

University of Massachusetts Medical School

Canavan's Disease as a model for developing efficacious, sustained and safe rAAV gene therapeutics for CNS disorders

Guangping Gao, PhD

Penelope Booth Rockwell Professor in Biomedical Research

Director, Gene Therapy Center

University of Massachusetts Medical School, USA

Advanced Therapeutics Cluster

Gene Therapy Center

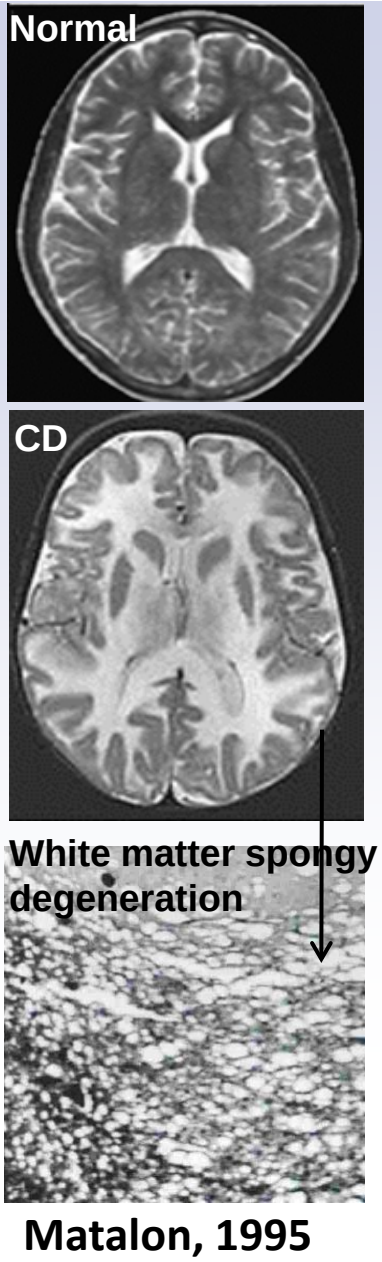
"Moving Research from the Bench to the Bedside"

DISCLOSURE

G.Gao is a founder of *Voyager Therapeutics* and holds equity in the company. G.Gao is an inventor on patents with potential royalties licensed to *Voyager Therapeutics* and other biopharmaceutical companies.

CANAVAN'S DISEASE (CD)

- First reported by Dr. Myrtelle Canavan in 1931
- Genetic defect
  - Caused by rare autosomal recessive mutations in aspartoacylase (*AspA*)gene
- Inborn error of N-acetyl aspartic acid (NAA) Metabolism
  - AspA enzyme activity↓
  - NAA↑ in the CNS and urine
- Fatal neurological disorder
  - White matter degeneration
  - Hydrocephaly/leukodystrophy
  - Psychomotor defect
  - Early death (< age of 5)



Matalon, 1995

CANAVAN'S DISEASE

- STILL NO EFFECTIVE TREATMENT 80 YEAR LATER



Molecular Studies of Canavan's Disease

- From bedside to test tubes

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GUANGPING GAO, PHD

NEUROLOGY 2014 INT'L CONFERENCE, PHILADELPHIA, 9/8/2014

Molecular Etiology of Canavan's Disease - the Biochemical/Metabolic Defect discovered 26 years ago

- First Reported by Matalon et al in 1988

  - *Matalon, R., et al., Aspartoacylase deficiency and N-acetylaspartic aciduria in patients with Canavan disease. Am J Med Genet, 1988. 29(2): p. 463-71.*

- Later confirmed by Divry and Mathieu in 1989

  - *Divry, P. and M. Mathieu, Aspartoacylase deficiency and N-acetylaspartic aciduria in patients with Canavan disease. Am J Med Genet, 1989. 32(4): p. 550-1.*

Molecular Etiology of Canavan's Disease - the Gene and Causative Mutations discovered 20 years ago

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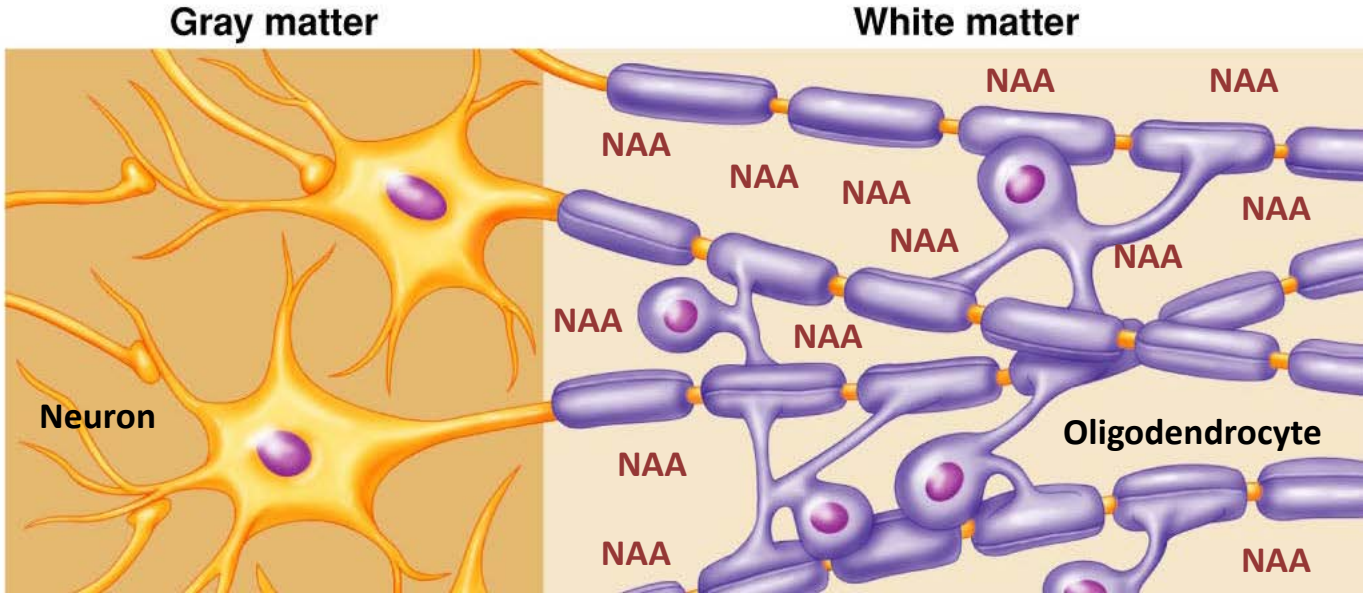
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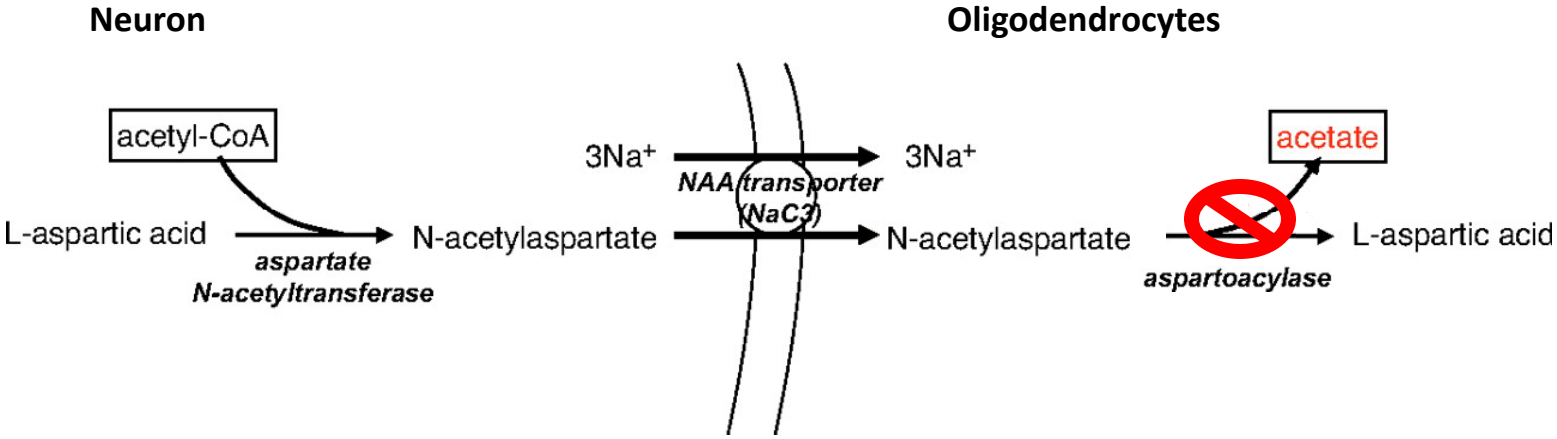
Cloning of the human aspartoacylase cDNA and a common missense mutation in Canavan disease

Rajinder Kaul, Guang Ping Gao, Kuppareddi Balamurugan & Reuben Matalon

Canavan disease, an autosomal recessive leukodystrophy, is caused by deficiency of aspartoacylase and accumulation of N-acetylaspartic acid in brain. We have cloned the human aspartoacylase (*ASP*) cDNA spanning 1,435 basepairs, and show that the isolated cDNA expresses aspartoacylase activity in bacteria. Furthermore, an A to C base change, at nucleotide 854, has been found in 85% of the 34 Canavan alleles tested so far. This base change results in a missense Glu285Ala mutation that is predicted to be part of the catalytic domain of aspartoacylase. The data suggest that the catalytic centre of aspartoacylase involves a triad of Ser, His and Glu residues. Our findings have implications for diagnosis and screening of Canavan disease.

Molecular Etiology of the Disease





Gene Therapy for Canavan's Disease

- From bench top to bedside: clinically testing the 1st generation CD gene therapy

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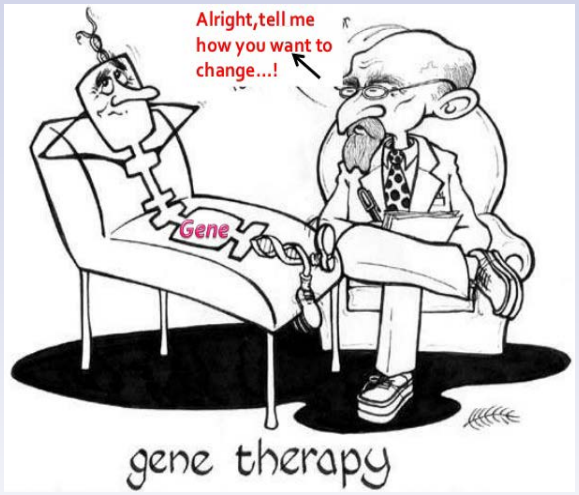
What is gene therapy?



Treatment, cure or prevention of disease by modification of the expression of one's genes or addition of a new gene



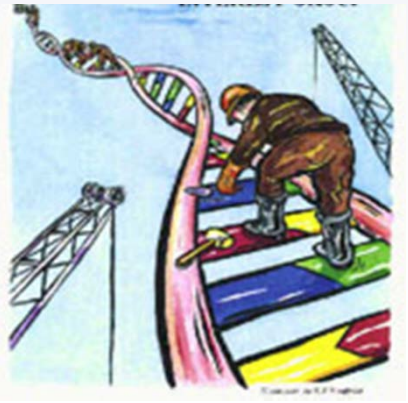
How Does Gene Therapy Work?



- **Gene Replacement**
  - ✓ To replace nonworking mutant gene (***loss - of - function***) with a healthy one
- **Gene Silencing**
  - ✓ To inactivate a mutated gene that becomes toxic to cells (***gain - of - function***)
- **Gene Addition**
  - ✓ To introduce a new gene to help fight a disease (infectious disease , cancer)
- **Gene Editing**
  - ✓ To correct genetic errors on patient genome directly (delivery & erasing?)



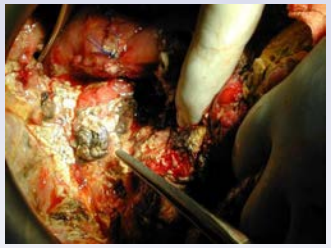
Gene Addition



Gene Editting

RAPID PROGRESS IN GENE THERAPY IN THE PAST TWO DECADES





- ✓ **~2,000 human clinical trials**
- ✓ **>2,000 patients received rAAVs in 109 trials**
- ✓ **Among all CNS gene therapy trials from 2000 to 2009, 76% used rAAV**
- ✓ **Recently showed efficacy in**
  - LCA : an inherited childhood blindness
  - Hemophilia B: a defect in blood clotting factor

The New York Times

July 21, 2012

European Agency Backs Approval of a Gene Therapy ✓





Willy Slingerland

Technicians at uniQure working on Glybera, a gene therapy that is closing in on a regulatory breakthrough.

By ANDREW POLLACK

Published: July 20, 2012

Three commercialized Gene Therapy Drugs in the world to date

- Adenovirus
  - Gendicine (Ad-p53, China, 2004)
  - Oncrine (Oncolytic Ad, China, 2006)
- Aedno-associated virus
  - Glybera (rAAV1 to treat Lipoprotein Lipase Deficiency, the first commercialized gene therapy drug in Europe, 2012)

$1.6 M per shot! therapy drug in Europe, 2012)

"Gene Therapy's Big Comeback"
3/26/2014, *Forbes* magazine



GOING VIRAL

COMPANIES THAT USE VIRUSES TO REPLACE DISEASE GENES HAVE BEEN BOOMING—AT LEAST WHEN IT COMES TO FUND-RAISING. LOOK HOW MUCH THESE FIRMS HAVE RAISED SINCE THE BEGINNING OF 2013.

