Qualifying Biomarkers to Support Rare Disease Regulatory Pathways

Case example: Heparan sulfate in neuronopathic lysosomal storage diseases

February 21, 2024

10am-4pm *(eastern)*

The public meeting will begin shortly

Denali Therapeutics, Orchard Therapeutics, REGENXBIO Inc., and Ultragenyx provided funding for this event.

REAGAN-UDALL FOUNDATION FOR THE FDA

Welcome

Susan C. Winckler, RPh, Esq.
Chief Executive Officer
Reagan-Udall Foundation for the FDA

Hybrid Meeting

REAGAN-UDALL FOUNDATION FOR THE FDA

Joining online:
Microphone and video will remain off during the meeting
Share your questions using the Zoom Q&A function

Joining in-person:
Please write your questions on the index cards provided

This public meeting is being recorded. The slides, transcript, and video will be available at www.ReaganUdall.org.

Today's Agenda (Eastern Time)

REAGAN-UDALL FOUNDATION FOR THE FDA

10 a.m.	Welcome & Opening Remarks
10:10 a.m.	Biomarkers in Rare Diseases
10:40 a.m.	Case Study: Understanding Neuronopathic Mucopolysaccharidoses (MPS)
11:20 a.m.	Case Study: Measuring Glycosaminoglycans, including Heparan Sulfate (HS)
11:50 a.m.	Q & A Session with Morning Case Study Presenters
12:10 p.m.	Lunch (provided for in-person attendees)
12:40 p.m.	Case Study: Animal Model Translation to Human Application
1:20 p.m.	Case Study: Relationship Between Cerebrospinal HS Levels and Clinical Outcomes
2:05 p.m.	Q & A Session with Afternoon Case Study Presenters
2:35 p.m.	Break
2:45 p.m.	Panel Discussion: Challenges in Qualifying Biomarkers to Support Rare Disease Approvals
3:55 p.m.	Closing Remarks & Adjourn

REAGAN-UDALL FOUNDATION FOR THE FDA

Biomarkers in Rare Genetic Diseases

Peter Marks, MD, PhD
Director
Center for Biologics Evaluation and Research
U.S. Food and Drug Administration

U.S. FOOD & DRUG ADMINISTRATION

Biomarkers in Rare Genetic Diseases

Peter Marks, MD, PhD

RUF Biomarkers Meeting

February 21, 2024

Importance of Therapies for Disorders that are Rare

- Out of thousands of rare hereditary and acquired diseases there are hundreds of disorders affecting one to thousands per year that could be addressed with novel therapies
  - Addressing molecular defects may reduce some more common diseases to very rare diseases

U.S. Approved Gene Therapies

- Tisagenlecleucel (2017)
- Axicabtagene (2017)
- Voretigene (2017)
- Onasemnogene (2019)
- Brexucabtagene (2020)
- Lisocabtagene (2021)
- Idecabtagene (2021)
- Ciltacabtagene (2022)
- Betibeglogene (2022)
- Elivaldogene (2022)
- Etranacogene (2022)
- Nadofaragene (2022)
- Beremagene (2023)
- Delandistrogene (2023)
- Valoctocogene (2023)
- Lovotibeglogene (2023)
- Exagamglogene (2023)

Stem cell T cell Directly administered

First names only provided for products due to space limitations

Current Challenges

- Gene therapy is currently at a critical juncture due to a combination of factors
  - Manufacturing challenges
  - Clinical development timelines
  - Different global regulatory requirements

Rare Disease Development Issues

- Natural history of disease may be limited
- Frequent diversity of disease manifestations
- Time course of illness can be prolonged
- Disease manifestations may be irreversible

Leveraging Accelerated Approval

- The science inherent in the development of many gene therapies potentially facilitates the use of biomarkers as endpoints that are *reasonably likely* to predict clinical outcomes
  - Enzyme activity levels, structural protein levels can be measured and correlated with clinical endpoints in model systems or even in humans

Connecting Biomarkers with Gene Therapy Clinical Outcomes

Animal Models

- Disease model reflects aspects of human pathology
- Administration of therapy associated with achievement of a specific protein level ameliorates disease

Human Observations

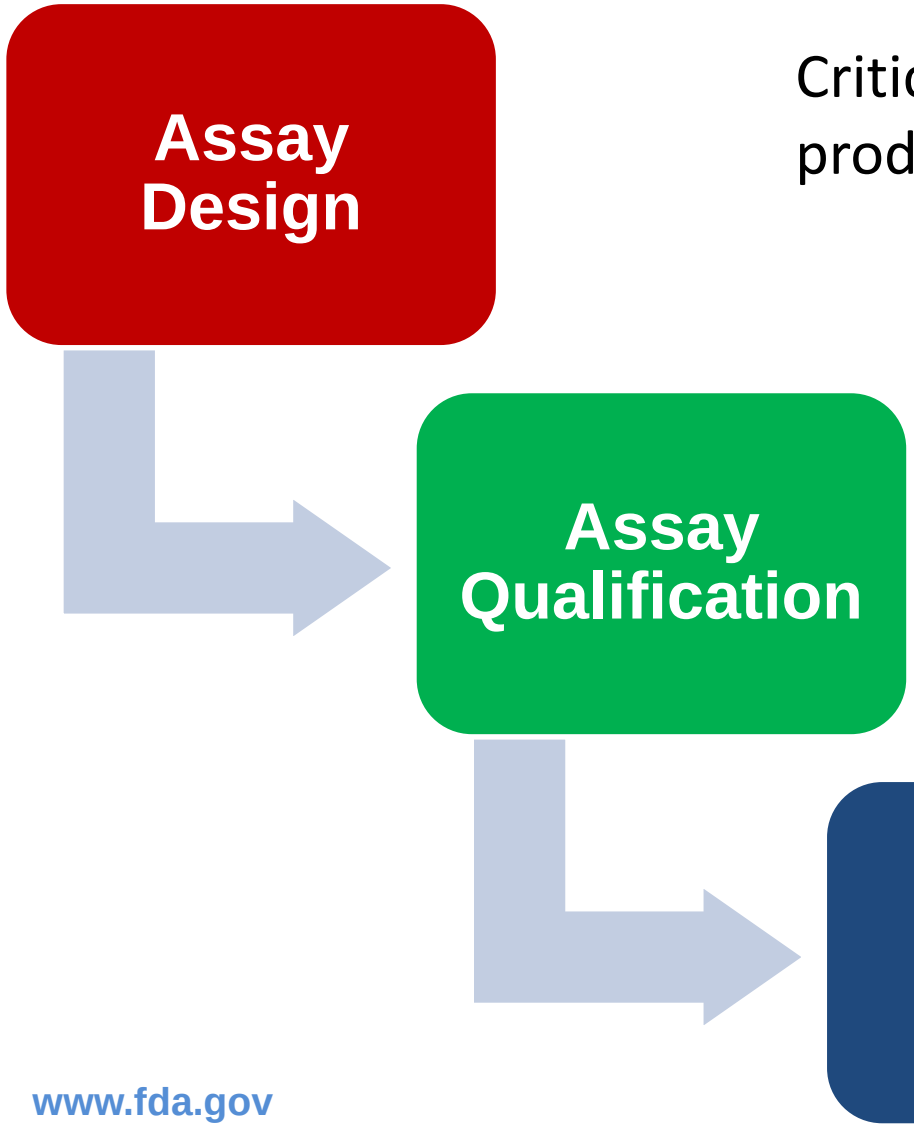
- Disease state is associated with protein levels above or below a certain range
- Certain protein levels are associated with disease absence or minimal disease

Demonstrate that equivalent protein levels can be achieved in humans affected by the disease

Importance of Biomarkers

- Along with intermediate clinical endpoints, biomarkers play a critical role in facilitating application of the accelerated approval pathway
- Whether directly connected to the therapeutic intervention (e.g., factor activity in hemophilia) or indirectly (e.g., reduction in heparan sulfate in MPS), accuracy and precision of measurements are crucial

Stages of Analytical Method Validation



Critical components for successful assay design and products critical quality attributes are identified

Optimization of assay design variables. Suitability of the assay for its intended purpose based on limited, pre-determined performance criteria

Specifications are pre-established, documented, and confirmed

Assay Qualification versus Validation

<u>Assay Qualification</u> = suitable for its intended purpose

- Documented testing demonstrating the assay method will provide meaningful data for the specific conditions, matrix and samples that the procedure is intended for
- Prove that the assay works correctly and leads to the expected results
- Limited pre-determined performance criteria

<u>Assay Validation</u> = suitable for its intended purpose on a routine basis

- Process of establishing documented evidence providing a high degree of assurance that a specific process such as analytical test method will consistently meet predetermined specifications and quality attributes (i.e., accuracy, precision, etc.)

Validation

- **Analytical Validation**
  - Demonstrates the accuracy, precision, reproducibility of the test and how well tests measure what it claims to measure
  - How accurate are the results of measuring the analyte?
  - What is the risk to patient if wrong result will be generated?
  - Wrong diagnosis vs. wrong dose determination for therapeutic applications
- **Clinical Validation**
  - Does biomarker link biological processes and clinical endpoints
  - Is the test result relevant to the clinical condition?

Analytical Assay Validation Explores all Aspects of Assay Performance

- **Selectivity and Specificity**
- **Accuracy**
- **Precision**: including Repeatability and intermediate precision
- **Reproducibility**
- **Linearity**
- **Range**
- **Sensitivity**: Limits of Blank, Detection, Quantitation, Limit of Quantification
- **Stability:** In-process stability analyte stability, freeze/thaw stability, processed sample stability , stability of reagent, controls, calibrators etc.,
- **Robustness**
- **Software Validation if applicable**
- **Cross-validation**: when two or more analytical methods are used to generate data within the same study

Accuracy

- Accuracy refers to the closeness of a measured value to a standard or known value
  - Reference Standard
  - Reference Controls
  - Clinical Truth, Sample with known diagnosis
  - Reference Methodology
  - Reference Laboratory

Precision

Precision refers to the closeness of the measurements to each other

Repeatability (Intra-assay):

- Repeat test under the **same conditions**: same location; the same measurement procedure; the same operator; the same measuring instrument over a short period of time

Reproducibility

- The degree of agreement under **different conditions** different operator, different locations, different instruments, different lots etc.
  - Within-laboratory Reproducibility (intermediate precision)
  - Multi-Site Reproducibility: precision between the measurement results obtained at different laboratories

Linearity

- Relationship between the observed values and the true concentration
- Linearity assesses the ability of the method to obtain test results that are directly proportional to the concentration of the analyte in the sample
- Demonstrate linearity within the claimed/established range using the same Intended Use specimen(s)

Specificity and Interference

- **Analytical Specificity:** the ability of the assay to detect the intended target (analyte of interest) in the presence of other analytes in the sample matrix

Assay Sensitivity

- **Limit of Blank (LOB):** is the highest signal expected in absence of the measurand

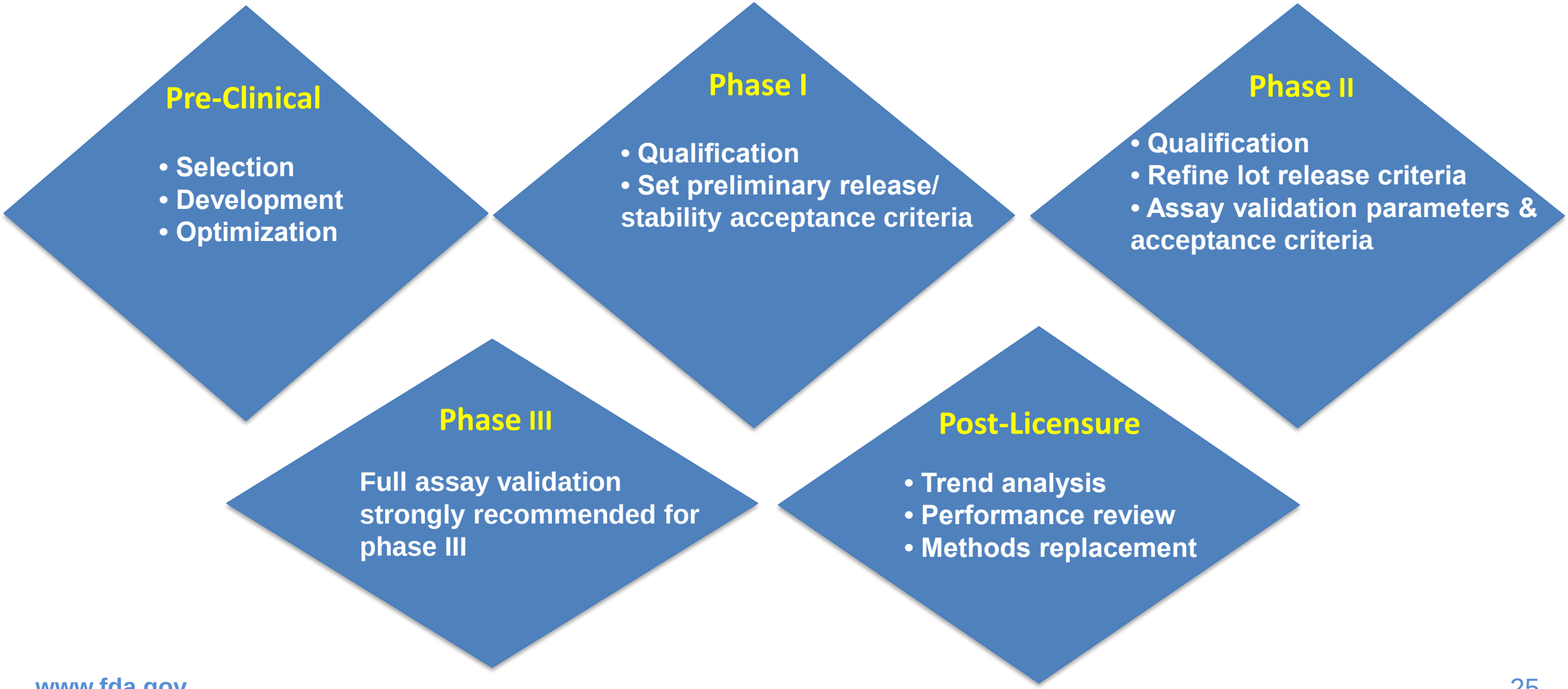
- **Limit of Detection (LOD):** is the ability of the assay to distinguish signal from background

- **Limit of Quantification (LOQ):** is the ability to precisely and accurately measure low amounts of the measurand

Stability

- Stability of the analyte in the matrix
- In-process stability
  - Stability of analyte under the conditions of sample preparation
- Processed sample stability
  - Stability of an analyte in the prepared samples under conditions of analysis
- Reagent Stability
- Calibrator Stability
- Controls Stability
- Freeze/thaw stability

Assay Development in Clinical Trials



Summary

- The Center for Biologics Evaluation and Research aims to make 2024 a breakout year addressing key challenges to the development of gene therapies, especially for rare disorders, and reliance on increased use of biomarkers may play a critical role in accomplishing this objective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Case Study: Understanding Neuronopathic Mucopolysaccharidoses (MPS)

- **Mark Dant**, Ryan Foundation
- **Joseph Muenzer, MD, PhD**, University of North Carolina at Chapel Hill

REAGAN-UDALL FOUNDATION FOR THE FDA

Neuronopathic MPS and Treatment Challenges

Joseph Muenzer, M.D., Ph.D.

Bryson Distinguished Professor in Pediatric Genetics

Director, Muenzer MPS Research and Treatment Center

Professor of Pediatrics and Genetics

Division of Genetics and Metabolism

Department of Pediatrics

University of North Carolina at Chapel Hill

Chapel Hill, NC

Feb 21, 2024 Washington, DC

Regan Udall Foundation Meeting

“Qualifying Biomarkers to Support Rare Disease Regulatory Pathways”

Disclosures

- I have been a consultant and/or served on advisory boards for Takeda, Sanofi, Regenxbio, Denali Therapeutics and JCR Pharmaceuticals.
- I am currently the principal investigator for a PTA program for intrathecal enzyme replacement clinical trials for severe MPS II, a Phase I/II gene editing clinical trial for MPS II and a Phase I/II and a Phase II/III IV ERT clinical trial for MPS II.

Presentation Overview

- Overview of MPS
- Clinical features of MPS I, MPS II, MPS III and MPS VII
- Treatment Options for MPS
- Clinical trial challenges in neuronopathic MPS
- Why CSF heparan sulfate is a biomarker for neuronopathic MPS
- Two examples on MPS II clinical trials
- Ten reasons why the biomarker CSF heparan sulfate using the accelerated approval pathway should be utilized for neuronopathic MPS disorders

Overview of Mucopolysaccharidoses

- Lysosomal enzyme deficiencies
  - Twelve known enzyme deficiencies comprise eight different clinical types each involved in the breakdown or recycling of glycosaminoglycans (GAG).
- The hallmark of MPS disorders is increased urinary excretion of partially degraded glycosaminoglycans fragments due to the primary event of intra-lysosomal GAG accumulation.
- MPS are ultra-rare genetic disorders with an estimated US prevalence of < 2500 individuals.

Overview of Mucopolysaccharidoses

- The MPS disorders are heterogenous, progressive and clinically characterized by somatic and/or central nervous system involvement with premature death for most individuals.

Major Clinical Manifestations of MPS

- Developmental delay/cognitive impairment
- Communicating hydrocephalus
- Carpal tunnel syndrome
- Spinal cord compression
- Corneal clouding
- Combined conductive/ neurosensory hearing loss
- Obstructive sleep apnea
- Valvular heart disease
- Pneumonia and otitis media
- Joint stiffness & contractures
- Hepatomegaly
- Abnormal gums, teeth and enamel
- Inguinal/umbilical hernias

Overview of Mucopolysaccharidoses

- The MPS disorders are heterogenous, progressive and clinically characterized by somatic and/or central nervous system involvement with premature death for most individuals.
- In general, MPS patients appear normal at birth and subsequently develop somatic and/or cognitive impairment.

