

Phase I/II gene transfer clinical  
trial of rAAV9.CMV.hNAGLU for  
Mucopolysaccharidosis (MPS) IIII



# Key Personnel

<b>Kevin Flanigan, MD</b>	<b>Principal Investigator</b>
<b>Haiyan Fu, PhD</b>	<b>Pre-clinical</b>
<b>Doug McCarty, PhD</b>	<b>Pre-clinical</b>
<b>K. Reed Clark, PhD</b>	<b>CMC</b>
<b>Tim Miller, PhD</b>	<b>Pres. &amp; CEO, Abeona Therapeutics</b>
Jerry Mendell, MD	Consultant
Kim McBride, MD	Co-Investigator
Keith Yeates, PhD	Psychologist
Marco Corridore, MD	Anesthesiologist
Nicholas Zumberge, MD	Radiologist
Chris Shilling, MS	Director, Drug and Device Development
Krista Kunkler	Study Coordinator

# Rationale

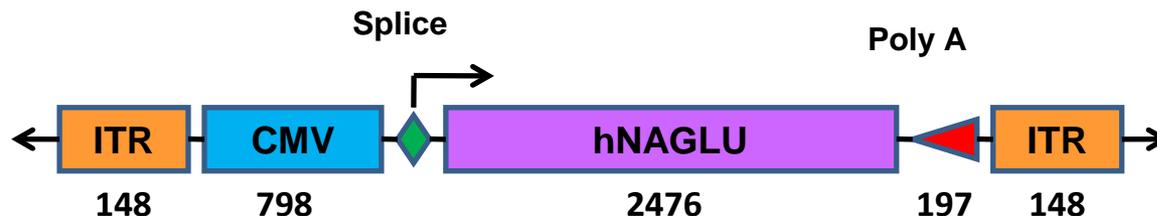
- MPS IIIB (Sanfilippo syndrome B) is a devastating lysosomal storage disease
- Monogenic – due to autosomal recessive defects in NAGLU
- It is relentlessly progressive and typically leads to death by the end of the second or the beginning of the third decade.
- **There is no treatment currently available for the disease.**
- We propose the first gene transfer of hNAGLU using recombinant adeno-associated virus serotype 9 (rAAV9) vector, targeting the root cause of the disease.

# Rationale for gene therapy

- We can correct the neurological *and* somatic features of MPS IIIB with a single intravenous transfer of rAAV9.CMV.hNAGLU in adult mice
- MPSIIIB is an ideal candidate for gene therapy because of the bystander effects of NAGLU.
  - Enzyme is partially secreted and can be taken up by neighboring cells.
  - Only very low (1-10% normal levels) enzyme activity is needed to normalize the metabolic function in bystander cells.
  - It is therefore not necessary to deliver the target gene to every cell for optimal benefits.
- We expect to take advantage of the trans-blood-brain-barrier (BBB) neurotropism of AAV9 to restore the NAGLU activity throughout the CNS and periphery by systemic vector delivery

# Advantages to this approach

- Potential for one-time dosing as opposed to enzyme replacement therapies
- Cassette uses a ubiquitously active CMV promoter that has demonstrate efficiency and long-term gene expression in the CNS and somatic tissues in numerous studies
- Although this is primarily a safety and tolerability study, based upon our preclinical studies in MPS IIIB mice and in non-human primates there is a potential for yielding clinical benefit in patient subjects.



# The three phases of mucopolysaccharidosis type III (MPS III) and associated signs and symptoms

Normal development for 1- 2 years, then:

1. ~1-2 year: Developmental delay becomes apparent
2. ~3-4 years: Severe behavioral problems, progressive mental deterioration → severe dementia
3. Behavioral problems slowly disappear; motor retardation with swallowing difficulties and spasticity

Death by end of second/beginning of third decade

Phase*	Signs/Symptoms <sup>†</sup>
Presymptomatic	Apparently normal development
Phase 1	Neurocognitive Developmental delay Speech delay Somatic Mild facial dysmorphism (can be very subtle) Frequent ear infections Frequent upper respiratory infections Cardiac valve disease Hernia (umbilical, inguinal) Hepatomegaly Diarrhoea

Phase 2	Neurocognitive Progressive cognitive decline/mental retardation Decline in speech/lack of speech Behavioural disturbances Hyperactivity Impulsivity Aggression Restlessness Anxious behaviour Compulsive behaviour Autistic-like behaviour Decline in motor skills Seizures Somatic (those in phase 1, plus the following) Hearing loss Orthopaedic manifestations Scoliosis Kyphosis Lumbar lordosis Hip dysplasia and pain Carpal tunnel syndrome Trigger digits Joint contractures
Phase 3	Neurocognitive Profound mental retardation progressing to vegetative state Lack of speech or communication Behavioural disturbances cease Difficulty swallowing progressing to inability to swallow Spasticity Seizures Somatic Those in phases 1 and 2

\*The timing of the disease course in attenuated patients is more variable than that seen in severe patients, but progression through these phases is common to all MPS III patients.

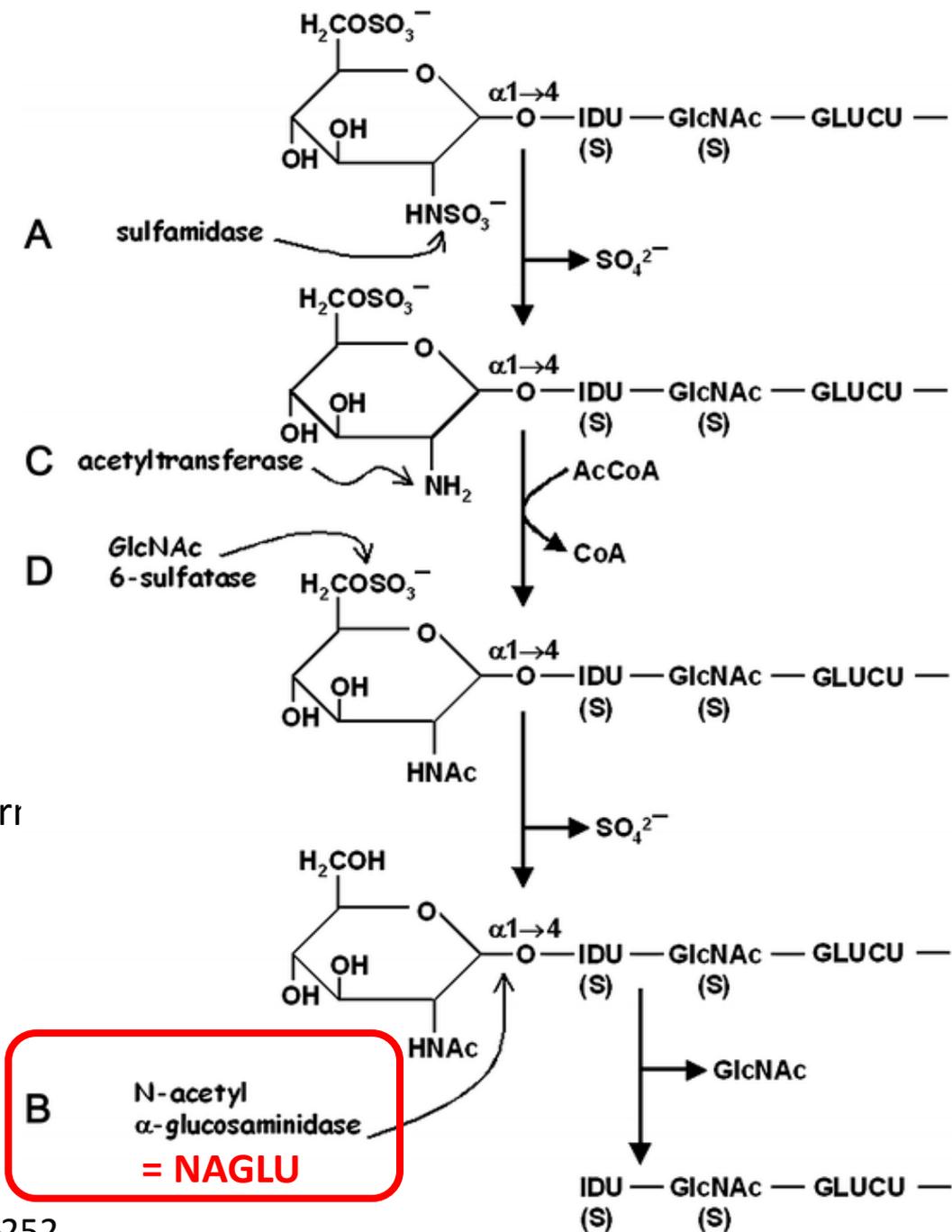
<sup>†</sup>Not all signs and symptoms may be present in any individual patient.

