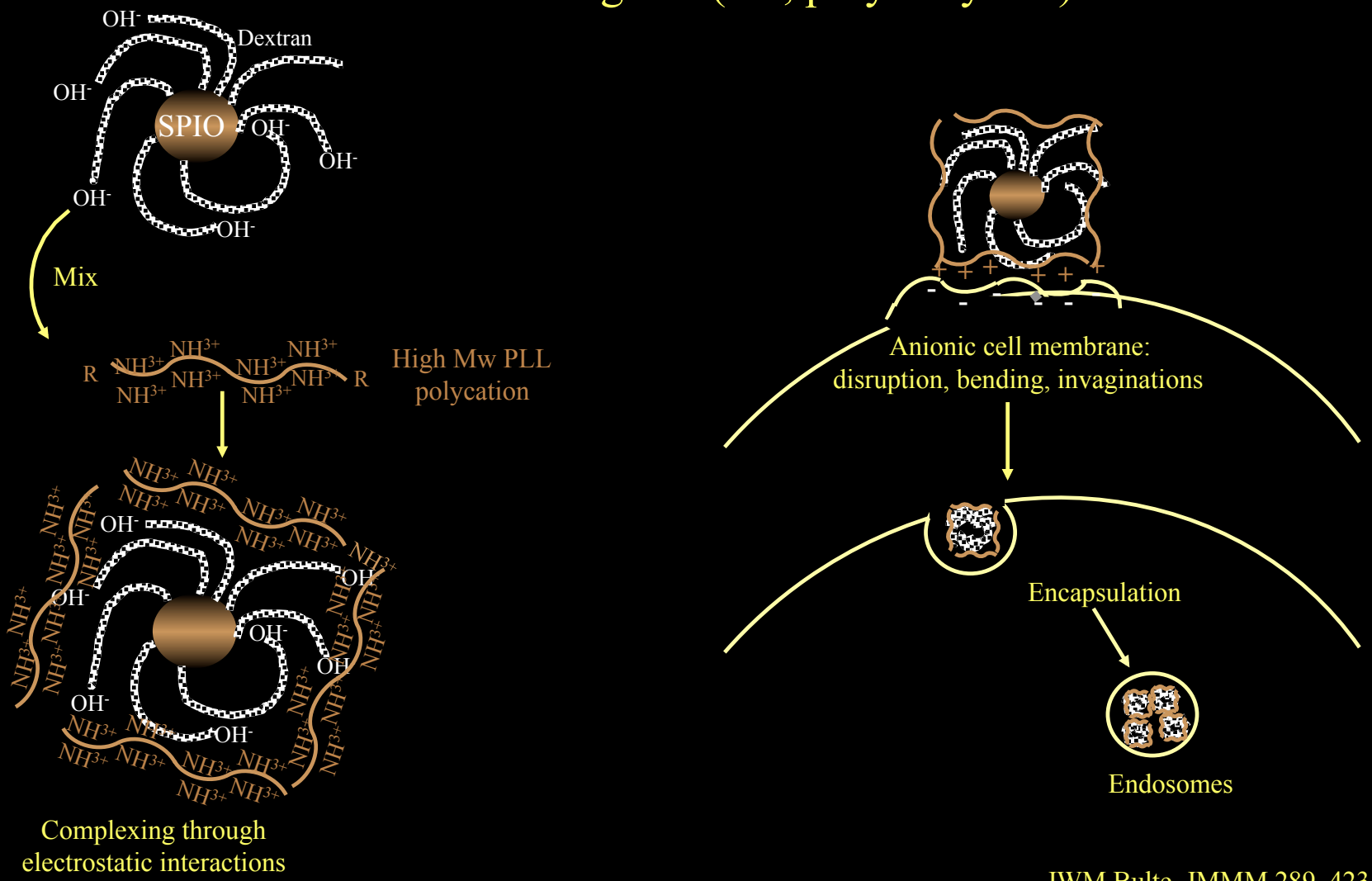
Feridex®: FDA-approved SPIO since 1996 (as Liver Agent, not for Cell Tracking) same as Endorem® in Europe



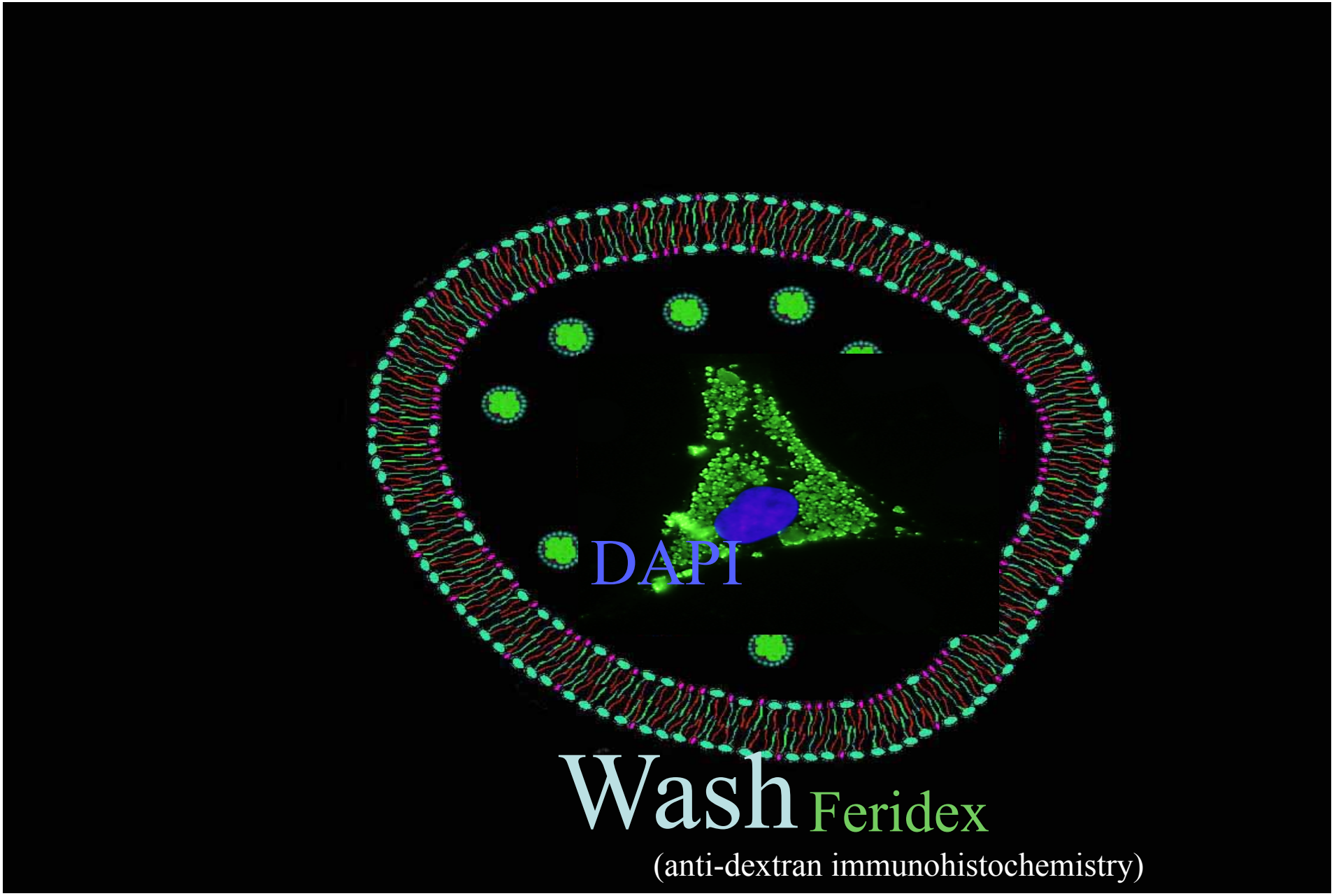
Pre

Post

Intracellular Magnetic Labeling using Transfection Agents (i.e., poly-L-lysine)

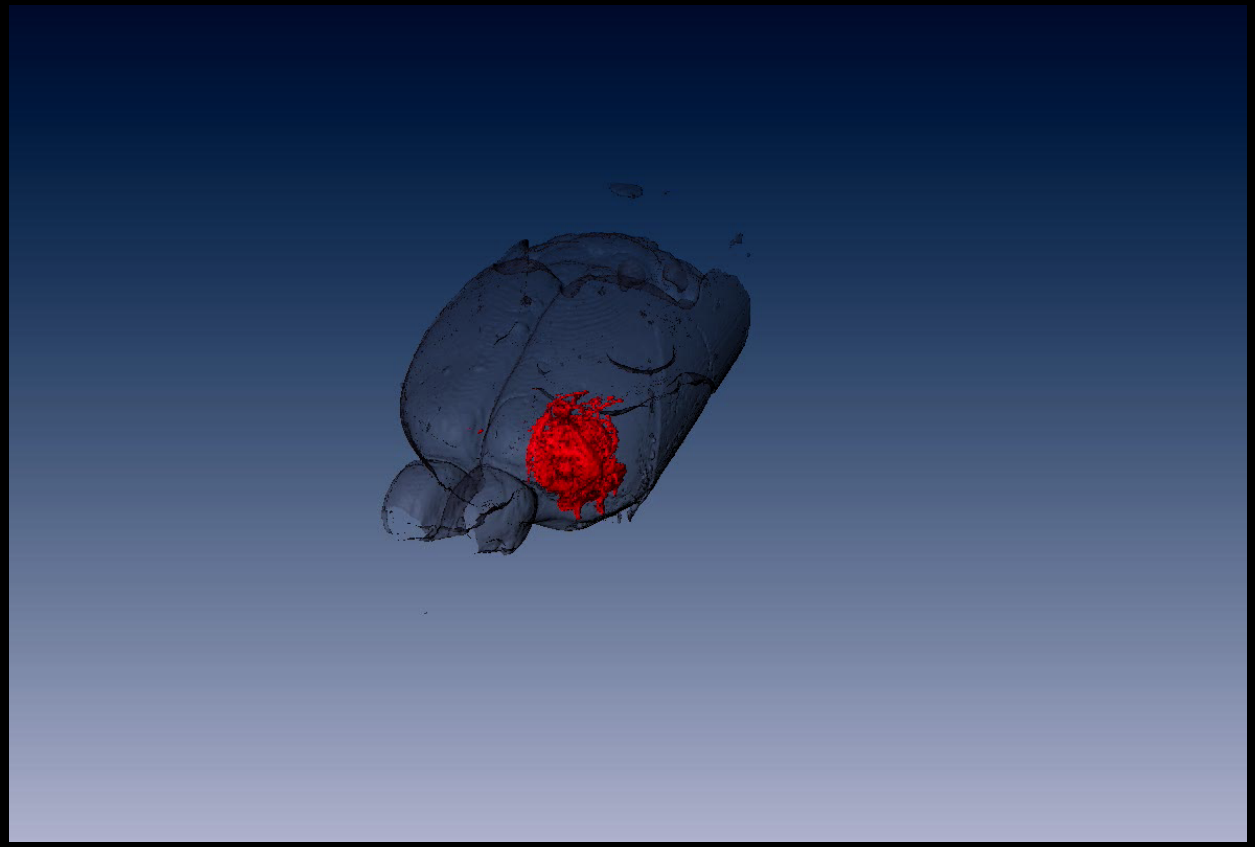


JWM Bulte, JMMM 289, 423 (2005).


DAPI
Wash Feridex
(anti-dextran immunohistochemistry)

JOHNS HOPKINS
MEDICINE

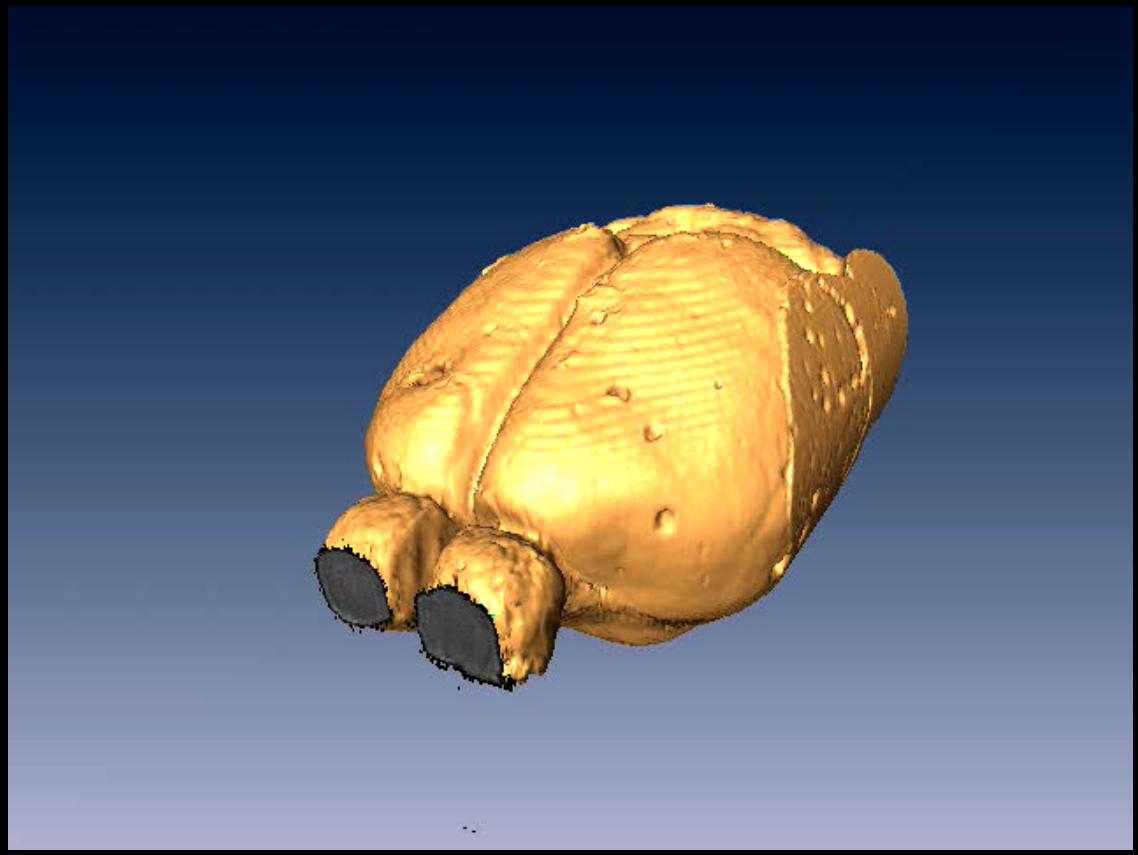
MR Imaging of Magnetically Labeled Neural Stem Cells



P. Walczak et al. Nanomedicine 2, 89-94, 2006

JOHNS HOPKINS
MEDICINE

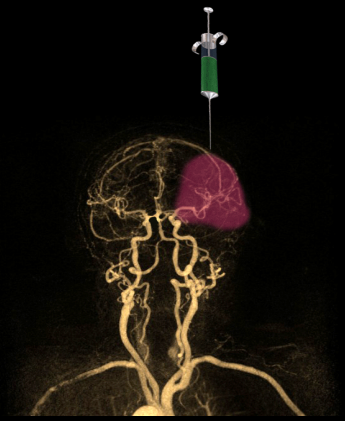
MR Imaging of Magnetically Labeled Neural Stem Cells



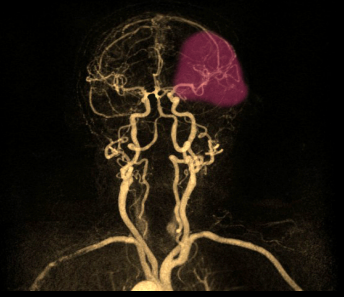
P. Walczak et al. MRM 54, 769-774, 2005.

JOHNS HOPKINS
MEDICINE

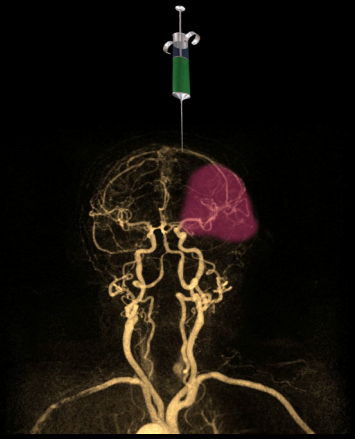
Routes of Cell Delivery



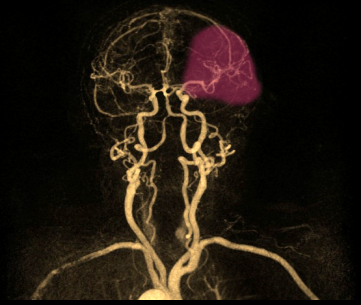
Stereotactic parenchymal injection



Intravenous infusion



Intraventricular injection



Targeted intra-arterial infusion

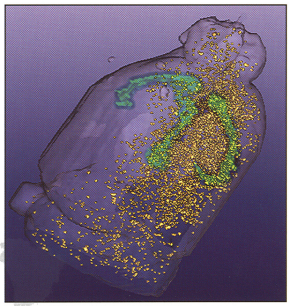
JOHNS HOPKINS
MEDICINE

Volume 39, Number 5, May 2008
ISSN 0039-2499
http://stroke.ahajournals.org

American Heart Association. | American Stroke Association.
Learn and Live.

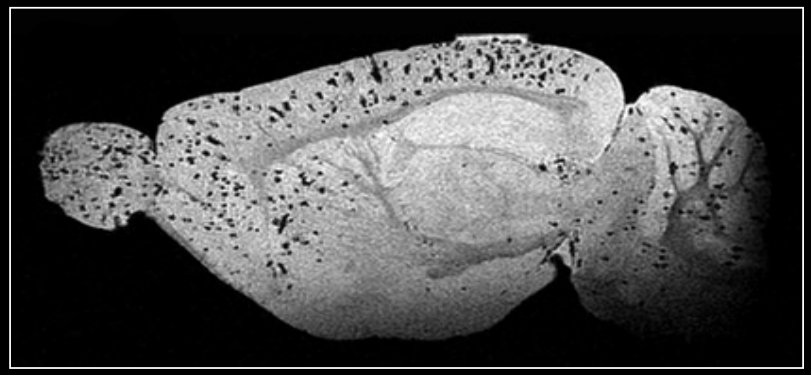
Stroke

JOURNAL OF THE AMERICAN HEART ASSOCIATION

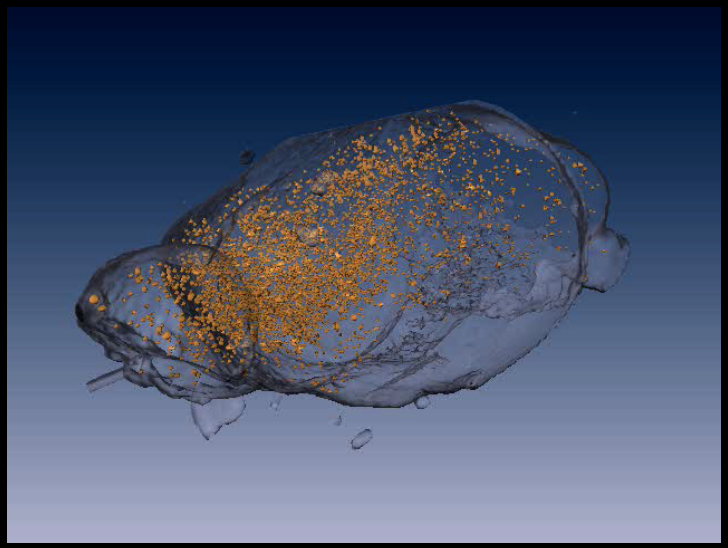


Targeted Intracarotid Delivery of Magnetically Labeled Mesenchymal Stem Cells

2D



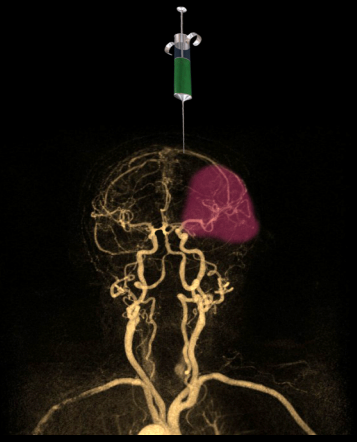
3D



P. Walczak et al., Stroke, 39, 1569-1574, 2008.

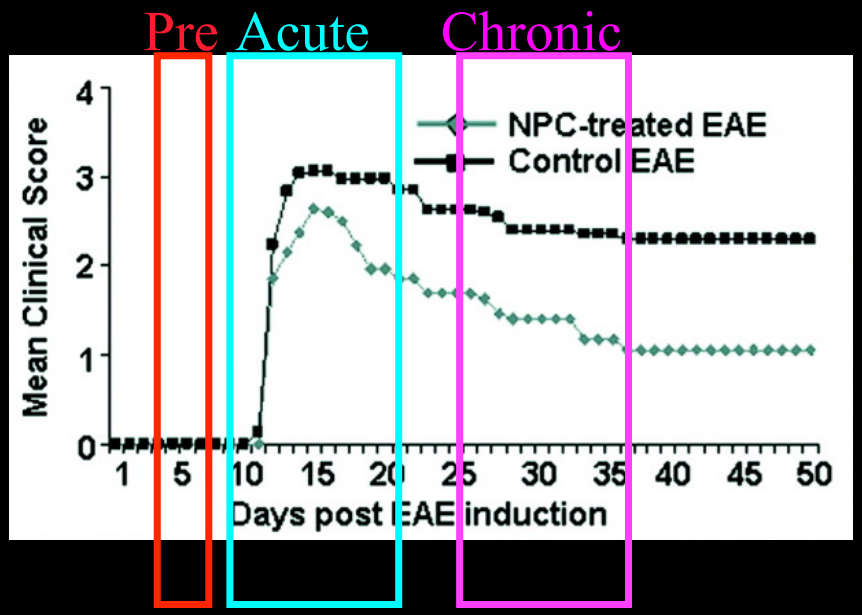
JOHNS HOPKINS
MEDICINE

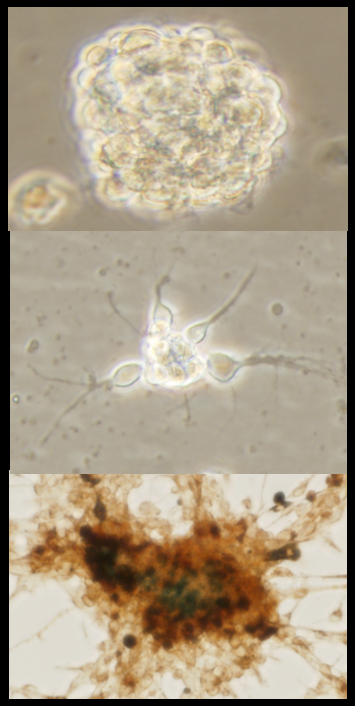
Our Injection Approach for EAE: Disseminate Cells throughout the entire Neuroaxis



Intracerebroventricular injection

Tx of Feridex-labeled neuro-spheres at various stages of EAE





5,000 Feridex labeled neurospheres injected ICV

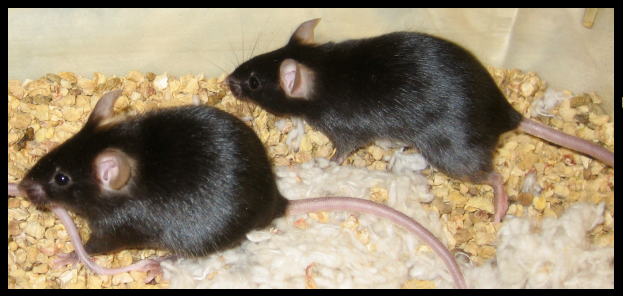
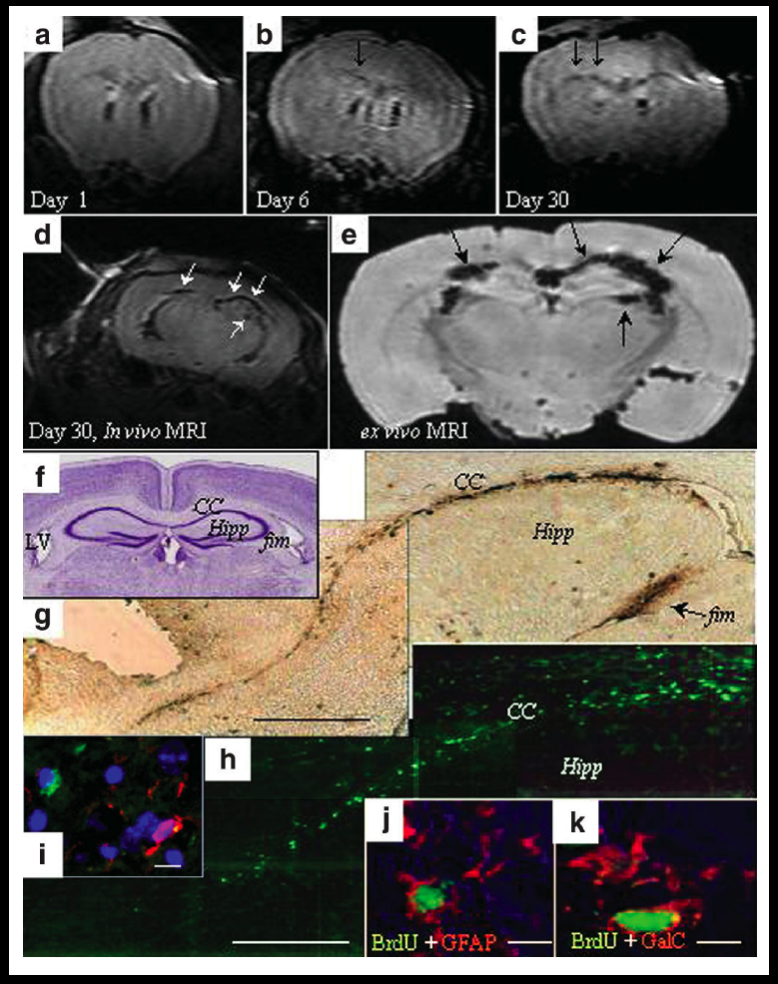


Image CNS *in vivo* at 9.4T after cell tx

Days 1, 4, 7, 14, and 28.