COMPANY AIMS TO TREAT	RAISED ($MIL)
GENSIGHT BIOLOGICS GENETIC BLINDNESS	$41
BLUEBIRD BIO "LORENZO'S OIL" DISEASE	$116
AUDENTES THERAPEUTICS X-LINKED MYOTUBULAR MYOPATHY	$30
SPARK THERAPEUTICS GENETIC BLINDNESS	$50
DIMENSION THERAPEUTICS HEMOPHILIA	>$10
EDITAS MEDICINE YET TO BE DETERMINED	$43
JUNO THERAPEUTICS CANCER	$120
CELLADON ADVANCED HEART FAILURE	$51
UNIQURE LIPOPROTEIN LIPASE DEFICIENCY	$92
VOYAGER THERAPEUTICS PARKINSON'S DISEASE	$45
NIGHTSTARX RETINAL DYSTROPHIES	$20

VENTURE CAPITAL INITIAL PUBLIC OFFERING

After the *Forbes* report :

AGTC went public, the stock went up from IPO $10 to $32.

KITE went public, the stock went up from IPO $13 to > $30.

Chathem for gene therapy of hemophilia, Initially invested $30 M and then purchased by Baxter with $70M。

Avalanche received $6.4 M from Regeneron$6.4M for gene therapy。

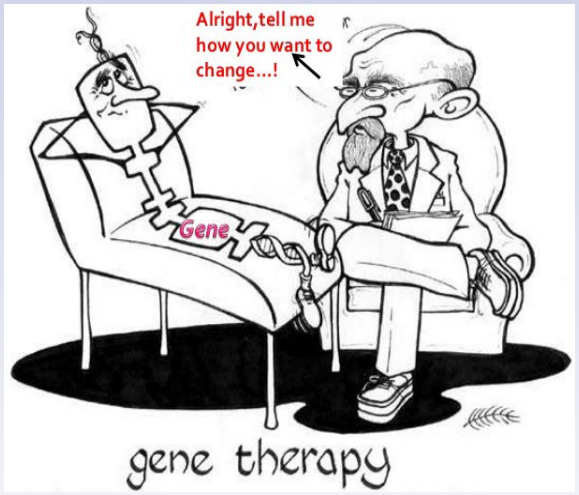
Received additional $80M VC in 6/2014

Additional $30M VC + $230 M from Bayer for Hemophilia A gene therapy

Additional $145M from Amazon a few month ago

Gene Therapy, particularly rAAV gene therapy is very HOT now!

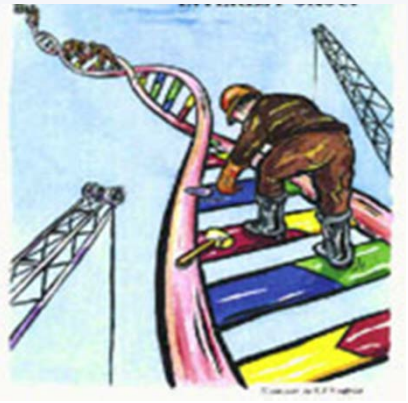
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Gene Addition



Gene Editting

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- Gene Editing
  - ✓ To correct genetic errors on patient genome directly (delivery & erasing?)

GENE THERAPY FOR CANAVAN'S DISEASE - AN ATTRACTIVE THERAPEUTIC APPROACH

- **The clinical study of the 1st generation of Canavan gene therapy pioneered by Drs. During and Leone in early 2000**
  - ✓ Multiple site intracranial injections of rAAV2 through 6 burr holes
  - ✓ Limited clinical improvement
  - ✓ **Confirmed safety of rAAV in the CNS gene therapy** (*Leone et al, Translational Medicine, Science 2013*)
- **The lesson learned**
  - ✓ Diffused white matter degeneration throughout the CNS requires wide-spread <u>global CNS gene transfer</u>

Gene Therapy for Canavan's Disease

- *Bedside back to bench top: developing the 2nd generation CD gene therapy*

KEY CHALLENGES IN GENE THERAPY

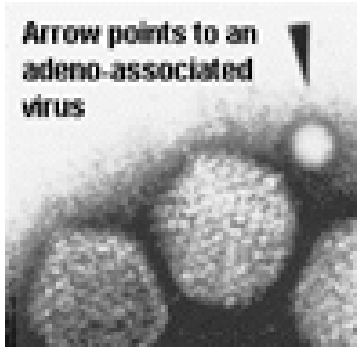
- Gene Delivery Vehicle –**Vector**
- **Method to deliver** vector
- Bona fide **Animal Model** for a disease

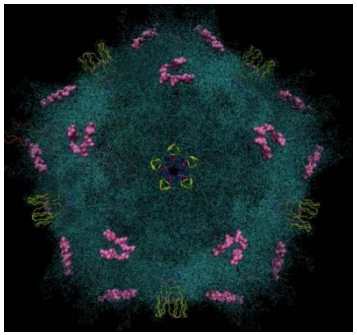
Desired features of An viral vector for in vivo gene transfer

- Efficiency
- Stability
- Low immunogenicity
- Low genotoxicity (episomal)

KEY CHALLENGES IN GENE THERAPY RESEARCH

- Gene delivery vehicle (i.e. vector)





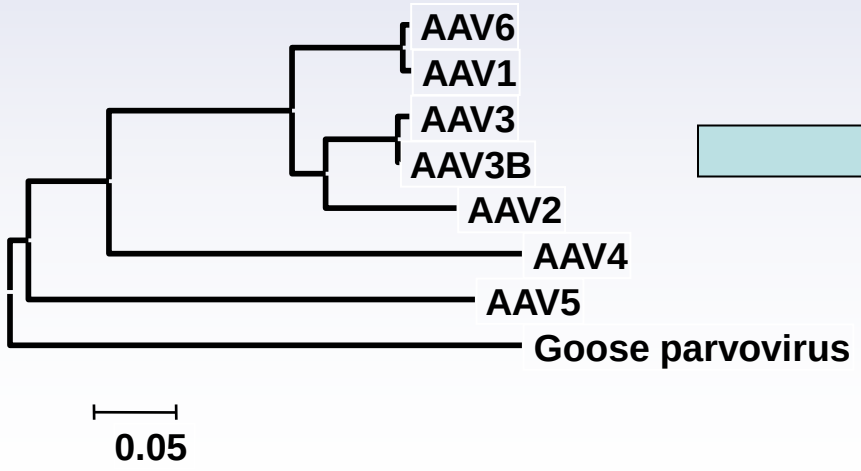
Adeno-Associated Virus (AAV)

- A contaminant in adenovirus preps
- A dependovirus that is Naturally replication defective
- One of the smallest mammalian viruses (26 nm)
- A small single stranded DNA genome (4.7 kb)
- A benign residential virus in humans
- rAAVs are safe in > 2,000 patients in > 100 clinical trials
- **2nd generation of rAAVs discovered in the past decade**

An Ever-growing AAV Tree

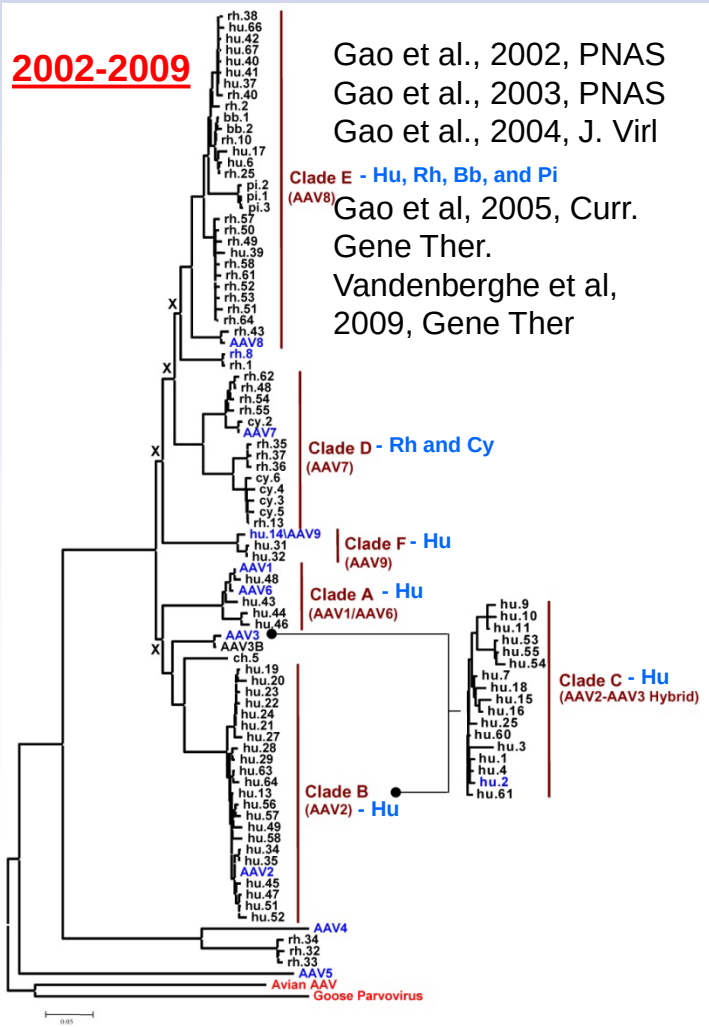
- AAV serotypes 1-9
- Over 250 genotypes
- Biodiversity/ 6 different clades
- Cross-species transmission

1965- 2001



2002-2009

Gao et al., 2002, PNAS
Gao et al., 2003, PNAS
Gao et al., 2004, J. Virl



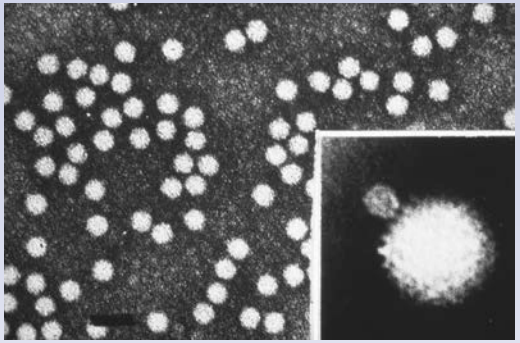
Gao et al, 2005, Curr. Gene Ther.
Vandenberghe et al, 2009, Gene Ther

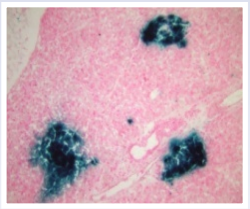
AAV INFECTION IN PRIMATES

- **Prevalent**
  - Humans: ~20% PCR (+), up to 80% sero (+)
- **Persistent**
  - Life time in animals primarily as episomes
- **Privileged**
  - Low immunogenicity
- **Pathogenicless**
  - No reported disease association