In Sanfilippo syndrome, glycosaminoglycans (GAGs) in the form of heparan sulfates accumulate within lysosomes, leading to pathology.

Absence of different enzymes responsible for heparan sulfate degradation result in different forms of Sanfilippo syndrome.

Clinical Diagnosis:

- 1.) Urinary GAG quantification
- ↓
- 2.) Serum/leukocyte enzyme level deterri
- ↓
- 3.) Genomic mutational analysis



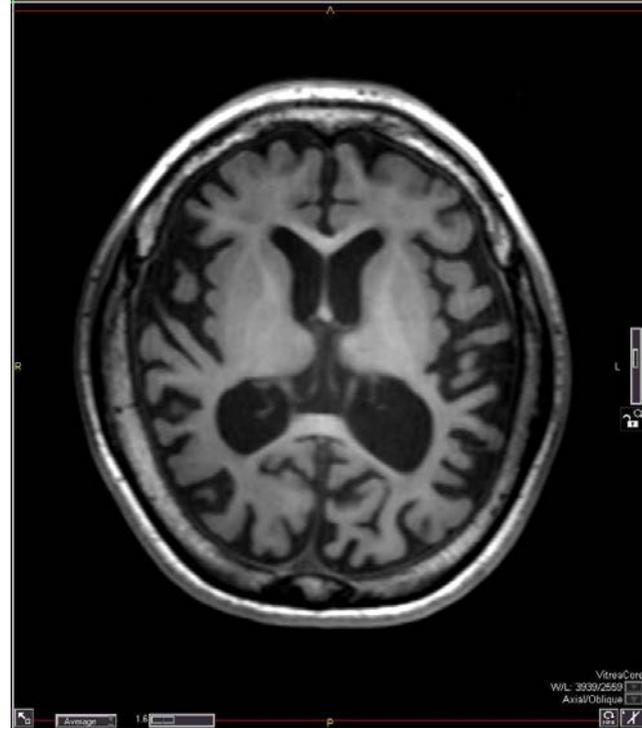
# Progressive cortical volume loss

6 y.o.

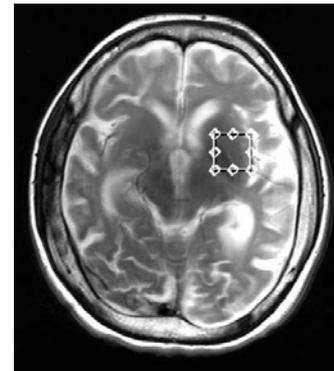
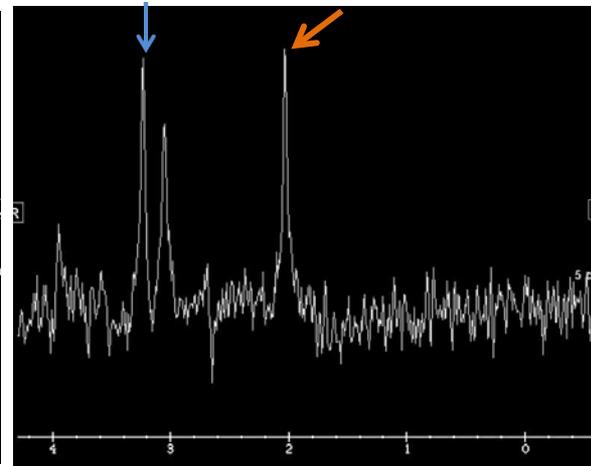
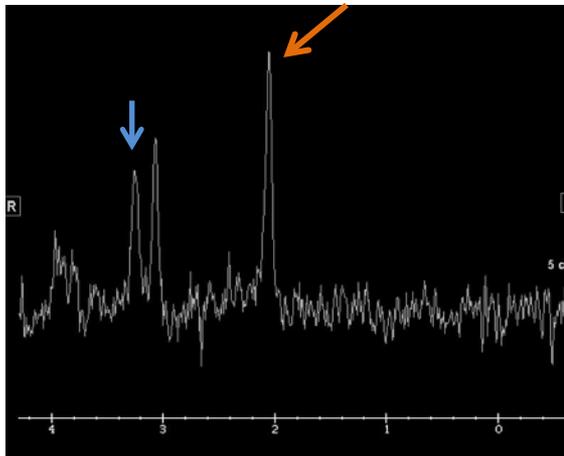


3D SPGR

9 y.o.



## Decreasing NAA:Choline ratio



# AAV9 crosses the blood-brain barrier and demonstrates neurotropism

- Foust et al, Nat Biotechnol 2009
- Duque et al, Mol Ther, 2009
- Bevan et al, Mol Ther, 2011

## **Correction of Neurological Disease of Mucopolysaccharidosis IIIB in Adult Mice by rAAV9 Trans-Blood–Brain Barrier Gene Delivery** Mol Therapy, 2011

Haiyan Fu<sup>1,2</sup>, Julianne DiRosario<sup>1</sup>, Smruti Killedar<sup>1,2</sup>, Kimberly Zaraspe<sup>1</sup> and Douglas M McCarty<sup>1,2</sup>

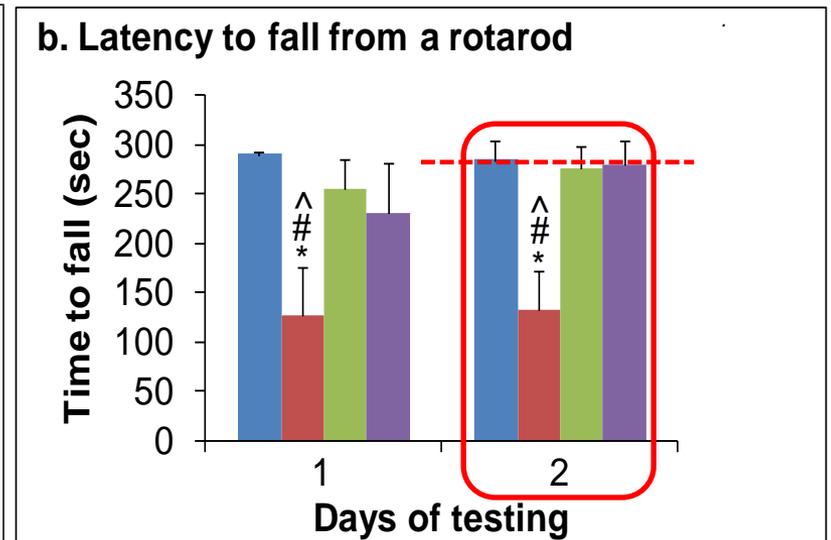
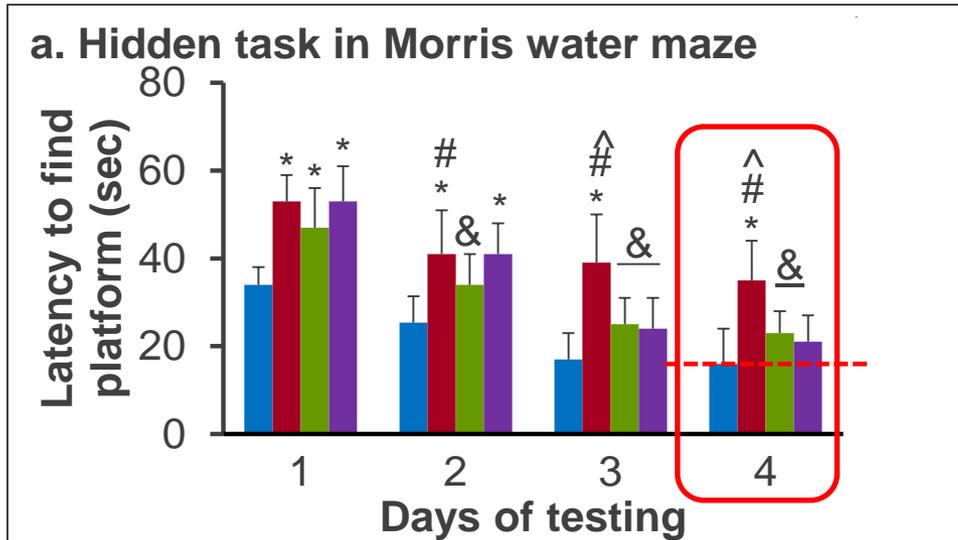
*<sup>1</sup>The Center for Gene Therapy, The Research Institute at Nationwide Children's Hospital, Columbus, Ohio, USA; <sup>2</sup>Department of Pediatrics, College of Medicine and Public Health, The Ohio State University, Columbus, Ohio, USA*