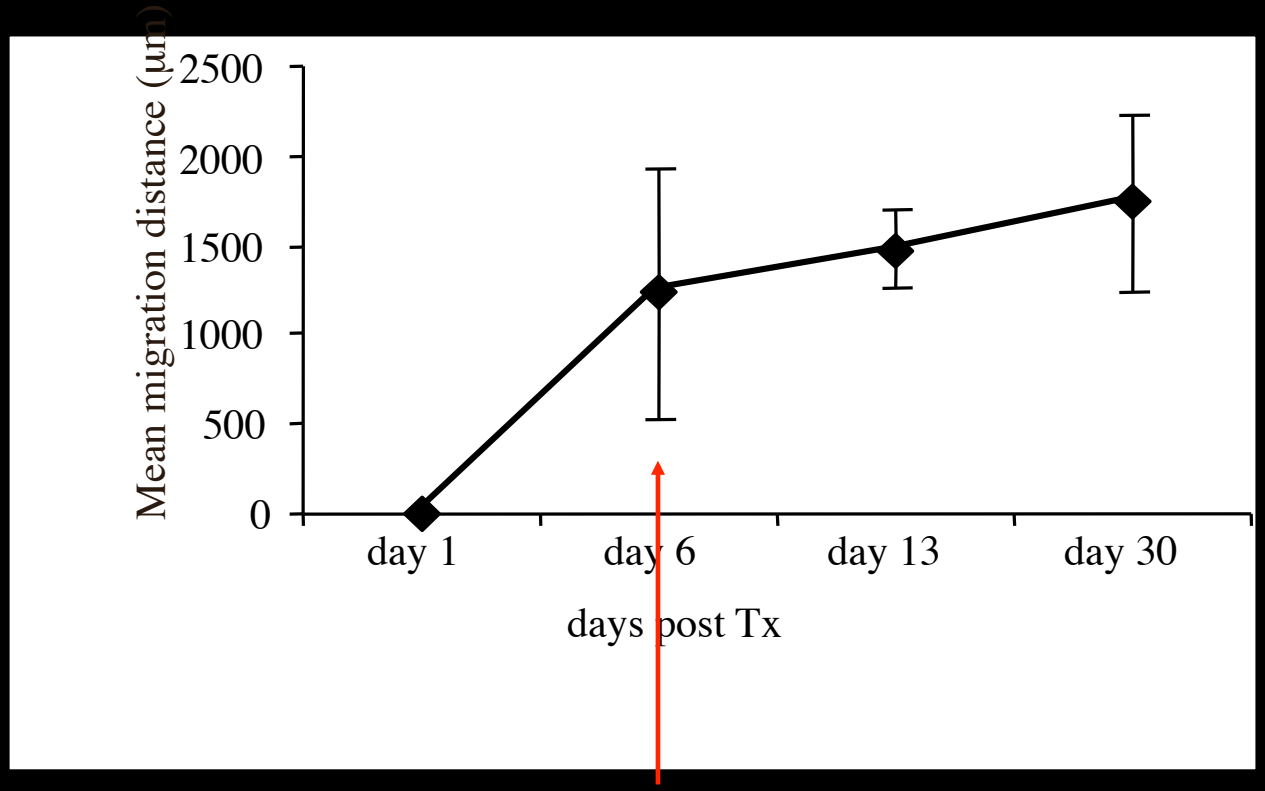
Tx during Pre-onset of EAE



T. Ben-Hur et. al., Magn. Reson. Med. 57, 164-171 (2007).

JOHNS HOPKINS
MEDICINE

Most migration occurs during the first days after transplantation, and within the first days of clinical disease

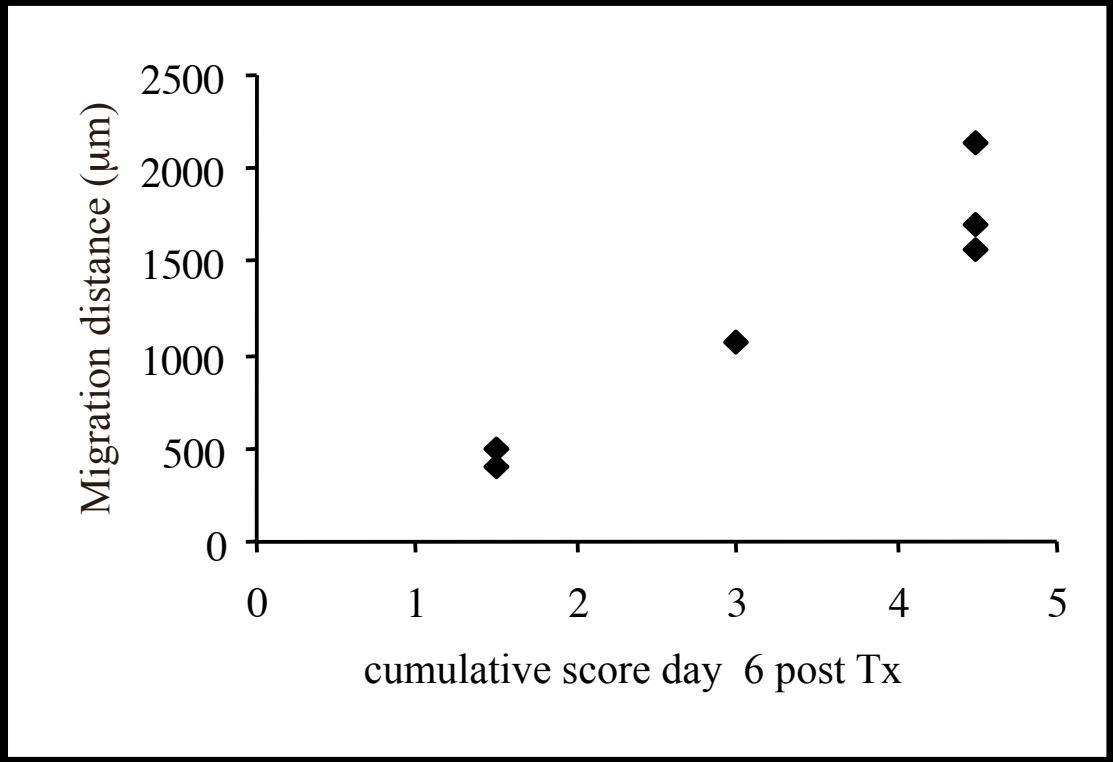


Variability at day 6

T. Ben-Hur et. al., Magn. Reson. Med. 57, 164-171 (2007).

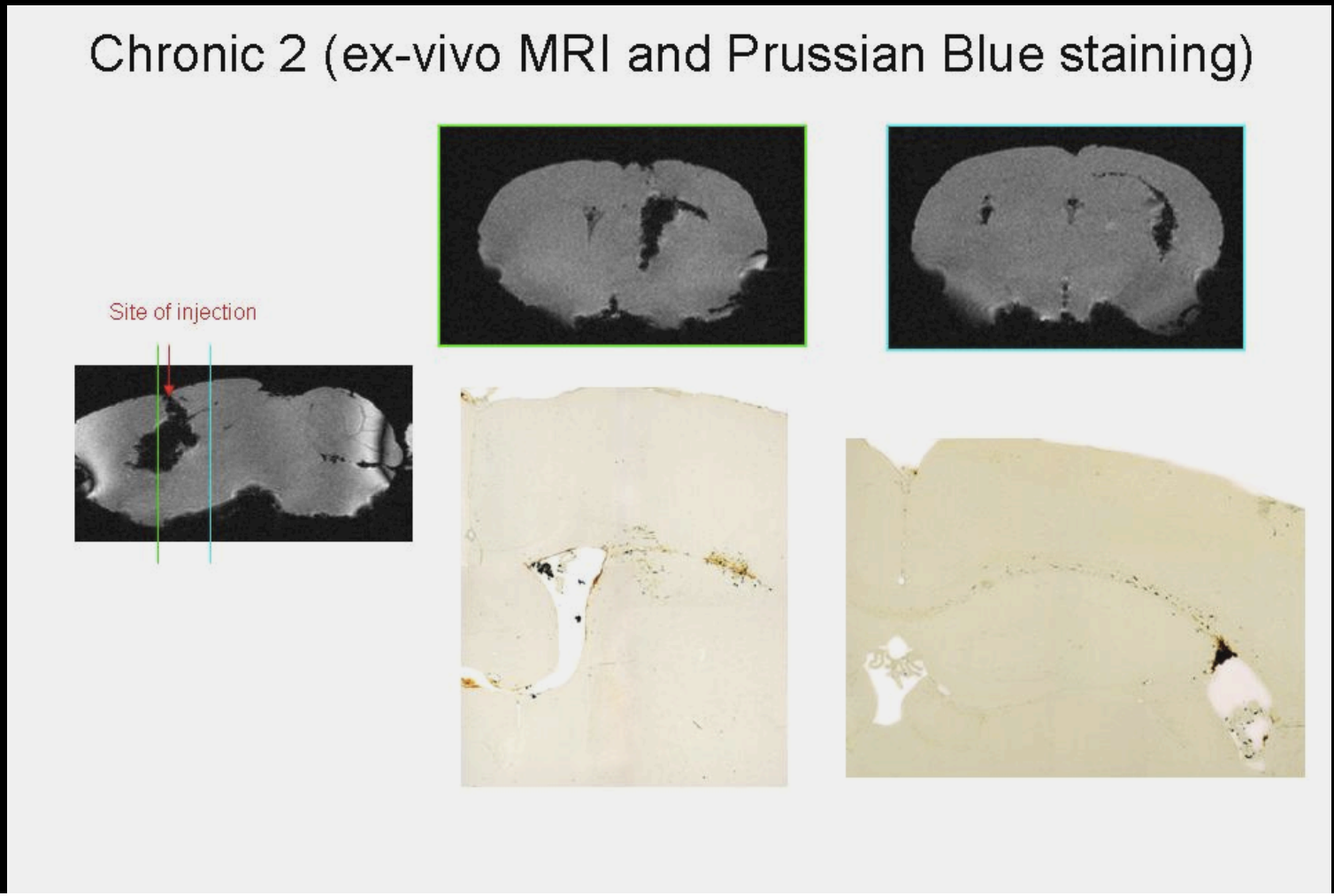
JOHNS HOPKINS
MEDICINE

Good correlation between cumulative disease score and distance of MRI migration during the first week post Tx