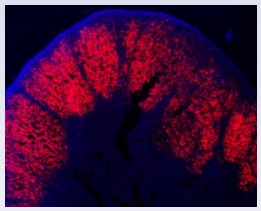
Novel rAAV Vectors

-The smallest bio-nanoparticles

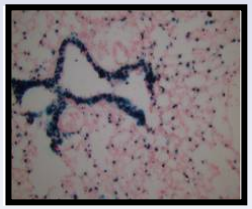




Islet



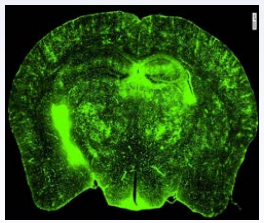
Adrenal gland



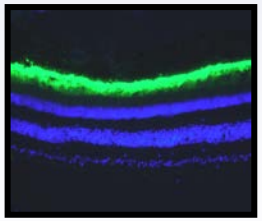
Lung



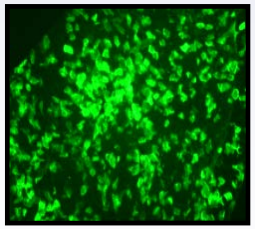
Heart



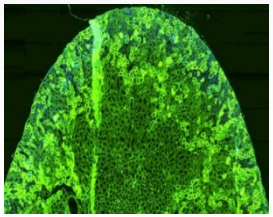
CNS



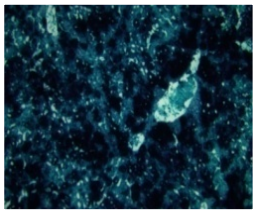
Eye



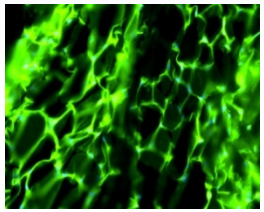
Pancreas



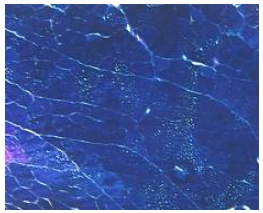
Kidney



Liver



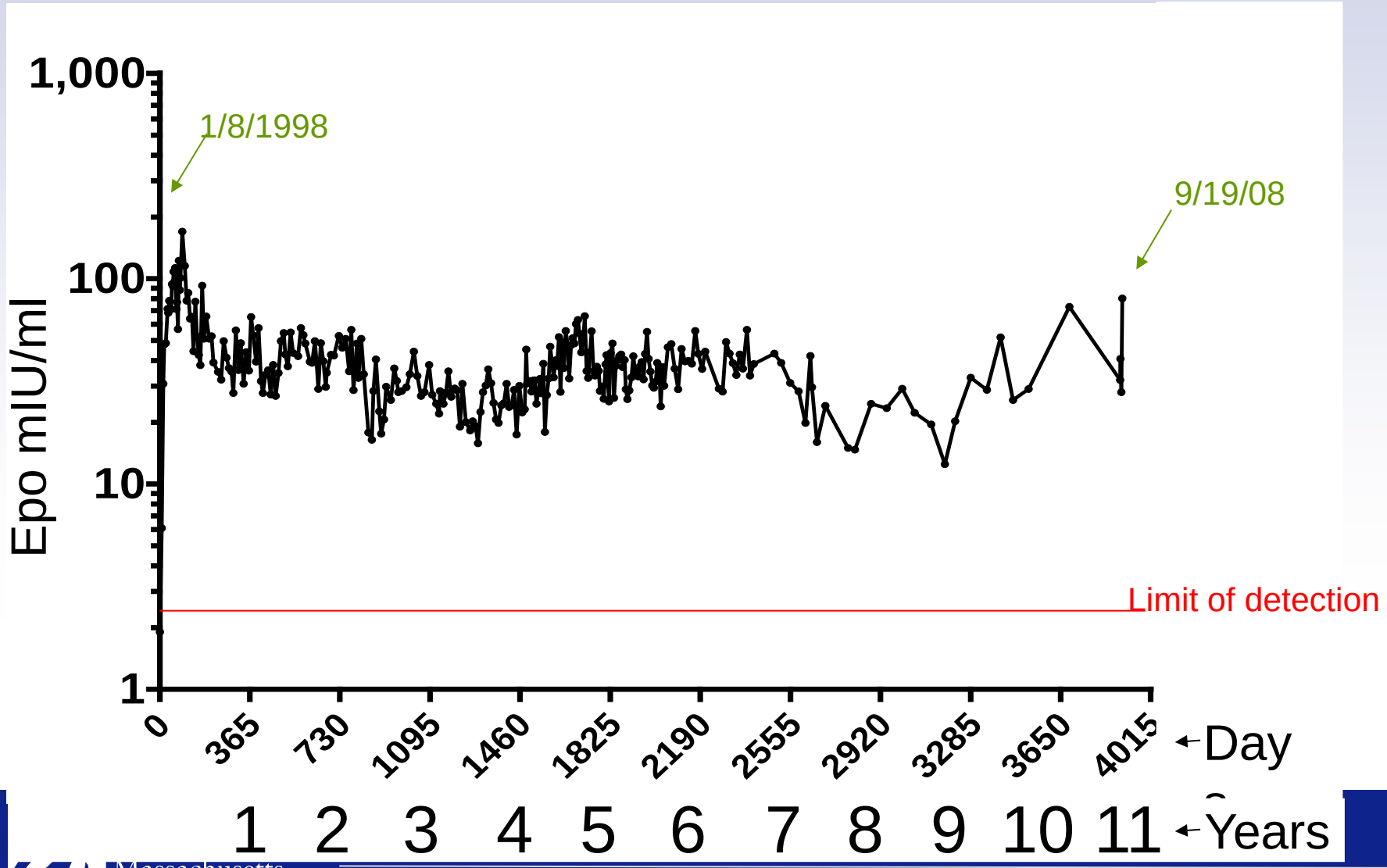
Fat



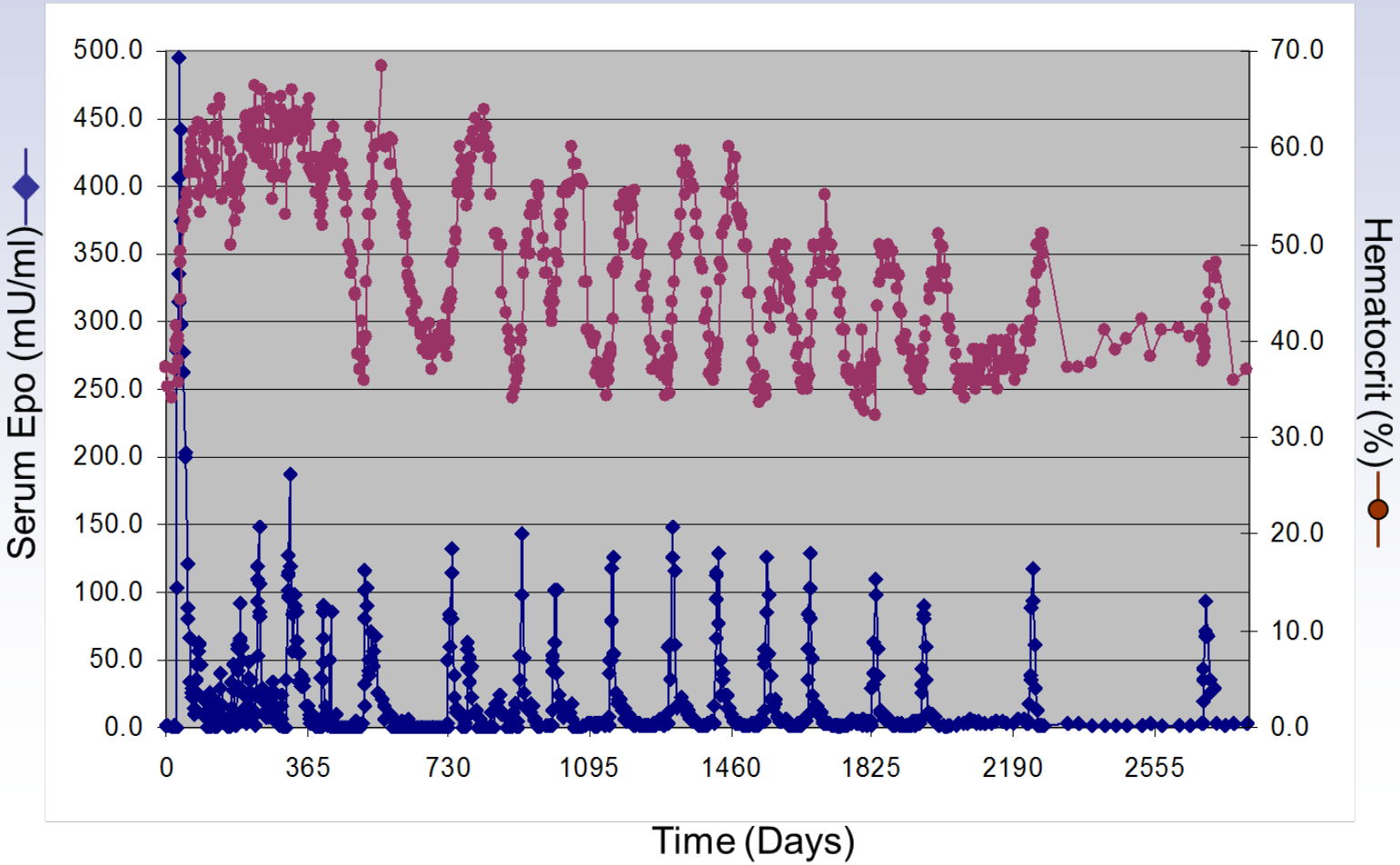
Muscle

- ★ Simple
- ★ Savvy
- ★ Stealthy
- ★ Stable
- ★ Safe (BSLI)

11 Years of Constitutive rAAVEpo Expression in NHP



9 YEARS OF REGULATED EPO IN NHP

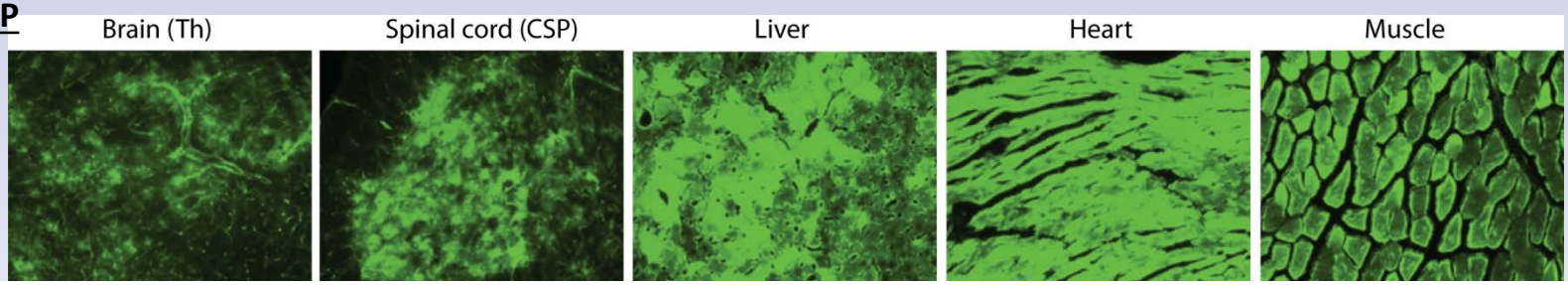


Gene • Rhesus monkey injected i.m. with 2.1 x 10^{13} GC/kg on day 1

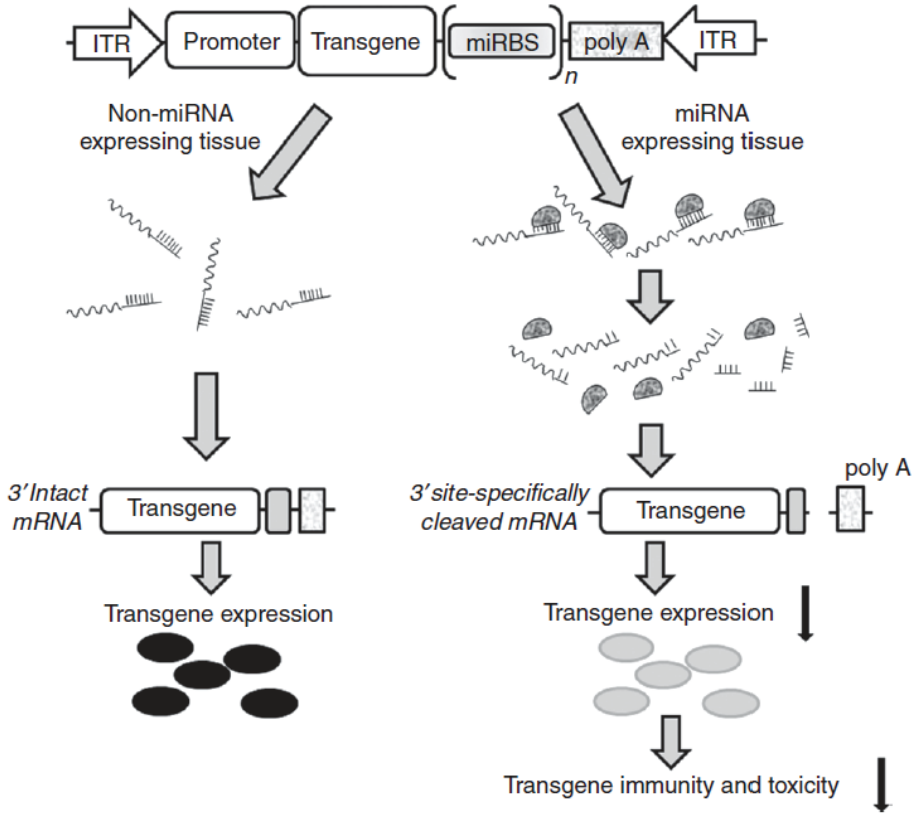
Delivery • Rapamycin administered i.v. ( ) at various dose levels

Tissue Specific posttranscriptional Regulation of rAAV Expression by Endogenous miRNAs

rAAV9-GFP



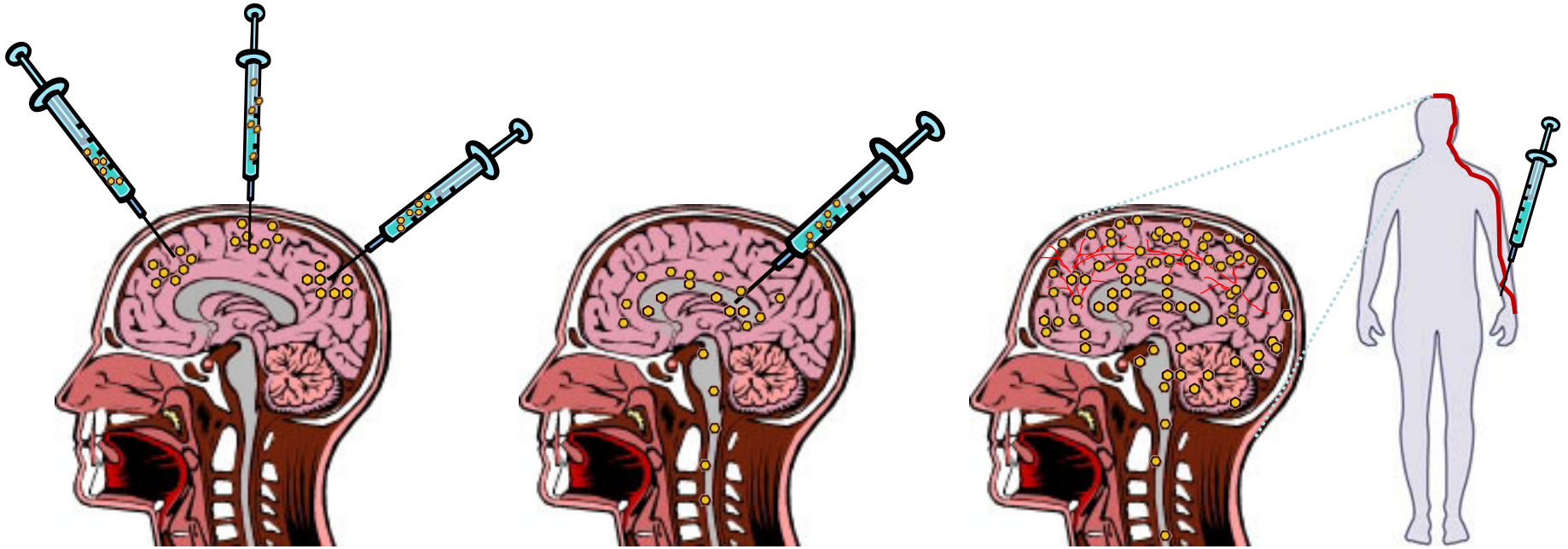
+ miRBS



Xie et al., 2011, Mol. Ther.