### **Systemic AAV9 delivery has advantages over other approaches, specifically:**

- In the CNS, more widespread distribution than intrathecal or intracranial delivery
- Simultaneous systemic delivery to somatic tissues

# Significant behavioral correction in 4-6 week old MPS IIIB mice after a single IV injection of rAAV9-CMV-hNAGLU

5.0 - 5.5 months old (4 months post-injection)

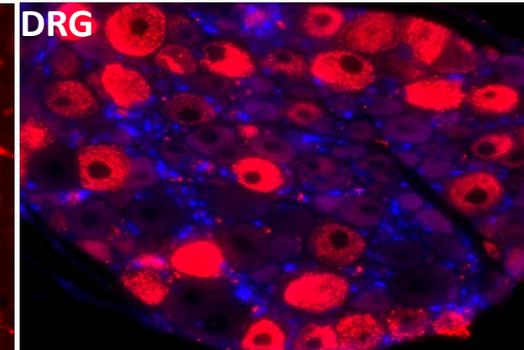
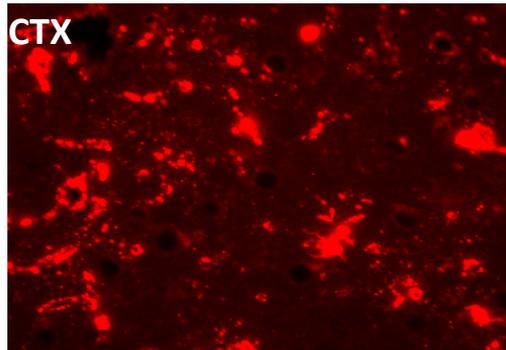
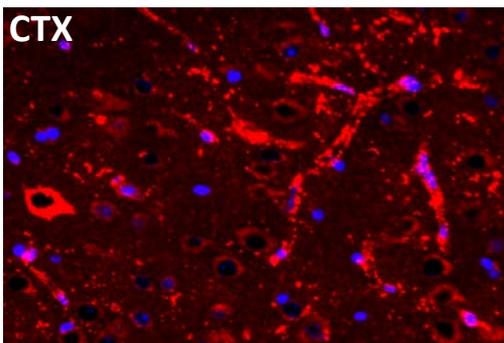
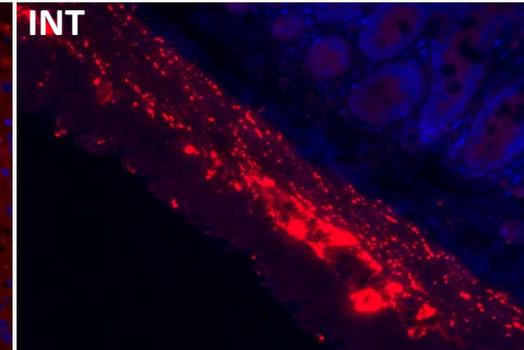
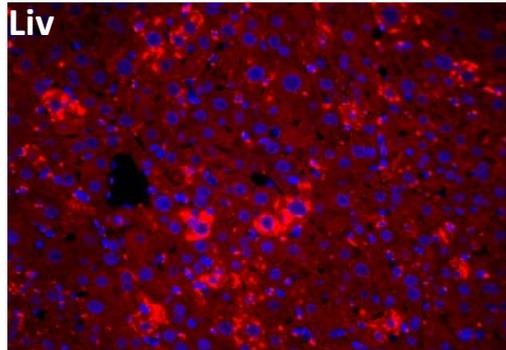
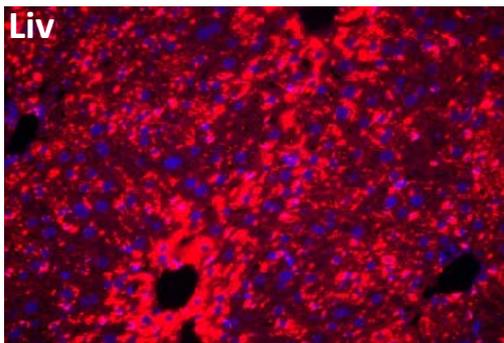


- +/+ = unaffected mice
- -/- = IIIB mice (untreated)
- AAV9-L =  $5 \times 10^{12}$  vg/kg
- AAV9-H =  $1.5 \times 10^{13}$  vg/kg

# IV delivery to MPSIIIB mice demonstrates persistent global CNS, PNS, and widespread somatic transduction

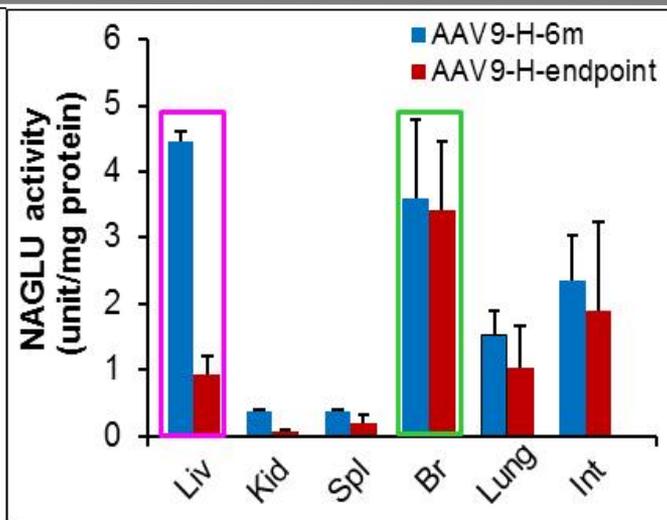
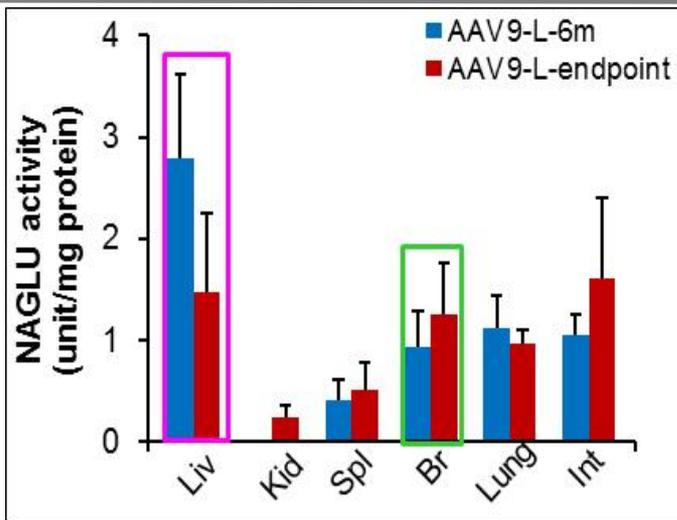
**6m pi**