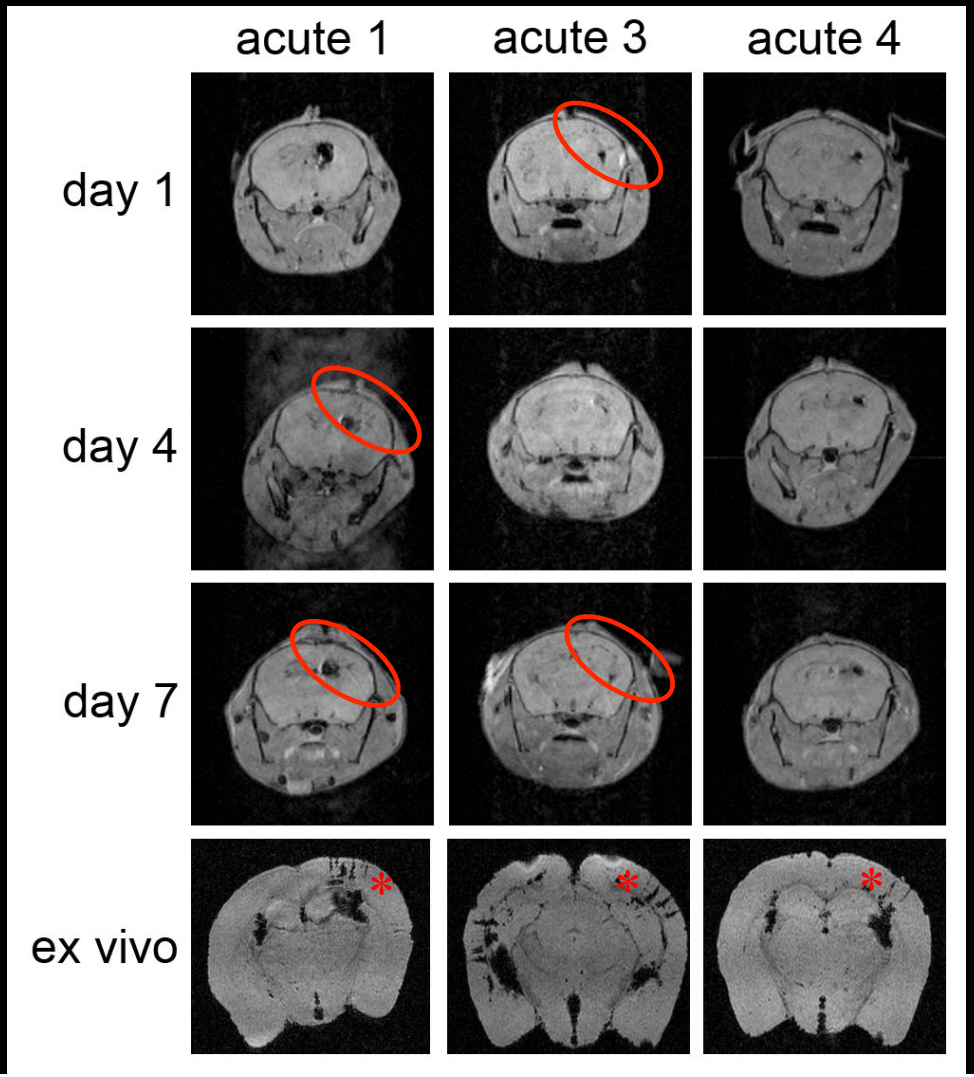


T. Ben-Hur et. al., Magn. Reson. Med. 57, 164-171 (2007).

Tx During Chronic Phase of EAE

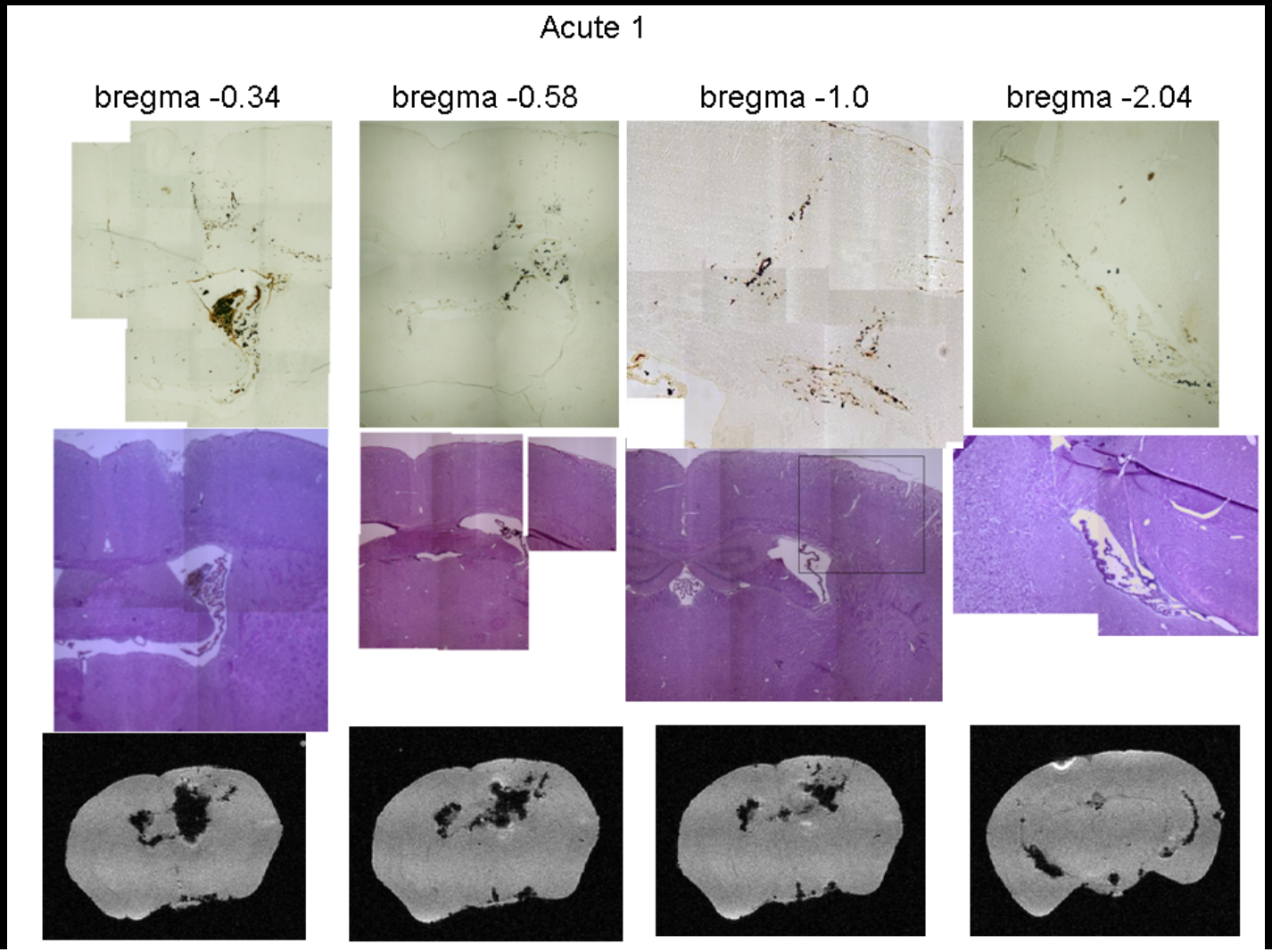


Tx During Acute Phase of EAE

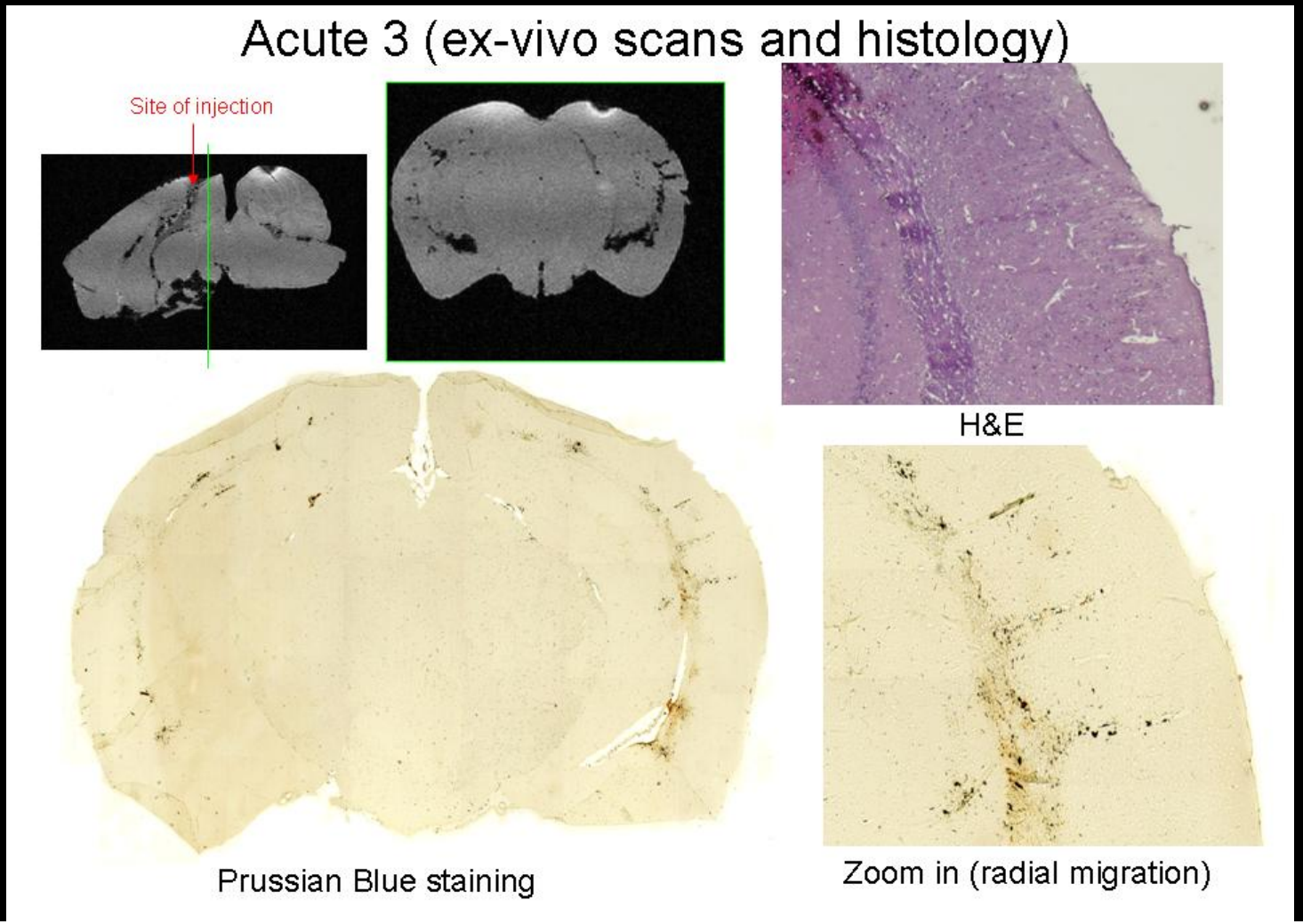


N. Muja,
M. Cohen et al.

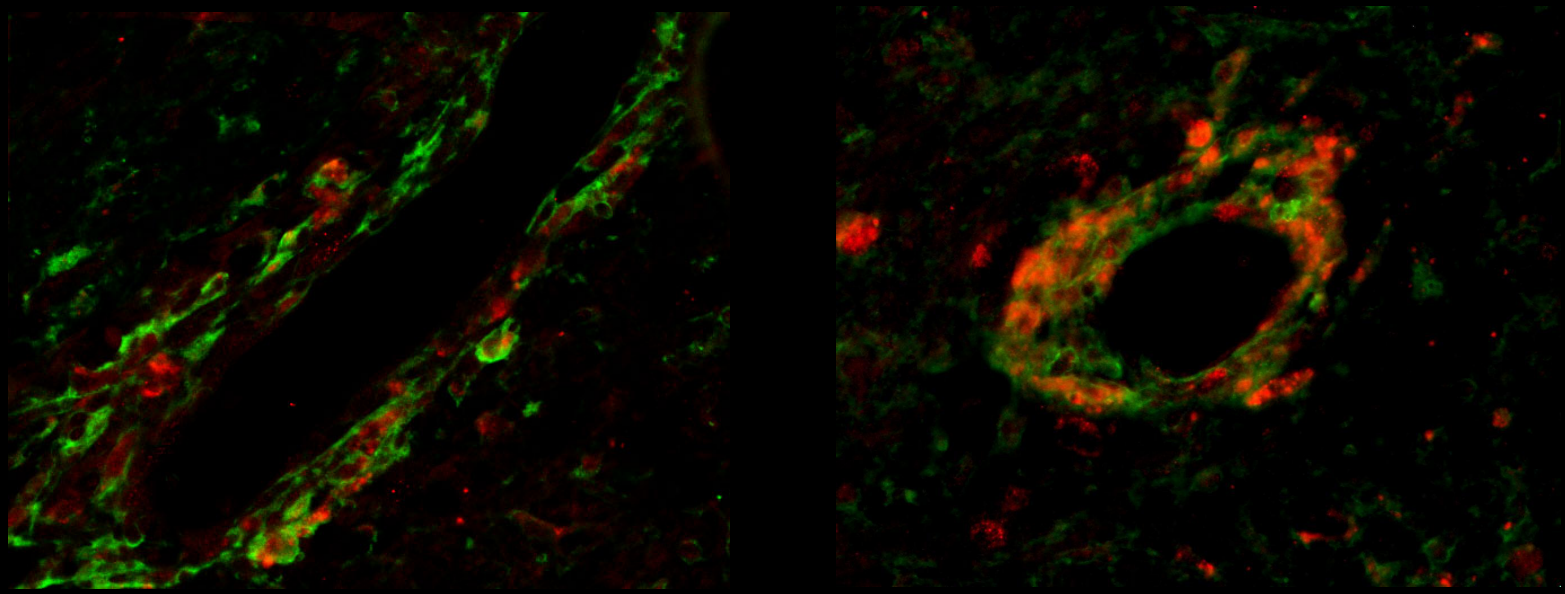
Tx During Acute Phase of EAE



Tx During Acute Phase of EAE



Radial migration along blood vessels

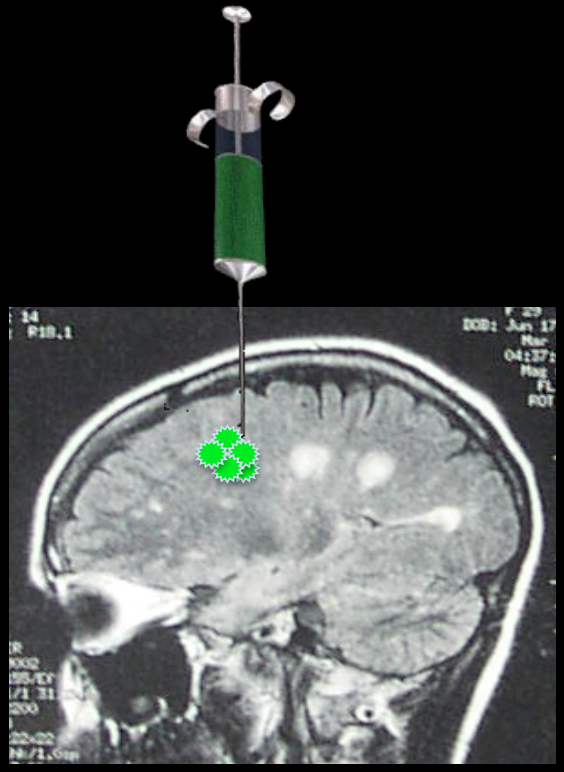


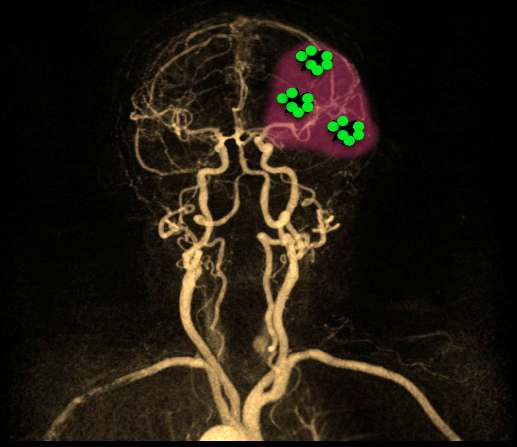
Reca-1 (blood vessel) / anti-BrdU (transplanted neural precursors)

JOHNS HOPKINS
MEDICINE

Cell-based therapy for neuroimmunological disease

How to most effectively and safely deliver stem cells?





JOHNS HOPKINS
MEDICINE

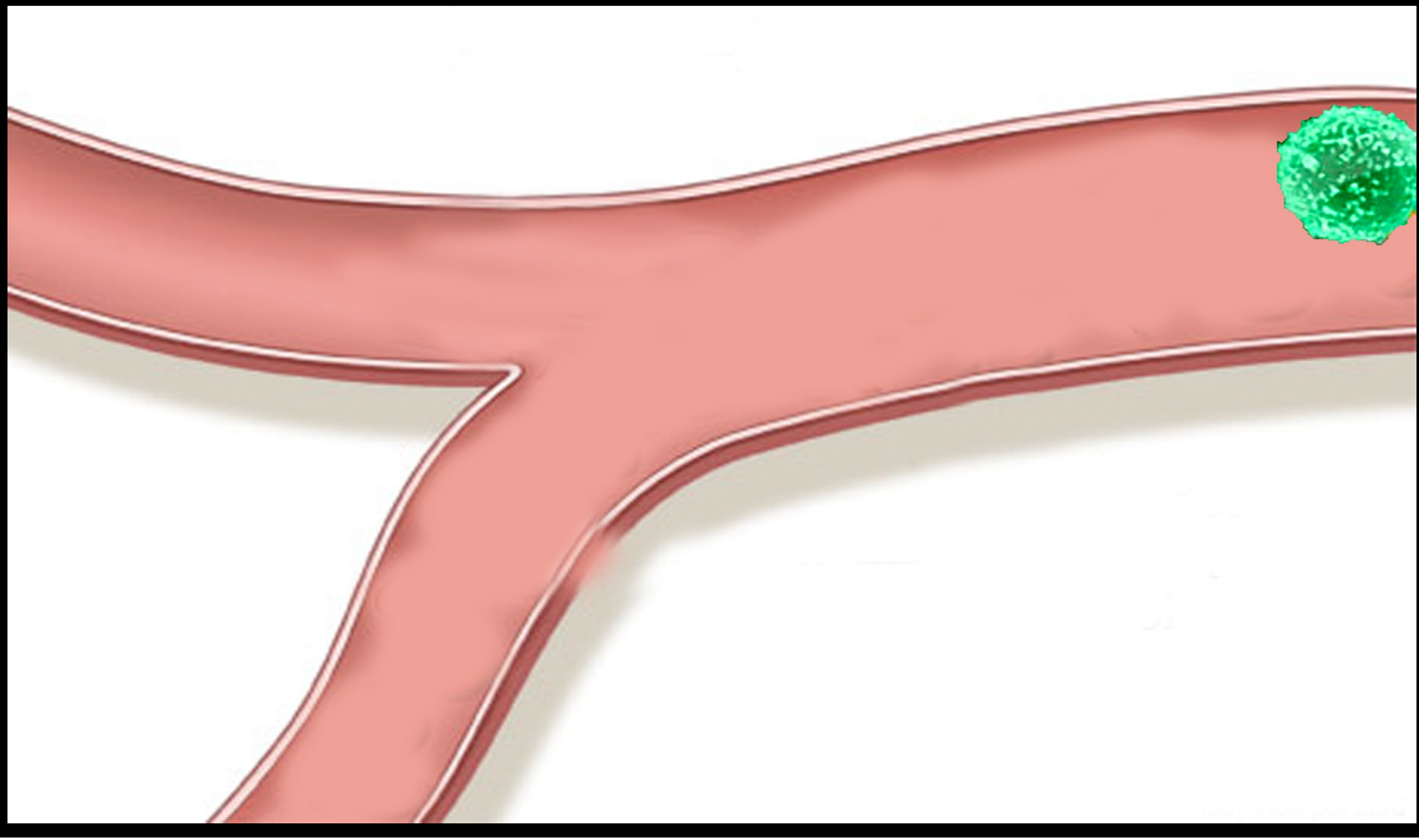
Exploiting the VLA-4 / VCAM-1 Pathway

($\alpha 4\beta 1$) VLA-4: Very Late Antigen-4
VCAM: Vascular Cellular Adhesion Molecule

Well-characterized "integrin" system involved in trafficking of leukocytes towards inflammatory sites

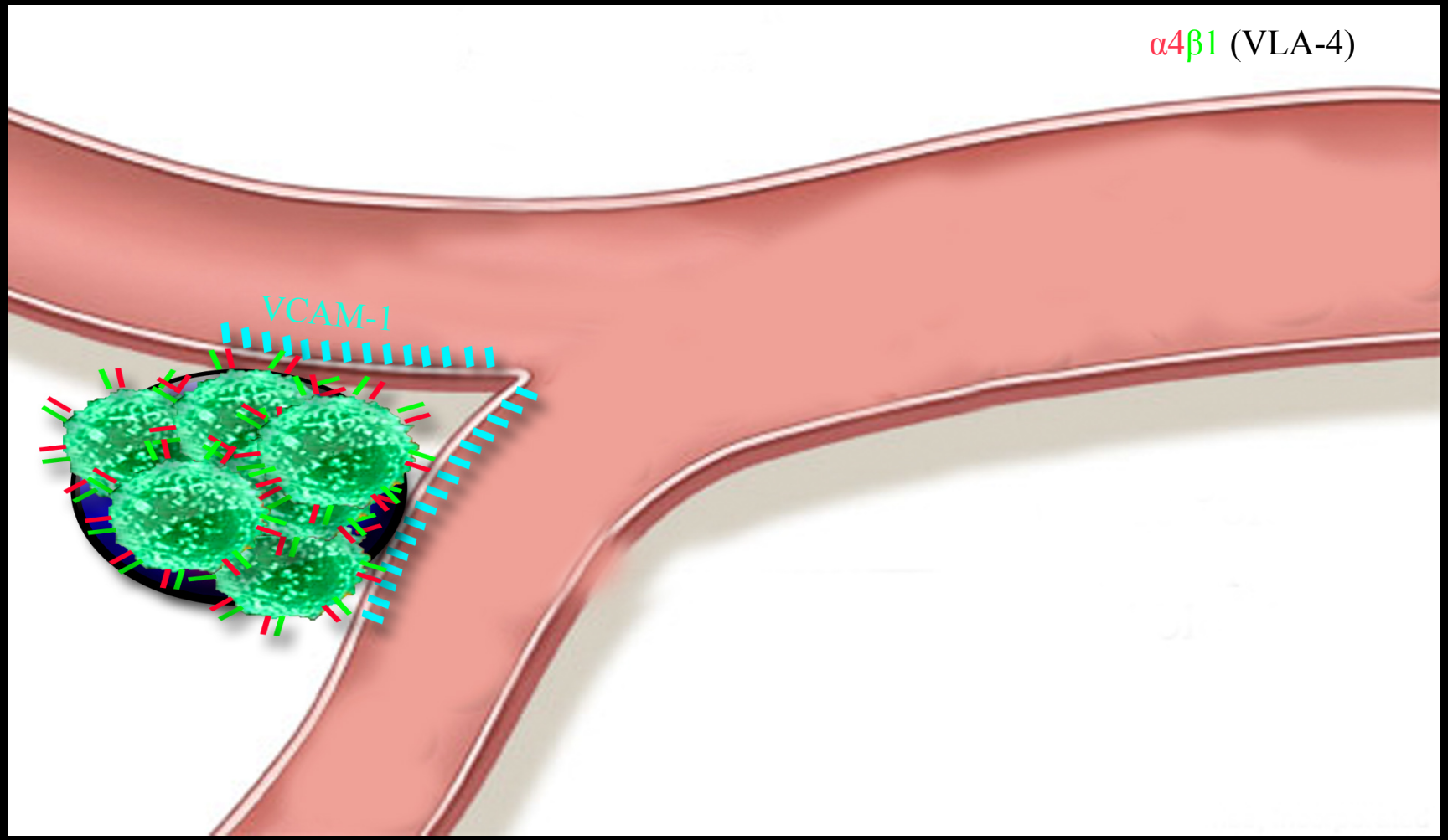
JOHNS HOPKINS
MEDICINE

Non-Inflammatory Conditions



JOHNS HOPKINS
MEDICINE

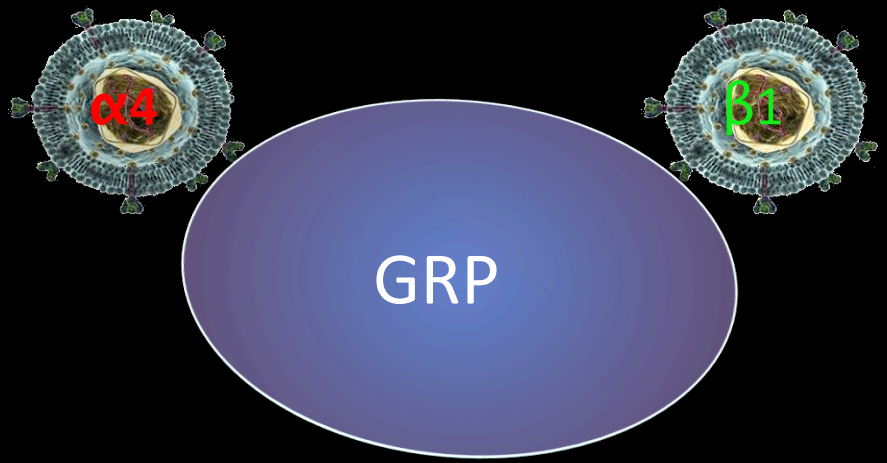
Inflammation



JOHNS HOPKINS
MEDICINE

1. Genetic engineering of glial restricted precursor cells (GRPs)

   Lentiviral transduction with two vectors, inducing expression of both subunits of VLA-4 integrin

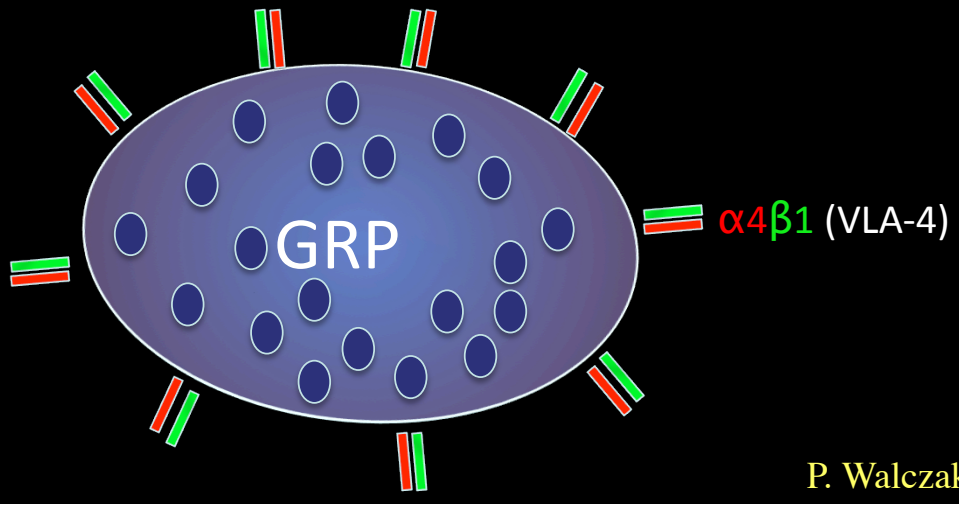


P. Walczak, D.A. Kerr et. al.

JOHNS HOPKINS
MEDICINE

2. Magnetic labeling

Feridex+PLL

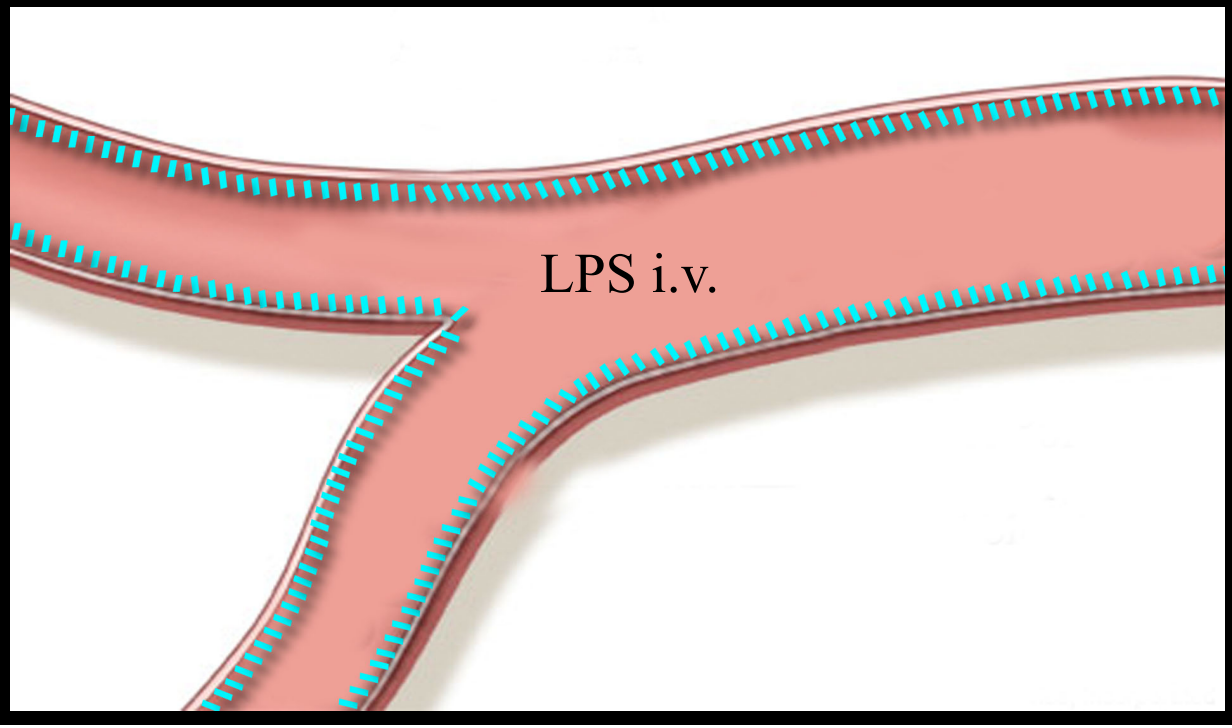


P. Walczak, D.A. Kerr et. al.

JOHNS HOPKINS
MEDICINE

3. LPS treatment or recipient animals

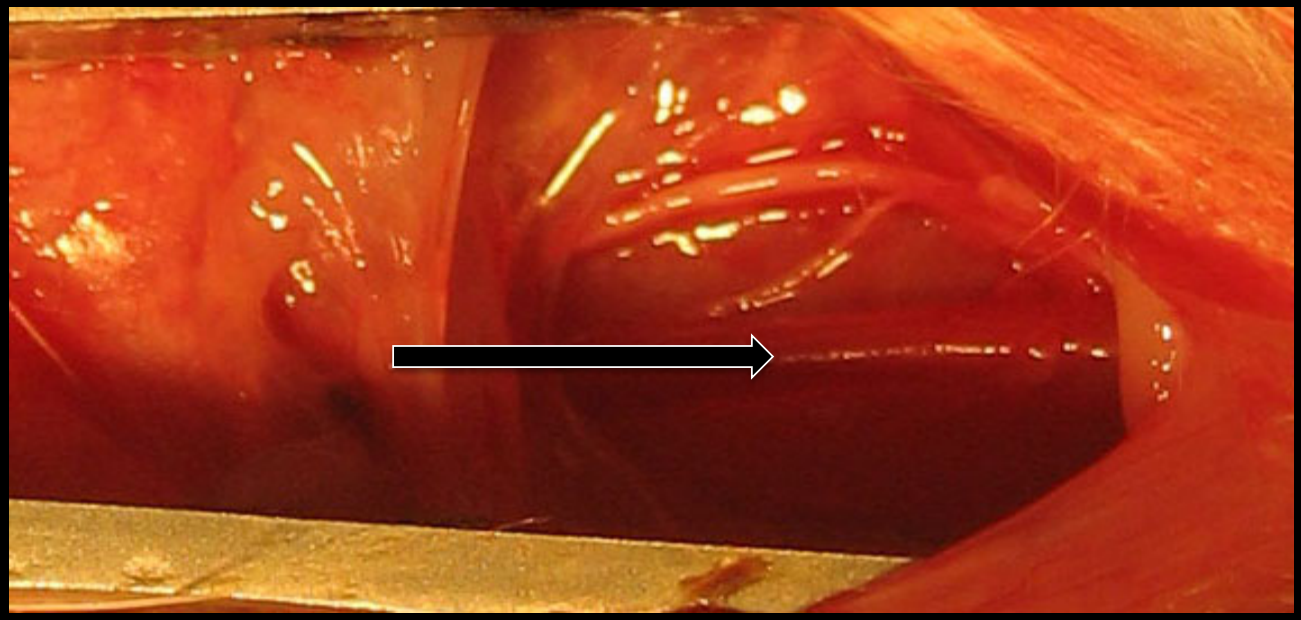
Global induction of endothelial adhesion molecules