Mucopolysaccharidoses (MPS)

#	Name	Enzyme defect	GAG
I-H	Hurler	Iduronidase	DS,HS
I-H/S	Hurler-Scheie	Iduronidase	DS,HS
I-S	Scheie	Iduronidase	DS,HS
II	Hunter-severe	Iduronate sulfatase	DS,HS
II	Hunter-attenuated	Iduronate sulfatase	DS,HS
III-A	Sanfilippo A	Heparan N-sulfatase	HS
III-B	Sanfilippo B	N-acetylglucosaminidase	HS
III-C	Sanfilippo C N-acetyltransferase	Acetyl CoA:α-glucosamine	HS
III-D	Sanfilippo D 6-sulfatase	N-acetyl-α-glucosamine	HS

Muenzer J. Rheumatology (2011)

Mucopolysaccharidoses (MPS)

#	Name	Enzyme defect	GAG
IV-A	Morquio-A	N-acetylgalactosamine 6-sulfatase	KS
IV-B	Morquio-B	β-galactosidase	KS
V	No longer used		
VI	Maroteaux-Lamy	N-acetylgalactosamine 4-sulfatase (arylsulphatase B)	DS
VII	Sly	β-glucuronidase	DS,HS
VIII	No longer used		
IX		Hyaluronidase	Hyaluronan
X*		Arylsulfatase K	DS,HS

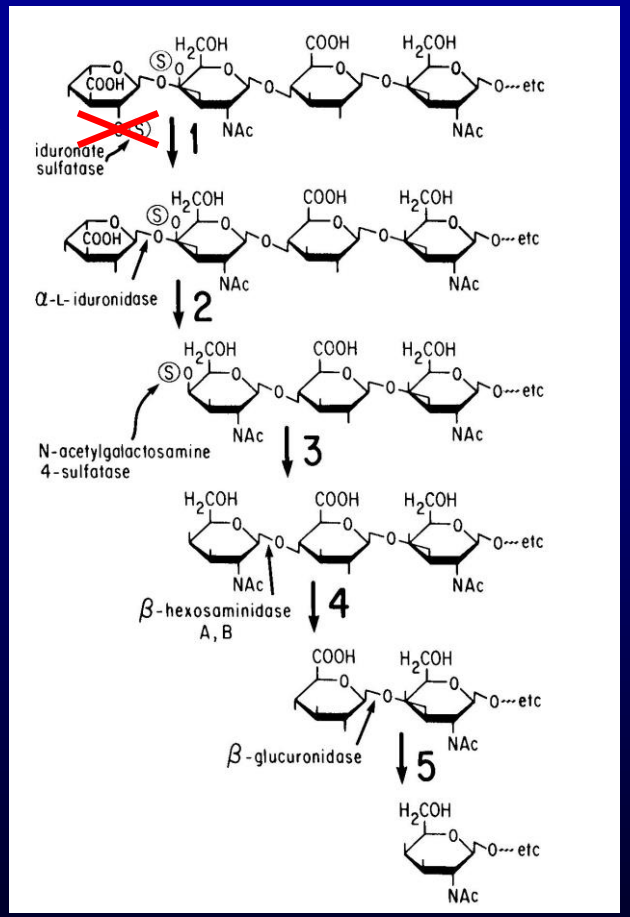
Muenzer J. Rheumatology (Oxford). 2011; 50 Suppl 5:v4-12 *Verheyen S et al. J Med Genet (2022); 59:957-964

MPS Clinical Nomenclature

- The description of the MPS clinical features has evolved over last 30 years.
- Initially, MPS patients were describe as having either severe or mild disease.
- About 20 years ago, I first heard Dr. Ed Wraith use the terms severe and attenuated to better describe the clinical spectrum in MPS.
- I initial proposed in 2015 at a Berlin MPS II meeting to use “neuronopathic” to better describe the individuals with progressive cognitive impairment.

Biochemistry of the MPS Disorders (Example – MPS II)

MPS II →



Iduronate-2-sulfatase deficiency causes a block in the sequential steps in glycosaminoglycans (GAG) degradation resulting in the lysosomal accumulation of GAG.

e.g. Dermatan Sulfate Degradation

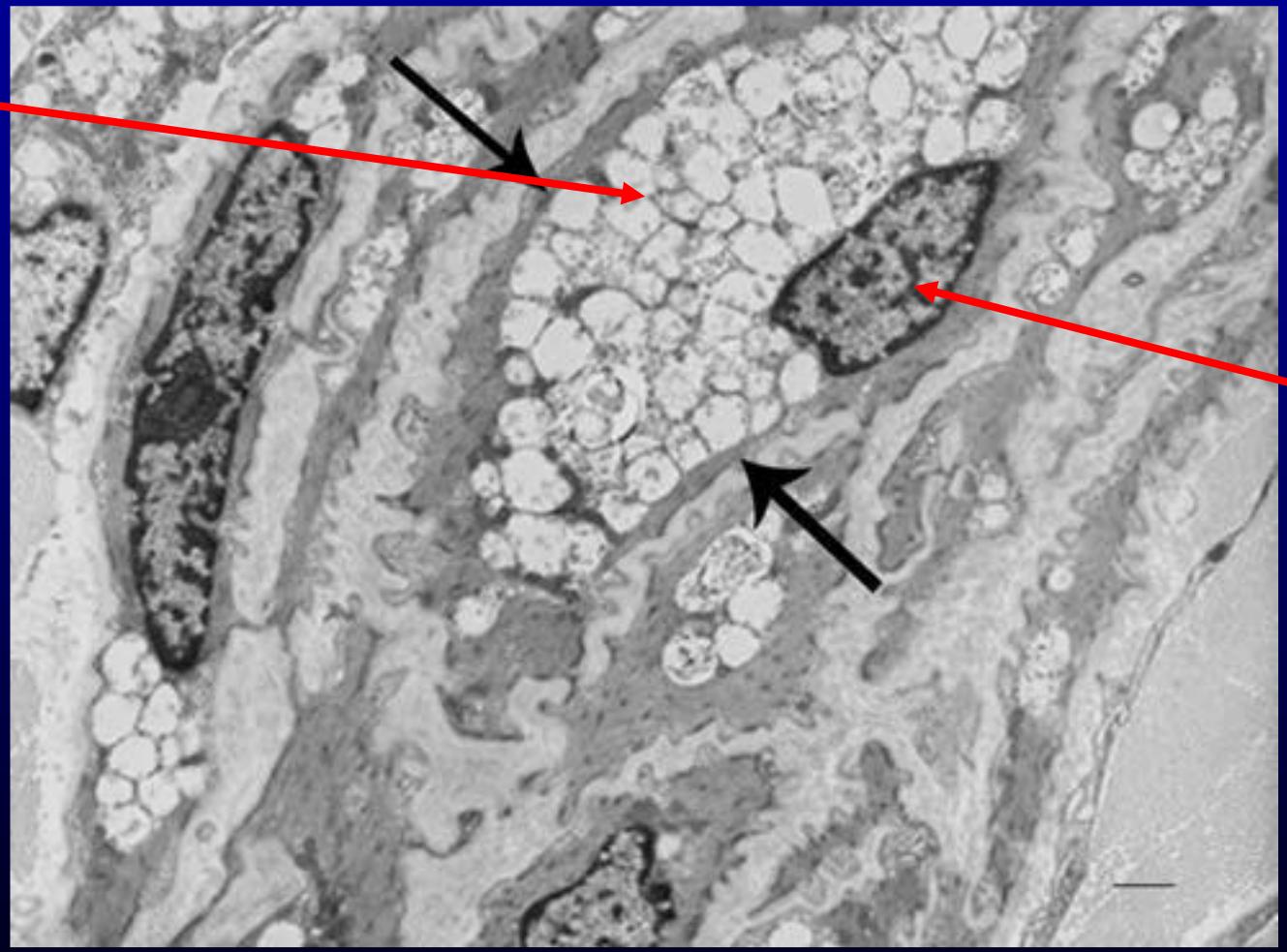
Muenzer J et al. Pediatrics (2009) 124:1228-39

Lysosome Function

- The major function of the lysosome is the breakdown and recycling of macromolecules and organelles into basic precursors.
- A defect in the activity of a lysosomal enzyme results in either non-degraded or partially degraded substrate and typically expansion of the size and number of the lysosomes.
- In MPS disorders the resulting intralysosomal GAG storage results in cell, tissue and organ dysfunction.

MPS I Peripheral Blood Sample Demonstrating Lysosomal Storage

Enlarged Lysosomes



Nucleus

V. Pala et al. Ultrastructural Pathology, iFirst1-9, 2020

MPS Disease Pathophysiology

- The amount of residual enzymatic activity appears to be one of the main drivers of clinical severity.
- The major classes of accumulating glycosaminoglycan are not equaling distributed throughout the body.
  - Heparan sulfate – CNS
  - Dermatan sulfate – Somatic
  - Keratan sulfate – Bone

Presenter's own opinion

Glycosaminoglycan Urinary Excretion Patterns in MPS

	Dermatan Sulfate	Heparan Sulfate	Keratan Sulfate
MPS I	+	+	
MPS II	+	+	
MPS VII	+	+	
MPS III		+	
MPS VI	+		
MPS IV			+

All the MPS disorders that have progressive cognitive impairment (neuronopathic) have elevated urinary and CSF heparan sulfate.

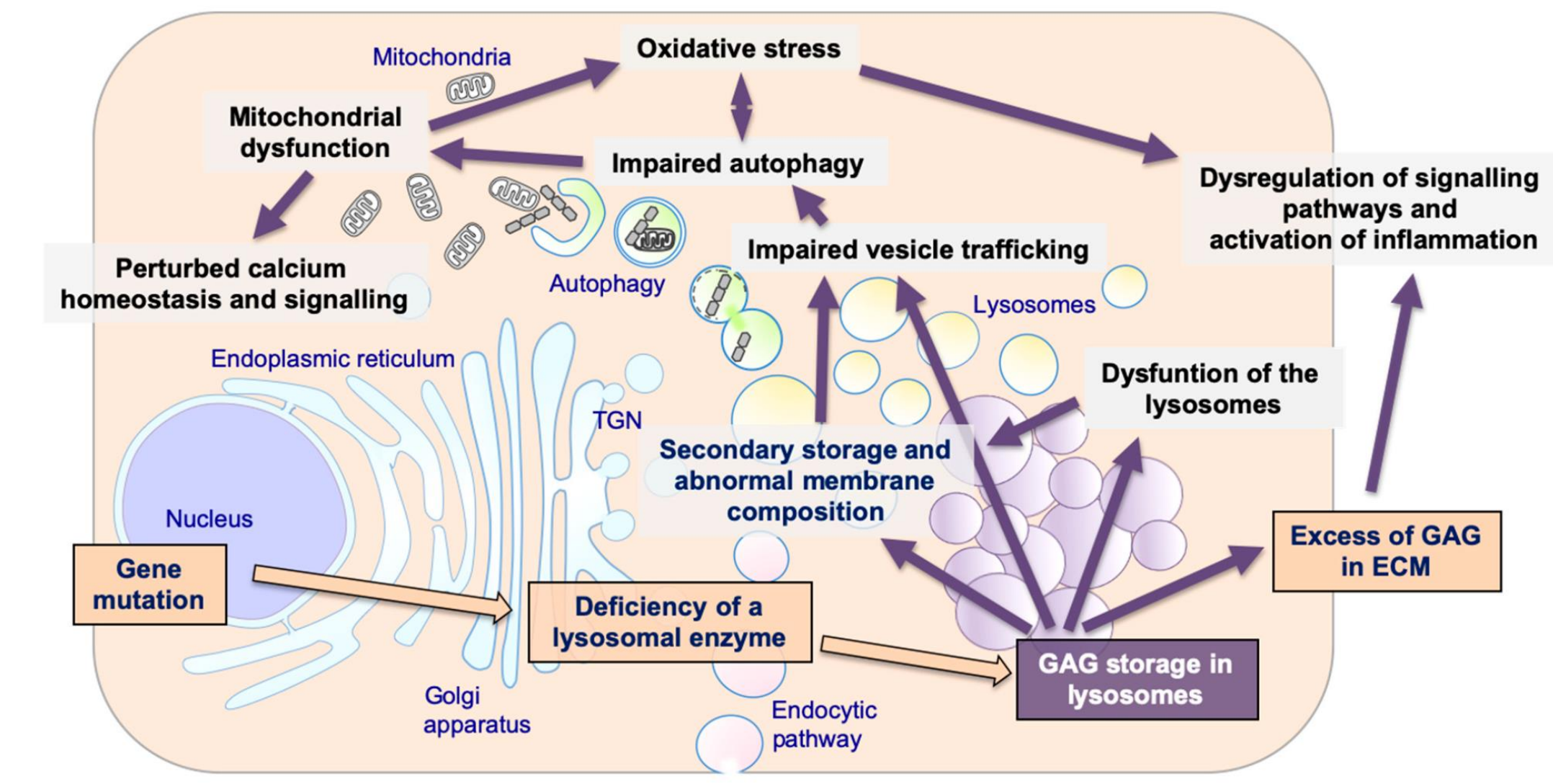
Presenter's own opinion

MPS Disease Pathophysiology

- The amount of residual enzymatic activity appears to be one of the main drivers of clinical severity.
- The major classes of accumulating glycosaminoglycan are not equaling distributed throughout the body.
  - Heparan sulfate – CNS
  - Dermatan sulfate – Somatic
  - Keratan sulfate – Bone
- The unique glycosaminoglycan storage for each MPS disorder results in a wide range of clinical disease.
- A variety of secondary events result in a complex cascade of disruption of cellular pathways.

Presenter's own opinion

MPS Disease Pathophysiology



Fecarotta S et al. Int. J. Mol. Sci. (2020) 21: 2515

Mucopolysaccharidosis II (MPS II) (Hunter Syndrome)

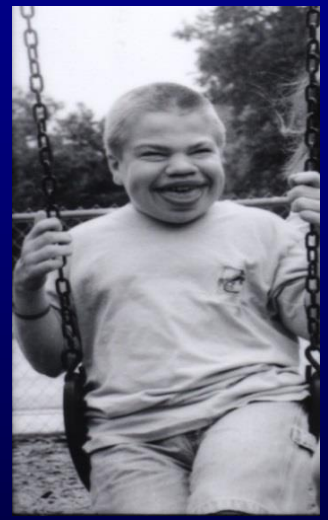
- MPS II is a rare X-linked recessive disorder (est. incidence 1:100,000)[1] due to the deficiency of lysosomal enzyme iduronate-2-sulfatase.
- MPS II is an ultra-orphan disorder with an estimated US prevalence of 450 to 500 patients.[2]
- About 2/3 of MPS II patients develop cognitive impairment with onset of symptoms between 1 to 3 years of age in the neuronopathic form.
- Premature mortality (teenage years) occurs in the neuronopathic form secondary to overwhelming neurologic progression.
- Although intravenous enzyme replacement is available for somatic disease, there is a high unmet medical need for treatment of the CNS disease in MPS II.[2]

[1]Muenzer J et al. Pediatrics (2009) 124:1228-39 [2]Presenter's own opinion

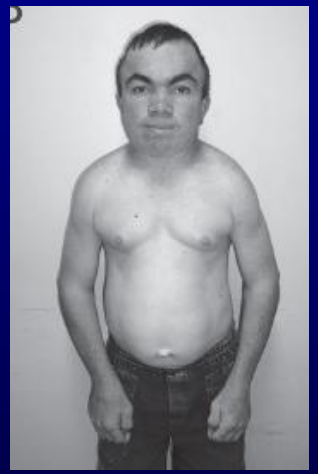
Image used with permission

Spectrum of Disease in MPS II

Neuronopathic ⟷ Non-neuronopathic



- Onset of symptoms from 1 to 3 years of age
- Progressive cognitive impairment
- Life expectancy 10 to 20 years without treatment



- Insidious onset
- Normal intelligence
- Variable life expectancy secondary to airway and heart disease

Presenter's own opinion

Giugliani R, Genetic & Mol Biol June 2014

Neuronopathic MPS II

- A devastating somatic and neurologic disorder with progressive cognitive impairment with onset between 1 to 3 years of age and start of regression by 3 to 6 years of age.
- Common CNS features that impact quality of life:
  - Severe behavior problems including aggression, hyperactivity and obstinacy
  - Seizures
  - Communicating hydrocephalus
  - Hearing loss
- No approved treatment is available for the CNS disease

Muenzer J et al. Pediatrics (2009) 124:1228-39

Sanfilippo Syndrome (MPS III)

- MPS III comprises four different enzymatic disorders all with a similar clinical phenotype.
- MPS III is characterized by childhood onset, progressive neurocognitive deterioration with rapidly (severe) or slowly (attenuated) progressing phenotypes.
- However, adult onset-phenotypes (primarily with MPS III A) with mild cognitive impairment or non-neuropathic phenotypes have been identified.[1]
- Major clinical manifestations of classical MPS III include; mental deterioration, hyperactivity, relatively mild somatic features and death typically in the teenage years in severe/neuronopathic form.