**Endpoint** (16.1 – 27.2 months)



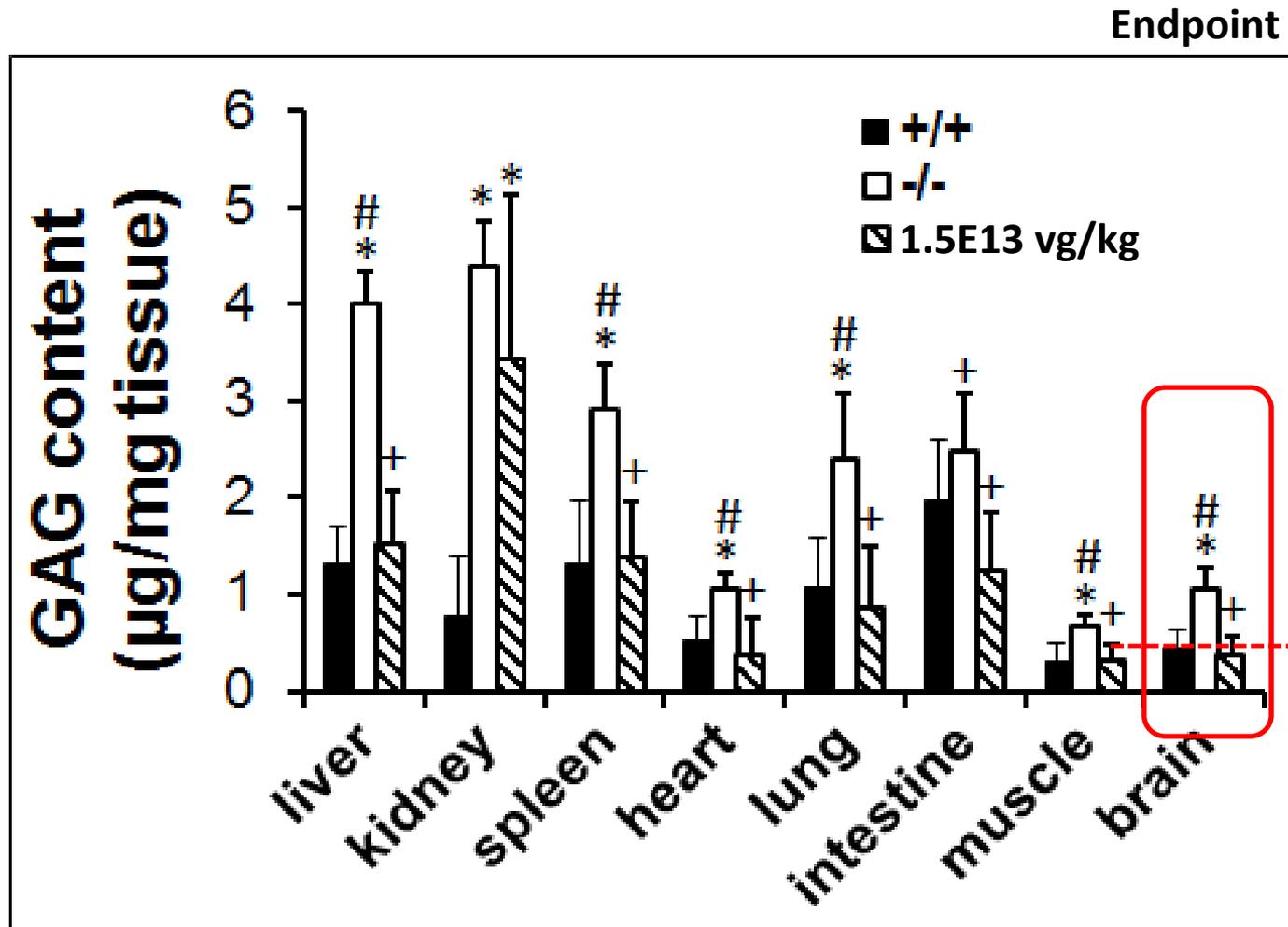
Red: **NAGLU**  
Blue: **DAPI**

$5 \times 10^{12}$   
vg/kg

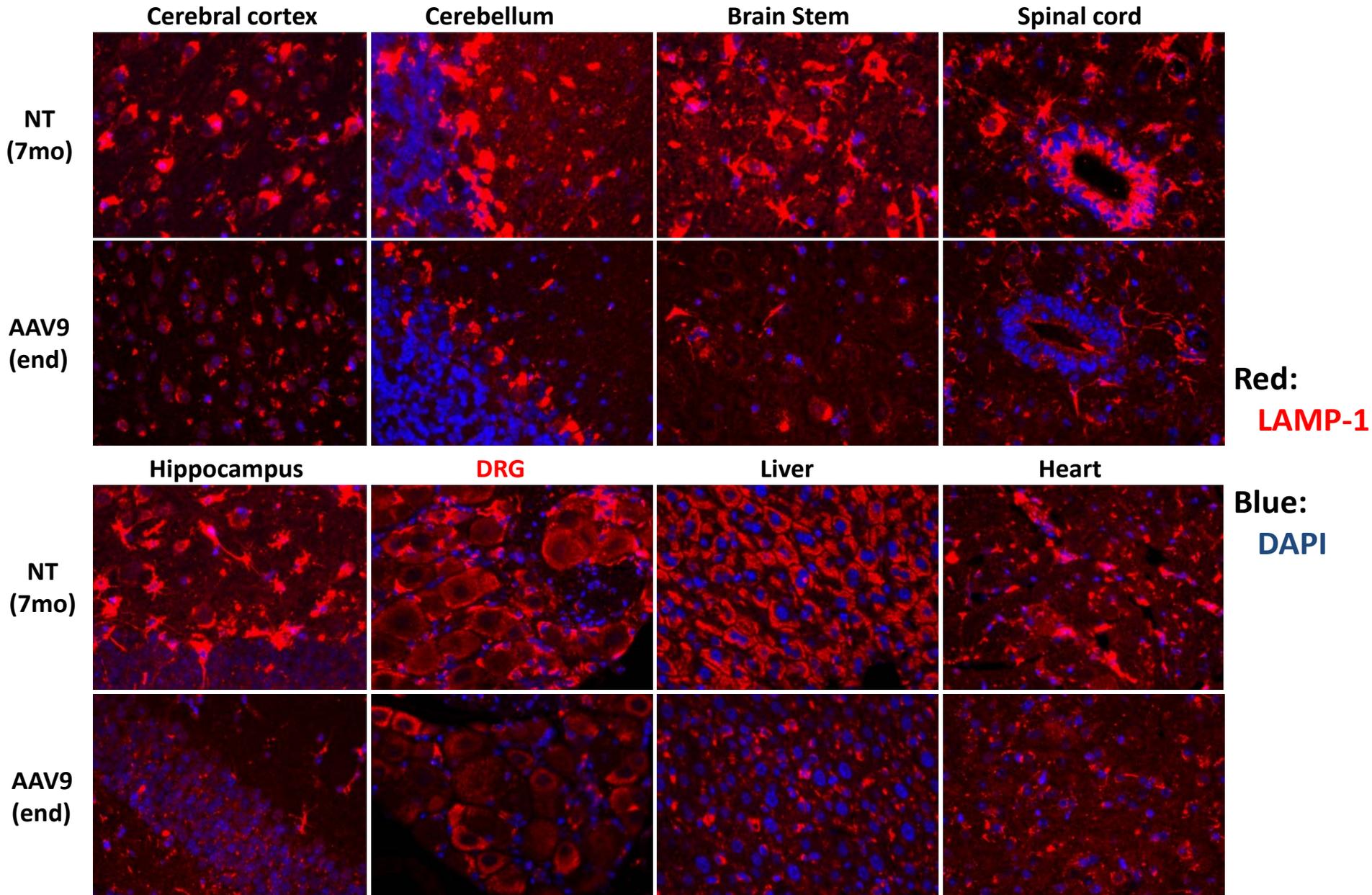


$1.5 \times 10^{13}$   
vg/kg

# IV delivery of rAAV9.CMV.NAGLU induces clearance of lysosomal GAG storage in the CNS and somatic tissues



# Correction of lysosomal storage pathology: reduction of LAMP-1



5 x 10<sup>12</sup> vg/kg; 6 mo post-infusion

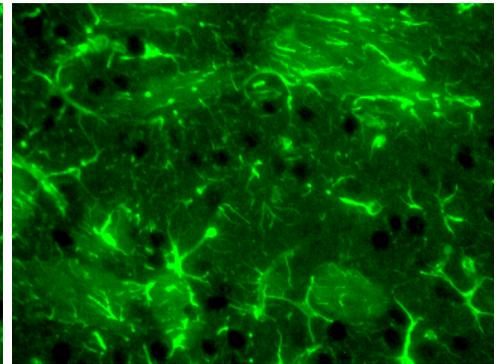