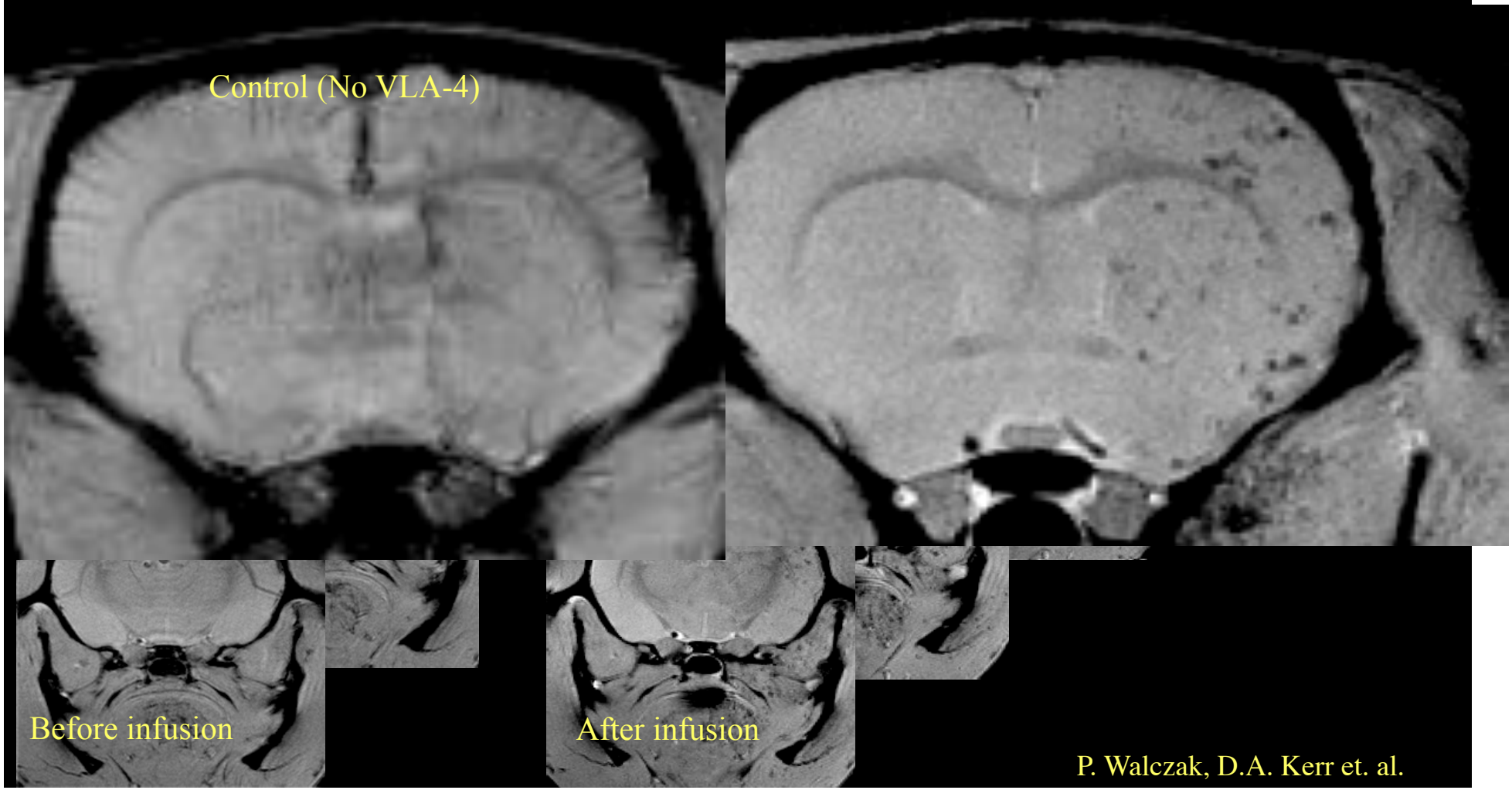
P. Walczak,
D.A. Kerr et. al.

JOHNS HOPKINS
MEDICINE

4. Cannulation of right carotid artery

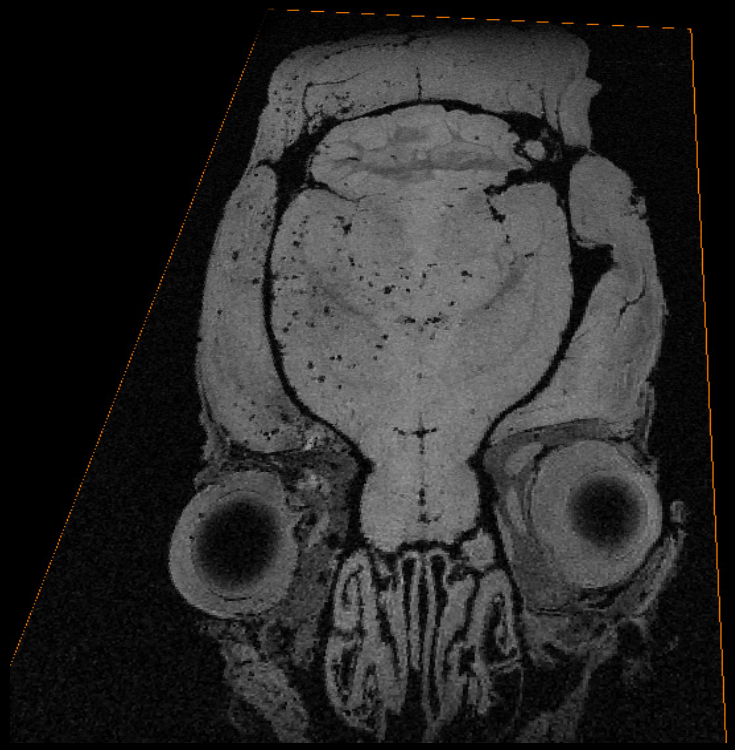


MR monitoring of targeted cell delivery *in vivo*



JOHNS HOPKINS
MEDICINE

MR monitoring of cell infusion *ex vivo*



P. Walczak, D.A. Kerr et. al.

JOHNS HOPKINS
MEDICINE

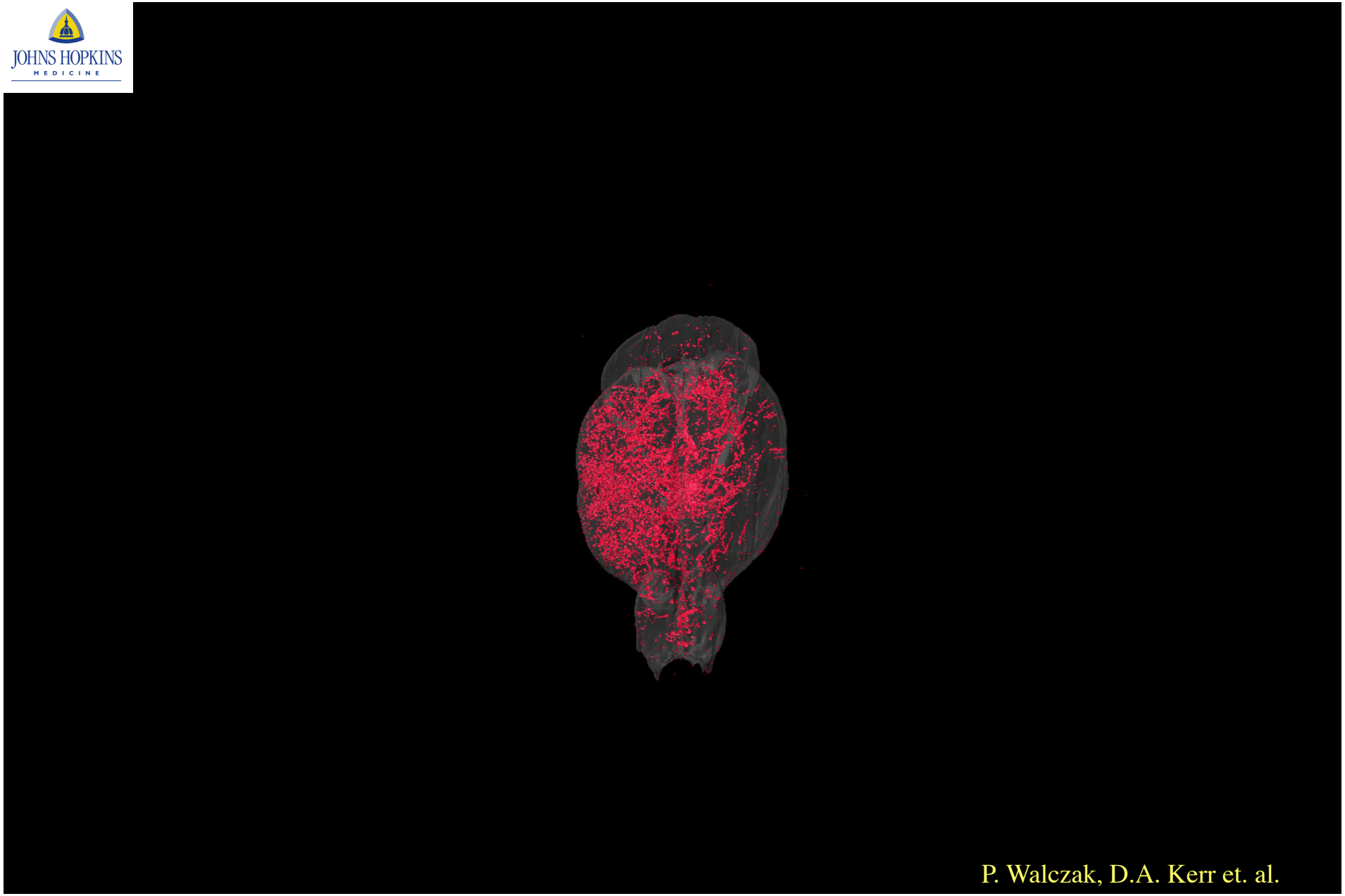
P. Walczak, D.A. Kerr et. al.

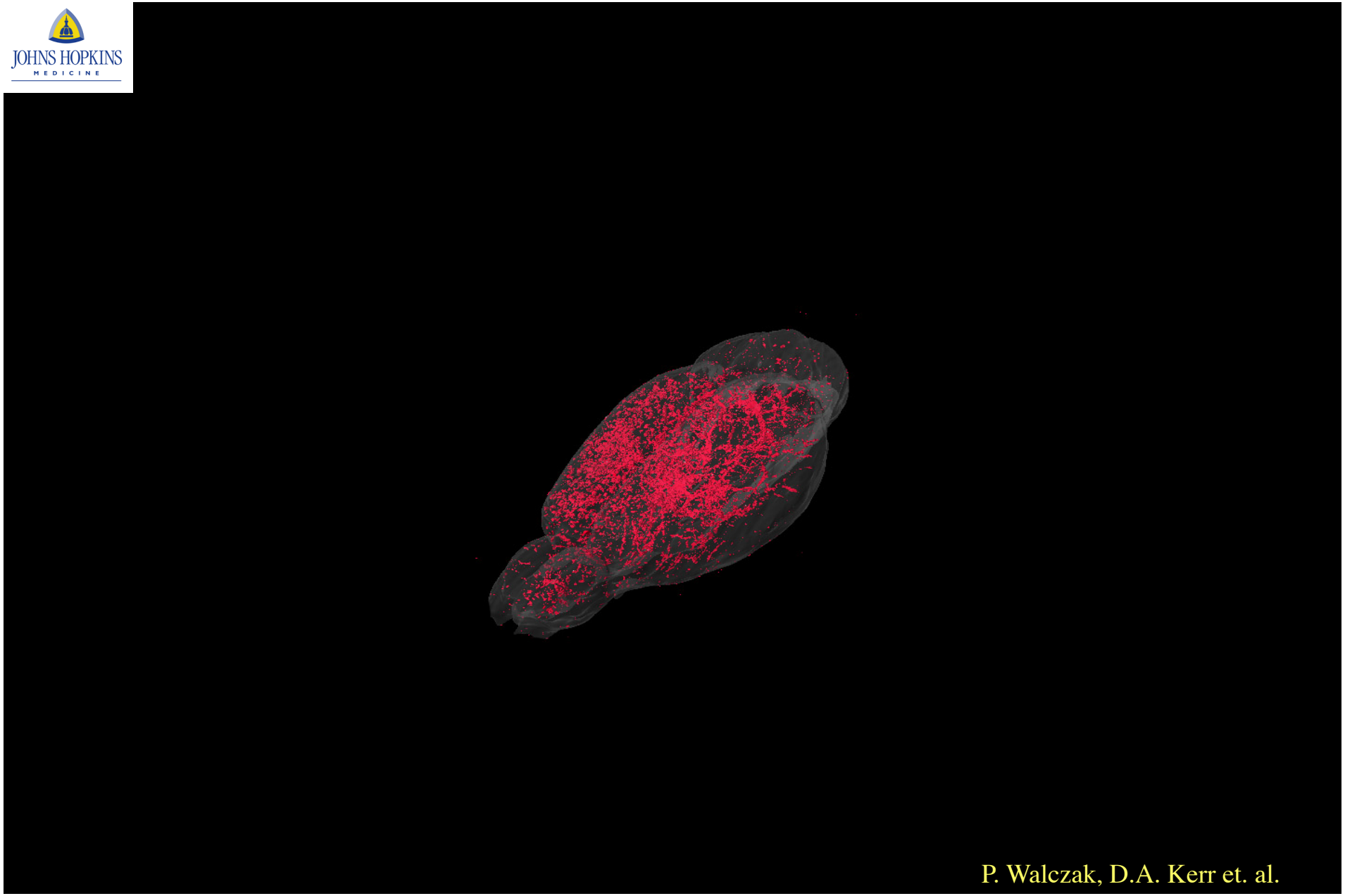
JOHNS HOPKINS
MEDICINE

P. Walczak, D.A. Kerr et. al.

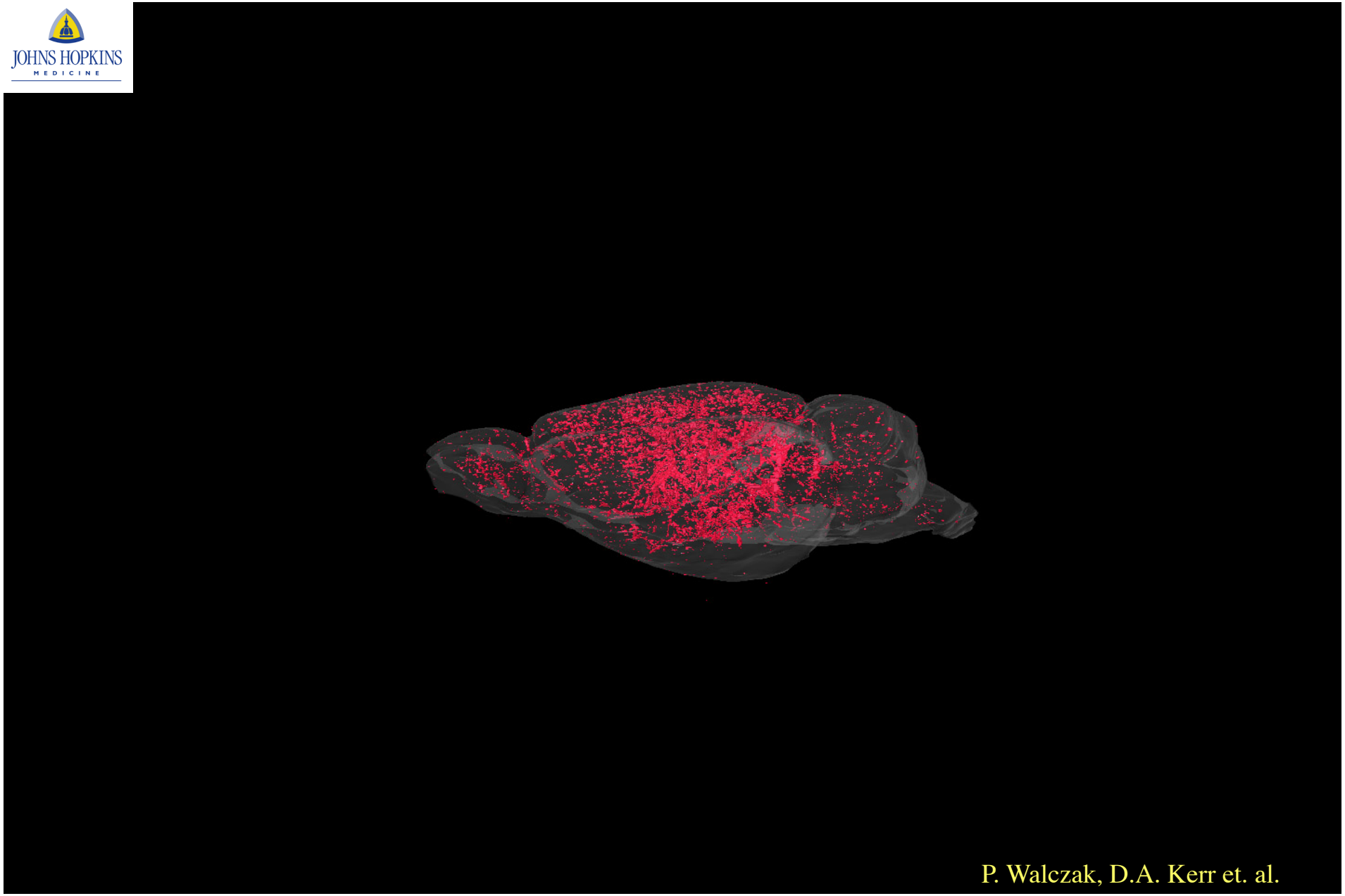
JOHNS HOPKINS
MEDICINE

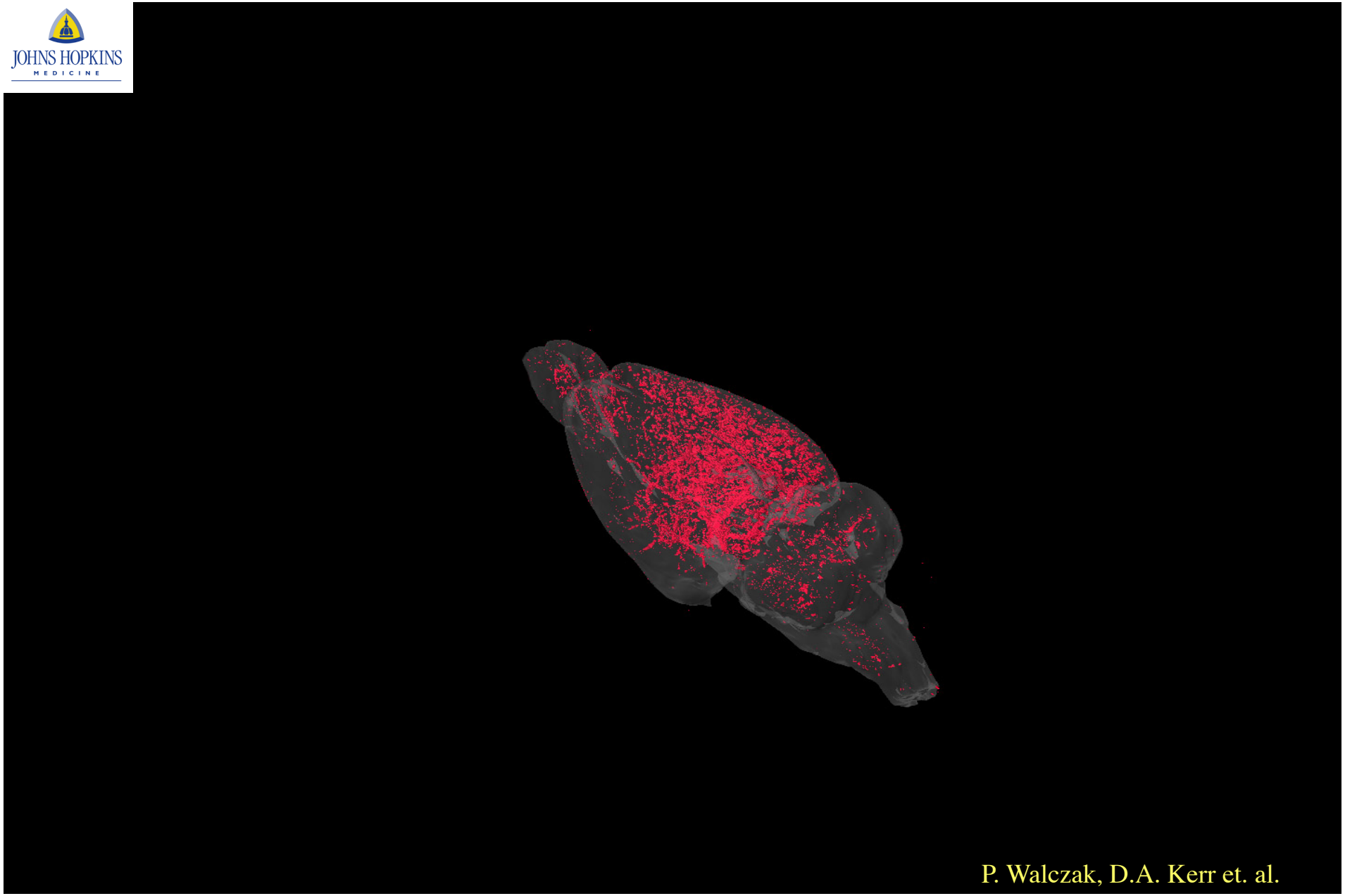
P. Walczak, D.A. Kerr et. al.

