Case Study: Animal Model Translation to Human Application

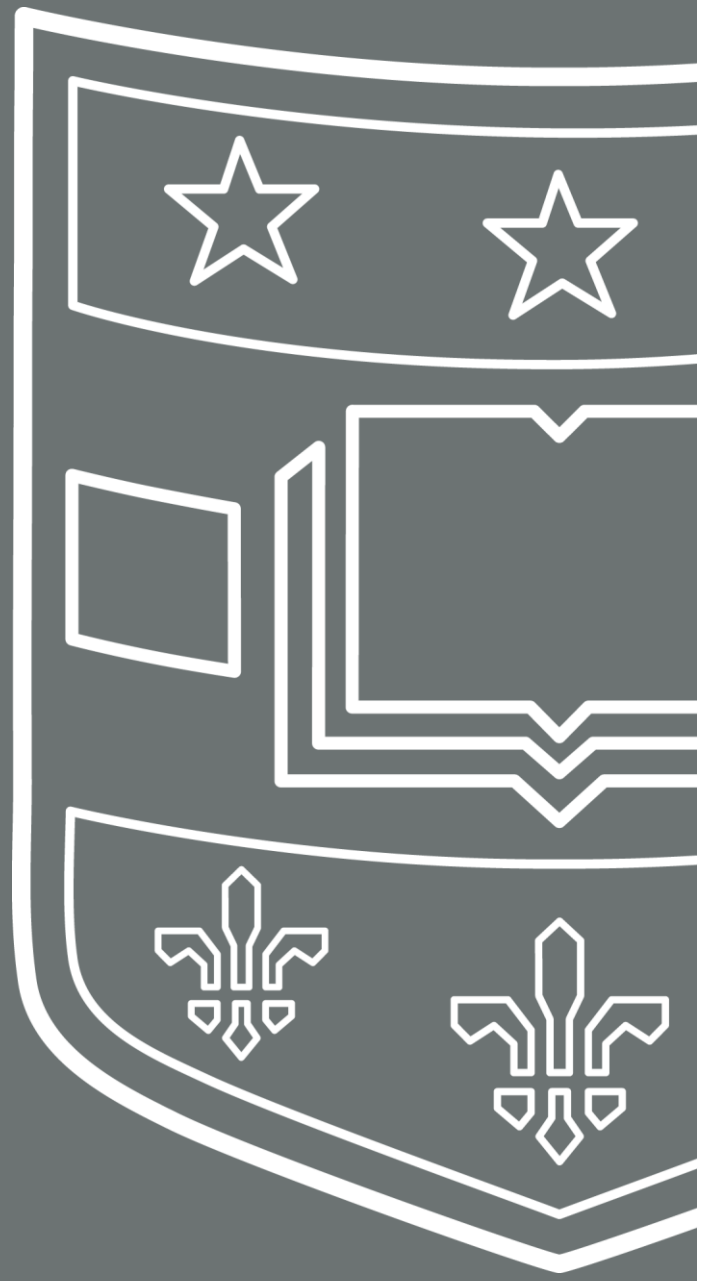
- **Nidal Boulos, PhD**, REGENXBIO, Inc.
- **Patricia Dickson, MD**, Washington Univ. School of Medicine, St. Louis
- **Matthew Ellinwood, DVM, PhD**, National MPS Society

REAGAN-UDALL FOUNDATION FOR THE FDA

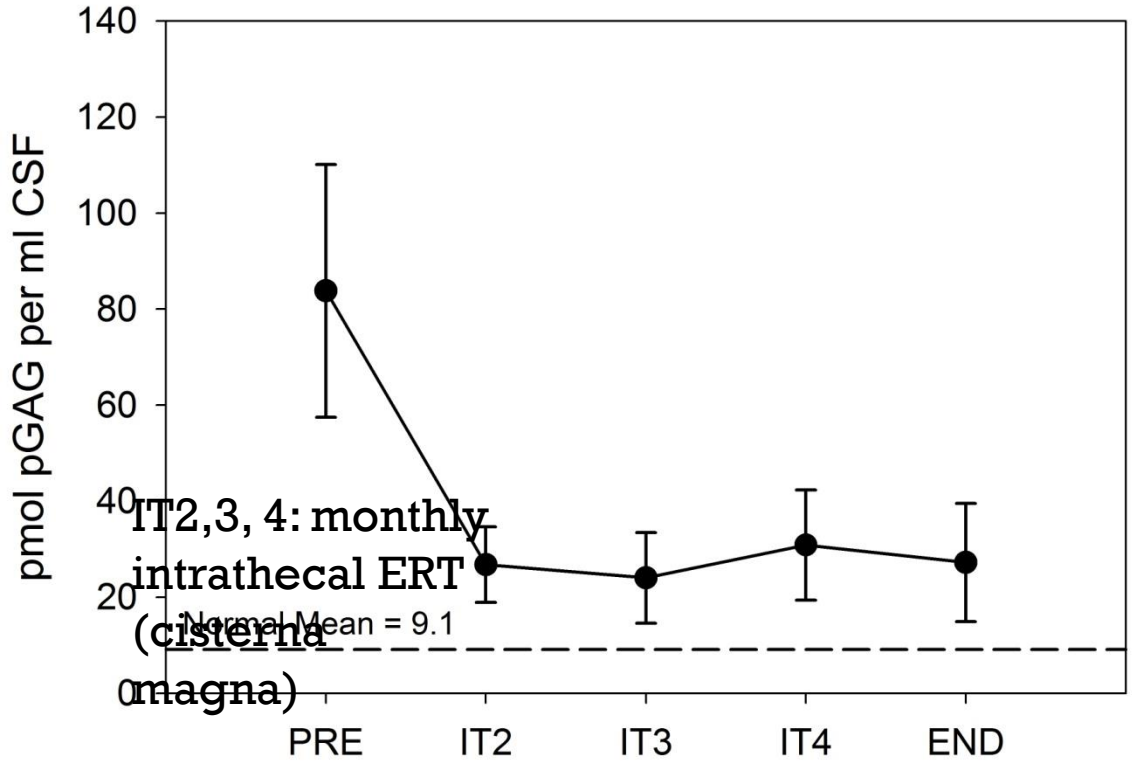
Membrane-tethered NAGLU to explore origins of CSF heparan sulfate

Patricia Dickson, MD

Washington University School of Medicine in St. Louis



Intrathecal enzyme replacement therapy reduces heparan sulfate glycosaminoglycans in CSF in MPS I dogs

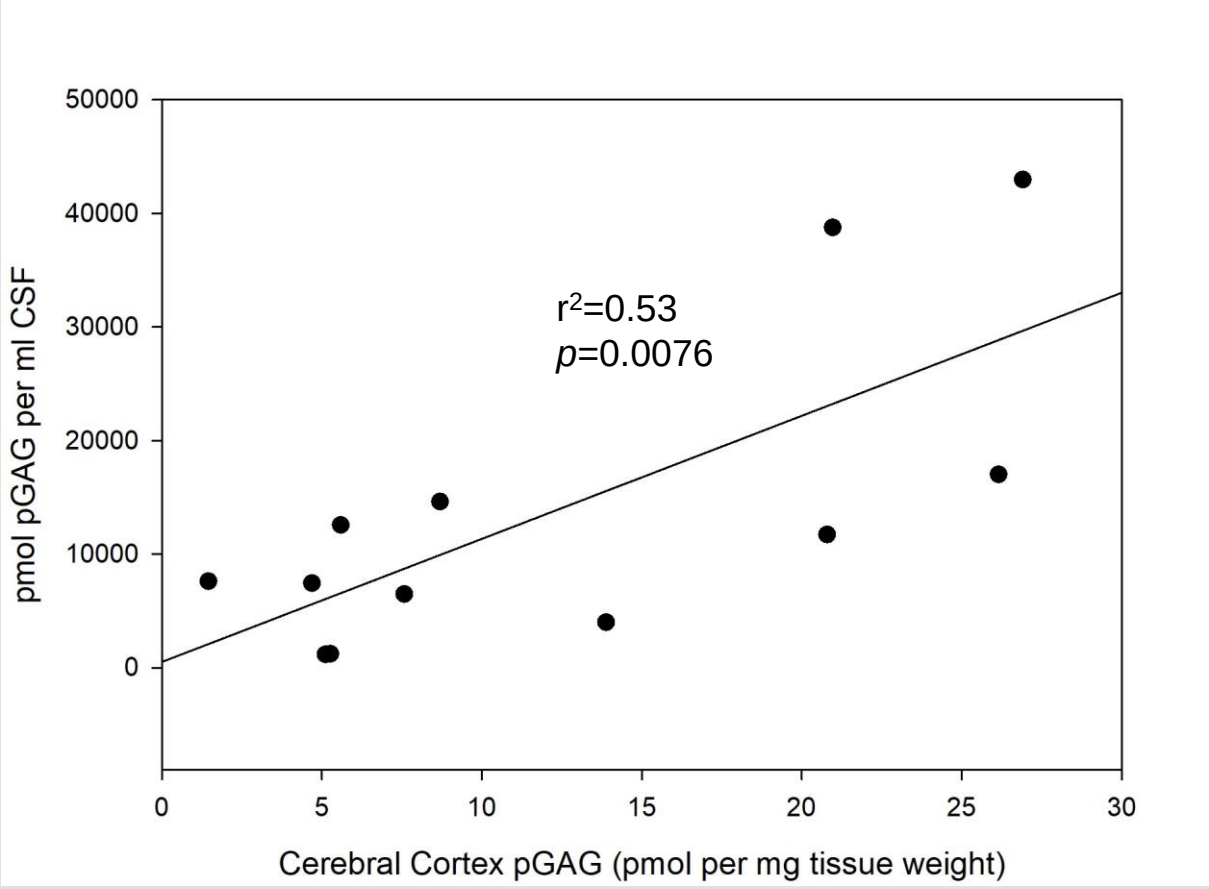


IT2,3, 4: monthly intrathecal ERT (cisterna magna)

pGAG = "pathologic GAG"
A previous term for specific GAG measured by the non-reducing end (NRE) method. The method used here measured HS only. GAG were purified and digested with heparin lyases and labeled for NRE, then measured by HPLC.

Dickson et al, Mol Genet Metab 2012

Heparan sulfate GAG in CSF correlates with heparan sulfate GAG in brain in MPS I dogs



pGAG = "pathologic GAG"
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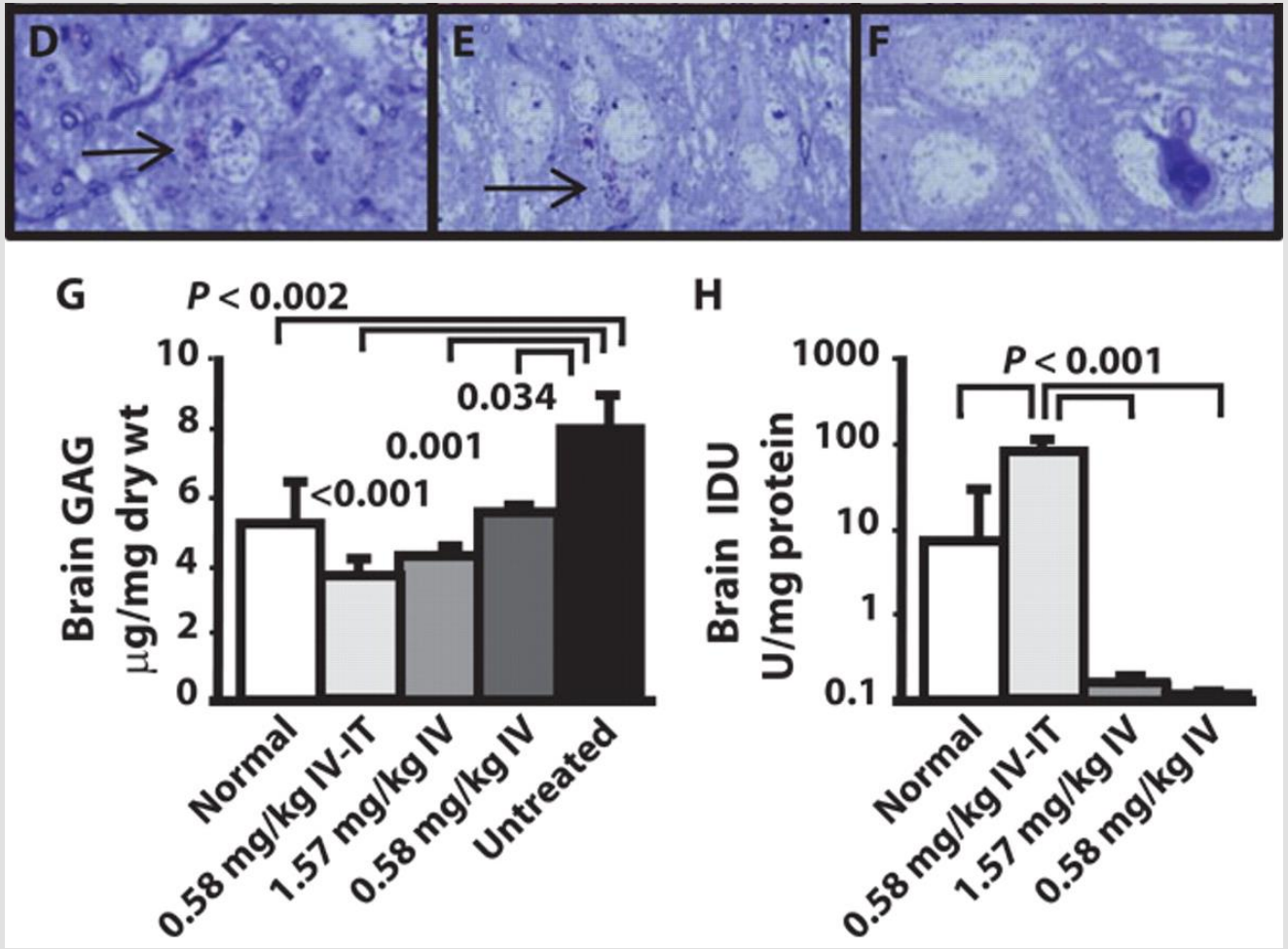
Unpublished. Data are from Dickson et al Mol Genet Metab 2012

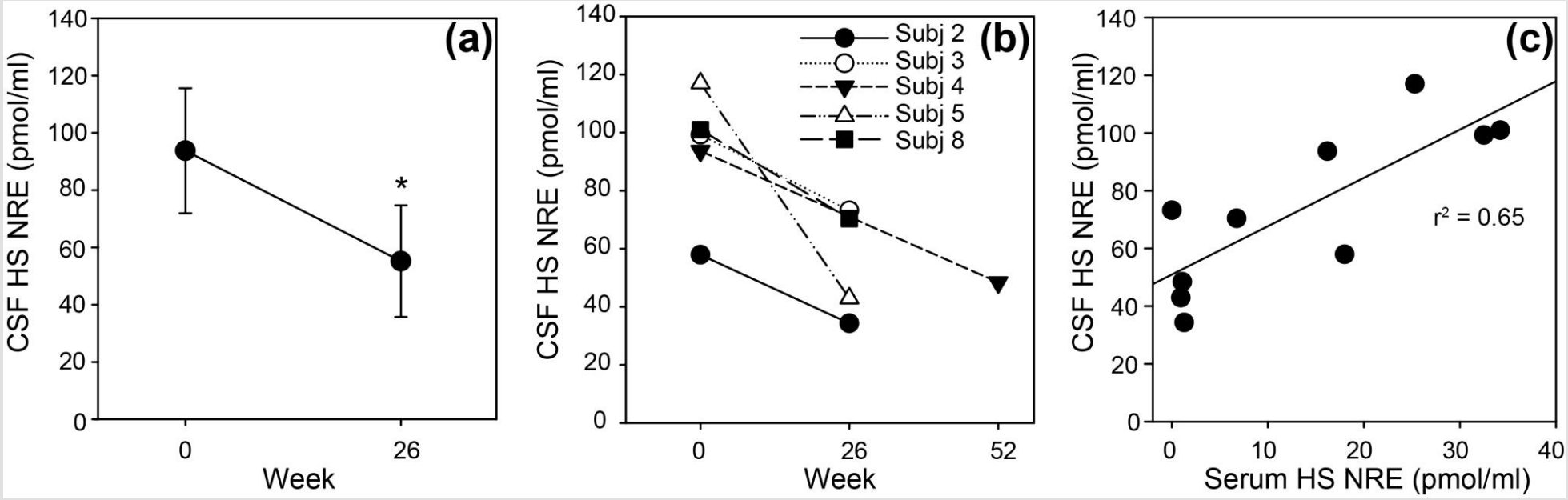
Intravenous enzyme replacement therapy reduces GAG and storage in brain of MPS I dogs



GAG was measured with dye-binding assay

Dierenfeld et al, Sci Transl Med 2010

Intravenous enzyme replacement therapy reduces CSF heparan sulfate in MPS I *patients*



GAG were purified and digested with heparin lyases (only cleaves HS) and labeled for NRE, then measured by HPLC.

Vera et al, Mol Genet Metab 2020

Hypothesis 1: CSF heparan sulfate originates from brain.
Implies that intravenous enzyme therapy *does* cross the blood brain barrier, at least in MPS I.

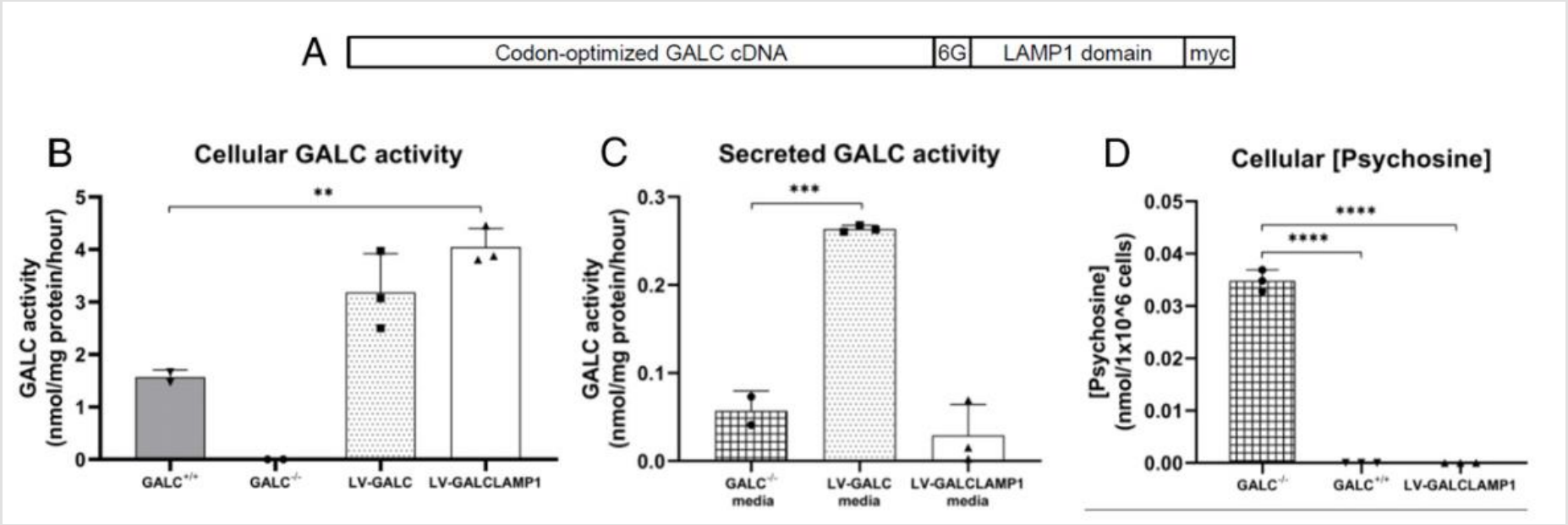
Hypothesis 2: CSF heparan sulfate does not reflect brain.
Could CSF heparan sulfate originate in the bloodstream?

Problem: How do we test these hypotheses?

Cell-autonomous expression of the acid hydrolase galactocerebrosidase

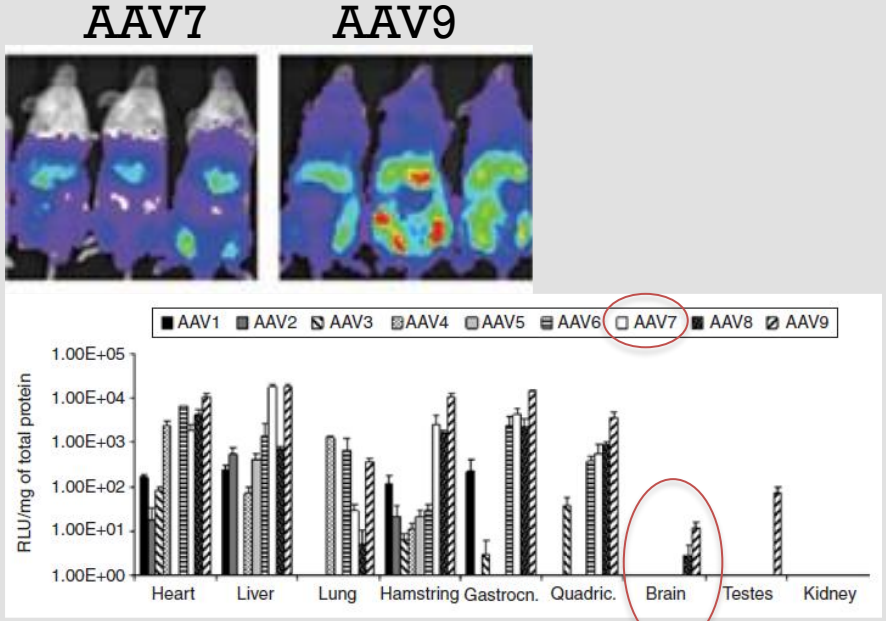
Christina R. Mikulka[a], Joshua T. Dearborn[a], Bruno A. Benitez[b], Amy Strickland[c], Lin Liu[a,1], Jeffrey Milbrandt[c], and Mark S. Sands[a,c,2]

[a]Department of Medicine, Washington University School of Medicine, St. Louis, MO 63110; [b]Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110; and [c]Department of Genetics, Washington University School of Medicine, St. Louis, MO 63110

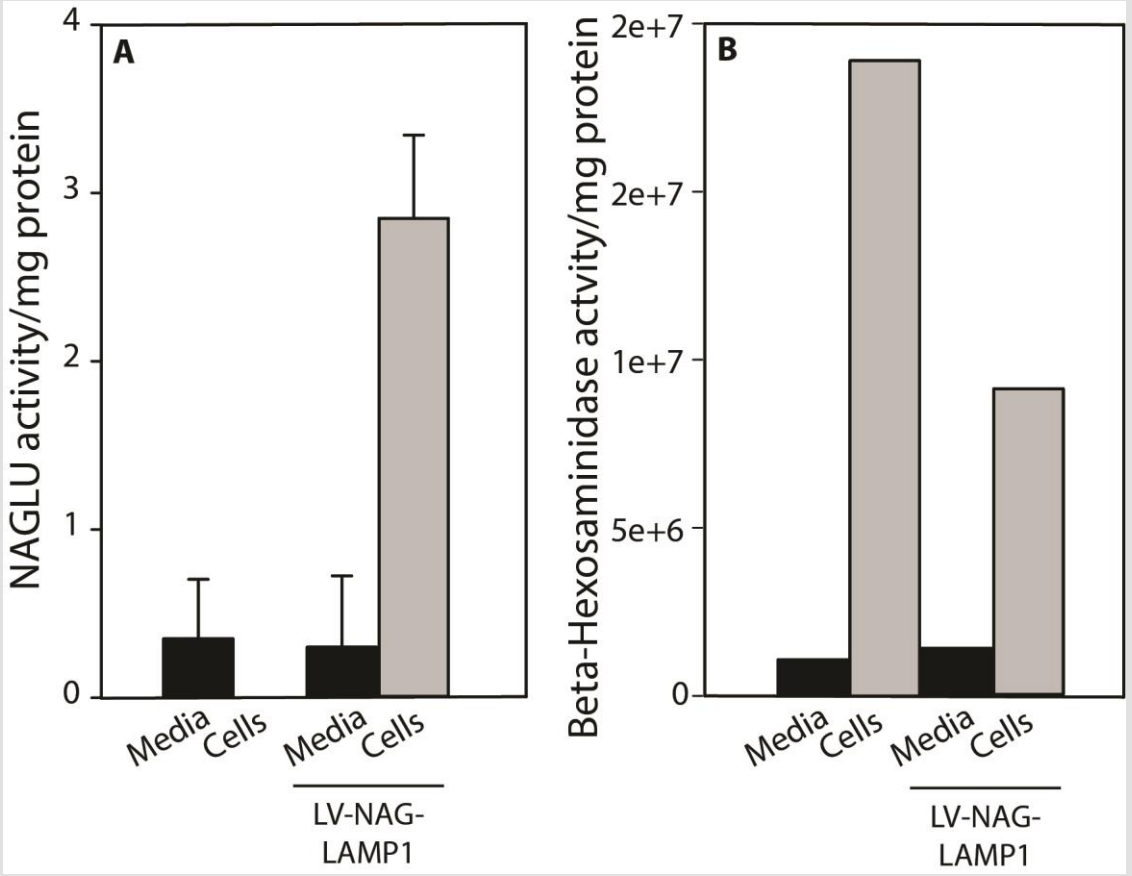


Mikulka et al, PNAS 2020

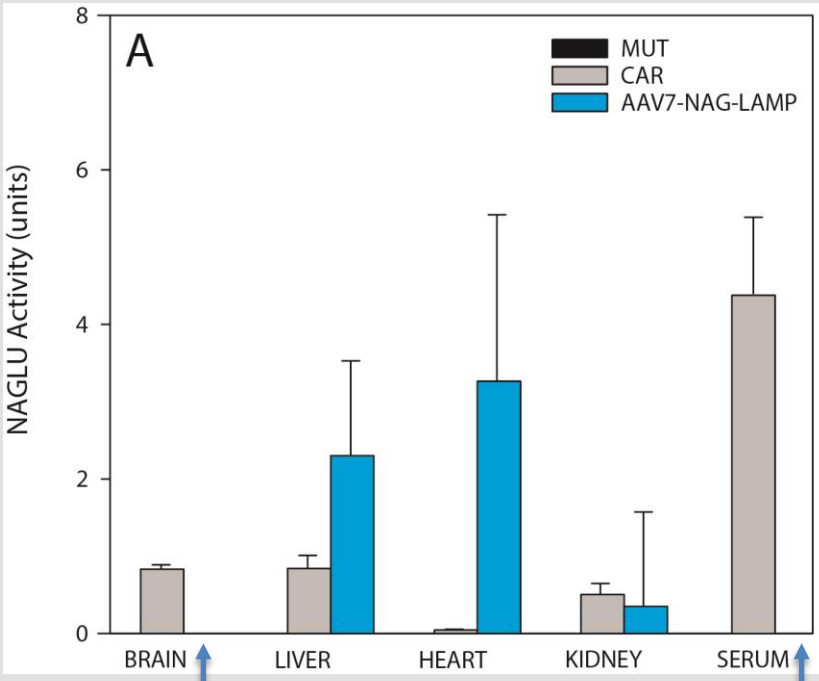
Membrane-tethered NAGLU

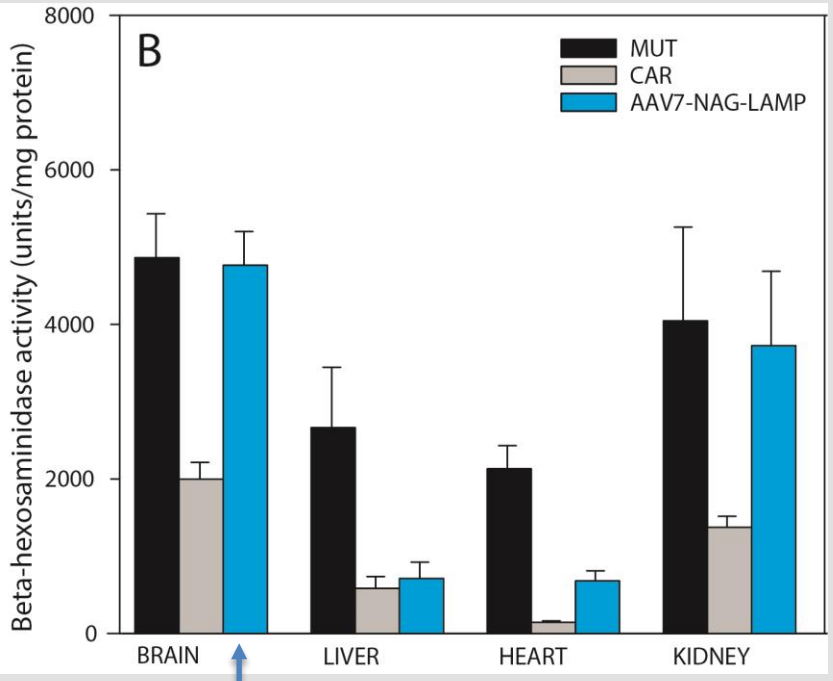


From Zincarelli et al, Mol Ther 2008 (not our work)



Intravenous AAV7 NAGLU-LAMP1: isolated systemic treatment





No NAGLU activity in brain

No NAGLU activity in serum

No treatment effect in brain

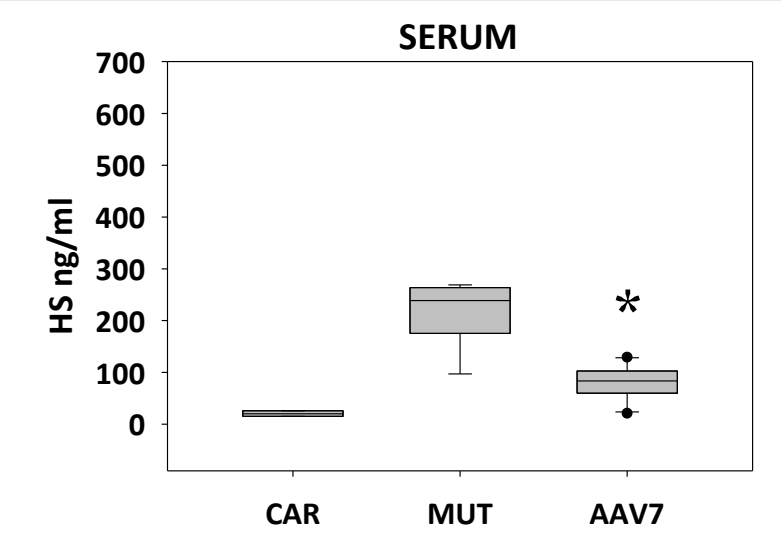
Sanfilippo B (Naglu-/-) mice received $1.5x10^{11}$ vg/mouse IV AAV7 NAGLU-LAMP1 (CBA promoter) and were compared to untreated affected and carrier mice (n=10-12 per group).