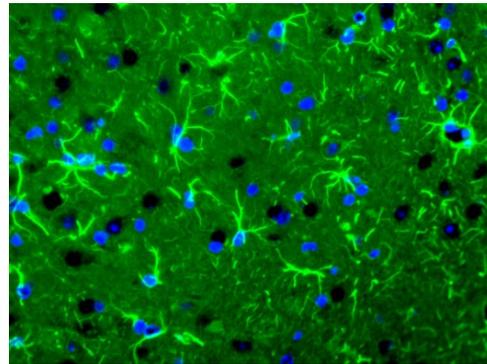
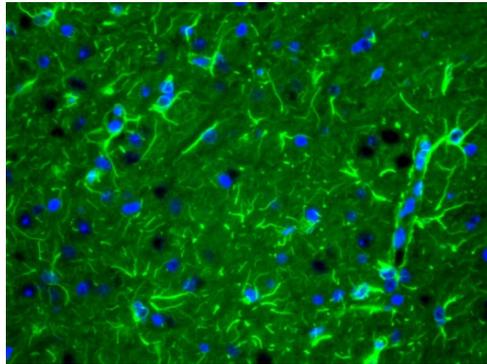
# Correction of secondary neuropathology in IIIB mice: astrocytosis and neurodegeneration

Cerebral cortex

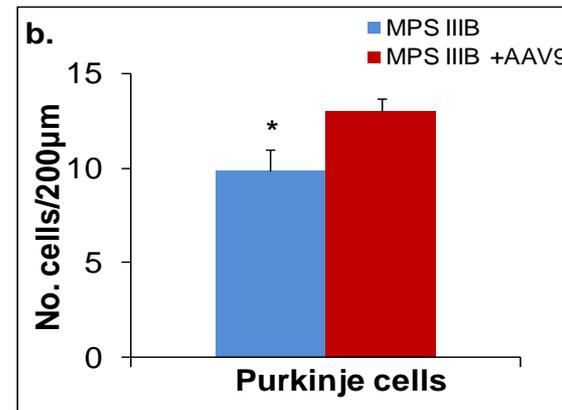
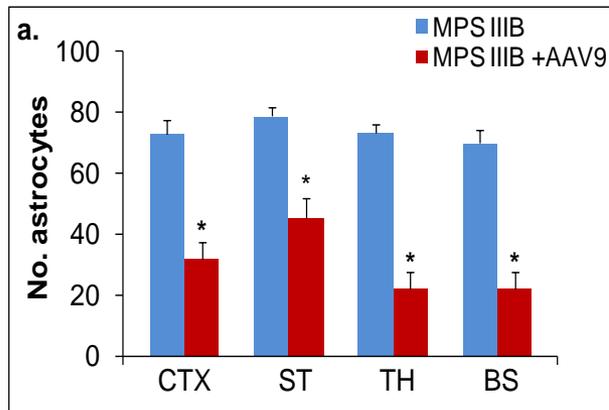
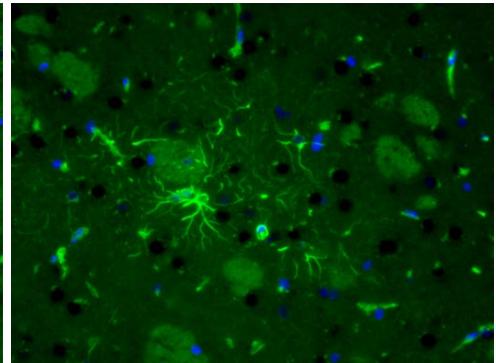
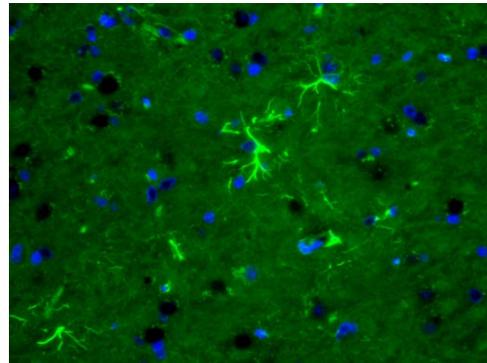
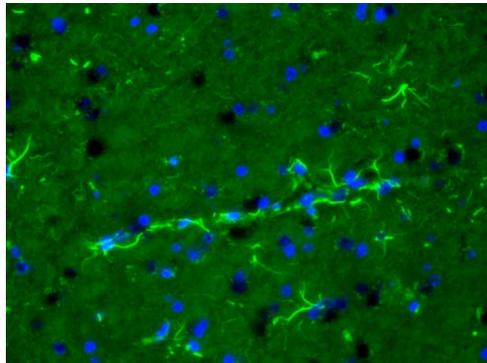
Thalamus

Striatum

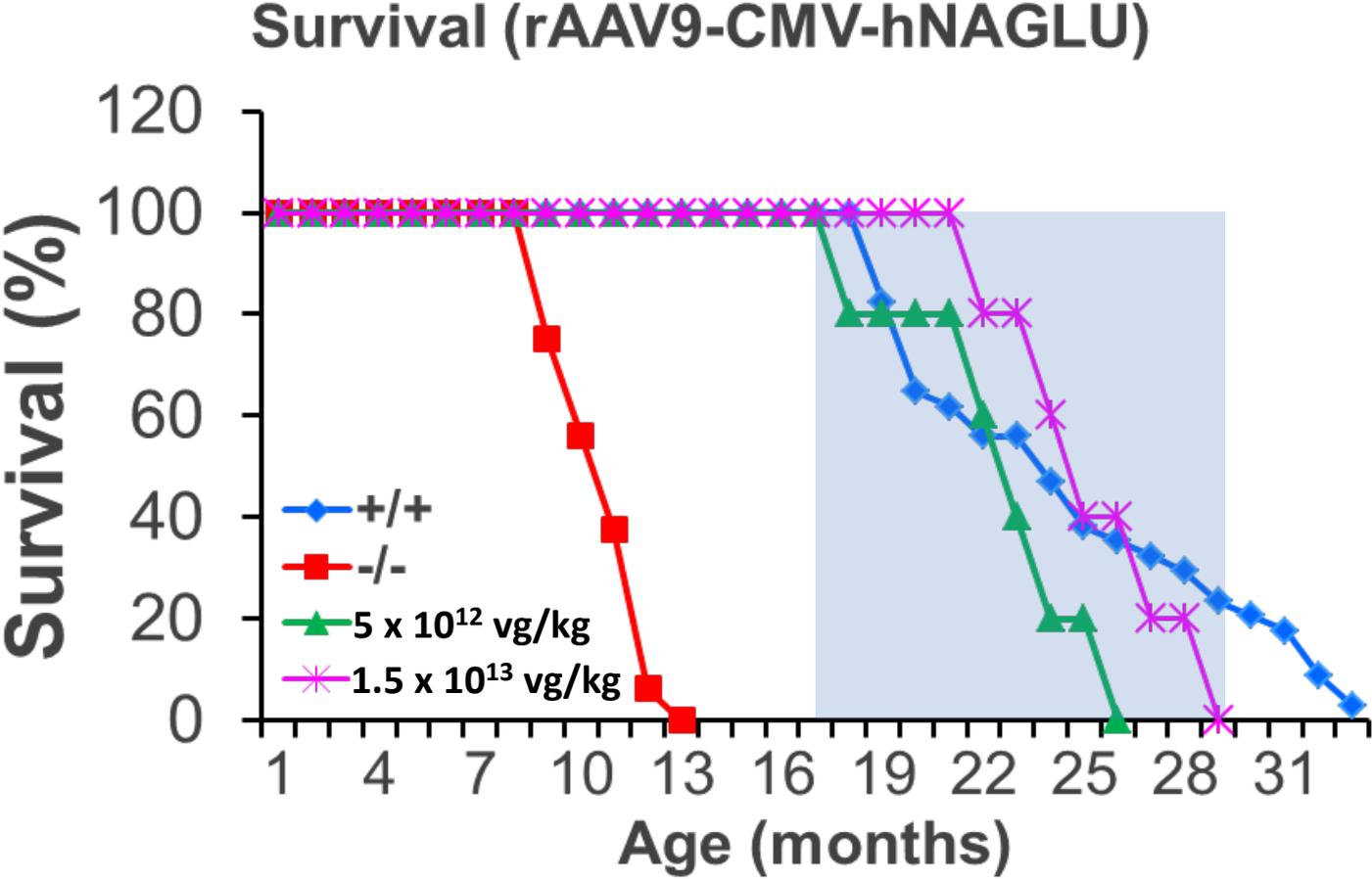
No treatment  
(7month)