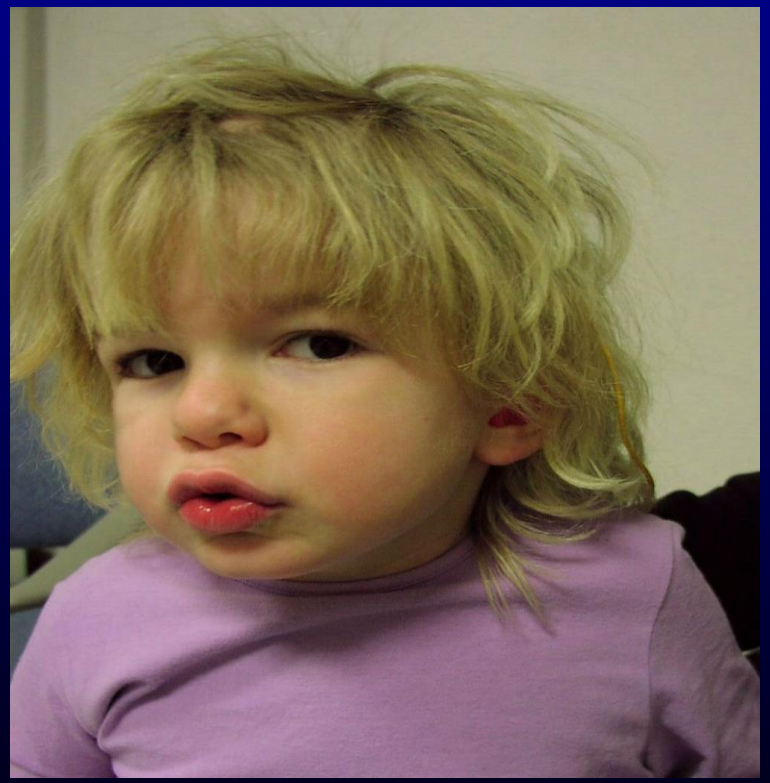
[1]Nijmeijer SCM et al. Orphanet J Rare D (2019) 14:249

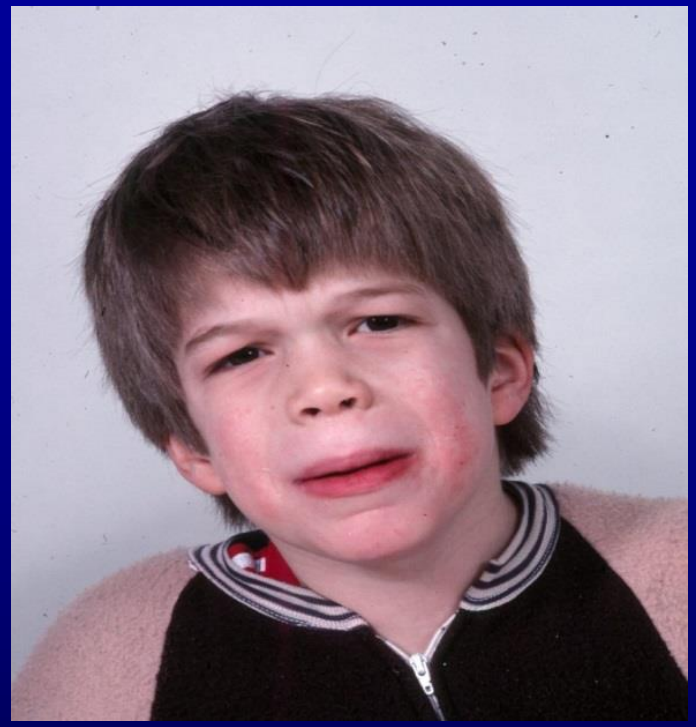
Sanfilippo Syndrome (MPS III)

- **Classical MPS III is clinically divided in 3 disease phases:[1]**
  - **First phase – After an initial symptom-free period, developmental delay is generally noted at 2 to 6 years of age**
  - **Second phase – Progressive loss of cognition with onset of behavioral and sleeping issues**
  - **Third phase – Progressive motor deterioration, profound cognitive impairment and death in the second or third decades due to overwhelming neurological disease**
- **In general, all classical MPS III individuals follow the same disease course, a progressing phenotype with variable rates of disease progression.**
- **Delayed diagnosis is common in attenuated patients with a slowly progressive disease course.**
- **No treatment is approved for individuals with any type of MPS III.**

[1]Nijmeijer SCM et al. Orphanet J Rare D (2019) 14:249

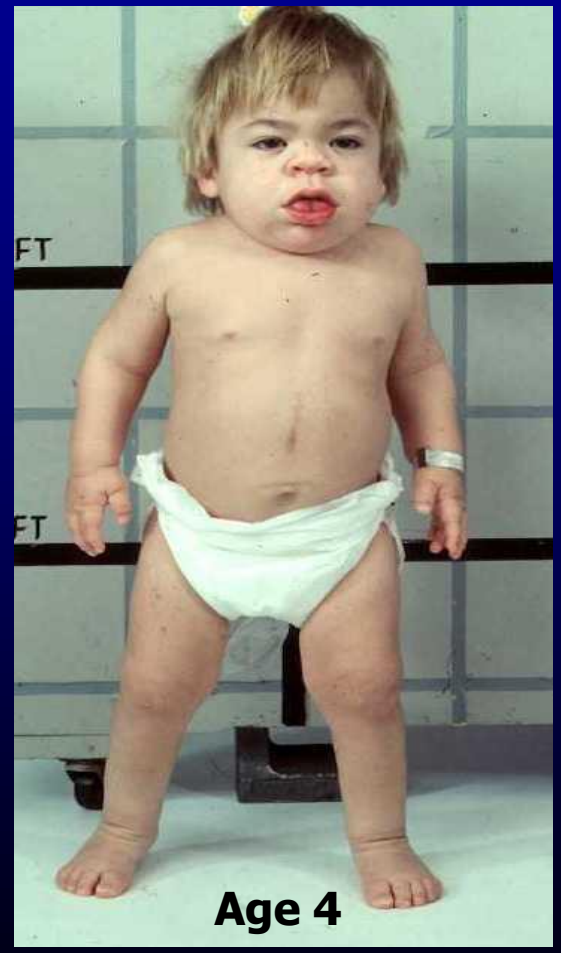
MPS III





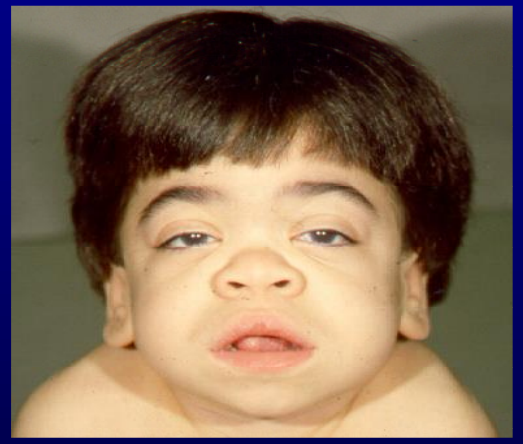
Mucopolysaccharidosis I (MPS I)

- Deficiency of lysosomal enzyme α-L-iduronidase
- Onset of symptoms before 6 months of age in severe form (Hurler syndrome)
- Early mortality in severe form (3 to 10 years of age)
- Rare (est. incidence 1:100,000)
- Autosomal recessive disorder
- Transplantation is the treatment of choice for individuals with Hurler syndrome < 2 years of age



MPS I: Iduronidase Deficiency

Hurler



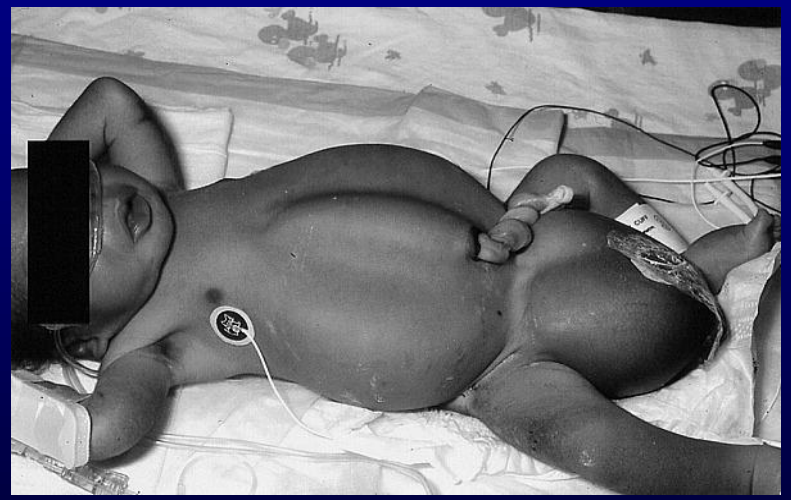
Hurler-Scheie

Scheie

All patients typically have <1% of normal enzyme levels

Mucopolysaccharidosis VII (MPS VII) (Sly Syndrome)

- Deficiency of lysosomal enzyme beta-glucuronidase
- Somatic and CNS involvement is similar but can be more severe than MPS I
- Non-immune hydrops fetalis is a common presentation in North America
- Rare (est. incidence > 1:500,000) in North American
- Autosomal recessive disorder
- No treatment for the CNS in MPS VII



Age 4

Treatment of Mucopolysaccharidoses

- **Enzymatic correction is possible at the cellular level in MPS fibroblasts secondary to the following observations:**
  - **Cultured cells release small amounts of lysosomal enzymes “correction factors”.**
  - **Efficient mannose-6-phosphate receptor-mediated enzyme uptake occurs in fibroblasts.**
  - **Correction of GAG metabolism may occur with only 1 to 2% of residual enzyme activity.**

Presenter’s own opinion

Current Treatment Options for MPS

- Hematopoietic stem cell transplantation (HSCT)
- Intravenous (IV) enzyme replacement therapy (ERT)

Treatment Options for MPS

	HSCT		IV ERT
	Somatic*	CNS*	Available
• MPS I	Yes	Yes	Yes
• MPS II	Yes	?	Yes
• MPS IIIA		No	No
• MPS IIIB		No	No
• MPS IVA	No		Yes
• MPS VI	Yes		Yes
• MPS VII	?	?	Yes

*Proven clinical benefit

Hematopoietic Stem Cell Transplantation (HSCT)

Enzyme Replacement Therapy (ERT)

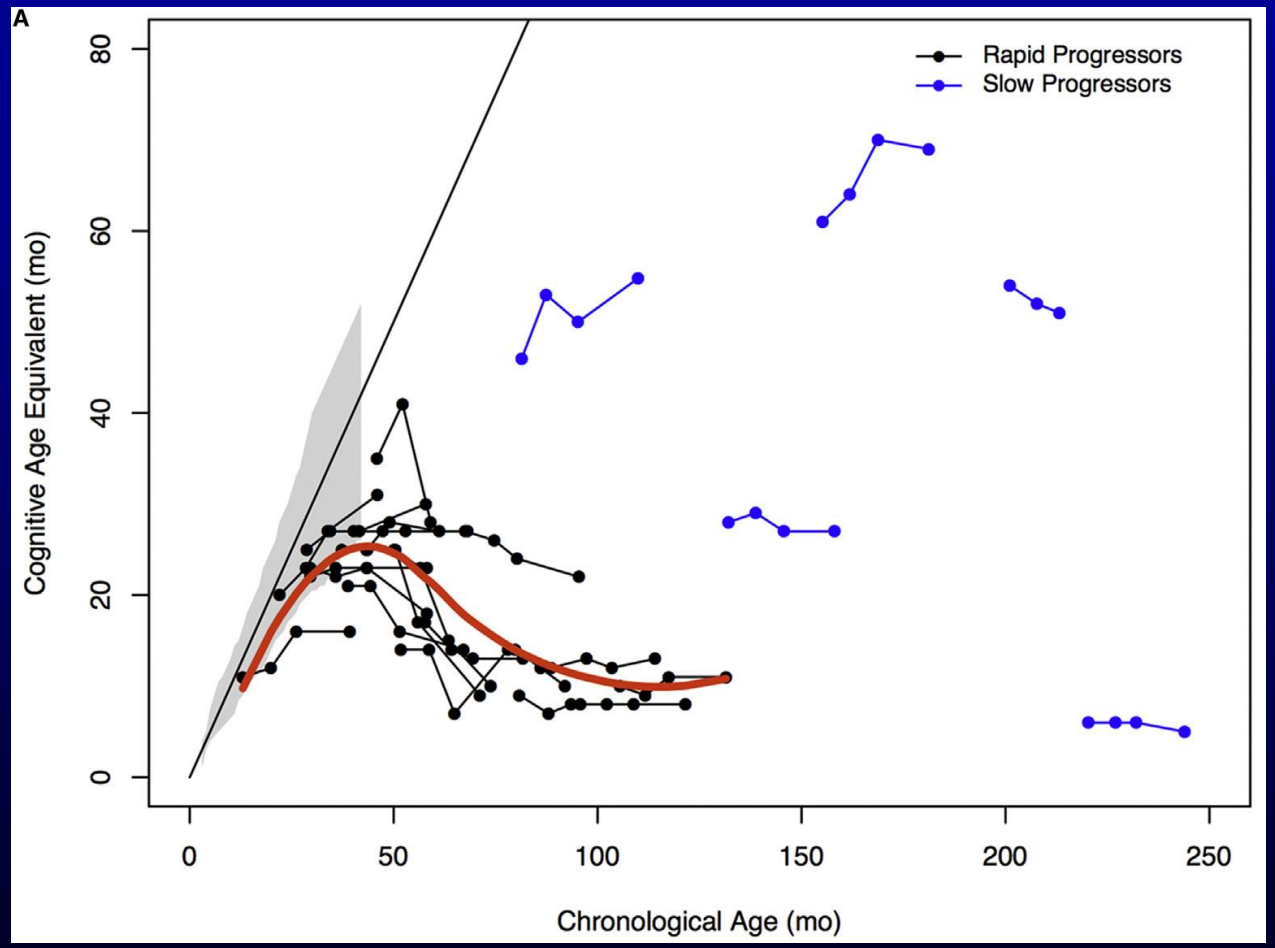
Presenter's own opinion

Clinical Trial Challenges in Neuronopathic MPS

- Heterogeneity in the onset and course of disease in neuronopathic individuals occurs, but all will develop CNS disease and die premature if untreated.
- Because of the slow and variable disease course it may take 4 to 6 yrs or more to observe the results of a successful intervention.

Presenter's own opinion

MPS IIIA Natural History



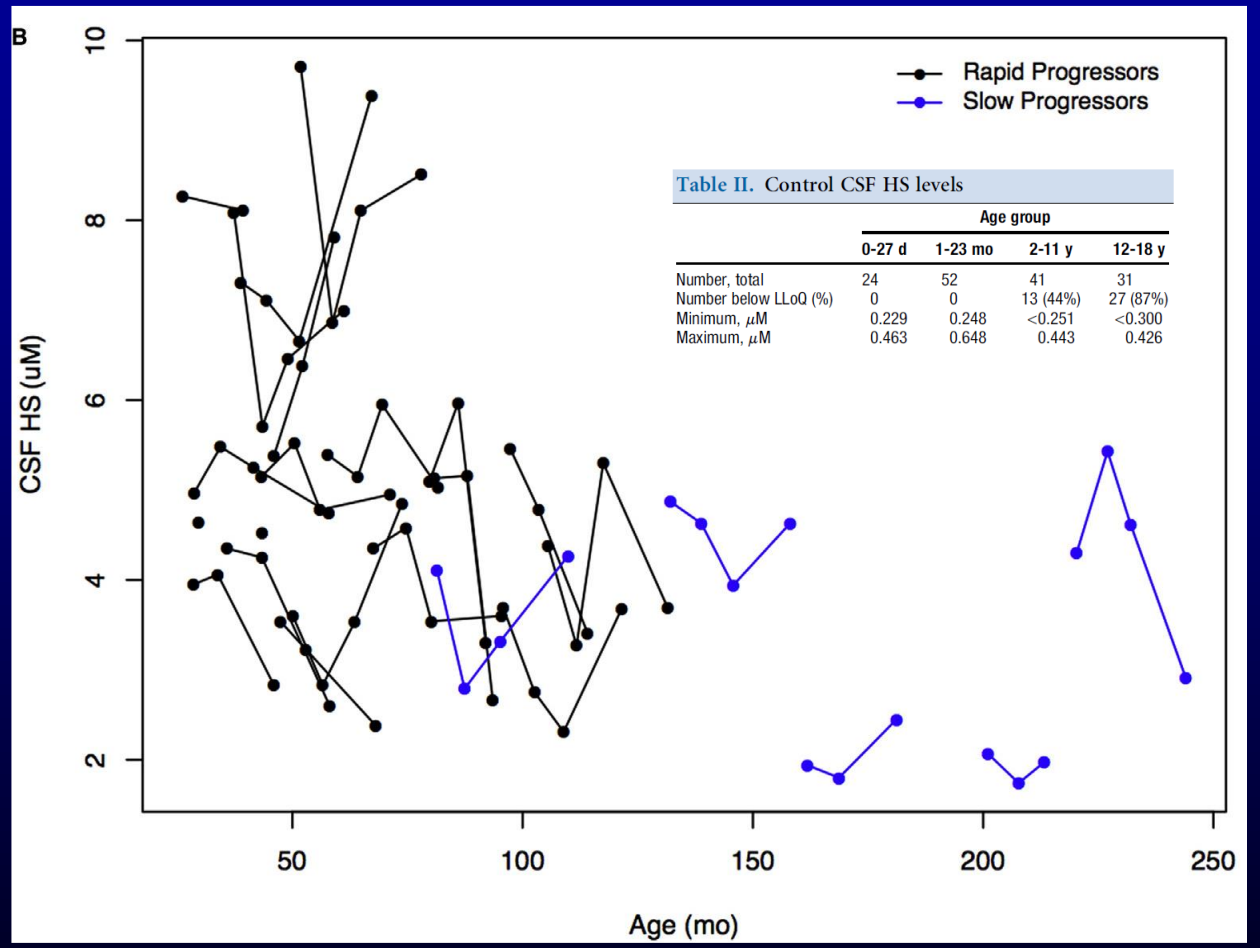


Table II. Control CSF HS levels

	Age group			
	0-27 d	1-23 mo	2-11 y	12-18 y
Number, total	24	52	41	31
Number below LLoQ (%)	0	0	13 (44%)	27 (87%)
Minimum, μM	0.229	0.248	<0.251	<0.300
Maximum, μM	0.463	0.648	0.443	0.426

Shapiro EG et al. J Pediatr (2016) 170:278

Clinical Trial Challenges in Neuronopathic MPS

- Clinically diagnosed MPS patients with developmental delays/cognitive impairment already have significantly impaired neuronal function that is typically irreversible.
- Replacement of the missing MPS enzyme in the brain of a neuronopathic individual with cognitive impairment will not result in cognitive improved, but at best clinical stability.
- Placebo controlled clinicals trials of greater than 1 to 2 years for a progressive neuronopathic disorder are unethical.
- Utilizing CSF HS as the biomarker and the accelerated approval pathway is the logical solution with long-term follow-up (5-10 yrs).

Presenter's own opinion

Why CSF Heparan Sulfate Should be a Biomarker for Accelerated Approval

- Lysosomal enzymes are only active within the acidic lysosome.
- Heparan sulfate is a primary substrate that accumulates in neuronopathic MPS individuals.
- CSF heparan sulfate levels correlates with brain tissue heparan sulfate in MPS animals.
- CSF is a dynamic fluid that turns over about 4 times per day.
- The only way for CSF heparan sulfate to be decreased is that enzyme enters brain cells and reduces brain heparan sulfate content.
- Lowering CSF heparan sulfate is “reasonable likely” to predict clinical benefit.

Presenter’s own opinion

Phase II/III Intrathecal ERT Clinical Trial for Severe MPS II

- MPS II males with cognitive impairment who continued on weekly IV idursulfase.
- A one-year placebo-controlled trial evaluating 10 mg monthly IT injections of idursulfase-IT via an IDDD or by lumbar puncture.
- The phase II/III data for the first year demonstrated safety, but the study did not meet its pre-specified primary or key secondary endpoints.
- Although the less involved and younger patients appear to have significant clinical benefit*, Takeda is no longer seeking market approval, however the study is continuing to monthly dose patients.

