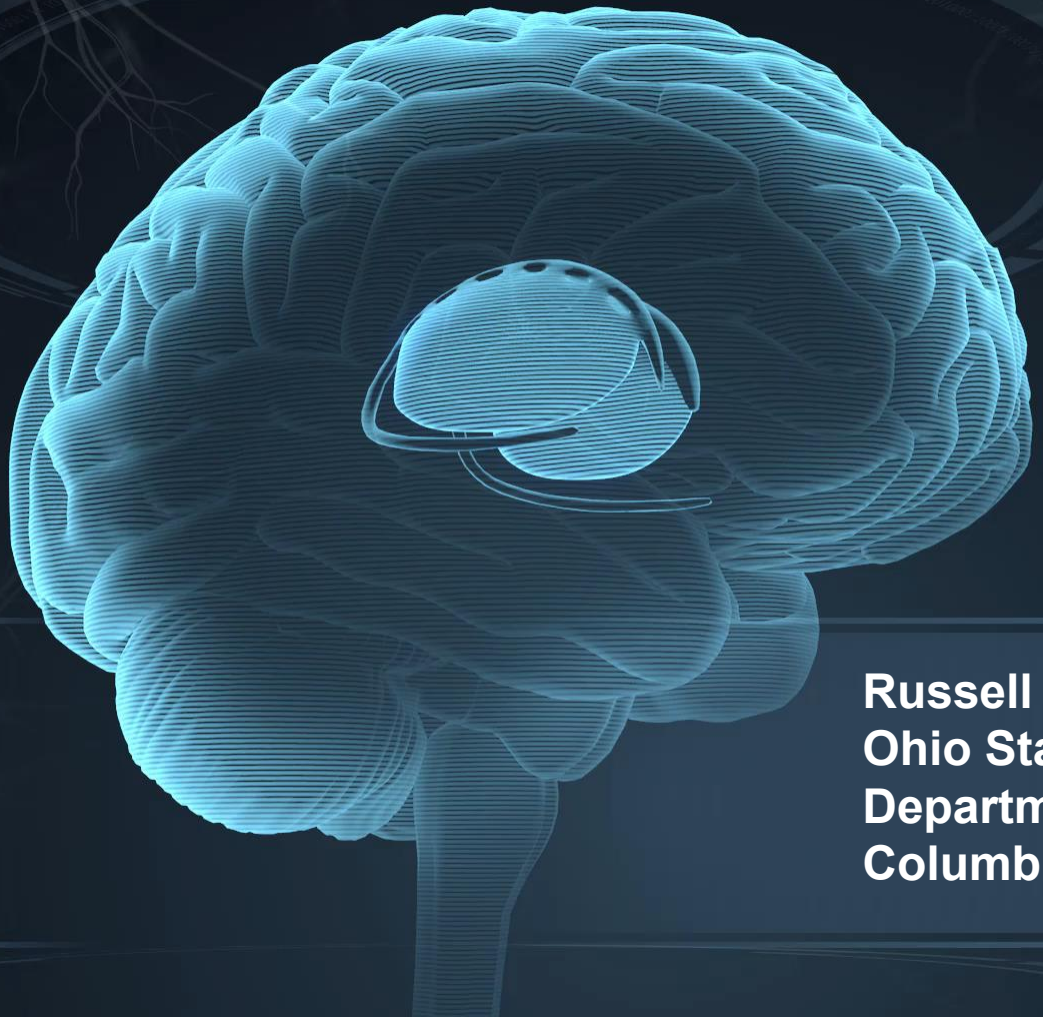
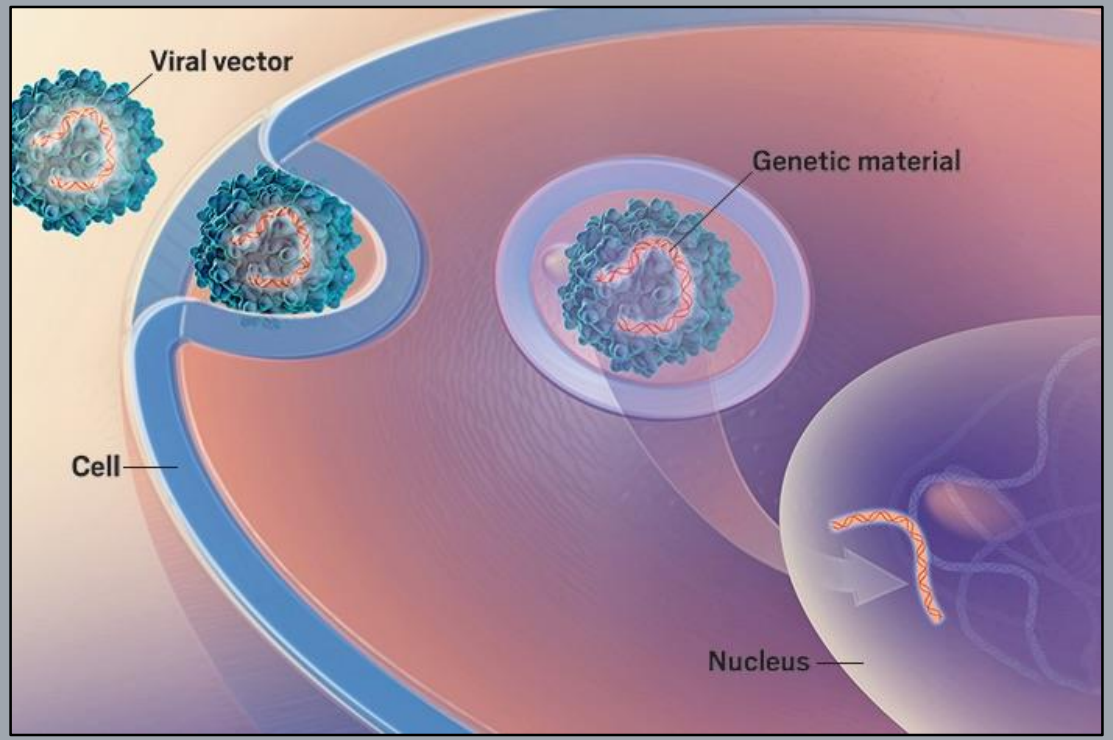


Direct convective delivery for nervous system gene therapy

Russell R. Lonser, M.D.
Ohio State University
Department of Neurological Surgery
Columbus, Ohio

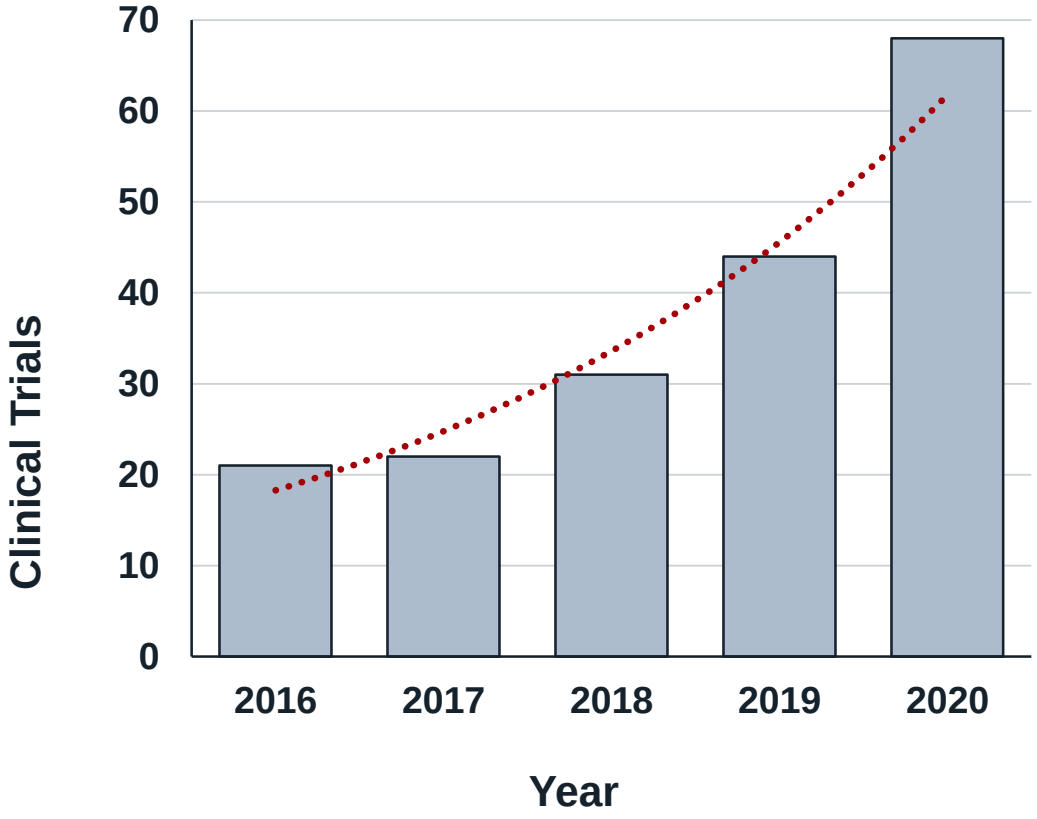
Gene therapy

Gene therapy is based on viral vector carriers of therapeutic gene



Nervous system clinical trials

Gene therapy clinical trials are growing at an exponential rate



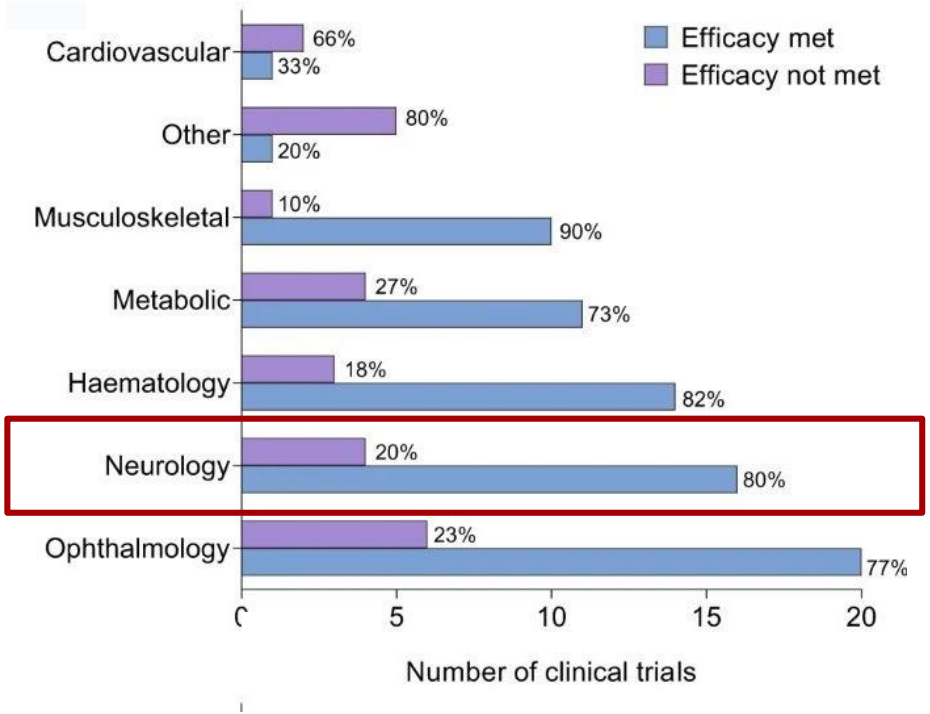
Gene therapy trials

80% of nervous system gene therapy trials have met efficacy end points

ARTICLE

Changing trends in the development of AAV-based therapies: a meta-analysis of past and present therapies

Tamara Burdett [1]✉ and Samir Nuseibeh[1]



Delivery routes

Route of Administration	Distribution	Advantages	Limitations
Systemic	Widespread (systemic)	□ Non-invasive □ Systemic impact	□ Blood-brain barrier □ Non-targeted coverage (off-target effects) □ Immune response
Intrathecal/Intraventricular	Widespread (nervous system)	□ Minimally invasive □ Widespread nervous system impact	□ Blood-ependymal barrier □ Non-targeted coverage (off-target effects) □ Immune response
Direct convective delivery	Limited (perfused area)	□ Targeted □ Selective manipulation of disease neuronal circuits and treatment regions □ Bypasses blood-brain barrier	□ Most invasive (placement of infusion cannula in nervous system) □ Requires significant infrastructure

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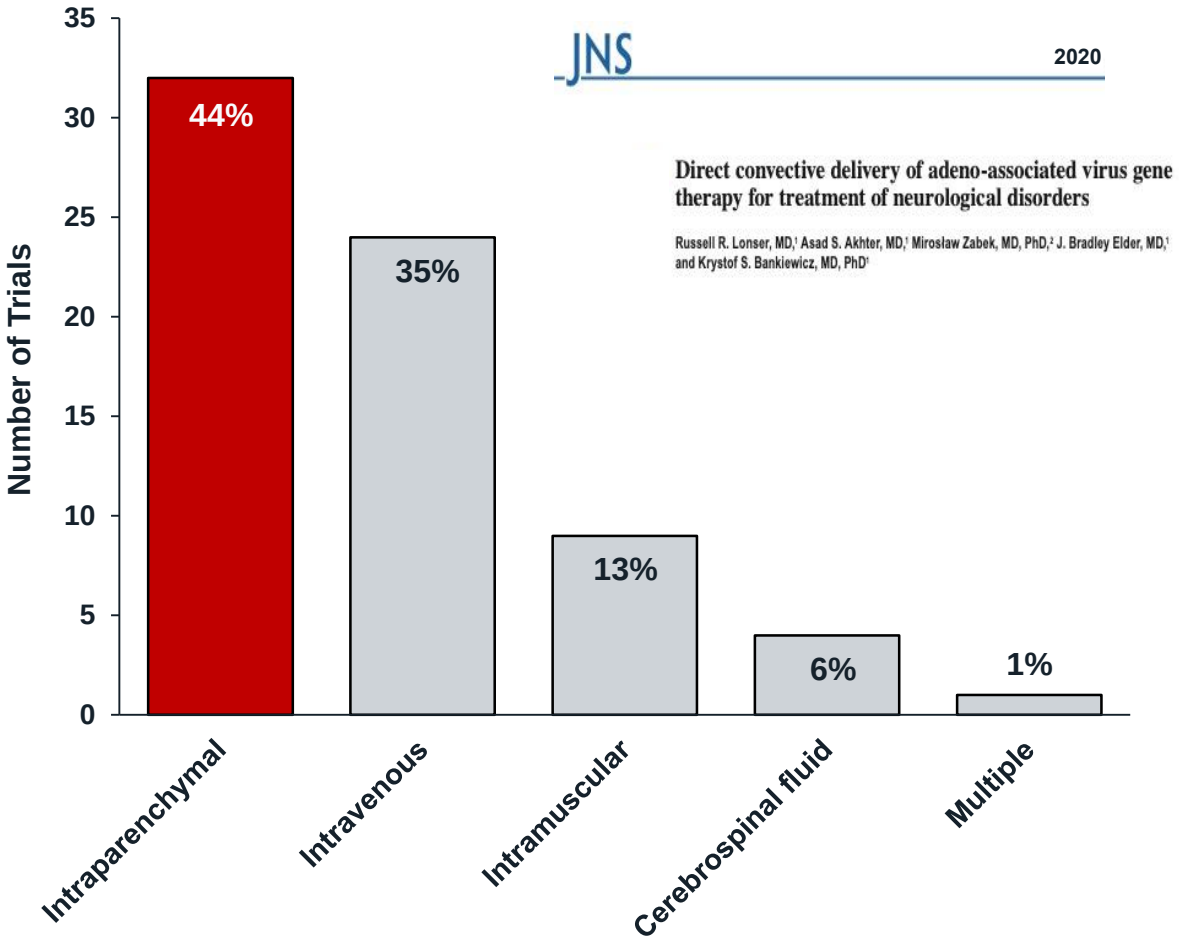
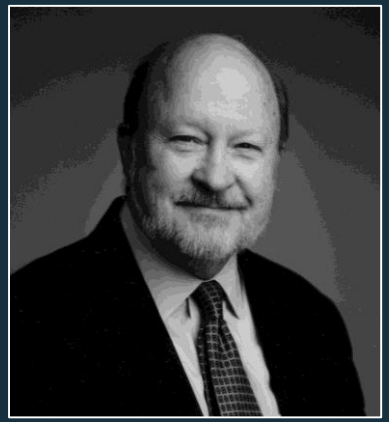
Routes of delivery