$5 \times 10^{12}$  vg/kg  
(Endpoint)

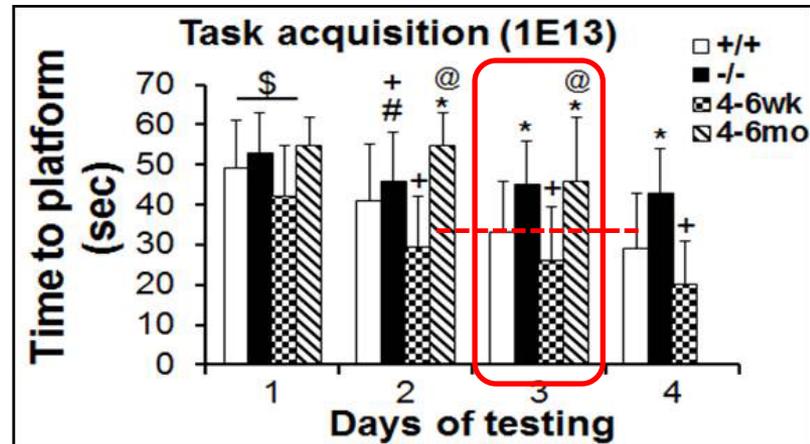


# rAAV9.CMV.hNAGLU vector at 4-6 weeks of age normalizes survival in MPS IIIB mice

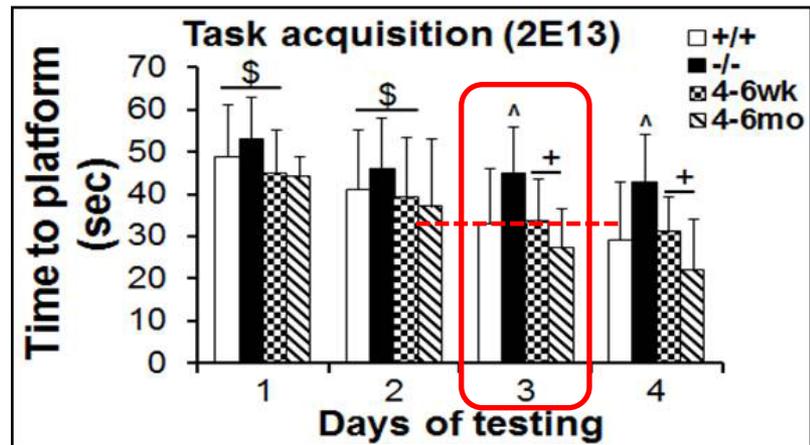


# Higher doses of rAAV9.CMV.hNAGLU are needed to improve motor function in MPS IIB mice treated at older age (4-6 months)

$1 \times 10^{13}$  vg/kg

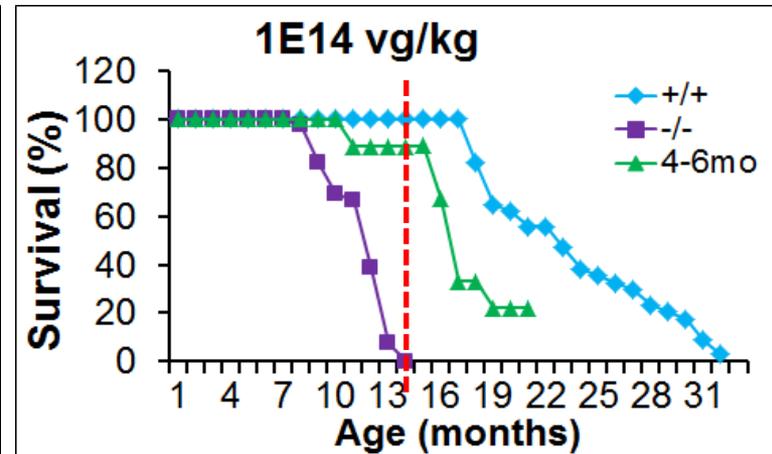
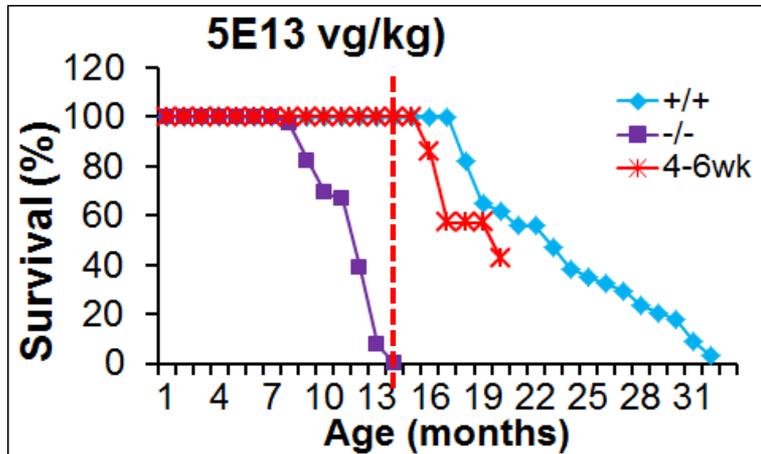
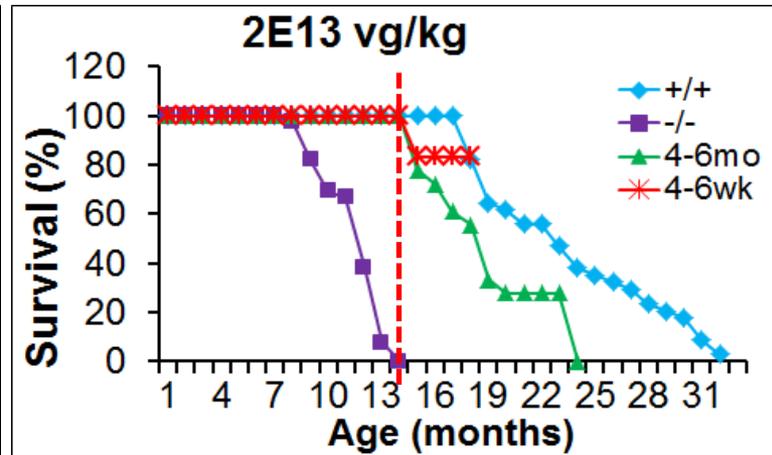
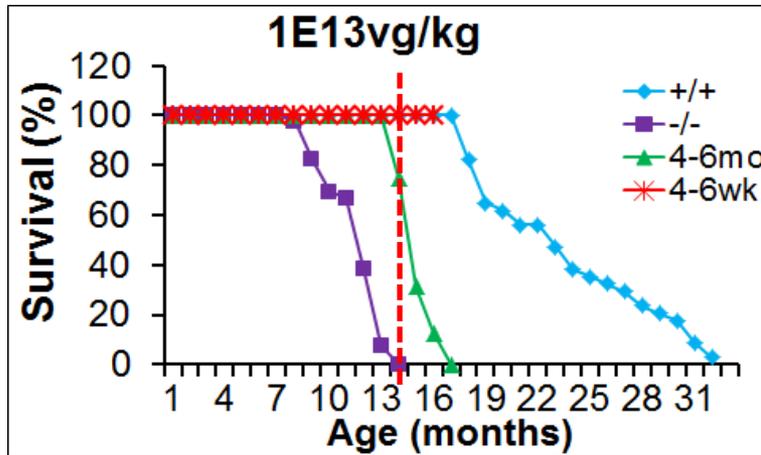


$2 \times 10^{13}$  vg/kg



*This cohort of older mice more likely reflects the typical clinical situation, as patients are typically have significant functional impairment by the time of diagnosis*

# Higher doses of rAAV9.CMV.hNAGLU are needed to increase survival in MPS IIIB mice treated at older age (4-6 months)



Therefore the minimal efficacious dose we have defined – and the lowest proposed dose for our trial – is **2 x 10<sup>13</sup> vg/kg**