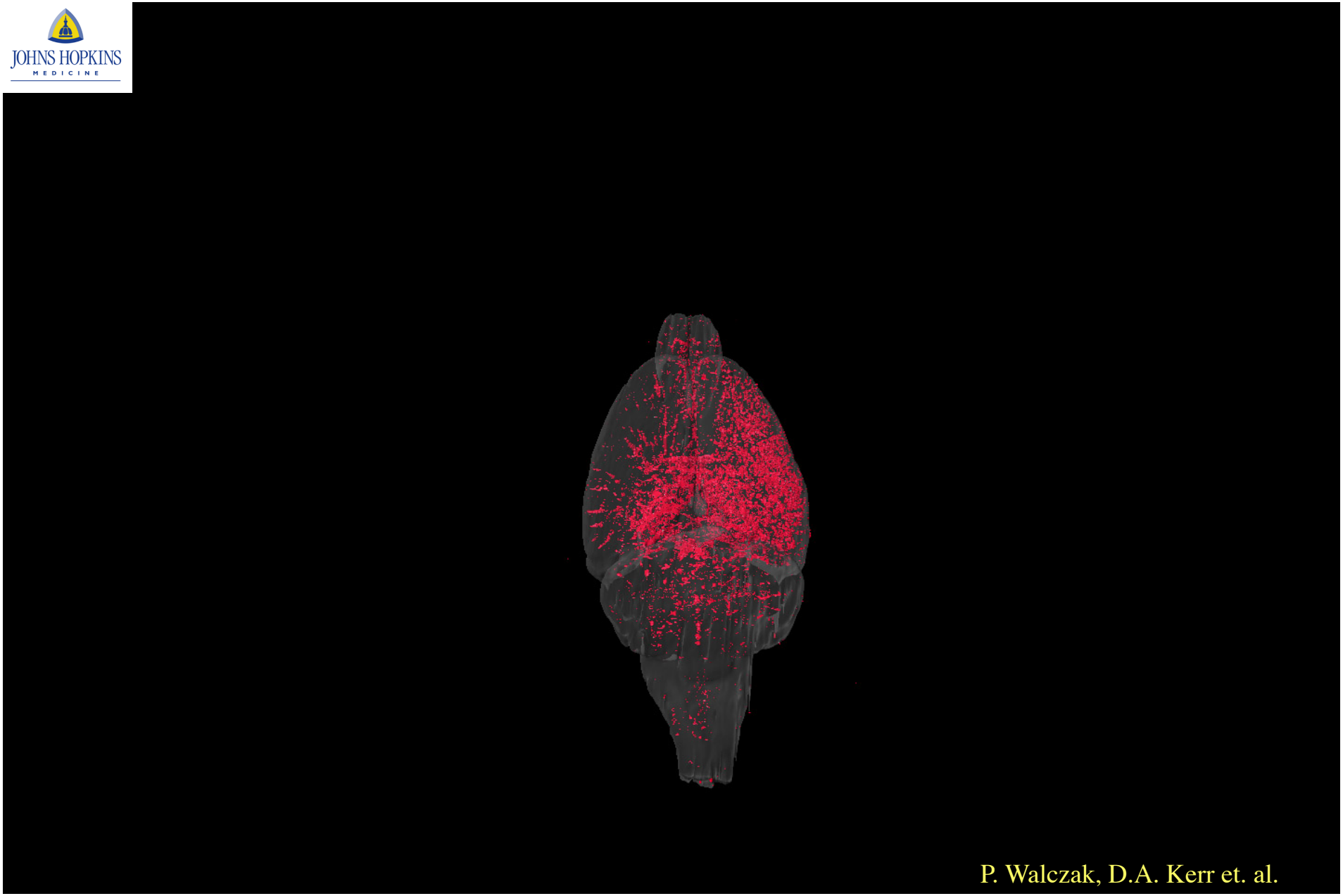

P. Walczak, D.A. Kerr et. al.



P. Walczak, D.A. Kerr et. al.


JOHNS HOPKINS
MEDICINE
P. Walczak, D.A. Kerr et. al.


P. Walczak, D.A. Kerr et. al.

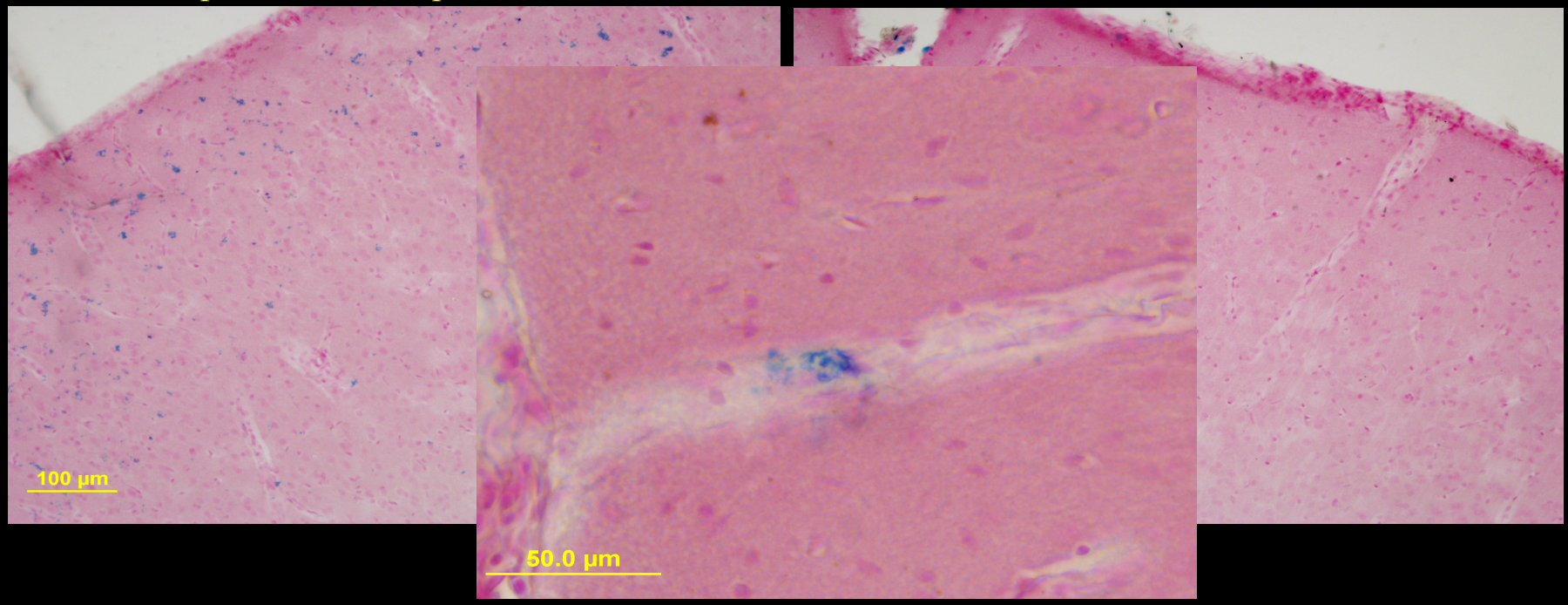

JOHNS HOPKINS
MEDICINE
P. Walczak, D.A. Kerr et. al.

JOHNS HOPKINS
MEDICINE

Histology after transplantation

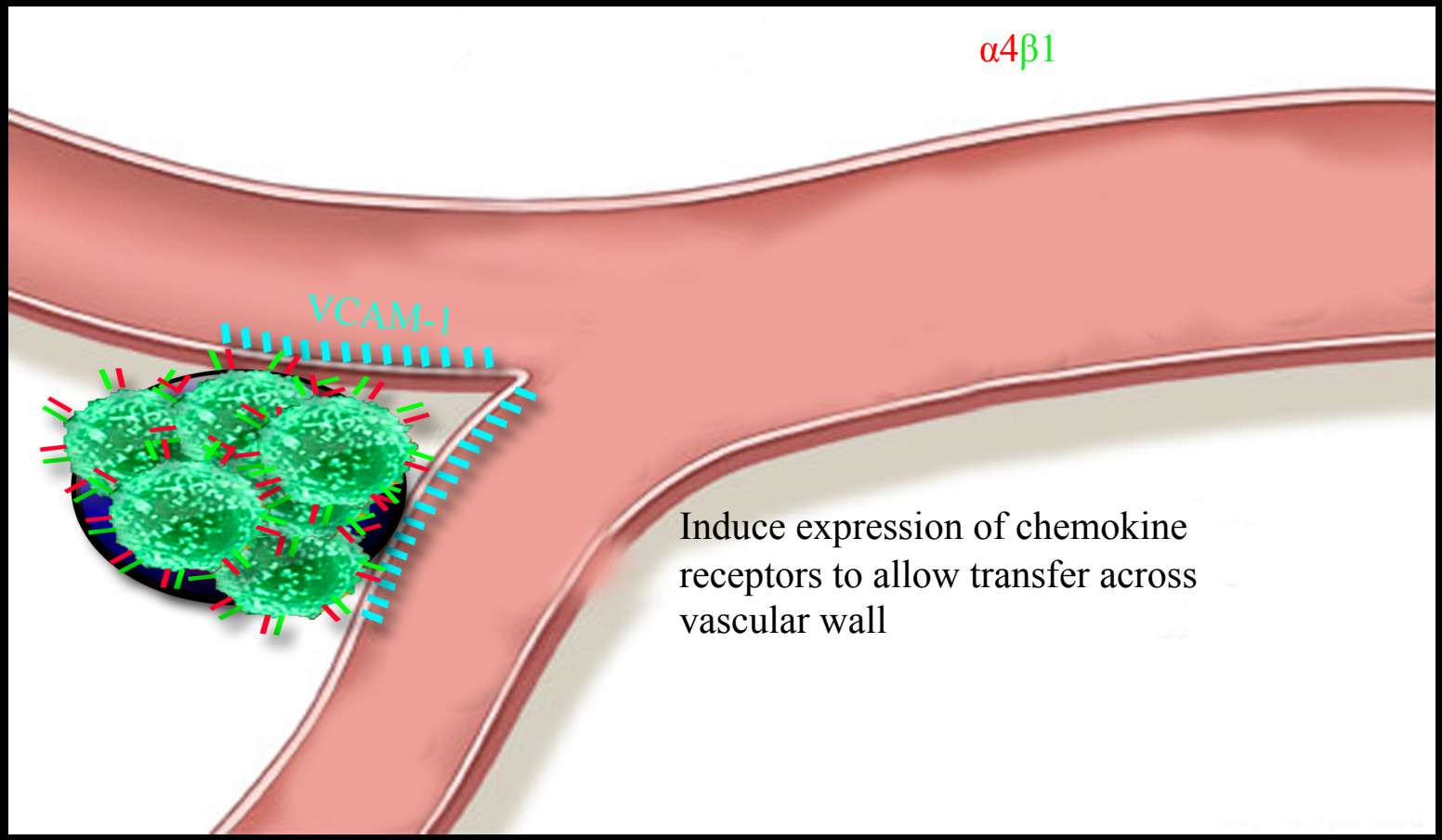
Ipsilateral hemisphere

Contralateral hemisphere



P. Walczak, D.A. Kerr et. al.

Enhanced Targeted Delivery of Stem Cells towards Inflammatory Lesions (such as those occurring in MS)



P. Walczak, D.A. Kerr et. al.

JOHNS HOPKINS
MEDICINE

AJR FOCUS ON:

In Vivo MRI Cell Tracking: Clinical Studies

Jeff W. M. Bulte[1]

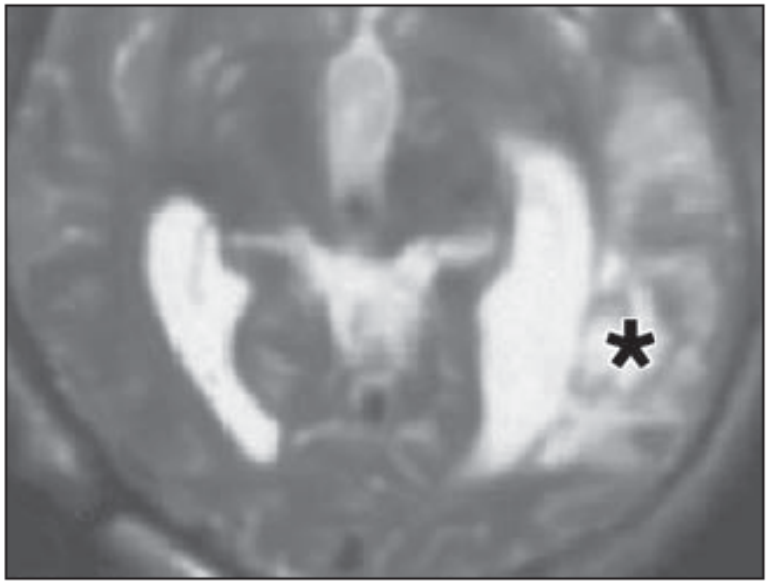
OBJECTIVE. The purpose of this review is to describe the principles of MRI cell tracking with superparamagnetic iron oxides and the four clinical trials that have been performed.

CONCLUSION. Clinical MRI cell tracking is likely to become an important tool at the bedside once (stem) cell therapy becomes mainstream. The most prominent role of this technique probably will be verification of accurate cell delivery with MRI-guided injection, in which interventional radiologists will play a role in the near future. All clinical studies described as of this writing have been performed outside the United States.

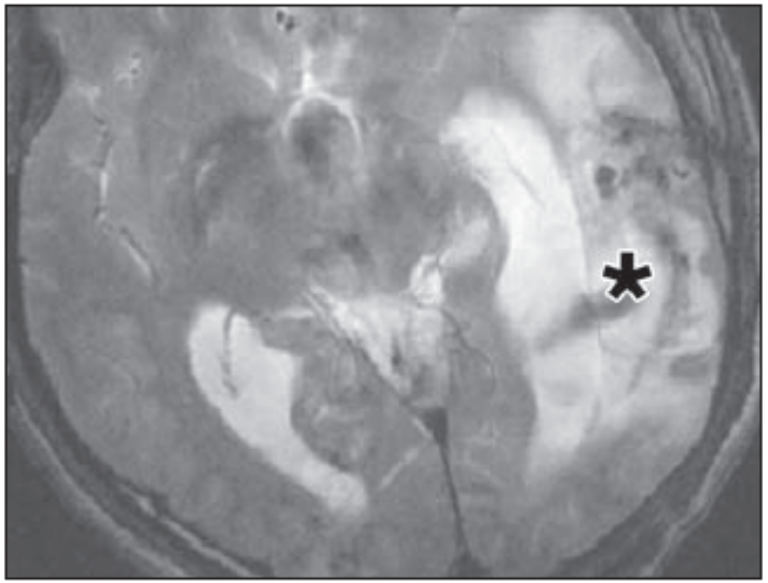
Amer. J. Roentgenol. 193, 314-325 (2009).

JOHNS HOPKINS
MEDICINE

Neural Stem Cell in Brain Trauma Patients (Shanghai)



A



B

J. Zhu et al., N. Engl. J. Med. 355, 2376-2378 (2006).

Clinical experience with the use of Feridex-labeled autologous MSCs in MS patients

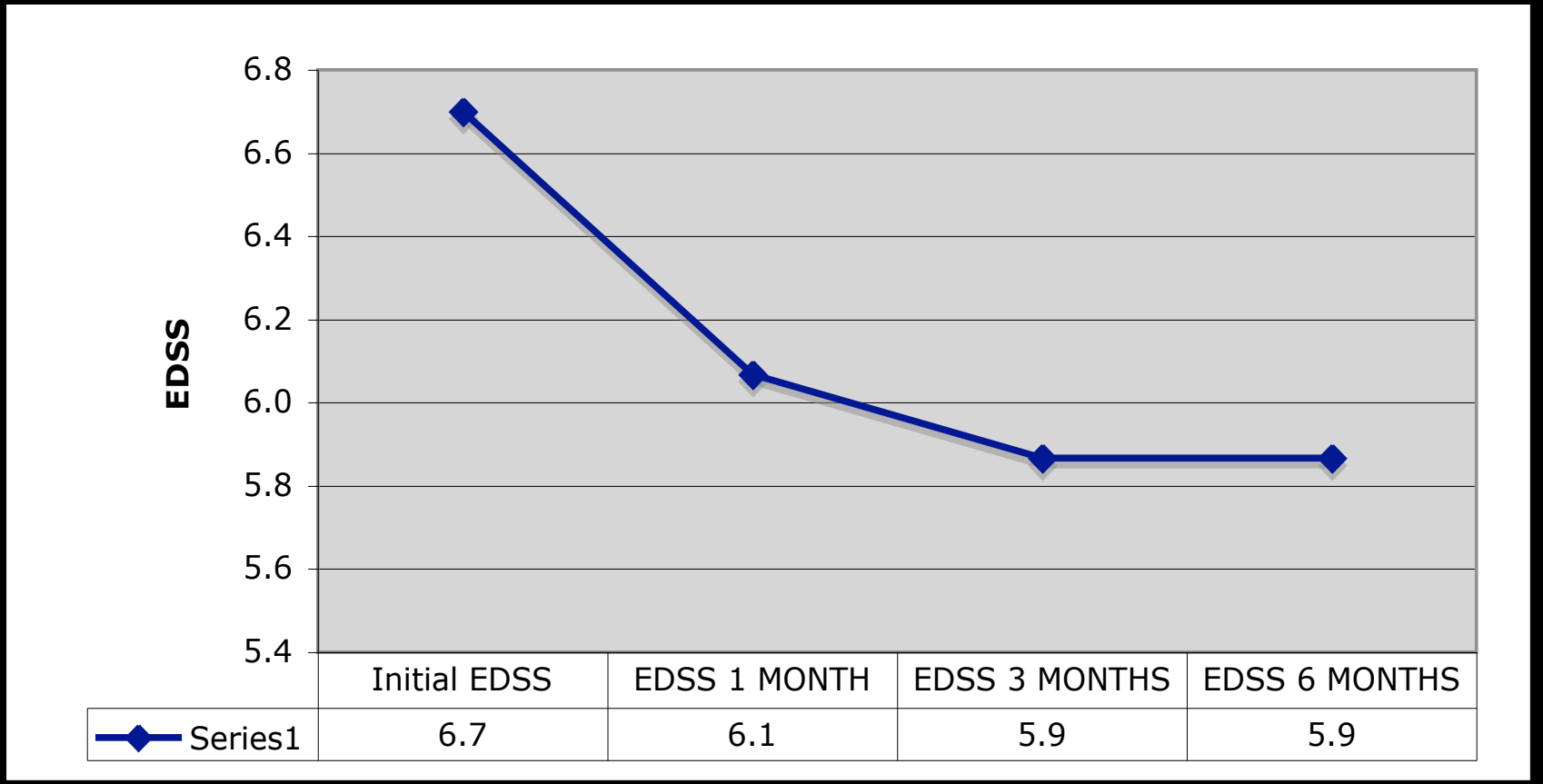
D. Karussis, J.W.M. Bulte, T. Ben-Hur et al.,

Arch. Neurol., in press

Clinical follow up of MS patients following MSC transplantation (6 months)

PT INITIALS	GENDER	AGE	DISEASE DURATION	TYPE OF MS	Initial EDSS	EDSS 1 MONTH	_DSS 3 MONTHS	EDSS 6 MONTHS
TI	F	47	12	SP	7.5	6.5	6	6
MH	M	25	14	SP	6	5	3.5	3.5
PD	M	35	13	RP	6.5	5.5	5.5	6
SE	F	36	15	SP	8	7.5	7	7
AI	F	34	8	SP	5.5	4	4.5	5
KE	F	30	5	SP	7	6.5	6.5	6.5
MDC	F	37	9	RP	6.5	6.5	6.5	6.5
ML	F	52	12	SP	7.5	7	7	7.5
SH	M	35	12	RP	7	7	7	7
PM	F	28	10	SP	6.5	5.5	5.5	5
PMT	M	32	10	SP	6	5	4.5	4.5
IO	M	28	8	SP	8	7.5	7.5	7.5
LZ	F	32	7	PP	4	3.5	3	2
CY	M	52	15	PP	7	7	7	7
TK	M	27	10	SP	7.5	7	7	7
		35.3	**10.7**		**6.7**	**6.1**	**5.9**	**5.9**
		8.58	**2.97**		**1.05**	**1.25**	**1.41**	**1.59**

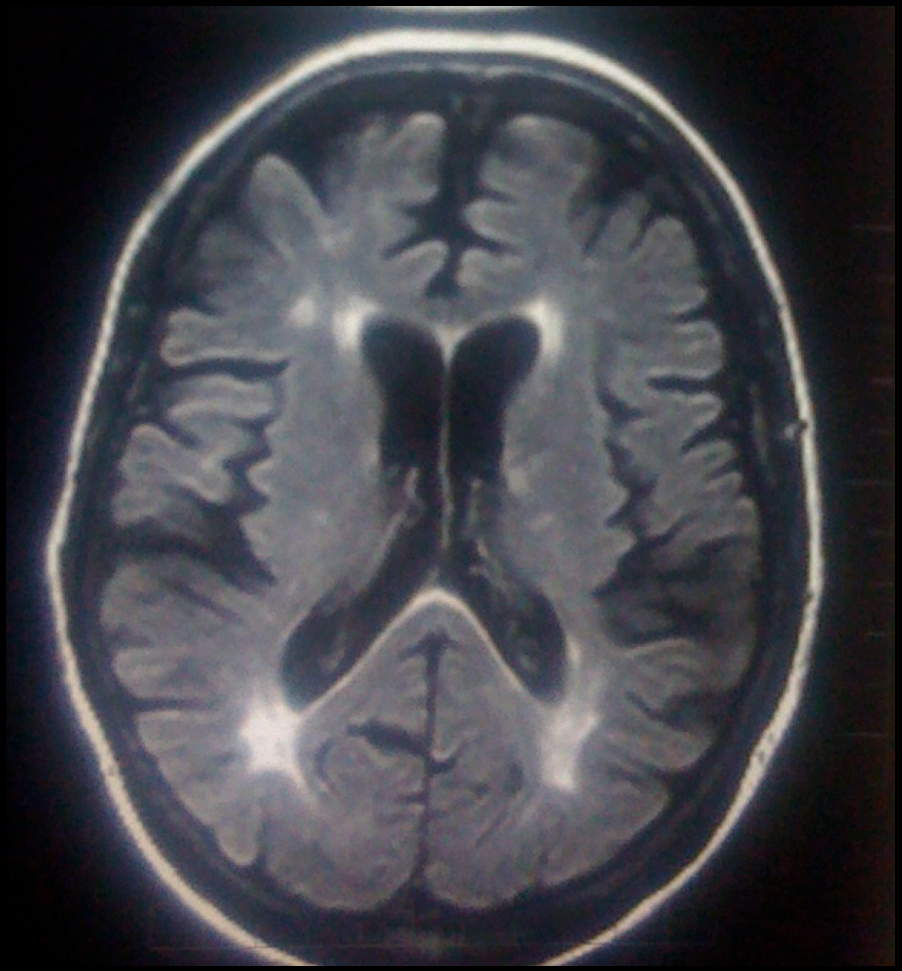
Clinical follow up of MS patients following MSC transplantation (6 months)