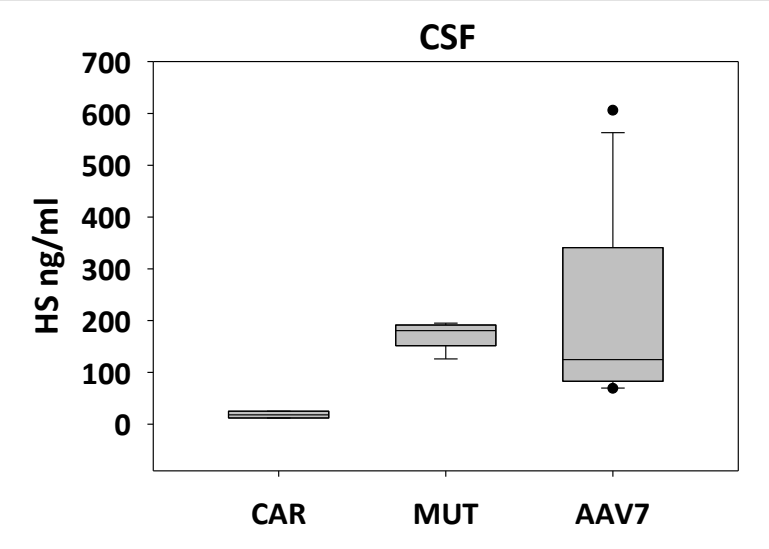
Treated at age 4 weeks
Studied at age 8 weeks

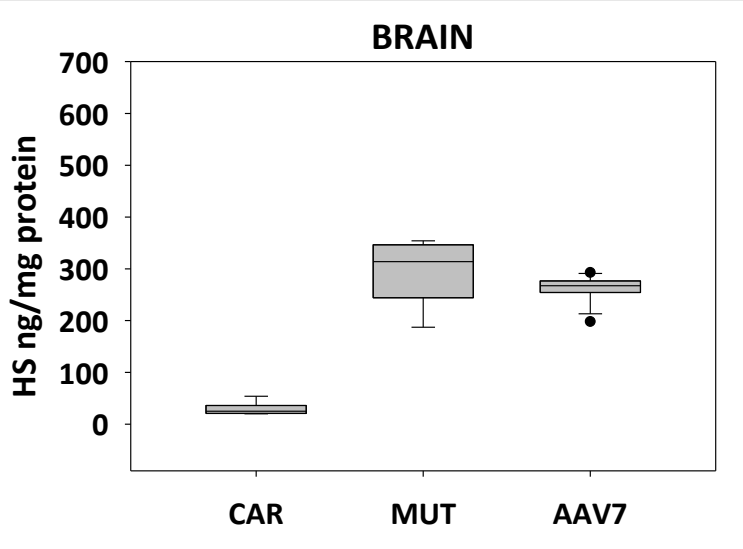
Steven Le, abstract WORLD 2023

Reduction in serum HS was not accompanied by reduction in brain or CSF HS

in Sanfilippo B mice treated with IV AAV7 NAGLU-LAMP1





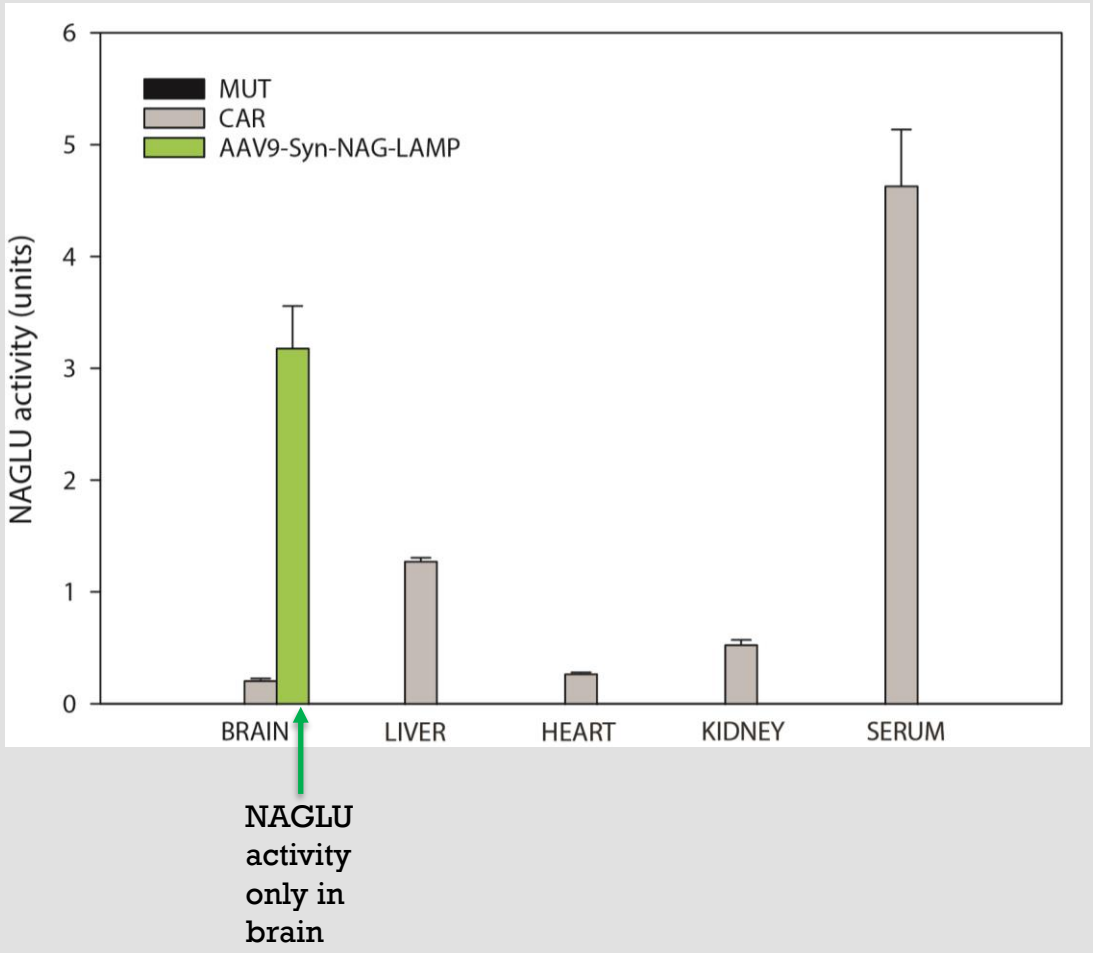


	Female	Male
CAR	5	5
MUT	5	5
AAV7	8	4

Total HS was measured by mass spectrometry (GRIL LC-MS) at the UCSD GlycoAnalytics Core. GAG were purified, digested with heparinases, tagged with $^{12}C_6$-aniline, and analyzed by LC-MS in negative ionization mode.

Steven Le, abstract WORLD 2023

Intraventricular AAV9 Syn-NAGLU-LAMP1: isolated CNS treatment

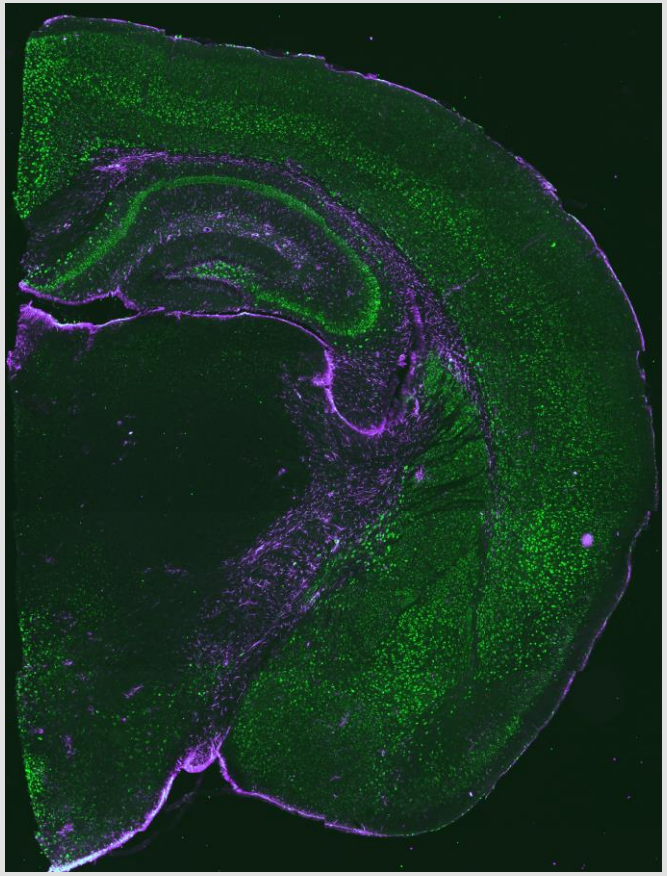


We designed an AAV9 viral vector with NAGLU-LAMP1 under a Synapsin-1 promotor to express NAGLU in neurons and delivered 6.5E+10 vg/mouse ICV to Sanfilippo B (Naglu-/-) mice in order to confine NAGLU restoration to the brain.

Treated at PND 1 or 2
Studied at age 4 weeks

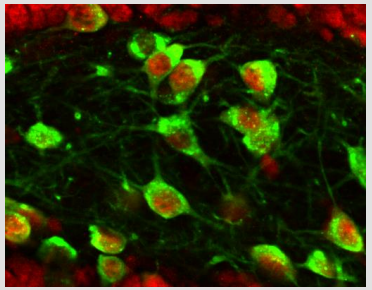
Steven Le, abstract WORLD 2024

Intracerebroventricular AAV9 Syn-NAGLU-LAMP1 is expressed in neocortical neurons



Green: NAGLU; Magenta: GFAP; Cyan: CD68

Intracerebroventricular AAV9 Syn-NAGLU-LAMP1 distributed widely in neonatal mice



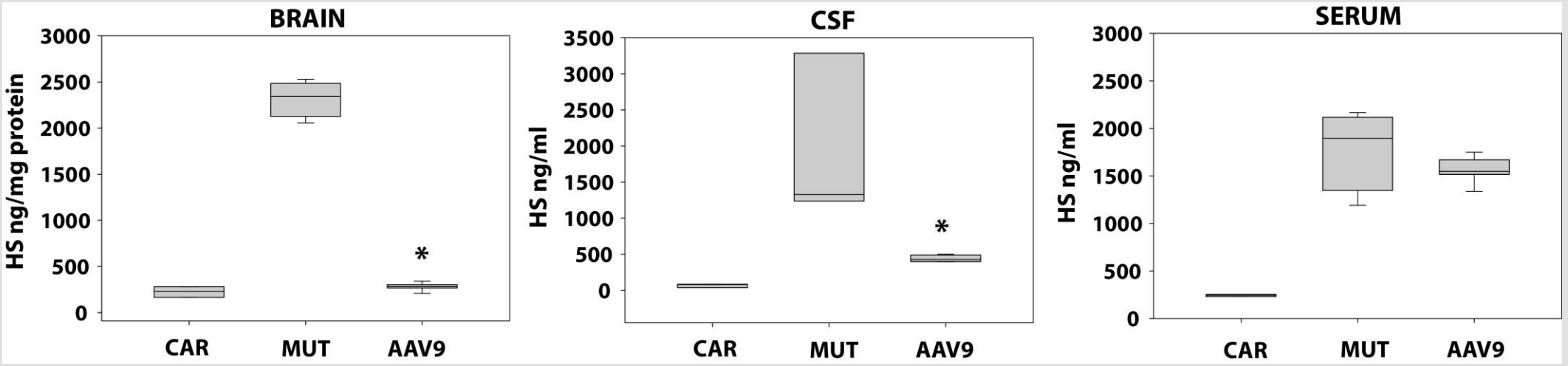
Green: NAGLU
Red: NeuN

Immunofluorescence showed expression of NAGLU-LAMP1 in brain neurons (NeuN) but not in microglia (CD68) or astrocytes (GFAP)

Steven Le, abstract WORLD 2024

Restoring NAGLU in brain neurons reduces CSF HS without reduction in serum HS

in Sanfilippo B mice treated with ICV AAV9-Syn-NAGLU-LAMP1



	Female	Male
CAR	1	2
MUT	1	3
AAV9	1	7

Total HS was measured by mass spectrometry (GRIL LC-MS) at the UCSD GlycoAnalytics Core. GAG were purified, digested with heparinases, tagged with $^{12}C_6$-aniline, and analyzed by LC-MS in negative ionization mode.

Steven Le, abstract, WORLD 2024

Summary

- Intravenous AAV7 NAGLU-LAMP1
  - Delivered systemically with a vector that does not cross the BBB
  - NAGLU activity in liver, heart, kidney, but not serum or brain
  - HS reduced in serum but not CSF or brain
- Intracerebroventricular AAV9 Syn-NAGLU-LAMP1
  - Delivered to the brain and expressed in neurons
  - NAGLU activity in brain but not liver, heart, kidney or serum
  - HS reduced in brain and CSF but not serum

Acknowledgements

- Steven Le
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- Marie Roberts Nuñez
- Mark Sands
- Jonathan D. Cooper

Biswa Choudhury,

University of California San Diego
GlycoAnalytics Core

- Washington University Institute of Clinical and Translational Sciences “Just-In-Time” grant (NIH/NCATS UL1 TR002345)
- Hope Center Viral Vectors Core

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Support for Families, Research for a Cure

Cerebrospinal Fluid Heparan Sulfate: MPS IIIB Dogs Treated with Brain Directed Therapy

Qualifying Biomarkers to Support Rare Disease Regulatory Pathways

February 21, 2024, Reagan-Udall Foundation

N. Matthew Ellinwood, DVM, PhD

CSO, National MPS Society

Professor Emeritus, Iowa State University

Disclosures

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- No personal conflict of interests to disclose
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  - Association of Public Health Laboratories
  - Denali Therapeutics
  - EdiGene Biotechnology USA
  - EveryLife Foundation for Rare Diseases
  - Global Genes (Rare Drug Dev. Symp.)
  - Guidepoint Global, LLC
  - PRECISIONadvisors
  - REGENXBIO, Inc.
  - Terrapin (World Orphan Drug Cong-USA)
  - WORLD*Symposium*™

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Need for Translation Pre-clinical Models in MPS IIIB

- Challenges of ultra rare genetic pediatric neurodegenerative disease
  - Incidence
    - Spinal muscular atrophy (1:12,000 births) versus MPS IIIB (~1:100,000 births)
  - Diagnostic delay yields a patient population with established clinical disease
    - Treating of clinical stage patients is likely associated with complex cognitive-endpoint variability
  - Without therapy, public health newborn screening can't identify pre-clinical patients
    - Even with preclinical treatments, patients may require very long trial periods to reach endpoint
- Moderately progressive neuropathic disease manifesting as developmental delay and cognitive dysfunction
  - Age at onset and course of disease complicates unified systems to evaluate cognitive outcomes
- Ultra rare status precludes large and efficient enrollment to power conventional clinical trial approaches

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Animal Models of Neuropathic MPSs

- Spontaneous models
  - Bovine MPS IIIB
  - Canine MPS I, IIIA, IIIB, and VII
  - Caprine MPS IIID
  - Emu MPS IIIB
  - Feline MPS I and VII
  - Murine MPS IIIA and VII
  - Swine MPS IIIB
- Genetically engineered
  - Murine MPS I, II, IIIA, IIIB, IIIC, and IIID
- Conclusion
  - All models share consistent homologous genetic, enzymatic, and pathological findings of intra-lysosomal HS accumulation and neuropathology
  - Lysosomal storage of HS and neuropathology is conserved across 640 million years of evolutionary time
    - Divergence of sauropsids (ancestral to aves) and therapsids (ancestral to mammalia) 320 million years ago

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Canine Models of MPS III

- Canine MPS III models
  - Canine models of human MPS III are spontaneous
    - Overt clinical disease leads to clinical and model characterization
      - MPS IIIA dachshund model
      - MPS IIIA hunt away model
      - MPS IIIB schipperke model
  - All forms present with an early adult onset of cerebellar ataxia
    - Canine MPS III models are severe forms of canine MPS III despite early adult onset
    - All three models manifest similar clinical signs and time course
- Canine MPS IIIB characterized at the pathologic and molecular level
  - Ellinwood et al., J Inherit Metab Dis. doi: 10.1023/a:1025177411938
  - Egeland et al., Sci Rep. 2020. doi: 10.1038/s41598-020-77032-y
  - Raj et al., Sci Rep. 2020. doi: 10.1038/s41598-020-60121-3
  - Harm et al., Vet Pathol. 2021. doi: 10.1177/0300985820960128

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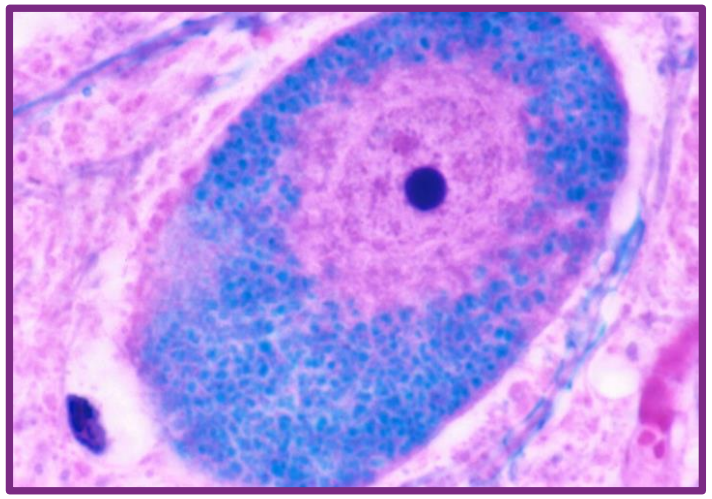
Clinical Manifestations of Canine MPS IIIB

- Severe cerebellar ataxia
  - Onset at 24-30 months of age
  - Hind and forelimb hypermetria and dysmetria
  - Truncal swaying
  - Postural instability
  - Positive cerebellar rebound reflex

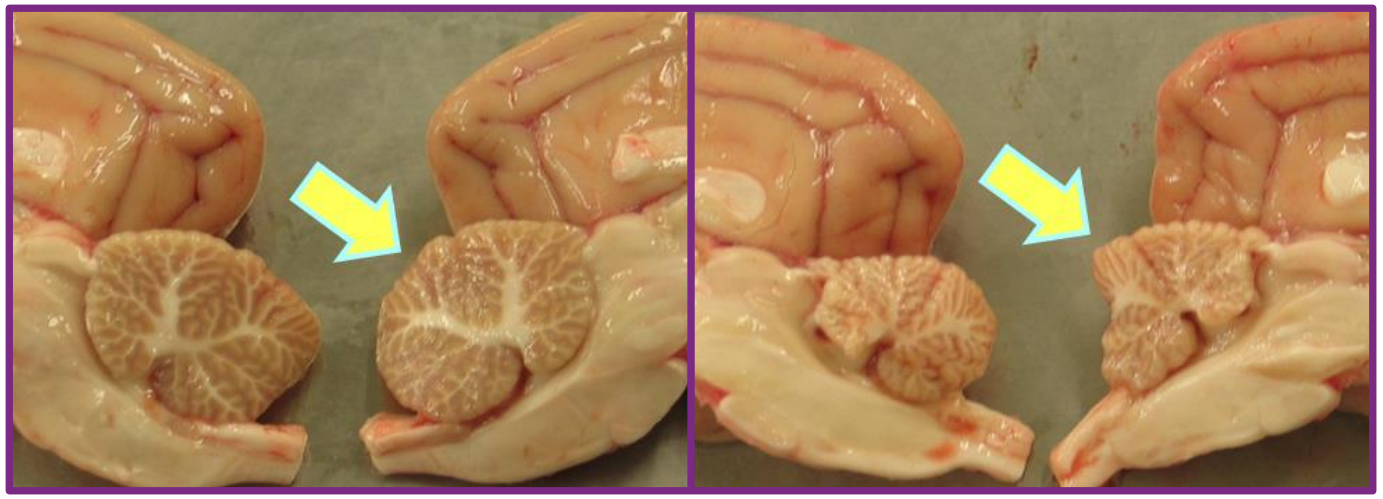
Clinical Progression in Canine MPS IIIB

- Humane euthanasia 12-18 months from onset of clinic signs
- Widespread neuronal storage, microgliosis, and astrocytosis, with pronounced Purkinje cell loss and cerebellar atrophy



Trigeminal Nucleus (LFB)

Ellinwood et al., J Inherit Metab Dis. doi: 10.1023/a:1025177411938



Wild Type

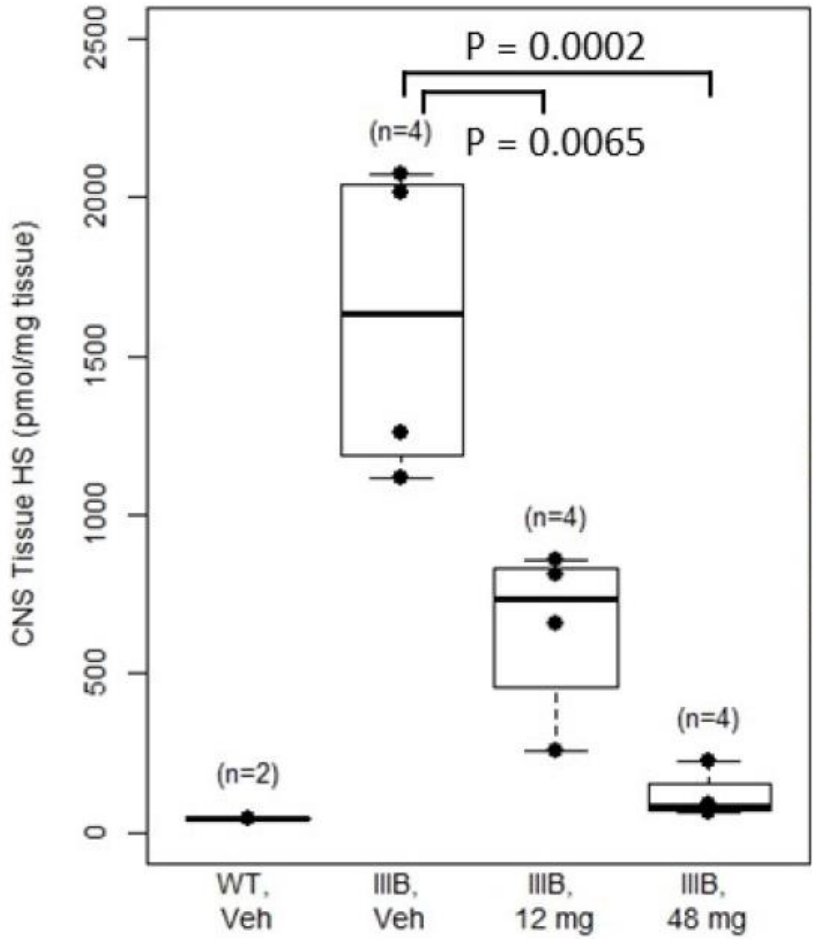
Ellinwood, Unpublished data

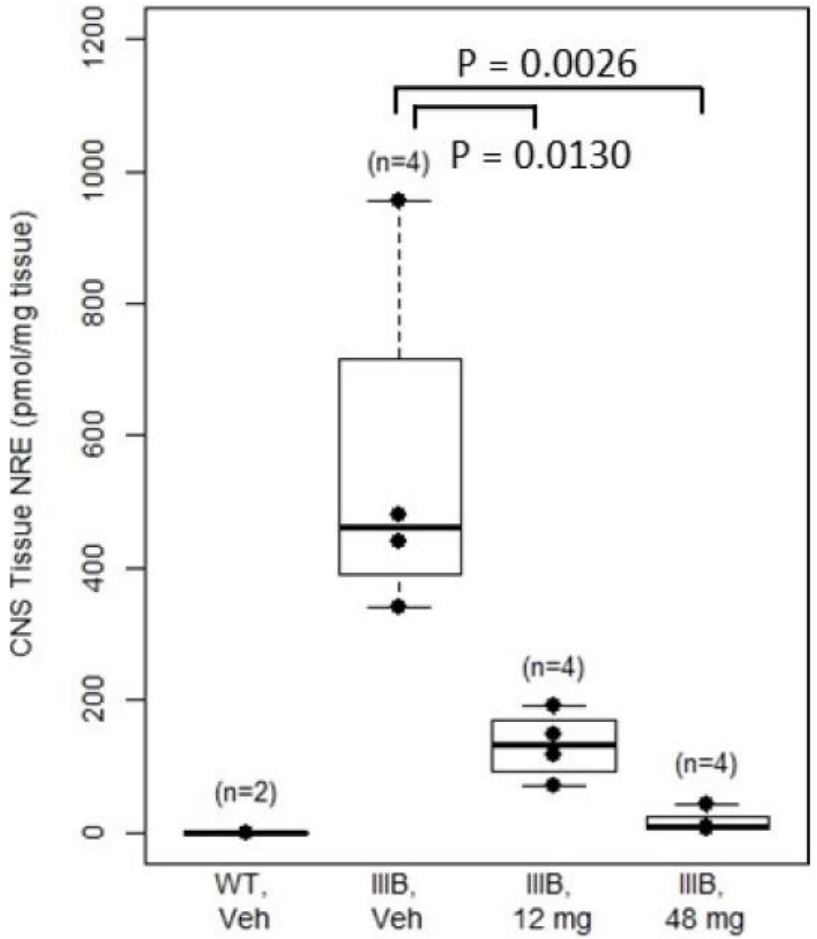
End Stage MPS IIIIB

Intraventricular/Intracisternal ERT in Canine MPS IIIB

- Route of infusion designed to overcome blood brain barrier
  - Approach equivalent to that for Brineura®, approved by the FDA to treat tripeptidyl peptidase 1 deficiency in CLN2 children
- Infusions of 12 or 48 mg of AX 250 in an artificial CSF vehicle
  - Recombinant human *N*-acetyl-alpha-D-glucosaminidase (NAGLU)
    - Cis fusion of a IGF2 receptor ligand tag
      - Ligand tag used to overcome the well-documented poor mannose 6 phosphorylation of conventual methods of recombinant NAGLU production
  - Up to 42 infusions over 20 months (24 months of age at last dose)
    - Intracerebroventricular infusions followed by isovolumetric intracisternal infusions beginning with dose 5 to 24

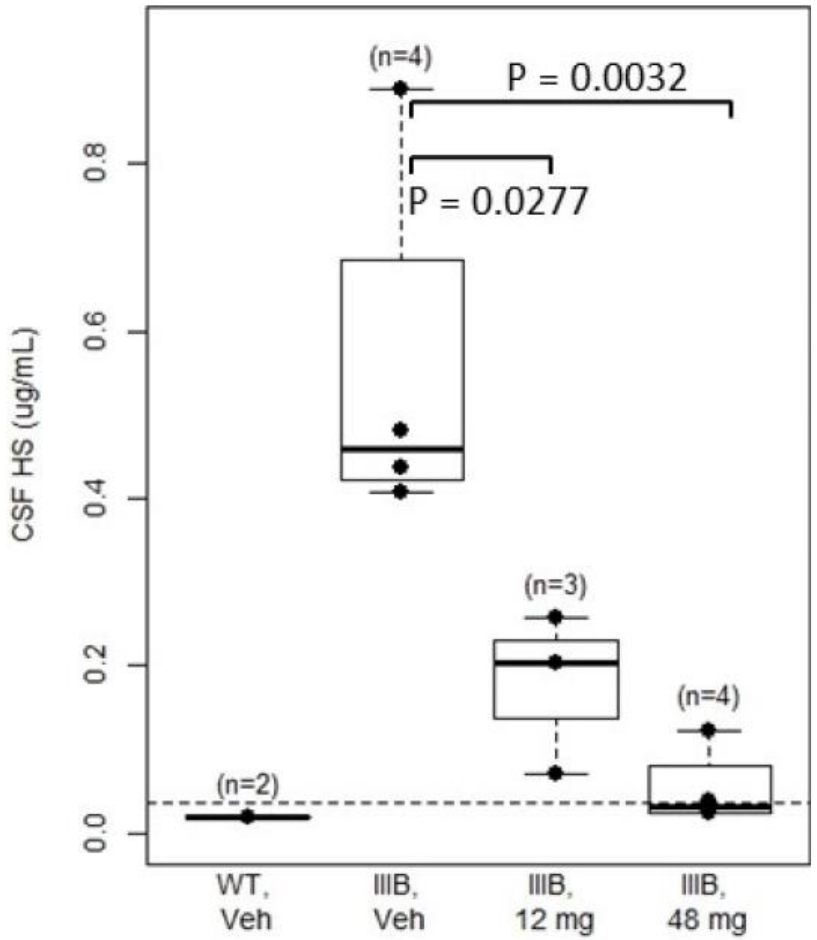
Tissue HS Derived Disaccharides (HS) and MPS IIIB Non-reducing End Oligos (NREs)