# Prolonged expression of NAGLU expression in MPSIIIB mouse brain

**Table 13. rAAV9-mediated rNAGLU expression in MPS IIIB mouse brain**

Vector dose (vg/kg)	AOI	NAGLU activity (% of wt levels)				
		1mo pi	3mo pi	6mo pi	12mo pi	End
<b>1x10<sup>13</sup></b>	4-6wk	60-560%	80-440%	60-312%	44-159%	268-721%
<b>1x10<sup>13</sup></b>	4-6mo	n/a	n/a	n/a	0-40%*	-
<b>2x10<sup>13</sup></b>	4-6wk	40-440%	50-674%	40-440%	55-419%	377-621%
<b>2x10<sup>13</sup></b>	4-6mo	n/a	n/a	n/a	n/a	560-1,549%

- NAGLU expression at or above heterozygote (and often above wild-type)
- Comparison of lifetime exposure at lower levels (as in heterozygotes) versus treated patients may not be relevant – because CNS and somatic tissues have abundant storage of GAGs that have to be cleared

# No evidence that overexpression is harmful

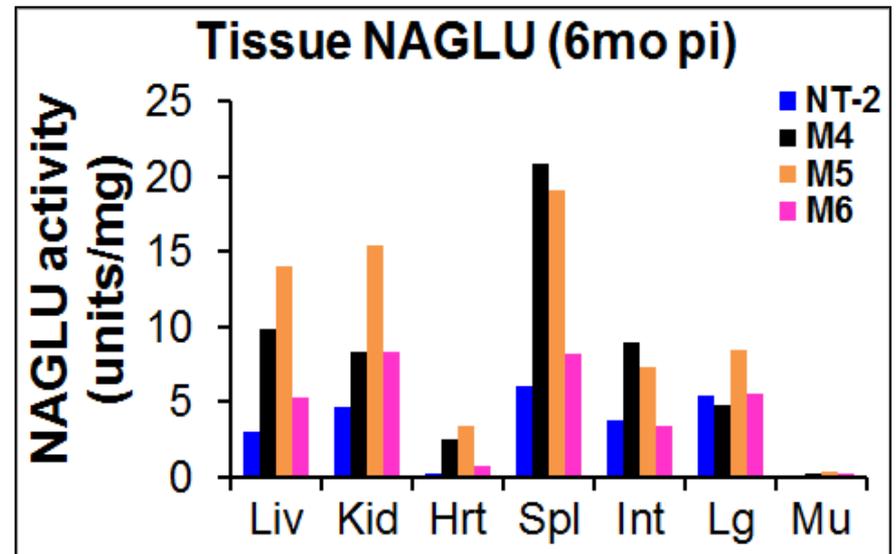
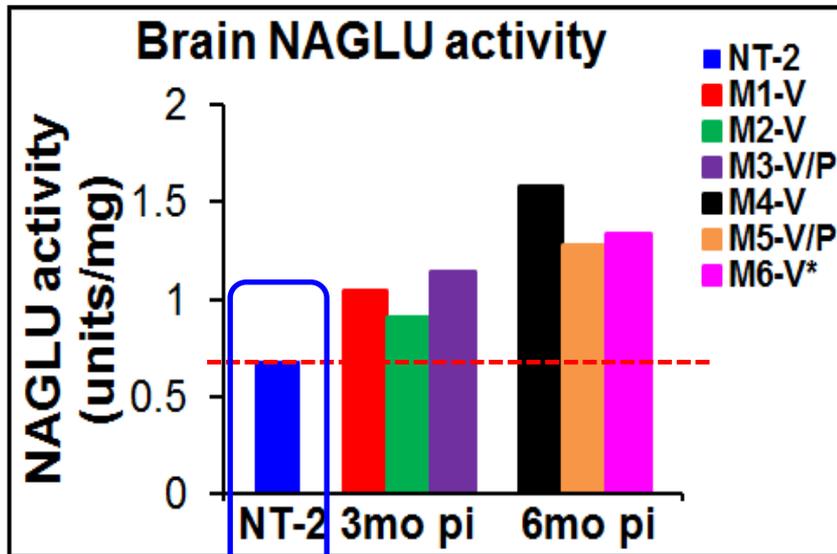
- No abnormalities in hematology or blood chemistry studies in mice and NHP (Figs. 27 and 28)
- Enzyme replacement therapies in similar lysosomal storage diseases result in peak enzyme levels thousands of times above normal without consequences

# Persistence of vector genomes in CNS at 6 mo in NHP

## Biodistribution of rAAV9-CMV-hNAGLU vector in NHP by systemic delivery

NHP	Time (pi)	$10^5$ vg/ $\mu$ g gDNA							
		Liver	Spleen	Muscle	Heart	Lung	Kidney	Intestine	Brain
<b>Group 1 (10 yr old, <math>1 \times 10^{13}</math> vg/kg)</b>									
NT-1	6wk	<0.001	0.006	0.008	<0.001	0.001	0.003	0.003	0.008
S1-V	6wk	3.172	1.307	0.012	0.005	0.015	0.017	0.009	0.012
S2-V	3mo	15.45	1.016	6.105	3.18	0.31	0.069	0.012	0.021
<b>Group 2 (2 yr old, <math>2 \times 10^{13}</math> vg/kg)</b>									
NT-2	3mo	0.002	<0.001	0.003	0.001	0.001	0.001	<0.001	0.002
M1-V	3mo	115.654	1.157	1.321	13.755	0.405	0.105	0.142	0.367
M2-V	3mo	26,070	1.084	1.123	5.435	0.176	0.240	0.136	0.223
M3-VP	3mo	156.750	3.647	4.275	9.996	0.430	0.116	0.116	0.317
M4-V	6mo	157.350	0.158	0.285	10.062	0.060	0.297	10.062	0.149
M5-VP	6mo	47.802	0.120	0.117	1.762	0.077	0.094	0.068	0.185
M6-V*	6mo	0.869	12.195	0.878	0.050	0.015	0.074	0.331	0.063