*Presenter's own opinion

Phase II/III Intrathecal ERT for Severe MPS II

	Idursulfase-IT 10 mg (95% CI)	No idursulfase-IT treatment (95% CI)	Estimated treatment difference (95% CI)	*p* value
ITT population	−4.6 (−9.4, 0.2)	−11.2 (−15.7, −6.6)	6.6 (−0.1, 13.2)	0.0530
Baseline DAS-II GCA score ≤ 70	−7.0 (−13.5, −0.6)	−13.6 (−21.1, −6.1)	6.6 (−3.3, 16.4)	0.1833
Baseline DAS-II GCA score > 70	−2.1 (−9.4, 5.1)	−8.7 (−14.4, −3.0)	6.6 (−2.6, 15.8)	0.1487
Baseline age < 6 years	−4.0 (−8.9, 1.0)	−15.4 (−21.1, −9.8)	11.5 (4.0, 19.0)	0.0037
Baseline age ≥ 6 years	−13.9 (−24.6, −3.3)	−3.2 (−7.7, 1.3)	−10.7 (−22.5, 1.1)	0.0649
Baseline age < 55 months	−7.1 (−12.3, −2.0)	−18.1 (−25.1, −11.1)	11.0 (2.4, 19.6)	0.0151
Baseline age ≥ 55 months	−3.0 (−11.1, 5.1)	−5.6 (−11.6, 0.4)	2.6 (−8.1, 13.3)	0.6212

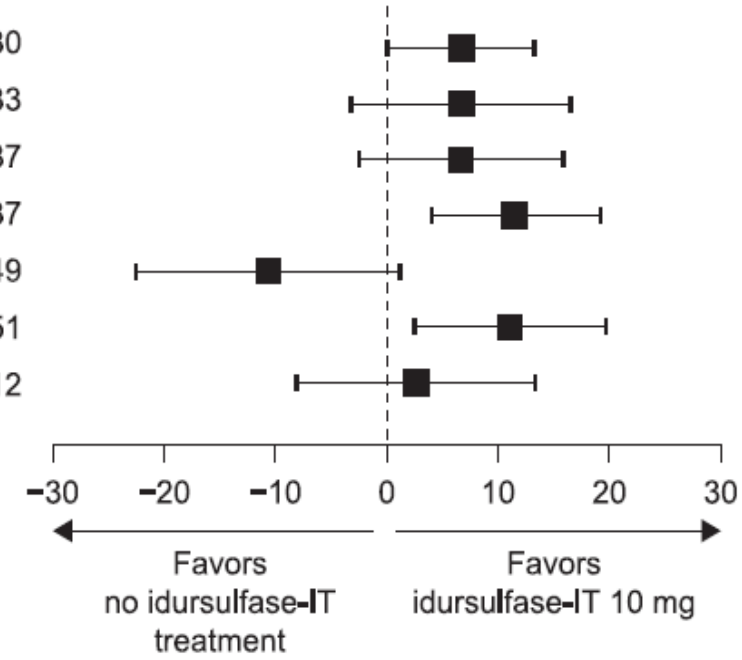
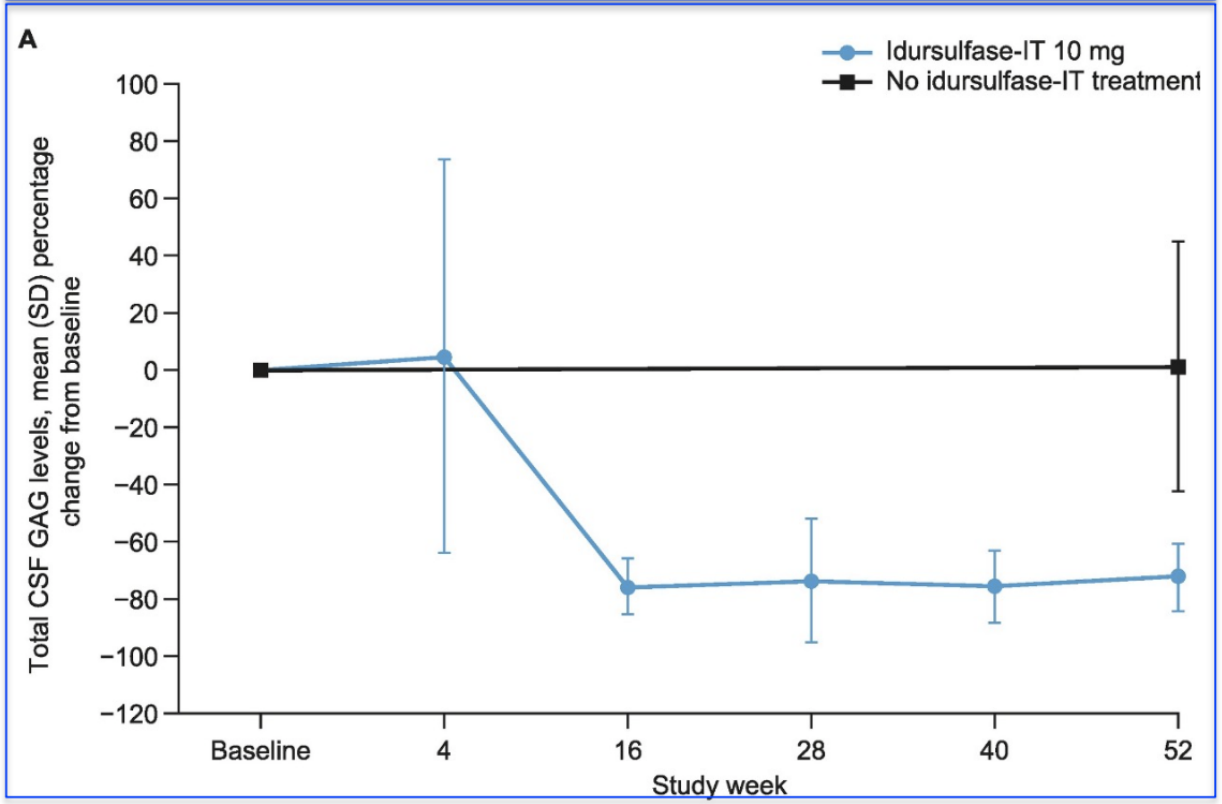


Fig. 4. Rate of change (weighted slope) in DAS-II GCA scores by prespecified subgroup. CI, confidence interval; DAS-II, Differential Ability Scales-II; GCA, General Conceptual Ability; IT, intrathecal; ITT, intention-to-treat; SE, standard error.

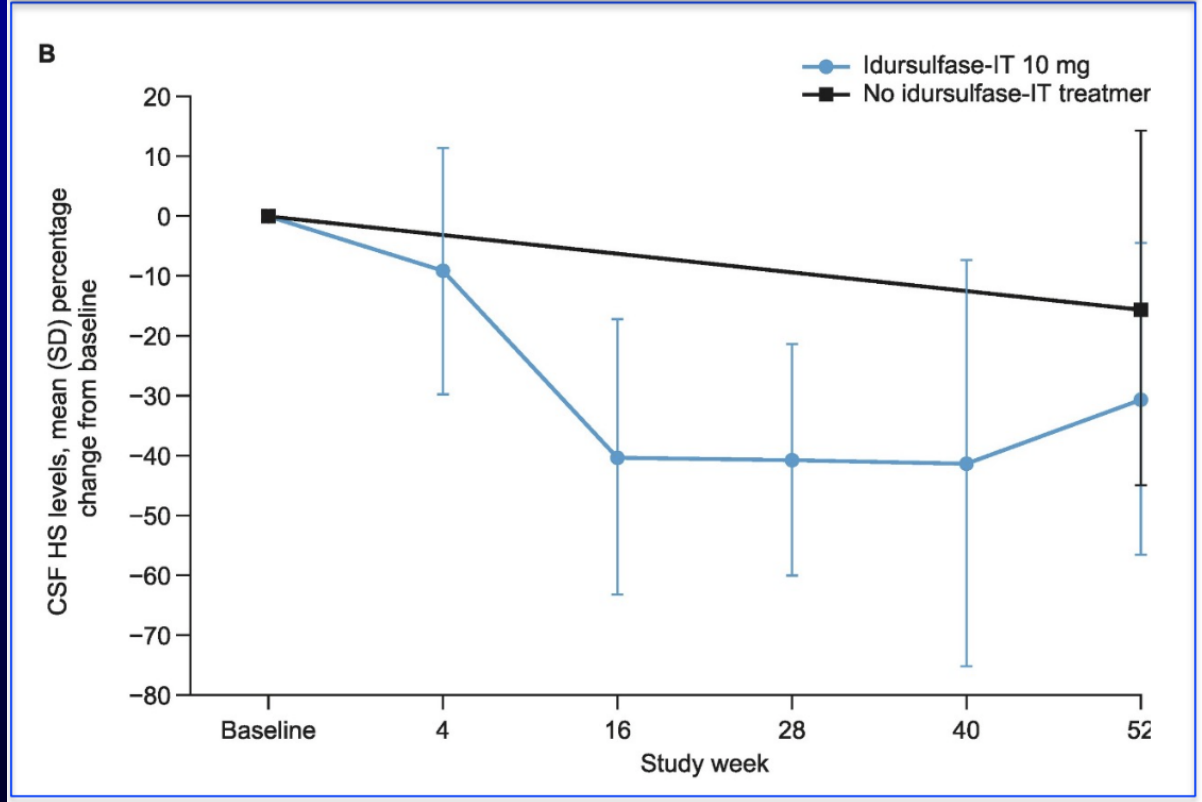
Muenzer et al. Mol Genet Metab (2022) 137:127-139

CSF GAG Analysis in the Phase II/III (AIM-IT study) IT ERT Clinical Trial

CSF GAG changes in the AIM-IT study



CSF HS changes in the AIM-IT study



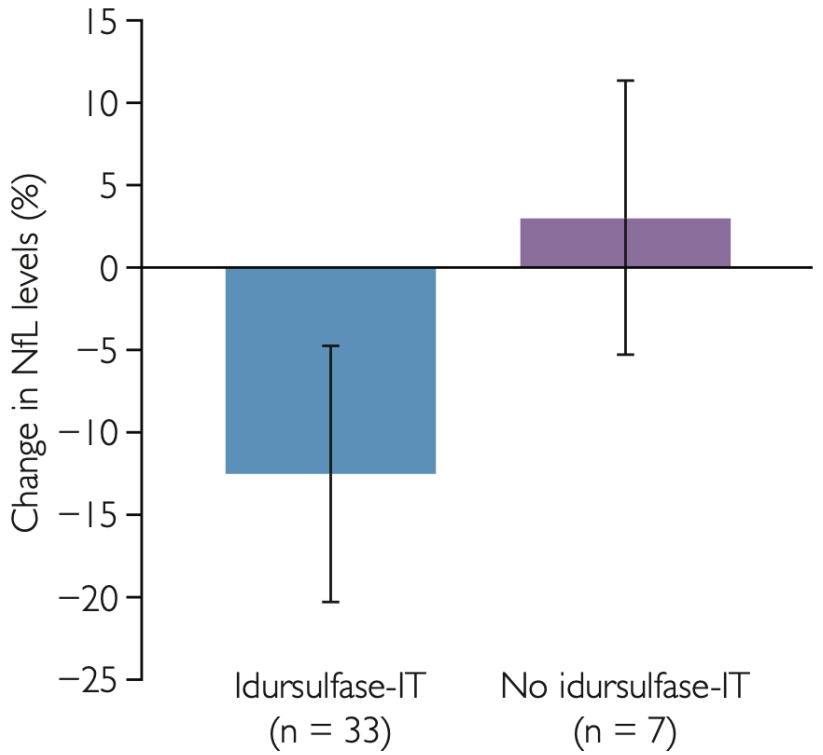
GAG measured by thrombin activity assay

GAG measured by mass spectrometry

Presented at the WORLD Symposium, San Diego, CA (Feb 2024) by C. Argueta

Neurofilament Light Chain (NfL) Analysis in the Phase II/III IT ERT Clinical Trial

Figure 1. Change in mean CSF NfL levels from baseline to week 48/52 by treatment status.



Error bars show the SEM. Data from weeks 48 and 52 were combined into a single time point for this analysis.
CSF, cerebrospinal fluid; idursulfase-IT, intrathecal idursulfase; NfL, neurofilament light chain; SEM, standard error of the mean.

Presented at the WORLD Symposium, San Diego, CA (Feb 2024) by C. Argueta

Neurofilament Light Chain (NfL) Analysis in the Phase II/III IT ERT Clinical Trial

- NfL levels may predict clinical severity and response to treatment.*

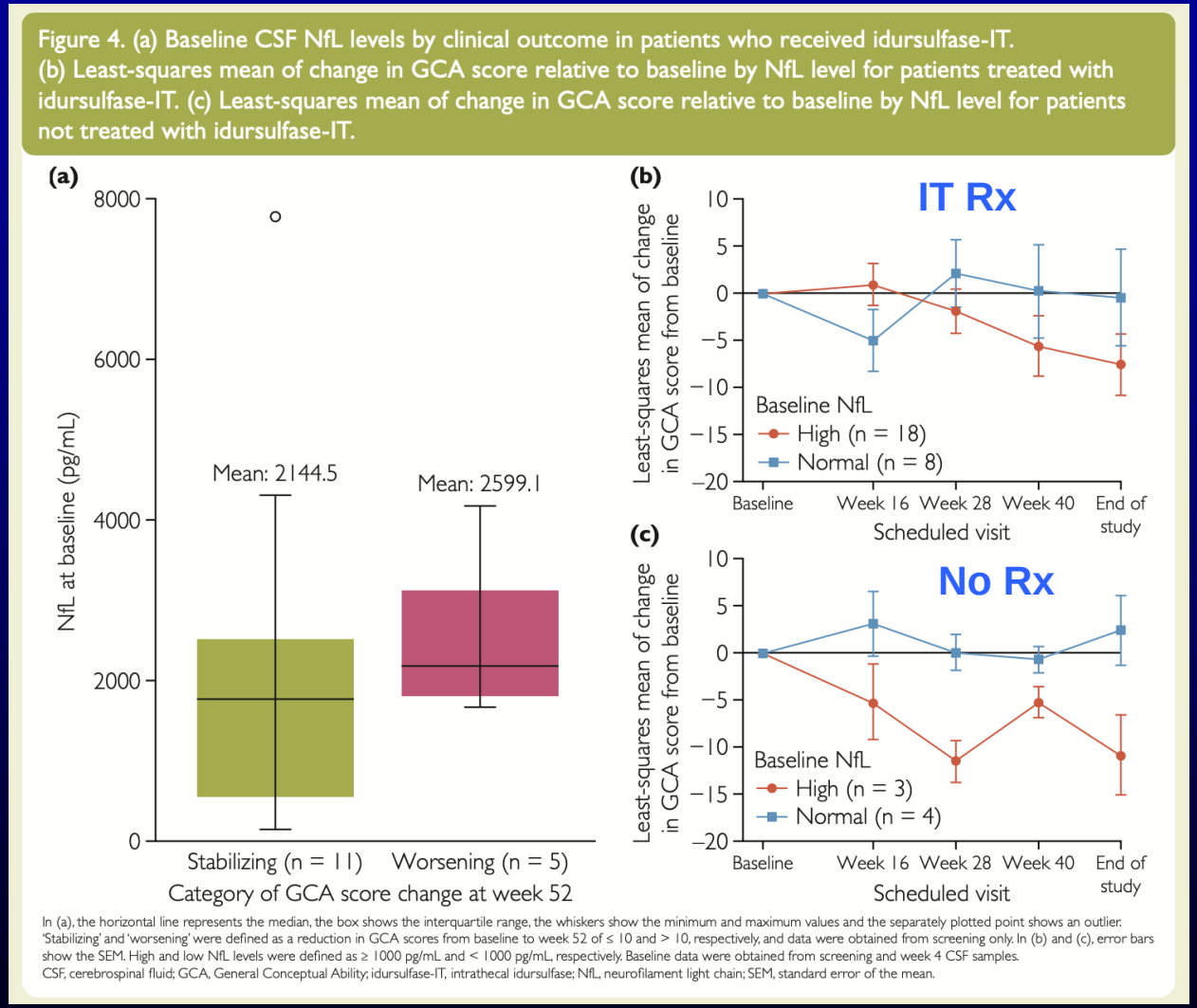


Figure 4. (a) Baseline CSF NfL levels by clinical outcome in patients who received idursulfase-IT. (b) Least-squares mean of change in GCA score relative to baseline by NfL level for patients treated with idursulfase-IT. (c) Least-squares mean of change in GCA score relative to baseline by NfL level for patients not treated with idursulfase-IT.

In (a), the horizontal line represents the median, the box shows the interquartile range, the whiskers show the minimum and maximum values and the separately plotted point shows an outlier. 'Stabilizing' and 'worsening' were defined as a reduction in GCA scores from baseline to week 52 of ≤ 10 and > 10, respectively, and data were obtained from screening only. In (b) and (c), error bars show the SEM. High and low NfL levels were defined as ≥ 1000 pg/mL and < 1000 pg/mL, respectively. Baseline data were obtained from screening and week 4 CSF samples.
CSF, cerebrospinal fluid; GCA, General Conceptual Ability; idursulfase-IT, intrathecal idursulfase; NfL, neurofilament light chain; SEM, standard error of the mean.

*Presenter's own opinion

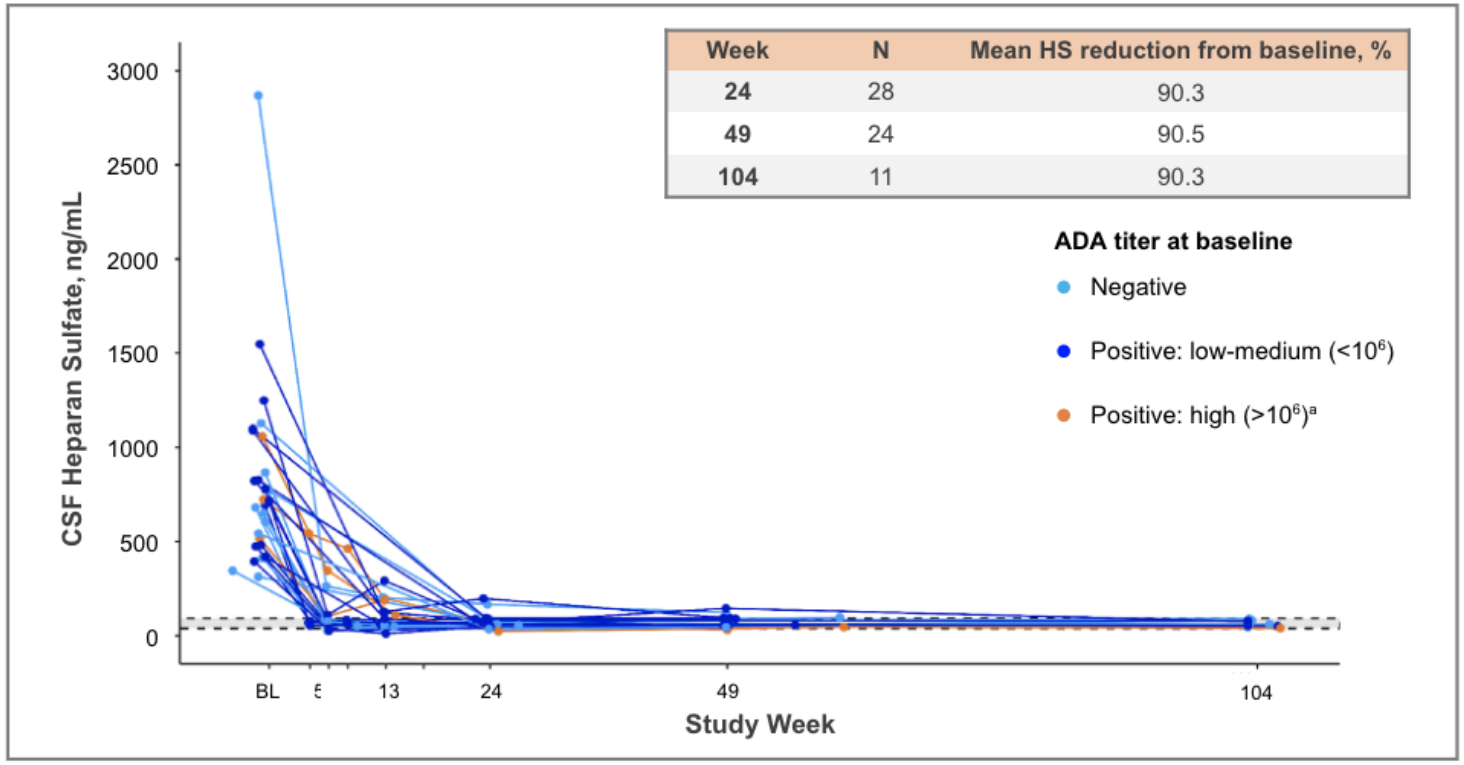
Presented at the WORLD Symposium, San Diego, CA (Feb 2024) by C. Argueta

DNL310 Phase I/II Study in Pediatric MPS II Patients

- Denali have developed a recombinant protein (DNL 310) consisting of an antibody fragment against the human transferrin receptor fused to iduronate-2-sulfatase as a treatment for the CNS disease in Hunter syndrome.
- 45 MPS II patients have received weekly IV infusions of DNL310 with dose ranging from 3 mg/kg to 30 mg/kg
- DNL310 was in general safe and well tolerated, but almost all patients had previously been on idursulfase.