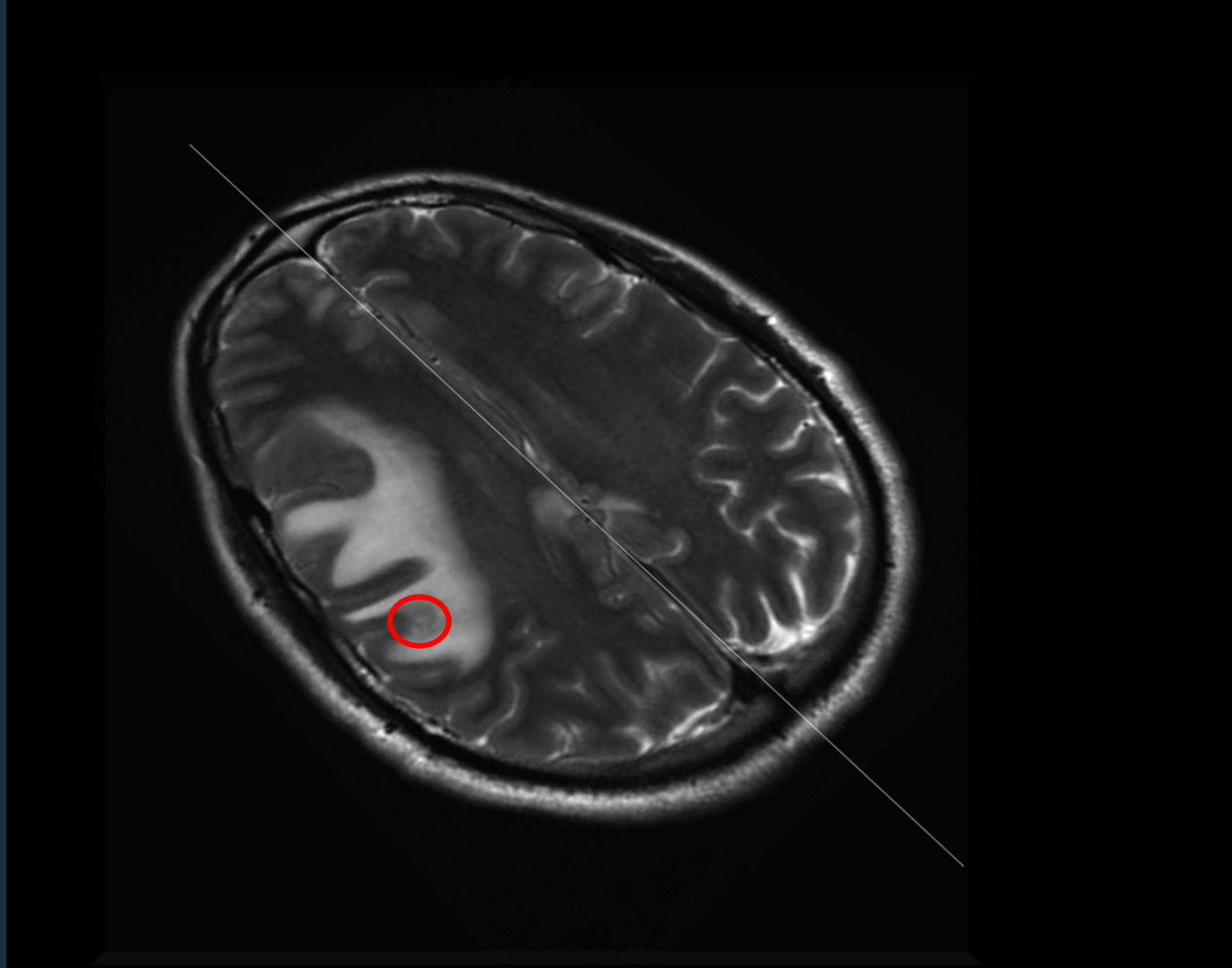
Most common route of gene distribution is direct convective delivery



Convective delivery



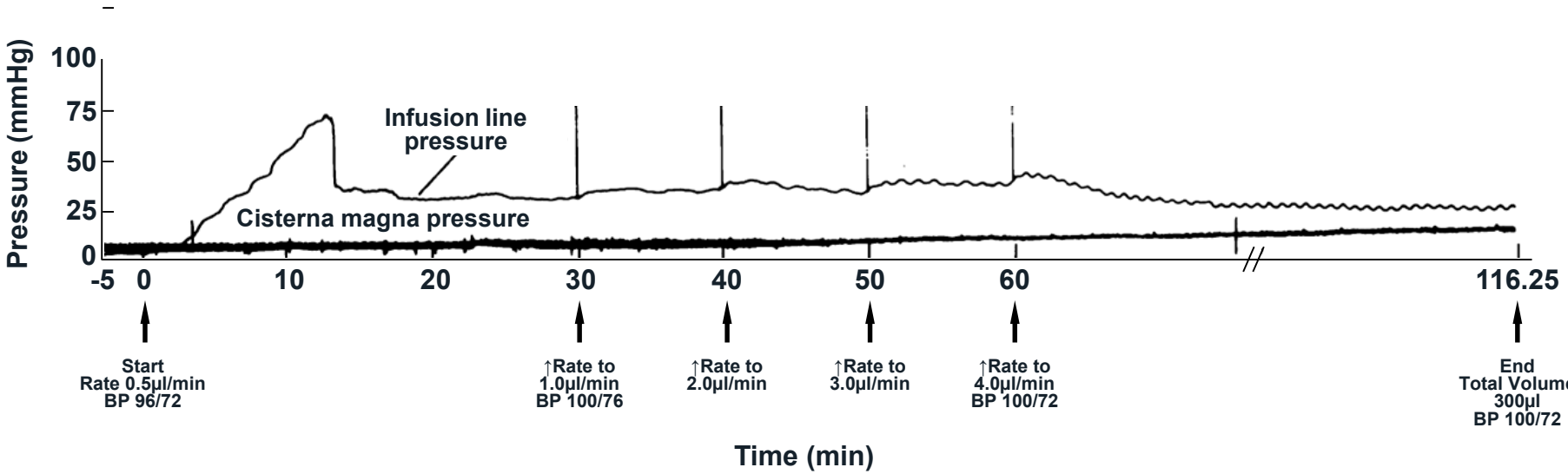
Edward H. Oldfield, M.D.



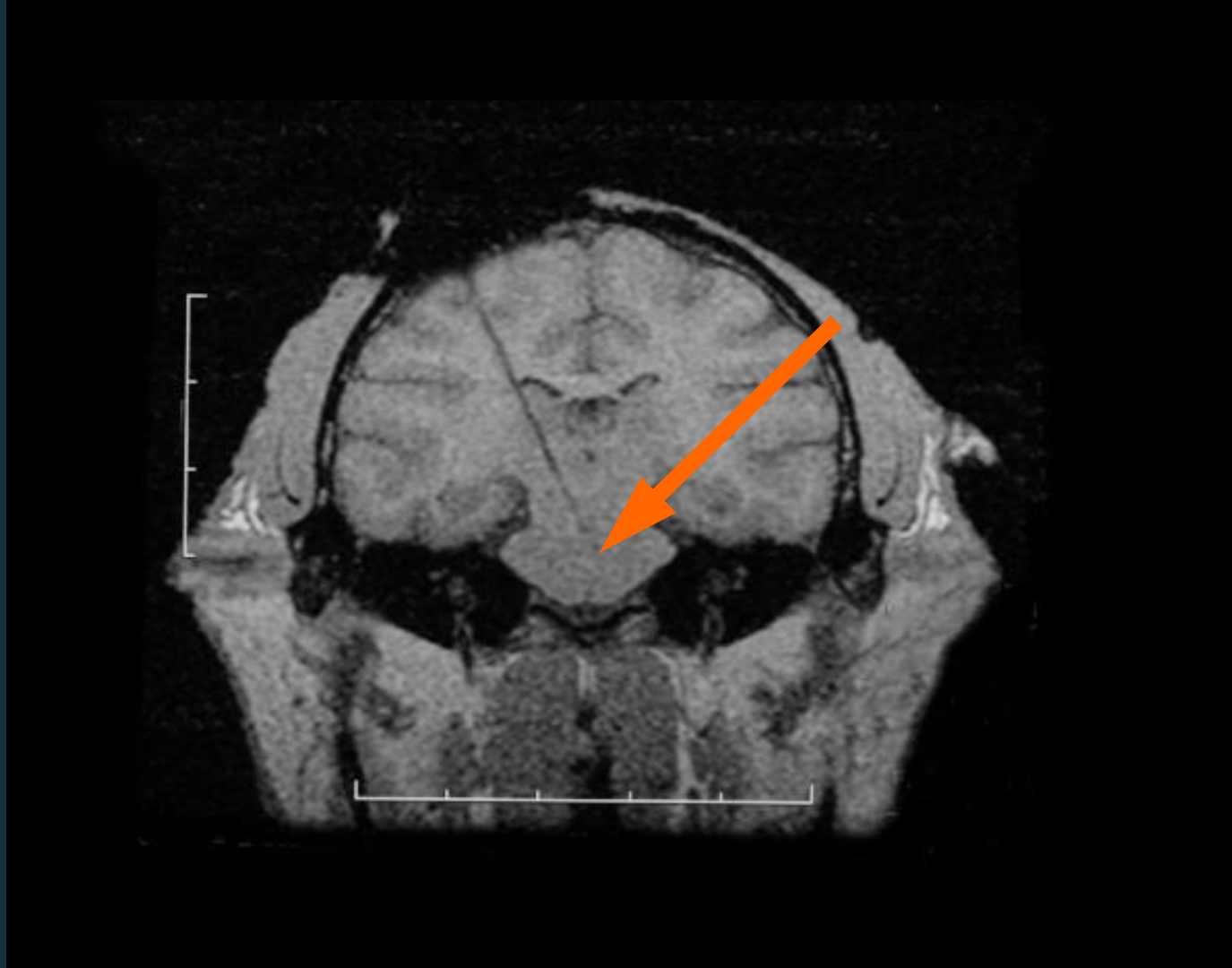
PNAS 1994

Convection-enhanced delivery of macromolecules in the brain

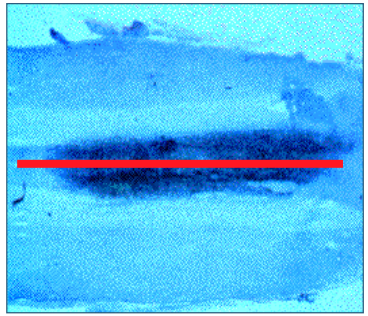
R. Hunt Bobo, Douglas W. Laske, Aytac Akbasak, Paul F. Morrison,
Robert L. Dedrick and Edward H. Oldfield

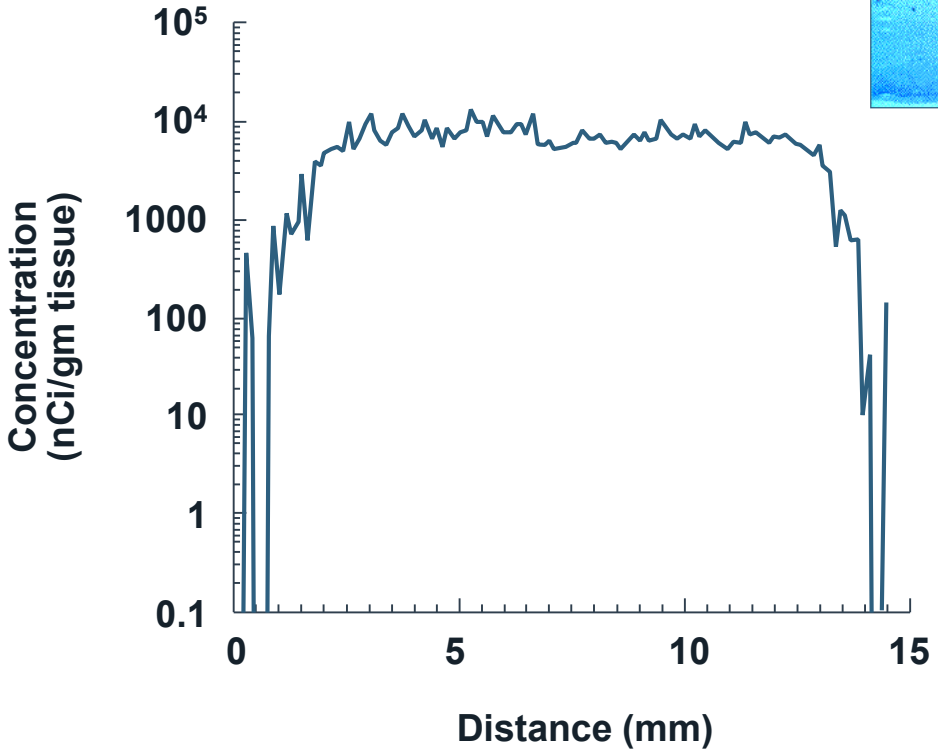


Bypasses blood-brain barrier



Uniform distribution

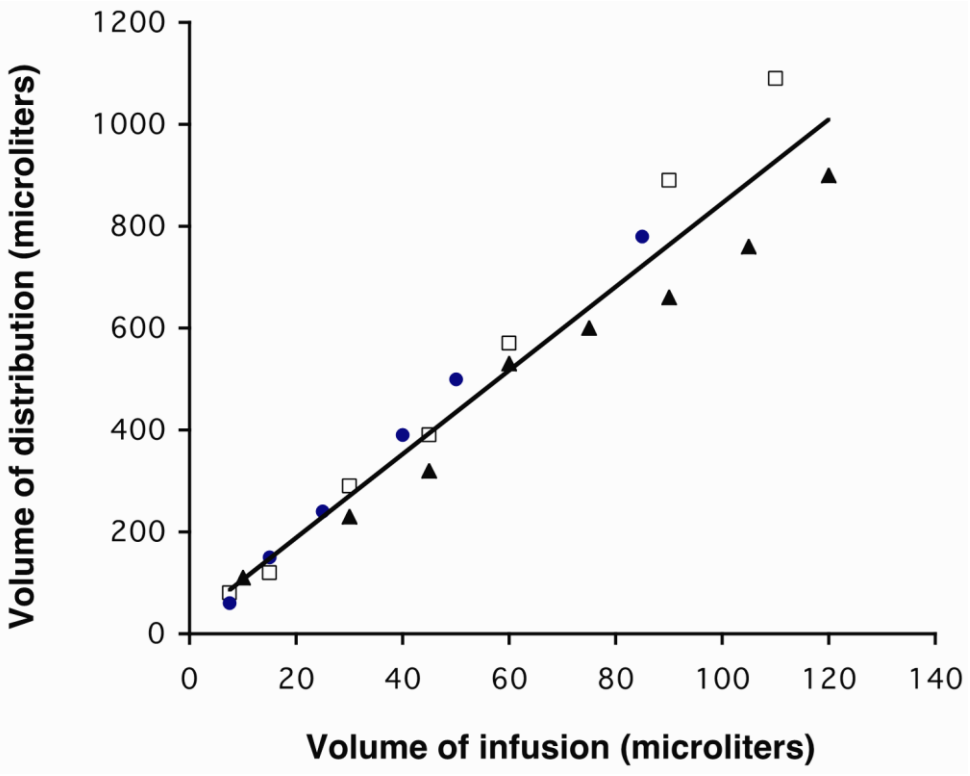




Reliable distribution

Linear relationship $R^2 = 0.94$ to 0.98

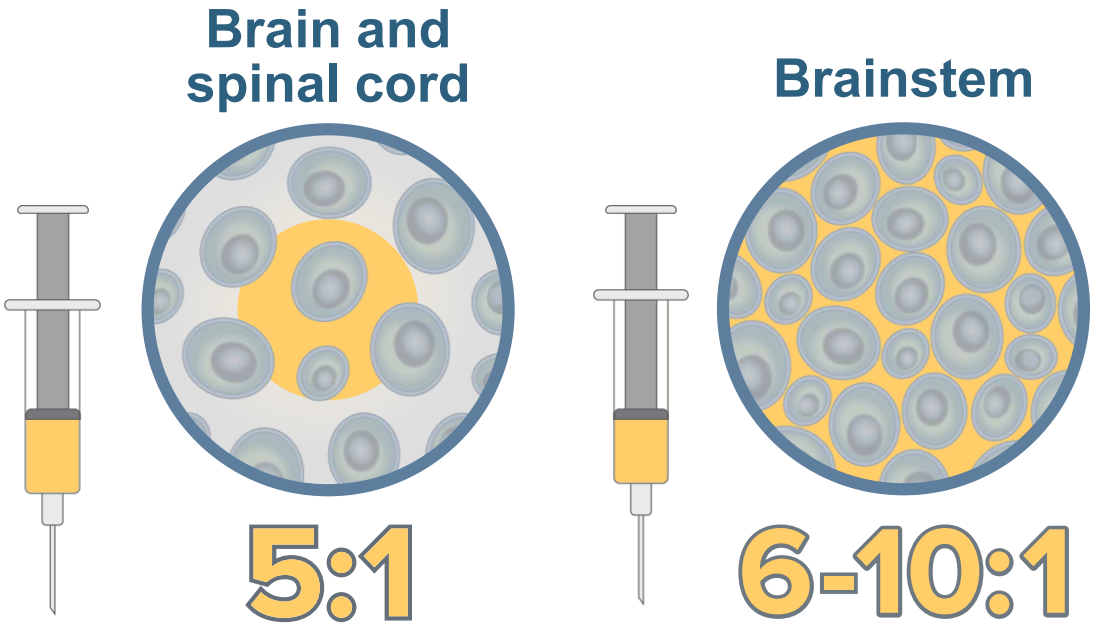
Volume of tissue distribution increases linearly with volume of infusion