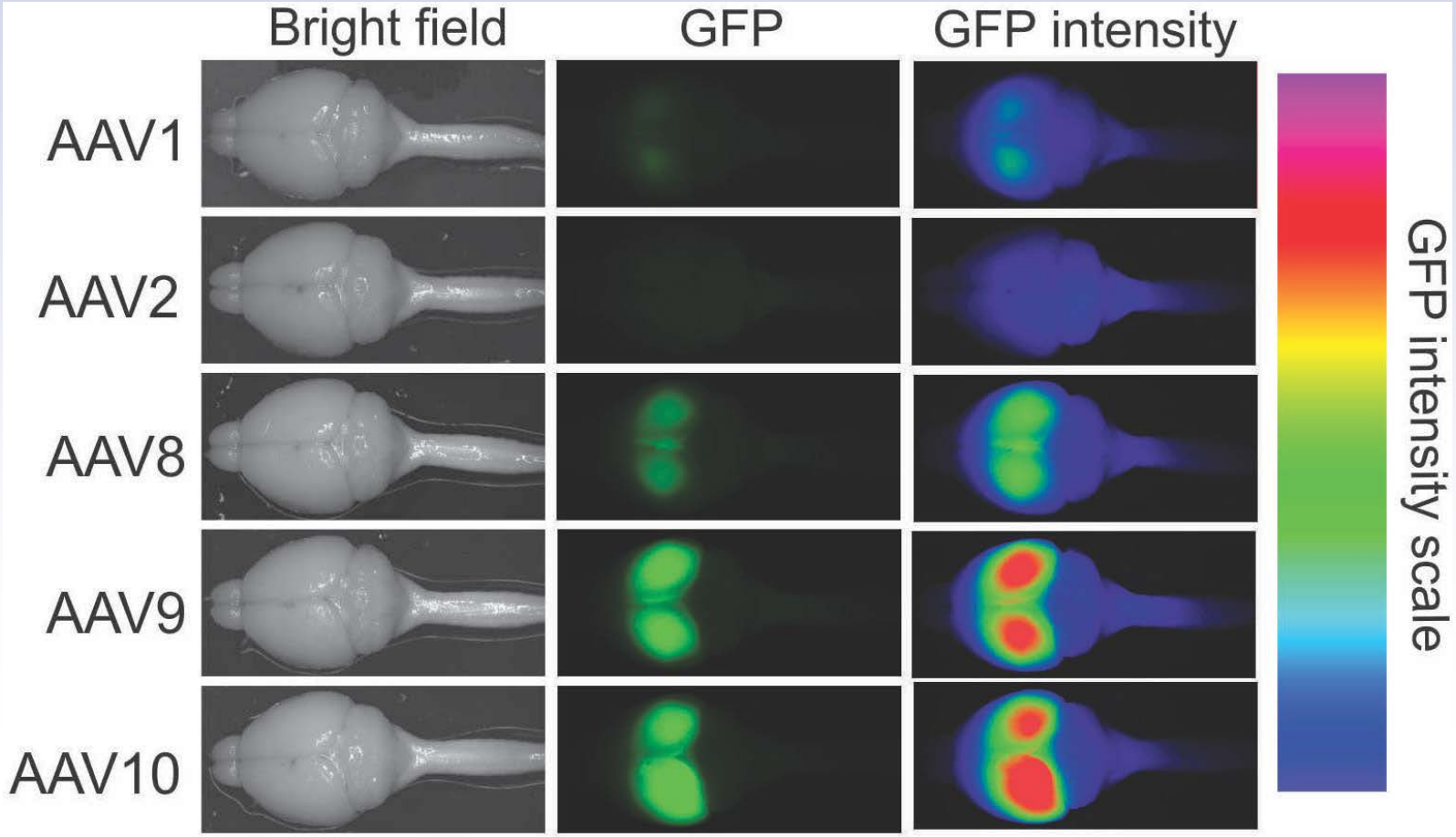
KEY CHALLENGES IN GENE THERAPY

- Gene Delivery vehicle – **Vector**
- **Method To Deliver** vector to the CNS: cross the BBB



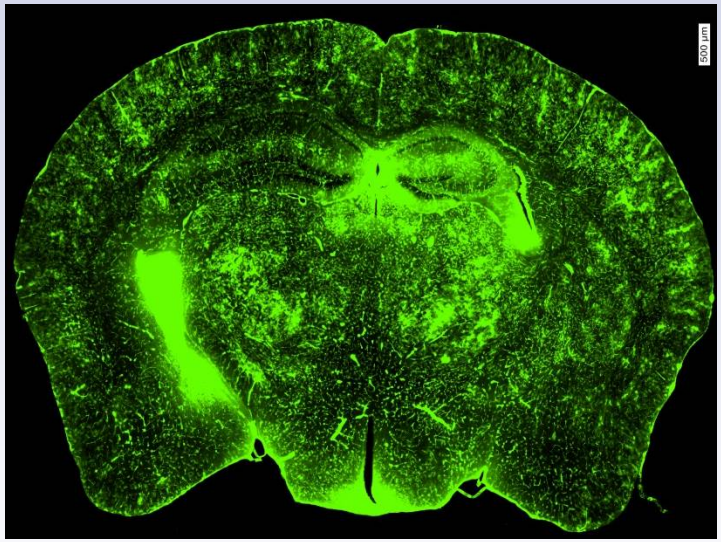
I.C.V. INJECTION OF rAAV IN ADULT MICE

- WIDE-SPREAD TRANSDUCTION IN THE FOREBRAIN

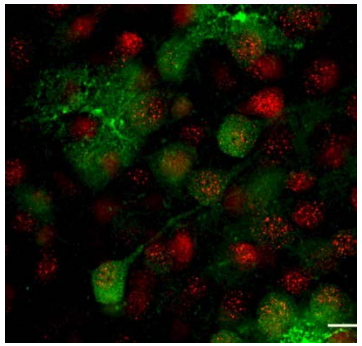


Wang, Yang, et al, 2013, Human Mol. Genet.

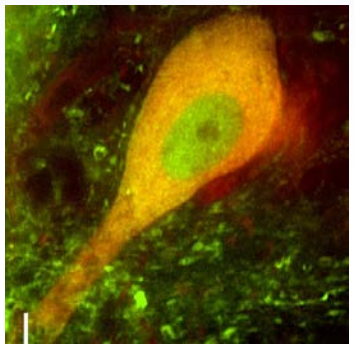
I.V. rAAV GENE TRANSFER TO NEONATAL CNS



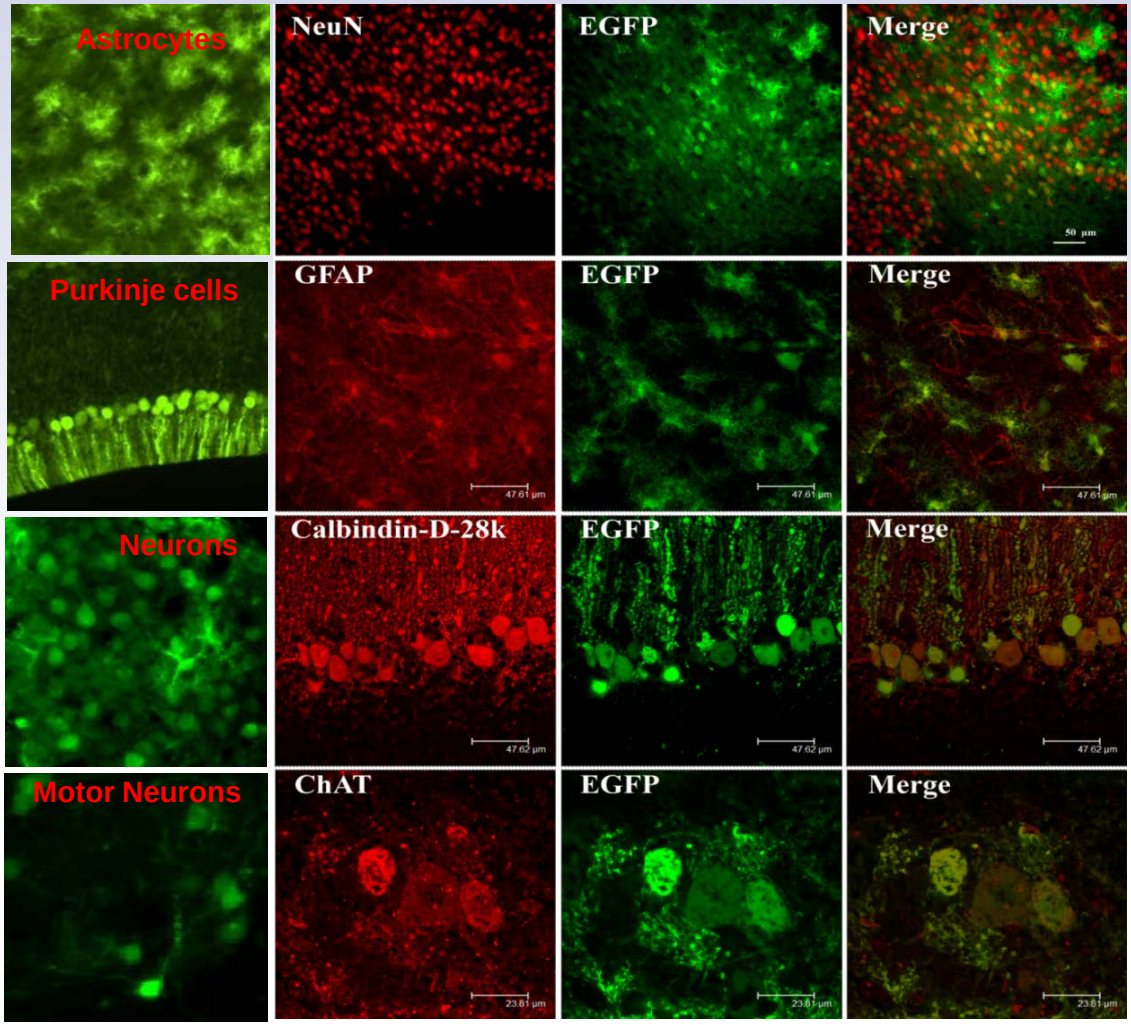
Cortical Neurons
(EGFP/ NeuN)



Dopaminergic Neurons in SN
EGFP/ TH

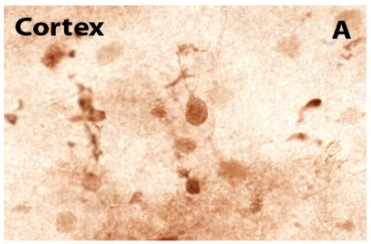


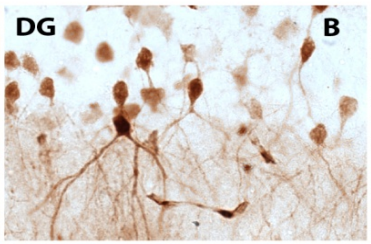
Zhang, Yang et al, 2011, Mol. Ther.