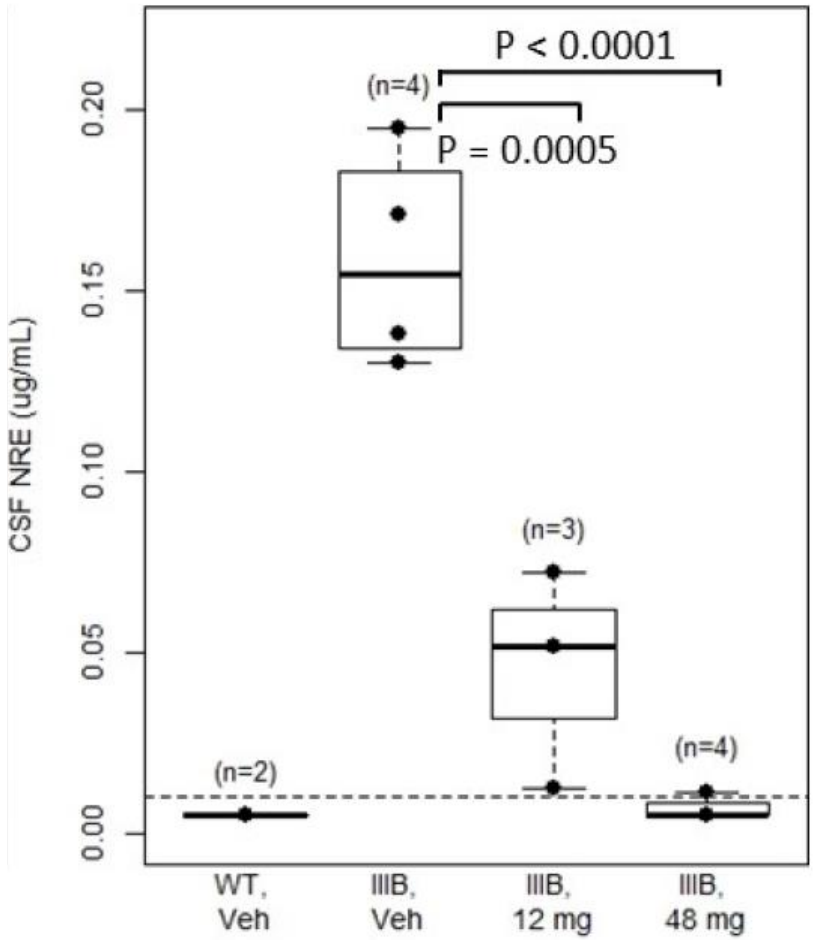




Ellinwood et al., J Pharmacol Exp Ther. 2022. DOI: 10.1124/jpet.122.001119

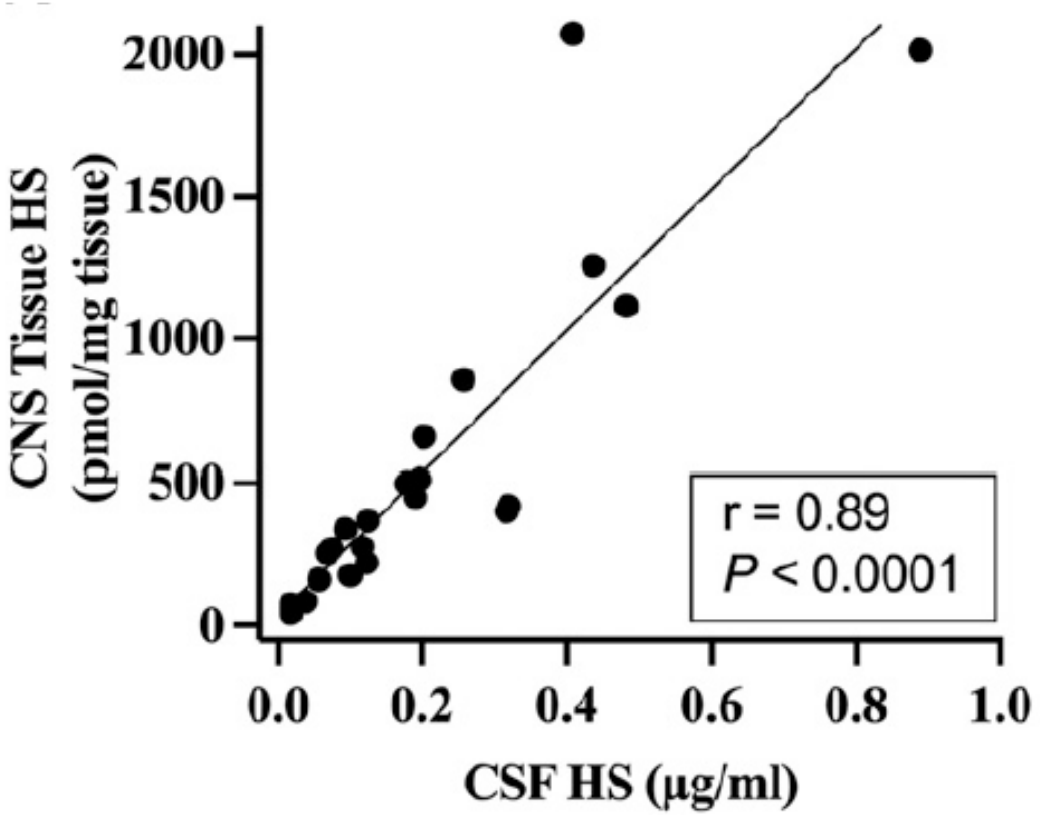
CSF HS and MPS IIIB NREs

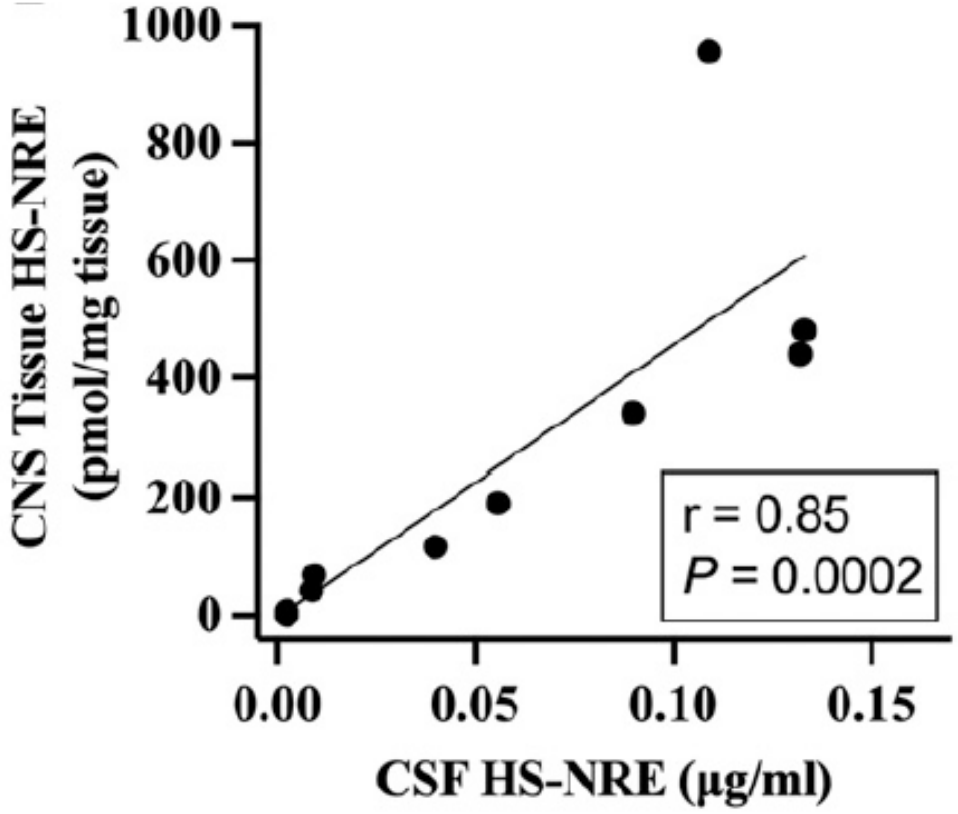




Ellinwood et al., J Pharmacol Exp Ther. 2022. DOI: 10.1124/jpet.122.001119

Tissue and CSF Correlations of HS and MPS IIIB NREs





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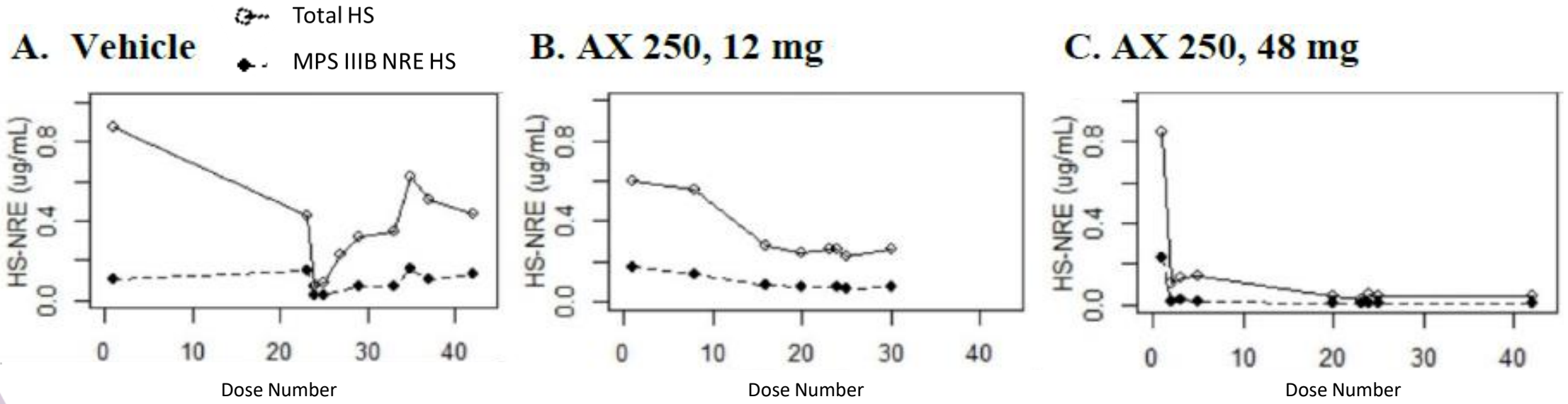
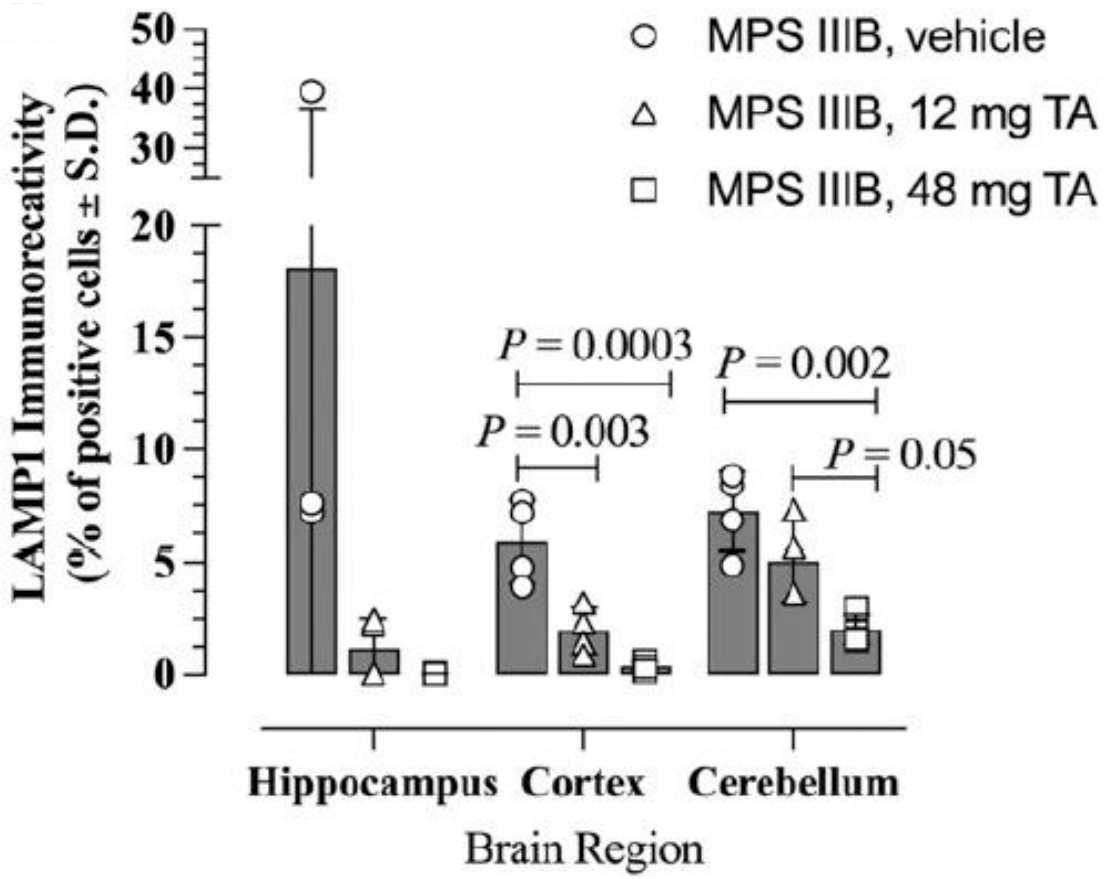
Dose Dependent Decrease of CSF HS and MPS IIIB NREs Over Time



Animal B538 inadvertently dosed with 48 ml AX 250 at ICV dose 23

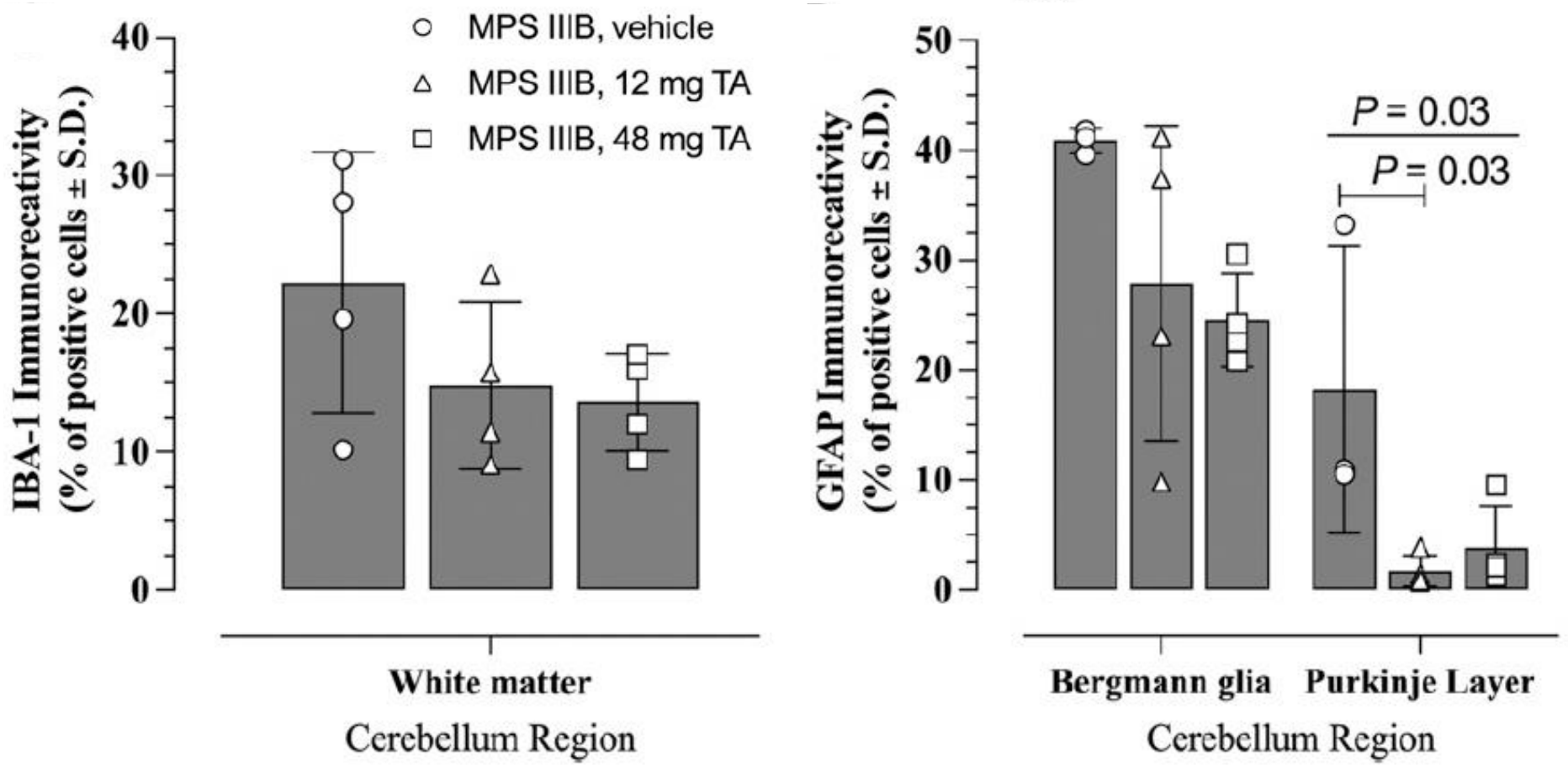
Ellinwood et al., unpublished data

Dose-Dependent CNS Decrease in a Marker for Lysosomal Storage: LAMP1 Immunoreactivity



Ellinwood et al., J Pharmacol Exp Ther. 2022. DOI: 10.1124/jpet.122.001119

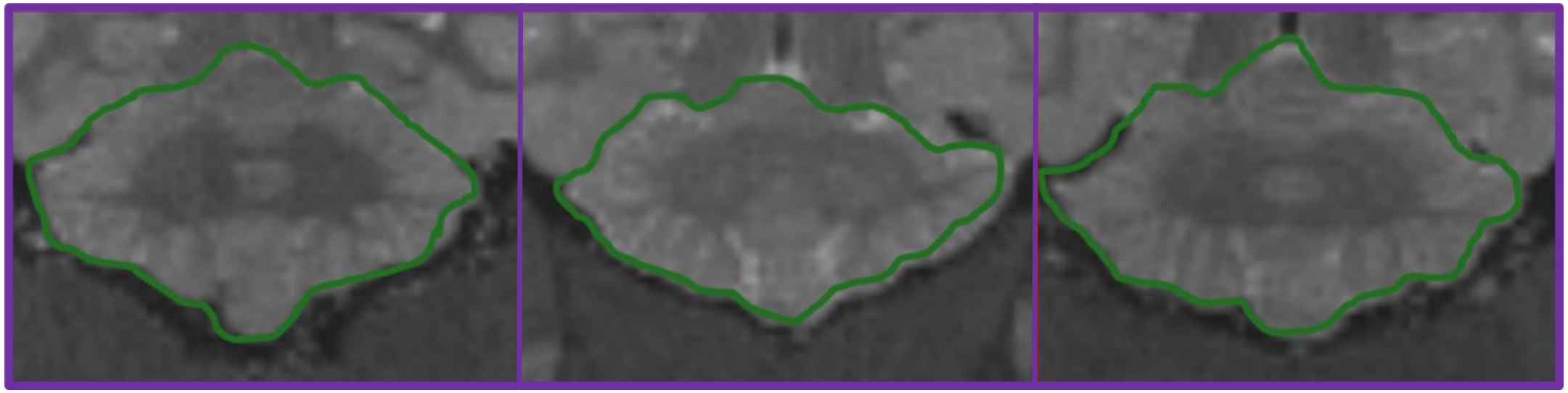
Decrease in Cerebellar Microglial Activation and Astrocytosis



Ellinwood et al., J Pharmacol Exp Ther. 2022. DOI: 10.1124/jpet.122.001119

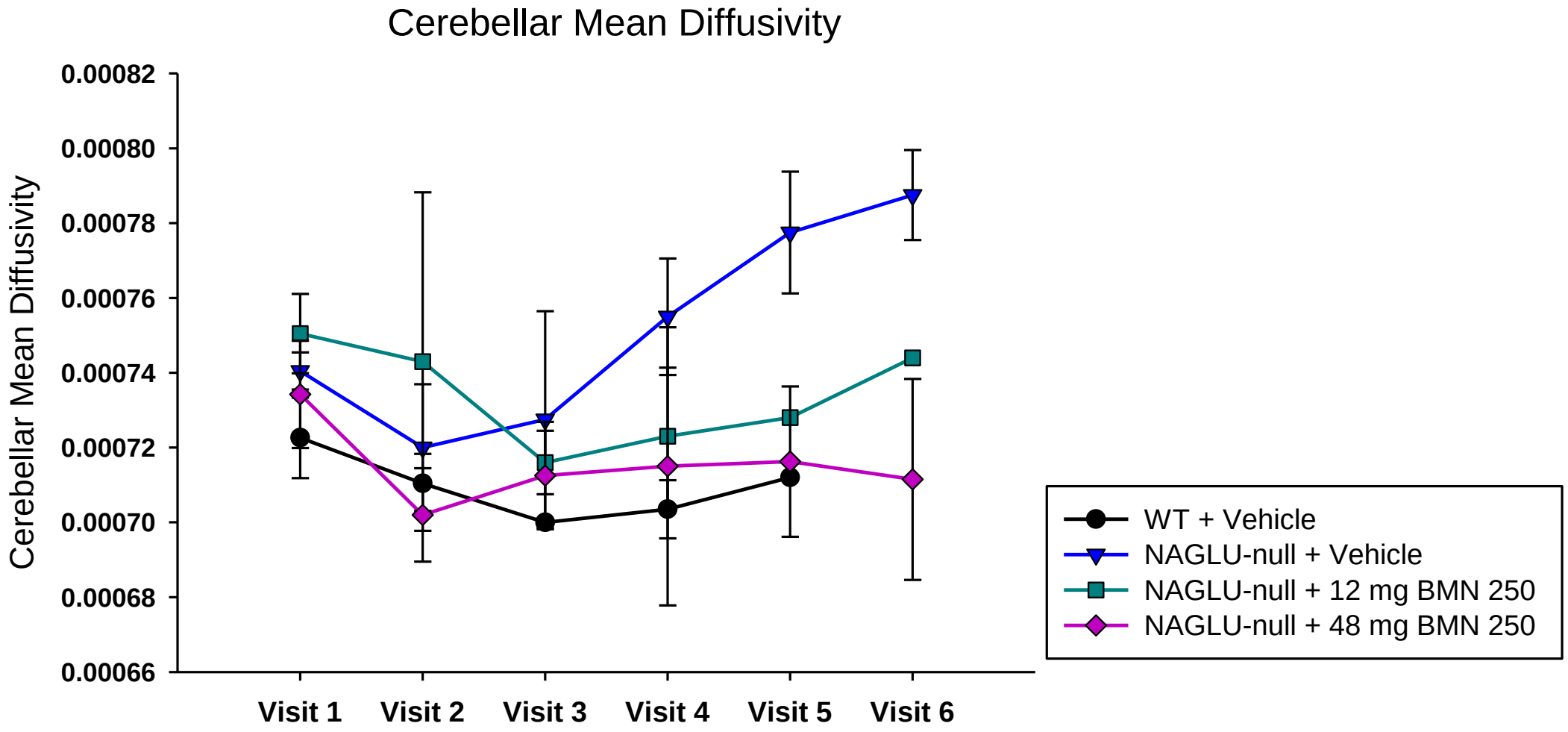
Biochemical and Histopathological Findings Support AX 250 Role in Prevention of Cerebellar Atrophy

Wild Type, Vehicle | MPS IIIB, Vehicle | MPS IIIB, AX 250



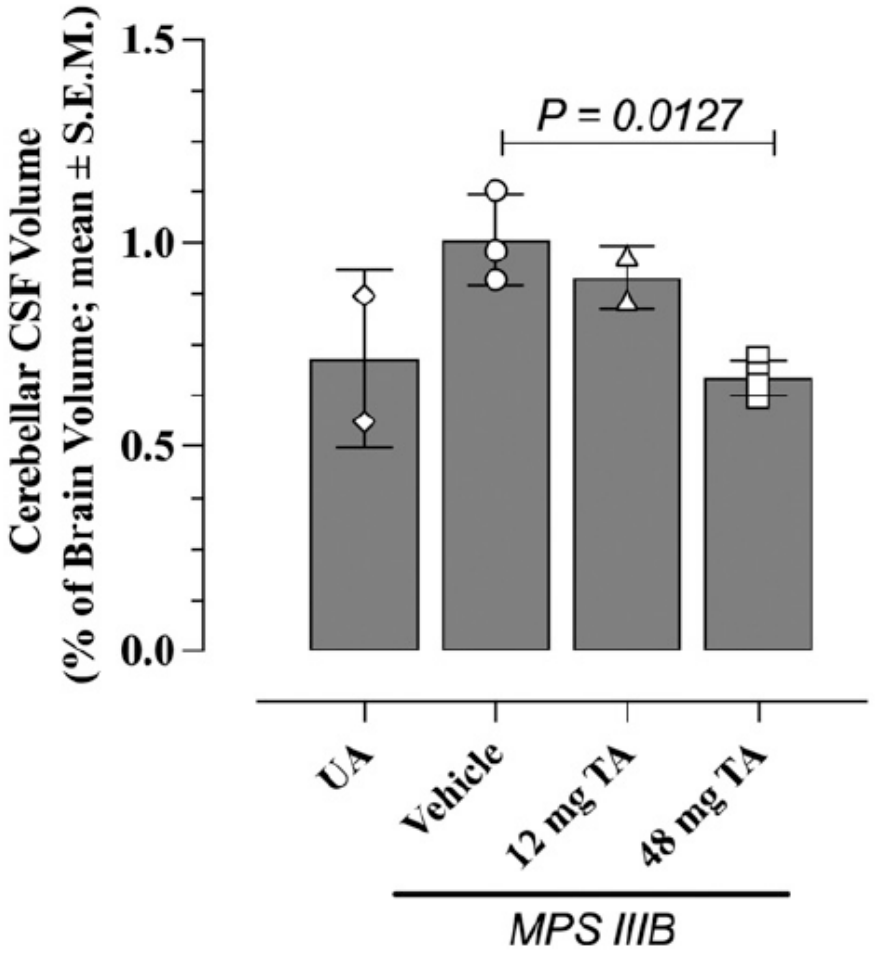
Ellinwood et al., unpublished data

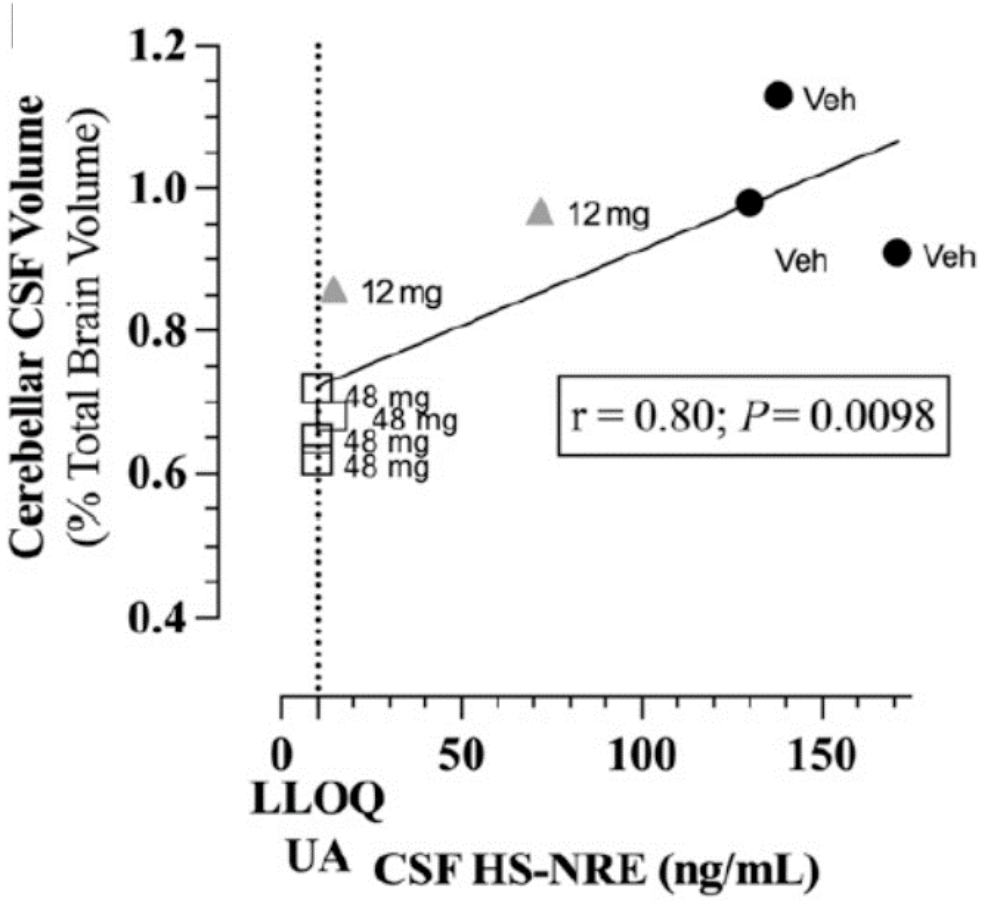
Dose-Dependent Preservation of Cerebellar Volume: CSF volume and mean diffusivity in cerebellum



Ellinwood et al., unpublished data

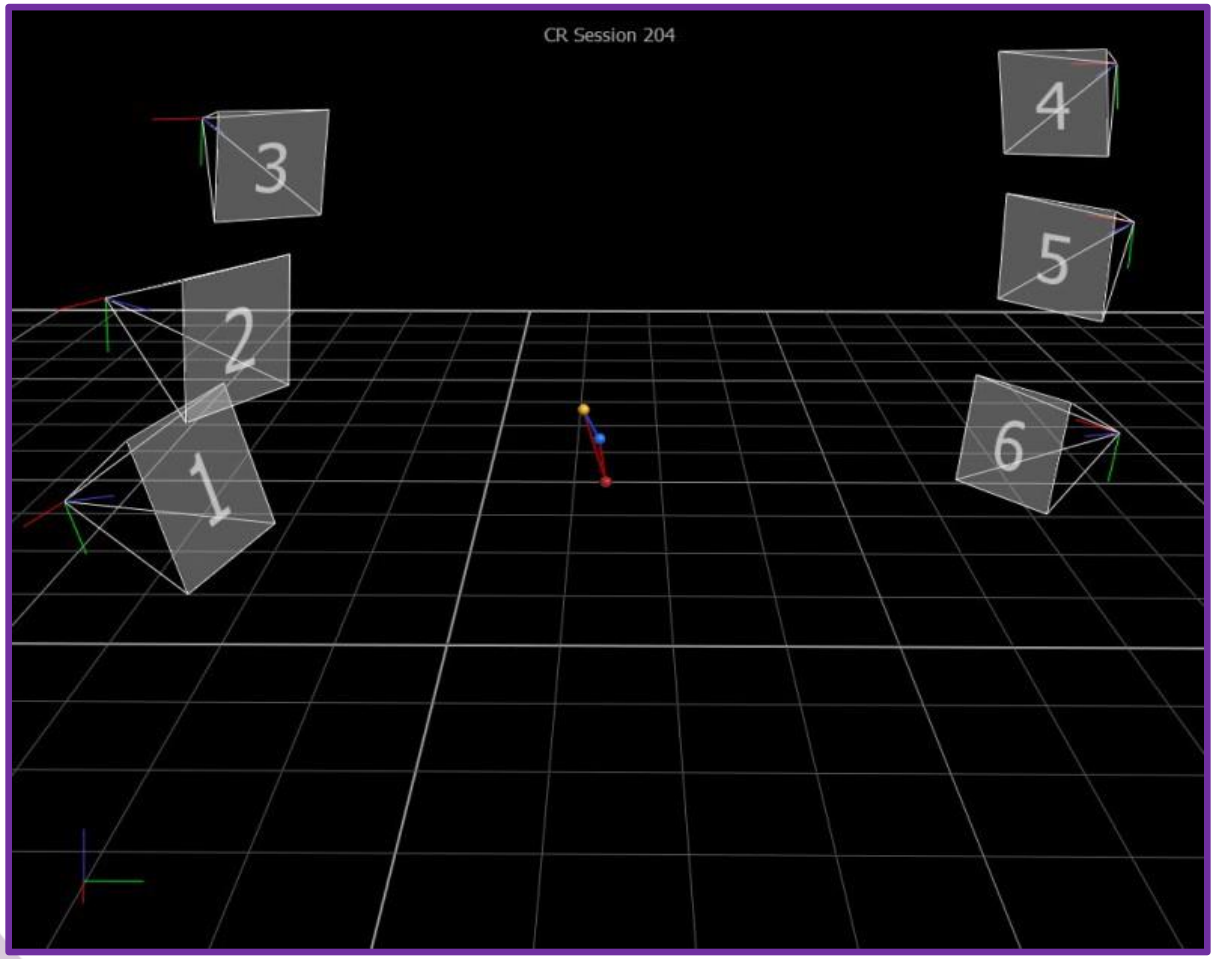
Pharmacokinetic Dose-Dependent Preservation of Cerebellar Volume: CSF volume and mean diffusivity in cerebellum





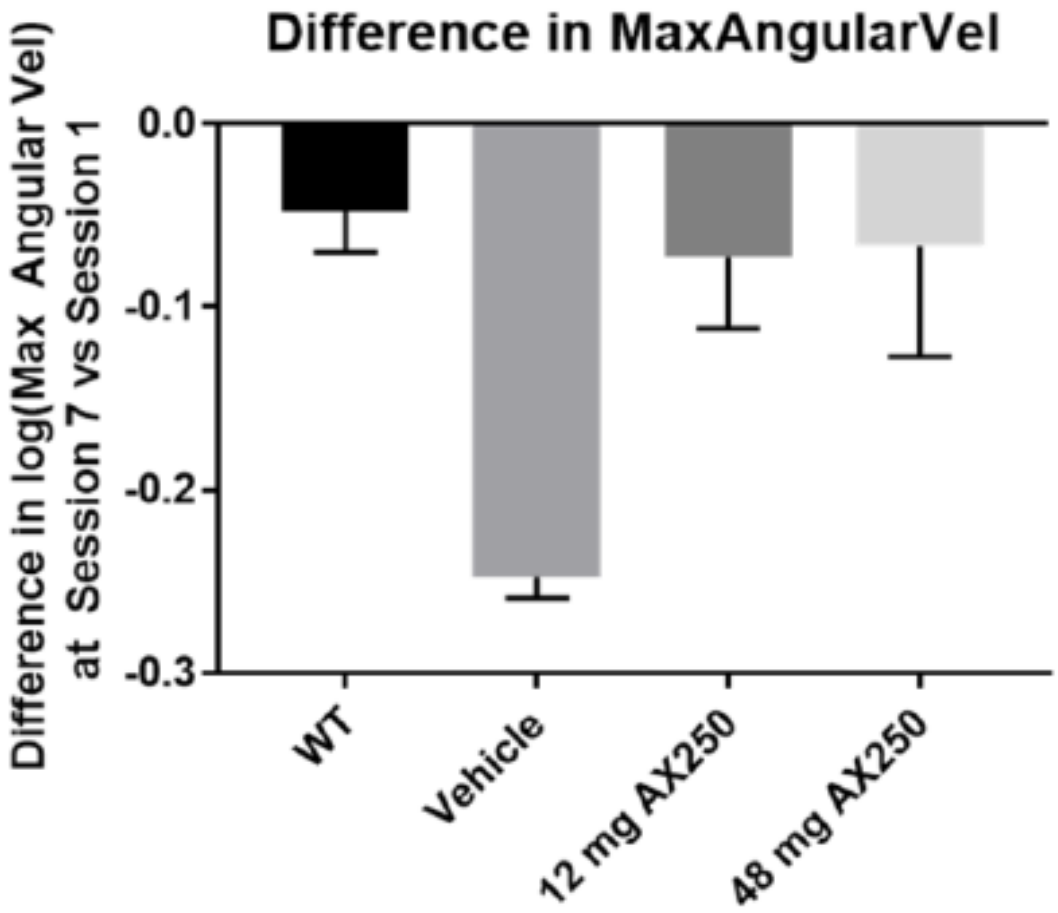
Ellinwood et al., J Pharmacol Exp Ther. 2022. DOI: 10.1124/jpet.122.001119