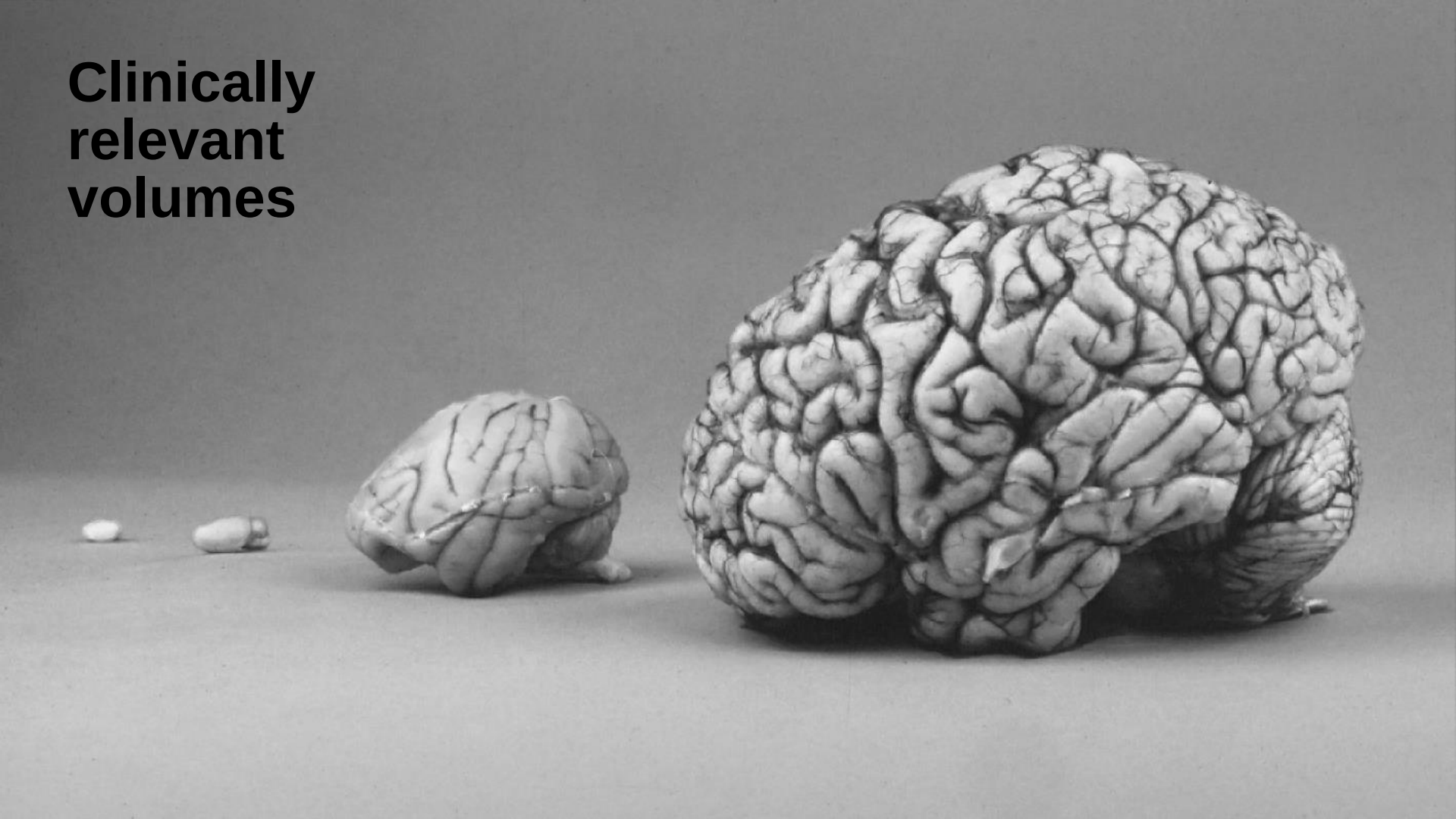
Reliable distribution

Inversely proportional to the interstitial space available for solute distribution

Vd:Vi Ratios



Clinically relevant volumes



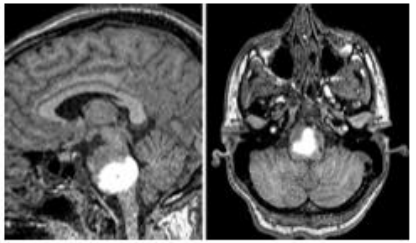
Targeted delivery

Various regions of the nervous system can be targeted

JNS 2015

Convection-enhanced delivery to the central nervous system

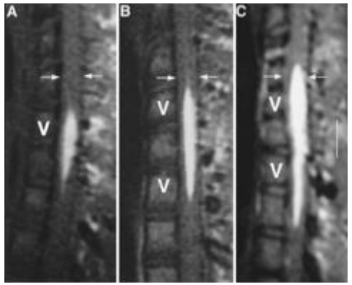
Russell R. Lonser, MD,[1,2] Malisa Sarntinoranont, PhD,[3] Paul F. Morrison, PhD,[4] and Edward H. Oldfield, MD[2,5]



JNS 1998

Direct convective delivery of macromolecules to the spinal cord

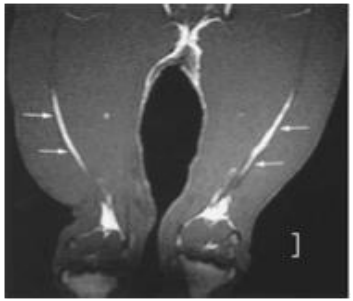
Russell R. Lonser, M.D., Nitin Gogate, M.D., Paul F. Morrison, Ph.D., J. David Wood, B.S., and Edward H. Oldfield, M.D.



JNS 1998

Direct convective delivery of macromolecules to peripheral nerves

Russell R. Lonser, M.D., Robert J. Weil, M.D., Paul F. Morrison, Ph.D., Lance S. Governale, and Edward H. Oldfield, M.D.



Targeted delivery

Less than 2 mm absolute error

Permits intraoperative MR-imaging during infusion

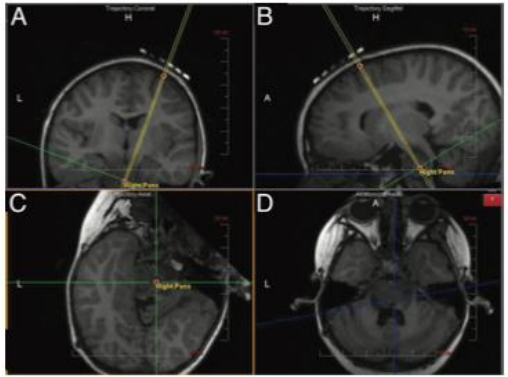
JNS

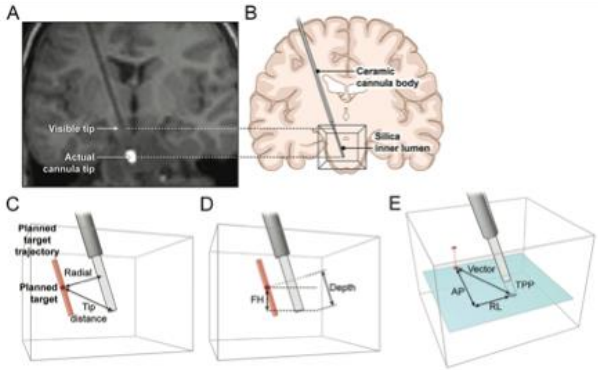
TECHNICAL NOTE

J Neurosurg 122:1173–1179, 2015

Accuracy of direct magnetic resonance imaging-guided placement of drug infusion cannulae

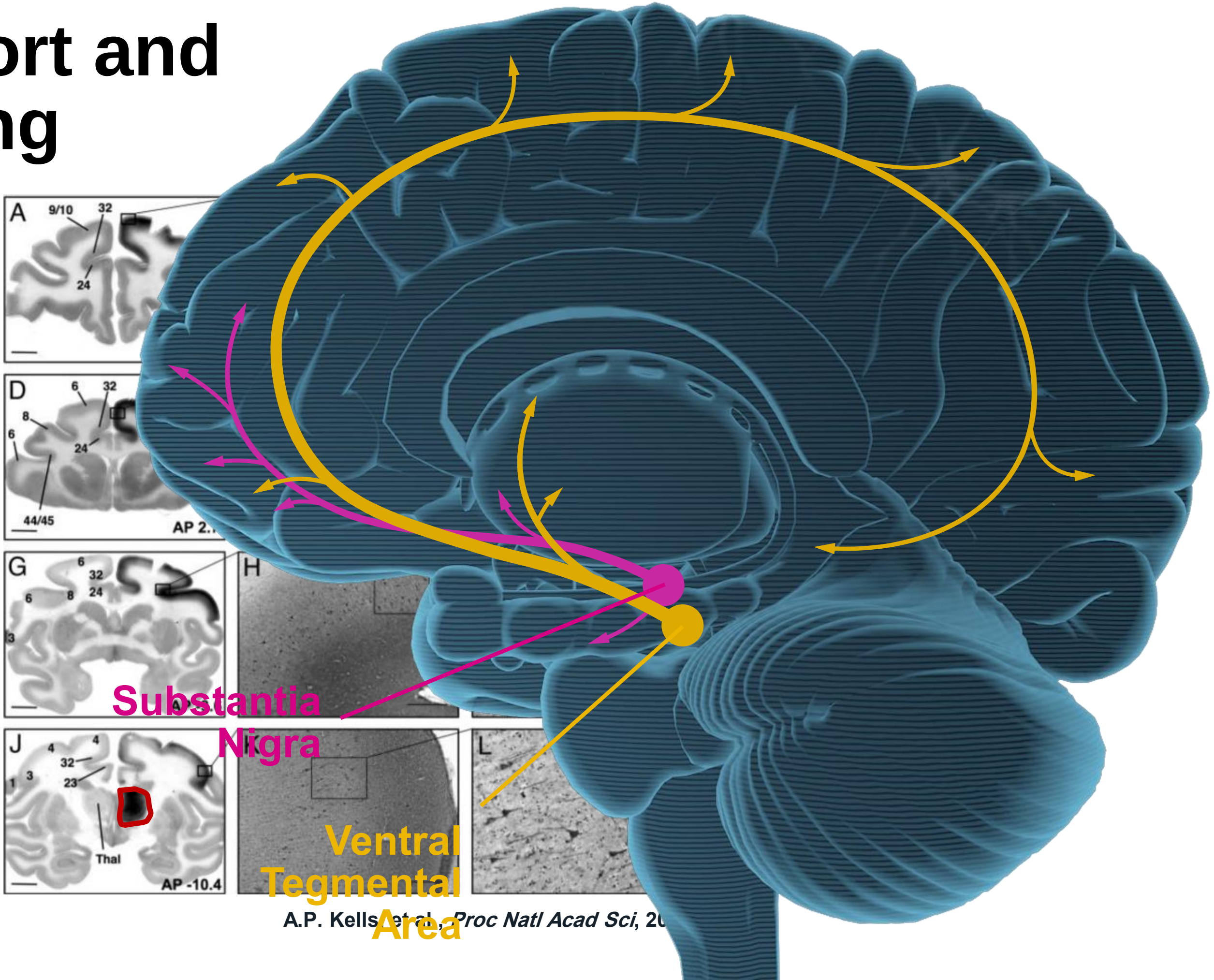
Prashant Chittiboina, MD,[1] John D. Heiss, MD,[1] and Russell R. Lonser, MD[1,2]





Vector transport and circuit targeting

Allows for focal or targeted widespread transport distribution in ability to target circuits from perfused region regions



A.P. Kells et al., *Proc Natl Acad Sci*, 20

Vector characteristics

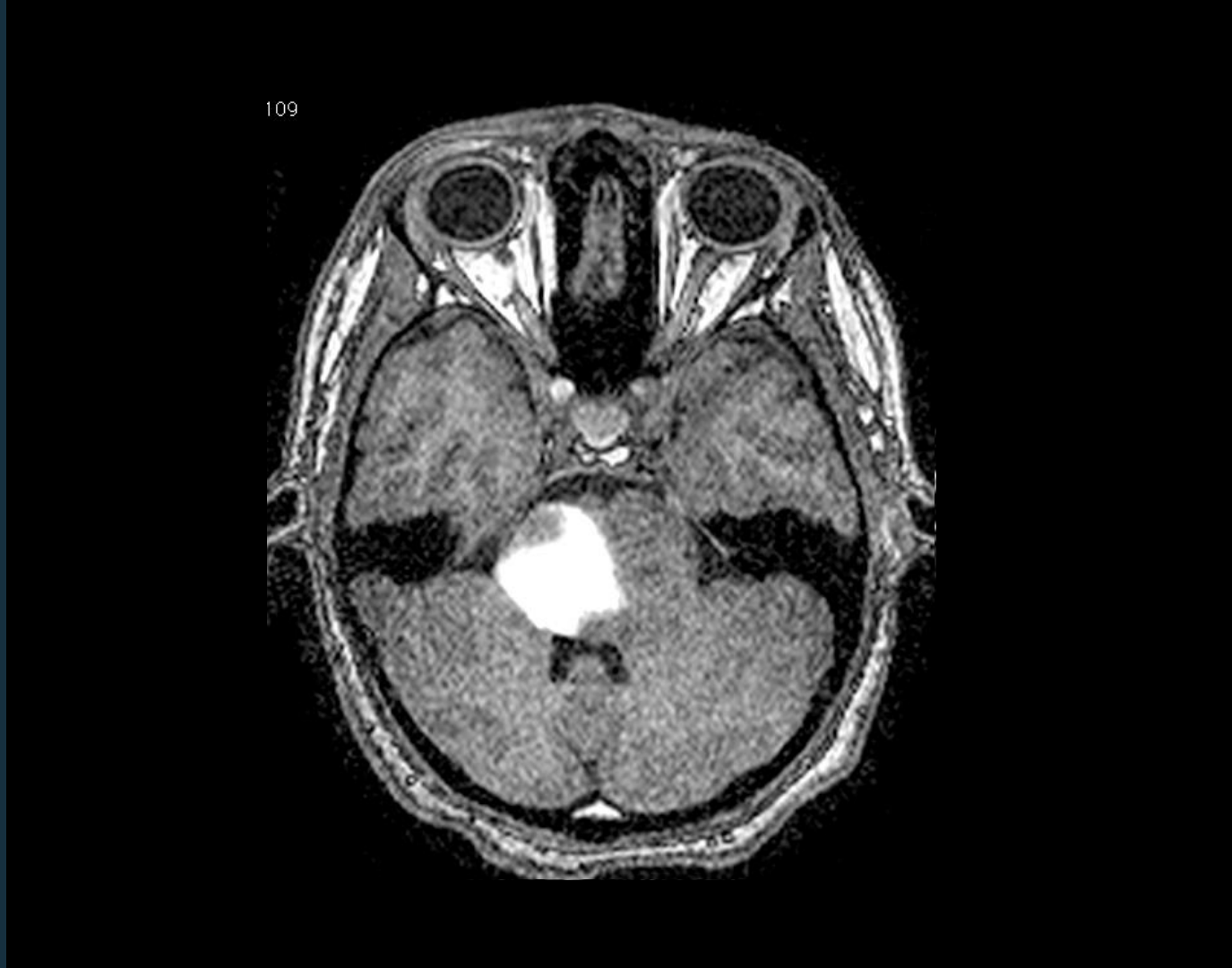
Viral Vector	Intravenous	Intramuscular	Cerebrospinal Fluid	Parenchymal	Cellular Trophism	Transport
Adeno-associated virus						
Serotype 1		X			N, A, NN	Ret, Ant
Serotype 2		X		X	N	Ant
Serotype 2/6	X				N, A	
Serotype 5				X	N, A, NN	Ret, Ant
Serotype 6	X	X			N, A	Ret
Serotype 8	X				N, A, NN	Ret, Ant
Serotype 9	X		X	X	N, A, NN	Ret, Ant
Serotype rh74	X	X				
Serotype rh10				X	N, A, NN	Ant
Lentivirus	X			X	A	Ret
Moloney leukemia virus				X	N	
Herpes Simplex 1*	X			X	N, A, NN	Ret, Ant