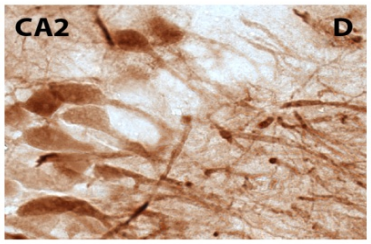
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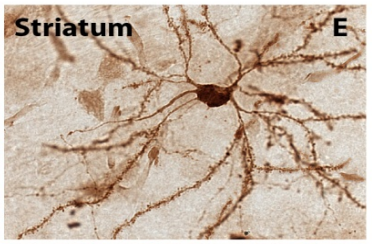
GUANGPING GAO, PHD

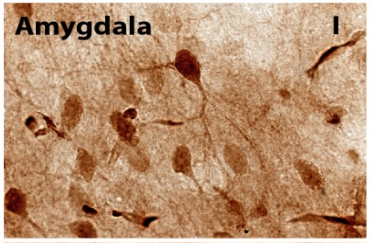


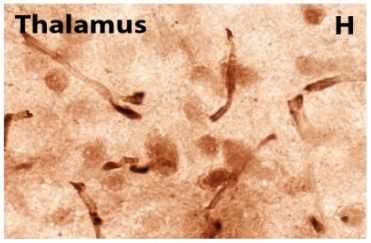


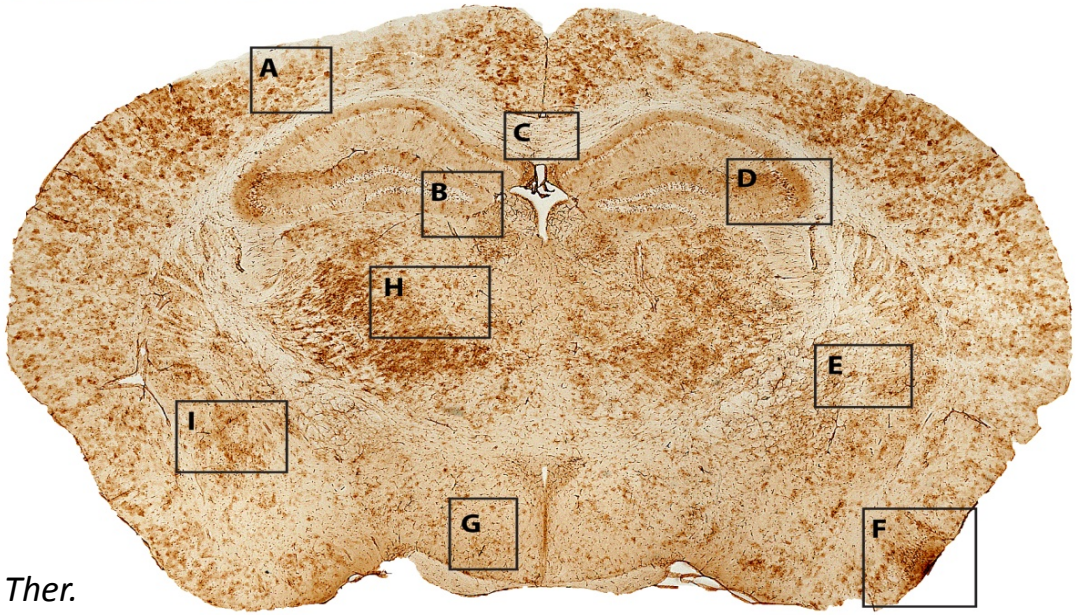


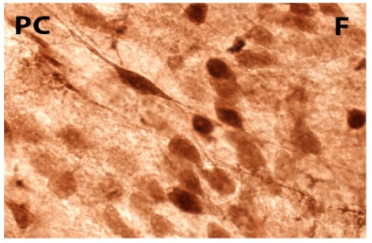


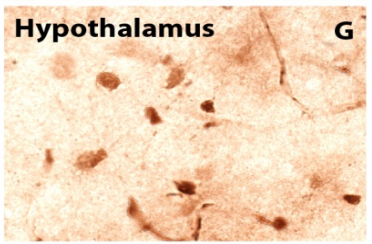




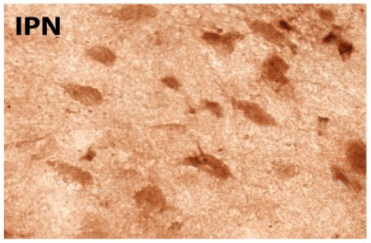


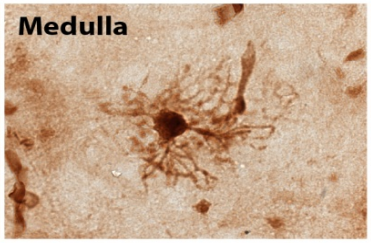


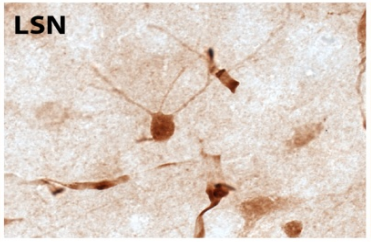


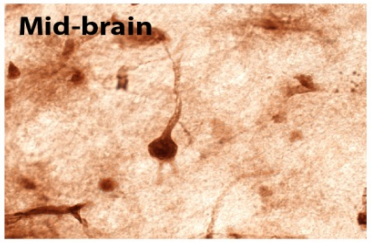


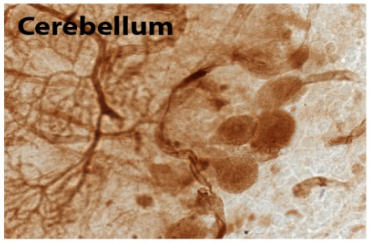
Yang et al, 2014, Mol. Ther.

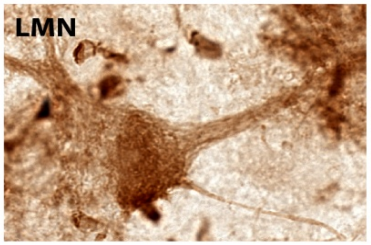


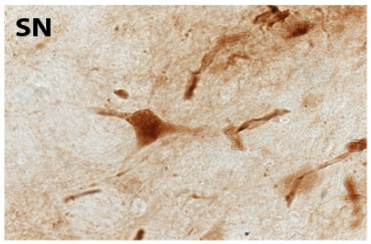








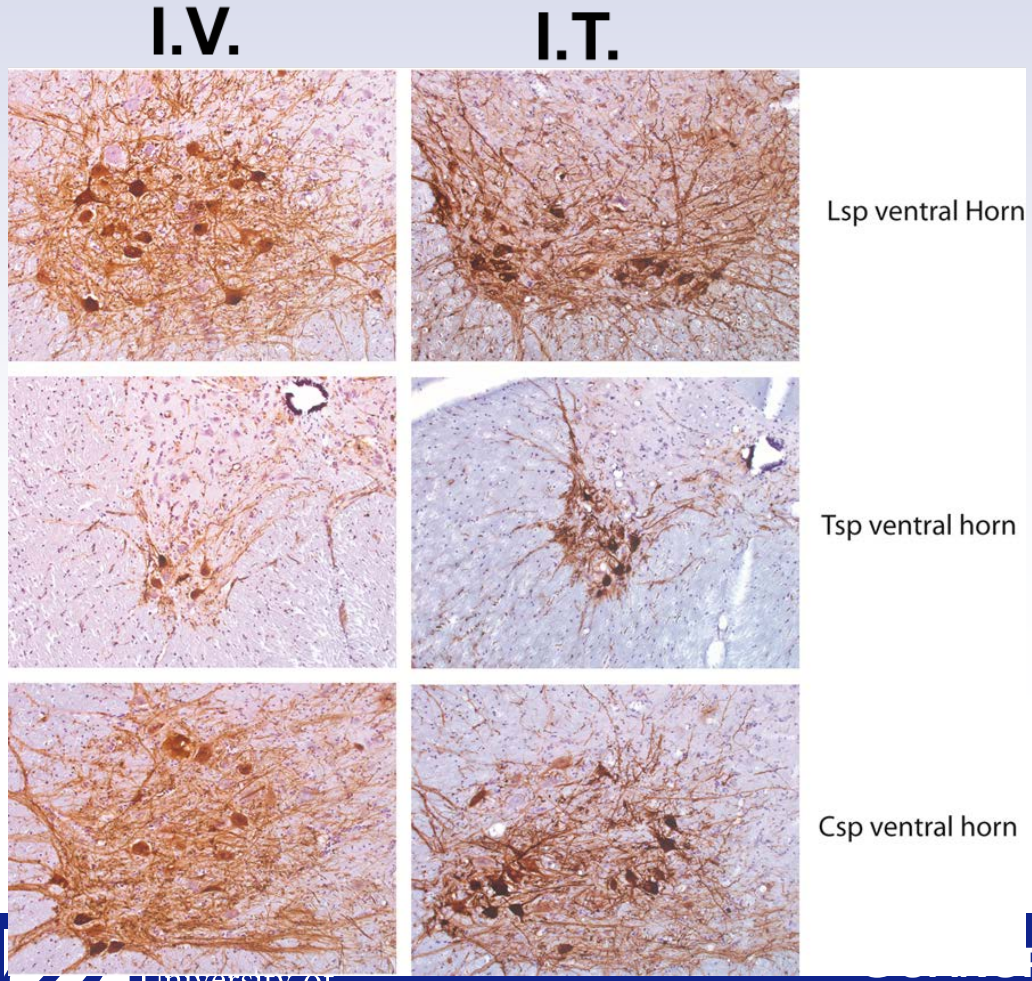




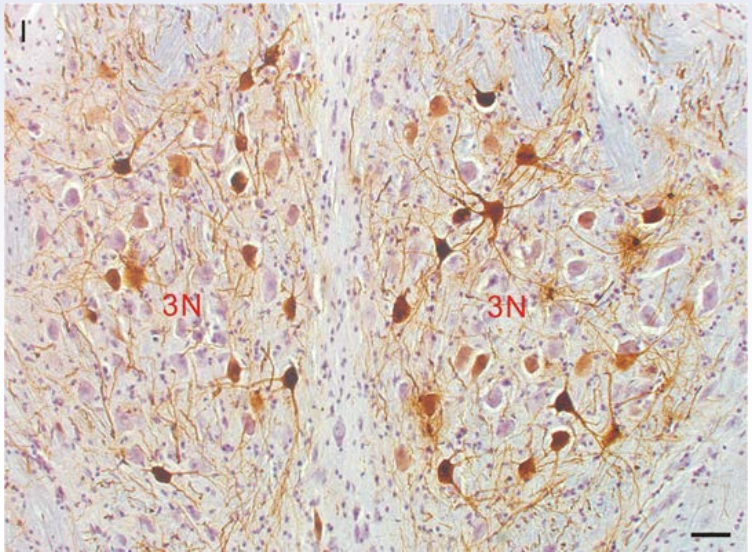
I.V. rAAV TO 10 WEEK OLD ADULT BRAIN

- *ROBUST TRANSDUCTION IN CLINICAL RELEVANT REGIONS/CELL TYPES*

Translation of i.v. & i.t. rAAV gene transfer to the CNS from mouse to Macaques



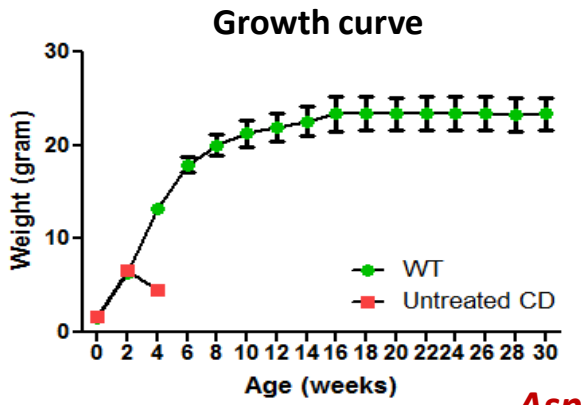
Ventral midbrain by i.v.
(Oculomotor nucleus)



Wang, Yang, et al, 2013, Human Mol. Genet.
Yang et al, 2014, Mol. Ther.

KEY ELEMENTS IN GENE THERAPY RESEARCH

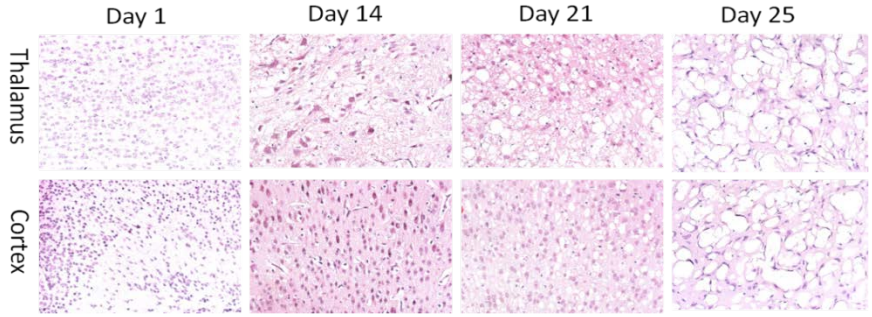
- Gene delivery vehicle – **Vector**
- **Method to deliver** vector
- Bona fide **animal model** for the disease
  - Tested for the first generation of CD gene Therapy(***Matalon** et al, 2005, MT*)



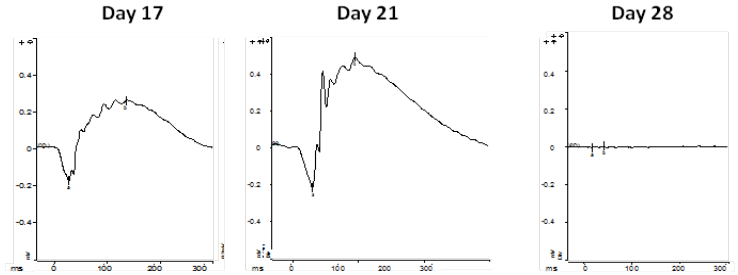


***AspA*$^{-/-}$ KO mice recapitulate the congenital and infantile forms of CD**
(Matalon et al., 2000, J. Gene. Med; Ahmed et al, 2013, Mol. Ther.)