Functional Response in Cerebellar Performance



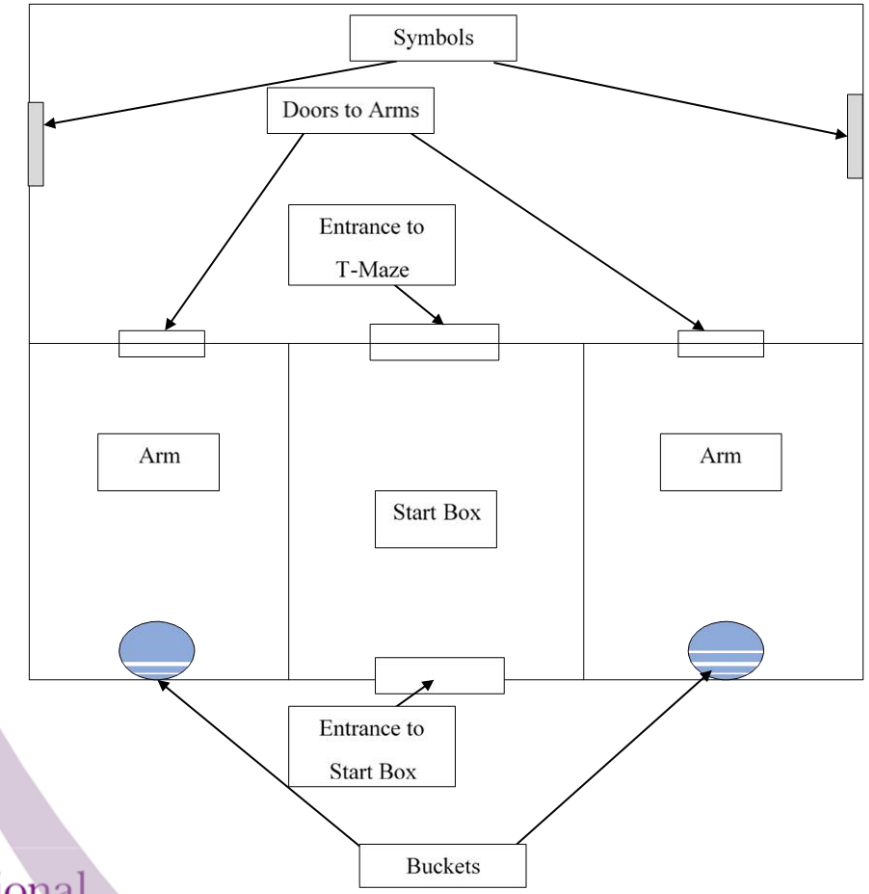
Ellinwood et al. Unpublished Data

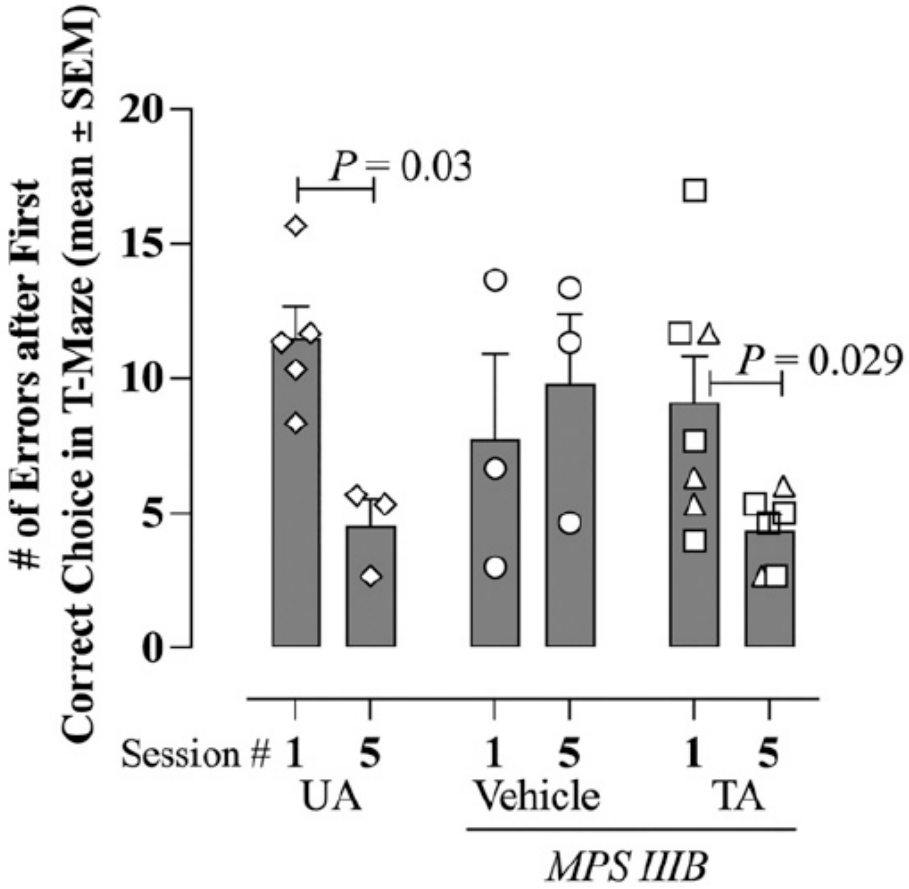
Functional Response in Cerebellar Performance



Ellinwood et al. Unpublished Data

AX 250 Response in Memory Performance T-Maze Reversal Learning Task



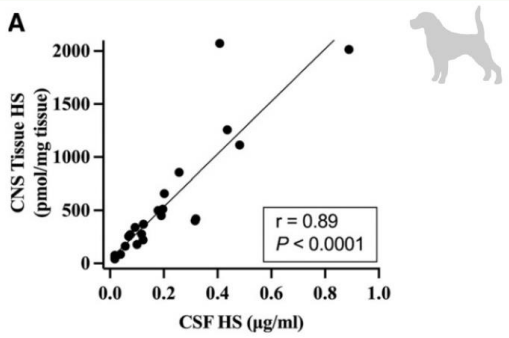


Ellinwood et al., J Pharmacol Exp Ther. 2022. DOI: 10.1124/jpet.122.001119

Comparative Tissue and CSF GAG Correlations:

Multiple therapeutic modalities and multiple neuropathic MPS murine and canine models (MPSII, MPSIIIA, and MPSIIIB)

Brain and CSF HS correlation in MPS IIIB dog model

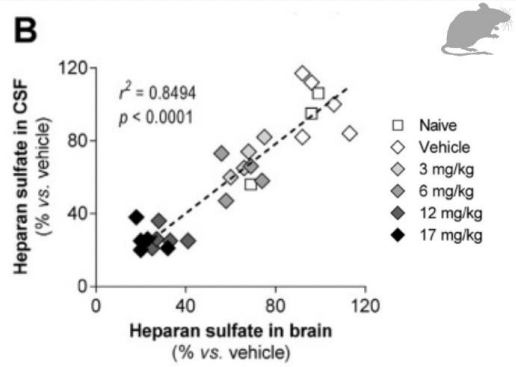


Notes: Dogs were administered vehicle, 12mg or 48mg test article ICV every other week for 83 weeks (n=12) or 15 or 30 mg ICV weekly for 8 weeks (n=11) with tralesinidase alfa, a fusion protein of recombinant human NAGLU and a modified human insulin-like growth factor 2.

CNS tissue was obtained by brain biopsy including from deep regions of the brain (frontal cortex, striatum, thalamus, midbrain, occipital cortex, cerebellum, and medulla).

Ellinwood et al 2022, J Pharmacology and Exp Therapeutics

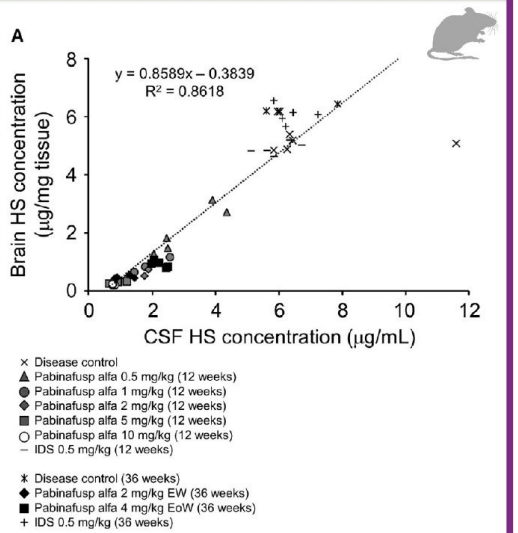
Brain and CSF HS correlation in MPS IIIA murine model



Notes: Vehicle or CM-rhSulfamidase (modified recombinant human sulfamidase subjected to a chemical procedure to disrupt glycan structures) were administered to mice IV, once weekly for 20 wks.

Gustavsson et al 2019, Mol Gen and Metabolism Reports.

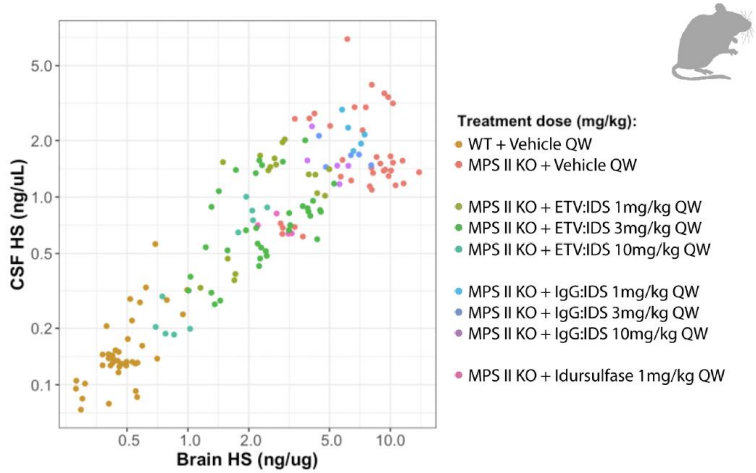
Brain and CSF HS correlation in MPS II murine model



Notes: Pabinafusp alfa was administered to mice IV in 12-week or 36-week repeated dose studies as indicated.

Morimoto et al 2021, Molecular Therapy.

MPS II murine model



Abbreviations: WT: Wild type mouse is a mouse expressing a chimeric human TfR apical domain and a murine TfR intracellular domain ($TfR^{mu/hu}$ KI) that facilitates binding and receptor-mediated transcytosis of ETV:IDS; MPS II KO: IDS knock out MPS II mouse model and harbors a chimeric transferrin receptor as described above for the $TfR^{mu/hu}$ KI mouse line.

Notes: Dosing regimen: Vehicle, ETV:IDS, Idursulfase, and IgG:IDS were given intravenously weekly.

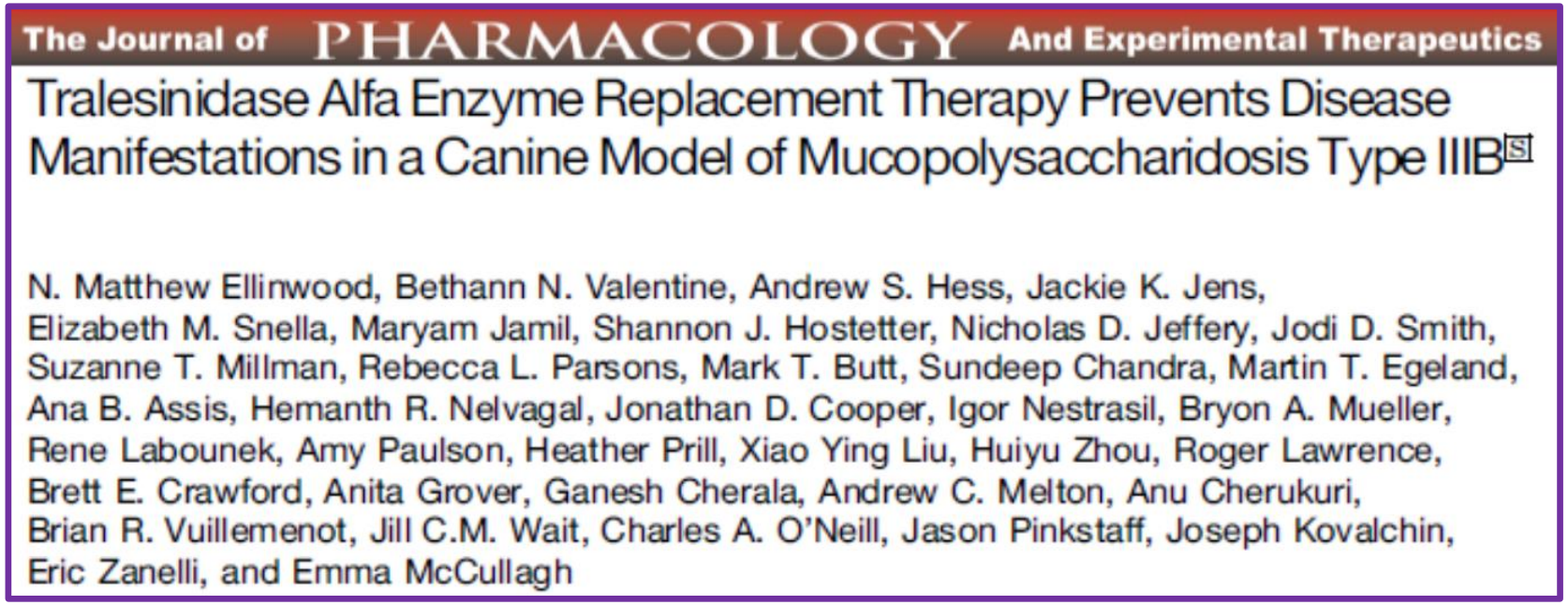
Conclusions

- Comparative biology and medicine confirm the neuropathologic nature of intra-lysosomal accumulation of HS
  - 7 genetically distinct disorders (MPSs I, II, IIIA, IIIB, IIIC, IIID, and VII)
  - 7 species spanning
    - 2 phylogenetic classes
    - 4 phylogenetic orders
    - At least 640 million years of conserved evolutionary biology
- Multiple modalities evaluating therapeutic intervention
  - Demonstrate highly correlated nature of CSF HS and CNS tissue storage of HS
  - Correlation of CNS and CSF HS decreases with:
    - Improved tissue pathology
    - Decreased neuroinflammation
    - Prevention of CNS atrophy
    - Improved behavior

National MPS Society

Acknowledgements

- Reagan-Udall Foundation
- Co-Authors and Collaborators


The Journal of PHARMACOLOGY And Experimental Therapeutics

Tralesinidase Alfa Enzyme Replacement Therapy Prevents Disease Manifestations in a Canine Model of Mucopolysaccharidosis Type IIIB[S]

N. Matthew Ellinwood, Bethann N. Valentine, Andrew S. Hess, Jackie K. Jens, Elizabeth M. Snella, Maryam Jamil, Shannon J. Hostetter, Nicholas D. Jeffery, Jodi D. Smith, Suzanne T. Millman, Rebecca L. Parsons, Mark T. Butt, Sundeep Chandra, Martin T. Egeland, Ana B. Assis, Hemanth R. Nelvagal, Jonathan D. Cooper, Igor Nestrasil, Bryon A. Mueller, Rene Labounek, Amy Paulson, Heather Prill, Xiao Ying Liu, Huiyu Zhou, Roger Lawrence, Brett E. Crawford, Anita Grover, Ganesh Cherala, Andrew C. Melton, Anu Cherukuri, Brian R. Vuillemenot, Jill C.M. Wait, Charles A. O'Neill, Jason Pinkstaff, Joseph Kovalchin, Eric Zanelli, and Emma McCullagh

- Fellow presenters, especially Drs. C. Ho and H. Lau
- Funding from NIH, Iowa State University, BioMarin, and Allievex

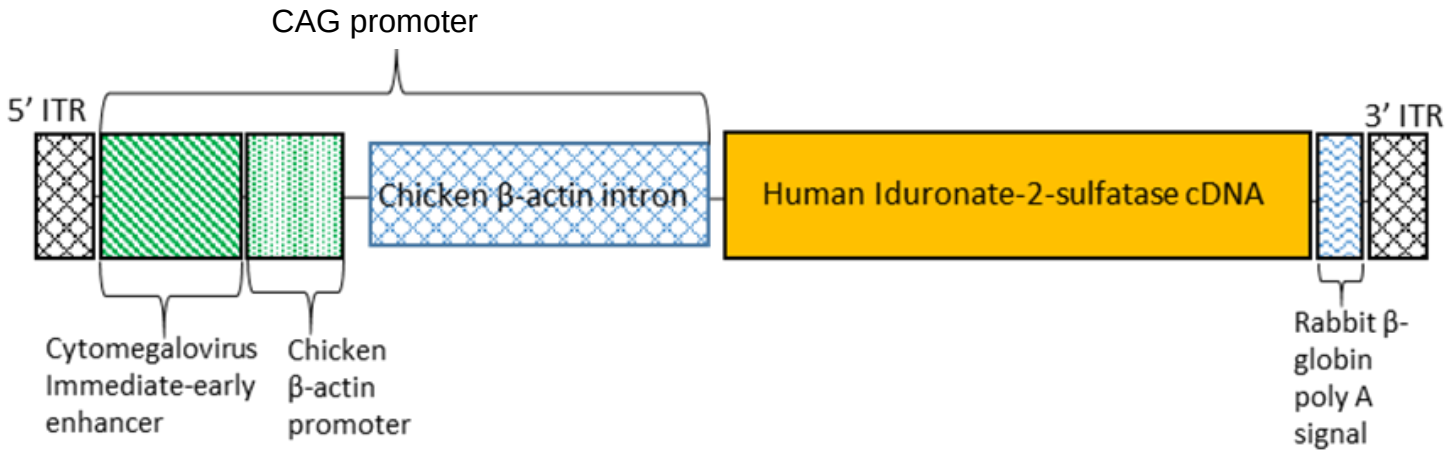
RGX-121 Gene Therapy Candidate for the Treatment of Neuronopathic MPS II

Case Study: Animal Model Translation to Human Application

Nidal Boulos, Ph.D.
Director, Clinical Science
February 21st, 2024

RGX-121 gene therapy candidate for the treatment of neuronopathic MPS II

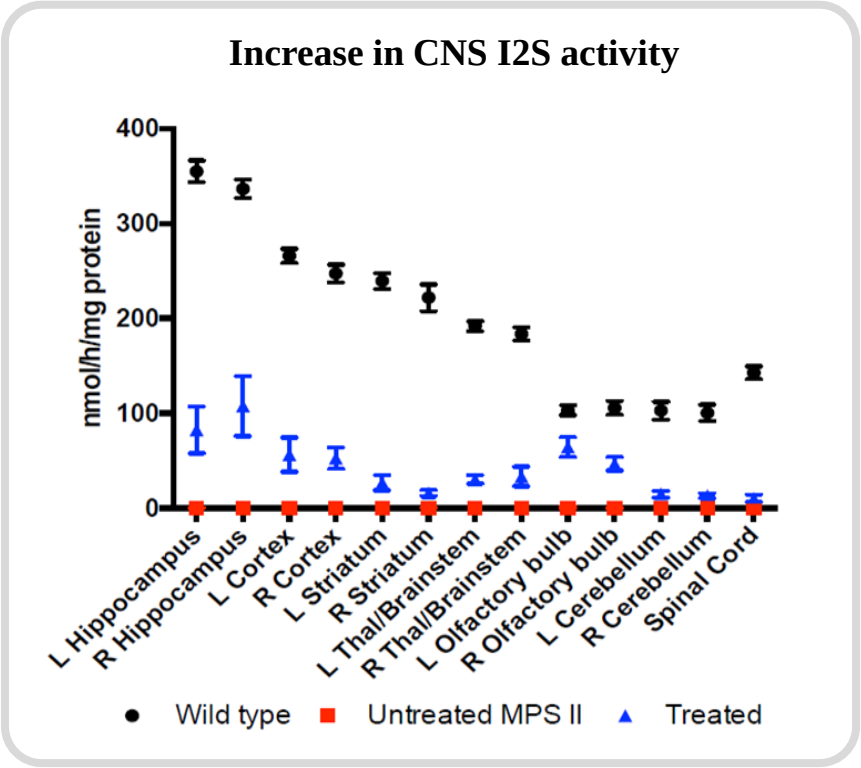
- RGX-121 is a non-replicating recombinant AAV9 containing human iduronate-2-sulfatase expression cassette.
- RGX-121 is designed for efficient expression of iduronate-2-sulfatase enzyme (I2S) in the CNS.
- RGX-121 is being investigated as a potential treatment for MPS II in a phase I/II/III clinical study (CAMPSIITE™) to address the unmet need of CNS disease involvement.*

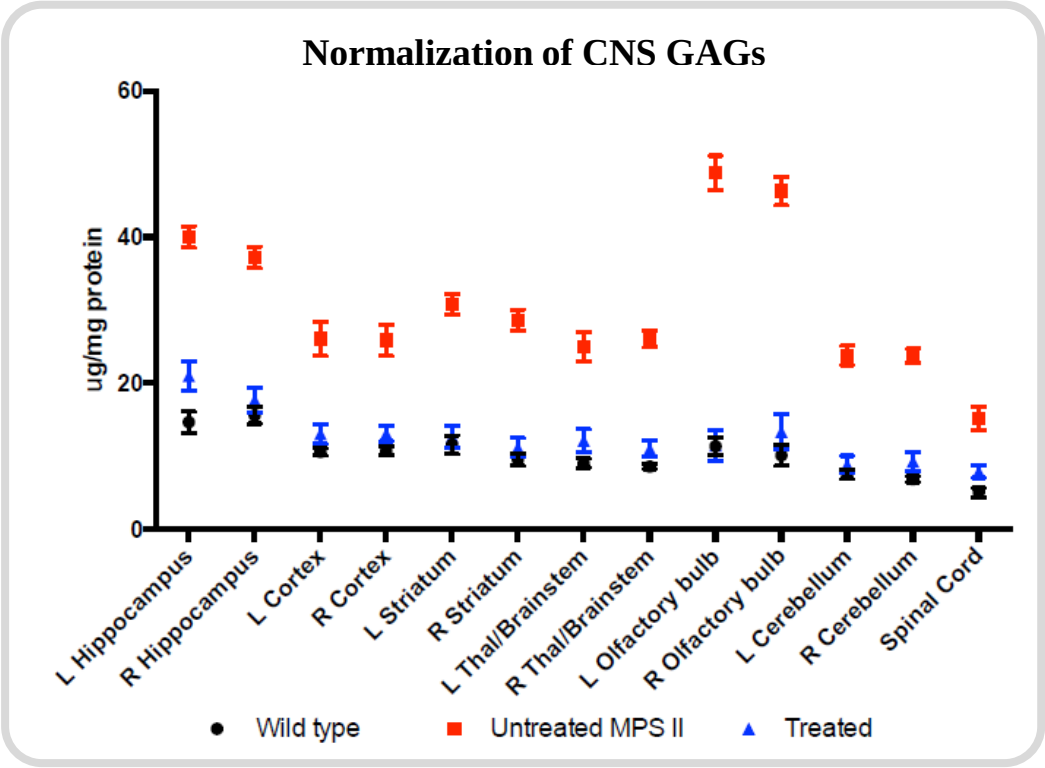


* RGX-121 is an investigational therapy and has not been approved by any regulatory authority.

Increase in I2S enzyme activity and normalization of CNS GAGs content in MPS II mice post RGX-121 gene therapy

- RGX-121 was administered into CSF (via intracerebroventricular (ICV) injection)

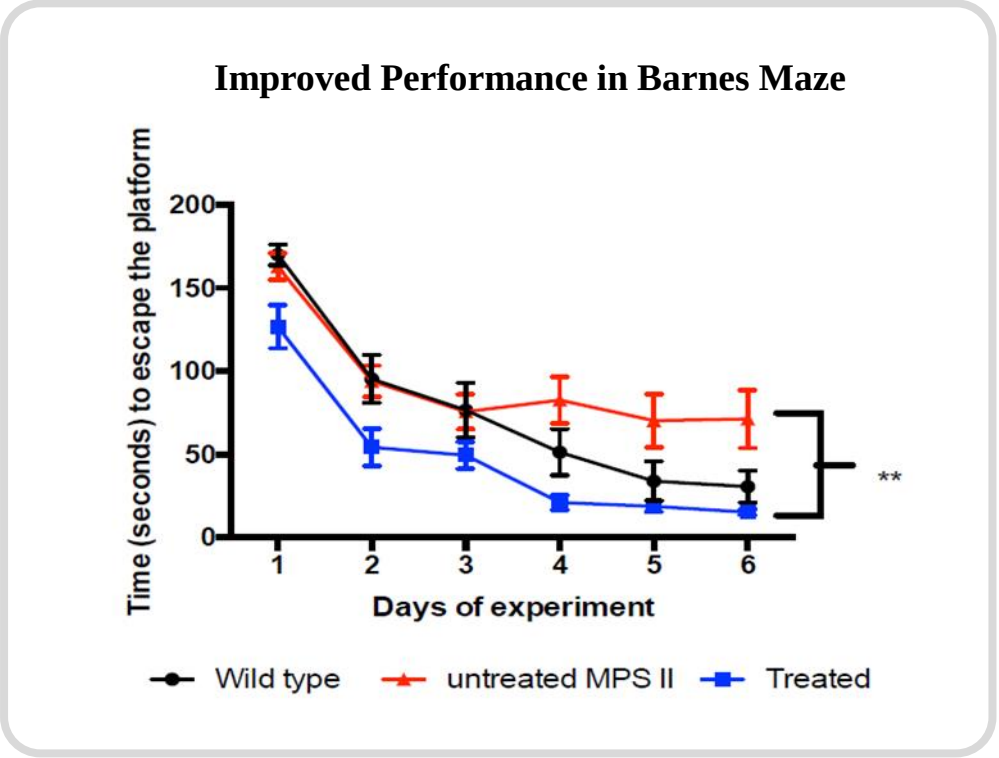




Laoharawee *et al*. Human Gene Ther 2017 28(8):626-638

Improved performance in Barnes maze in MPS II mice post RGX-121 gene therapy

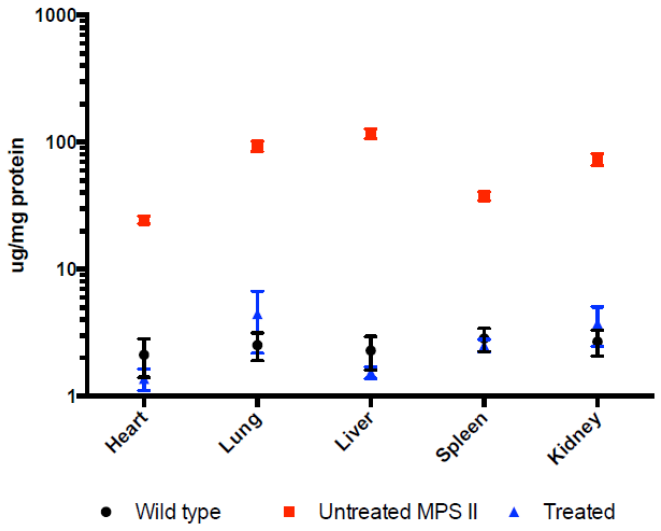
Neurobehavioral assessment of mice: spatial learning and memory

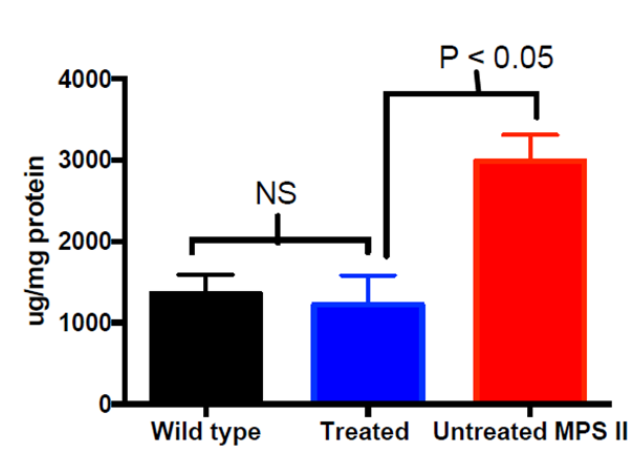


Laoharawee *et al*. Human Gene Ther 2017 28(8):626-638

Normalization of GAGs content in peripheral organs in MPS II mice post RGX-121 gene therapy

Systemic response was observed in peripheral organs





Laoharawee *et al*. Human Gene Ther 2017 28(8):626-638

Neuronopathic forms of MPS exhibit elevated Heparan Sulfate (HS) GAGs

Neuronopathic (severe) forms of MPS exhibit elevated concentrations of the GAG heparan sulfate (HS) in the brain leading to central nervous system abnormalities and neurocognitive impairment.

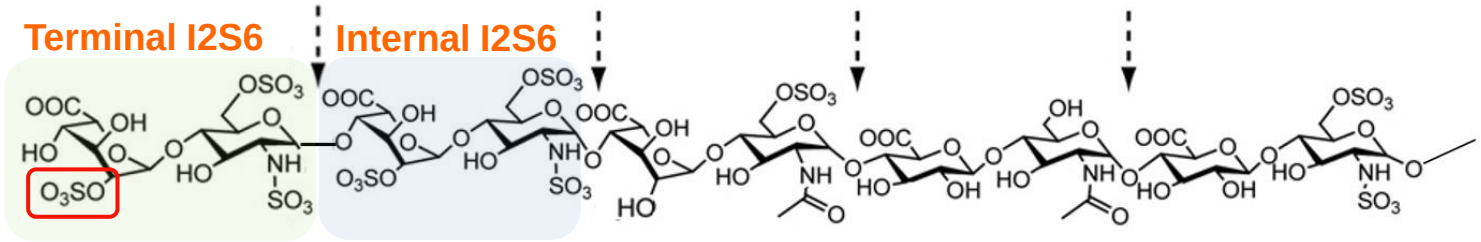
MPS Type	Main GAG stored	Neurologic Symptoms
MPS I	HS, DS	Hurler: Severe Hurler-Scheie and Scheie: mild to absent
MPS II	HS, DS	Severe (fast progressing phenotype) to mild or none (slow progressing phenotype)
MPS IIIA, B, C, D	HS	Severe
MPS IVA, B	KS	None
MPS VI	DS	None
MPS VII	HS, DS	Severe or mild to absent
MPS IX	Hyaluronan	None

Heparan Sulfate is a key biomarker in neuronopathic MPS types

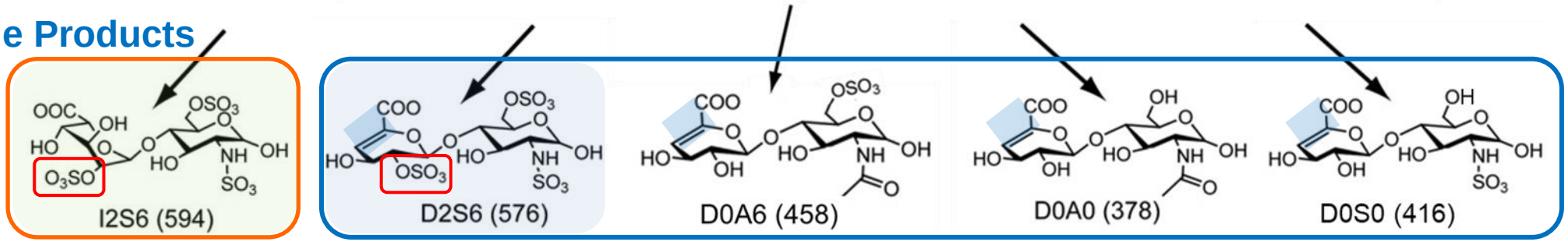
Heparan Sulfate digestion into disaccharides

Heparinase Sites for Cleavage

HS Chain



HS Disaccharide Products



Non-Reducing End (NRE)
MPS II disease-specific biomarkers
Extensively studied for NBS & diagnostics

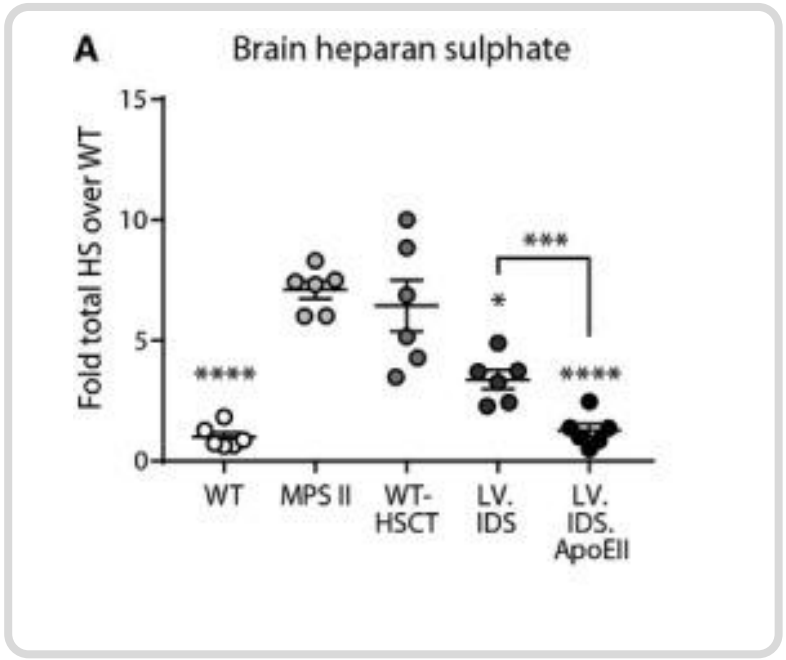
D2S6 contains 2-O-sulfated ends which are cleaved by I2S enzyme.