N – neuron, A – astrocyte, NN – non-neuronal, Ret – retrograde; Ant – anterograde
*Only current use is in neuro-oncology

R.R. Lonser et al., *J Neurosurg*, 2020

Real-time imaging of delivery

Co-infusion of surrogate tracer (Gd-DTPA) can be used to precisely define region of vector perfusion during real-time MR-imaging



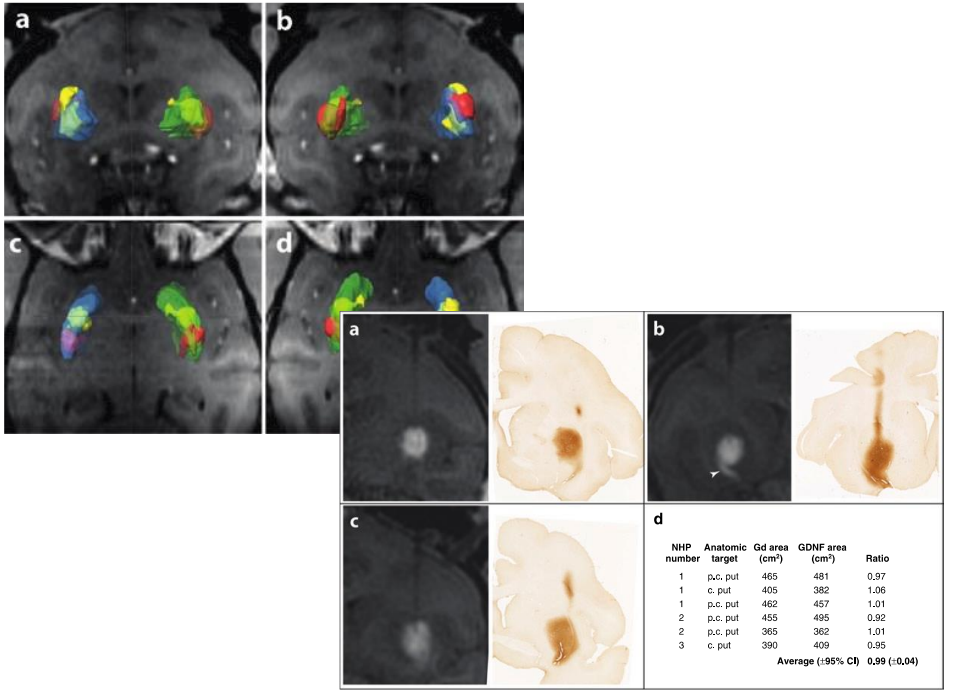
Imaging of vector delivery

Predicts transgene expression at site of infusion

Molecular Therapy
official journal of the American Society of Gene Therapy

Interventional MRI-guided Putaminal Delivery of AAV2-GDNF for a Planned Clinical Trial in Parkinson's Disease

R Mark Richardson[1], Adrian P Kells[1], Kathryn H Rosenbluth[1], Ernesto Aguilar Salegio[1], Massimo S Fiandaca[1], Paul S Larson[1], Philip A Starr[1], Alastair J Martin[2], Russell R Lonser[3], Howard J Federoff[4,5], John R Forsayeth[1] and Krystof S Bankiewicz[1]



Current clinical trials

Disorder	Putative Agent	Imaging Agent	Target
Parkinson's disease	AAV2-GDNF	Gd-DTPA	Putamen
Parkinson's disease	AAV2-AADC	Gd-DTPA	Putamen
AADC-deficiency	AAV2-AADC	Gd-DTPA	Brainstem
Multisystem Atrophy	AAV2-GDNF	Gd-DTPA	Putamen
Alzheimer's disease	AAV2-BDNF	Gd-DTPA	EC
Huntington's disease	AAV5-mRNA	Gd-DTPA	Putamen/Caudate
Malignant glioma	Various	Gd-DTPA	Tumor

Current clinical trials

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Huntington's disease	AAV5-mRNA	Gd-DTPA	Putamen/Caudate
Malignant glioma	Various	Gd-DTPA	Tumor

Regenerative therapy

Science
AAAS

1993

GDNF: A Glial Cell Line–Derived Neurotrophic Factor for Midbrain Dopaminergic Neurons

Leu-Fen H. Lin, Daniel H. Doherty, Jack D. Lile, Susan Bektesh, Frank Collins*

nature medicine

2003

Direct brain infusion of glial cell line–derived neurotrophic factor in Parkinson disease

Steven S. Gill[1], Nikunj K. Patel[1], Gary R. Hotton[2], Karen O'Sullivan[1], Renée McCarter[1], Martin Bunnage[1], David J. Brooks[2], Clive N. Svendsen[3] & Peter Heywood[1]

Regenerative therapy

Preclinical studies

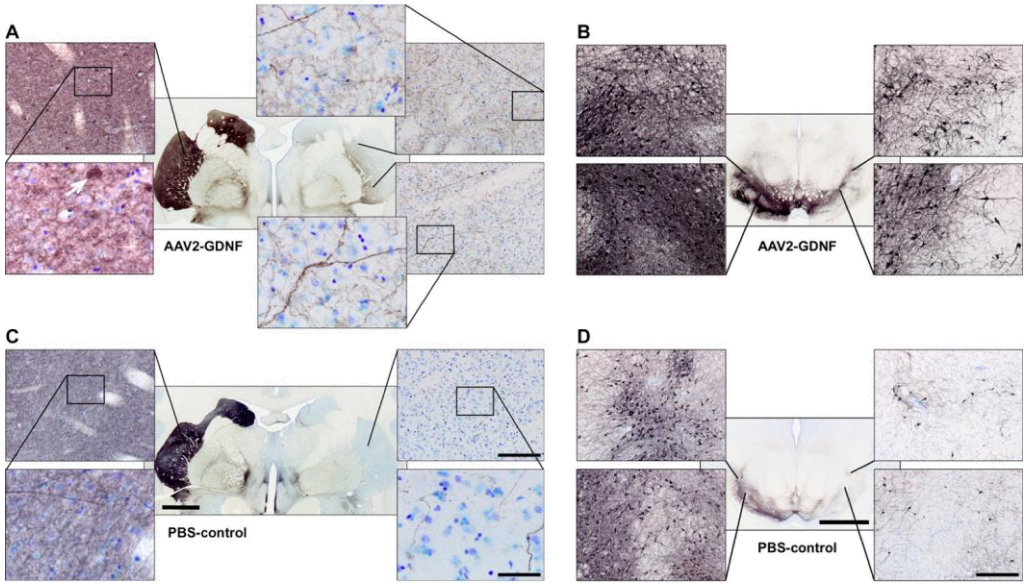
Anterograde transport of virus and regeneration

Human Gene Therapy

2009

Functional Effects of AAV2-GDNF on the Dopaminergic Nigrostriatal Pathway in Parkinsonian Rhesus Monkeys

Jamie L. Eberling, Adrian P. Kells, Philip Pivirotto, Janine Beyer, John Bringas, Howard J. Federoff, John Forsayeth and Krystof S. Bankiewicz



Axonal transport of AAV2-GDNF

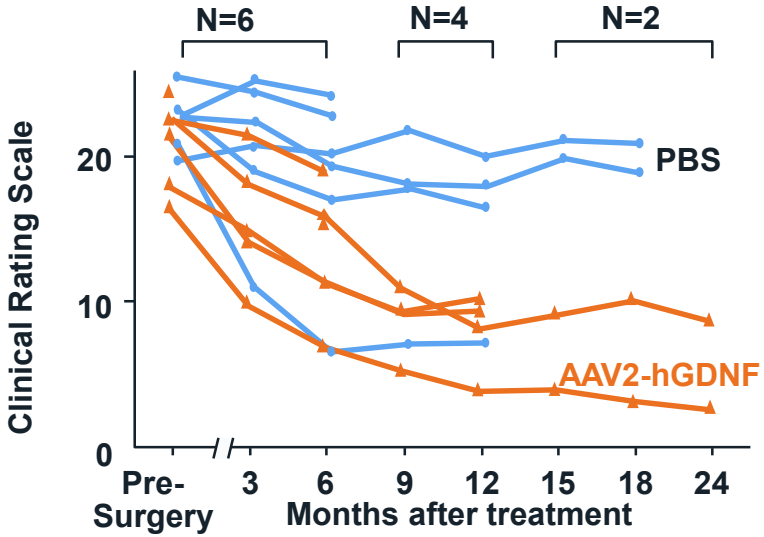
Axonal trafficking is critical for therapeutic effects of AAV2-GDNF. Vector undergoes anterograde transport via striatonigral pathway.

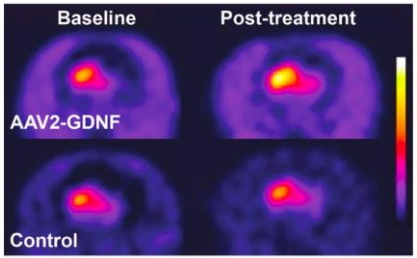
Preclinical studies

JNeurosci
THE JOURNAL OF NEUROSCIENCE
2010

Regeneration of the MPTP-Lesioned Dopaminergic System after Convection-Enhanced Delivery of AAV2-GDNF

Adrian P. Kells, Jamie Eberling, Xiaomin Su, Philip Pivirotto, John Bringas, Piotr Hadaczek, Wade C. Narrow, William J. Bowers, Howard J. Federoff, John Forsayeth and Krystof S. Bankiewicz





Phase I trial

AAV2-GDNF vector delivered to bilateral putamina of Parkinson's disease patients (moderate to severe) via a transfrontal approach.

Four dose levels (co-infusion with gadolium-DTPA):

Dose Cohort 1 (450 microliters; 3 x 10^{10} vg)
Dose Cohort 2 (450 microliters; 1 x 10^{11} vg)
Dose Cohort 3 (450 microliters; 3 x 10^{11} vg)
Dose Cohort 4 (450 microliters; 7 x 10^{11} vg)

Primary Objective:

- **Determine the safety and feasibility of treatment**

Secondary Objectives:

- **TH-fiber regeneration expression using ^{18}F-dopa PET**
- **Assessments of motor function and quality of life**
- **Assessments of daily requirements for levodopa**

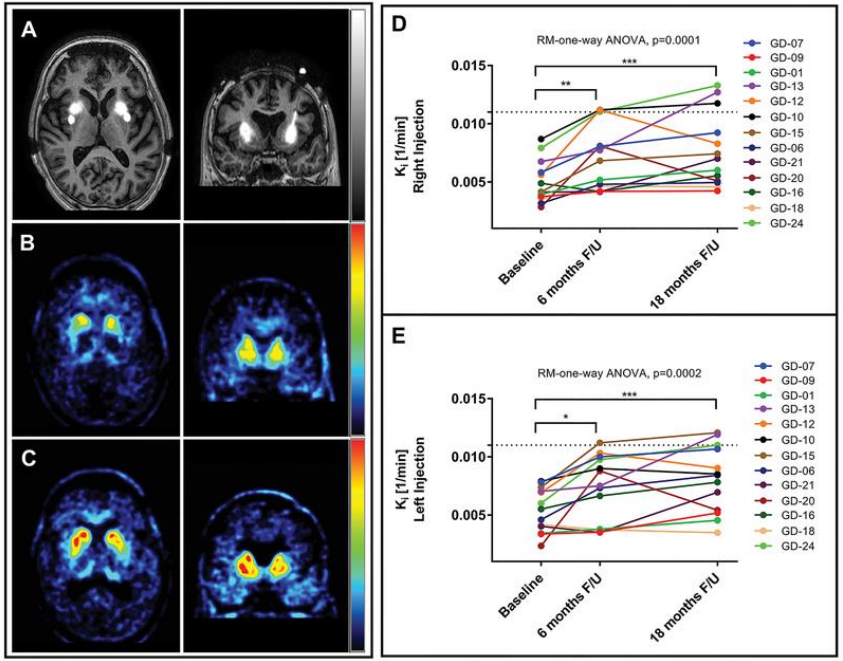
NIH National Institute of Neurological Disorders and Stroke

Phase I trial

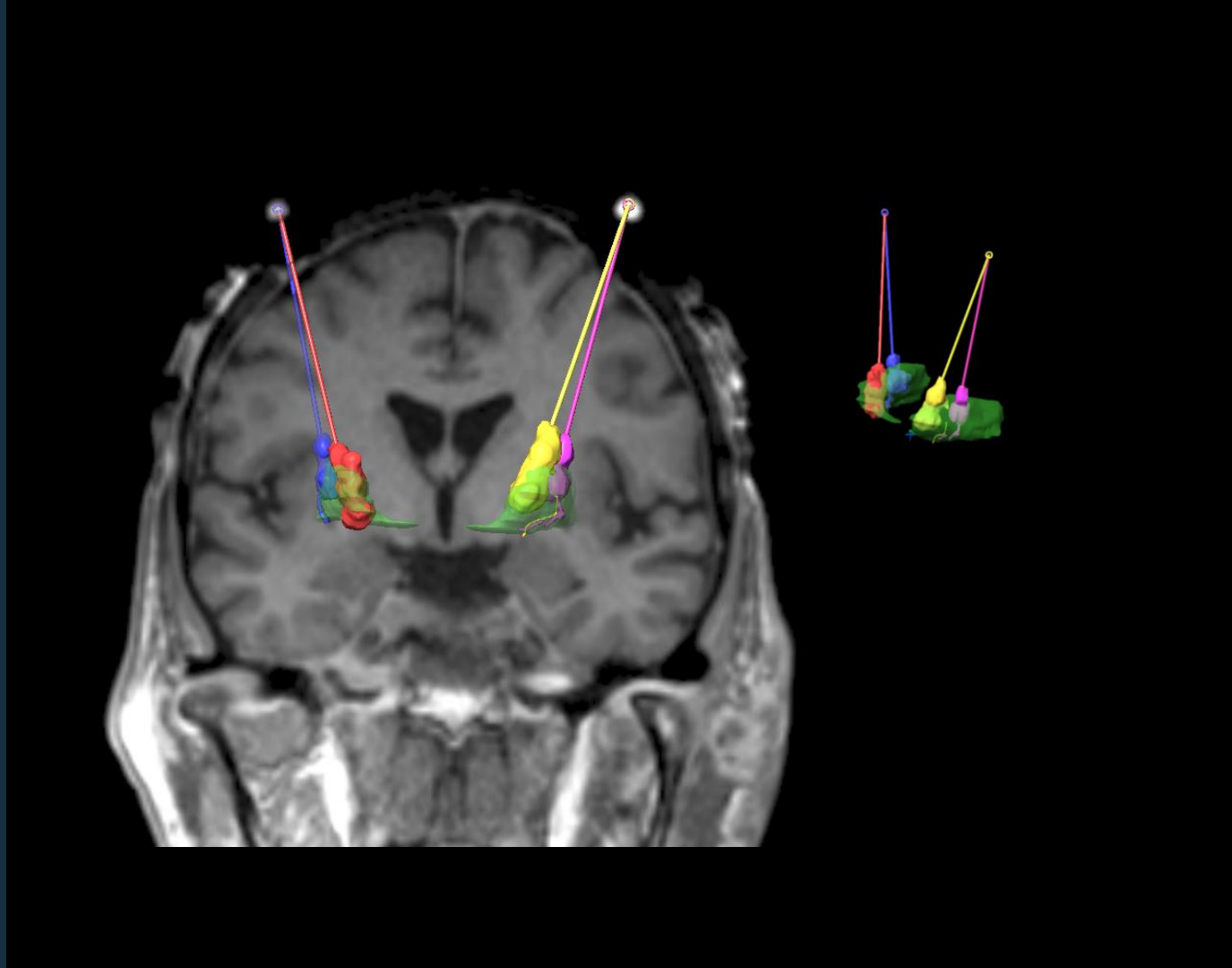
Movement Disorders

2019

Trial of Magnetic Resonance-Guided Putaminal Gene Therapy for Advanced Parkinson's Disease