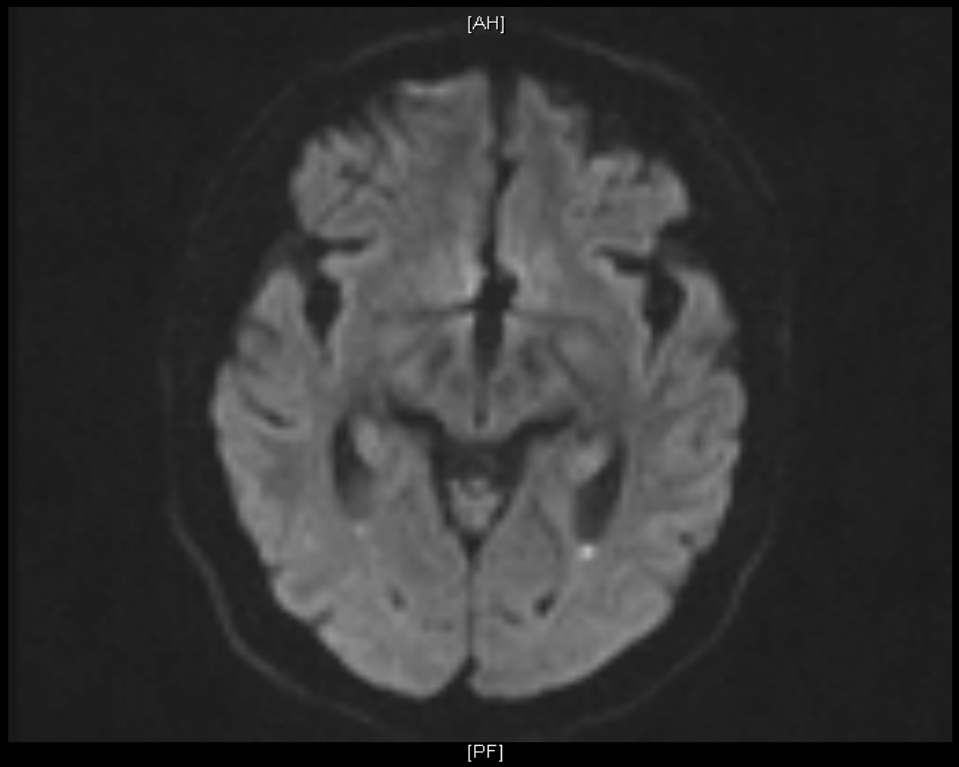
	Initial EDSS	EDSS 1 MONTH	EDSS 3 MONTHS	EDSS 6 MONTHS
Series1	6.7	6.1	5.9	5.9

PRE-MSC

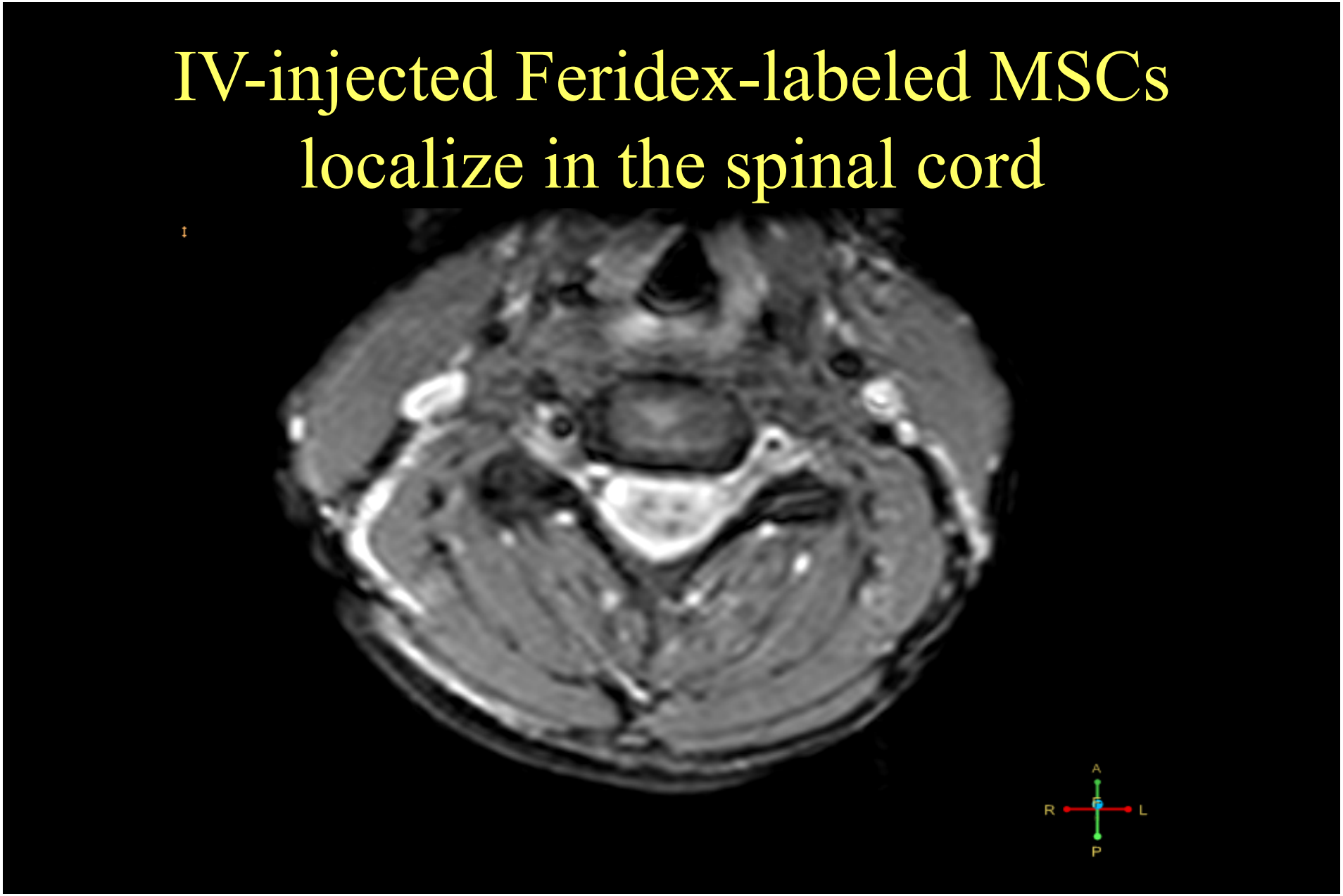
POST MSC



IV-injected Feridex-labeled MSCs localize in the occipital horns



IV-injected Feridex-labeled MSCs localize in the spinal cord



JOHNS HOPKINS
MEDICINE

"The number one clinical application of MRI cell tracking may not be monitoring cell migration following injection, but rather verifying the actual accuracy of the cell injection itself, at the target side, and then in real time, using MR-guided injectional procedures"

JOHNS HOPKINS
MEDICINE

MR-Guided Real-Time Injections Using MR Compatible Injection Catheters

- Modified loopless antenna design
- Matching, tuning, and decoupling circuitry
- Steerable guide catheter via nitinol pull wire
- 26 gauge nitonol injection needle

P. Karmarkar et al., MRM 51, 1163-1172 (2004).

First Clinical Trial Using MR Tracking of Magnetically Labeled Cells

First patient injected on April 26, 2004
(Collaboration between JHU and U of Nijmegen, NL)

ARTICLES

nature biotechnology

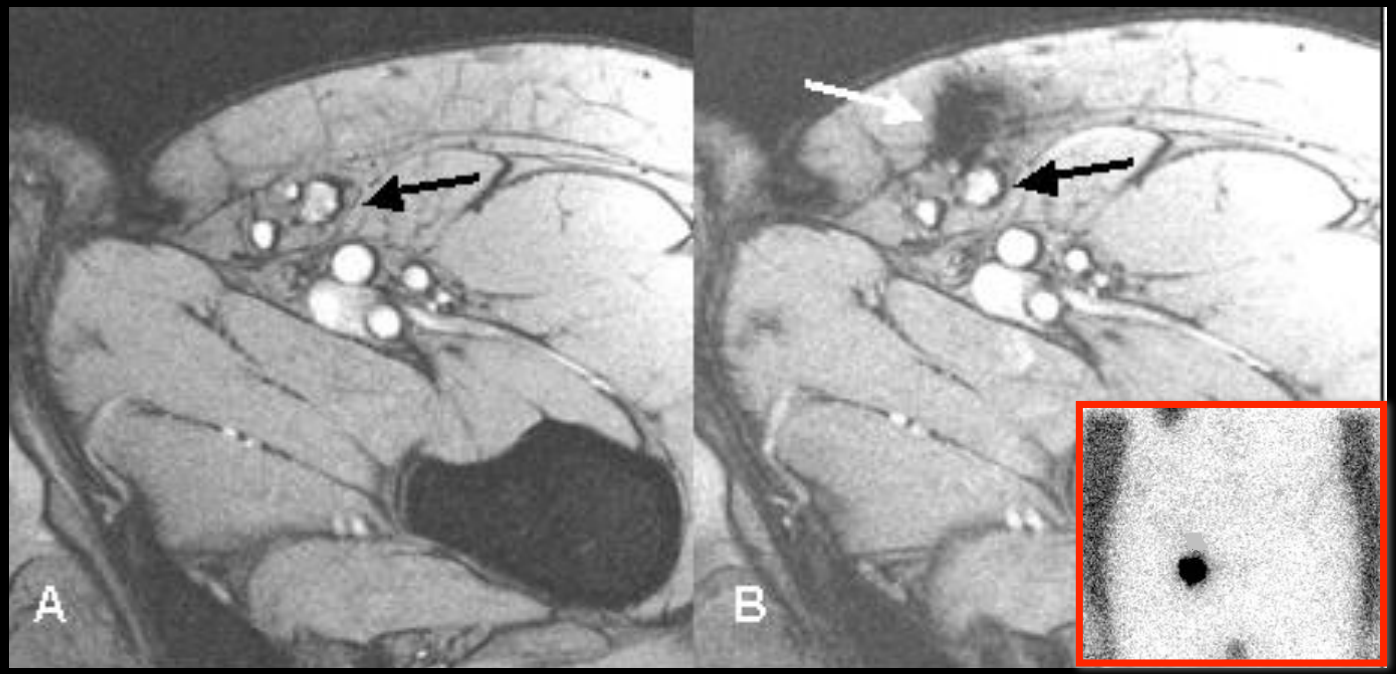
Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy

I Jolanda M de Vries[1,2], W Joost Lesterhuis[3], Jelle O Barentsz[4], Pauline Verdijk[1], J Han van Krieken[5], Otto C Boerman[6], Wim J G Oyen[6], Johannes J Bonenkamp[7], Jan B Boezeman[8], Gosse J Adema[1], Jeff W M Bulte[9], Tom W J Scheenen[4], Cornelis J A Punt[3], Arend Heerschap[4] & Carl G Figdor[1]

NATURE BIOTECHNOLOGY VOLUME 23 NUMBER 11 NOVEMBER 2005

What Did We Learn:
2) Accidental Misinjection in 4 out of 8 Patients

(Injections performed under US, not MR-Guided)



Pre

Post

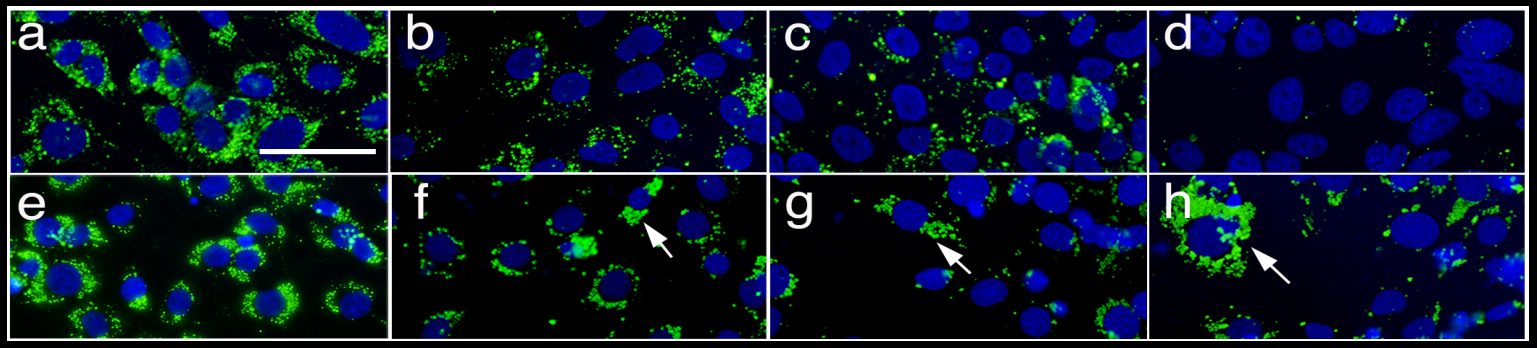
I. de Vries, et. al., Nat. Biotechnol. 23, 1407-1413 (2005).

JOHNS HOPKINS
MEDICINE

Limitations of Labeling with Exogenous MR Contrast Agents

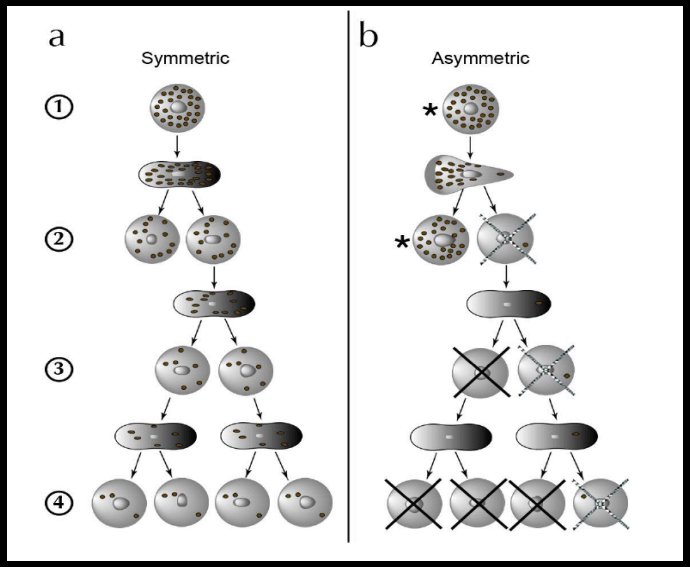
-No distinction between live and dead cells
-Contrast may change over time, affecting interpretation
-No imaging of cellular differentiation
-No imaging of rapidly dividing cells

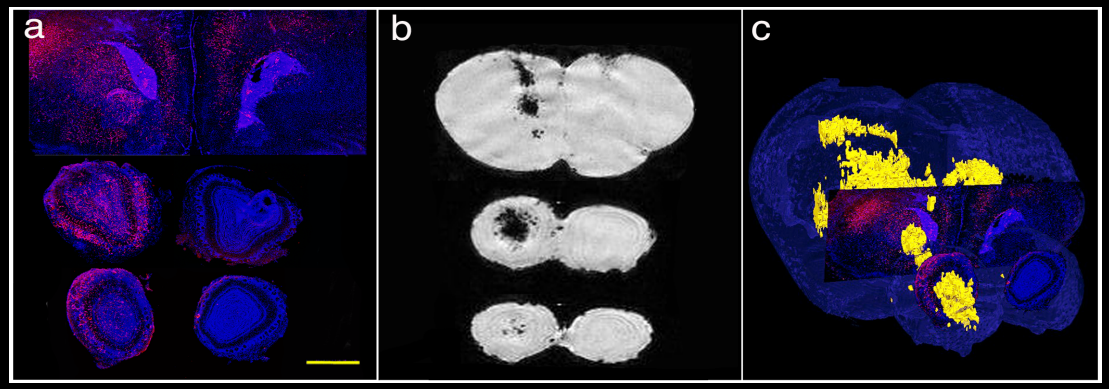
Asymmetric Cell Division and Dilution of Feridex Label



Symmetric

Asymmetric





LacZ reporter gene

Feridex

Lacz/Feridex overlay (→mismatch)

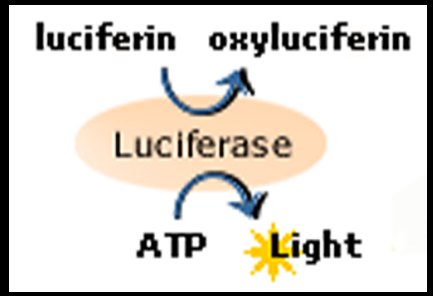
P. Walczak et al., MRM 58, 261-269, 2007.

JOHNS HOPKINS
MEDICINE

Reporter Genes

JOHNS HOPKINS
MEDICINE

American Firefly

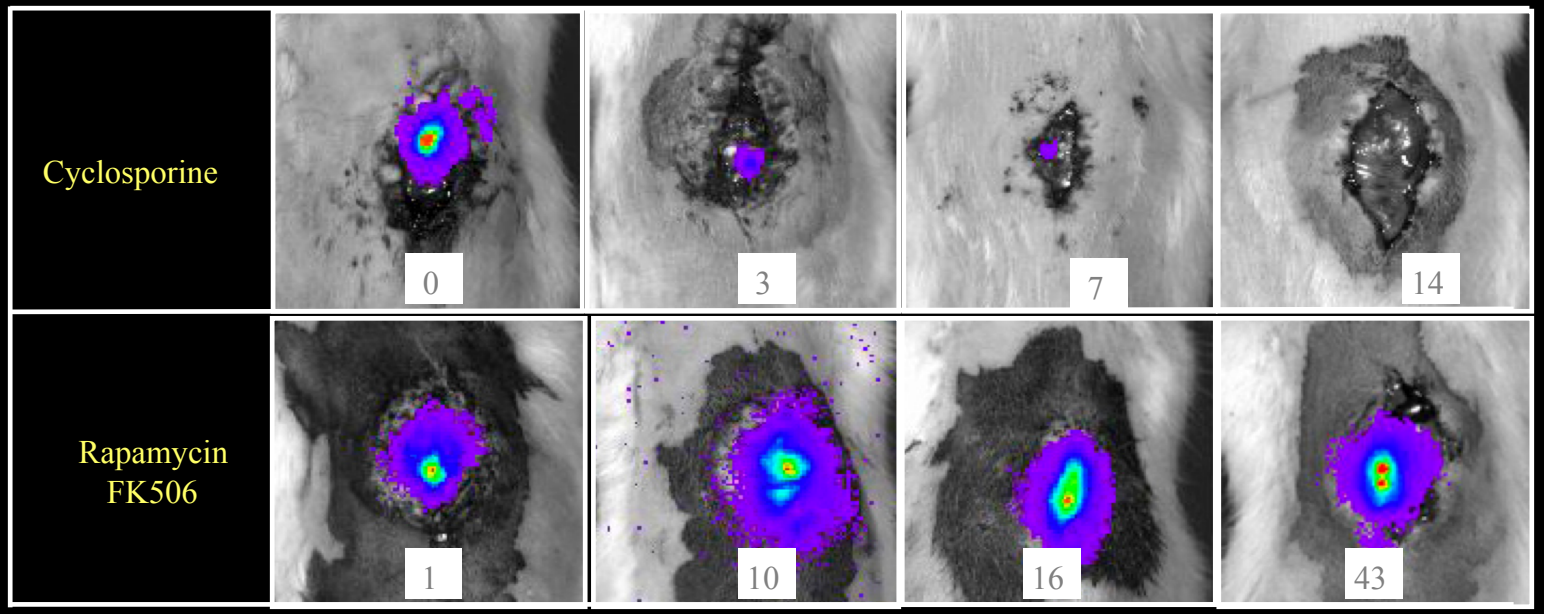


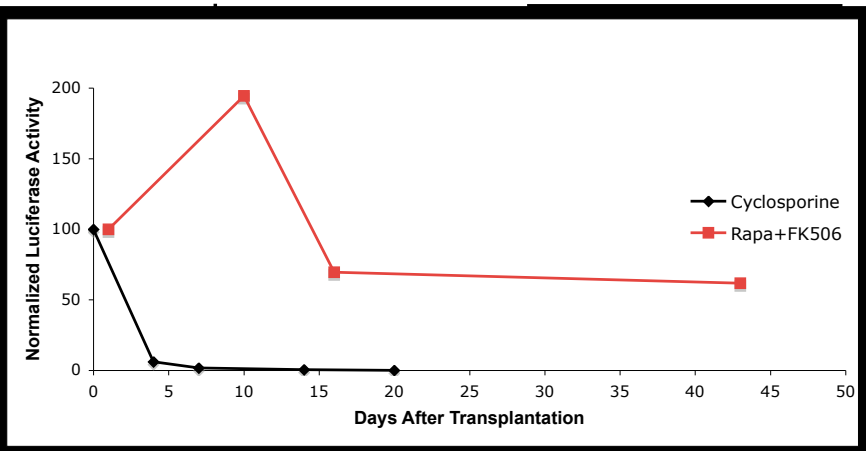
Photinus pyralis

JOHNS HOPKINS
MEDICINE

BLI - Monitoring of Cell Survival

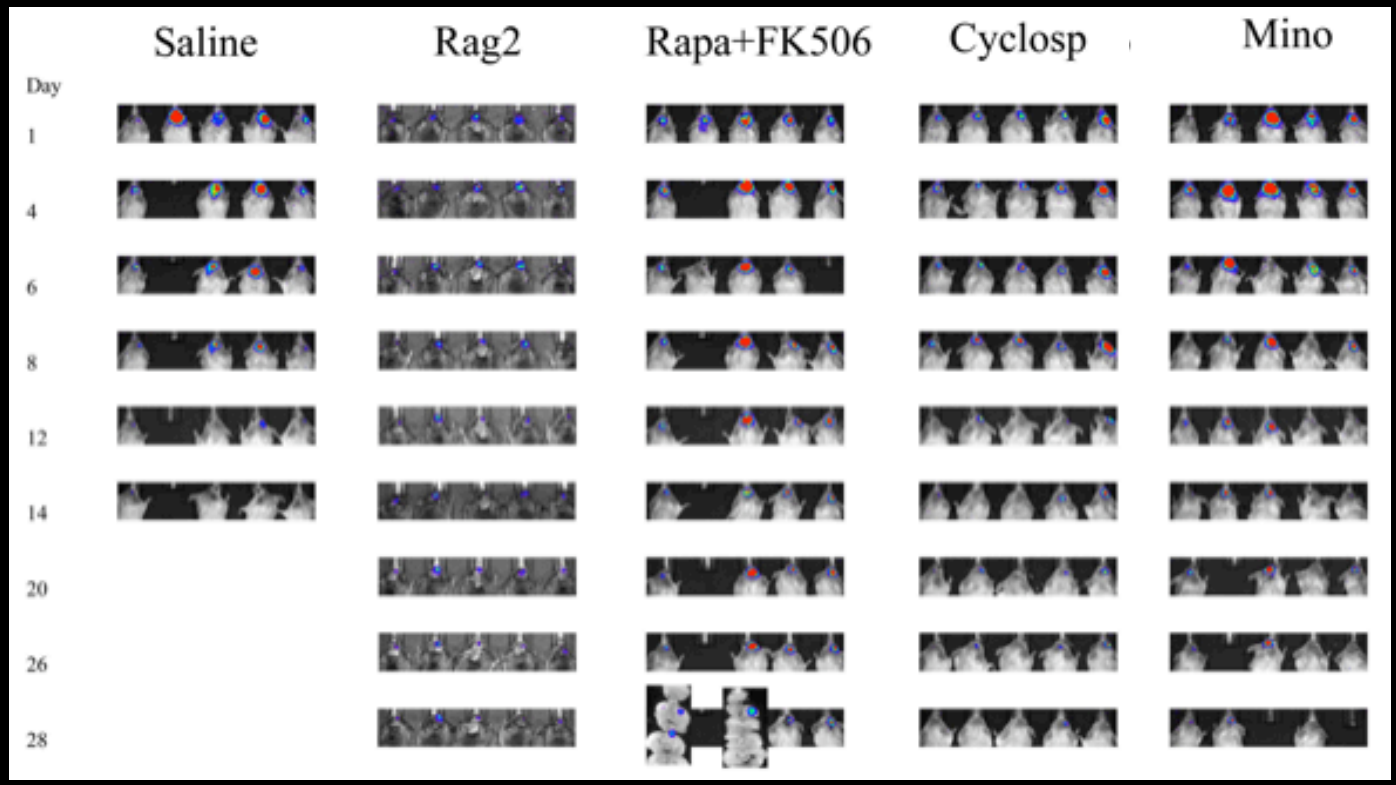
Evaluating Optimal Immunosuppresive Regimen