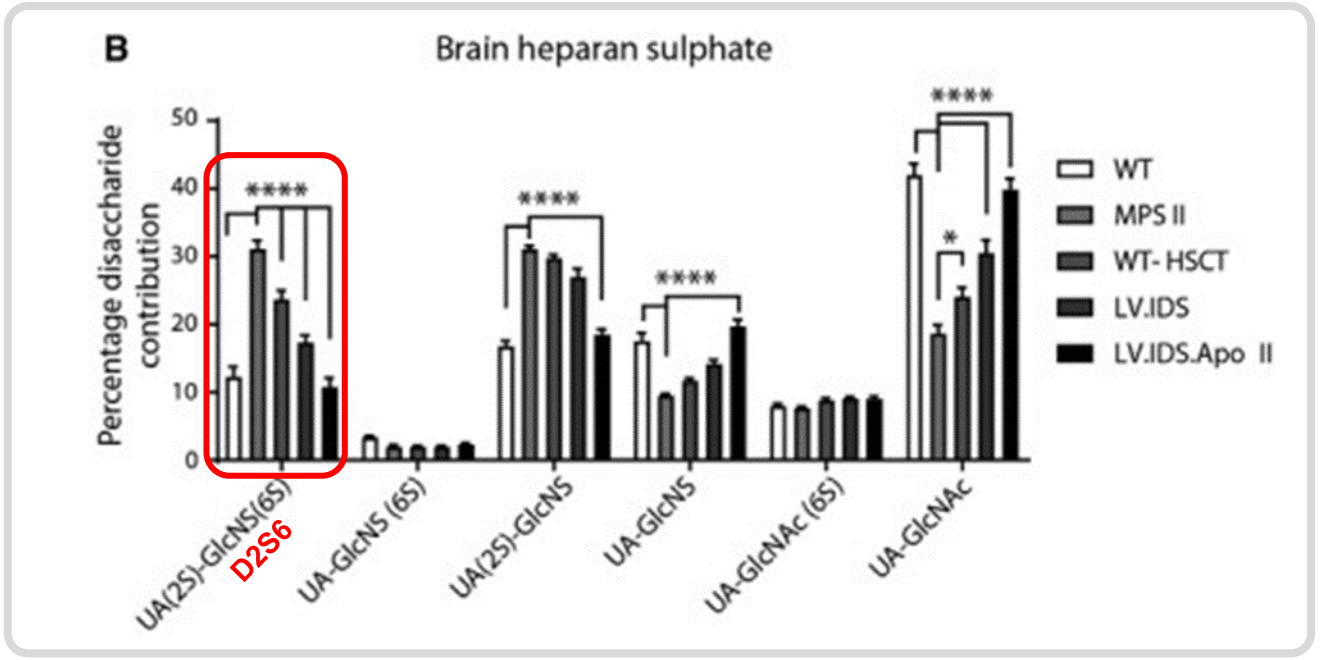
HS D2S6 disaccharide contains the 2-sulfate on non-reducing end = substrate for I2S enzyme (complicit with IDS gene deficiency)

Modified from Lawrence *et al*., 2014 *Molecular Genetics and Metabolism 111 (2014) 73–83*

HS D2S6 response to gene therapy in MPS II Mice

Brain-targeted hematopoietic stem cell gene therapy using lentiviral IDS fused to ApoEII





Accumulation of HS in brain is normalized post treatment

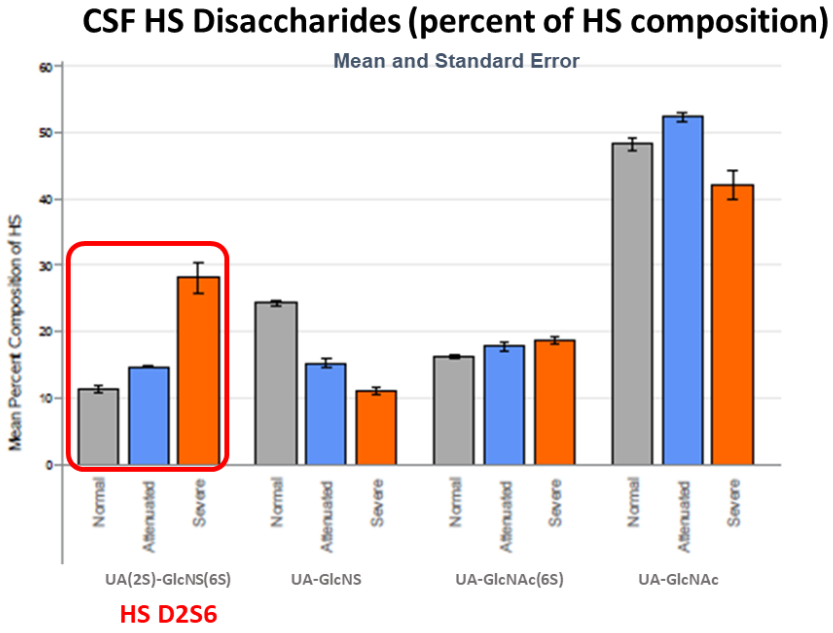
- **31% of total HS in the MPS II brain tissue shown to be HS D2S6**
- **HS D2S6 was the disaccharide most responsive to treatment**
- **Reductions in HS D2S6 associated with corrections in other disease markers, e.g., neuroinflammation, astrocytosis (GFAP, MCP-1, MIP-1α and IL-1α)**
- **Normalization in neurocognitive performance as assessed by behavior testing**

Gleitz *et al*. EMBO Mol Med 2018;e8730

Translation to Human Application

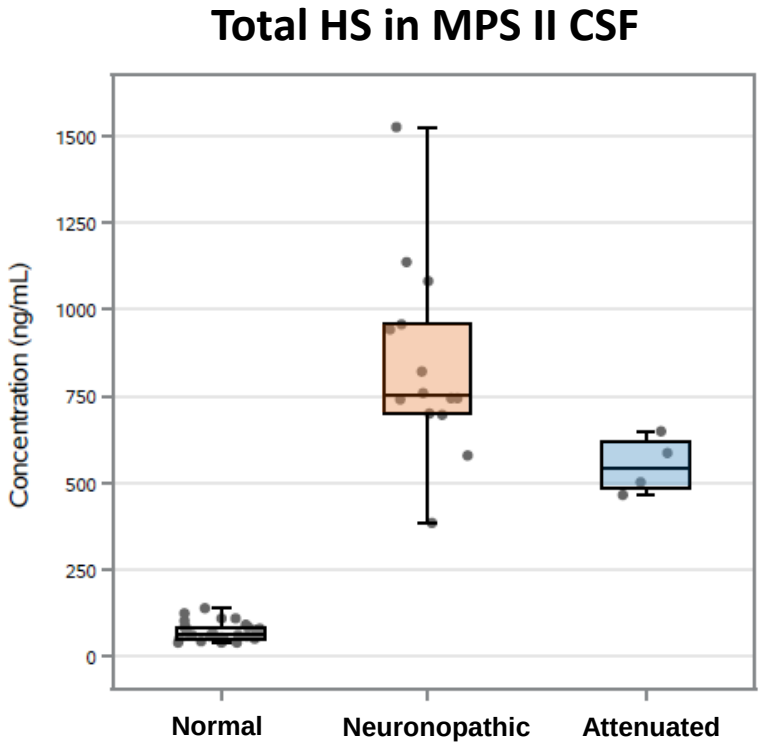
HS D2S6 is Increased in human CSF of neuronopathic MPS II compared to attenuated MPS II and Normal CSF (REGENXBIO generated data)

- Around 30% of total HS in neuronopathic MPS II CSF is HS D2S6
- HS D2S6 (% of total HS) was elevated in neuronopathic MPS II compared to normal and attenuated MPS II

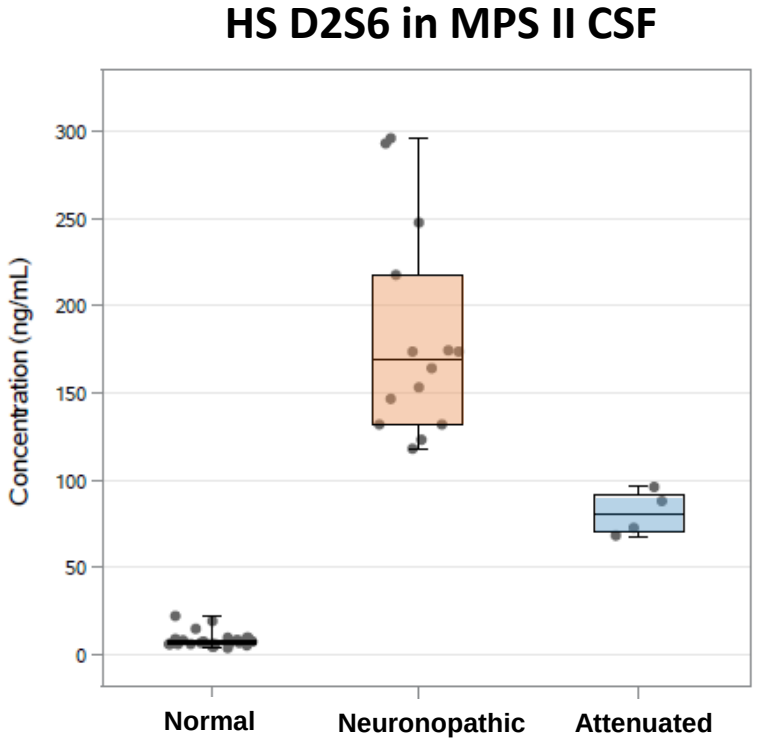


Boulos, WORLD*Symposium,* San Diego, CA 2020

HS D2S6 concentrations in MPS II CSF differentiated neuronopathic and attenuated MPS II phenotypes



	Normal	Neuronopathic	Attenuated
Sample size	29	14	4
Mean	72.3	843.5	551.7



	Normal	Neuronopathic	Attenuated
Sample size	29	14	4
Mean	8.1	181.9	81.2

HS D2S6 is reflective of disease pathology and can distinguish between disease phenotype

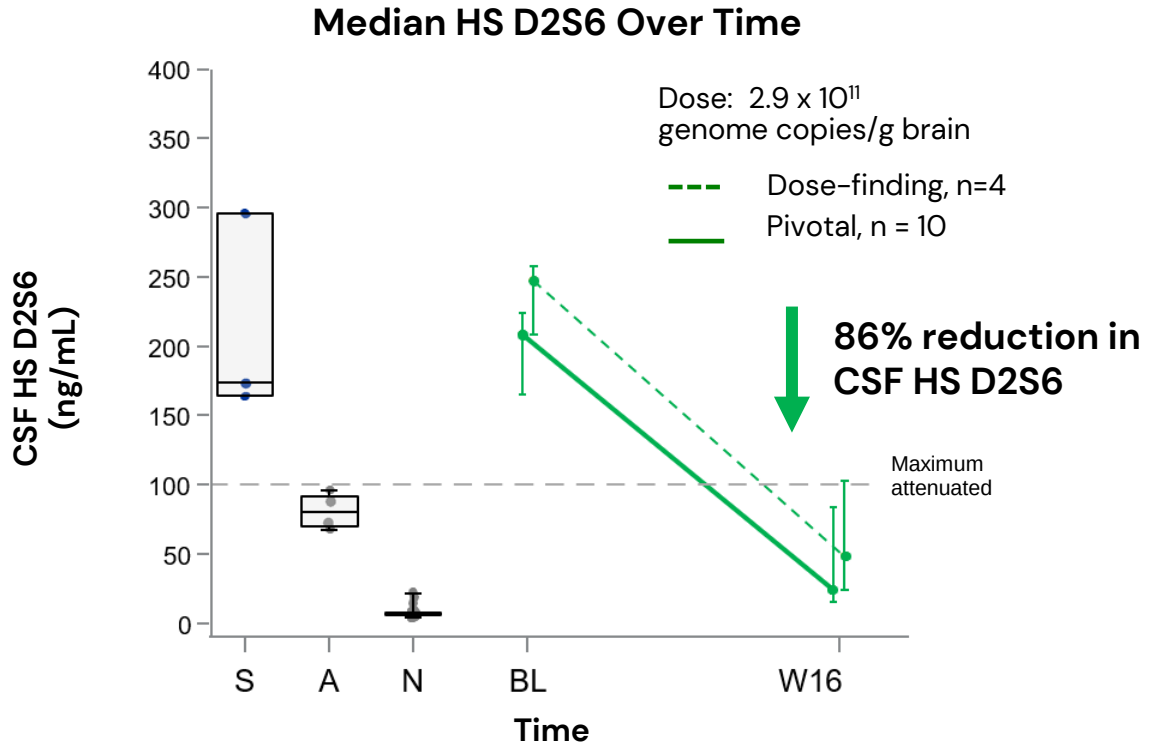
29 Normal CSF samples were purchased from BioIVT (n=20) and Discovery Life Sciences (n=6) or courtesy of Dr. Giugliani (n=3)
3 neuronopathic MPS II, 4 non-neuronopathic MPS II and all MPS I samples are courtesy of Dr. Giugliani
11 neuronopathic MPS II samples are from RGX-121-101

Boulos, WORLD*Symposium,* San Diego, CA 2020

Significant reductions in CSF HS D2S6 in pivotal trial for the treatment of neuronopathic MPS II (CAMPSIITE™)

HS D2S6 Disaccharide

- 2-sulfate on non-reducing end = IDS substrate (complicit with IDS deficiency)
- Correlates with total HS
- Correlates with other disease parameters in preclinical models



Normative data are based on 29 normal samples (N). Attenuated (A) defined as IQ > 70. The ages of 4 attenuated samples range from 11 years to 29 years old. Severe (S) defined as IQ < 70. The ages of 3 severe samples range from 4 years 8 months to 10 years old.

Harmatz, WORLD*Symposium,* San Diego, CA 2024

RGX-121 CAMPSIITE: HS D2S6 is a surrogate endpoint reasonably likely to predict clinical benefit in neuronopathic MPS II

Summary

- Heparan Sulfate (HS) is a surrogate endpoint that is reasonably likely to predict clinical benefit.
  - HS accumulation results from a missing enzyme (strong mechanistic rationale).
  - HS is the metabolite causing disease pathology in neuronopathic MPS types.
  - HS D2S6 disaccharide in CSF is reflective of disease pathology in MPS II patients and shows distinct concentrations between neuronopathic and attenuated MPS II phenotypes.
- In disease models reflecting aspects of clinical pathology, gene therapy expressing the missing enzyme:
  - Restored enzyme activity in relevant tissues
  - Associated with normalization of the pathologic substrate (HS GAG)
  - Improved neurocognitive performance as assessed by behavioral testing
- Translation of RGX-121 for the treatment of children with neuronopathic MPS II (CAMPSIITE™):
  - Accurate and validated method to measure HS D2S6 in CSF
  - Significant reductions in HS D2S6 in CSF with levels approaching normal in pivotal study
  - Accurate and sensitive measurements of CSF HS, such as HS D2S6, have the potential to be considered a surrogate endpoint that is reasonably likely to predict clinical benefit

Case Study: Relationship Between Cerebrospinal HS Levels and Clinical Outcomes

- **Simon Jones, MBChB**, St. Mary's Hospital, University of Manchester
- **Heather Lau, MD, MS**, Ultragenyx
- **Eric Zanelli, PhD**, Allievex

REAGAN-UDALL FOUNDATION FOR THE FDA

Relationship Between Cerebrospinal HS Levels and Clinical Outcomes?

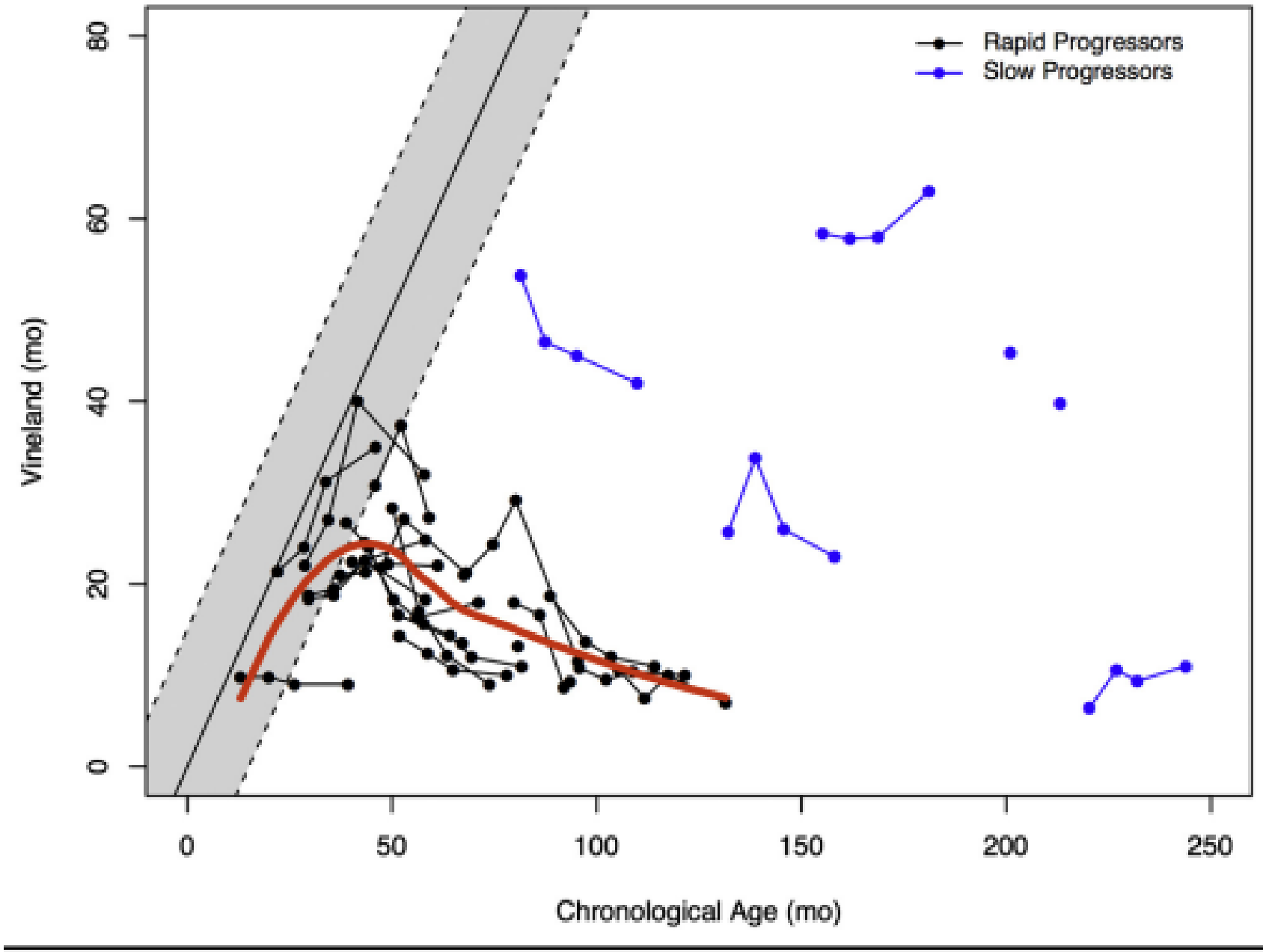
Simon Jones

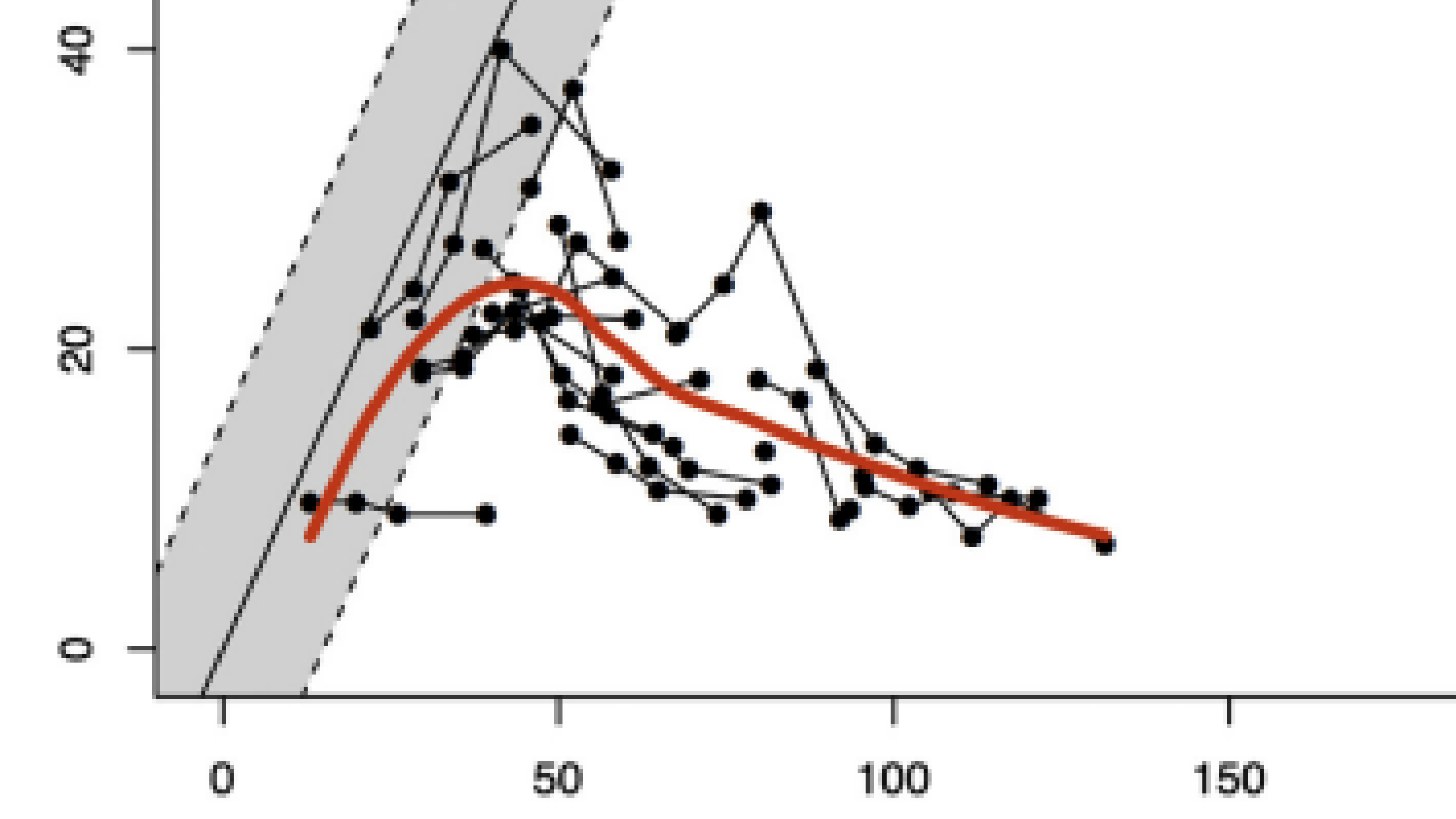
Consultant Paediatric Inherited Metabolic Disease, Manchester

Honorary Professor of Paediatrics and translational medicine

Medical Director, NIHR children's clinical research facility

Shapiro et al 2016

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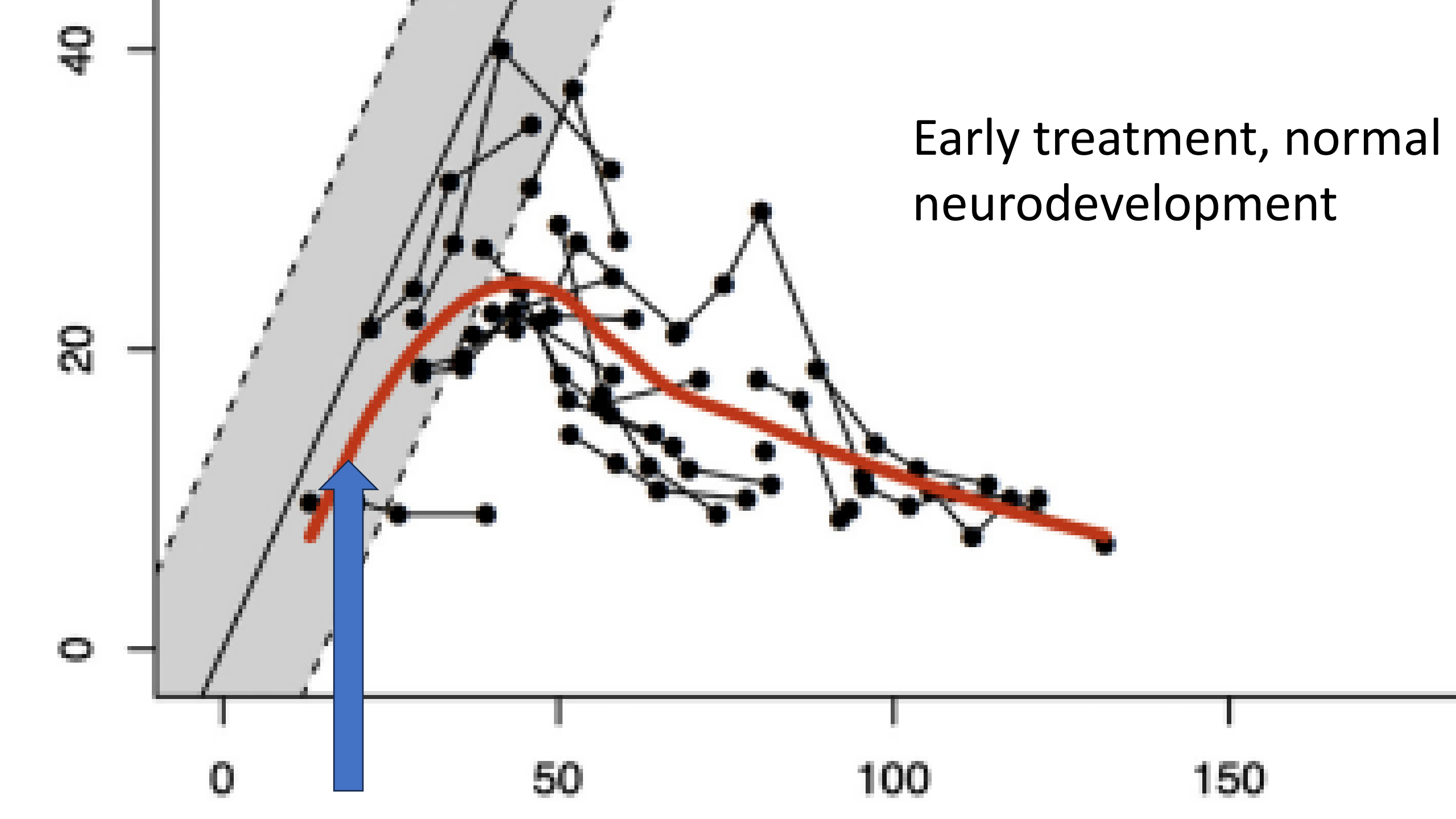
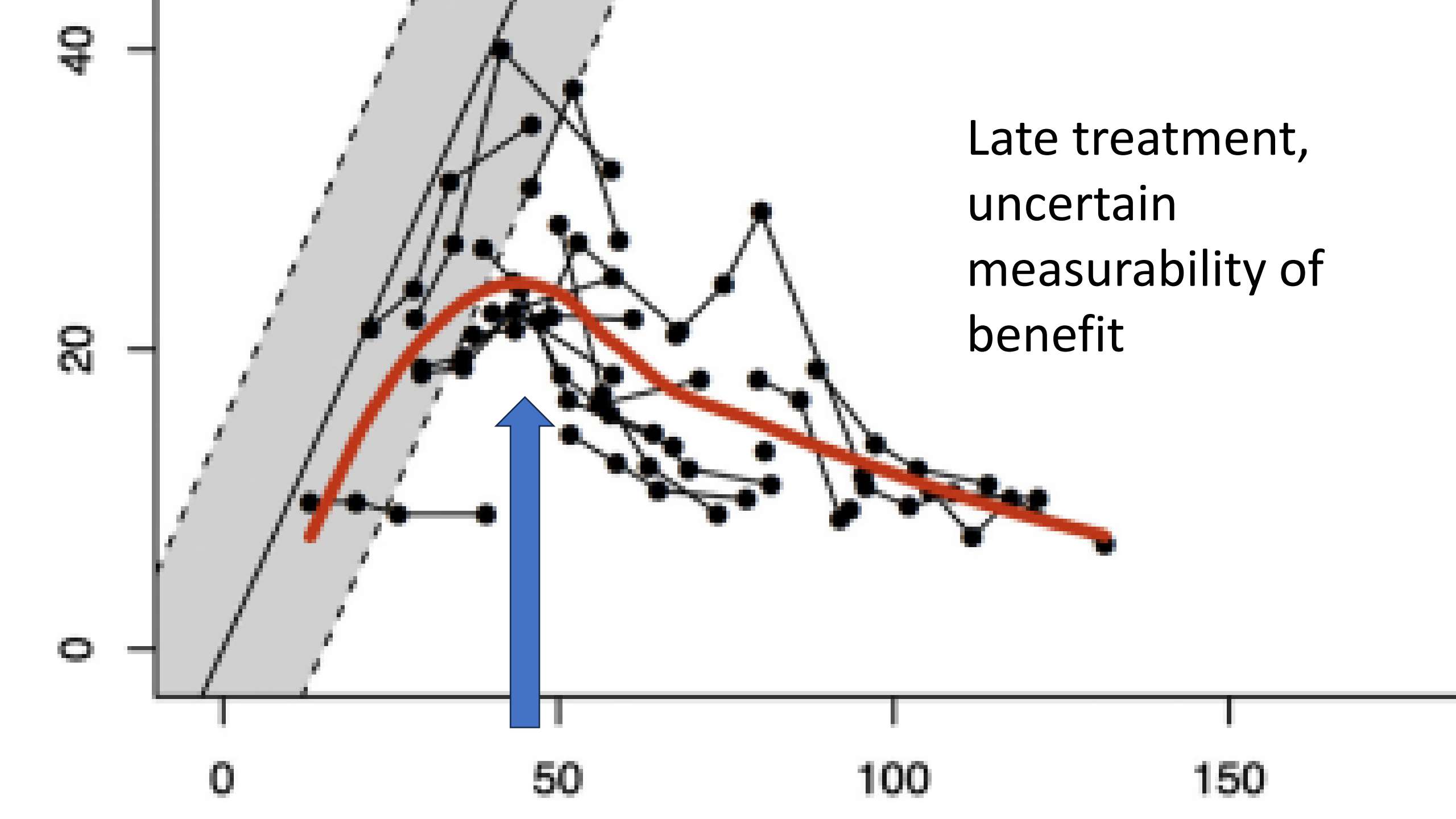