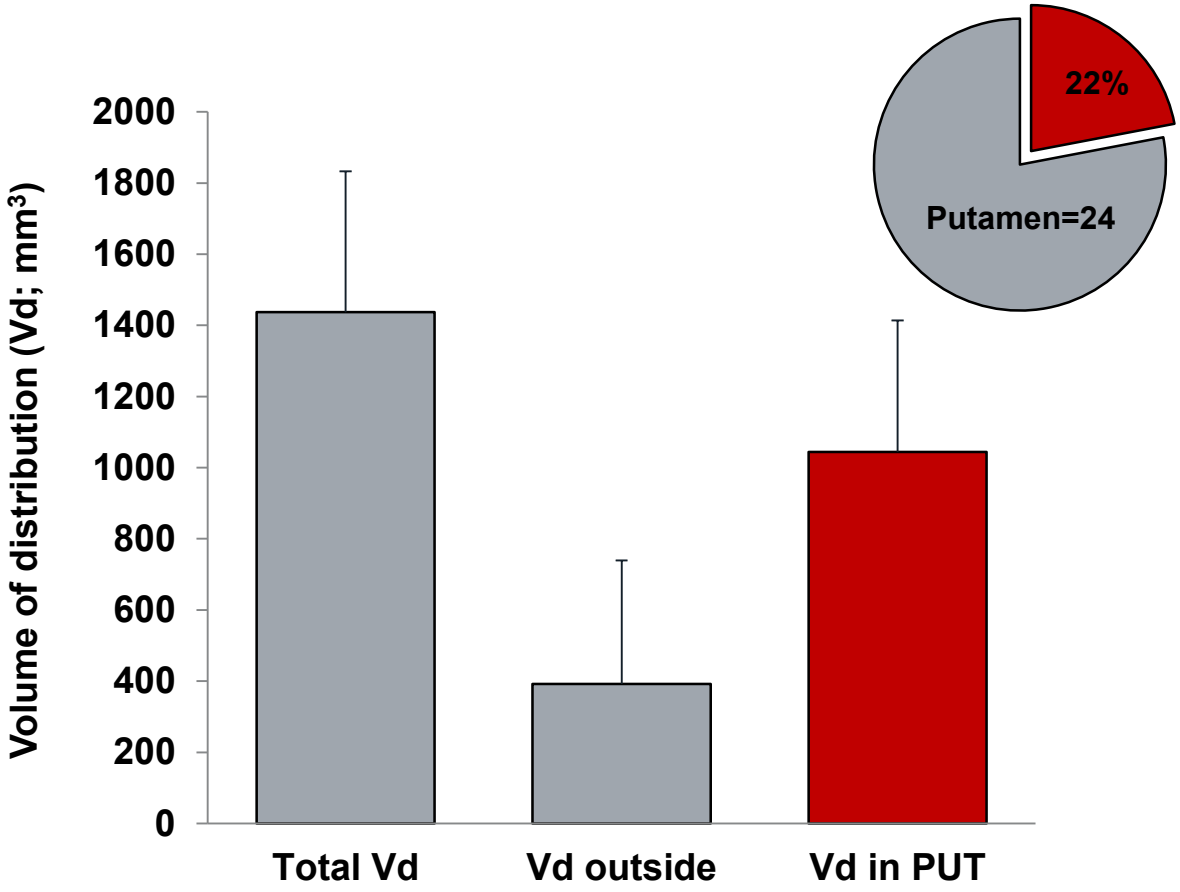


Imaging results



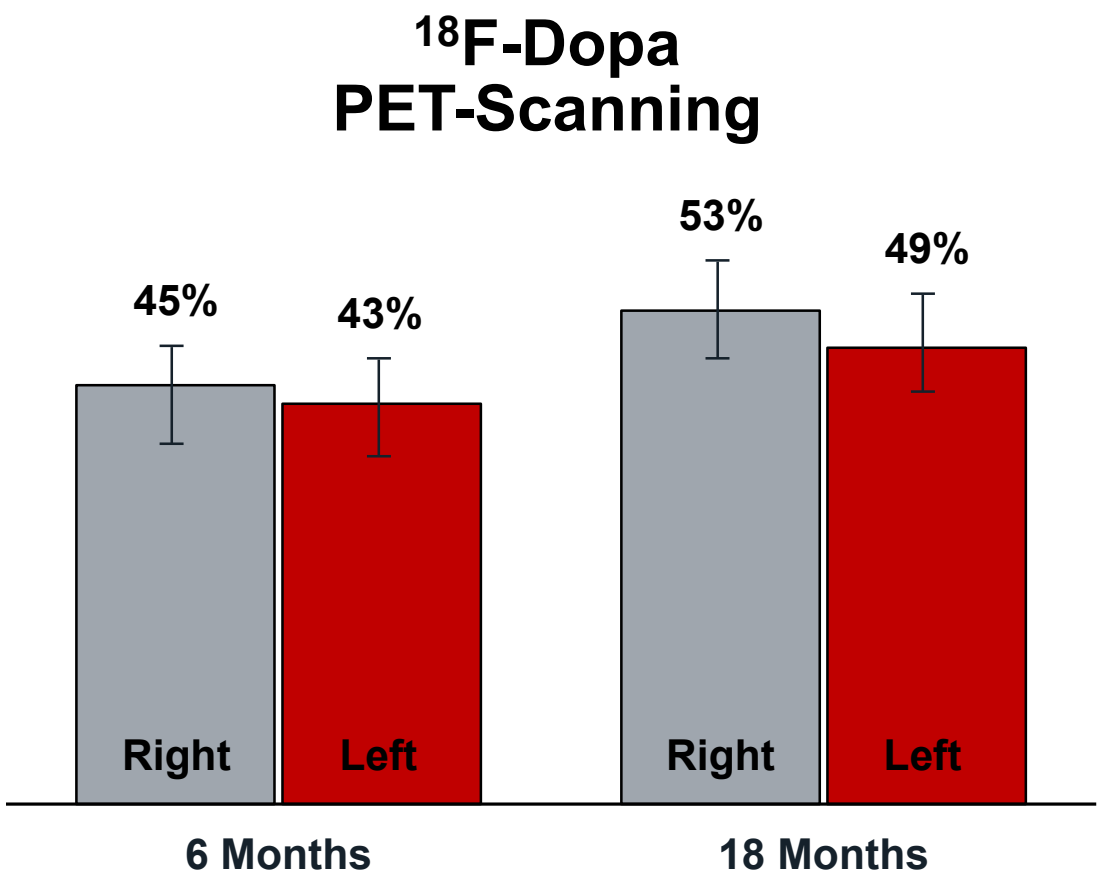
Total perfused putamen

On average 22% of the total putamen volume was perfused



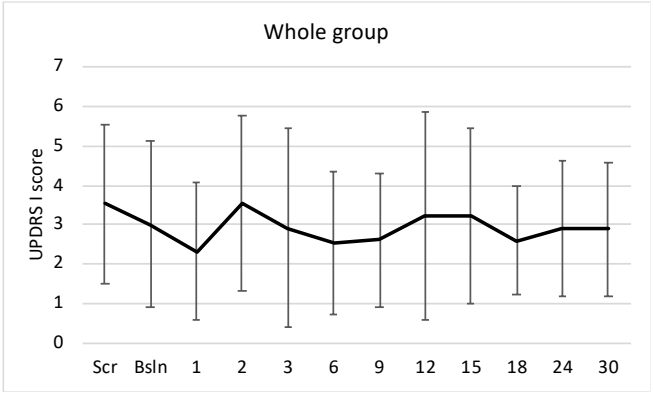
^{18}F-Dopa PET imaging

Patients showed increase in F-Dopa uptake after treatment

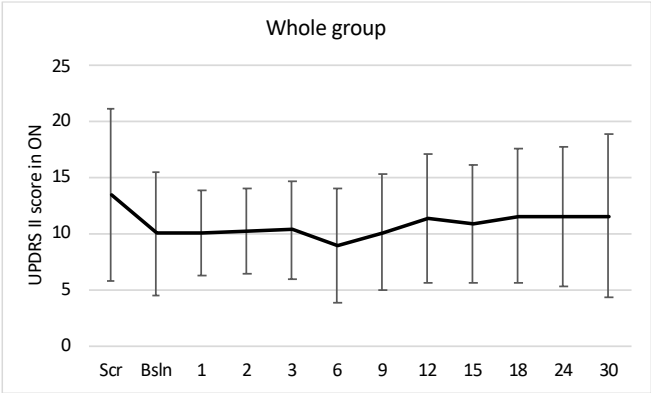


Patients remained stable

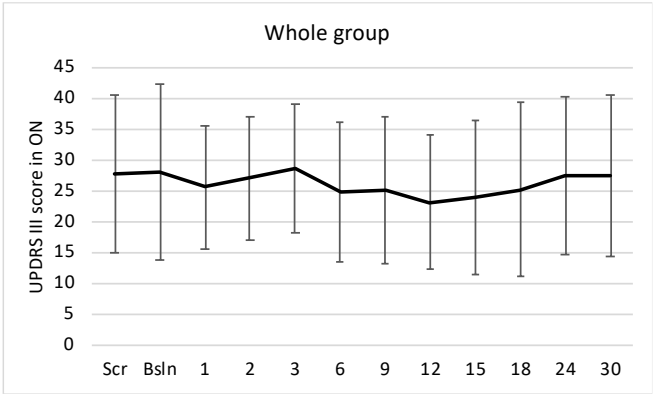
UPDRS Part 1 (mentation, mood, behavior)



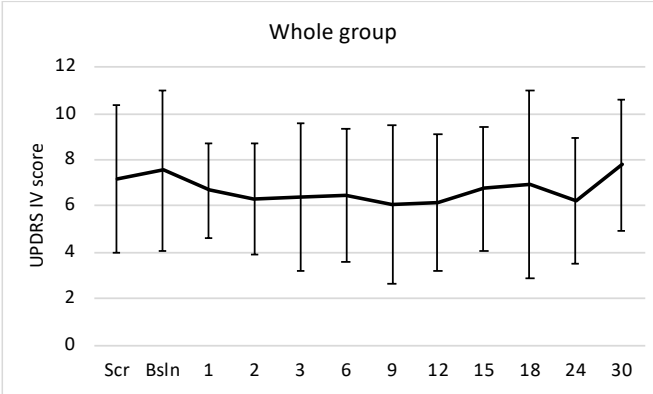
UPDRS Part 2 (activities of daily life)



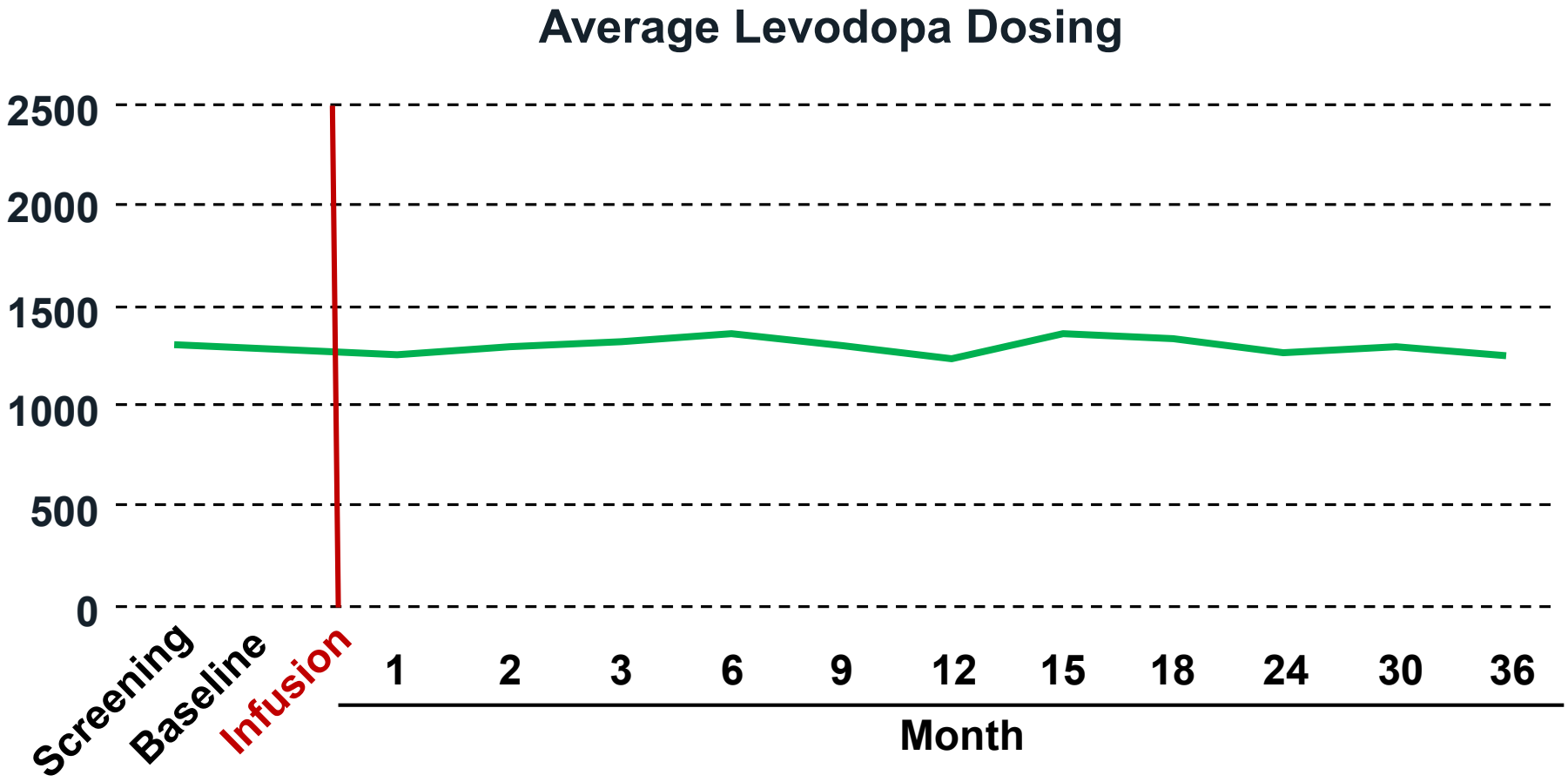
UPDRS Part 3 (motor function)



UPDRS Part 4 (Hoehn and Yahr)

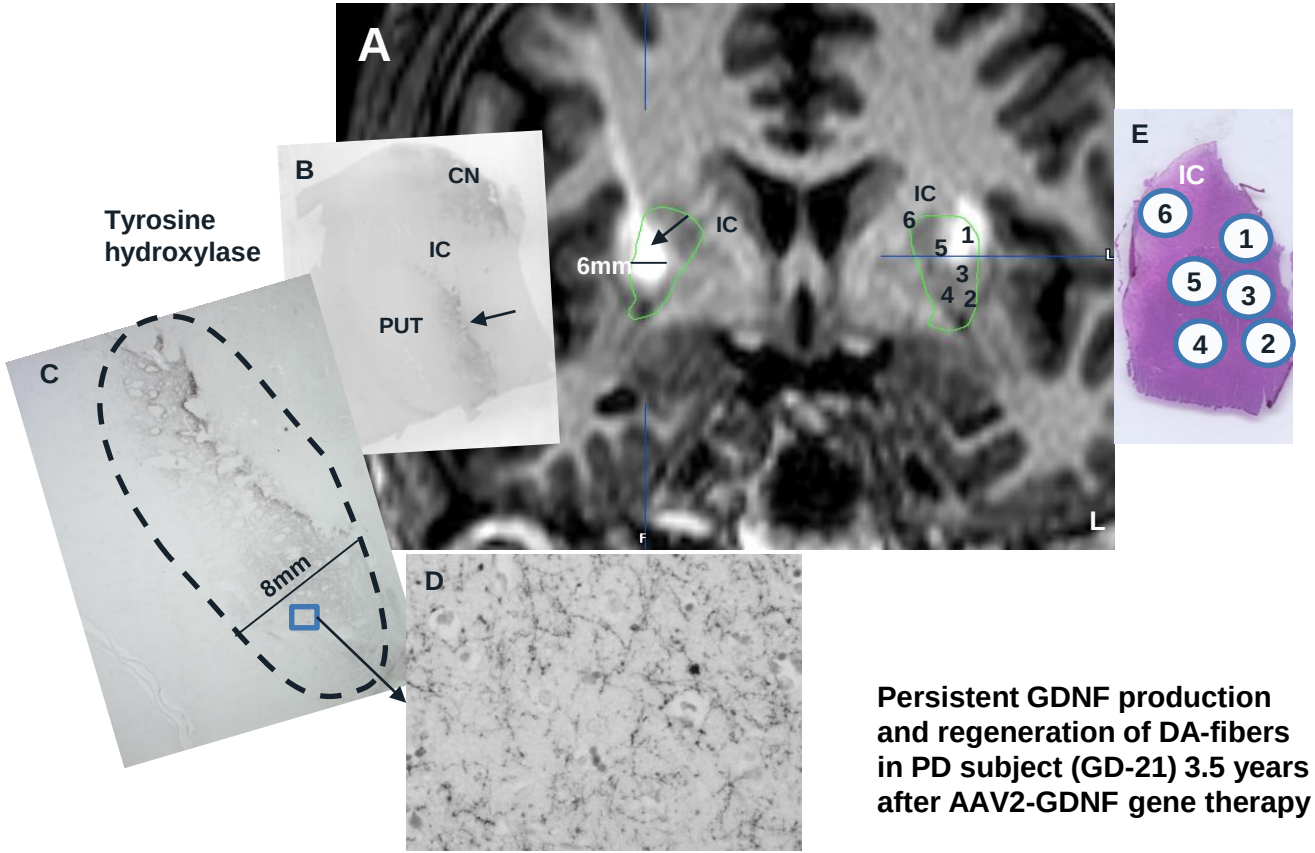


Levodopa stable



Post-mortem analysis

Informs biologic impact and early efficacy read out



Persistent GDNF production and regeneration of DA-fibers in PD subject (GD-21) 3.5 years after AAV2-GDNF gene therapy