CSF Heparan Sulfate Reduction with Weekly IV DNL310

CSF HEPARAN SULFATE



Week	N	Mean HS reduction from baseline, %
24	28	90.3
49	24	90.5
104	11	90.3

Normal levels of CSF HS[b] were achieved and sustained over time, including in those with pre-existing high ADA

Data cutoff: 2 Mar 2023. ADA, anti-drug antibody; BL, baseline; CSF, cerebrospinal fluid; HS, heparan sulfate.
[a]3 participants had high baseline ADA titer; [b]CSF HS was measured as a sum of the disaccharides D0A0, D0A6, D0S0, and D2S6 by mass spectrometry after enzymatic digestion. Preliminary normal range (10th-90th percentile) was based on analysis of CSF from healthy adults (n=30; median [range] age, 52 [18-80] years); 39.1-92.51 ng/mL. Total CSF GAG levels are similar in adults and children.[1]

1. Hendriksz CJ, et al. *Mol Genet Metab Rep*. 2015;5:103-106.

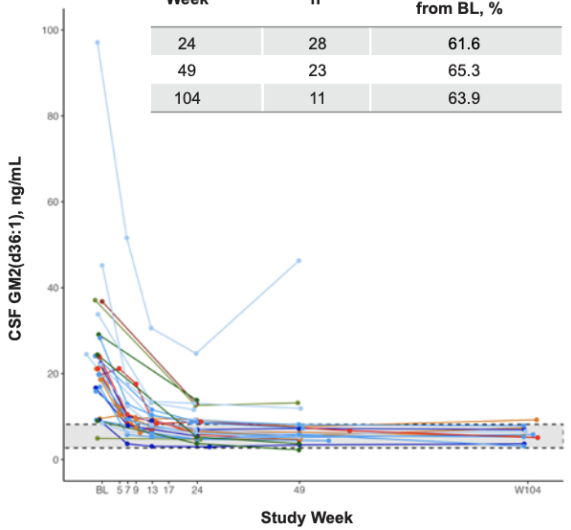
Data presented at the WORLD Symposium, San Diego, CA (Feb 2024)

CSF Lipid Reduction with Weekly IV DNL310

Figure 4. Lysosomal Lipids: Gangliosides and Glucosylsphingosine

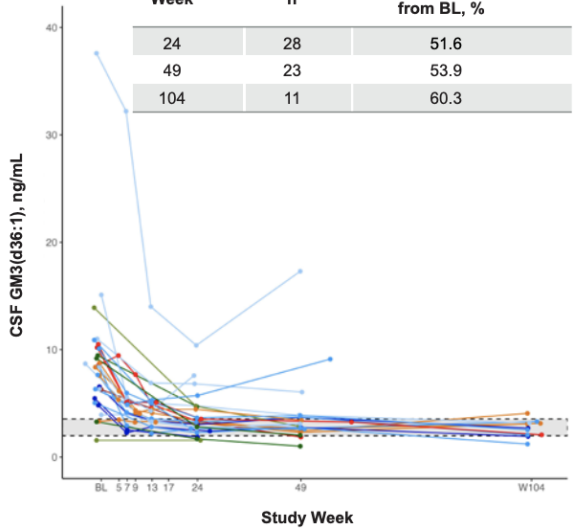
A GM2[a]

Week	n	Mean GM2 reduction from BL, %
24	28	61.6
49	23	65.3
104	11	63.9



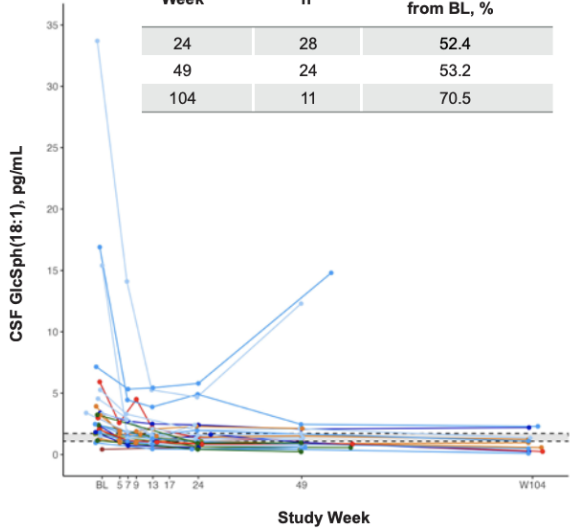
B GM3[a]

Week	n	Mean GM3 reduction from BL, %
24	28	51.6
49	23	53.9
104	11	60.3



C GlcSph[a]

Week	n	Mean GlcSph reduction from BL, %
24	28	52.4
49	24	53.2
104	11	70.5

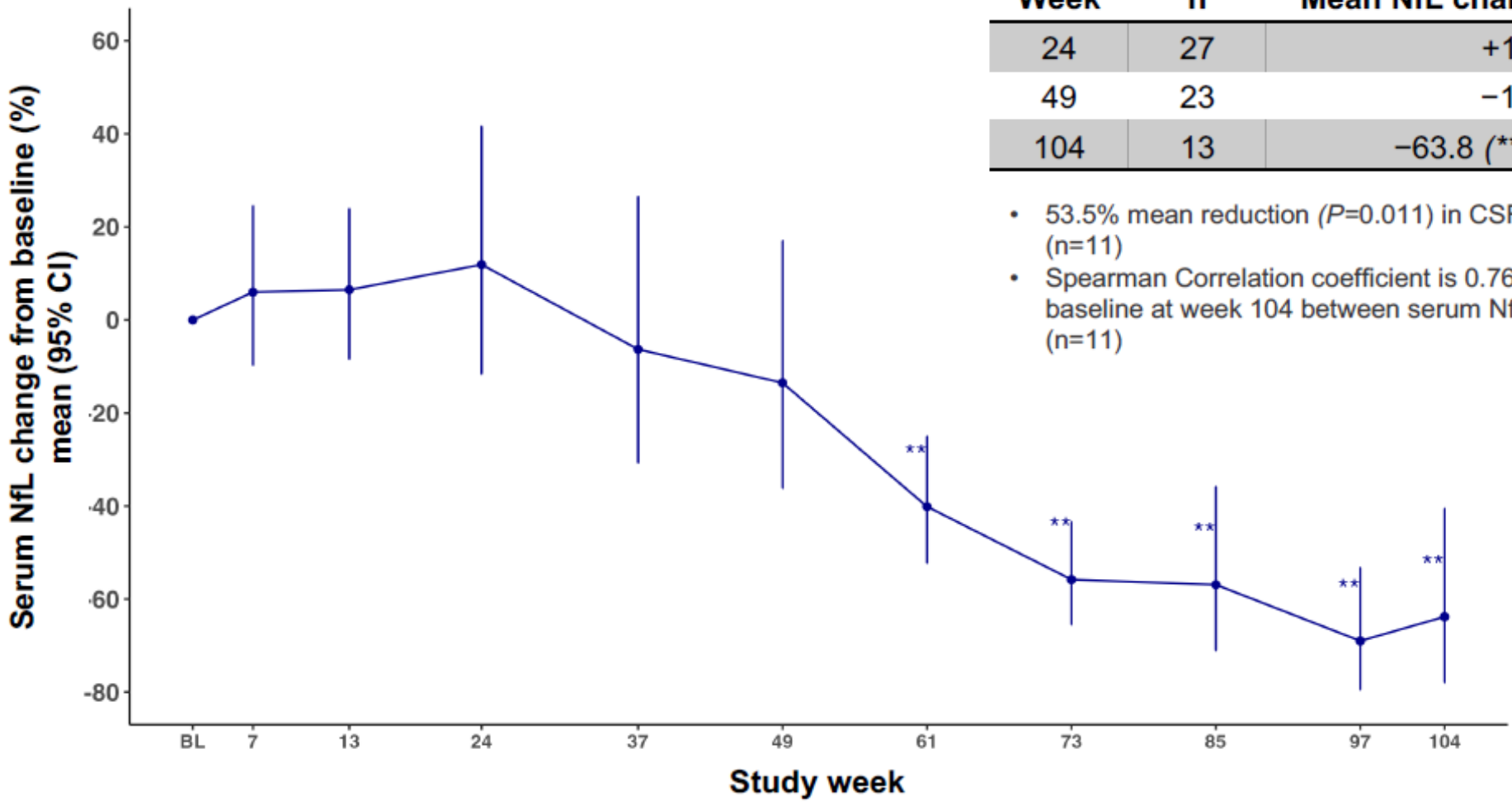


Cohort	Dose	Cohort	Dose	Cohort	Dose	Cohort	Dose
A	3 to 30 mg/kg	B2	7.5 to 15 mg/kg	C	15 mg/kg	E	15 mg/kg
B1	3 to 15 mg/kg	B3	15 mg/kg	D	15 mg/kg	High baseline ADA titer[b]	

Data cutoff: 2 Mar 2023.
ADA, anti-drug antibody; BL, baseline; CSF, cerebrospinal fluid; GlcSph, glucosylsphingosine; GM, ganglioside; IDS, iduronate-2-sulfatase; W, week.
[a]Preliminary GM3 normal range (gray dashed lines indicate 10th and 90th percentiles) was determined using CSF samples from 17 healthy adults (age range, 22-50 years; median, 27 years): 1.99-3.55 ng/mL. Preliminary GM2 and GlcSph normal ranges (gray dashed lines indicate 10th and 90th percentiles) were determined using CSF samples from 18 healthy adults (age range, 19-52 years; median, 24.5 years): GM3, 1.99-3.55 ng/mL; GM2, 2.72-8.2 ng/mL; GlcSph, 1.08-1.72 pg/mL. [b]Participants with high titers were defined as those with pre-existing ADA titers to IDS of >1:10^6; the 3 participants with high pre-existing ADA titers were from cohorts A, B1, and B2.

Data presented at the WORLD Symposium, San Diego, CA (Feb 2024)

Serum Neurofilament Light Chain (NfL) Reduction with Weekly IV DNL310



Week	n	Mean NfL change from BL, %
24	27	+11.9
49	23	−13.5
104	13	−63.8 (**P<0.001)

- 53.5% mean reduction (P=0.011) in CSF NfL at week 104 (n=11)
- Spearman Correlation coefficient is 0.76 for the change from baseline at week 104 between serum NfL and CSF NfL (n=11)

Data presented at the WORLD Symposium, San Diego, CA (Feb 2024)

Ten Reasons Why the Biomarker CSF Heparan Sulfate Using the Accelerated Approval Pathway Should be Utilized for Neuronopathic MPS Disorders

1. Neuronopathic MPS (nMPS) are ultra-rare (low-prevalence) disorders.
2. The biochemistry of MPS (single enzyme defects) is well understood.
3. The primary event in nMPS disorders is a defect in GAG metabolism resulting in intralysosomal substrate accumulation due to a deficient enzyme activity.
4. CSF heparan sulfate (HS) is always elevated in nMPS individuals.
5. CSF HS can be reliably measured using mass spectrometry.
6. CSF HS levels correlate with brain tissue HS.

Ten Reasons Why the Biomarker CSF Heparan Sulfate Using the Accelerated Approval Pathway Should be Utilized for Neuronopathic MPS Disorders

7. Reduction of CSF HS reflects reduction in brain tissue HS.
8. Reduction of secondary disease activity biomarkers of lysosomal dysfunction (GM2/GM3) and neuronal injury (NfL) support the relevance of CSF HS as the primary biomarker.
9. Reliance on clinical efficacy with placebo-controlled trials to demonstrate effectiveness is unethical.
10. Regulatory flexible is needed now to bring treatments to individuals with nMPS using the FDA 2020 industry guidelines.

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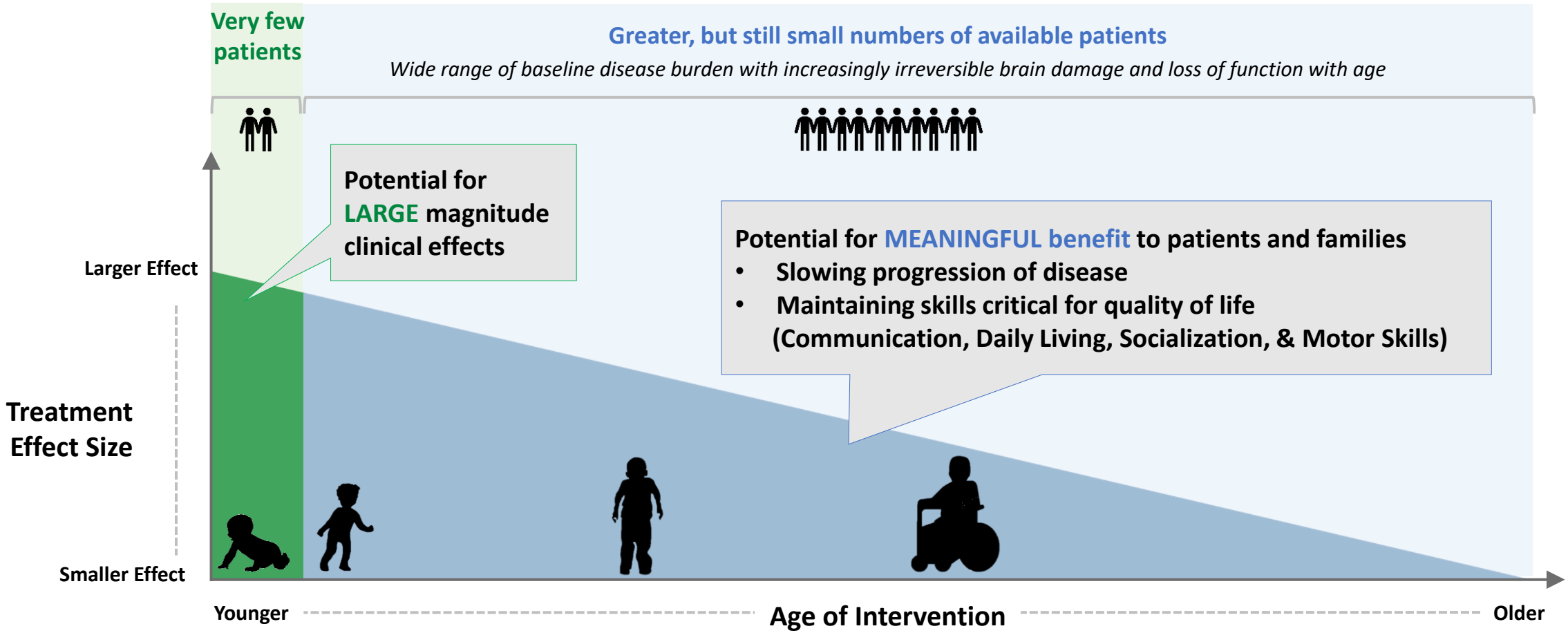
9. Reliance on clinical efficacy with placebo-controlled trials to demonstrate effectiveness is unethical.
10. Regulatory flexible is needed <u>now</u> to bring treatments to individuals with nMPS using the FDA 2020 industry guidelines.

Thank you for your attention!