# Persistence of NAGLU expression in CNS at 6 mo in NHP

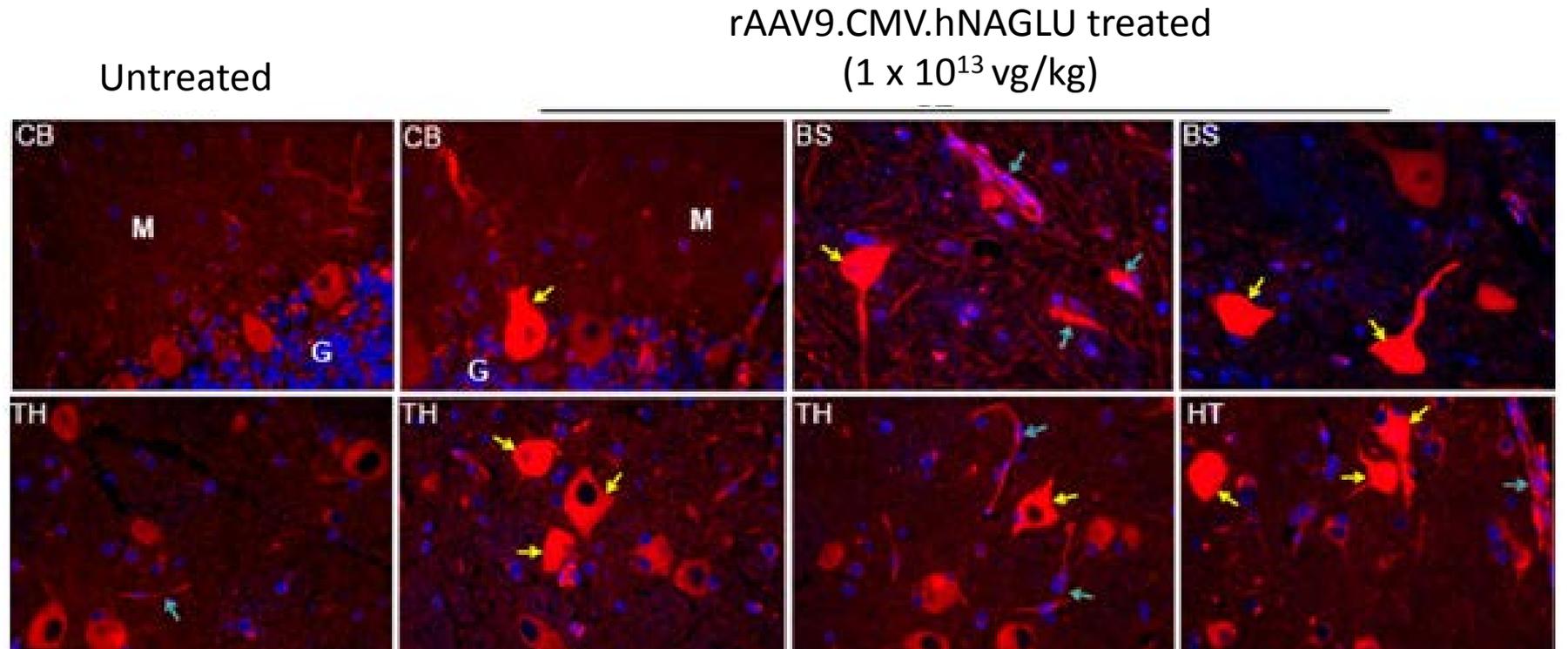


(2 yr old;  $2 \times 10^{13}$  vg/kg)

Untreated, wild type animal

- Brain and somatic NAGLU activity remains supraphysiologic at 6 months despite modest decreases in peripheral serum NAGLU levels

# Persistence of NAGLU expression in CNS at 6 mo in NHP



Red: NAGLU

Blue: DAPI

# No evidence for autoimmune responses

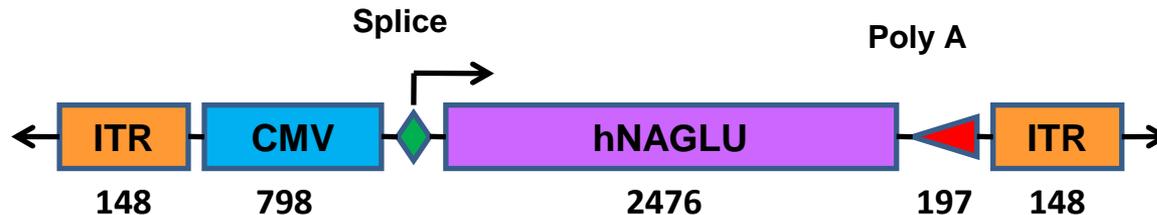
- Antibody responses to human NAGLU were demonstrated in NHP

However:

- No evidence for autoimmune pathology in any tissues at 6 months post-injection
- MPSIIIB mice – null for NAGLU – did not show evidence for clearance of transduced cells over two years despite Ab response
- No CTL responses by ELISpot in NHPs

# Proposed gene transfer trial

An open-label, dose-escalation clinical trial of rAAV9.CMV.hNAGLU injected intravenously through a peripheral limb vein



# Inclusion/Exclusion Criteria

- **Inclusion Criteria**

- Age 2 years old or greater
- Confirmed diagnosis of MPSIIIB by either of two methods:
  - No detectable or significantly reduced NaGlu enzyme activity by plasma, serum, or leukocyte assay.
  - Genomic DNA mutation analysis demonstrating a homozygous or compound heterozygous mutations in the NAGLU gene
- Clinical history of or examination features of neurologic dysfunction

- **Exclusion Criteria**

- Inability to participate in the clinical evaluation
- Presence of a concomitant medical condition that precludes lumbar puncture or use of anesthetics
- Inability to be safely sedated in the opinion of the clinical anesthesiologist
- Pre-existing antibodies to AAV9
- Active viral infection
- Concomitant illness or requirement for chronic drug treatment that in the opinion of the PI creates unnecessary risks for gene transfer.

# Dosing

- Cohort 1: three (3) subjects will receive a single infusion of the **Low Dose:  $2 \times 10^{13}$  vg/kg**
  - Minimum effective dose in older animals (treated at 4-6 months of age) in preclinical studies
  - Because the majority of MPSIIIB patients are diagnosed due to cognitive deficiencies, we believe these mice better model the clinical scenario
  - Considering disease severity and universal outcome, we cannot justify a subtherapeutic cohort.
- Cohort 2: three (3) subjects will receive a single infusion of the **High Dose:  $5 \times 10^{13}$  vg/kg**
  - Essential to exploration of a dose range (intrinsic to a phase I/II trial)
  - Preclinical data supports the safety of this dose

# Outcomes

<b>Primary Outcome</b>	Determination of safety based on the development of toxicity, defined by a grade III or higher event to be brought to the attention of the DSMB prior to approval for any additional dosing.
<b>Secondary Outcomes</b>	<ul style="list-style-type: none"><li>• Improved cognitive ability or stalled cognitive deterioration at 6 months after treatment, as measured by via direct testing of the child using the Leiter International Performance assessment.</li><li>• Parent ratings of adaptive functioning using the Adaptive Behavior Assessment System.</li><li>• Parental ratings of emotional/behavioral problems using the Child Behavioral Checklist</li><li>• Reduced hepatomegaly and splenomegaly at 6 months after treatment, as measured by MRI.</li><li>• Pediatric Quality of Life Inventory 4.0 (PedsQL)</li><li>• CSF and serum NaGlu enzyme activity levels</li></ul>

# Proposed gene transfer trial

- Intravenous infusion of AAV9.NAGLU at two doses (n=3 each)
- Primary outcome: safety
  - Absence of inflammatory response assessed by lumbar puncture at 1 month, 6 months, and 12 months
  - CSF for protein, glucose, cell count and differential
- Assessments at baseline, 6 months, and 12 months:
  - Urine GAG levels
  - Hepatosplenomegaly by abdominal MRI
  - Neurocognitive and parental rating assessments (Leiter, ABAS, Child Behavioral Checklist)
  - Timed functional motor tests
  - Standard laboratory assessments
  - Serum and CSF NAGLU activity
  - Brain MRI (including DTI and  $^1\text{H}$  spectroscopy)

# Study procedure summary

Study Interval	Baseline Screening	Vector Infusion (Inpatient)			Follow Up (Outpatient)						
	Visit	1	2			3	4	5	6	7	8
Days in Study	-30 to -1	0	1	2	7	30	90	180	360	540	720
Informed Consent	X										
Medical History	X	X	X	X	X	X	X	X	X	X	X
Physical Exam + Vitals	X	X	X	X	X	X	X	X	X	X	X
Safety Labs (Blood & Urinalysis)	X		X	X	X	X	X	X	X	X	X
Research Blood Labs	X				X	X	X	X	X	X	X
Neurocognitive Exam	X						X	X	X	X	X
Echocardiogram	X						X	X	X	X	X
Abdominal MRI	X							X	X		
Timed Functional Tests	X						X	X	X	X	X
Lumbar Puncture	X						X	X	X		
Brain MRI	X							X	X		
Gene Transfer		X									
Adverse Events	Any adverse events must be discussed and reviewed at every visit										
Review of Current Medications	A list of current medications must be collected at every visit										

# Summary

- MPSIIIB is a disease with a devastating impact on patients and families
- No treatment is currently available
- AAV9 has undergone significant preclinical testing and is entering clinical trials for another CNS disease (SMA; RAC Protocol 1210-1188)
- We believe a phase 1 safety trial is supported by the preclinical data



**NATIONWIDE  
CHILDREN'S**

*When your child needs a hospital, everything matters.™*

**Haiyan Fu, PhD**

**Doug McCarty, PhD**

K. Reed Clark, PhD

Tim Miller, PhD

Jerry Mendell, MD

Chris Shilling

Kim McBride, MD

Kristin Bain, MD

Keith Yeates, PhD

Marco Corridore, MD

Nick Zumberge, MD

William Sheils, MD

Krista Kunkler

Susan Gailey

**NIH/NINDS (U01NS069626)**

**The Sanfilippo Children's  
Research Foundation**



**Ben's Dream**



**THE CHILDREN'S  
MEDICAL RESEARCH  
FOUNDATION, INC.®**

*A Cure for Kirby*



**NATIONAL INSTITUTE OF  
NEUROLOGICAL  
DISORDERS AND STROKE**