Phase IB Trial

AAV_2-GDNF vector delivered to each Parkinson's disease patient (mild to moderate) via a posterior approach:

- **Recently diagnosed (4 years or more) with mild motor symptoms (Prevention; n=6)**
- **More than 4 years since diagnosis with moderate motor symptoms (Restoration; n=4)**

Primary Objective:

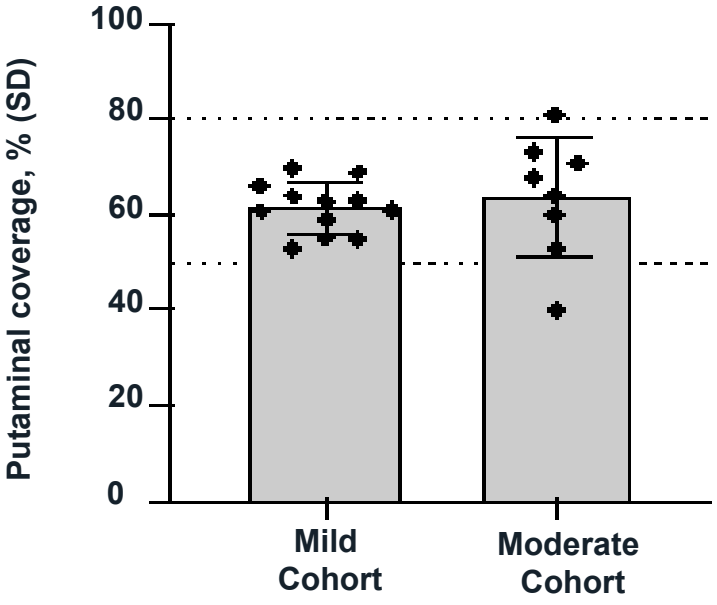
- **Determine the safety of treatment**

Secondary Objectives:

- **TH-fiber regeneration expression using ^{18}F-dopa PET**
- **Assessments of motor function and quality of life**
- **Assessments of daily requirements for levodopa**

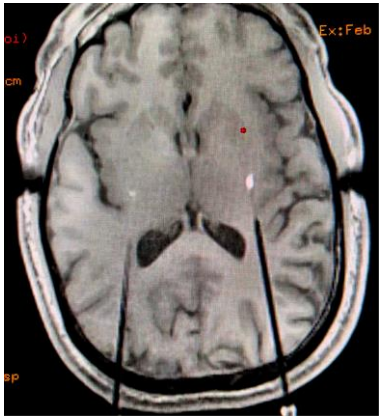
Results

Excellent putaminal coverage



Precise and reproducible delivery

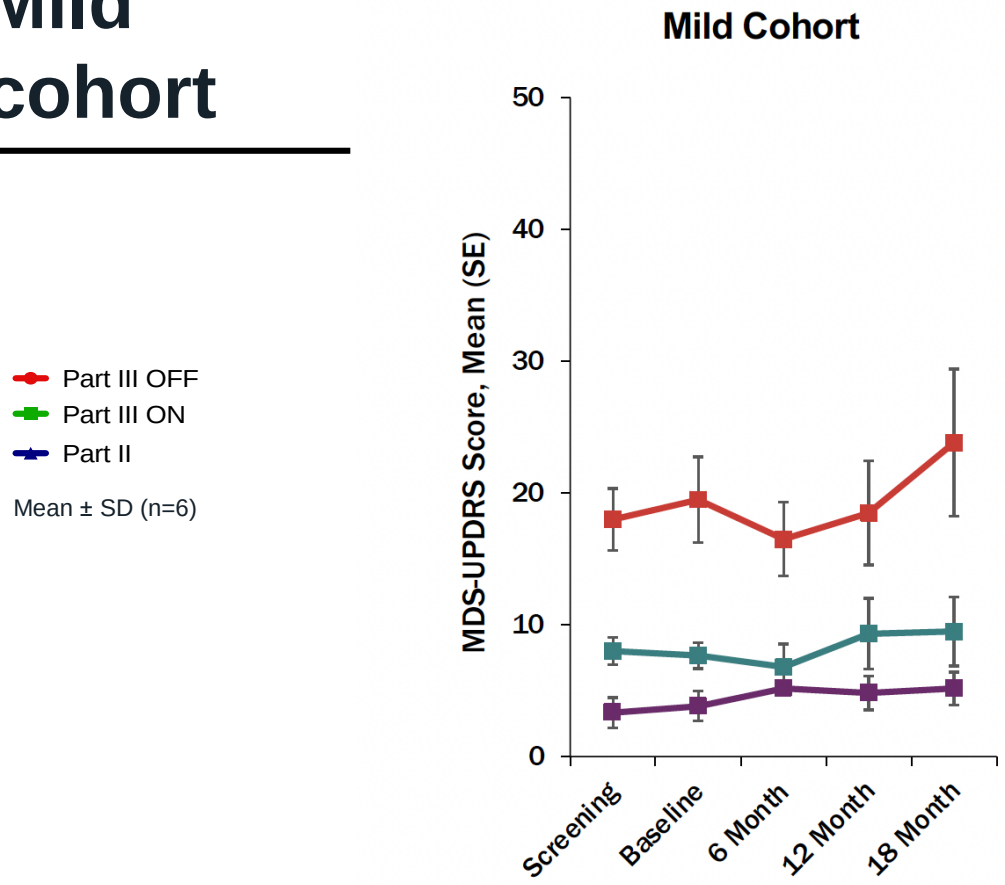
Mean, putaminal coverage, 63%



Results

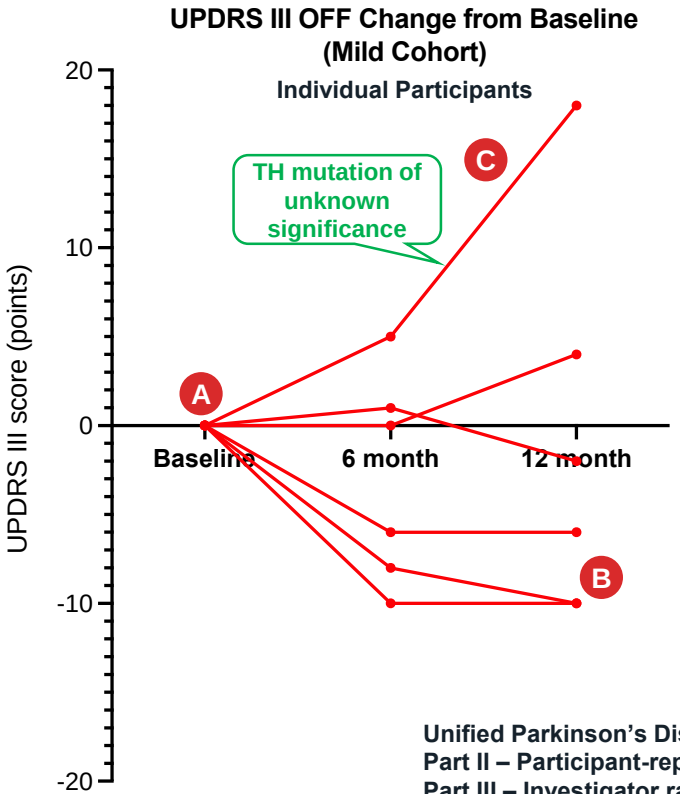
Parkinson's disease motor scores

Mild cohort



Results

Parkinson's disease motor scores



Key points

- **A** Stability demonstrated over 12 months in Mild PD
- **B** Limited window to measure large magnitude of functional improvements in the Mild PD
- **C** One outlier may represent potential risk of atypical PD when enrolling early-stage PD

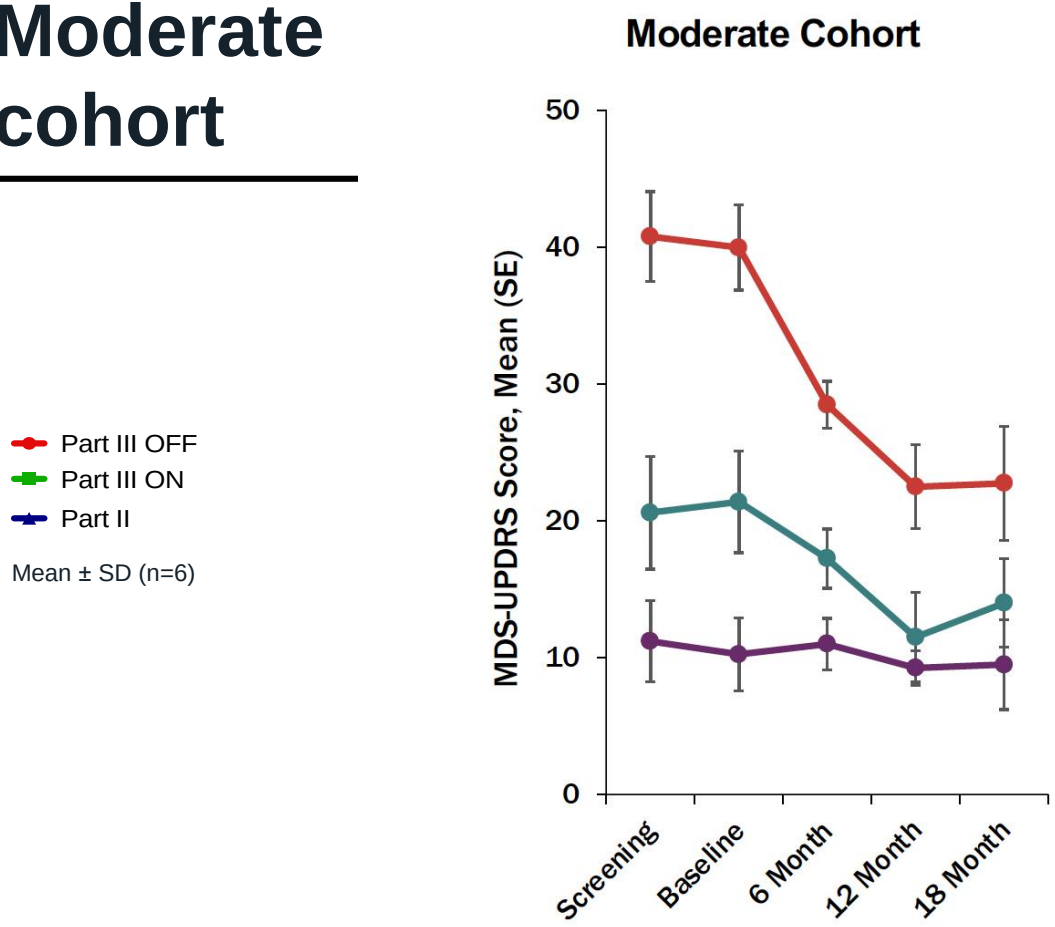
Long-term follow up needed to assess modification of disease progression

Unified Parkinson's Disease Rating Scale (UPDRS)
Part II – Participant-reported motor impacts on activities of daily living
Part III – Investigator rating of motor function OFF or ON medication

Results

Parkinson's disease motor scores

Moderate cohort



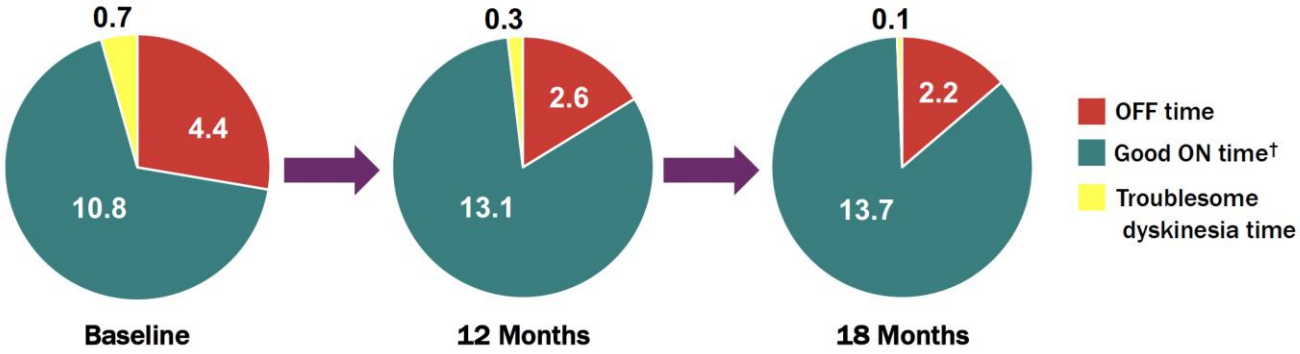
Results

Parkinson's disease motor scores

Moderate cohort

Key points

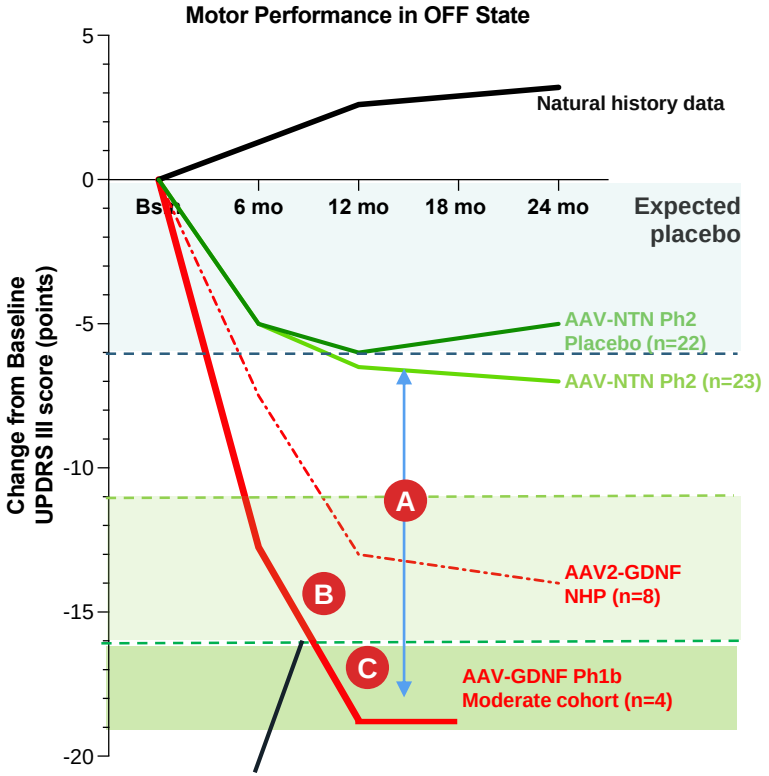
50% decrease in 'off' time
86% decrease in dyskinesia time
27% increase in 'on' time



PD, Parkinson disease.
*Data are averaged over 3 days and normalized to a 16-hour waking day.
†Time ON without dyskinesia + time with non-troublesome dyskinesia.