Demonstrating Effectiveness in Clinical Trials for Neuronopathic MPS Children is Challenging



Demonstrating evidence of effectiveness for therapies in neuronopathic MPS is **extremely challenging** given the low prevalence, baseline disease burden of children at time of entry into clinical trials and long timespan of symptom evolution

Slide created by Dr. Cara O'Neill

Mark Dant
Volunteer Executive Director
Ryan Foundation


Age 22
months

Benefit-Risk Calculation
In the real world

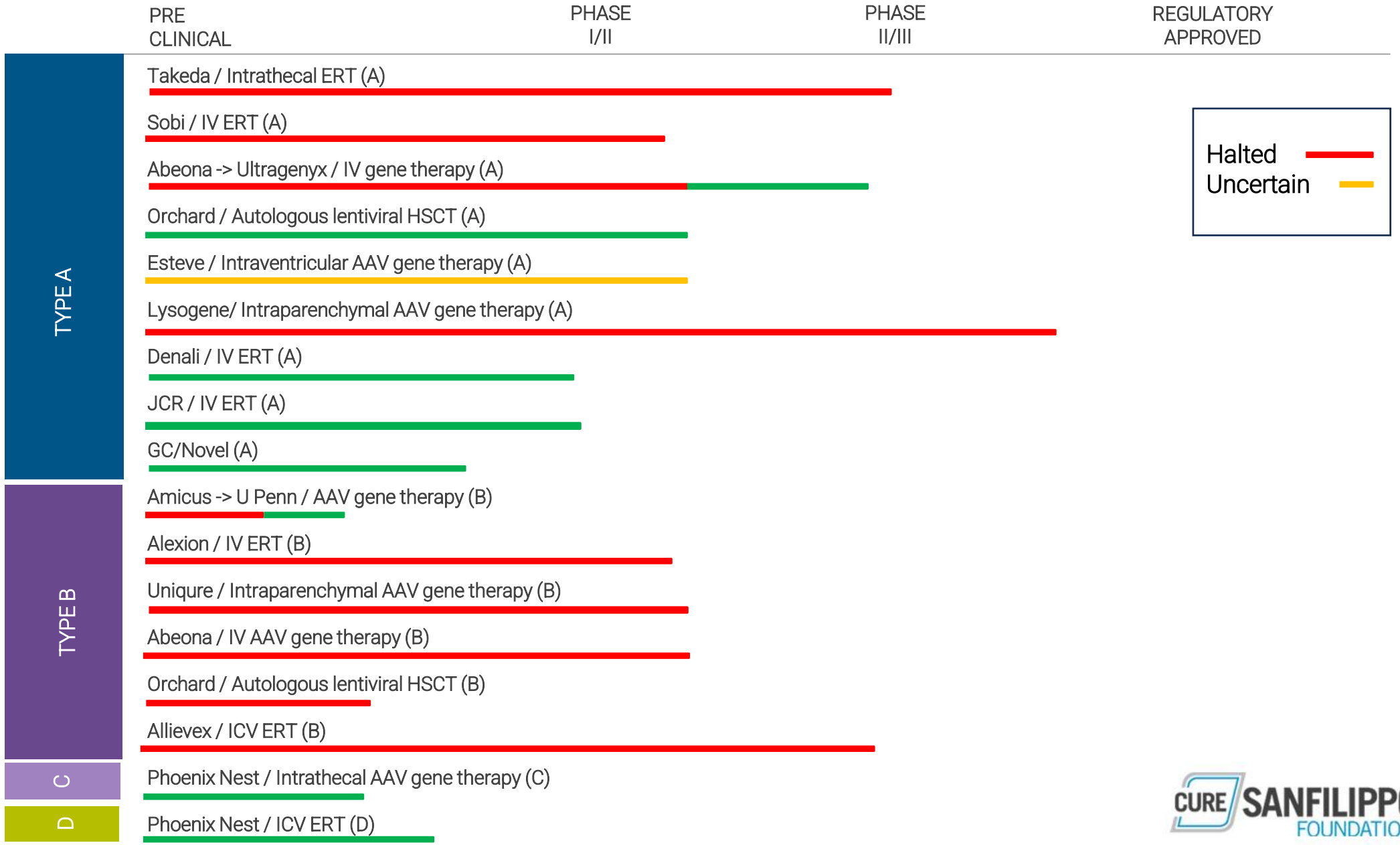
"I'll take that [my child] can sit and enjoy doing something for three more minutes than before. **I'll even take an intensive invasive medical procedure to get me six more months.**"

Porter KA, et al. ***Parent Experiences of Sanfilippo Syndrome Impact and Unmet Treatment Needs: A Qualitative Assessment****. Neurol 2021 Jun;10(1):197-212.*

CURE SANFILIPPO FOUNDATION

Liv (age 8) & her mom Becky
Sanfilippo syndrome (MPS IIIB)
Intracerebroventricular Enzyme Replacement

Sanfilippo Syndrome (MPS III) Therapeutic Pipeline



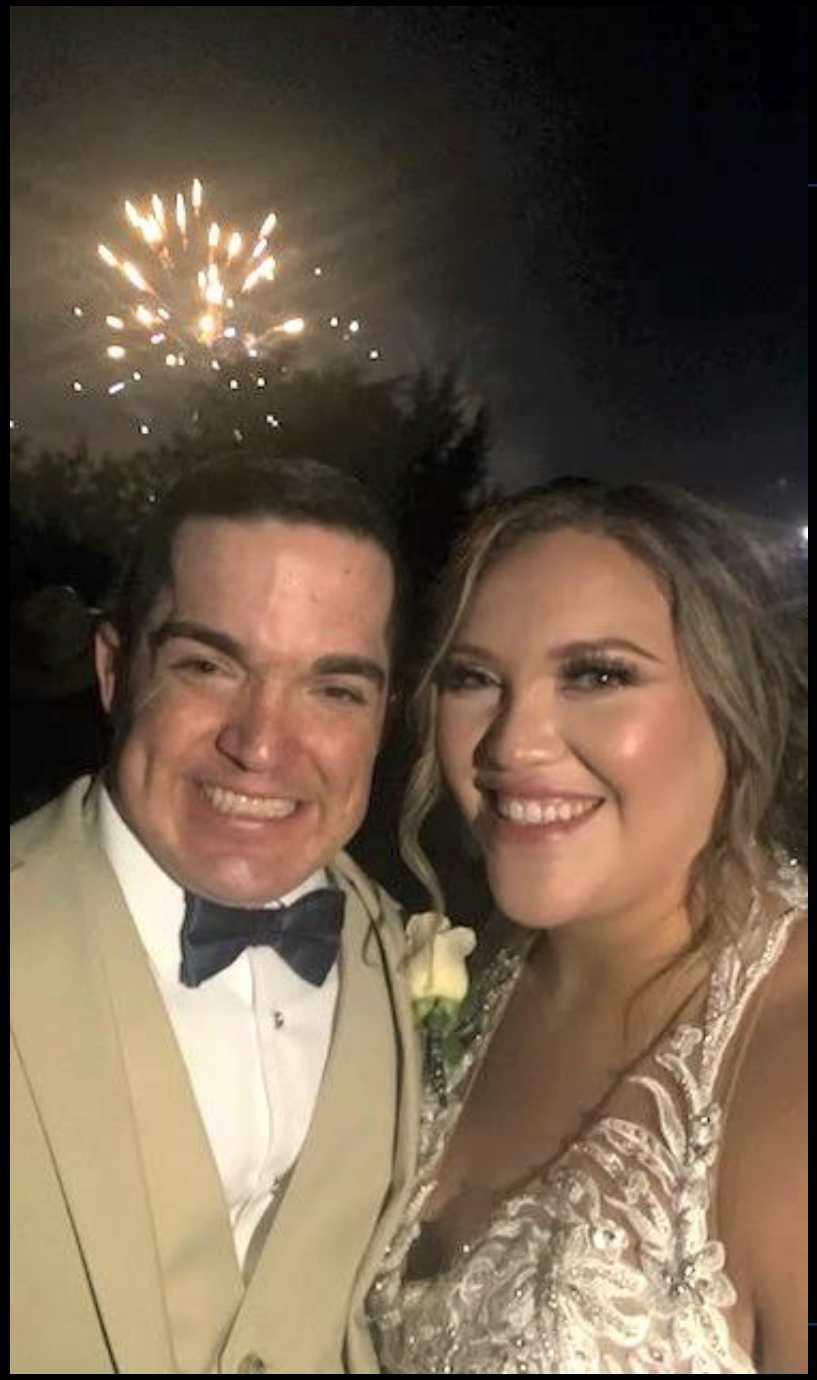
CURE SANFILIPPO FOUNDATION

March 2020 FDA Guidance

Slowly Progressive, Low-Prevalence Rare Diseases With Substrate Deposition That Result From Single Enzyme Defects: Providing Evidence of Effectiveness for Replacement or Corrective Therapies Guidance for Industry

SECTION III. TYPE AND QUANTITY OF EVIDENCE NECESSARY TO SUPPORT EFFECTIVENESS FOR REPLACEMENT OR CORRECTIVE THERAPIES

As discussed in section II., for certain slowly progressive, low-prevalence rare diseases, sponsors can pursue various treatment strategies to halt or slow the abnormal accumulation of substrate in tissues. **When the pathophysiology of a disease is well understood and the mechanism of action of the drug/biologic is well characterized, specific drug-induced substrate reduction in relevant tissue or tissues can have a *reasonable likelihood* of predicting clinical effectiveness.** In such a case, a clear demonstration in clinical trial or trials that an exogenously administered enzyme or drug results in substrate reduction **(i.e., it reaches the tissue of interest) can serve as the basis for accelerated approval.**




SADIE
LAST
INFUSION
104
4.22.21

Case Study: Measuring Glycosaminoglycans including Heparan Sulfate

- **Maria Fuller, PhD**, University of Adelaide

REAGAN-UDALL FOUNDATION FOR THE FDA

Measuring glycosaminoglycans (GAG), including heparan sulphate (HS)

Maria Fuller, National Referral Laboratory,

Genetics and Molecular Pathology, SA Pathology

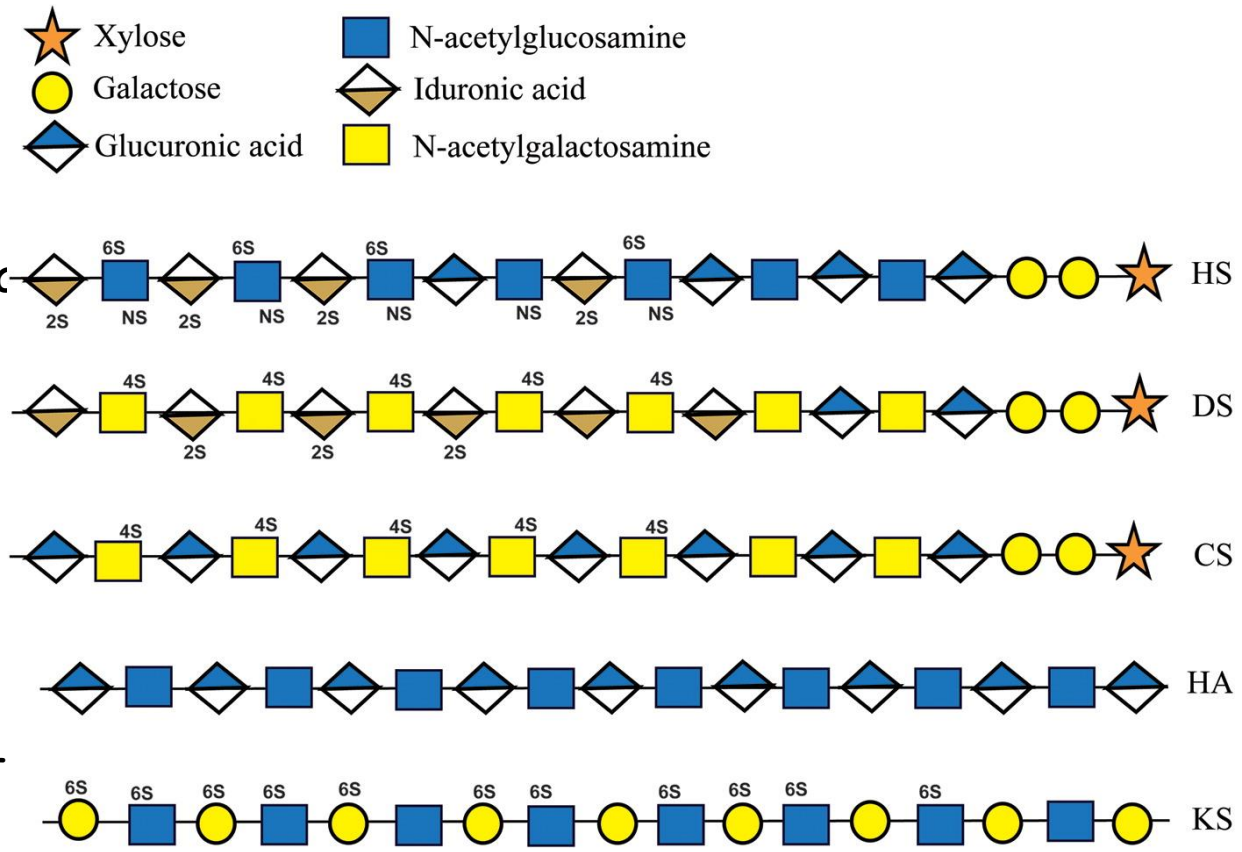
at Women's and Children's Hospital; Adelaide Medical School and School of Biological Sciences, University of Adelaide,

AUSTRALIA

SA PATHOLOGY

glycosaminoglycans (GAG) are complex sugars

- carbohydrate chains of proteoglycans
- covalently linked to protein core (except hyc
- repeating disaccharide units
- four/five main classes
- high degree of heterogeneity eg sulphat

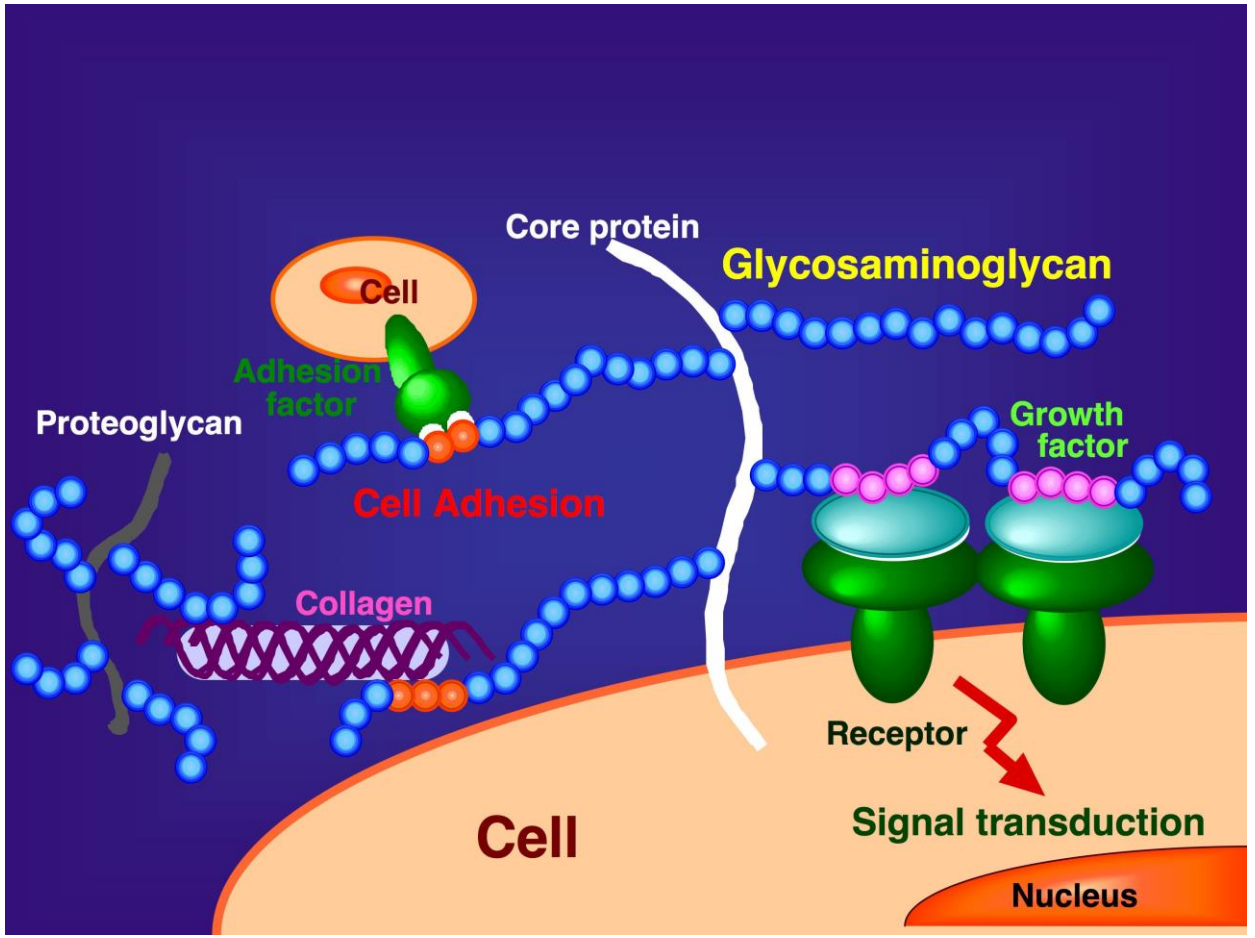


HS, heparan sulphate; DS, dermatan sulphate; CS, chondroitin sulphate
HA, hyaluronic acid; KS, keratan sulphate

Lindahl et al. In Essentials of Glycobiology 2015; pp 207-221

glycosaminoglycans (GAG) are essential for cell function

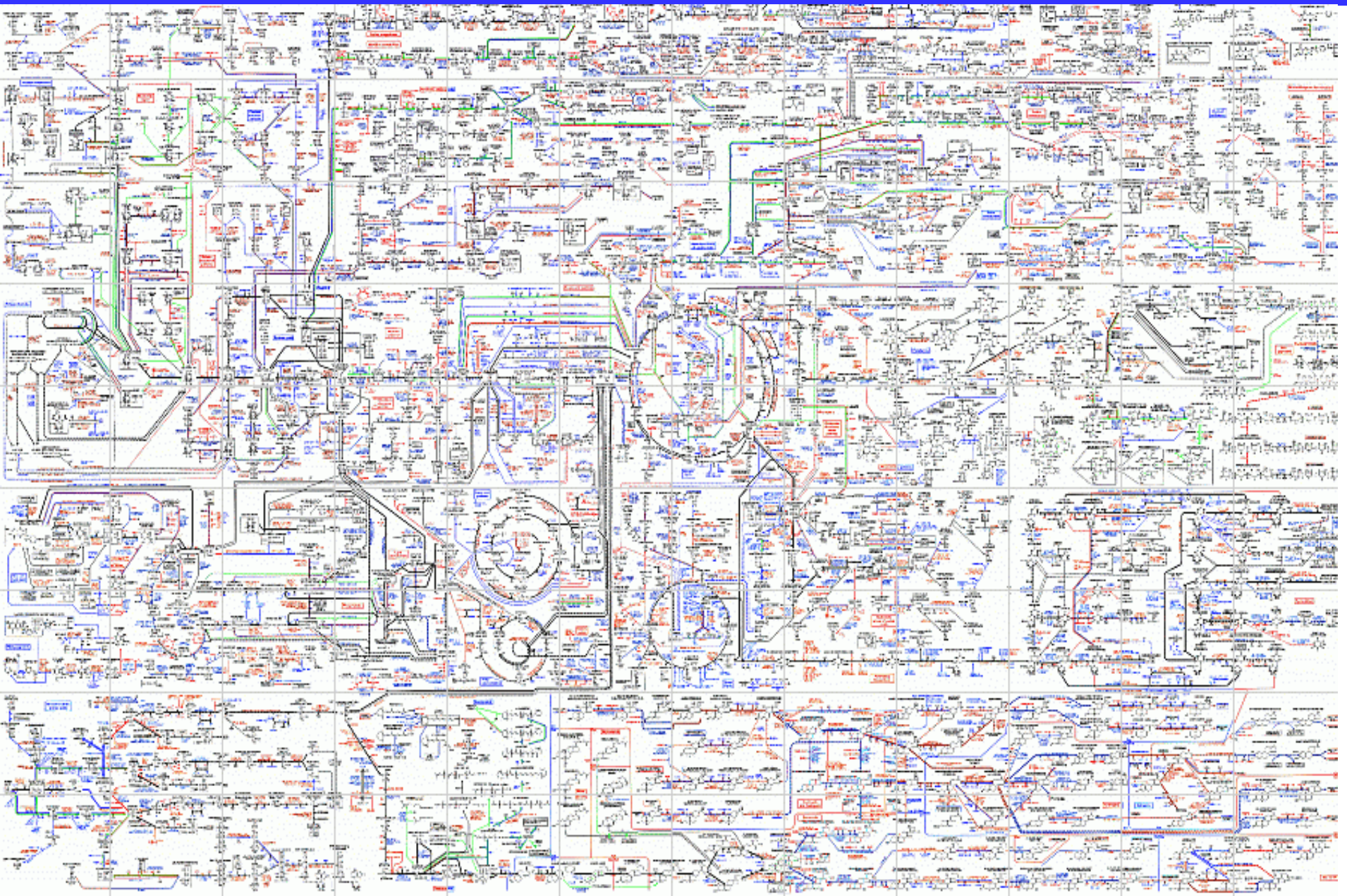
- present in all cells
- highly dynamic
- essential for proper development and function
- CS – cartilage, ligaments and tendons
- DS – skin, cartilage
- KS – connective tissue, cornea, cartilage
- **HS – cell signaling/transduction**
- HA – connective/epithelial tissue