Progressive neurodegeneration

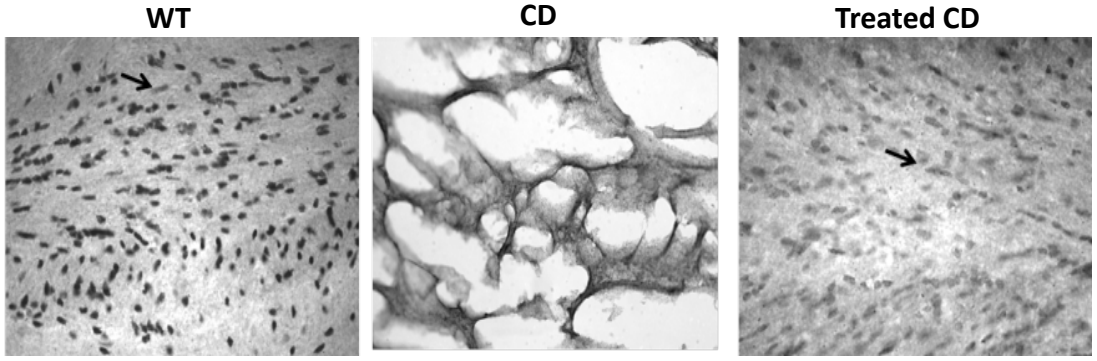


Progressive retinopathy (ERG)

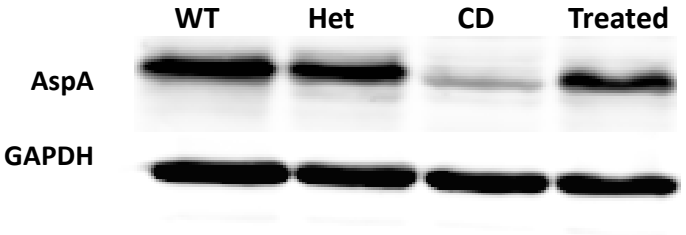


Proof-of-concept testing the 2nd generation of i.v. delivered gene therapy in CD mice
- Biochemical correction

ABC stain

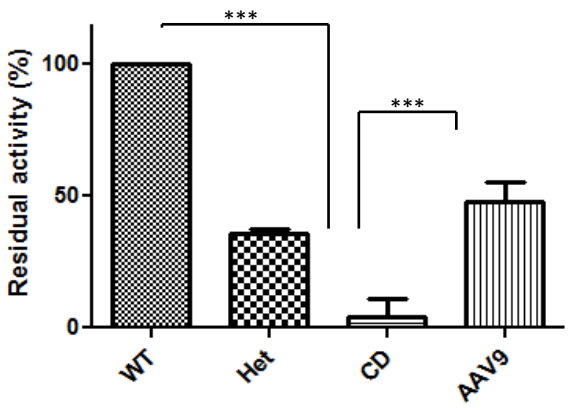


Western Blot



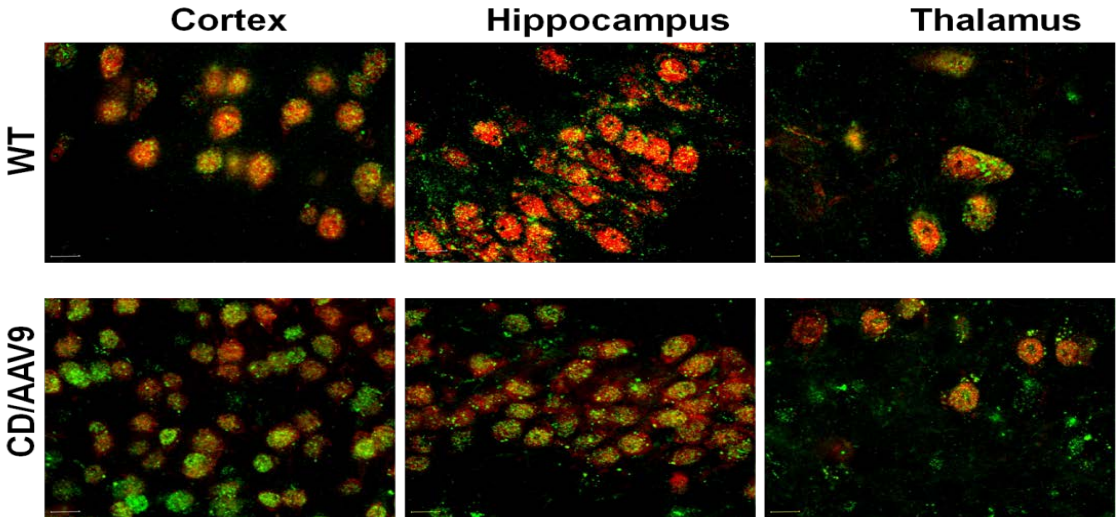
Ahmed et al., 2013, Mol Ther.

Enzyme Assay



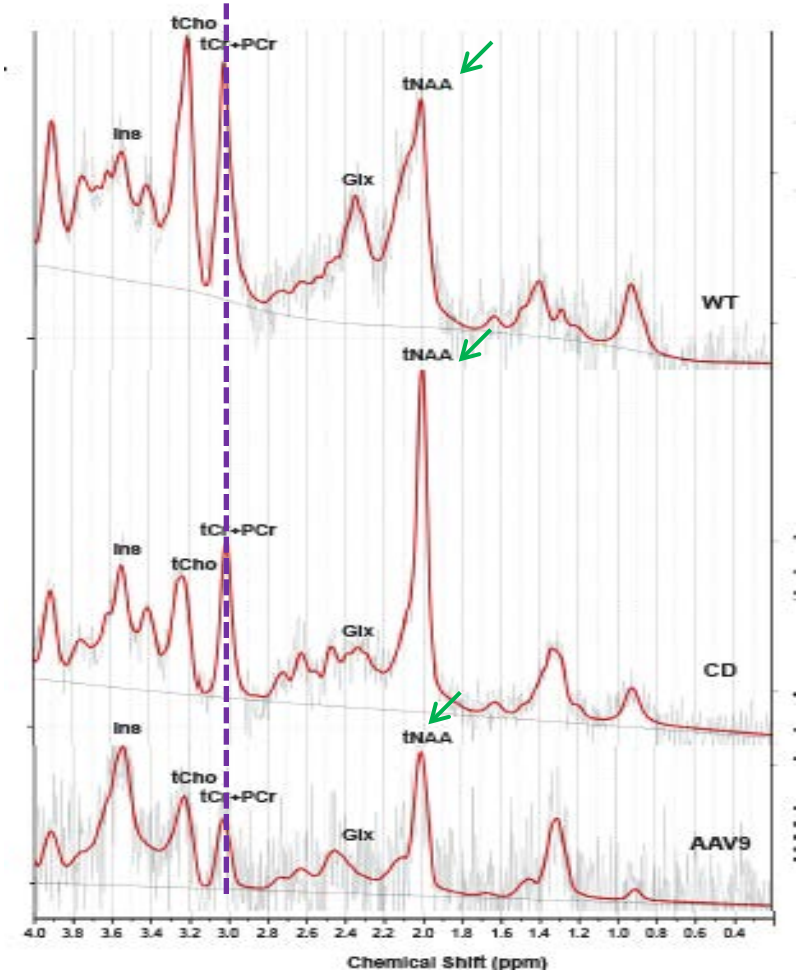
Immunofluorescence

[NeuN + AspA]

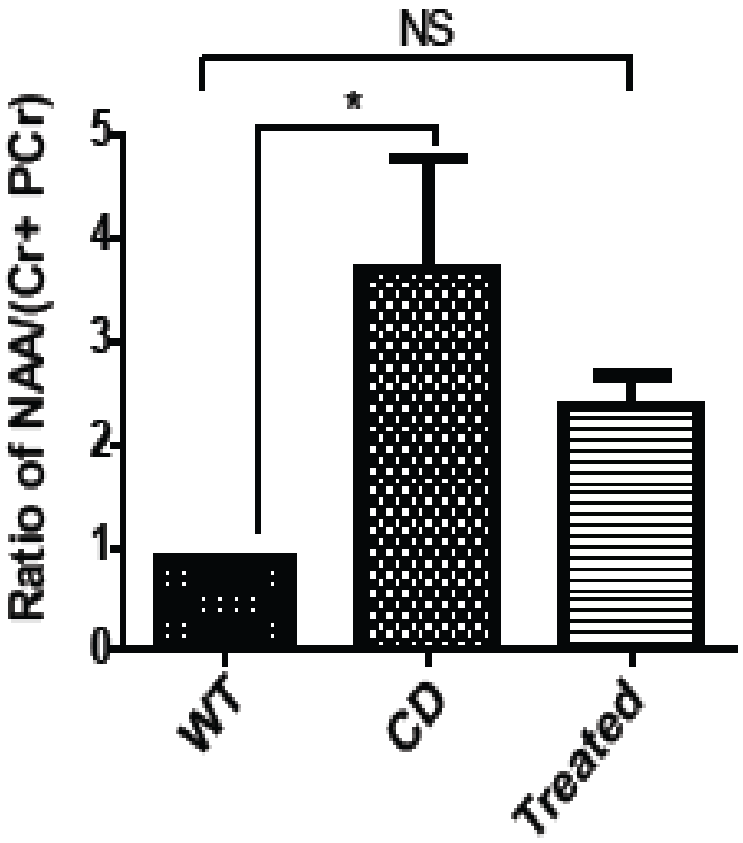


i.v. rAAV*AspA* removes excessive NAA from CD mouse brain - metabolic correction

MRS spectra



NAA quantification by MRS

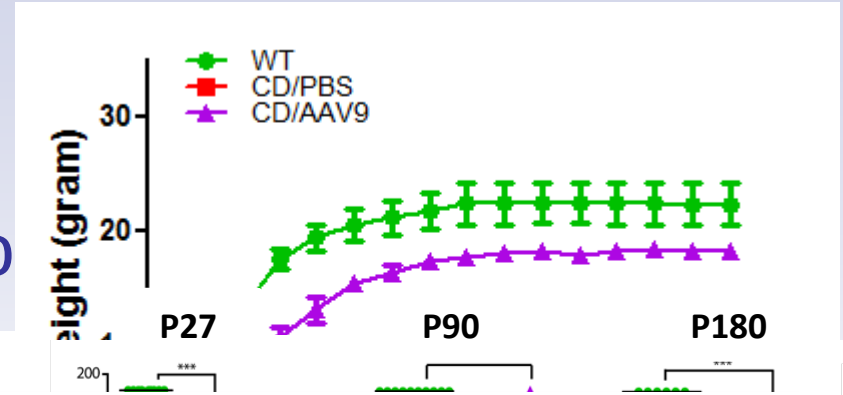


Ahmed et al. , 2013, Mol. Ther.