Results

Parkinson's disease motor scores

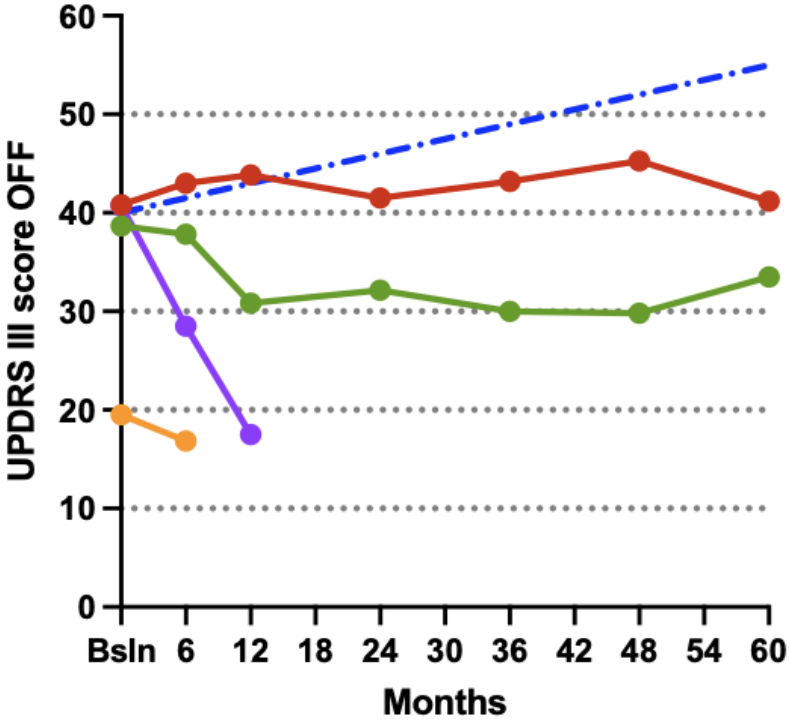


AAV2-GDNF with **20% L-dopa dose reduction**; different from previous CGT studies

Key points

- **A** 12-months clinical data **shows stronger improvements than previous neurotrophic CGTs**
- **B** **AAV-GDNF effects are more progressive than previous CGTs:** Continuous improvement after 6 months, unlike brief improvement in other CGTs
- **C** **Clinically meaningful improvements beyond 6-months consistent with anticipated MoA** – neuron regrowth and progressive restoration of dopamine function

Dose Response of AAV2-GDNF in Phase I vs Phase I^{B}



Projected Progression[2-6] (3pt/yr)

Low dose	9x10^{10}vg	Phase I 26% coverage
Med dose	3x10^{11}vg	
High dose - Moderate stage	1.2x10^{13}vg	Phase Ib 63% coverage
High dose - Early stage		

AAV2=adeno-associated virus serotype 2; GDNF=glial cell line–derived neurotrophic factor; UPDRS III=Unified Parkinson's Disease Rating Scale III.

1. Data on file. 2021. Research Triangle Park, NC. Asklepios BioPharmaceutical, Inc. 2. Alves G, Wentzel-Larsen T, Aarsland D, Larsen JP. *Neurology*. 2005;65(9):1436-41. 3. Holden SK, Finseth T, Sillau SH, Berman BD. *Mov Disord Clin Pract*. 2018;5(1):47-53. 4. Latourelle JC, Beste MT, Hadzi TC, Miller RE, Oppenheim JN, Valko MP, et al. *Lancet Neurol*. 2017;16(11):908-16. 5. Malek N, Kanavou S, Lawton MA, Pitz V, Grosset KA, Bajaj N, et al. *Parkinsonism Relat Disord*. 2019;65:55-61. 6. Mollenhauer B, Zimmermann J, Sixel-Döring F, Focke NK, Wicke T, Ebentheuer J, et al. *Mov Disord*. 2019;34(1):67-77.

Restorative therapy

Aromatic L-amino acid decarboxylase (AADC) deficiency

An ultrarare, inherited disorder that appears within the first year of life

nature COMMUNICATIONS

2021

Gene therapy for aromatic L-amino acid decarboxylase deficiency by MR-guided direct delivery of AAV2-AADC to midbrain dopaminergic neurons

Signs and symptoms

Developmental delay

Global hypotonia

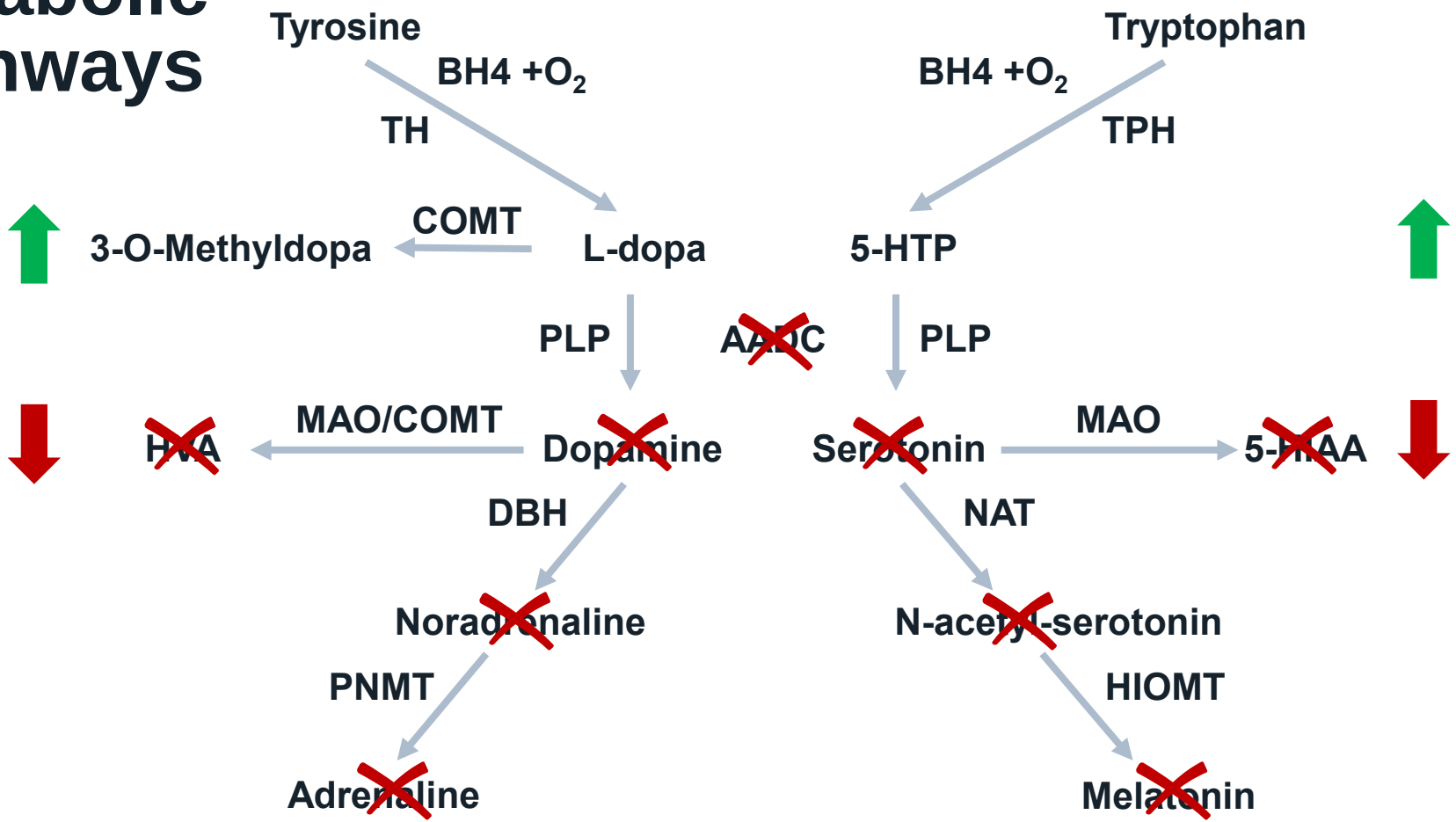
Non-verbal

Involuntary limb movement

Debilitating oculogyric crises

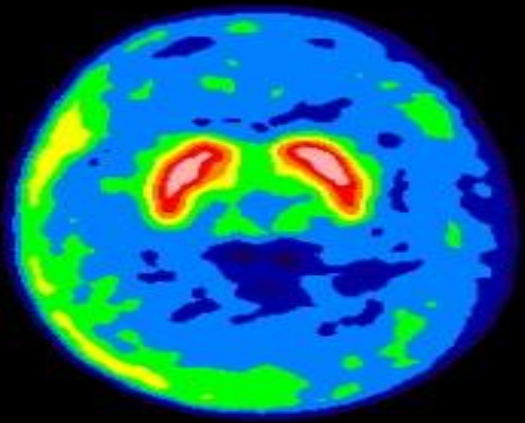
Patients die in first or second decade of life

Metabolic pathways

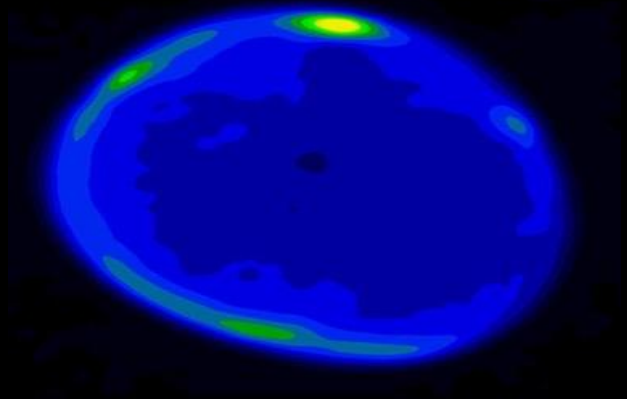


^{18}F-Dopa PET imaging

Normal Control



AADC deficiency



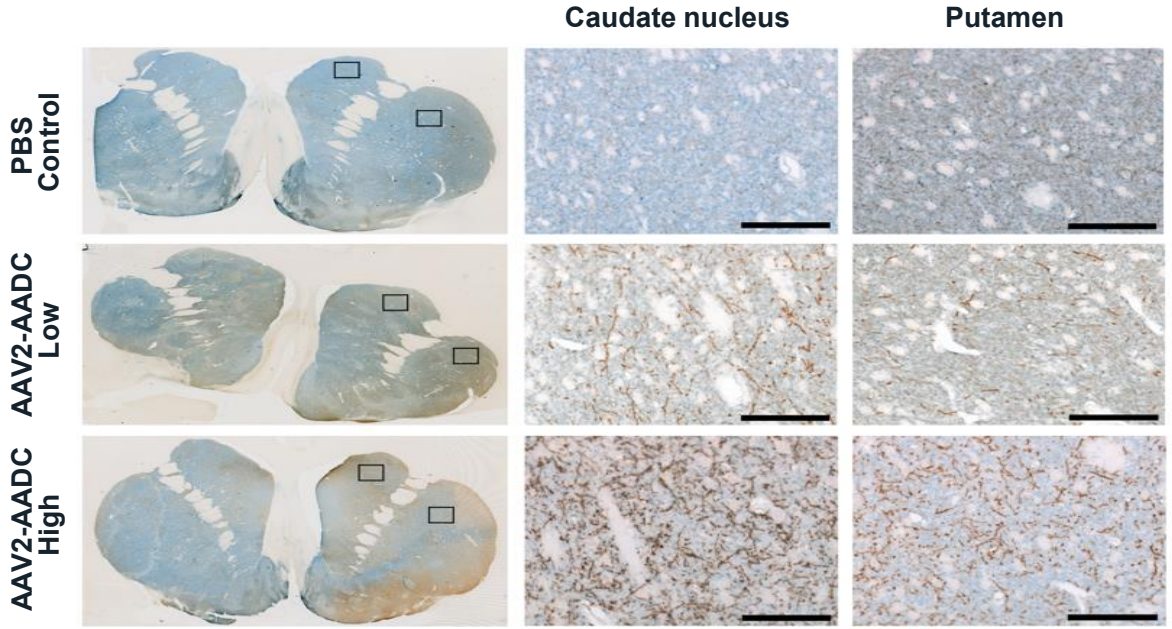
Anterograde axonal transport

Human Gene Therapy

Convection-enhanced delivery of adeno-associated type virus type 2 (AAV2) into the striatum and transport of AAV2 within the monkey brain

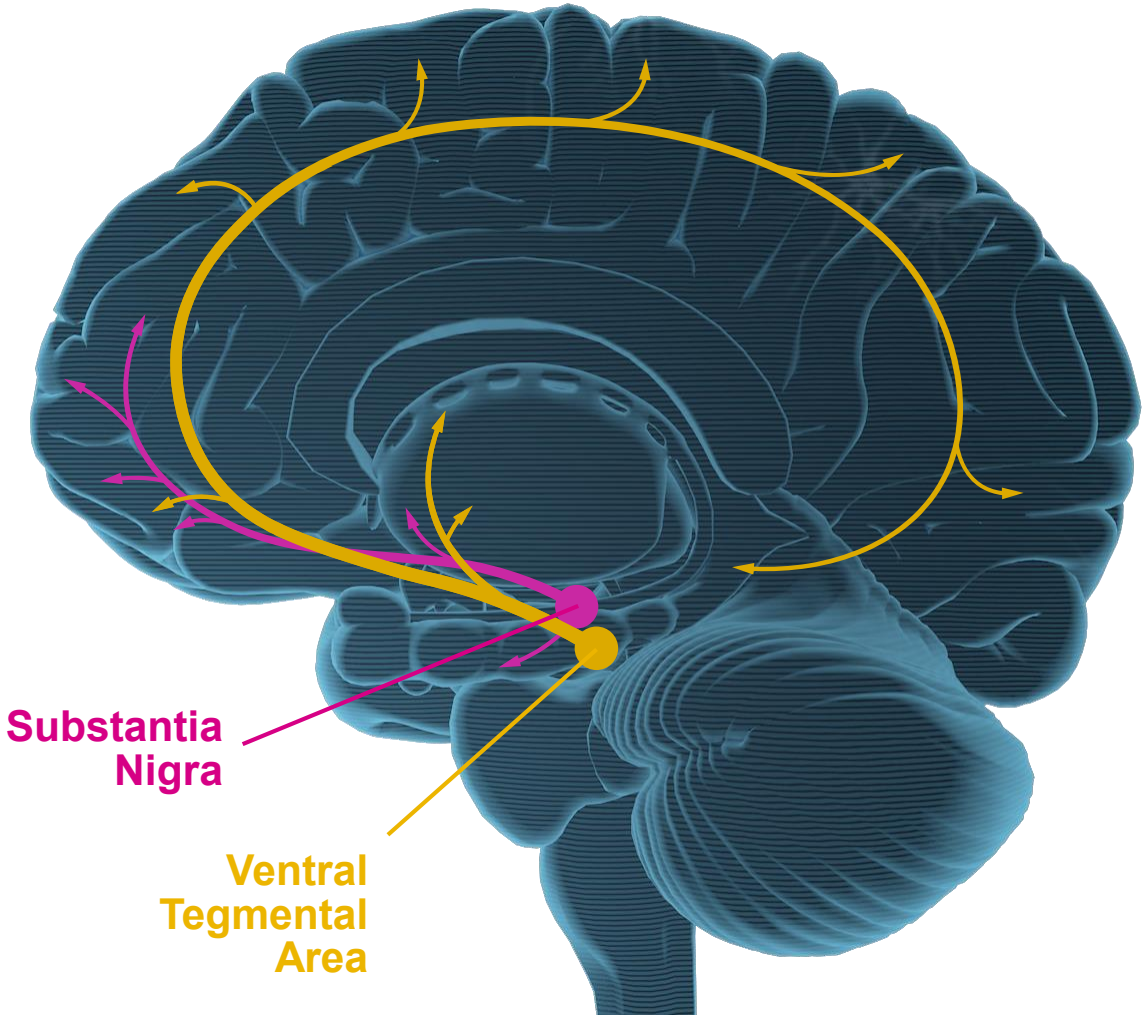
Piotr Hadaczek, Malgorzata Kohutnicka, Michal T. Krauze, John Bringas, Phil Pivirotto, Janet Cunningham, and Krystof Bankiewicz

2006



Midbrain infusion of AAV2-AADC

- Dopamine Pathways
- Serotonin Pathways



Phase I Trial

Pediatric AADC deficiency

AAV2-AADC vector will be delivered to bilateral substantia nigra and ventral tegmentum.