Early treatment, normal neurodevelopment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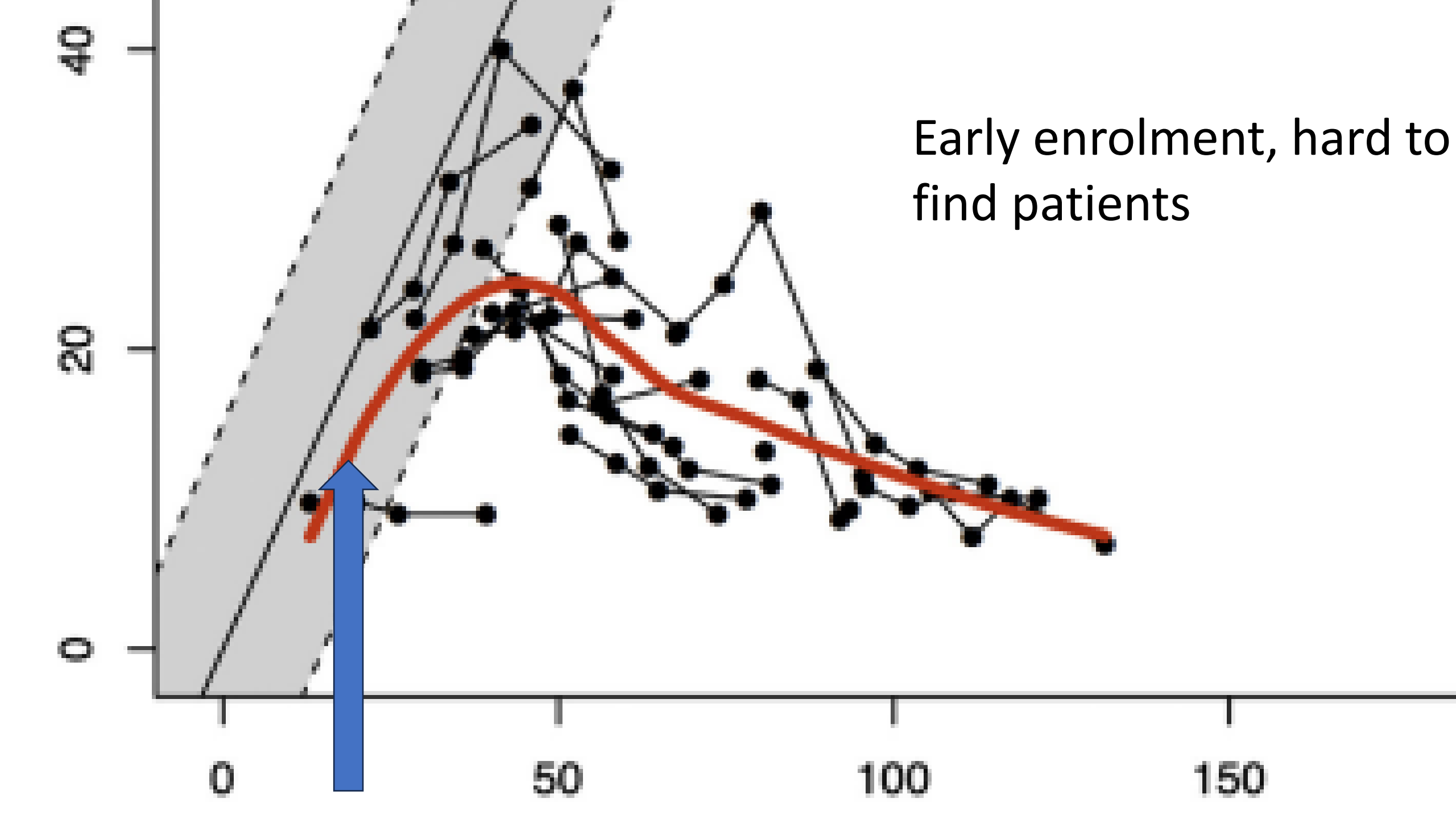
Late treatment, uncertain measurability of benefit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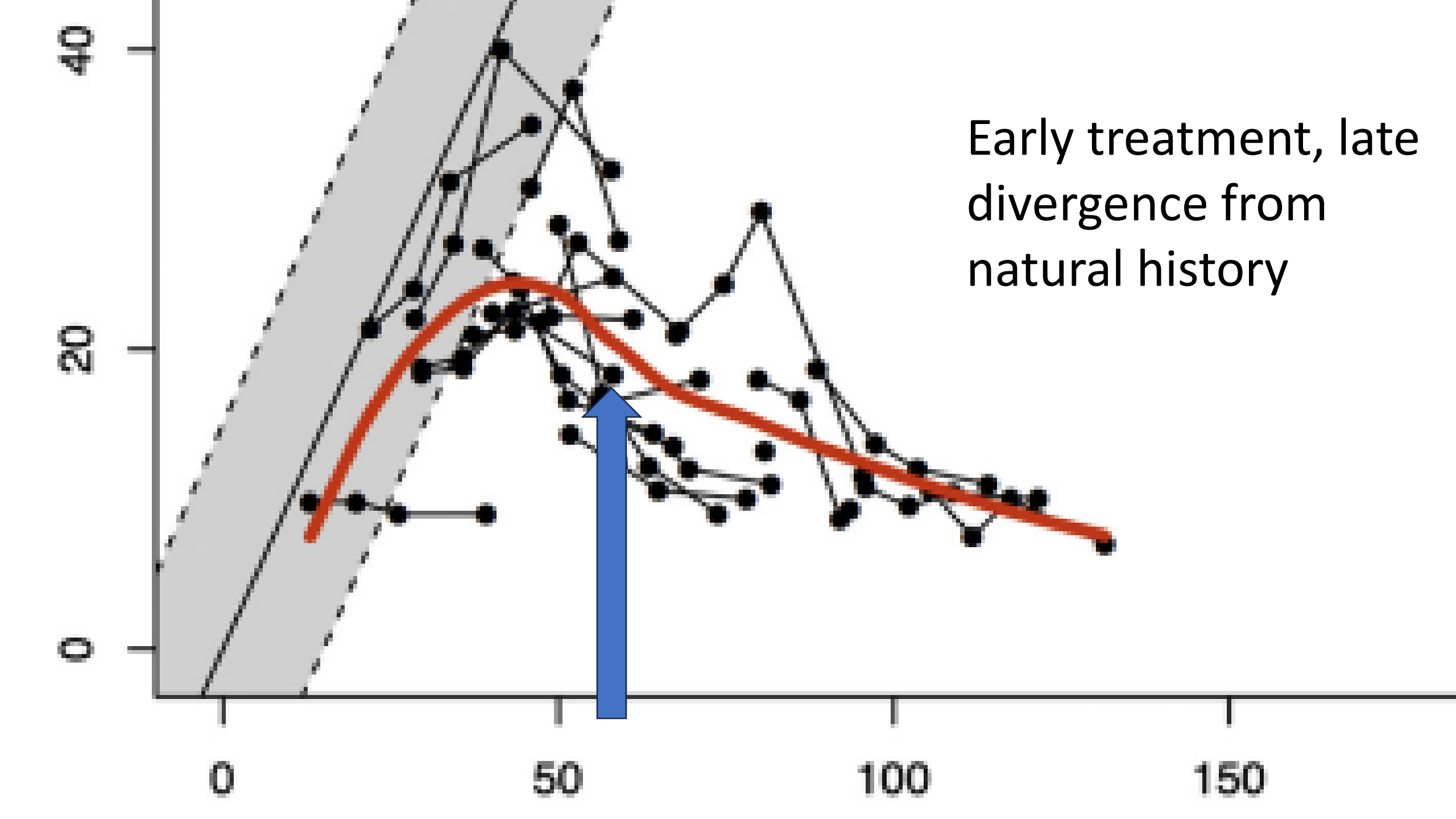
Early enrolment, hard to find patients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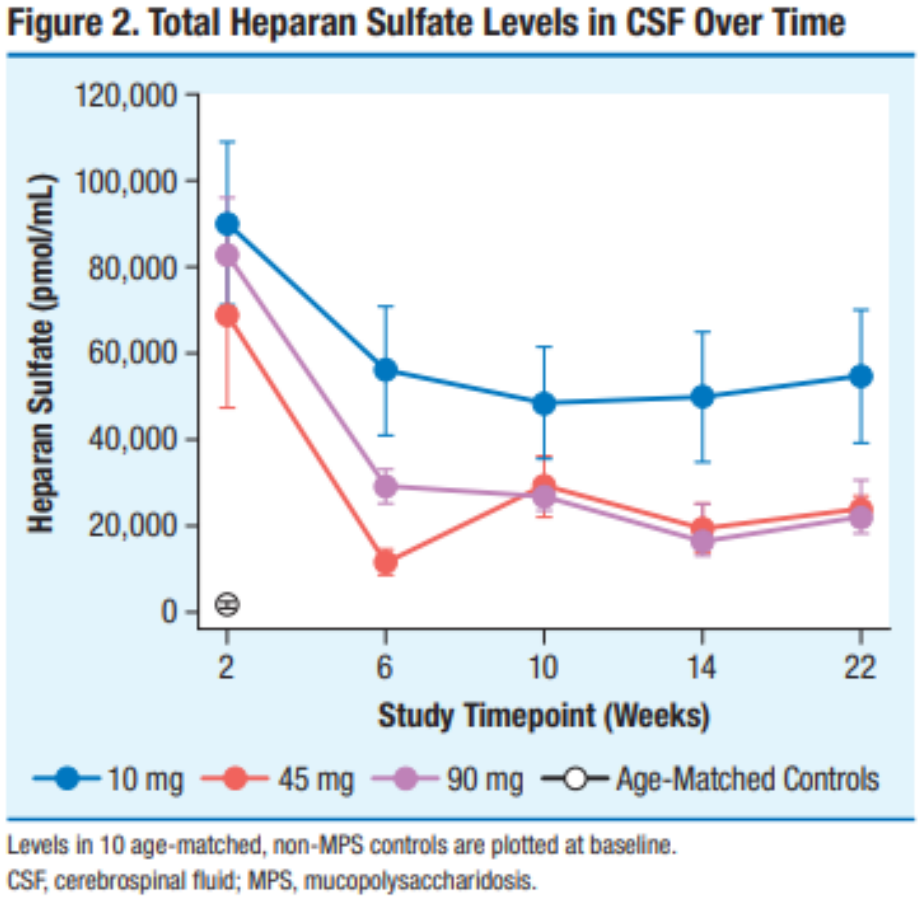
Early treatment, late divergence from natural history

A tale of three trials…..

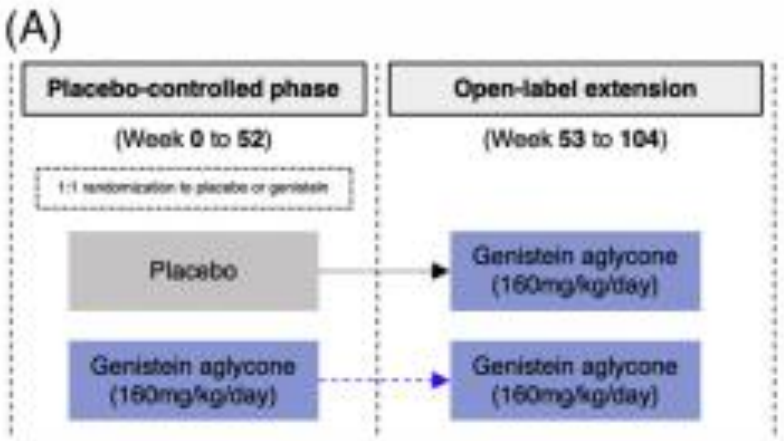
- Intra-thecal enzyme replacement therapy in MPSIIIA (Shire HGT/Takeda) phase I/II trial. Commenced 2010
- Genistein (isoflavone nutraceutical) in MPSIII phase III trial (academic), commenced 2015
- Lentiviral ex vivo stem cell gene therapy (academic but funded by Orchard Therapeutics) in MPSIIIA, commenced 2020

Shire/Takeda phase I/II trial in MPSIIIA



- Monthly intra-thecal rhSGSH delivery 10-90mg
- Early data using an early GAG methodology showed large reduction in CSF HS suggesting almost complete clearance.
- Later analysis (alternate methodology) suggested this was more like 60% reduction (Jones et al 2016)
- Compare with approved ERT for CLN2 (Brineura, 300mg delivered alternate weekly via icv port)

Wijburg et al presented at ACMG 2013



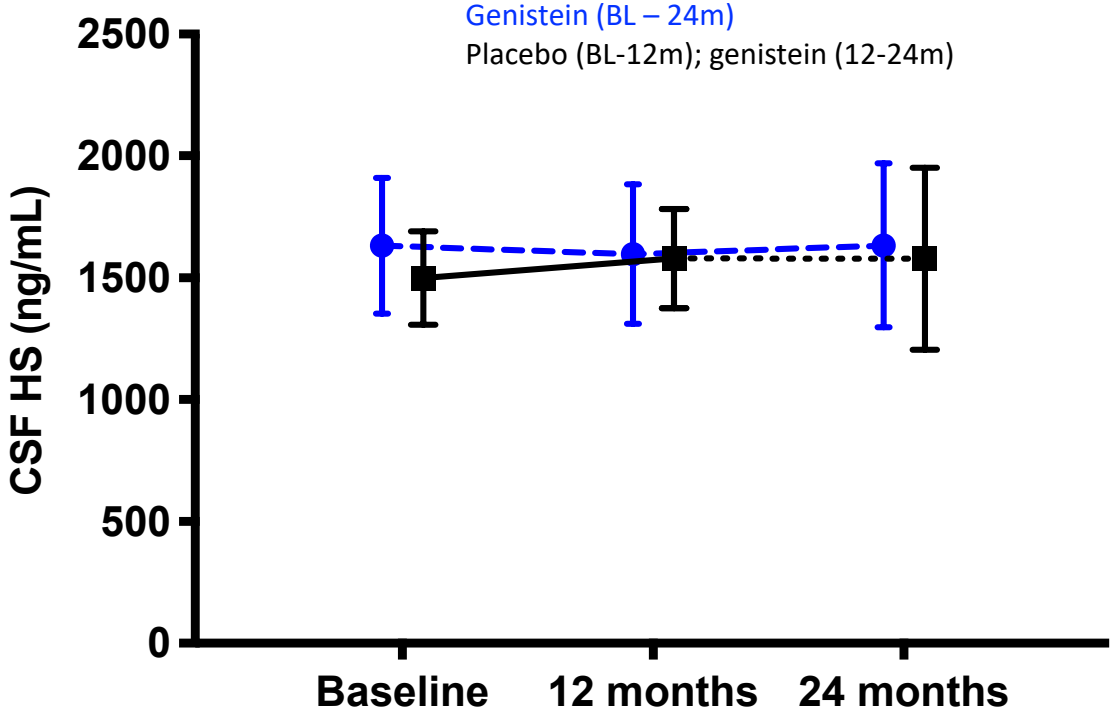
Received: 9 February 2021 | Revised: 25 May 2021 | Accepted: 27 May 2021
DOI: 10.1002/jimd.12407

ORIGINAL ARTICLE

JIMD SSIEM WILEY

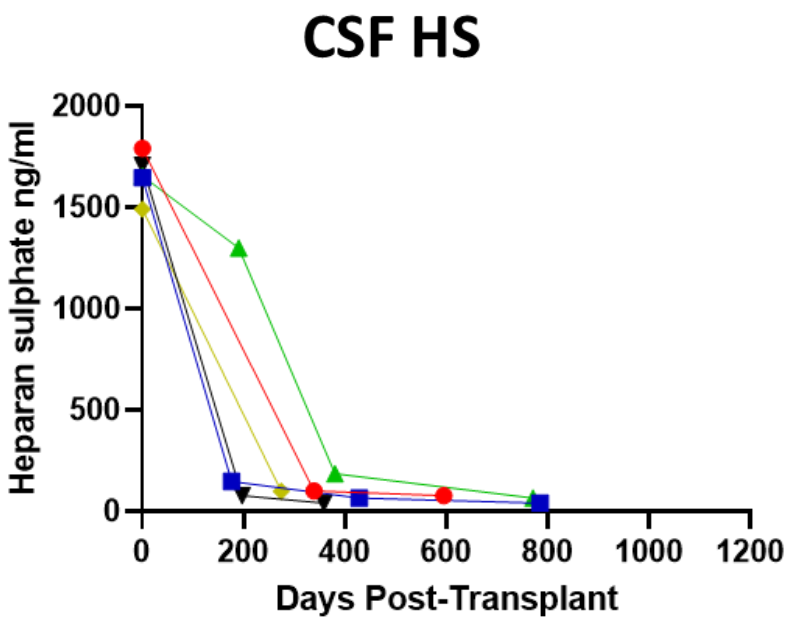
High dose genistein in Sanfilippo syndrome: A randomised controlled trial

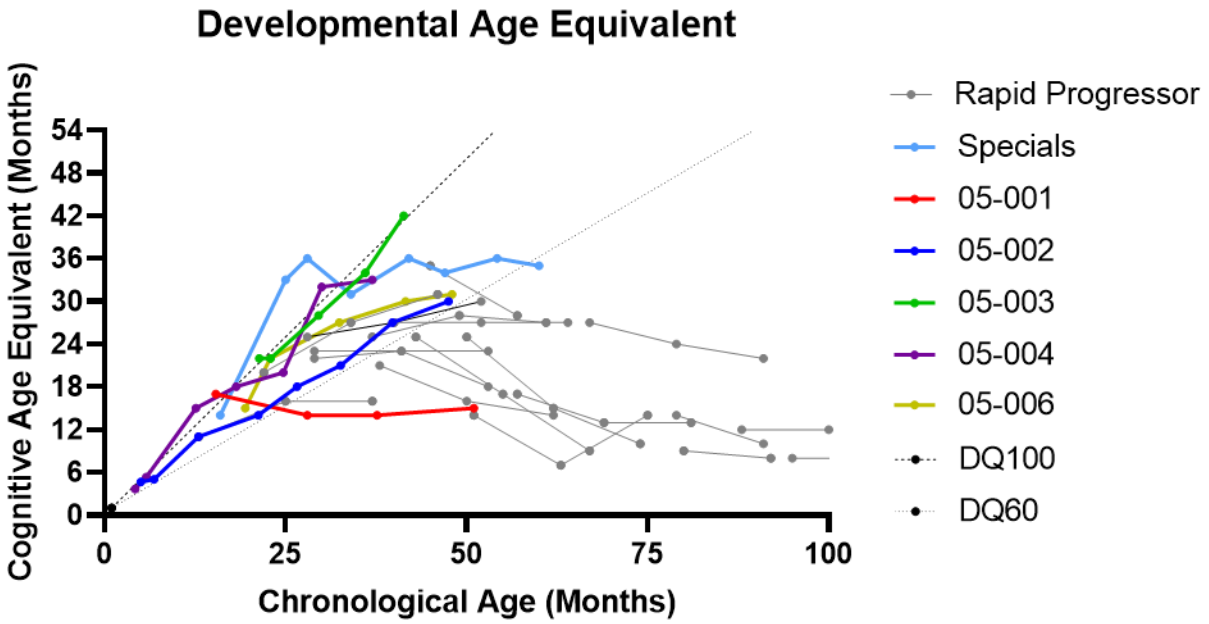
Arunabha Ghosh[1,2] | Stewart Rust[3] | Kia Langford-Smith[2] | Daniel Weisberg[3] | Maria Canal[4] | Catherine Breen[5] | Michelle Hepburn[6] | Karen Tylee[1] | Frédéric M. Vaz[7] | Andy Vail[8] | Frits Wijburg[9] | Claire O'Leary[2] | Helen Parker[2] | J. Ed Wraith[1†] | Brian W. Bigger[2] | Simon A. Jones[1]



- Why appropriate for perform a randomised trial in this disease then?
- MHRA discussion on CSF HS as primary endpoint
- CSF HS only 5.5% lower in treatment group – no evidence of likely clinically meaningful benefit

Lentiviral ex vivo stem cell gene therapy in MPSIIIA





Summary

- Trial design is highly challenging due to the natural history and nature of the clinical outcomes used in neuronopathic MPSs
- Early treatment (at birth) with long follow up (>5 years), plus a placebo group remains the ‘purest’ approach to demonstrate efficacy of a therapy however this is financially impossible and ethically inappropriate
- CSF HS can be closely linked to cognitive benefit but only in specific contexts (ie very early treatment)
- If we are to have therapies for neuronopathic MPS disorders we must approach clinical trials differently

Acknowledgements

Thanks to the MPSIII children and families and to the UK MPS society

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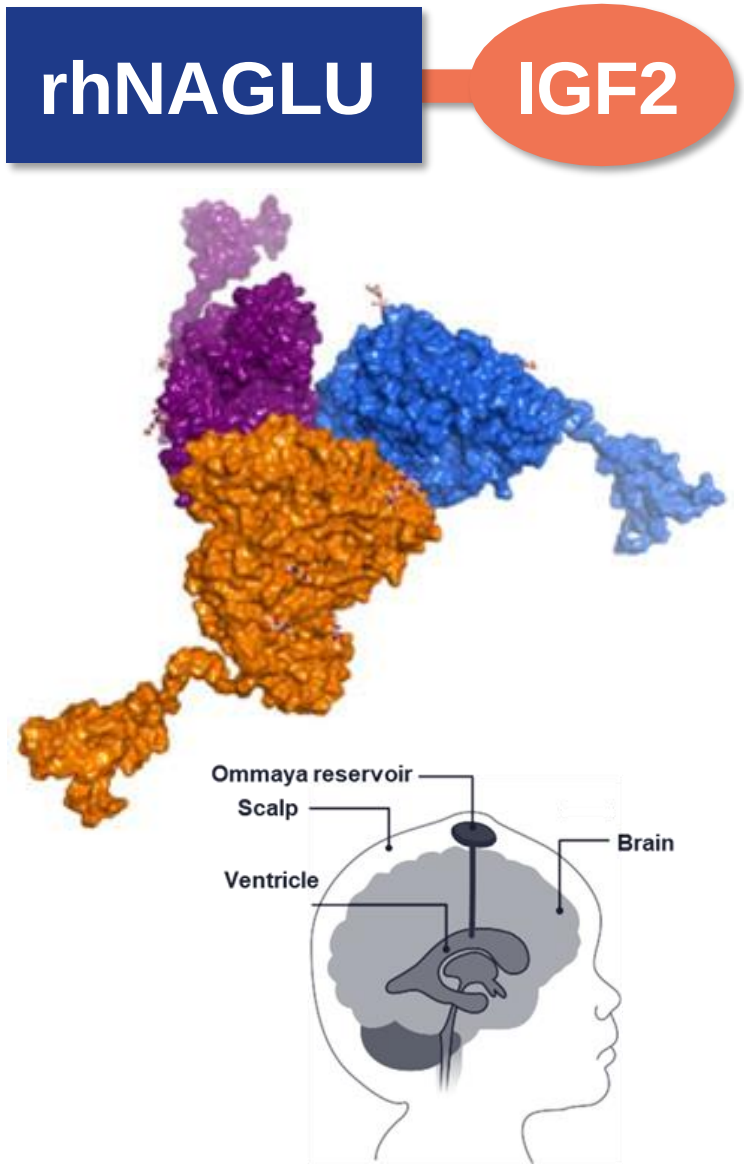
Tralesinidase Alfa protects Sanfilippo type B patients' cognitive functions by normalization heparan sulfate and preserving brain volumes

Eric Zanelli, PhD
Allievex Corporation

February 21, 2024

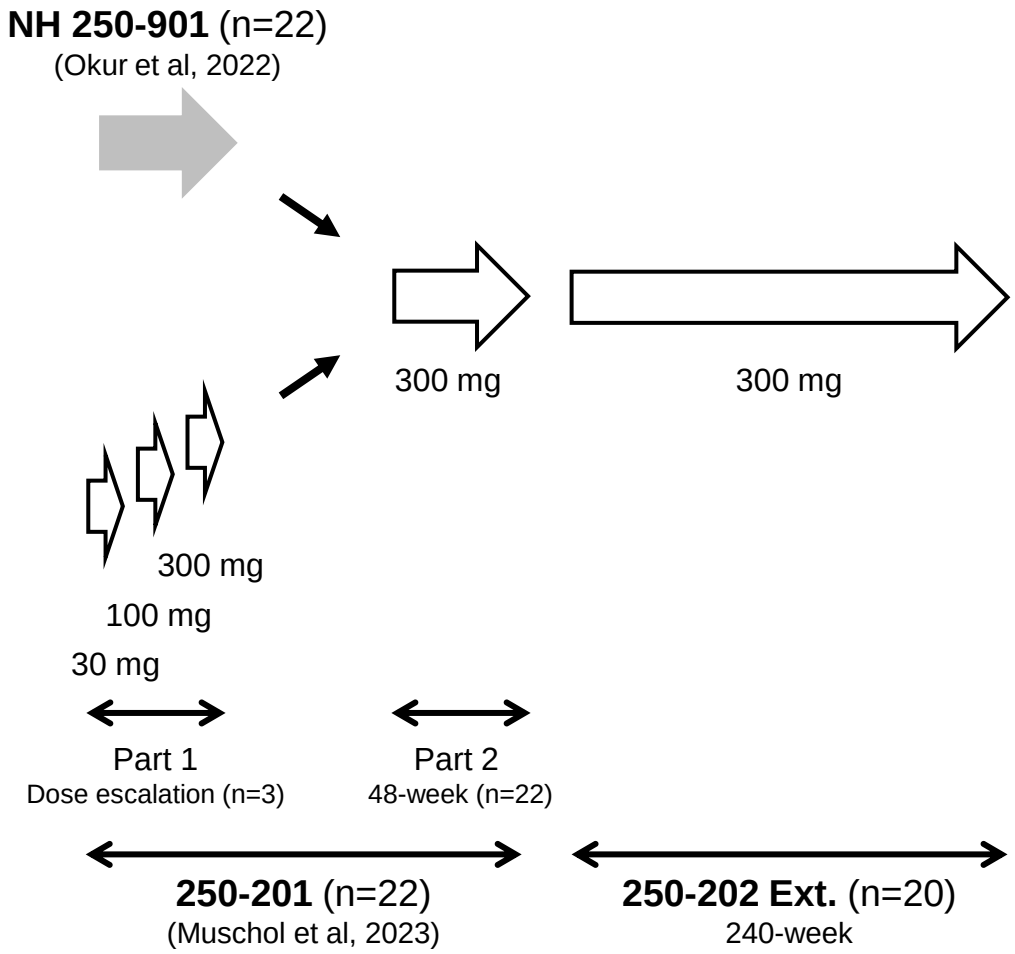
ALLIEVEX

TRALESINIDASE ALFA (TA) – AX 250



- Fusion protein trimer consisting of recombinant human alpha-N-acetylglucosaminidase (rhNAGLU) and truncated insulin-like growth factor 2 (IGF2)
- IGF2 tag allows glycosylation-independent lysosomal targeting (GILT) to enhance cellular uptake by cation-independent mannose 6-phosphate receptor (CI-MPR)
- Infused via Ommaya or Codman Holter Rickham reservoir bypasses the blood-brain barrier
- 300 mg delivered ICV once-a-week with infusion time of 5-10 minutes

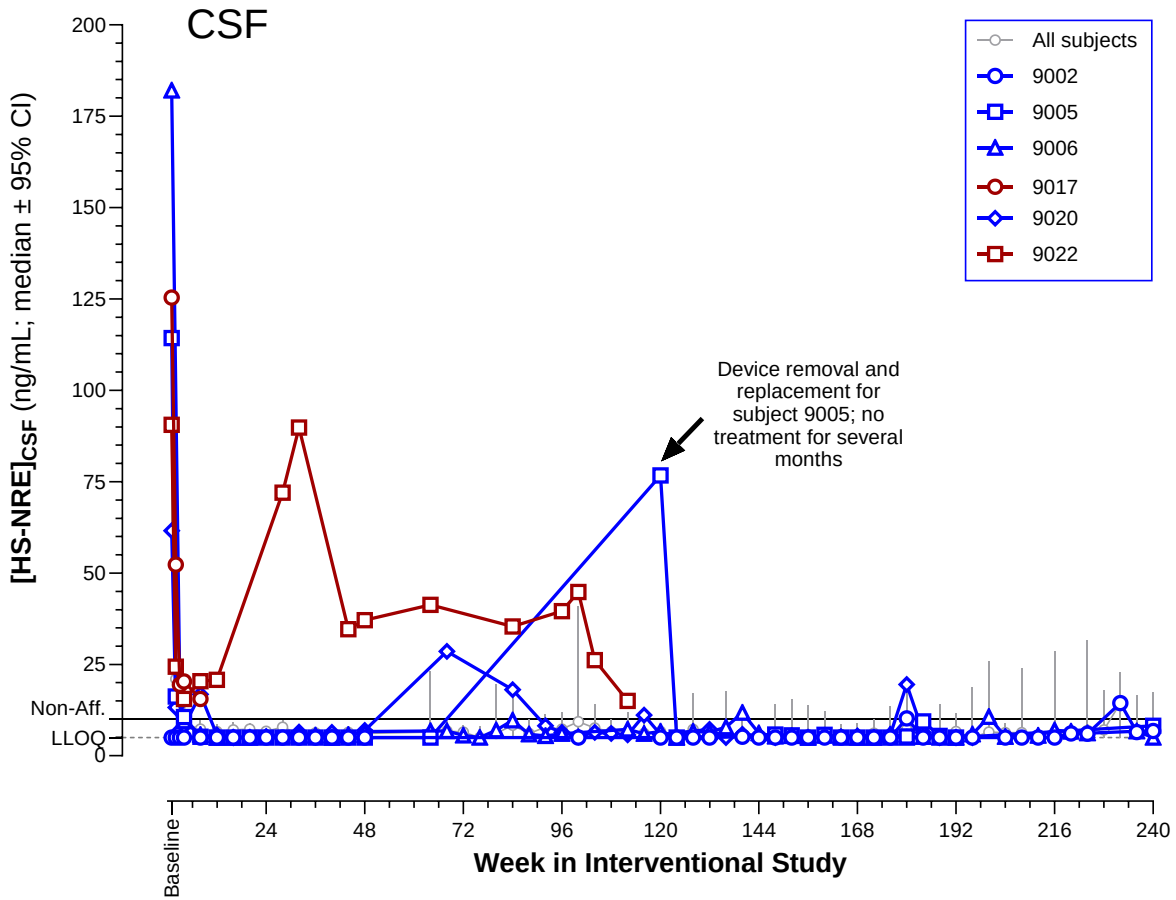
STUDY DESIGN AND ENDPOINTS

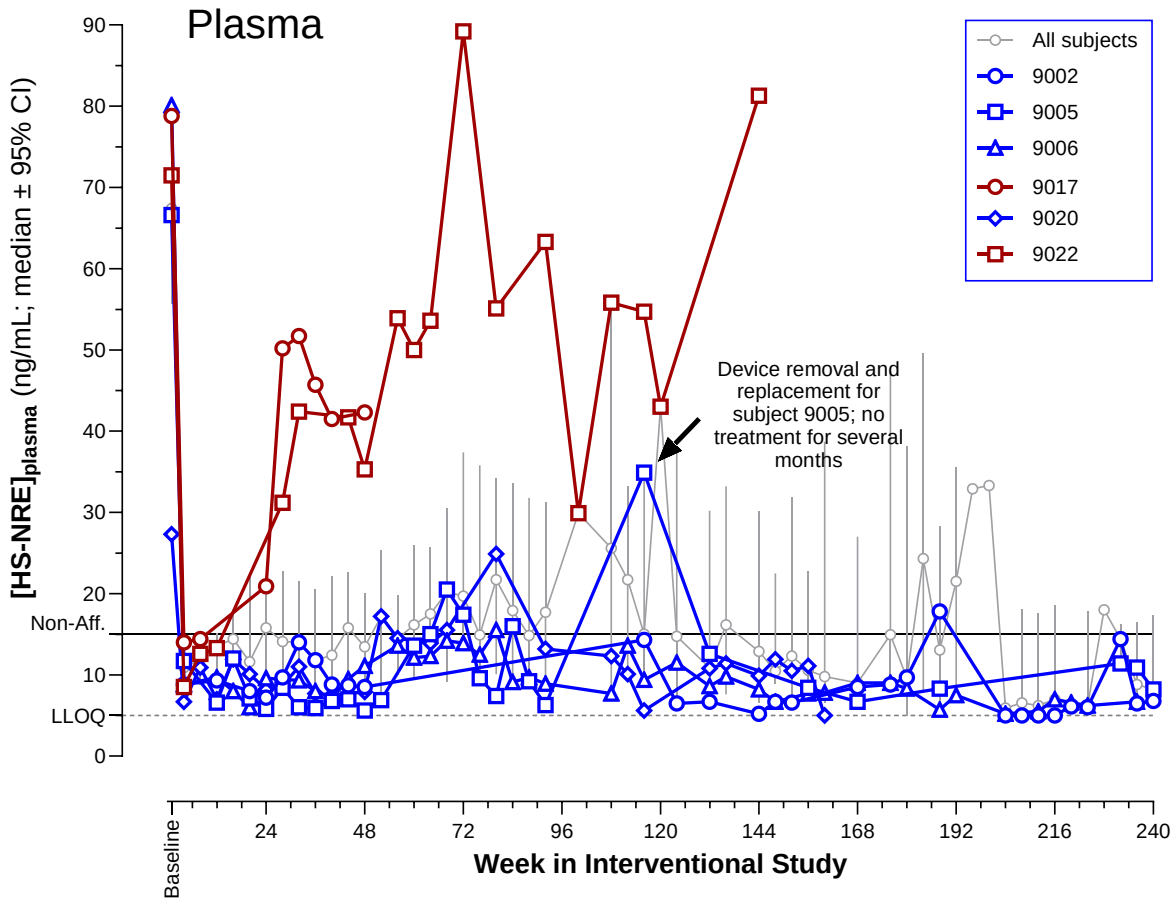


AX 250, 30, 100 or 300 mg weekly then bi-weekly

- **Cognition** (accepted primary endpoint by FDA)
  - Bayley Scales Of Infant and Toddler Development (BSID-III) ≤ 42 months or Kaufman Assessment Battery for Children (KABC-II) > 42 months
  - Data expressed as age-equivalent (AEq) to allow scoring continuity
- **Adaptive behavior**
  - Vineland Adaptive Behavior Scales (VABS-II) raw scores
- **Surrogate biomarkers**
  - Cortical grey matter volume (CGMV) measured by MRI
    - Average loss: **-35 mL/yr** in NH subjects (non-affected range: 489-648 mL)
  - CSF and plasma, MPS IIIB-specific, heparan sulfate non-reducing ends (HS-NRE) measured by LC-MS/MS method
    - Non-affected 95th percentiles:
      - CSF = **10 ng/mL**; plasma = **15 ng/mL**
    - Expressed as AUC from week 8 to last visit divided by week of follow-up to correct for missing values, differences in treatment duration and outliers