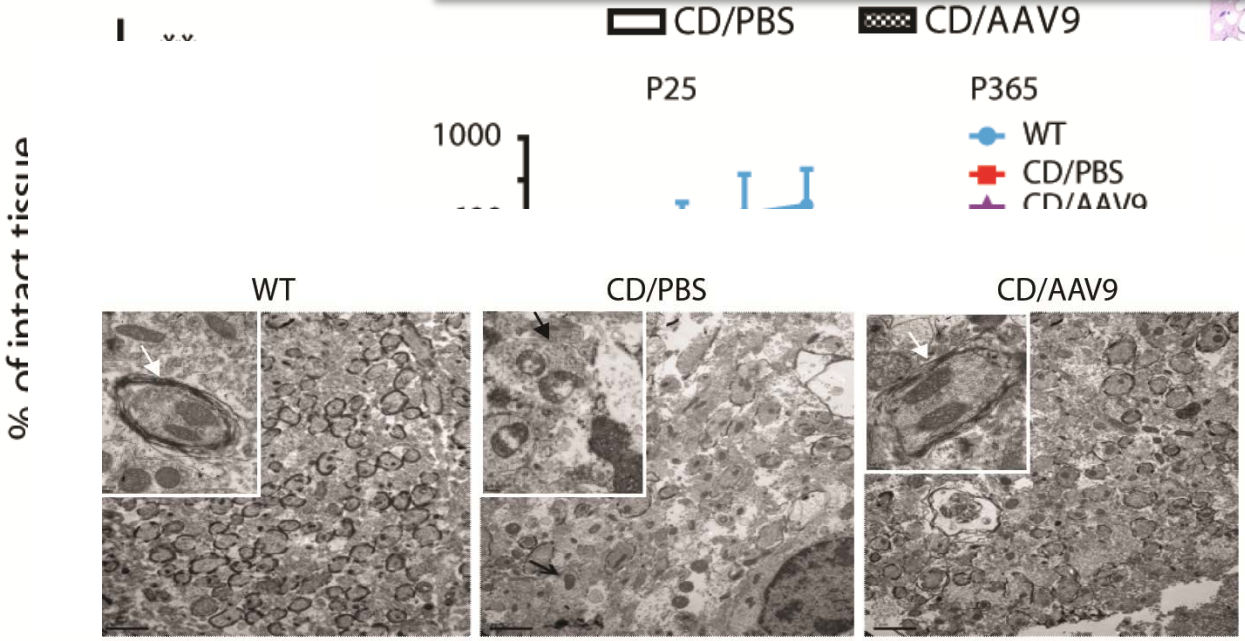
PROOF-OF-CONCEPT TESTING OF THE 2ND GENERATION CD GENE THERAPY

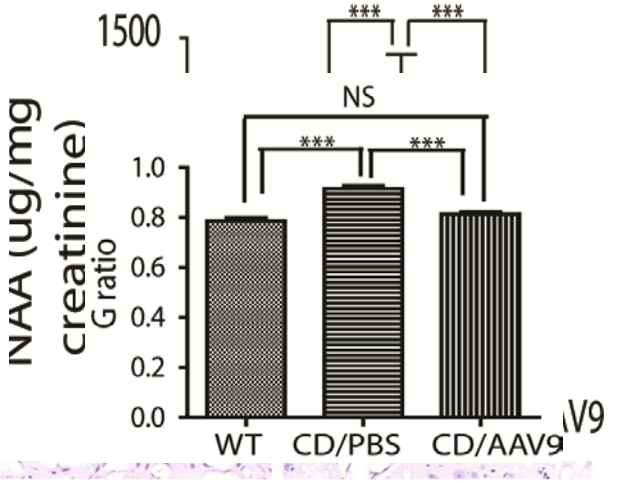
- THERAPEUTIC OUTCOMES

- Restored growth profile
- Significantly improved moto

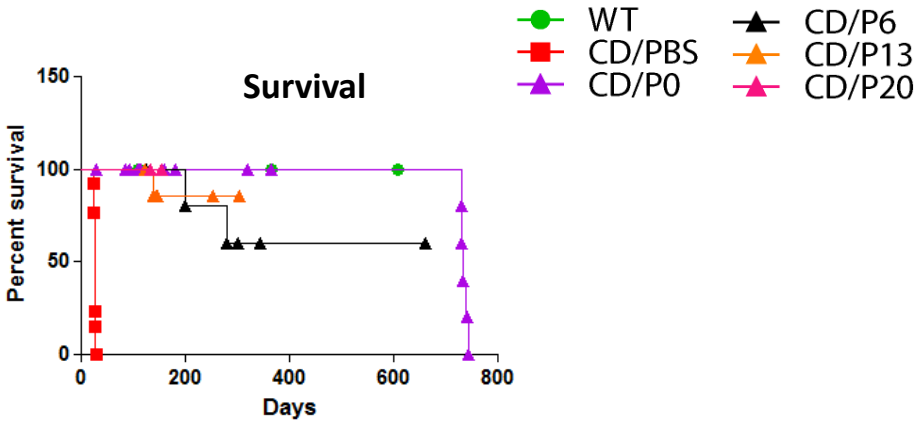


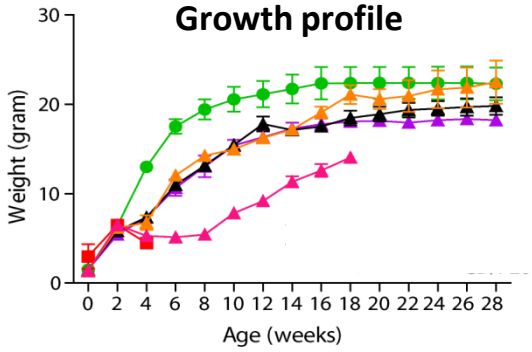
How late is too late? (Ahmed et al., 2013, Mol. Ther.)





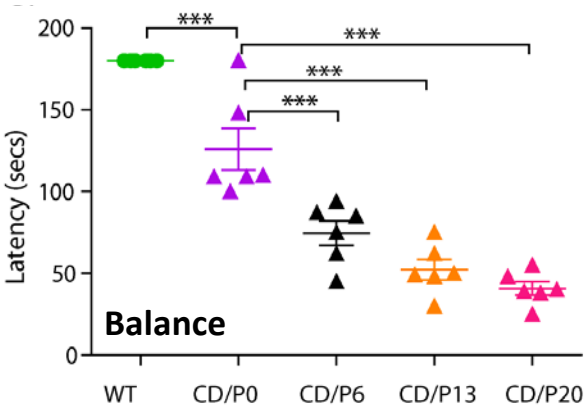
Defining the therapeutic window

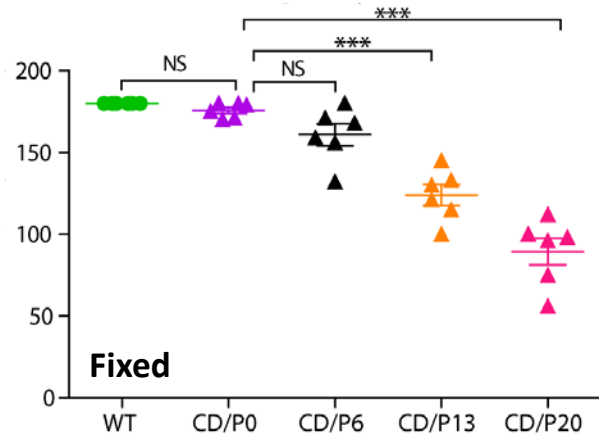


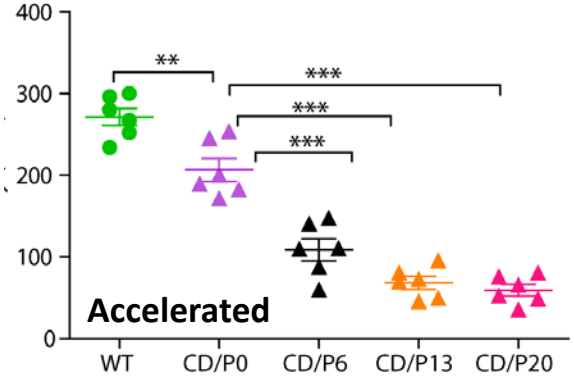


Gene Therapy is effective at ALL time points though EARLIER IS BETTER!

Rotarod







Fast therapeutic onset of the 2nd generation CD gene therapy

P0 treated mouse at P27



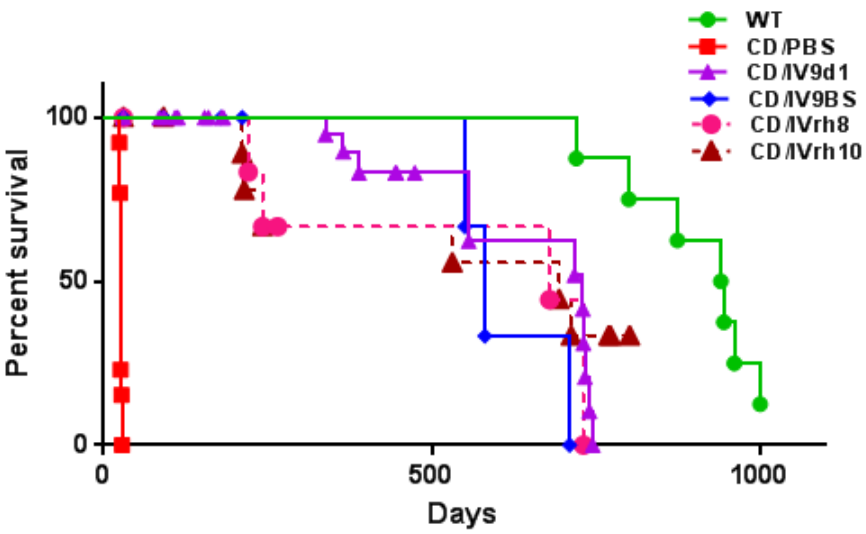
Gene Therapy Restored Reproductivity of CD mice



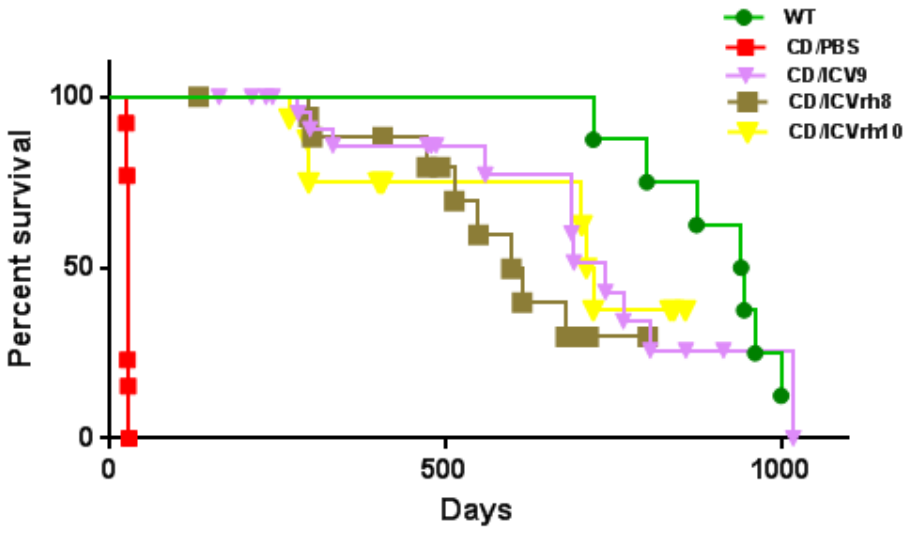
i.c.v *v.s.* i.v.: Lowering dose by a 50-fold

-Comparison for survival

i.v.



i.c.v.



- I.C.V delivery of rAAV AspA leads to longer survival (similar to wt)
- I.V. treated CD mice have a better quality of life

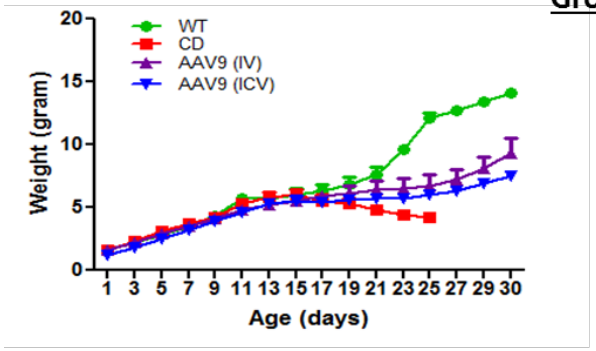
Ahmed et al., unpublished data

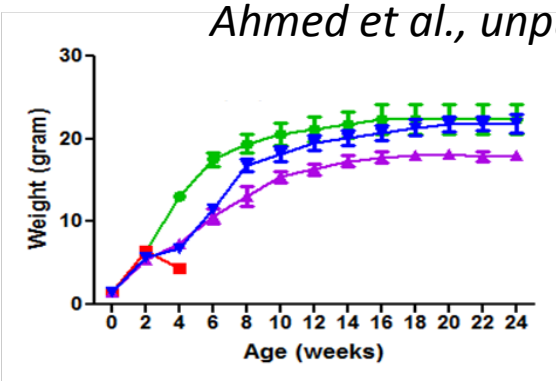
i.c.v *v.s.* i.v.: Lowering dose by 50-fold

-Comparing for growth and mobility

Ahmed et al., unpublished data

Growth Curve

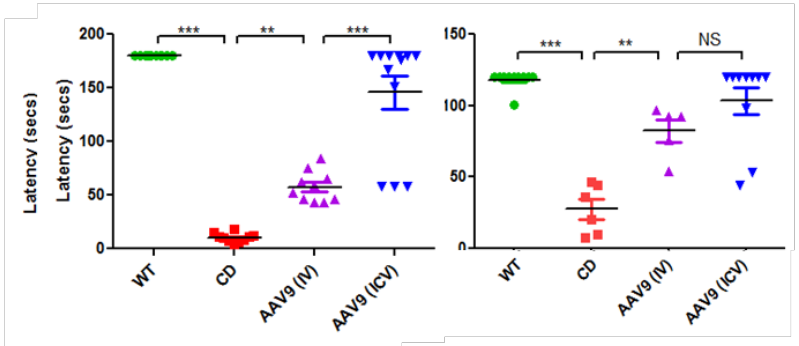


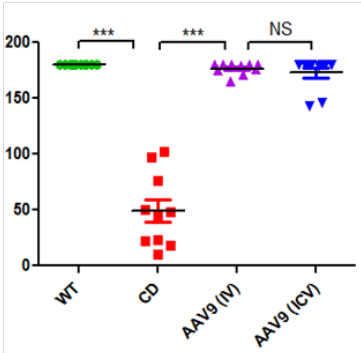


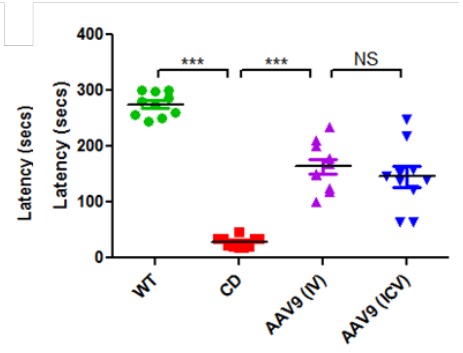
Balance Beam **Inverted Screen** **Rotarod**