2 dose levels will be employed:

- **Dose Cohort 1 = 1.3 x 10^{11} vg (160 microliters)**
- **Dose Cohort 2 = 8.3 x 10^{11} vg (160 microliters)**

Primary Objective:

Determine safety of the approach

Secondary Objectives:

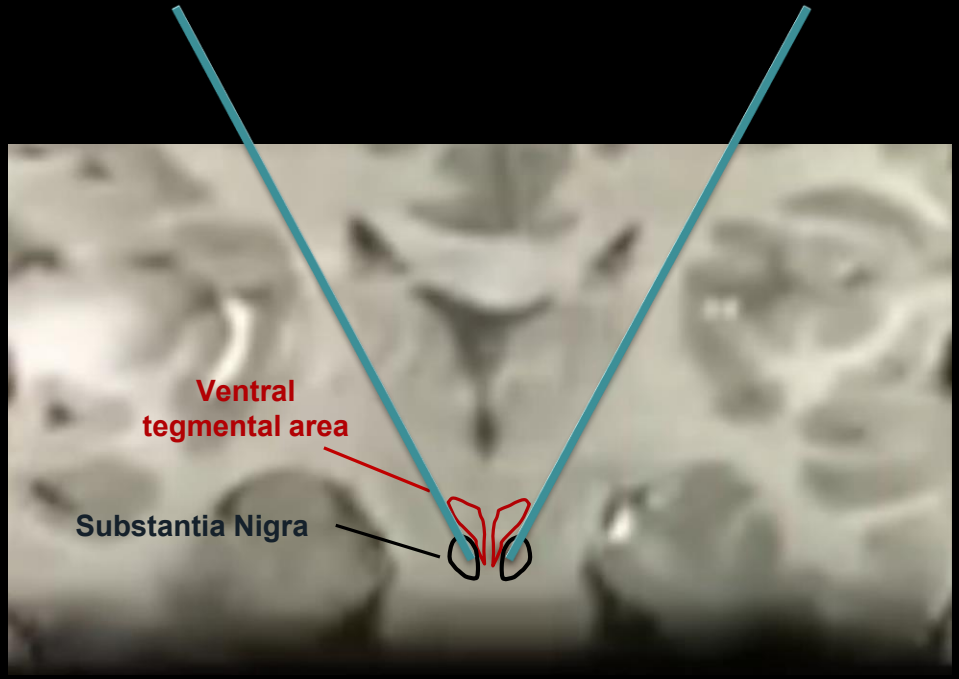
- **Define clinical effects**
- **Assess dopaminergic biologic changes with F-Dopa PET**
- **Assess accuracy and feasibility of MR-guided infusion**

THE OHIO STATE UNIVERSITY 1870

UCSF University of California San Francisco

Imaging results

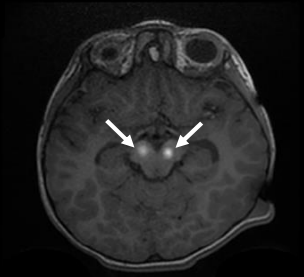
All patients received 160 microliters



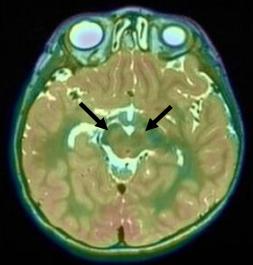
Mean coverage

- 98% substantia nigra
- 77% ventral tegmental area

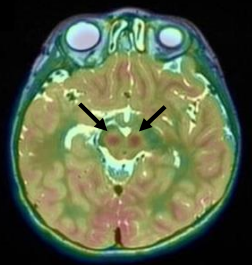
Imaging results



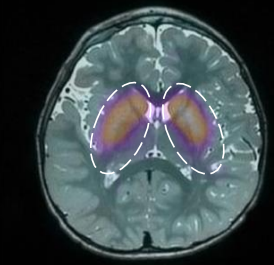
MRI of Vector Infusion



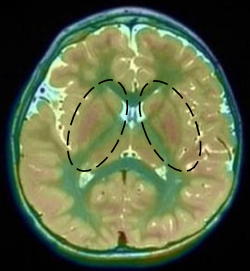
Baseline FDOPA PET



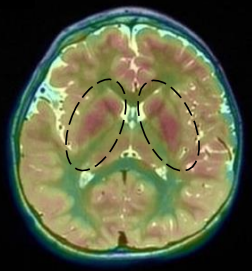
3M post FDOPA PET



Baseline DaTscan



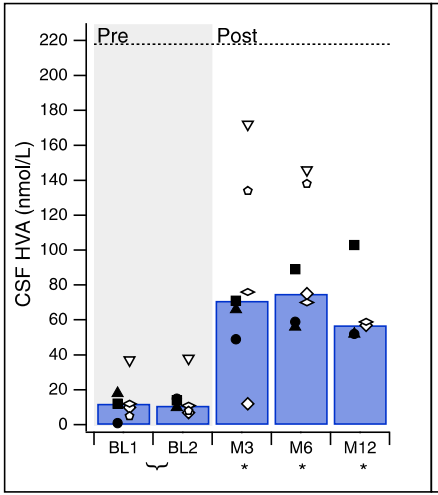
Baseline FDOPA PET



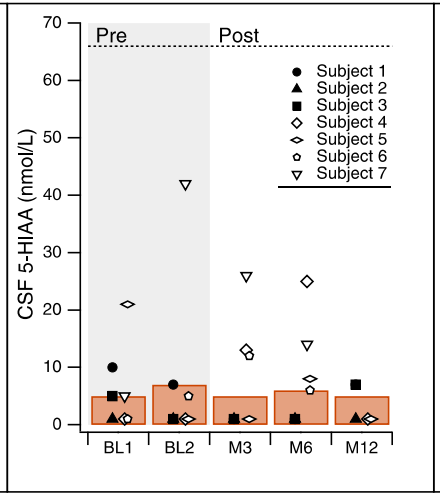
3M post FDOPA PET

Metabolic results

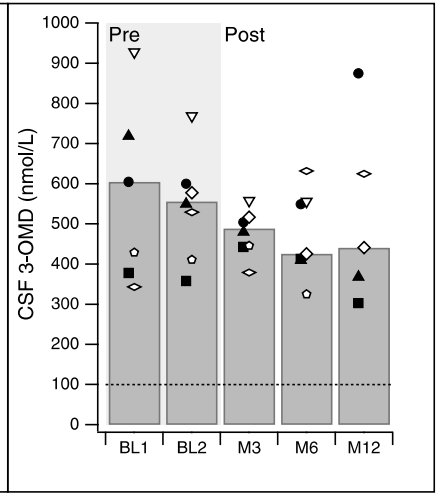
Downstream Dopamine Byproduct



Downstream Serotonin Byproduct



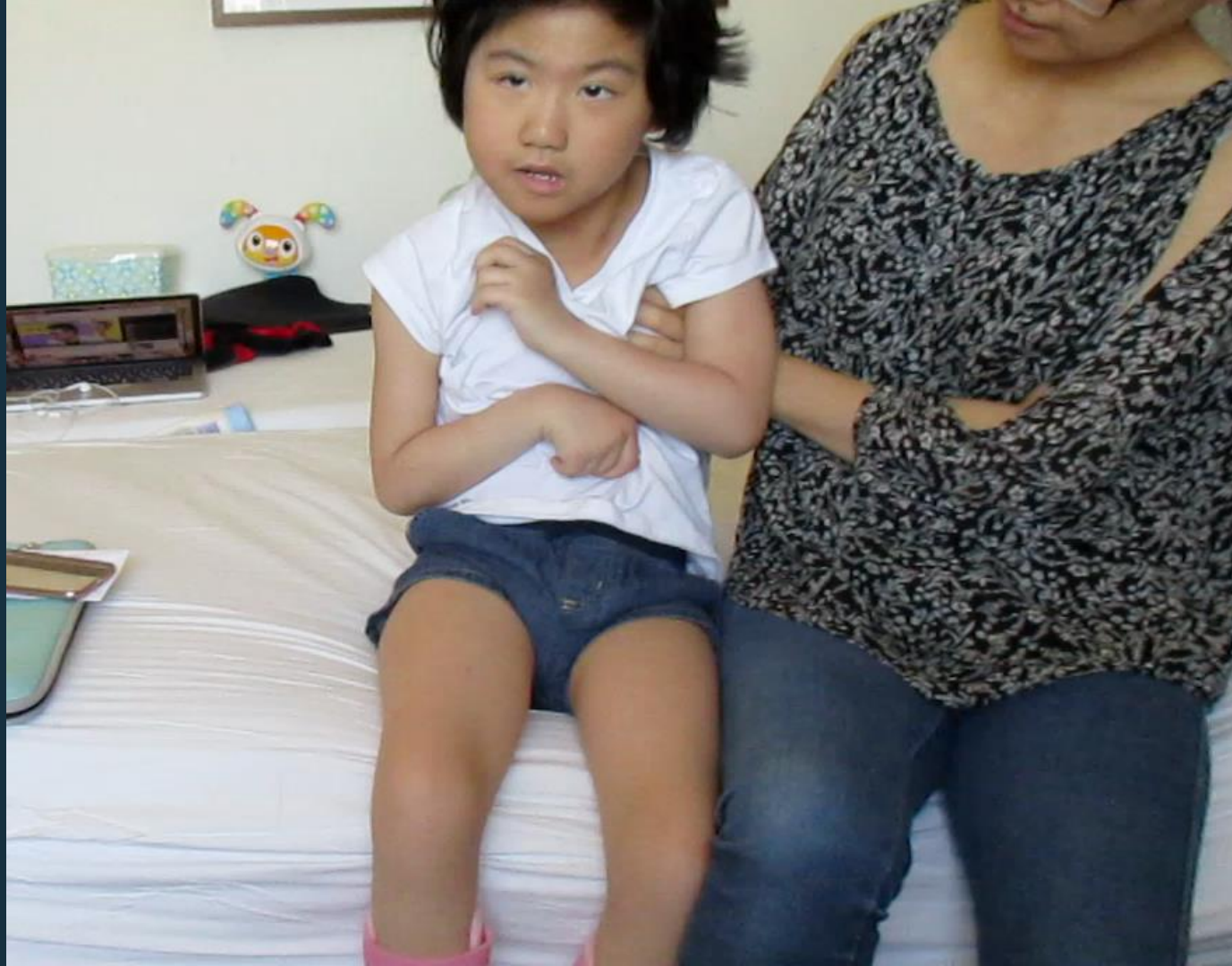
Upstream Dopamine Precursor



Clinical results

Baseline before infusion

Clinical results



5 weeks post infusion

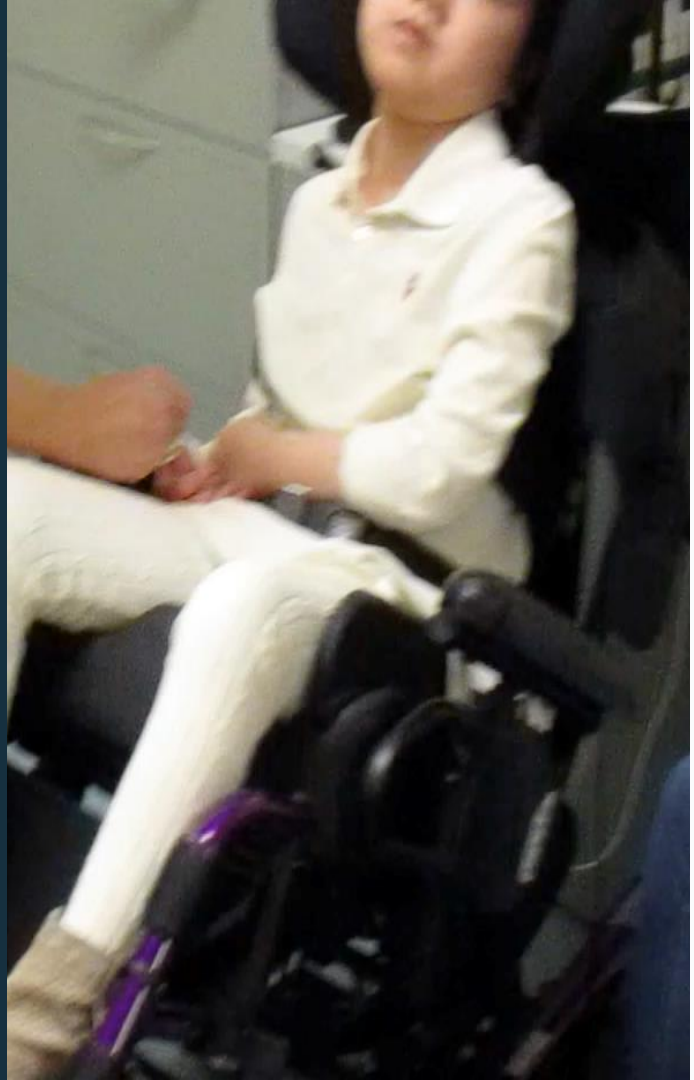
Clinical results

9 months post infusion

Clinical results

12 months after infusion

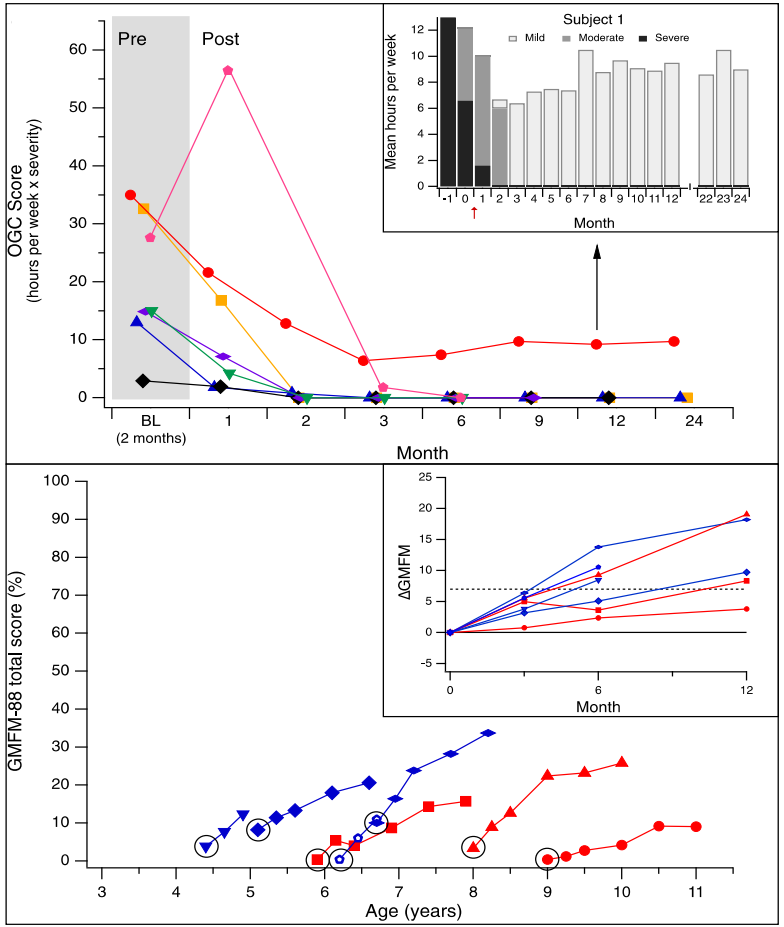
Clinical results



12 months post infusion

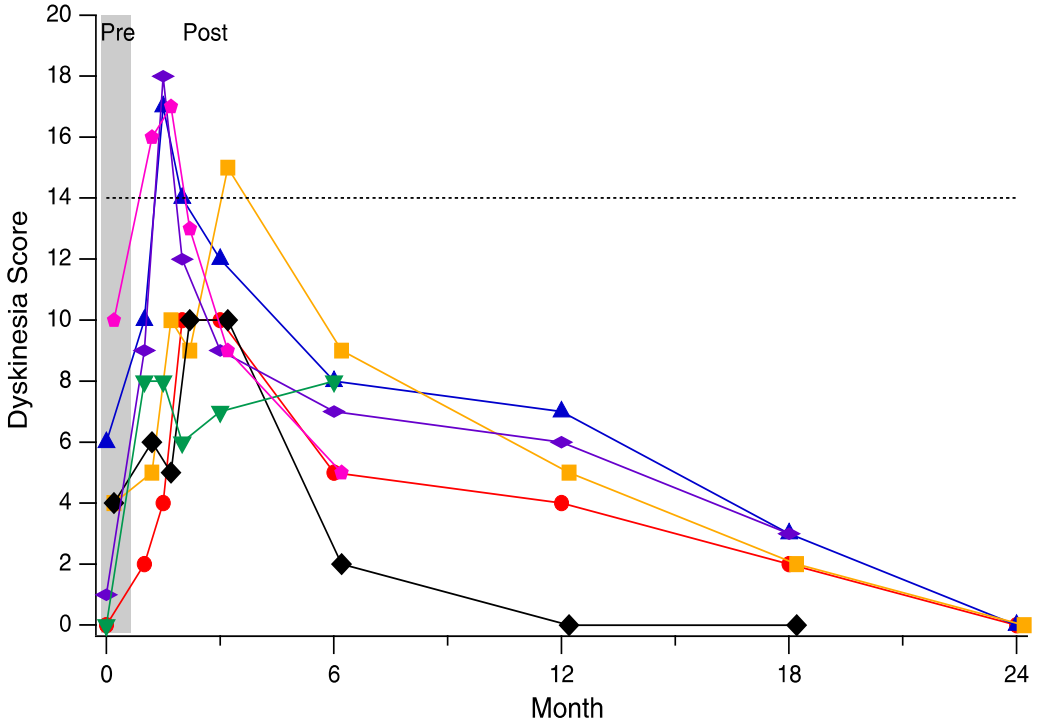
Clinical results

Motor cortex impairment in all patients except one (75% reduction) with 3 months



Clinical results

Dyskinesias occurred in all patients after treatment and resolved within 24 months



Conclusions

- Targeted coverage global effect
- MR-guided delivery essential
- Therapuetic coverage
- Significant clinical impact

Acknowledgements

Krystof Bankiewicz
Jeffrey R. Leonard
J. Bradley Elder
John D. Heiss
Toni Pearson
Andrea Davis
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