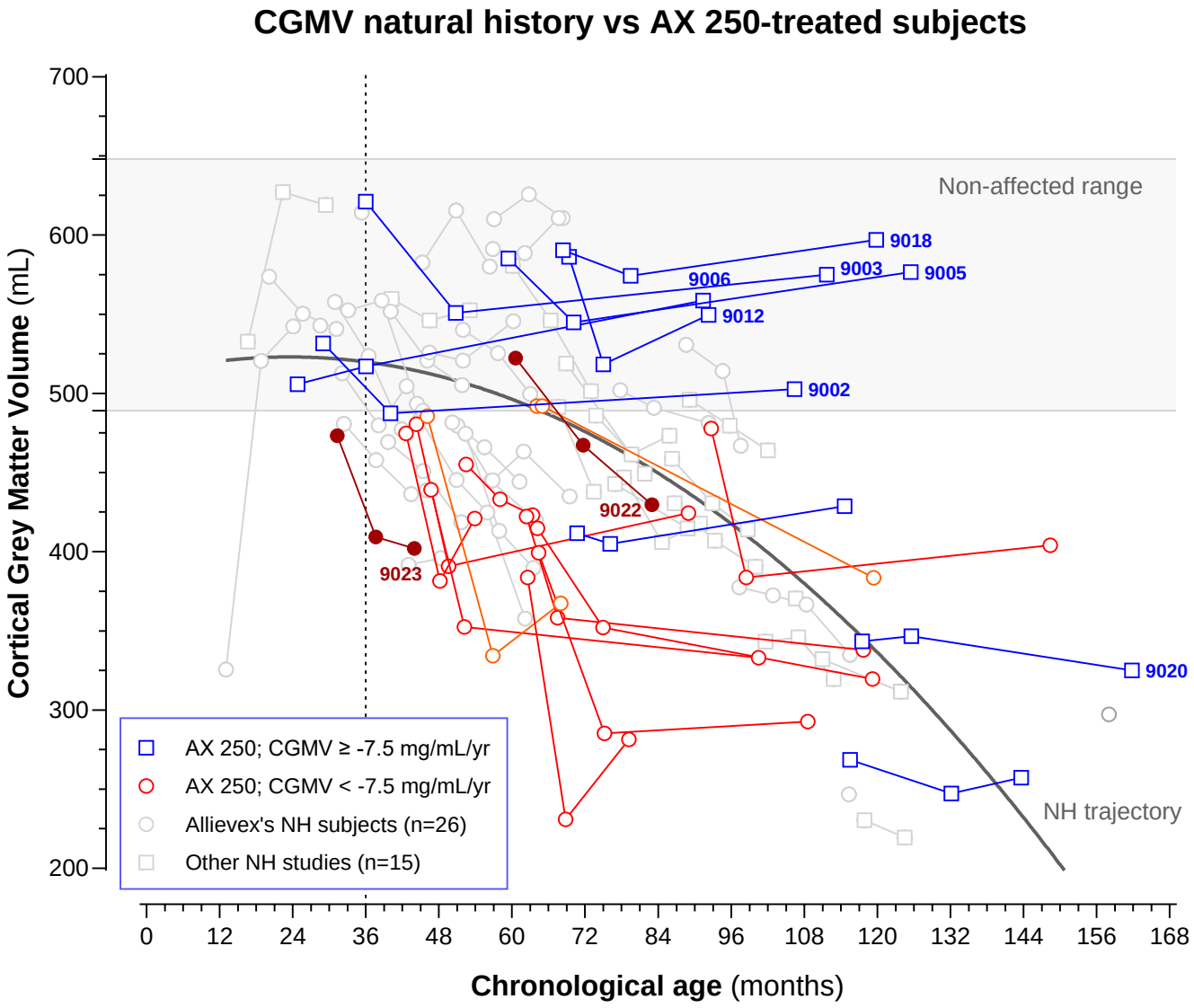
HS-NRE NORMALIZATION DEMONSTRATES TARGET ENGAGEMENT



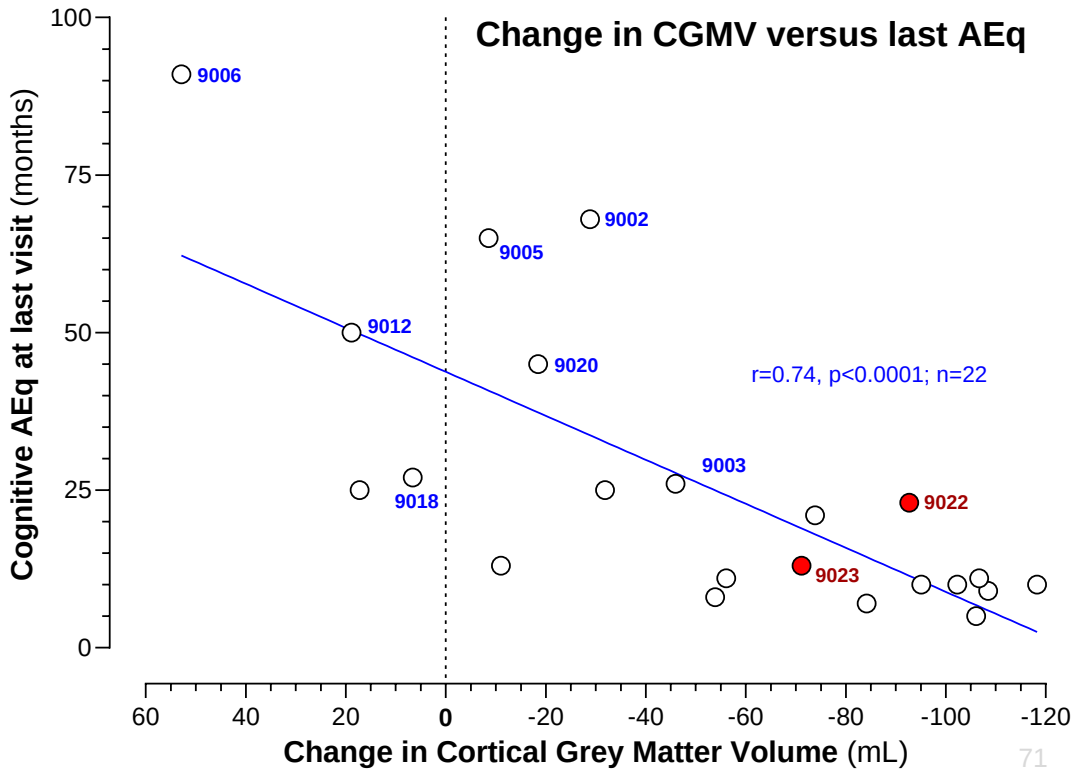


- Subjects with cognitive skills measured by KABC-II instrument, i.e., 9002, 9005, 9006 and 9020, sustain normal CSF and plasma HS-NRE levels. HS normalization is NOT sustained without treatment.
- Plasma HS-NRE has more dynamic range and can still be measured when subjects have treatment interruptions, e.g., device removal.

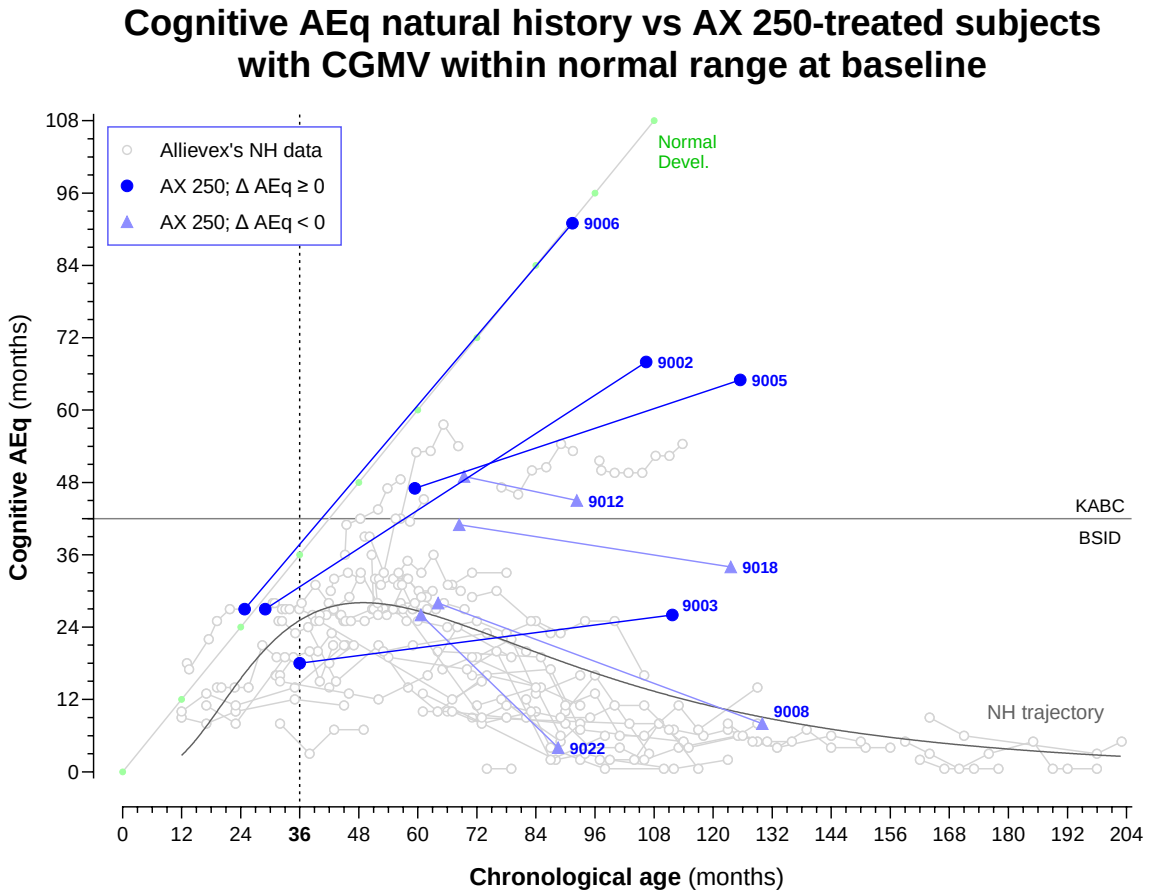
CGMV PRESERVATION CORRELATES WITH COGNITIVE OUTCOME

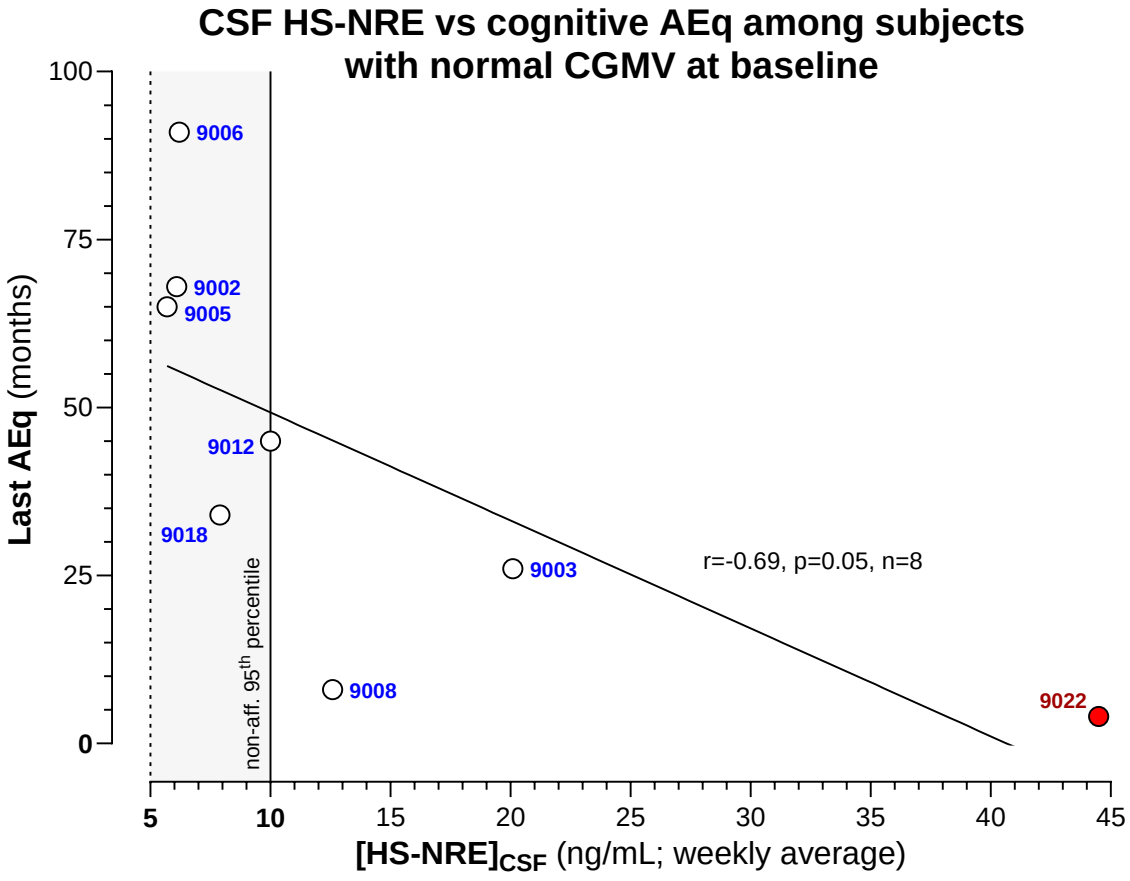


- 5 subjects have normal CGMV after > 3 years of treatment, sometimes after > 6 years.
- After an initial drop in CGMV due to acute elimination of HS from the brain tissue, tralesinidase alfa stops or limits brain atrophy in all subjects.
- There is a significant correlation between change in CGMV and AEq score at last visit.



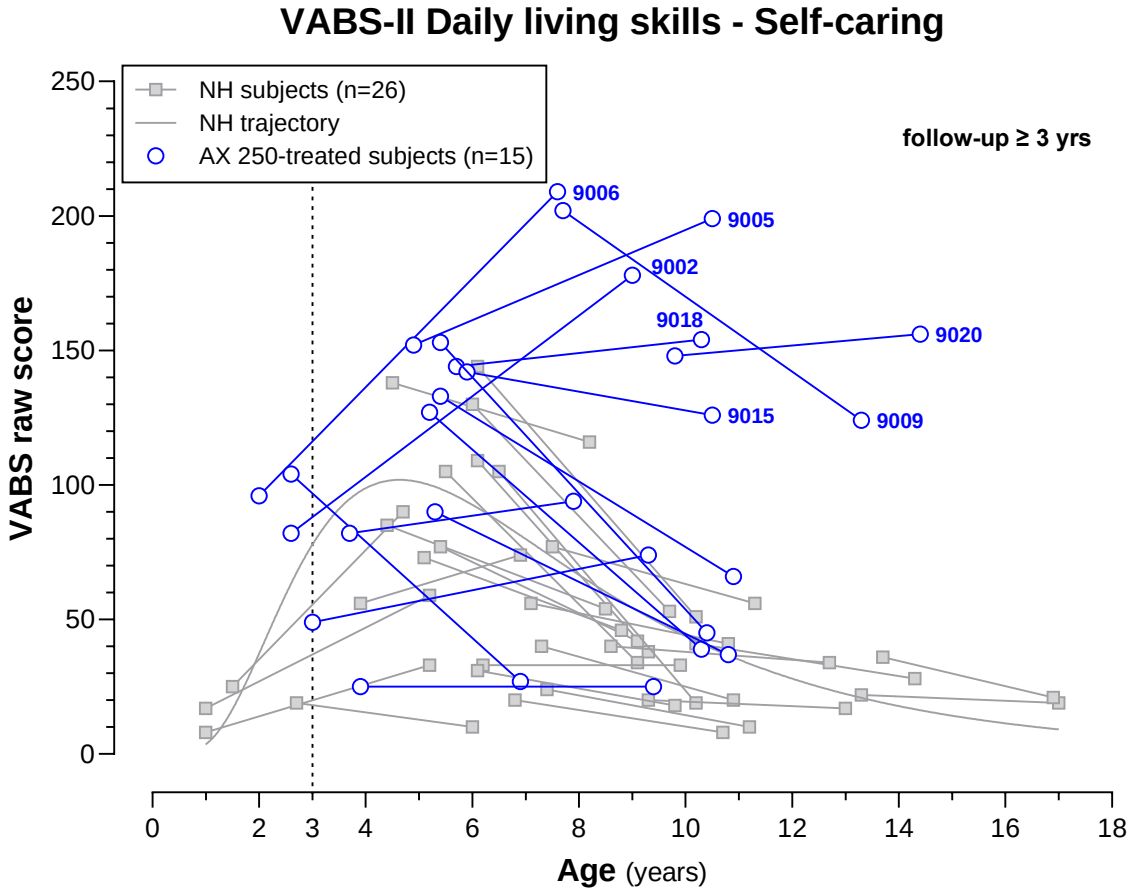
CSF HS-NRE NORMALIZATION ALONE PREDICTS GOOD COGNITION AMONG SUBJECTS WITH CGMV WITHIN NORMAL RANGE AT BASELINE

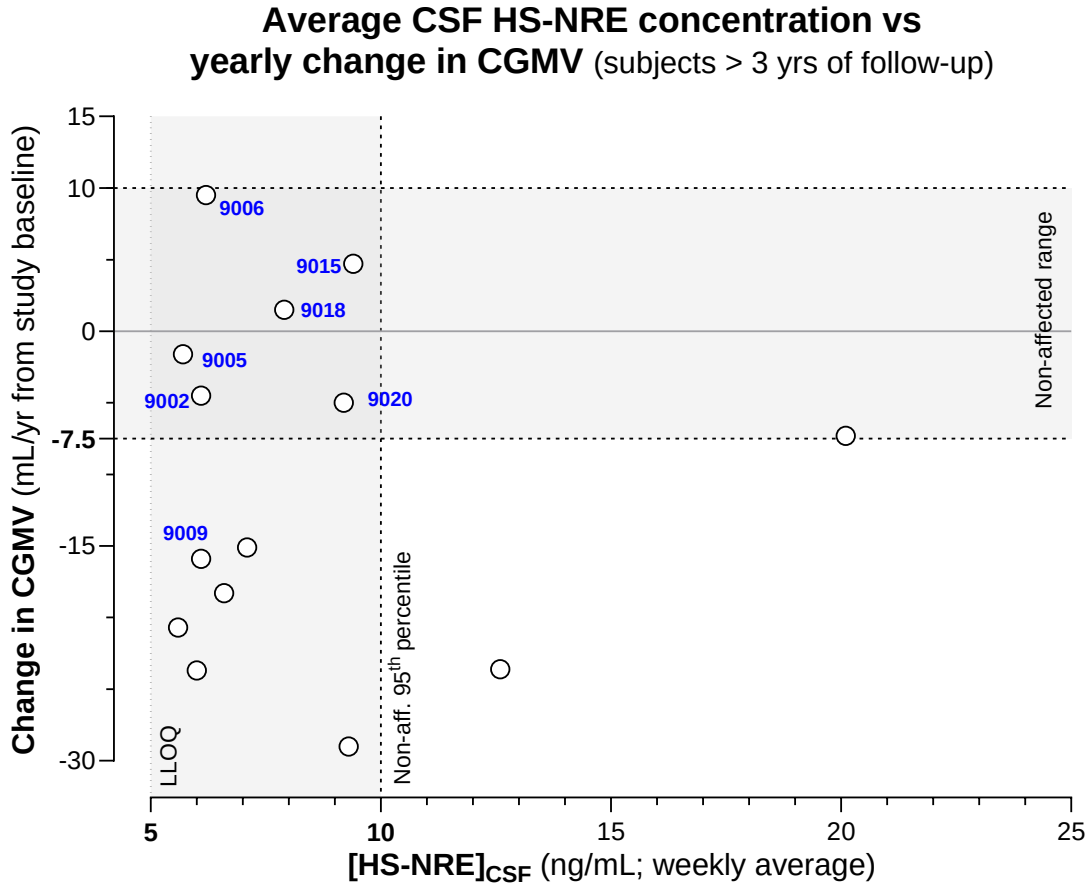




- 4 out of 8 subjects with normal CGMV at baseline have higher cognitive AEq after ≥ 6 years of treatment than at baseline; 3 of these 4 subjects were aged ≤ 3 at treatment initiation
- Subjects with highest AEq at last visit are subjects with sustained normalized HS-NRE in CSF

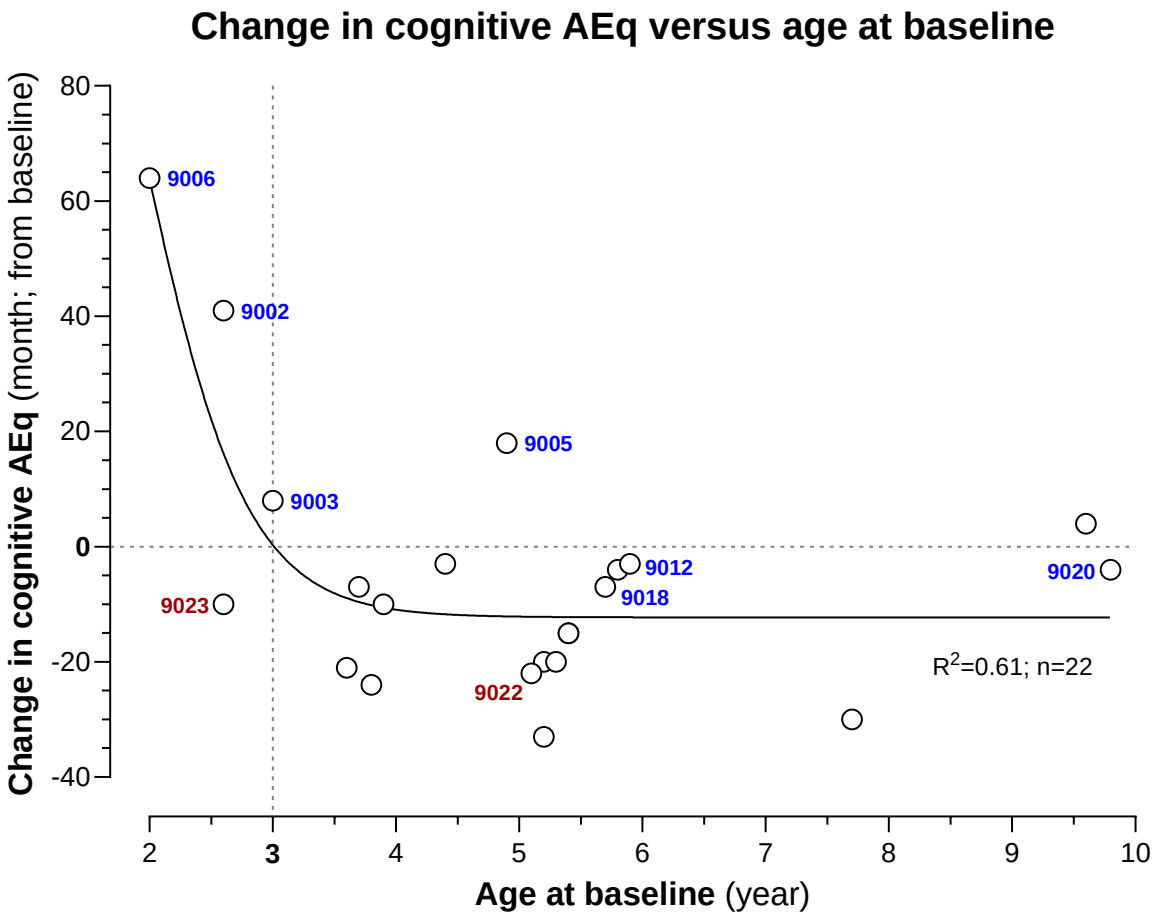
CSF HS-NRE NORMALIZATION COMBINED WITH LIMITED LOSS OF CGMV PREDICTS HIGH ADAPTIVE BEHAVIOR AMONG ALL SUBJECTS





- 7 AX 250-treated subjects have VABS-II self-caring raw score > 120 past 8 years of age after > 3 years of follow-up; only one of them (subject 9009) shows significant drop from baseline
- Preservation of CGMV and HS-NRE normalization in CSF combined correctly identify the 6 subjects with score > 120 without significant drop, i.e., 9002, 9005, 9006, 9015, 9018 and 9020

POINTS OF DISCUSSION



- Value of a surrogate marker *"reasonably likely to predict clinical effectiveness"* might depend on:
  - Age at baseline,
  - Preservation of brain volumes at baseline,
  - Route of administration,
  - Choice of clinical outcome assessment.
- If cognition measured as BSID-III raw scores is the only accepted clinical endpoint, only patients recruited ≤ 3 years of age are likely to consistently show best treatment benefits.
- CSF HS-NRE alone predicts best cognitive response in subjects ≤ 3 years of age and/or with normal CGMV at baseline.
- Cognition might not be the first choice for patients/caregivers. Patients affected with MPS III might benefit from treatment in other daily life assessments that are more important to the patients and their family.

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We owe an immense debt of gratitude to the subjects and their parents/caregivers for participating in this study, and we thank all the clinical staff, neuropsychologists and supporting individuals essential to the study implementation and execution. I would also like to express my gratitude to the individual clinical and research institutions and study staff of each of our collaborators for their support and commitment to the tralesinidase alfa program.

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- Joseph Kovalchin, Bernice Kuca, Uday Patel, Thomas Mathers, Sean Deller, Pat Gearing, Wendy Harrington, Allievex Corporation

Reduction of CSF HS exposure correlates with improved long-term cognitive function in patients with MPS IIIA following treatment with UX111 gene therapy

Heather A. Lau, MD, MS

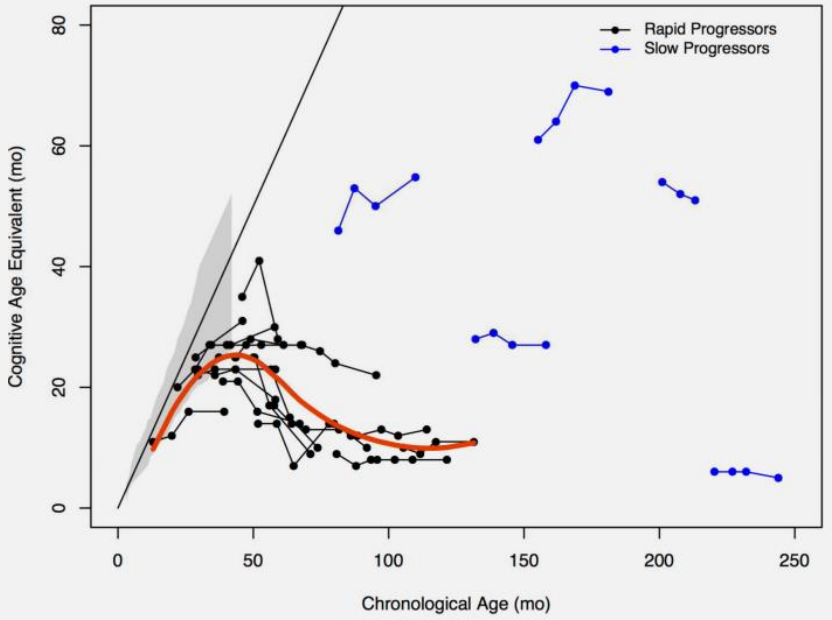
Ultragenyx Pharmaceutical Inc., Novato, CA

Presented at WORLDSymposium 2024; February 4-9, 2024; San Diego, CA, USA

Sanfilippo Syndrome Type A (MPS IIIA):
Irreversible neurodegeneration & early death

- Single enzyme defect leading to deficiency of sulfamidase (SGSH) and toxic accumulation of HS
- Triphasic clinical course
  - Age 0-24 mos: positive developmental slope
  - Age 24-48 mos: developmental slope approaching 0
  - Age >48 mos: negative developmental slope
    - Regression in all domains of development (language, cognition, and motor)

Trajectory of cognitive growth by age compared with published normative growth data (*gray-shaded area*)



Red line indicates the growth trajectory (slope) for the RP group only

Viana GM, Priestman DA, Platt FM, et al. (2020) Brain Pathology in Mucopolysaccharidoses (MPS) Patients with Neurological Forms. *J Clin Med* 9(2); Wagner V and Northrup H (2019) 2019 Sep 19. In: Adam MP, Mirzaa GM, Pagon RA, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle. *GeneReviews.*

Shapiro EG, Nestrasil I, Delaney KA, et al. (2016) A Prospective Natural History Study of Mucopolysaccharidosis Type IIIA. J Pediatr 170: 278-287.e271-274.

CSF HS is a primary disease activity biomarker for neuronopathic MPS

Late biomarkers herald irreversible cognitive decline

Early biomarkers that are proximal to the genetic defect

May herald metabolic dysfunction prior to irreversible damage

Qualified assay methodology that is sensitive and specific

Rapid Reduction in CSF HS reflects degradation of HS within lysosome of brain cells after treatment

Intermediate biomarkers: downstream to genetic defect

May not be involved in the primary affected metabolic pathway

Secondary storage of toxic GM2/GM3 gangliosides occurs in MPS

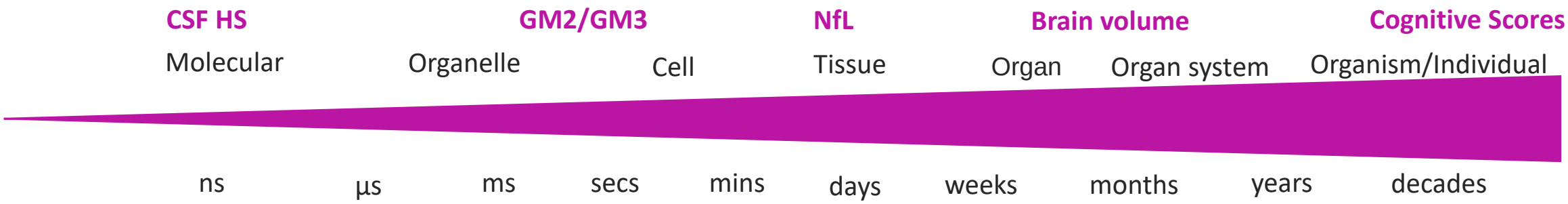
Reduction suggests recovery of organelle/cell function

Late biomarkers may reflect organ level function

May support correlation of primary disease activity marker to later clinical outcomes

Reduction in Neurofilament (NfL): reflects reduced neuronal tissue injury

Stable Brain volumes on MRI: reflect neuronal cell preservation



Minami Ket al. Int J Mol Sci. 2022 Oct 3;23(19):11724.
Saville et al. Genetics in Medicine Vol 21,:3,2019, 753-757
Kakkis ED et al. Orphanet J Rare Dis. 2015.
Figure adapted from Aßmus, Wet al. Expert review of molecular diagnostics. 6. 891-902, 2006.