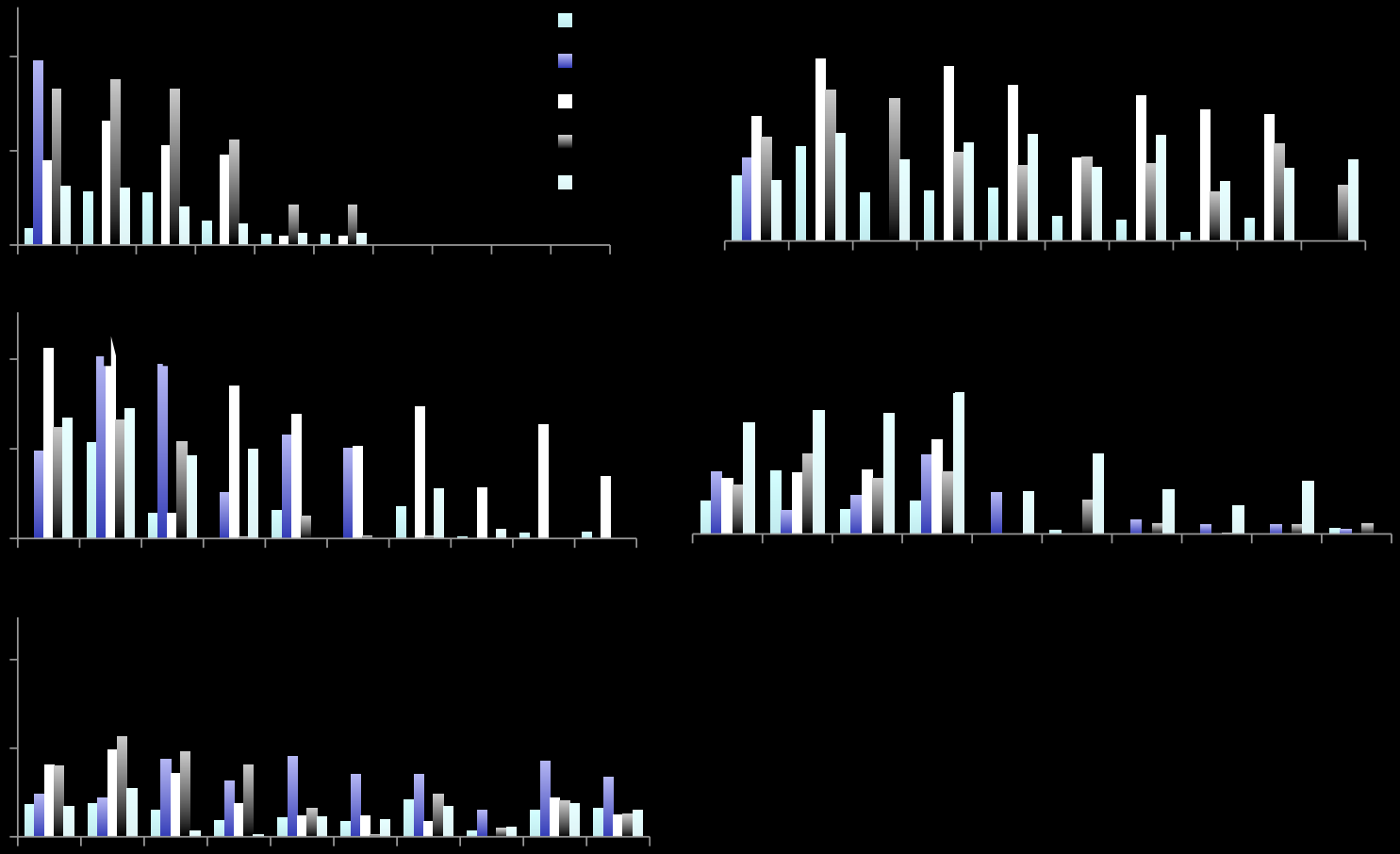


P Walczak et al.

BL Imaging of Survival of Allogeneic Mouse GRPs (*Luc* transgenic fvbn in Balb/c) Grafted in the Corpus Callosum



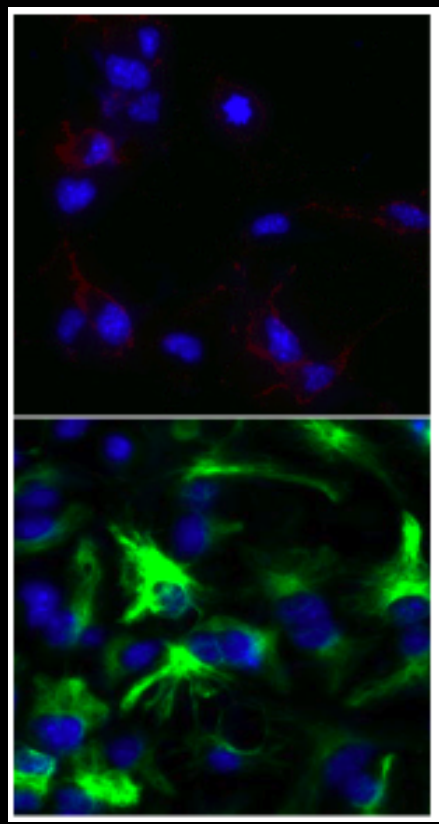
BL Imaging of Survival of Allogeneic Mouse GRPs Grafted in the Corpus Callosum



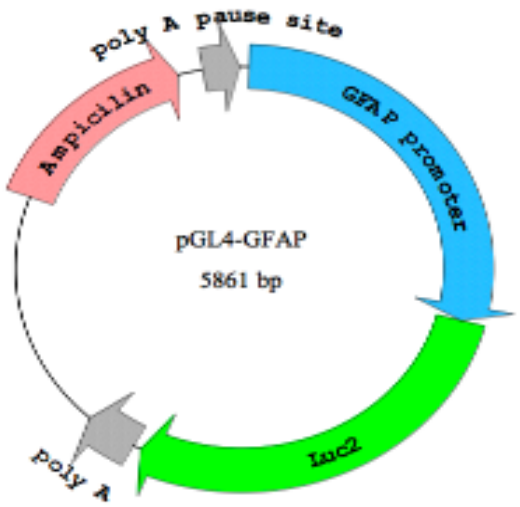
Imaging Cell Differentiation

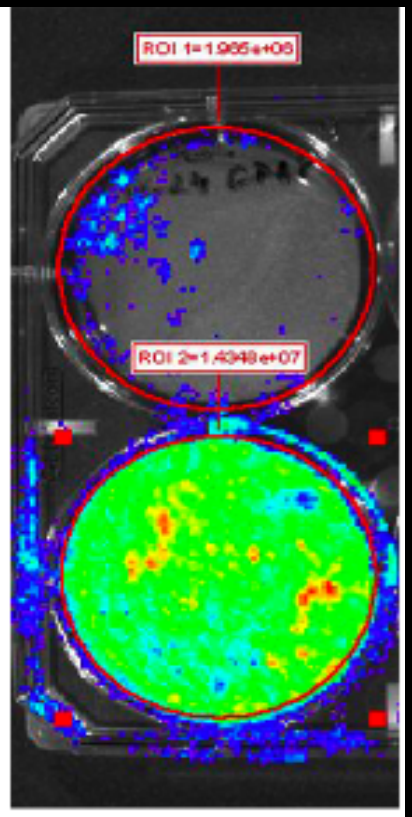
GRPs
Undifferentiated

GRP-derived
Astrocytes



GFAP
Immunostaining

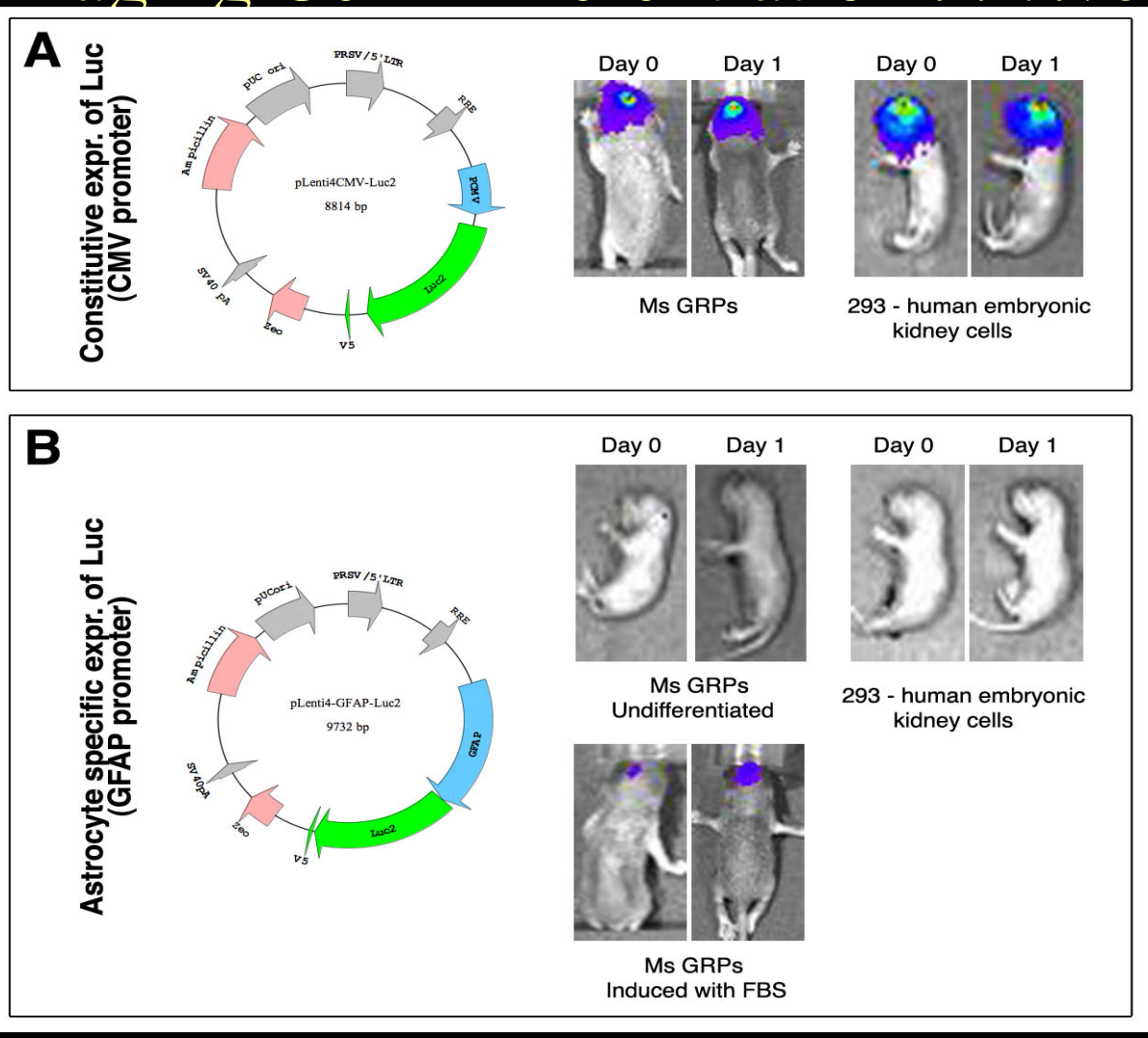




BL Imaging

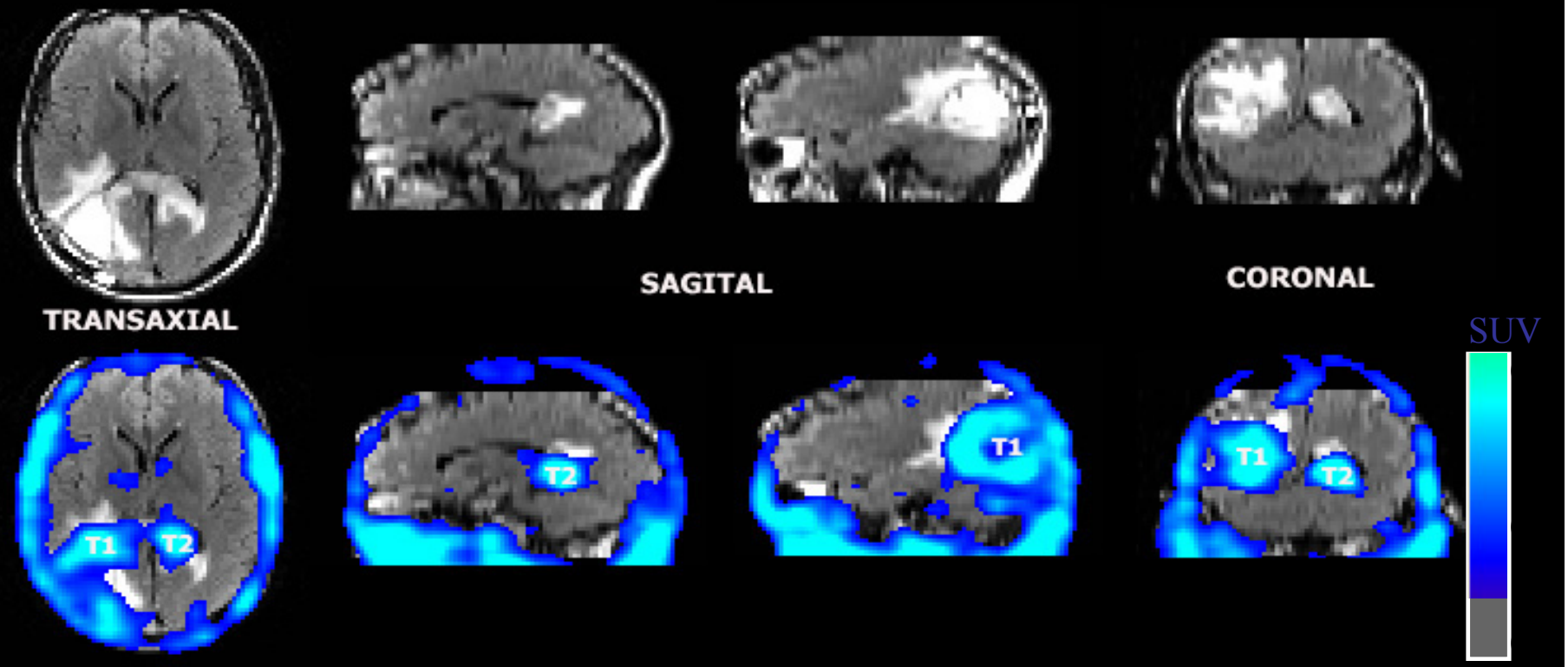
P. Walczak et al.

Imaging Cell Differentiation *in vivo*



P. Walczak et al.

T Cell Imaging with HSV-tk and ^{18}F-FHBG PET in a Glioma Patient



S.S. Yaghoubi et al., Nat. Clin. Pract. Oncol. 6, 53-58 (2009).

JOHNS HOPKINS
MEDICINE

MRI Reporter Genes: the Holy Grail

JOHNS HOPKINS
MEDICINE

NMR IN BIOMEDICINE
NMR Biomed. 2007; **20**: 275–290
Published online in Wiley InterScience
(www.interscience.wiley.com) DOI:10.1002/nbm.1134

WILEY InterScience®
DISCOVER SOMETHING GREAT

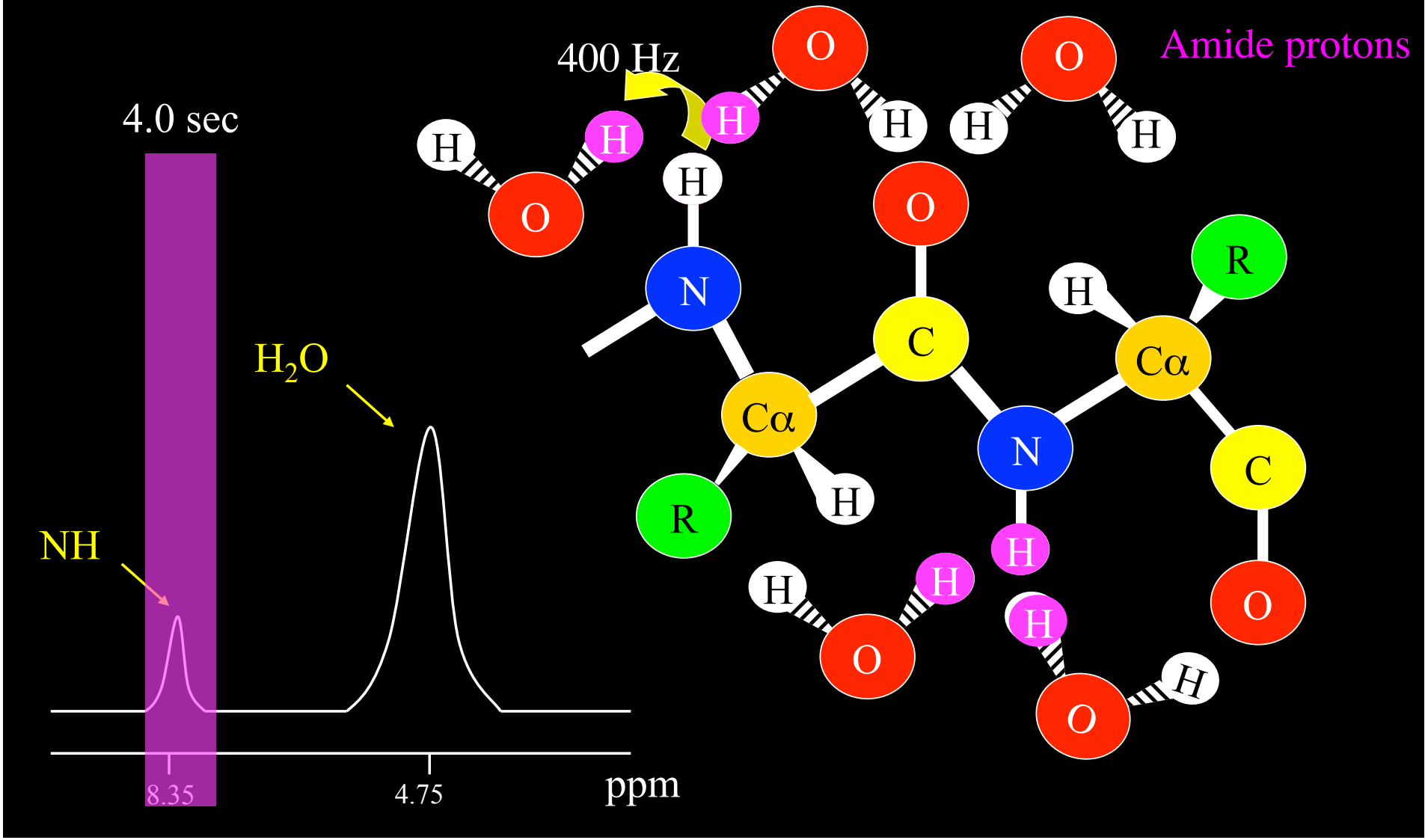
Review Article

Developing MR reporter genes: promises and pitfalls

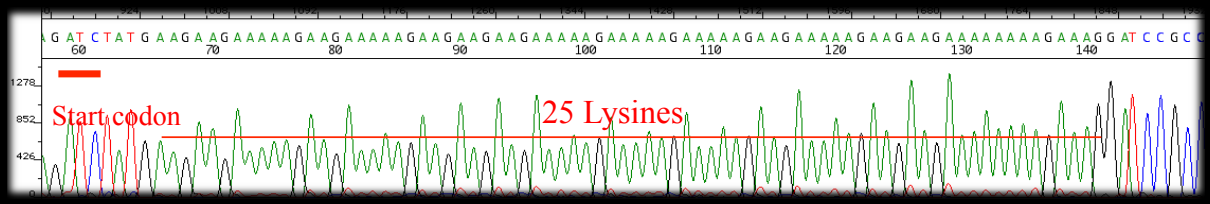
Assaf A. Gilad,[1,2†] Paul T. Winnard Jr,[1†] Peter C. M. van Zijl[1,3] and Jeff W. M. Bulte[1,2*]

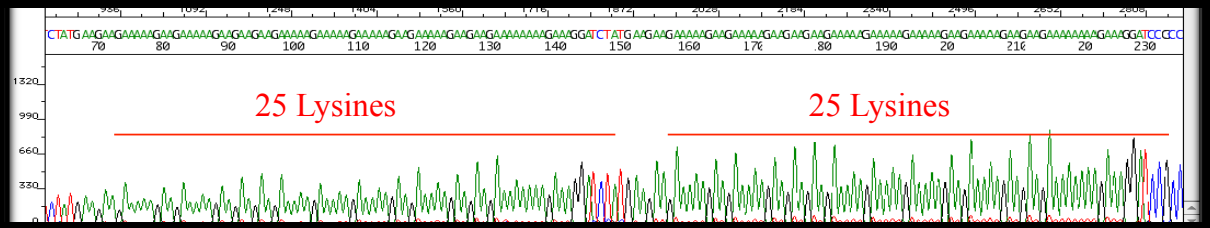
JOHNS HOPKINS MEDICINE

Developing a Reporter Gene based on Chemical Exchange Saturation Transfer (CEST)