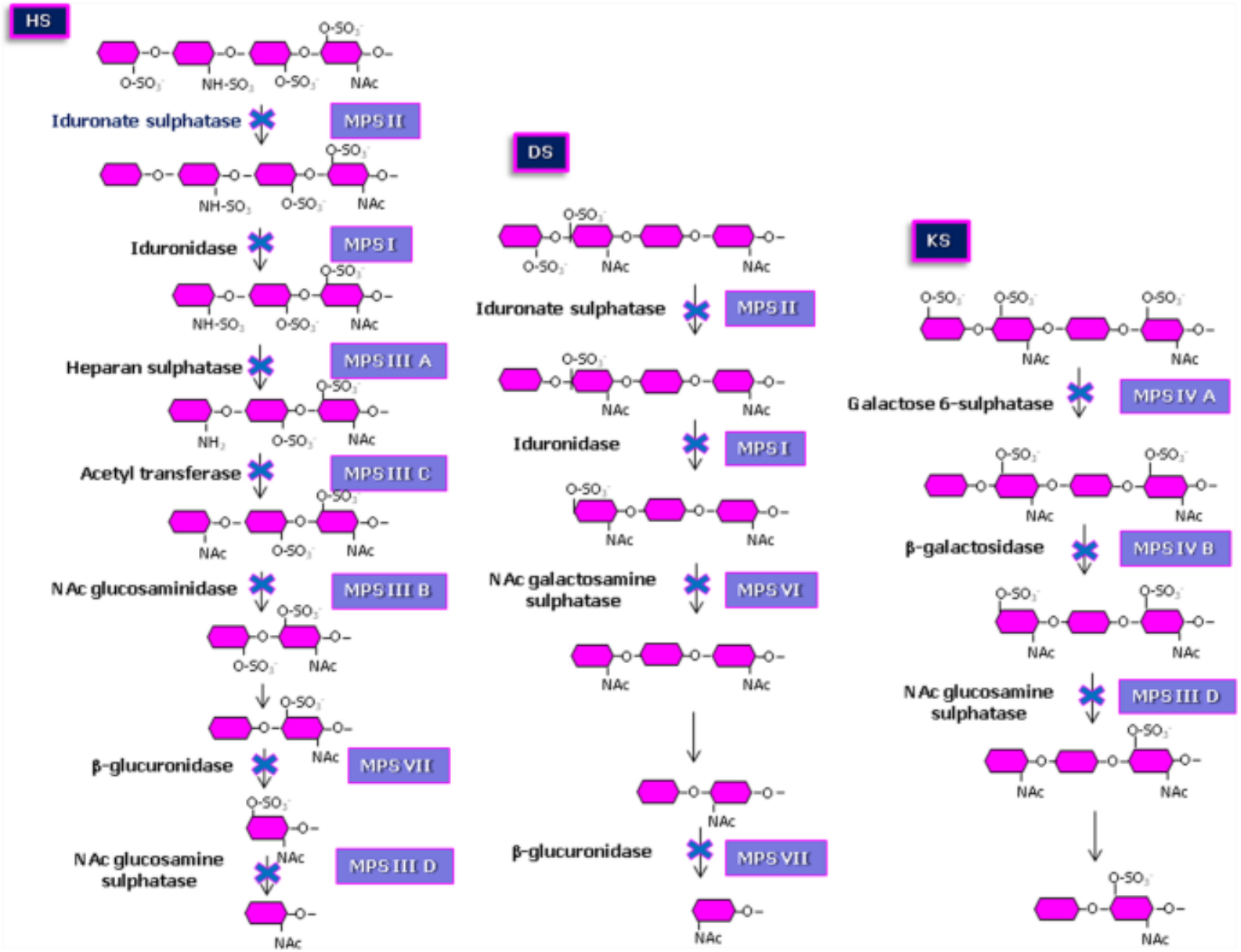
Lindahl et al. In Essentials of Glycobiology 2015; pp 207-221

HS, heparan sulphate; DS, dermatan sulphate; CS, chondroitin sulphate
HA, hyaluronic acid; KS, keratan sulphate

the quantity of glycosaminoglycans (GAG) is critical

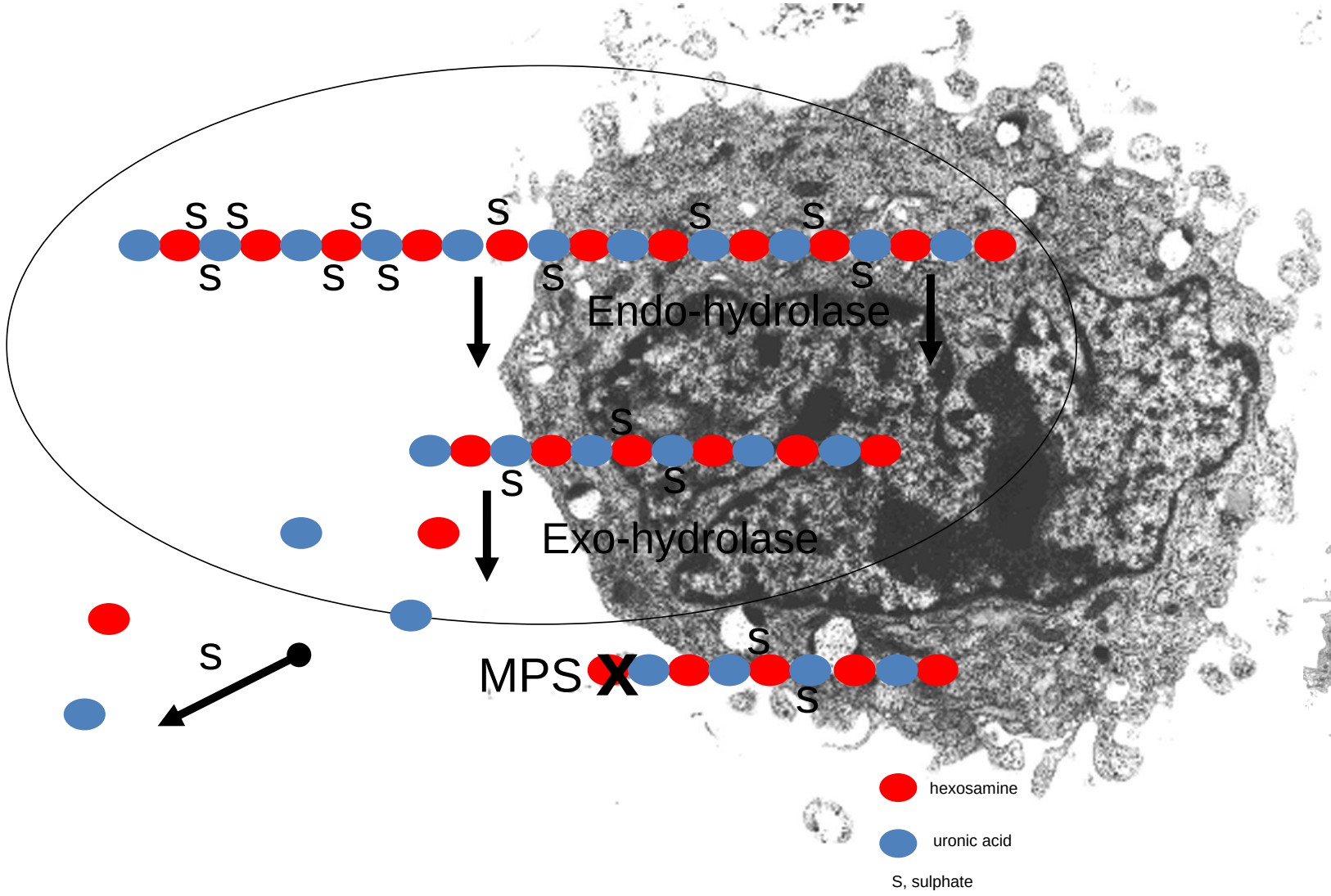


GAG degradation is sequential with no redundancy

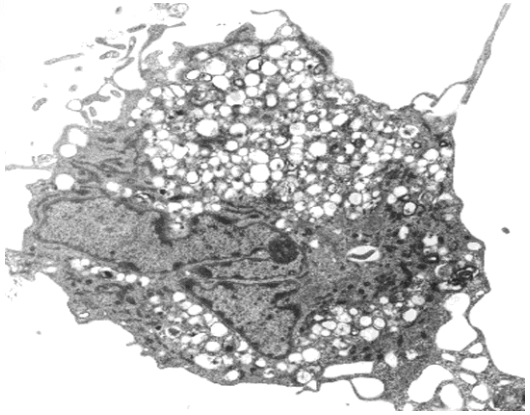


GAG, glycosaminoglycans
HS, heparan sulphate
DS, dermatan sulphate
KS, keratan sulphate
MPS, mucopolysaccharidoses

partially degraded GAG accumulate in lysosomes



unaffected cell



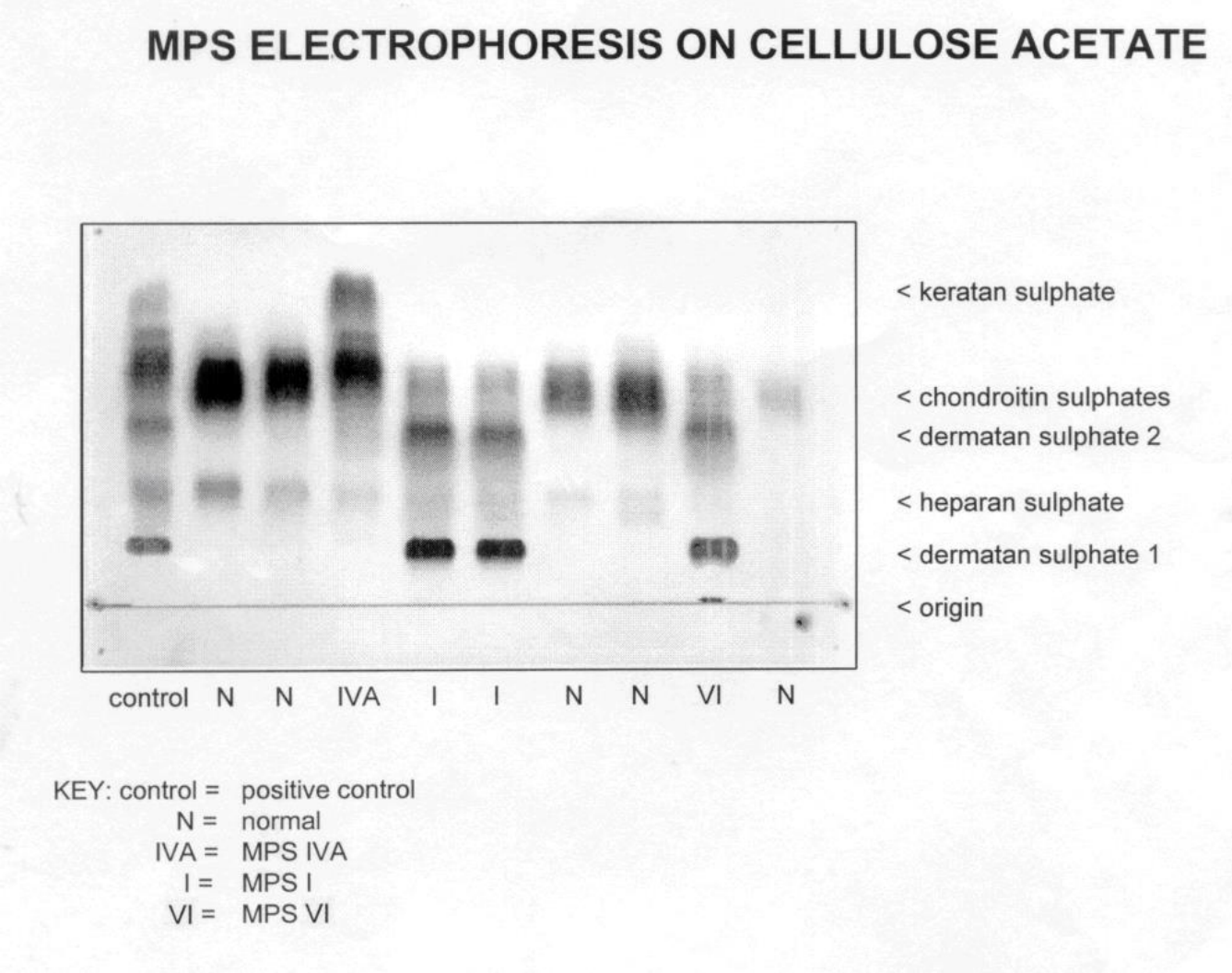
MPS cell

GAG, glycosaminoglycans; MPS, mucopolysaccharidosis

why and how have GAG been measured?

urine: dye binding

- poor precision
- poor sensitivity
- non-specific – total measurement
- concentrations vary with age
- not diagnostic



GAG, glycosaminoglycans; MPS, mucopolysaccharidosis

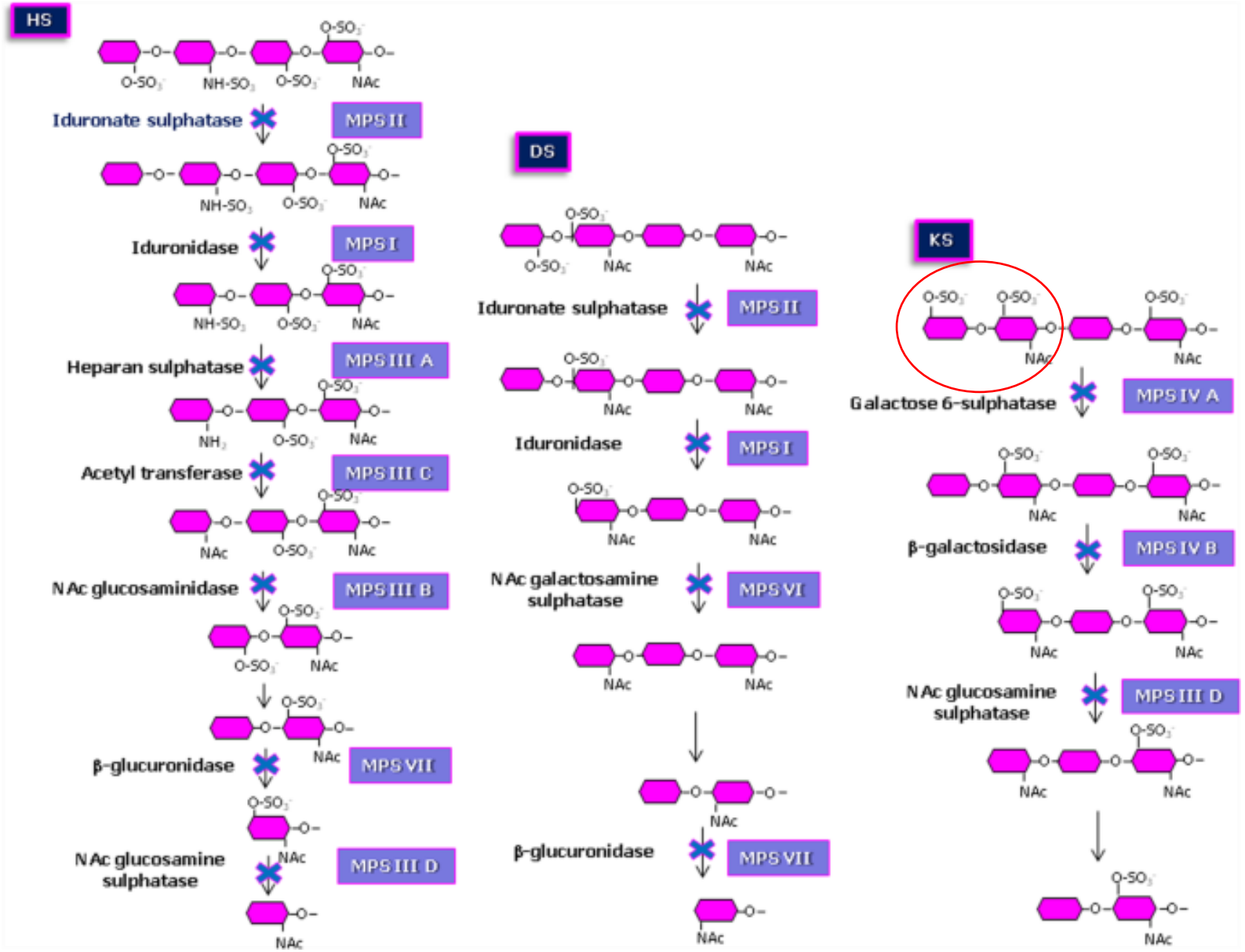
case presentation exemplifies the problem

Biochemical parameter	Reference range	Sibling 1	Sibling 2
Total urinary GAG (g/mol creatinine)	<6	11	6
One dimensional GAG high resolution electrophoresis	N/A	normal	normal
HNAc-UA (1S) (mmol/mol creatinine)	<0.1	0.24	0.28

Chin et al. JIMD Rep. 2020;55:68-74.