Fixed *Accelerated*

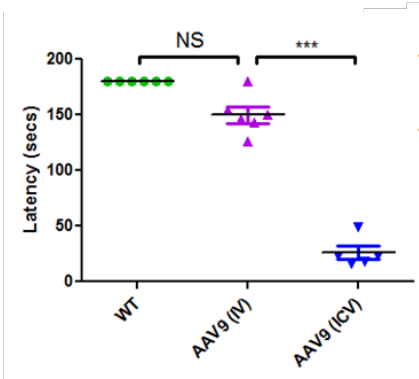
1 month

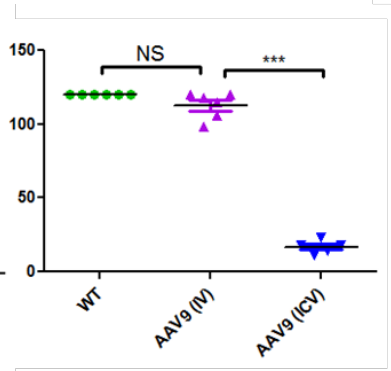


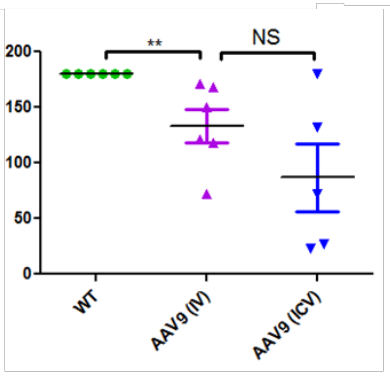


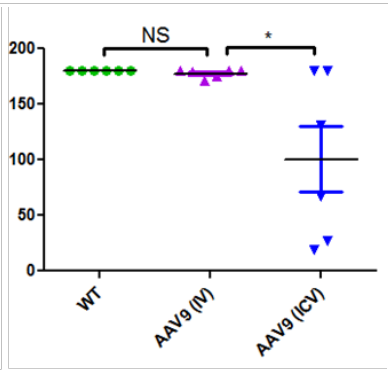


3 month









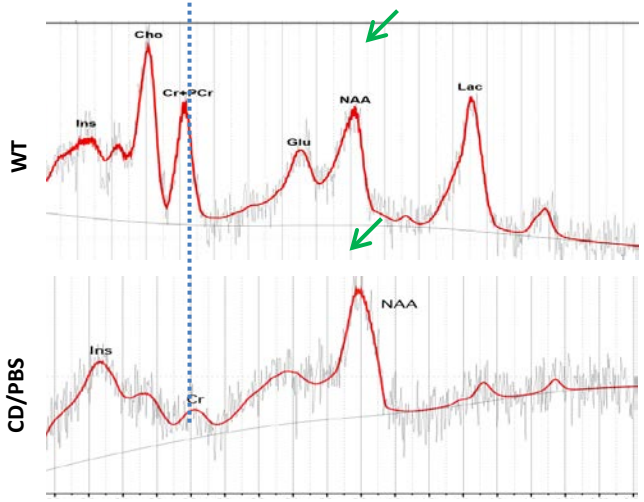
i.c.v *v.s.* i.v.: Lowering dose by a 50-fold

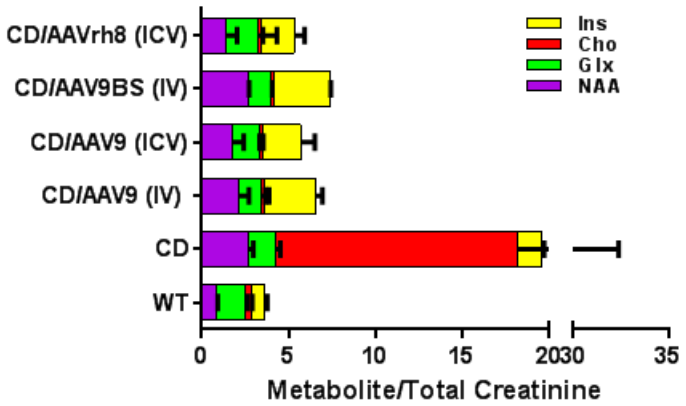
-Comparing for MRS and MRI

Ahmed et al., unpublished data

Reduced NAA level in the brain

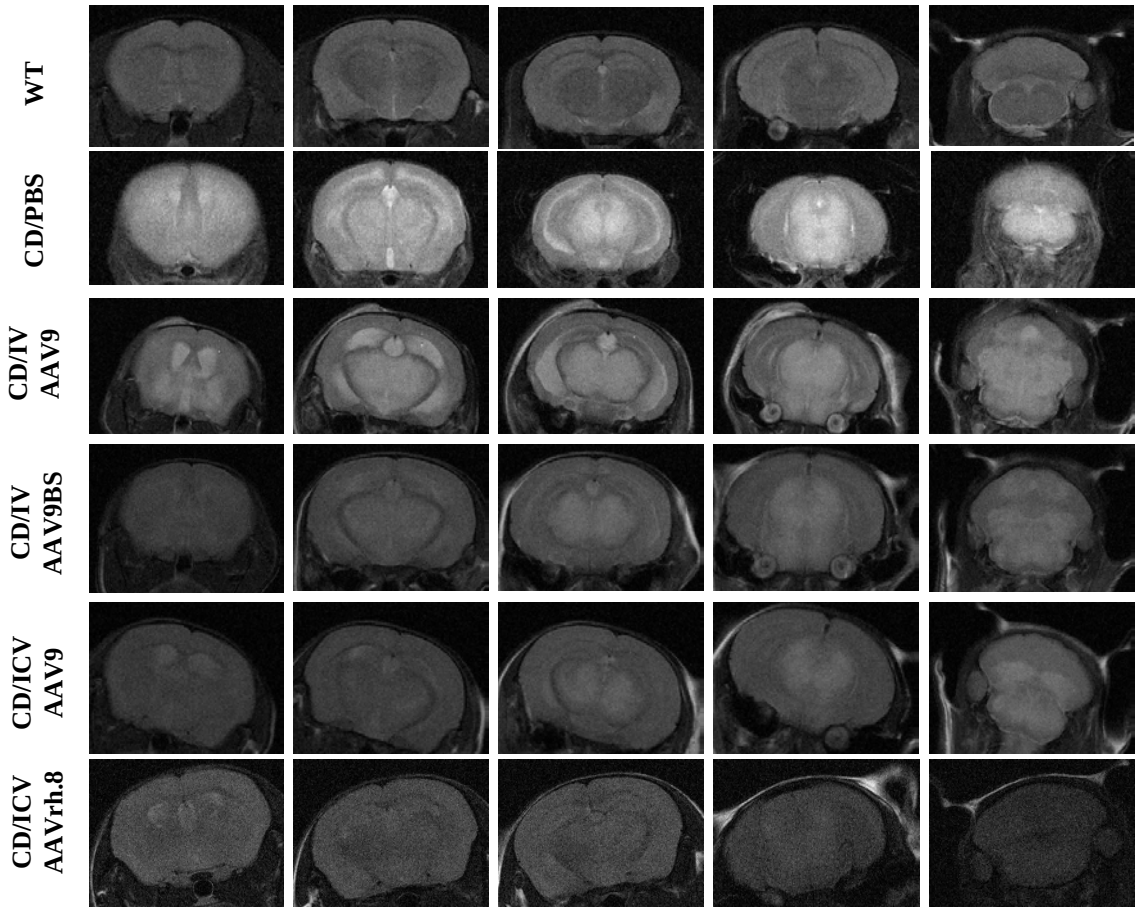
Magnetic Resonance Spectroscopy [MRS]





Reduced brain edema

Magnetic Resonance Imaging [MRI]



SUMMARY

- Single i.v. gene therapy as late as P20 or i.c.v. on P1 achieved
  - **100% rescue** of early lethality of AspA$^{-/-}$ mice (n>50)
  - **Extended survival** from **< 4 wks to > 24 months**
  - Normalized **AspA** activities, **NAA** metabolism and **myelination**
  - Mitigated **neuropathology and retinopathy**
  - Restored **motor function, vision and growth**
- What is the next?
  - To further improve vector potency and reduce effective dose
  - To perform formal toxicology/safety study in NHPs
  - To have Pre-IND meeting with FDA
  - To file IND and start clinical trial

Gene Therapy for Canavan's Disease

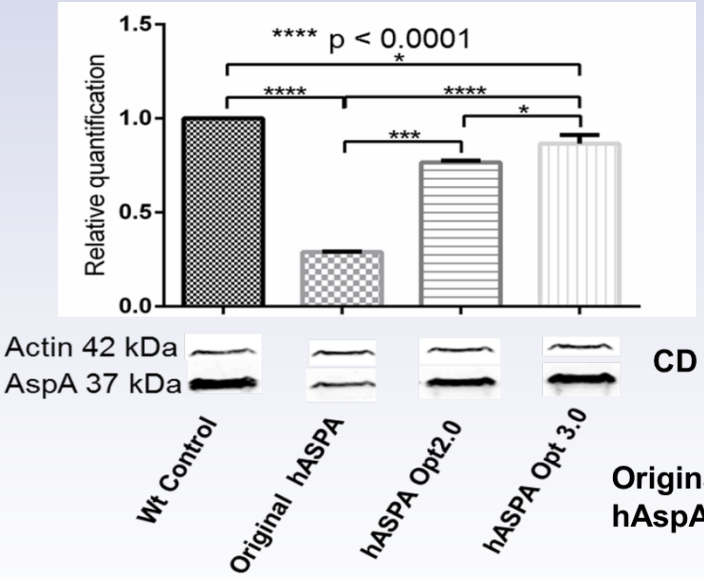
- *Developing the 3rd generation CD gene therapy*

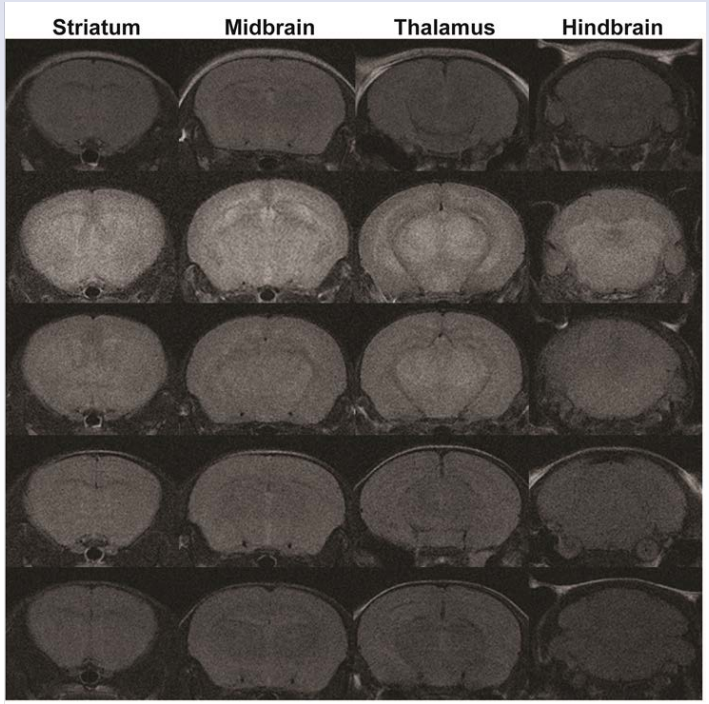
University of Massachusetts Medical School
UMASS

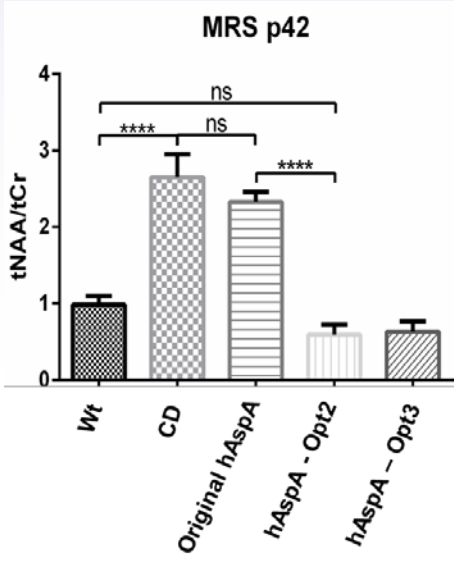
GUANGPING GAO, PHD

NEUROLOGY 2014 INT'L CONFERENCE, PHILADELPHIA, 9/8/2014

VECTOR OPTIMIZATION: LOWER THE DOSE FOR MORE EFFICACIOUS AND SAFER THERAPY







Gessler et al., unpublished data

Optimized vector transforms CD mice into supper-mice on Rotarod(4 wks old, 4wks after GT)



Gessler et al., unpublished data

ONE OPTIMIZED VECTOR IS BETTER IN BALANCING THAN THE OTHER ONE (GESSLER ET AL., UNPUBLISHED DATA)

hAspA – Opt3.0



hAspA - Opt2.0



Acknowledgements

UMassMed

Gao Lab
Seemin Ahmed
Dominic Gessler
Chunyan Hu
Dan Wang
Jun Xie

Xu Lab
Bing Yang

Mello Lab
Hiroki Kato

Zamore Lab
Li Zhong
Huapeng Li
Hongwei Zhang
Stefan Amerase
JenniferBroderick
Chengjian Li

Vector Core
Qin Su
Ran He

Flotte Lab
Terry Flotte

Esteves Lab
Miguel Esteves

Brown Lab
Robert Brown

Boston College
Tom Seyfridt
Dan Kirschner

U. Penn
Jim Wilson

UTBM

Matalon Lab
Sylvia Szucs

THE WILL FOUNDATION

National Institute of Allergy and Infectious Diseases
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National Institutes of Health

JACOB'S CURE