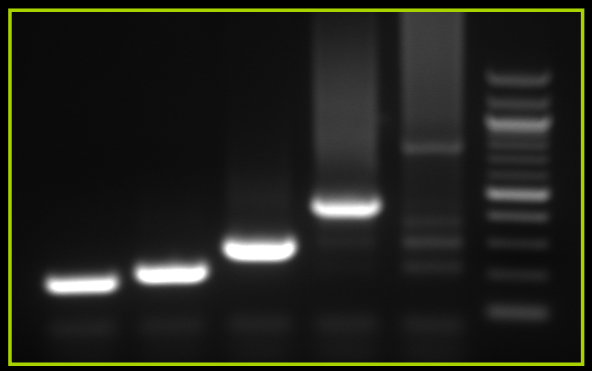


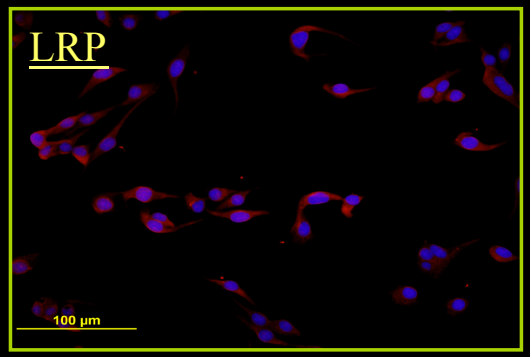
Cloning and Expression of a Lysine Rich Protein (LRP)

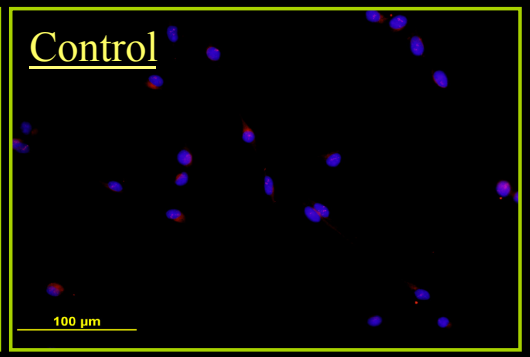




PCR

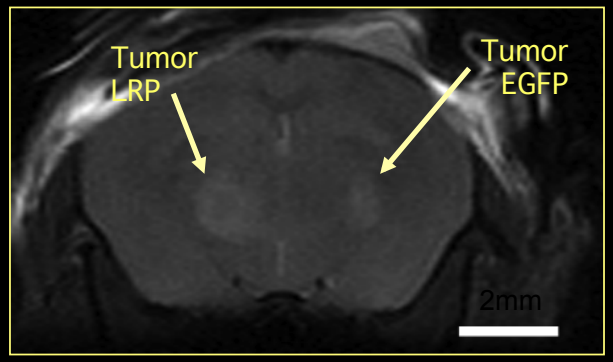




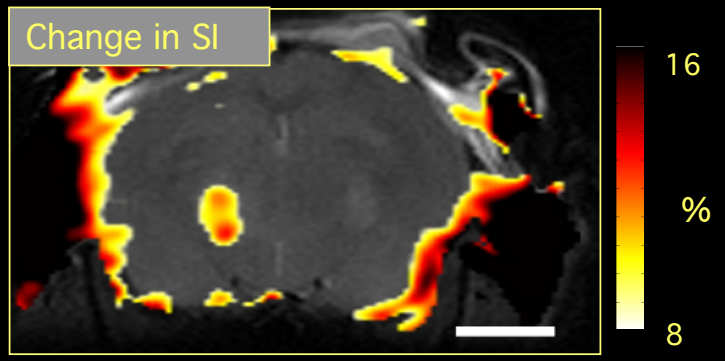


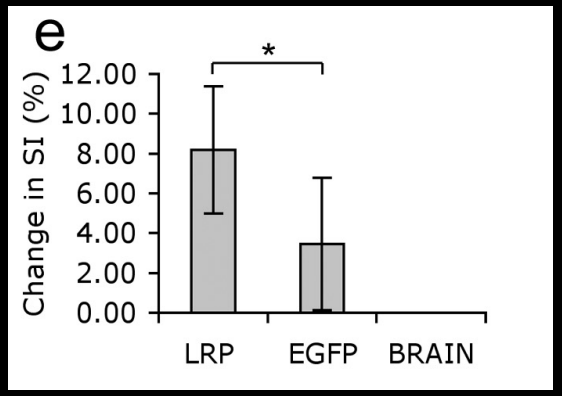
A.A. Gilad et al., Nat. Biotechnol. 25, 217-219 (2007).

Imaging of LRP-transfected glioma cells in vivo



CEST



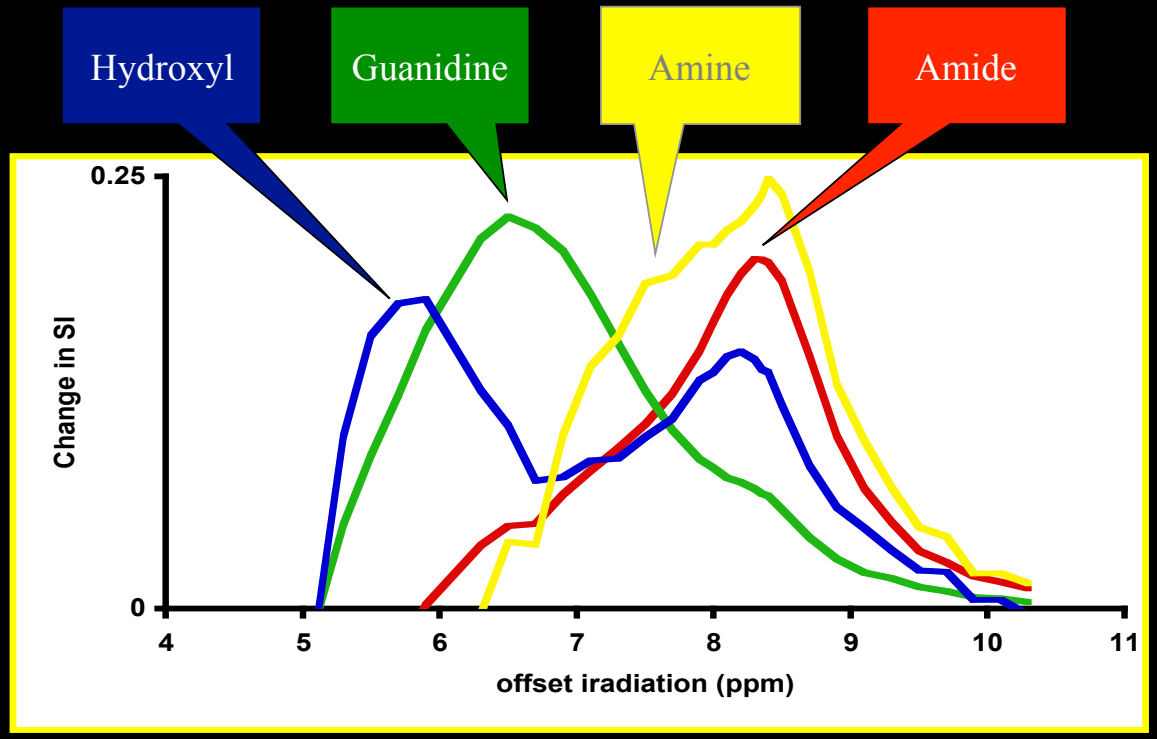


A.A. Gilad et al., Nat. Biotechnol. 25, 217-219 (2007).

JOHNS HOPKINS
MEDICINE

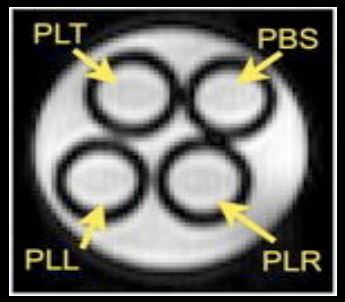
Multiple Colors

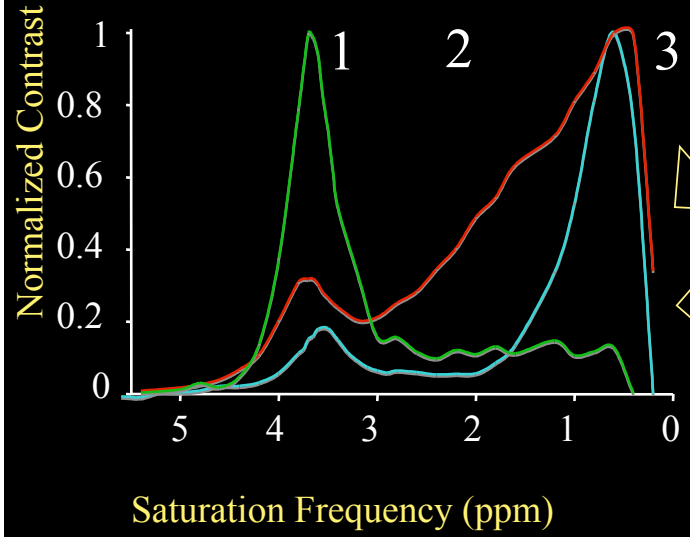
Frequency-dependent CEST MR contrast agents "Multi-color" peptides



M.T. McMahon et. al. Magn. Reson. Med., 60, 803-812 (2008).

JOHNS HOPKINS MEDICINE

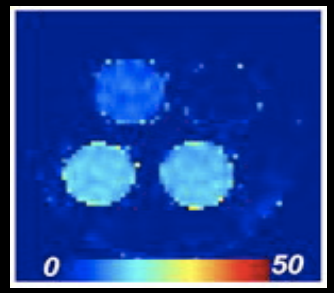


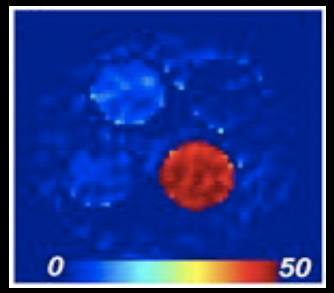


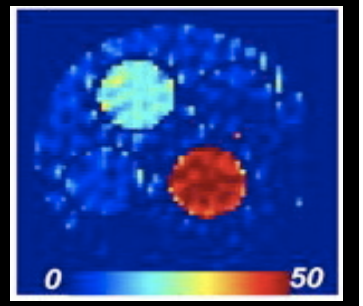
PLL
1

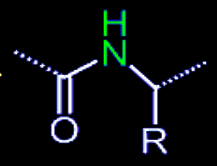
PLR
2

PLT
3

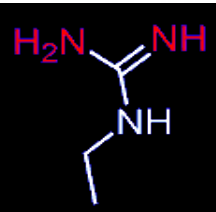




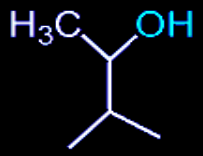




only



only

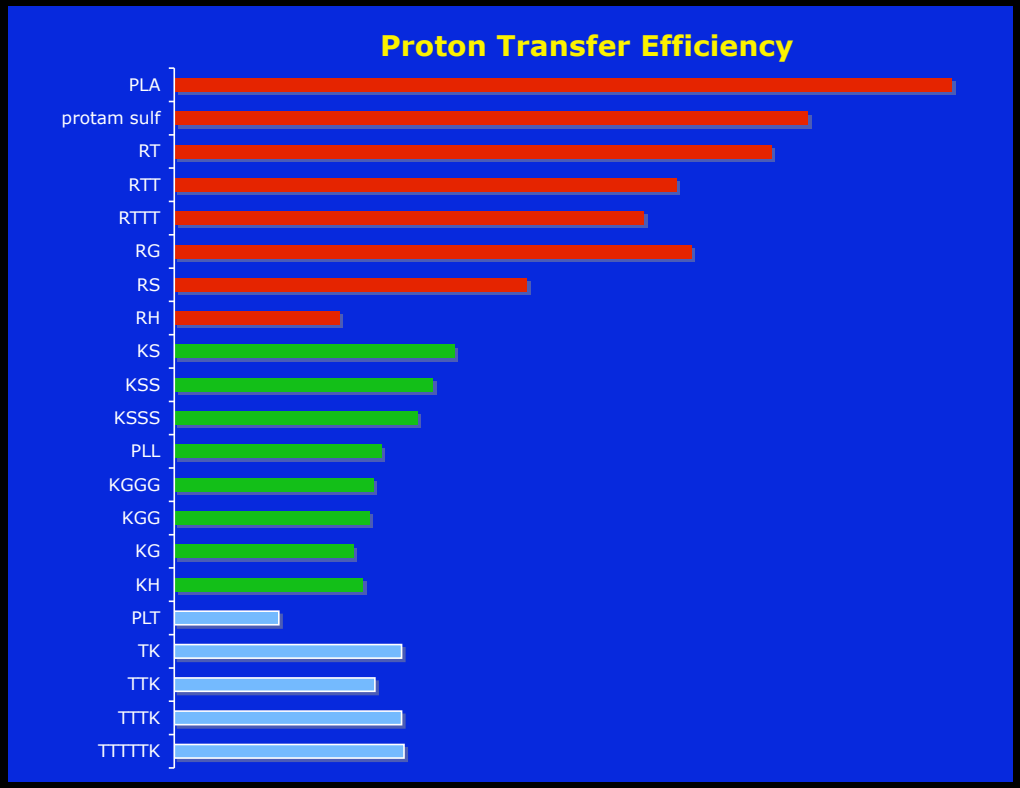


only

M.T. McMahon et. al. Magn. Reson. Med., 60, 803-812 (2008).

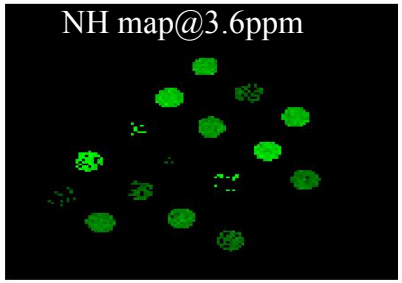
JOHNS HOPKINS
MEDICINE

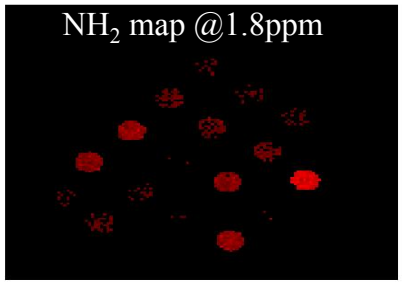
CEST Sensitivity for Different Peptide Sequences

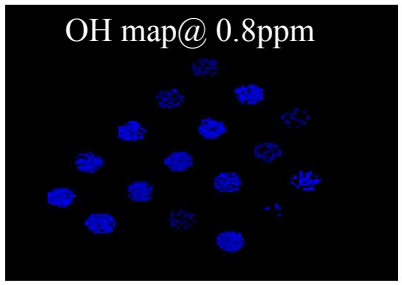


M.T. McMahon et. al. Magn. Reson. Med., 60, 803-812 (2008).

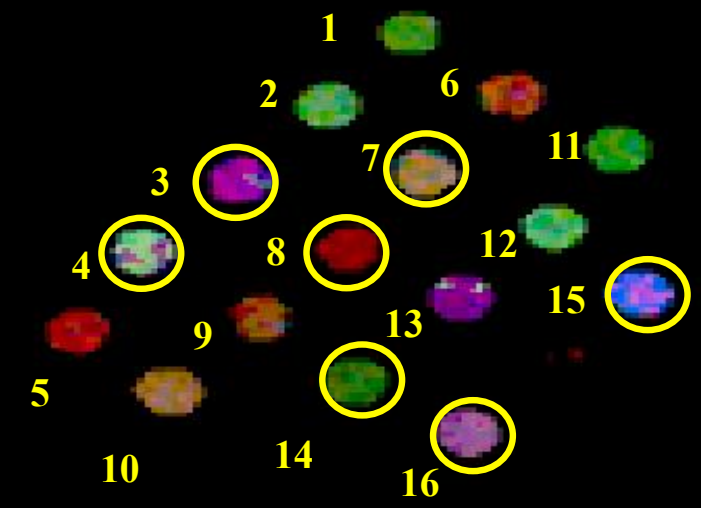
Peptide reporters with unique CEST 'codes'







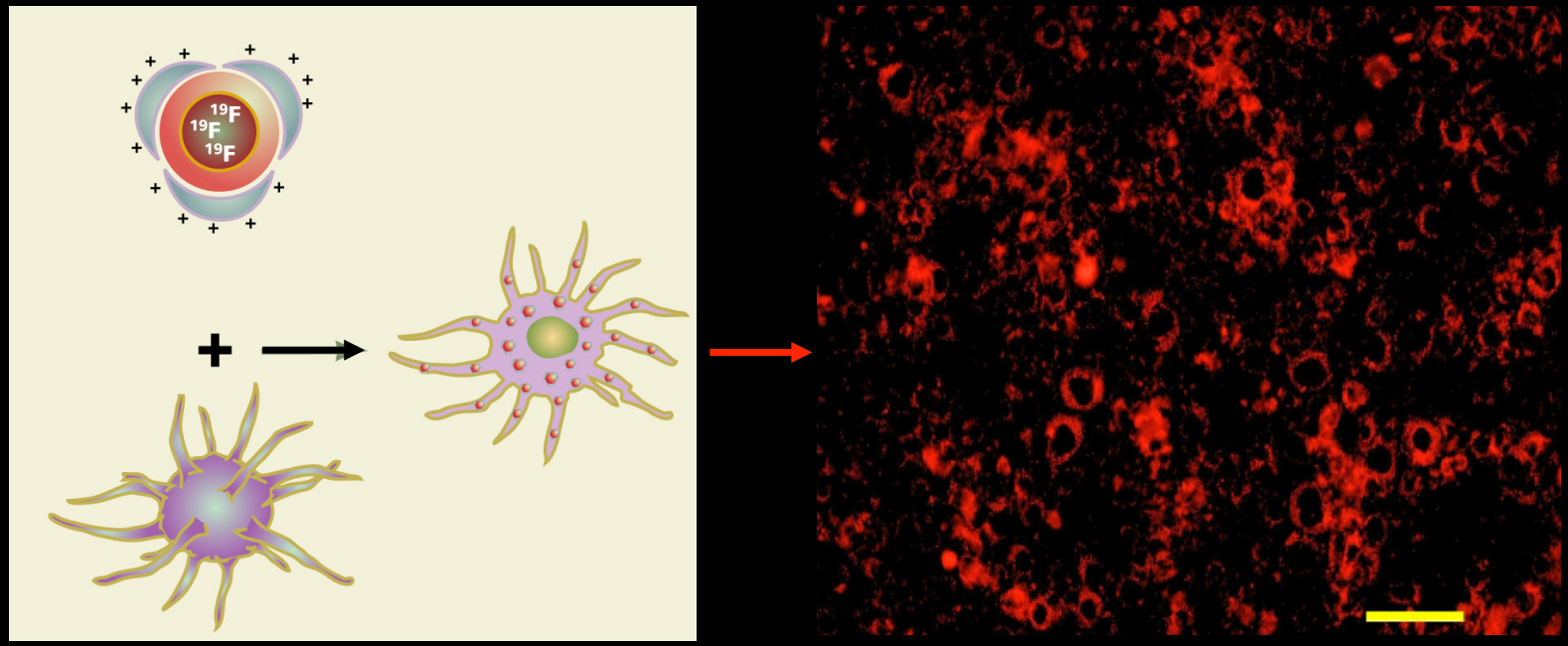
CEST RGB 'color'



3. $(DSSS)_3$
4. $(DSSSSS)_2$
7. $(DTTTTT)_2$
8. $(ETT)_4$
14. $(TTTTK)_2$
15. $(RT)_6$
16. $(RTTT)_3$

G. Liu, M.T. McMahon et. al.

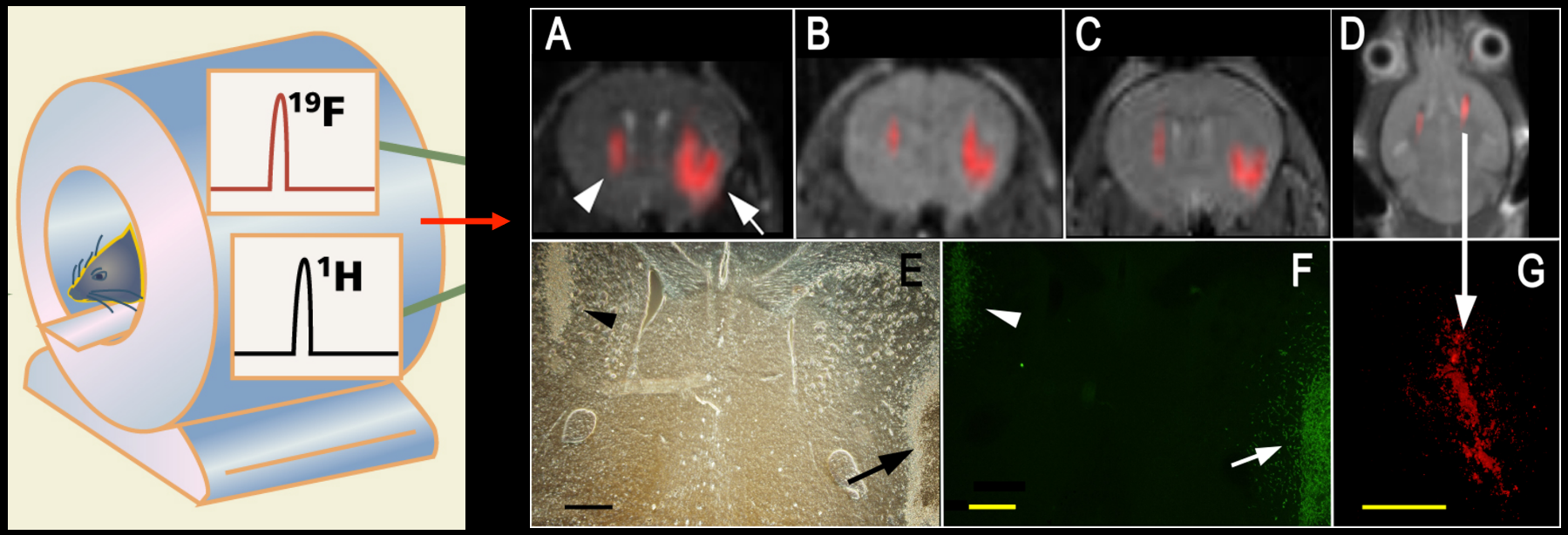
^{19}F "Hot Spot" MRI Cell Tracking



Jesus Ruiz-Cabello et al., Magn. Reson. Med., 60, 1506-15111 (2008).

JOHNS HOPKINS
MEDICINE

^{19}F "Hot Spot" MRI Cell Tracking



Jesus Ruiz-Cabello et al., Magn. Reson. Med., 60, 1506-15111 (2008).

Acknowledgments

Tamir Ben-Hur
Assaf Gilad
Doug Kerr
Heechul Kim
Michael Levy
Guanshu Liu
Michael McMahon
Naser Muja
Piotr Walczak
Peter van Zijl

Supported by 2RO1 NS045062, R21 EB005252, NMSS RG3630.

Dr. Bulte is a paid consultant for SurgiVision Inc.
This arrangement has been approvedby the Johns Hopkins University School of Medicine