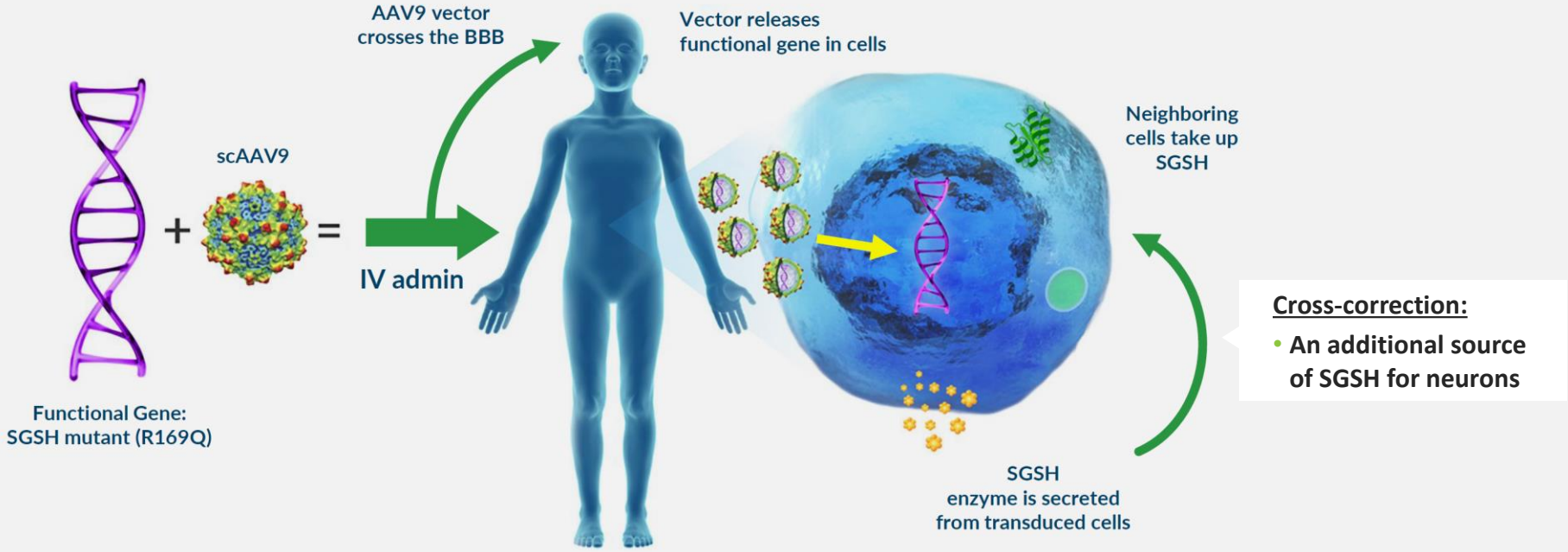
UX111 is designed to target underlying SGSH Enzyme Deficiency:

Expression of functional enzyme clears toxic levels of HS in cells

scAAV9.U1a.hSGSH MPS IIIA gene therapy encodes a full-length copy of *hSGSH* transgene with the ubiquitous murine U1 promoter

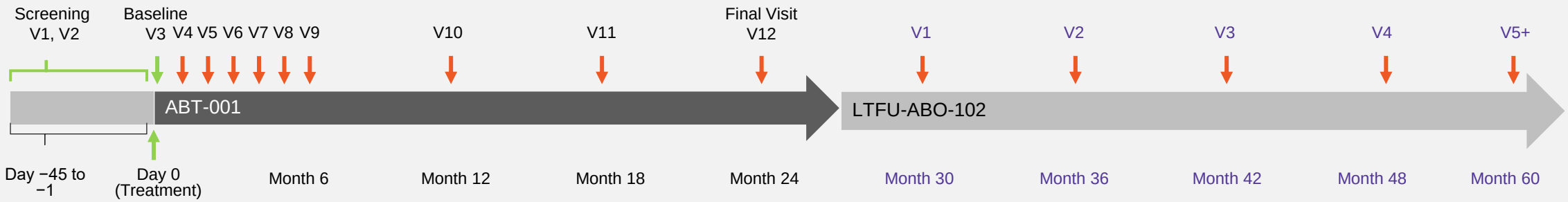
UX111 IV gene therapy results in reduction of the substrate, HS, based on both preclinical and clinical data

UX111 is under investigation as a therapy for children with MPS IIIA



Patients treated with UX111 are followed for 5+ years to see full clinical benefit

Visit Schedule for ABT-001 and LTFU-ABO-102 Studies



Enrollment

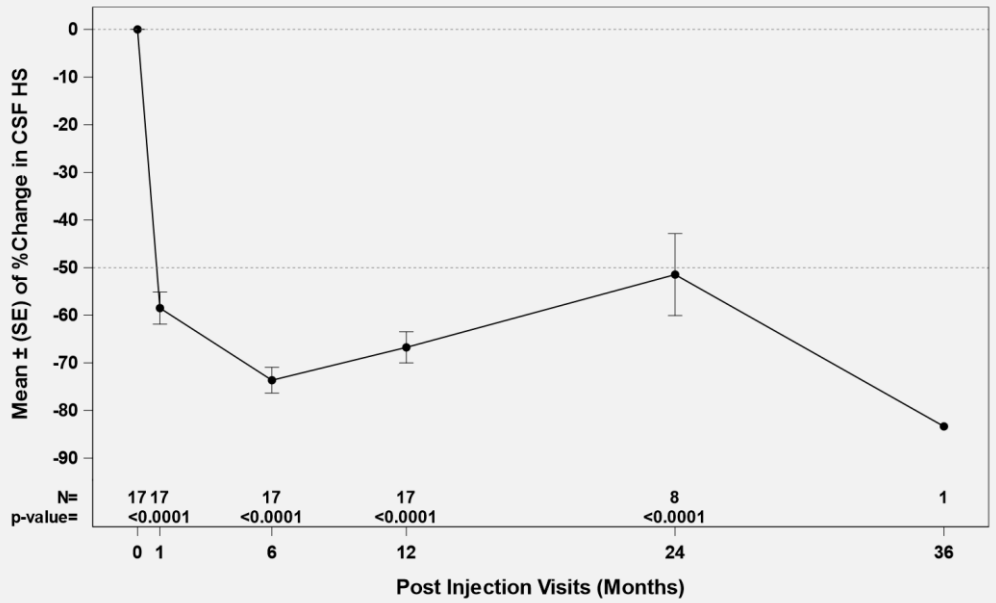
- The ABT-001 study designed as a 24-month Phase 1/2/3 study
  - Excluded attenuated genotypes
- Cohort 1 (0.5 x 1013 vg/kg): n = 3
- Cohort 2 (1 x 1013 vg/kg): n = 3
- **Cohort 3 (3 x 1013 vg/kg):** n = 22
- **Target population (mITT) defined as ≤2 years old or >2 years and cognitive DQ ≥ 60, n = 17**

As of 16 Aug 23 Data Snapshot:

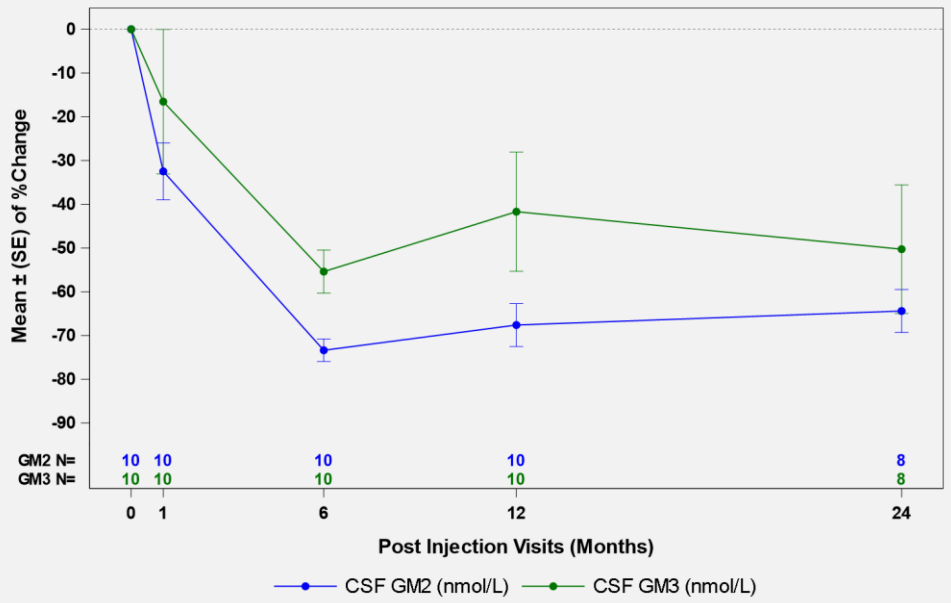
- 28 patients have been treated with UX111 in ABT-001
- 19 patients rolled into long term follow-up study (LTFU-ABO—102)
- 17 patients are in the mITT; at time of snapshot
  - 15 of the 17 mITT patients reached at least 30 months of age
  - 6 of 17 patients reached ≥ 60 months of age
  - Mean (SD) duration of follow-up of the mITT was 28.59 (17.05) months (range 11.3 to 60.1 months)

>50% Rapid and Sustained Reduction in CSF HS After Treatment with UX111

Primary Biomarker: CSF HS Mean Percent Change Over Time (%)



Secondary Biomarker: CSF GM2 and GM3 Mean Percent Change Over Time (%)



Reduction in CSF GM2/GM3 (toxic gangliosides) verifies that magnitude of CSF HS reduction is sufficient to resolve lysosomal dysfunction

mITT group represents the subset of Cohort 3 patients (Study ABT-001) with either age ≤24 months at treatment or age >24 months at treatment with baseline BSITD-III Cognitive DQ ≥ 60. If BSITD-III not performed at screening, Mullen Scales of Early Learning DQ used instead of BSITD-III DQ. P-values are based on MMRM (mixed models for repeated measures) using post-baseline visits as categorical visits. Reflects data through snapshot date of 16 August 2023.
CSF, cerebrospinal fluid; GM2, ganglioside type 2; GM3, ganglioside type 3; HS, heparan sulfate

Quantifying Toxic HS using Time Normalized Area Under the Curve (AUC)
Best Reflects Brain Injury Potential Like Phenylalanine in Phenylketonuria (PKU)

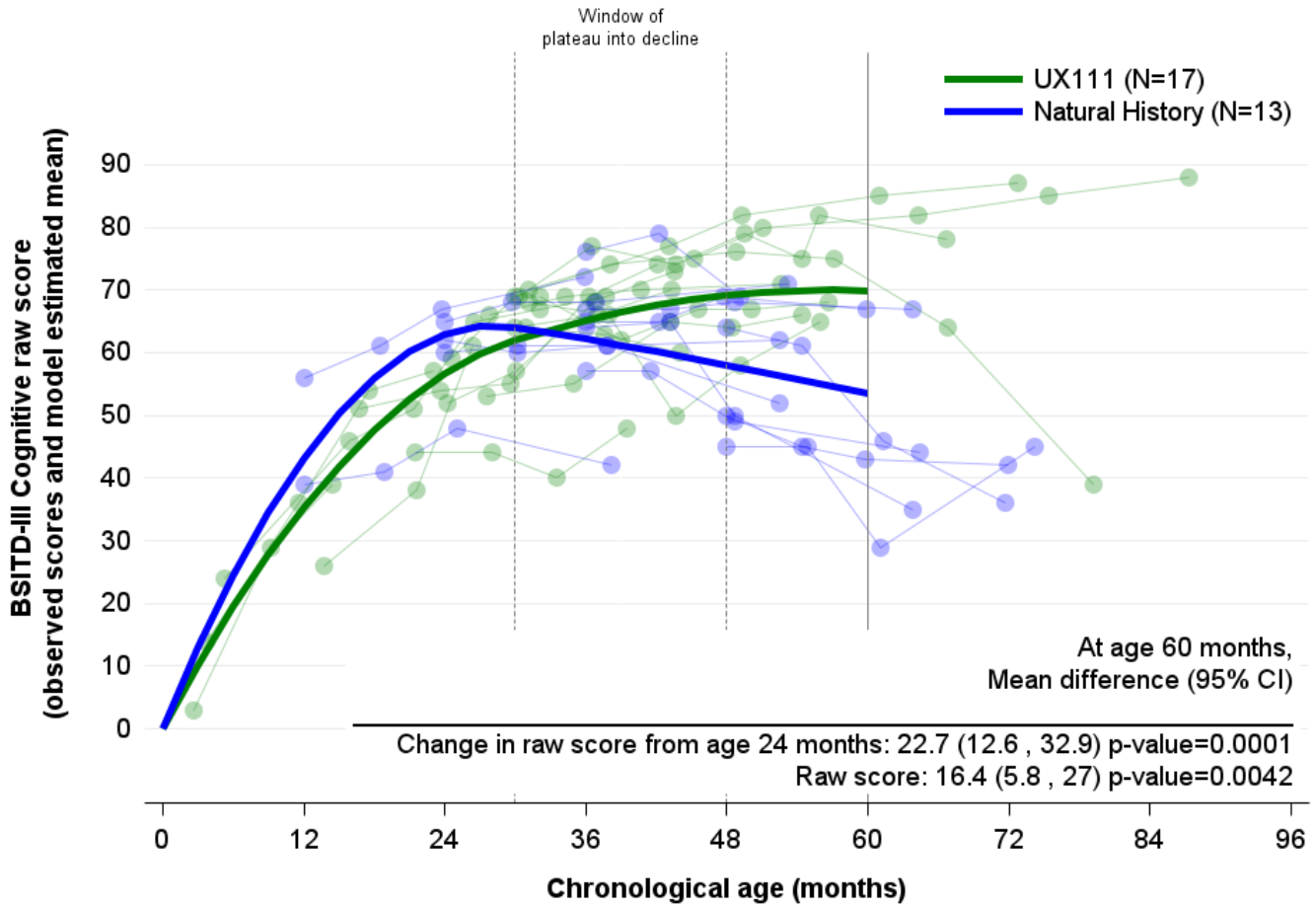
- Patient specific metric which accounts for fluctuations using all available CSF HS levels post-treatment
- May then be utilized to assess the relationship with cognitive outcomes
- **Mean reduction in CSF HS exposure was -63.3% (95% CI, -69.7%, -56.9%) in mITT**; over a median follow-up ~2 years

Example of CSF HS Exposure Calculation

Months since UX111 Administration	% Change in CSF HS	AUC	CSF HS Exposure (time-normalized AUC)
0.0	0.0	0.0	-
1.0	-50.0	-24.6	-25.0
5.6	-83.3	-329.1	-59.3
12.1	-66.7	-817.0	-67.8
30.4	-66.7	-2039.2	-67.1
60.1	**-83.3**	**-4264.2**	**-71.0**

AUC, area under the curve; Phe, phenylalanine; PKU, phenylketonuria.
Waisbren et al. Mol Genet Metab 91(1-2): 63-20. 2007

Significant Improvements in Cognitive Raw Scores in UX111 Treated vs. Untreated



- **0-24 mos old:** All gaining in cognitive skills; no differentiation as expected
- **>24 mos old:** Cognition stabilizes or improves in most UX111 treated patients; declines in untreated
  - 1 treated patient developed anti-SGSH immune response, CSF HS rebounded 1 year prior to cognitive decline
- **>48mos old**: **Clear differentiation**
- **24-60mos interval: there is a ~23 point mean increase in UX111 treatment group compared to untreated**
- **It takes years after CSF HS reduction to see this difference in clinical outcomes….**

Cognition measured by Bayley Scales of Infant and Toddler Development – Third Edition (BSITD-III)

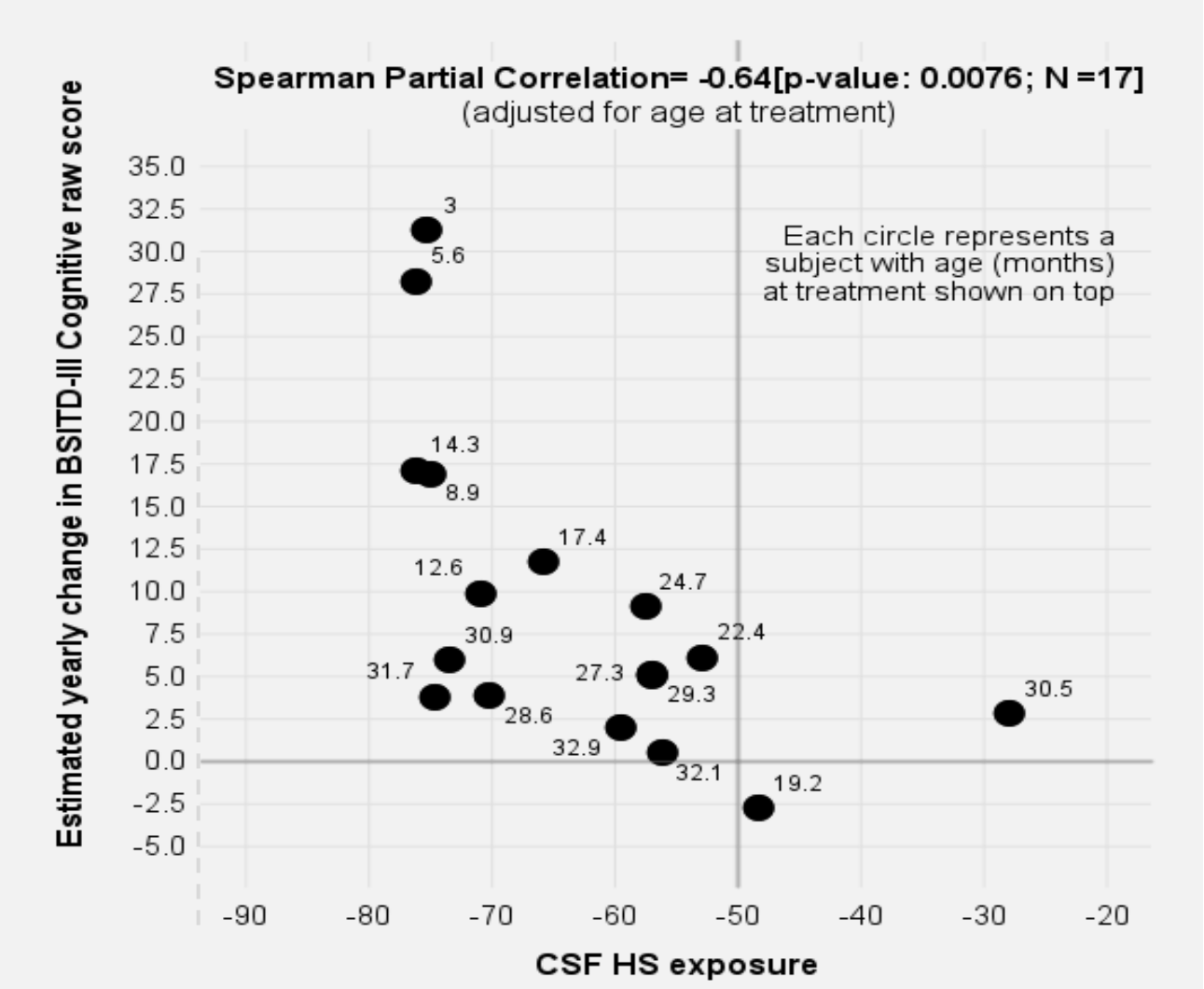
Natural history data reported in Shapiro EG, Nestrasil I, Delaney KA, et al. (2016) A Prospective Natural History Study of Mucopolysaccharidosis Type IIIA. J Pediatr 170: 278-287.e271-274 for which Sponsor has access to item level data.

Strong Predictive Relationship Between CSF HS Exposure and Change in Cognitive Raw Scores Over Time

Spearman's Rank-order Correlation Coefficient:

-0.72
(overall; P=0.0011)

-0.64
(adjusted for baseline age; P=0.0076)

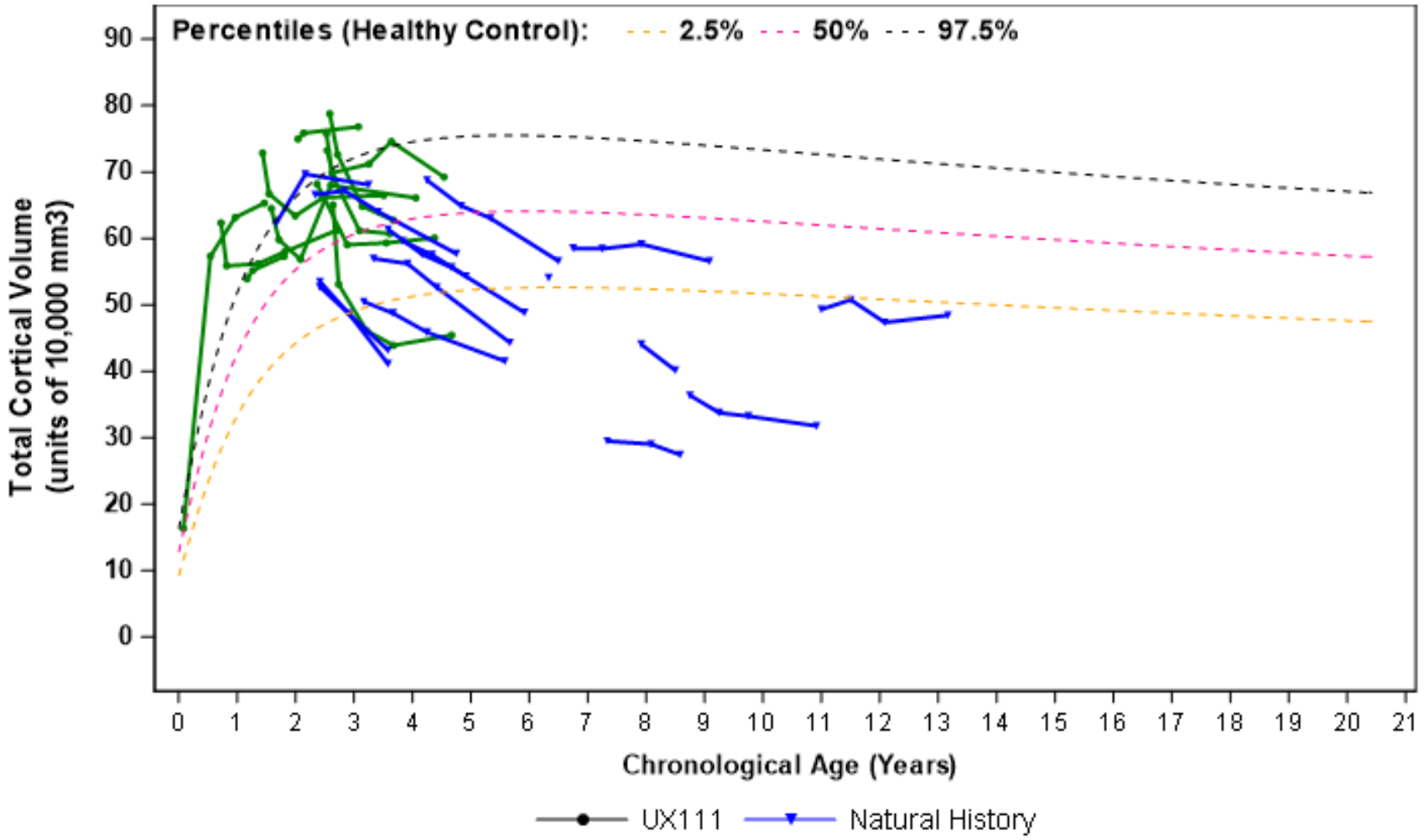


15 of 17 patients

simultaneously achieved CSF HS exposure reduction of > 50% and a positive estimated yearly change in Cognitive raw scores

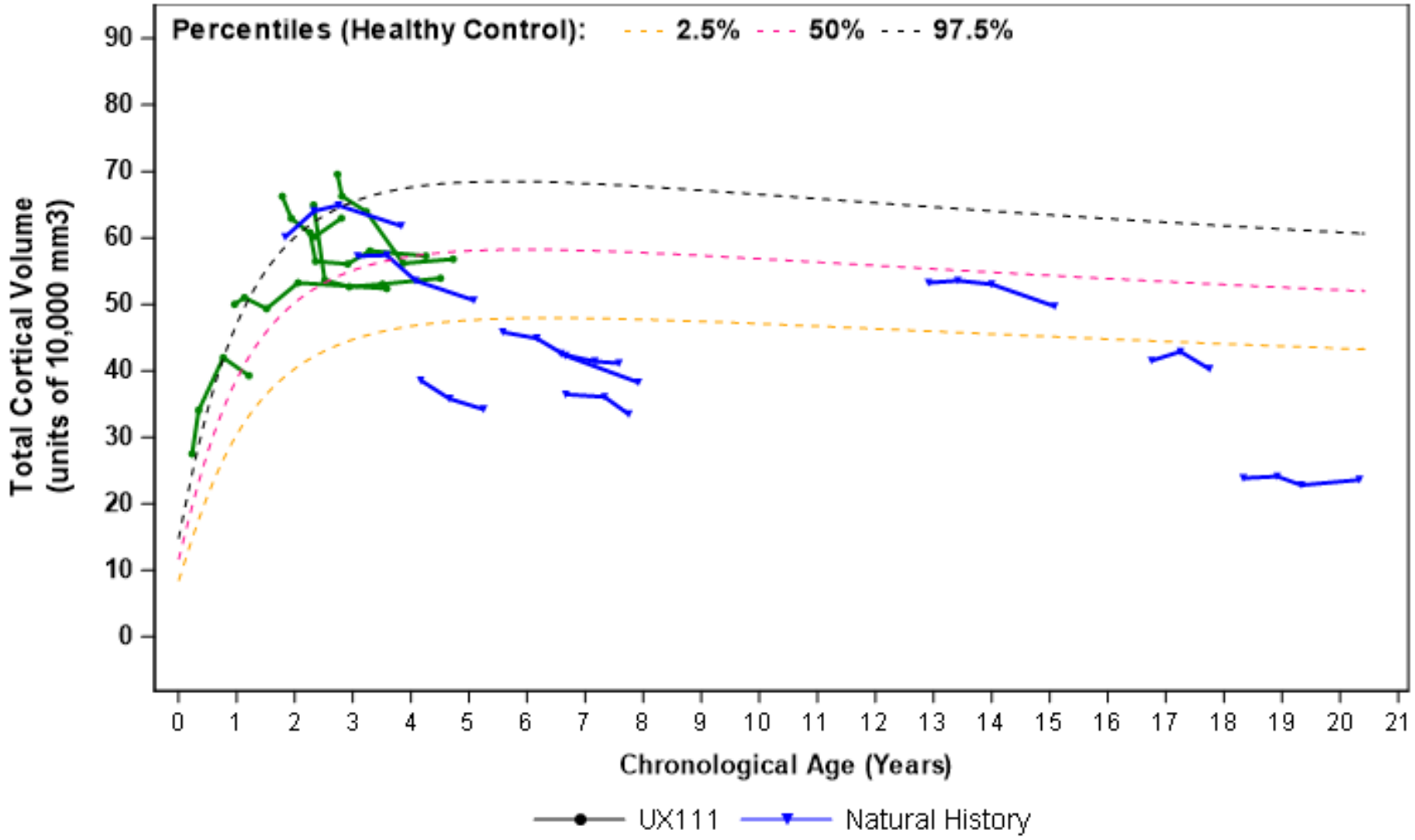
BSITD, Bayley Scales of Infant and Toddler Development – Third Edition; EYC, estimated yearly change

Late Biomarker: Total Cortical Brain Volumes normalize and are stable in UX111 Treated Compared To Untreated Male Patients with MPS IIIA



Natural history data reported in Shapiro EG, Nestrasil I, Delaney KA, et al. (2016) A Prospective Natural History Study of Mucopolysaccharidosis Type IIIA. J Pediatr 170: 278-287.e271-274 for which Sponsor has access to item level data.

Late Biomarker: Total Cortical Brain Volumes normalize and are stable in UX111 Treated Compared To Untreated Female Patients with MPS IIIA



Natural history data reported in Shapiro EG, Nestrasil I, Delaney KA, et al. (2016) A Prospective Natural History Study of Mucopolysaccharidosis Type IIIA. J Pediatr 170: 278-287.e271-274 for which Sponsor has access to item level data.

Safety profile of UX111 to date

- No treatment emergent adverse events (TEAEs) leading to study discontinuation or death have occurred
- The most frequently reported related TEAEs were elevations in liver enzymes; the majority of these events were mild (Grade 1) or moderate (Grade 2) in severity
- The only treatment-related adverse event ≥ Grade 3 was one event of increased alanine aminotransferase that resolved (a known class effect of AAV gene therapy)

Adverse Events	Total (N=28) n (%)
Treatment-Emergency Adverse Event (TEAE)	27 (96.4)
Serious TEAE	11 (39.3)
Related TEAE	21 (75.0)
Serious Related TEAE	0
TEAE Grade ≥3	12 (42.9)
Related TEAE Grade ≥3	1 (3.6)
TEAE Leading to Study Discontinuation	0
TEAE Leading to Death	0

Median follow up = 43.2 mos (range = 11.3, -71.1 mos)

Interim Data Showed a Positive Treatment Effect of UX111 in Pediatric Patients With MPS IIIA

Strong correlation between reduction in CSF HS exposure and stability or improvement in BSITD-III Cognitive raw scores

- **Rapid and sustained reduction** (≥ 50%) **in toxic *CSF HS exposure*** over a median follow-up period of approximately 2 years (23.9 months) after treatment with UX111
- **Gain or stability in *BSITD-III Cognitive raw scores*** observed during the expected window of plateau into decline in the majority of patients

Intermediate biomarkers: Post-treatment reduction in secondary biomarkers CSF gangliosides (GM2 and GM3) in line with results for reduction in CSF HS and reflect restoration of cellular function

Late biomarkers: Total cortical volume on brain MRI are stabilizing and within normal limits (relative to gender-matched healthy controls) in the majority of UX111 treated patients

Promising interim results suggest **a favorable benefit-risk profile** of UX111 for the treatment of pediatric patients with MPS IIIA

Conclusion

The advent of validated and high precision assays for HS provide the dynamic range specificity and reliability to allow the use of CSF HS as a predictive biomarker in contrast to the less specific older GAG assays.

Changes in CSF HS can occur rapidly in the neuronopathic MPS disorders, and indicate biochemical efficacy or failure of a potential therapy, which in turn informs a clinical development program

In contrast, clinical outcomes assessing neurodevelopment in a therapeutic trial for nMPS may take years to be fully realized and the magnitude and type of clinical benefit may be different depending on age of intervention.

The **totality of preclinical and clinical evidence** presented today **support the role of CSF HS** as a **biomarker reasonably likely to predict** clinical outcomes in neuronopathic MPS

Pursuing accelerated approval using CSF HS as a "reasonably likely surrogate endpoint" is critical to advance development of life saving therapies for progressive fatal diseases like the neuronopathic MPS disorders

Acknowledgements

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Q & A SESSION

- In person: Write your questions on the index card provided
- Virtual: Use the Q & A function on Zoom

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BREAK

The meeting will resume at 2:45 pm ET

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Panel Discussion: Challenges in Qualifying Biomarkers to Support Rare Disease Approvals

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John Crowley, JD, MBA, Amicus Therapeutics, Inc.

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Carole Ho, MD, Denali Therapeutics

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Edward Neilan, MD, PhD, National Organization of Rare Diseases

Cara O'Neill, MD, Cure Sanfilippo Foundation

James Wilson, MD, PhD, University of Pennsylvania

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Thank you!

Meeting materials will be posted on our website: www.reaganudall.org

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