GAG, glycosaminoglycans

GAG degradation is sequential with no redundancy



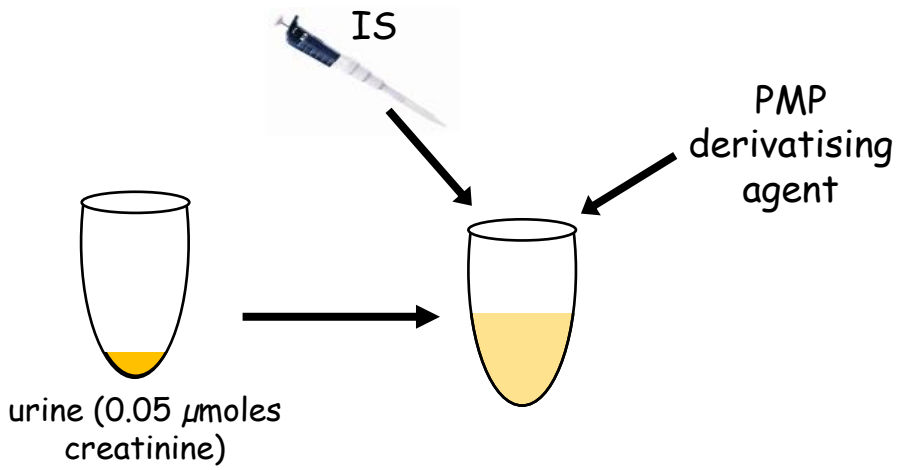
GAG, glycosaminoglycans
HS, heparan sulphate
DS, dermatan sulphate
KS, keratan sulphate
MPS, mucopolysaccharidoses

mass spectrometry: a game changing measuring tool

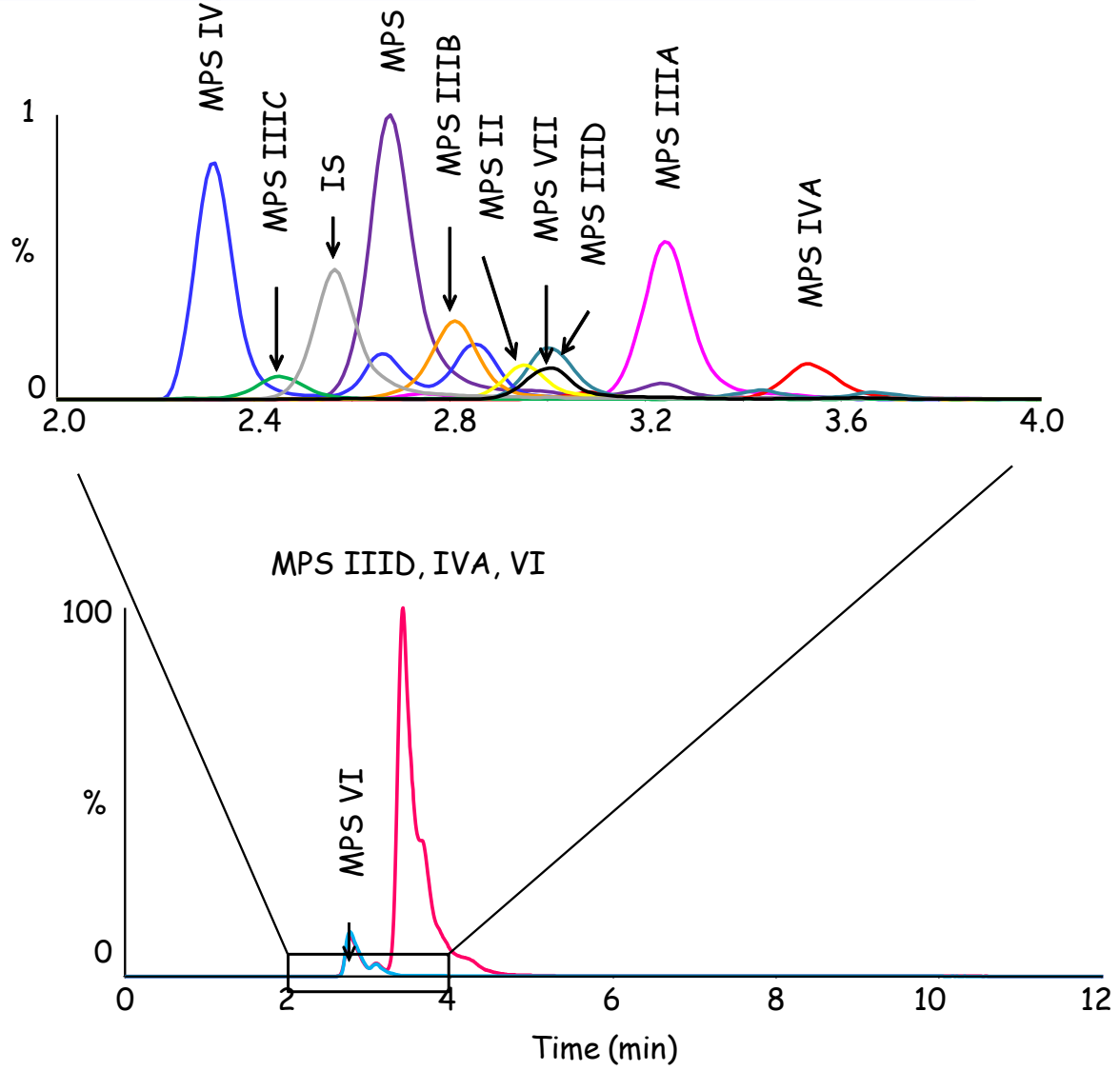
- measures compounds in the femtomole ra
- mass spectrometry has been a game chan
  affords partial structural elucidation – w
- internal standards have allowed absolute
- dye binding for total GAG and electropho

GAG, glycosaminoglycans

signature oligosaccharides identify each MPS



oligosaccharide derivatisation



Fuller, Clin Biochem Rev. 2020;41:53-66; Saville et al. Genet Med 2019;21:753-7.

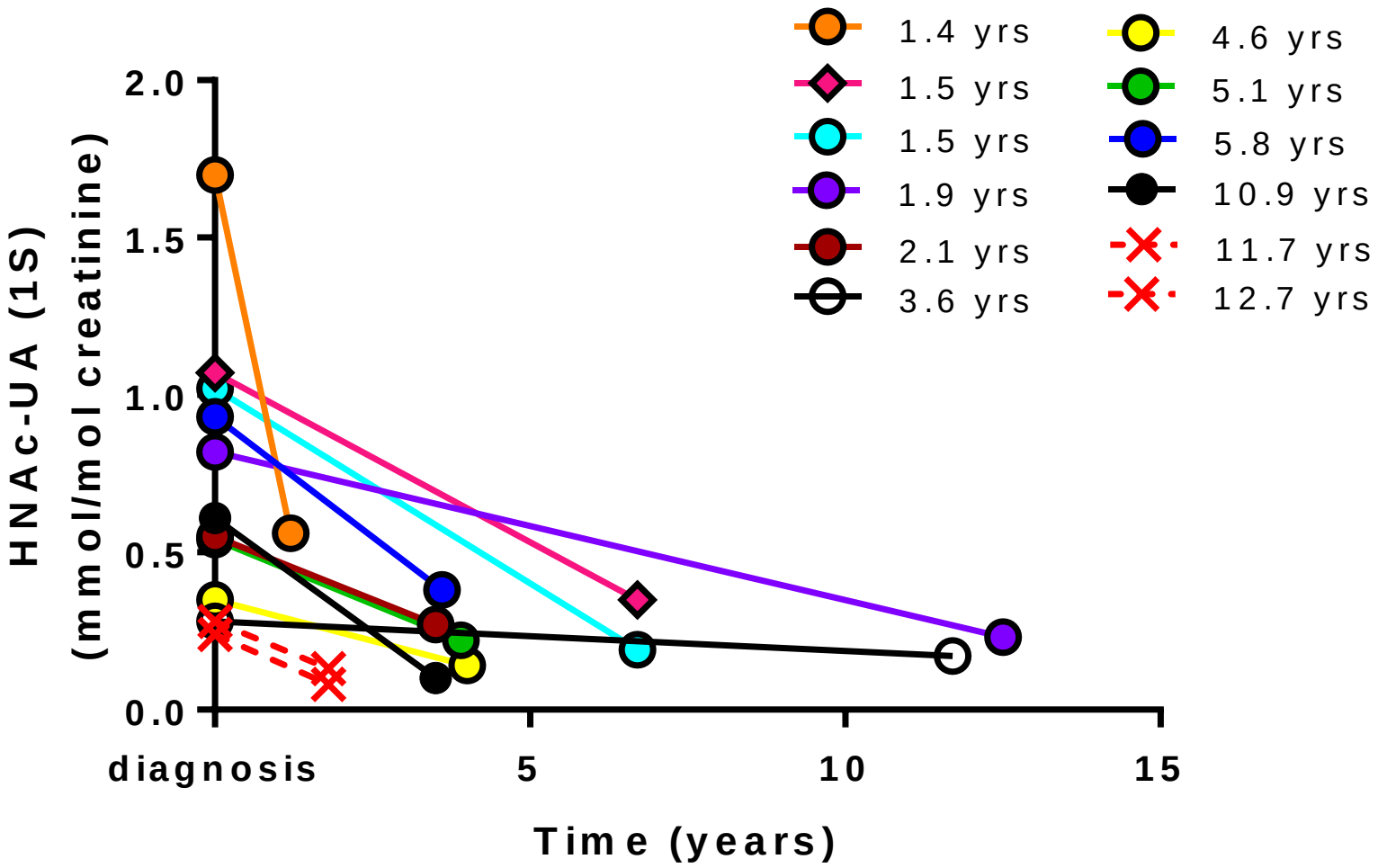
MPS, mucopolysaccharidosis; IS, internal standard; PMP, 1-phenyl 3-methyl 5-pyrazolone

demonstrated real-world utility: post-implementation

- NPAAC validated to ISO 15189 pathology standards
- introduced into our diagnostic service in 2016 (NATA accredited)
- 55 positives in the last eight years:
    - 9 x MPS I
    - 13 x MPS II
    - 9 x MPS IIIA
    - 7 x MPS IIIB
    - 2 x MPS IIIC
    - 9 x MPS IVA
    - 3 x MPS IVB/GM1
    - 2 X MPS VI
    - 1 x MPS VII
- two "false" positives: MPS IIIC and MPS II = laboratory errors = 0 false positives
- perfect external quality control (ERNDIM)

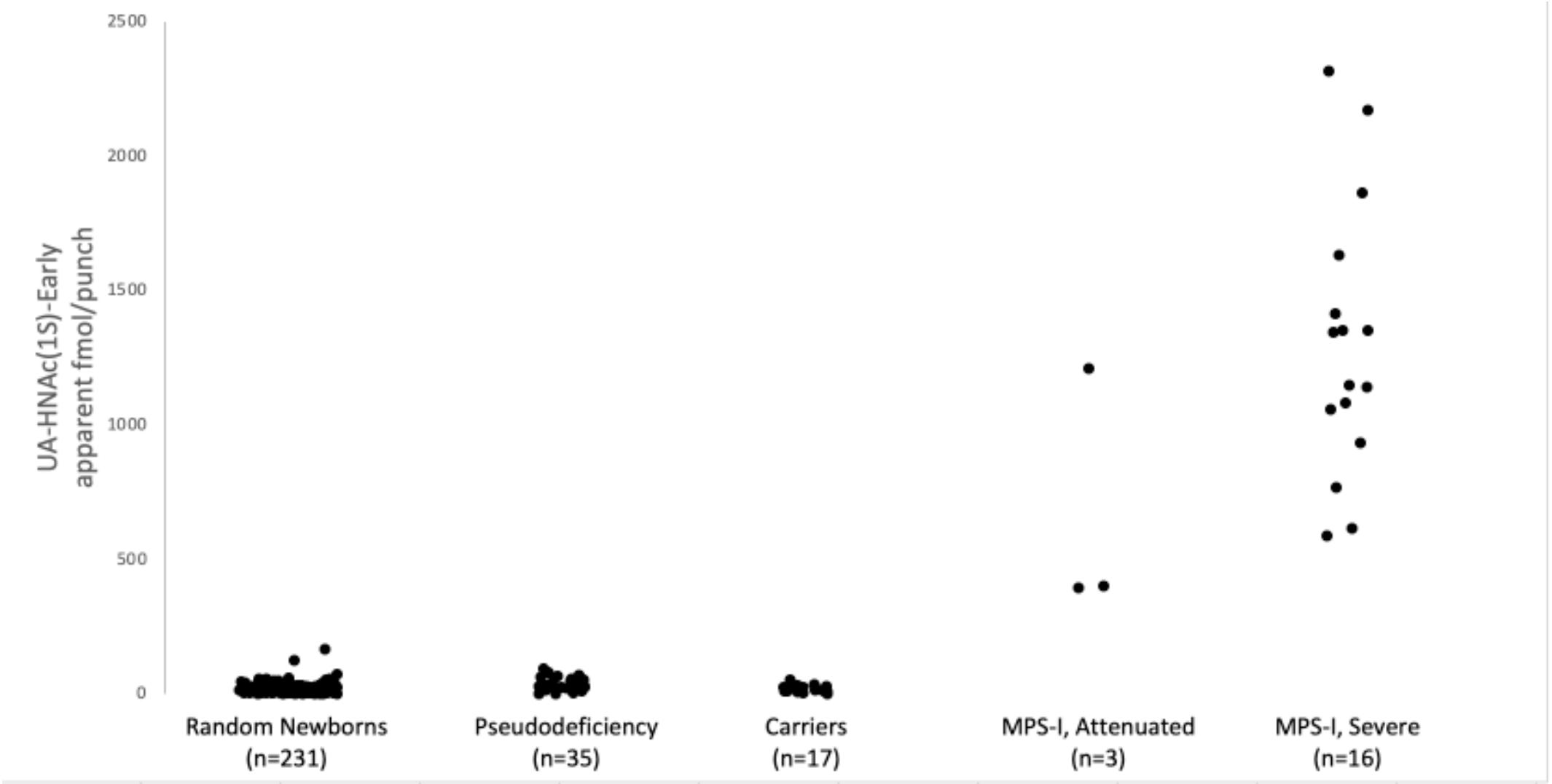
NPAAC, National Pathology Accreditation Advisory Council;
ERNDIM, European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inborn errors of Metabolism;
MPS, mucopolysaccharidosis

monitoring enzyme replacement therapy



Chin S et al., JIMD Rep 2020;55:68-74

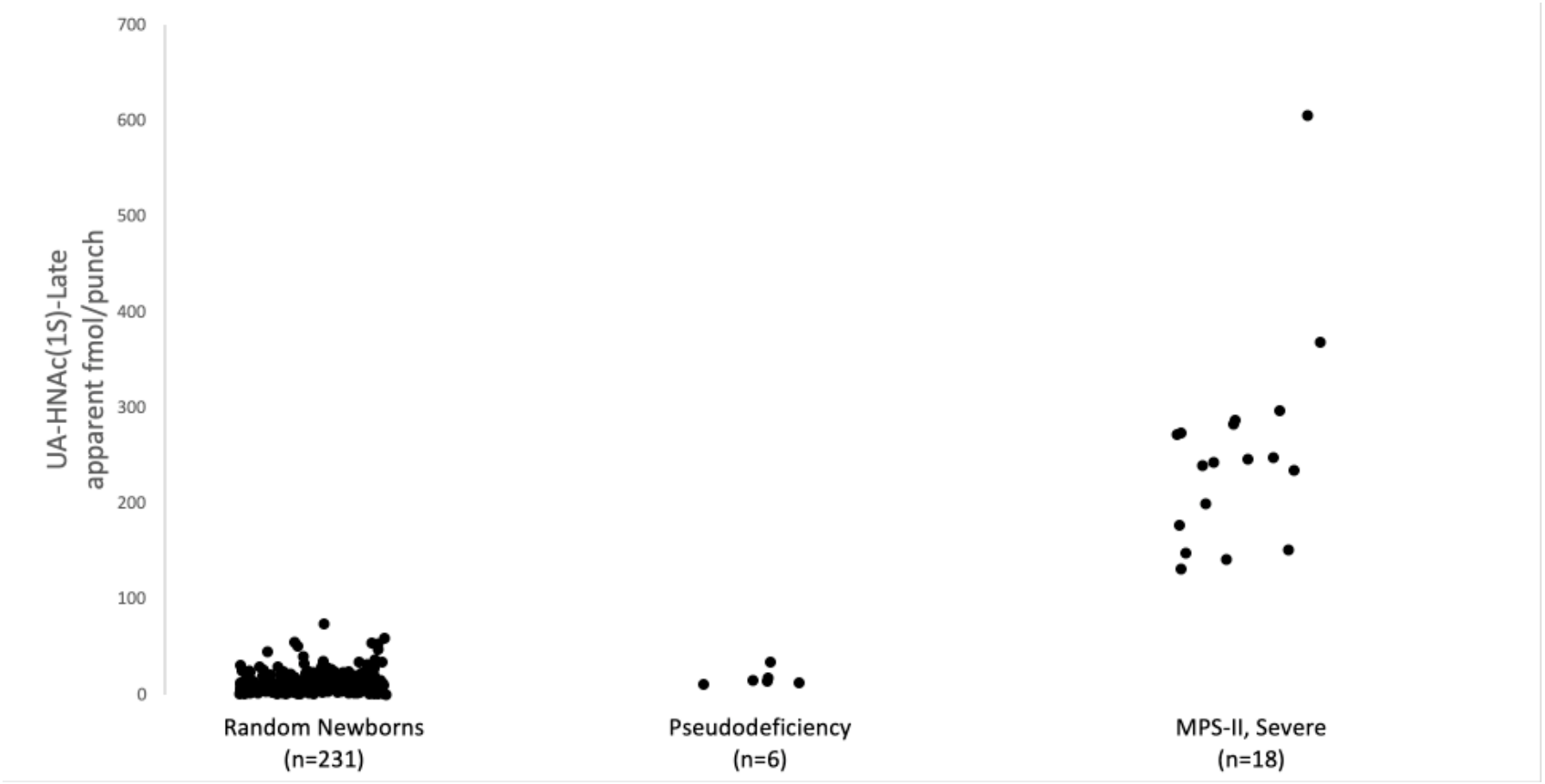
utility for newborn screening: MPS I



Herbst et al. Int J Neonatal Scr 2020;6:69

MPS, mucopolysaccharidosis

utility for newborn screening: MPS II



MPS, mucopolysaccharidosis

Herbst et al. Int J Neonatal Scr 2022;8:9

newborn screening for all the mucopolysaccharidoses

Molecular Genetics and Metabolism 140 (2023) 107632

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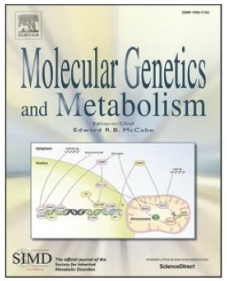

Check for updates

Endogenous, non-reducing end glycosaminoglycan biomarkers are superior to internal disaccharide glycosaminoglycan biomarkers for newborn screening of mucopolysaccharidoses and GM1 gangliosidosis

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depolymerisation of the polymer to disaccharides

1 Chemical depolymerisation

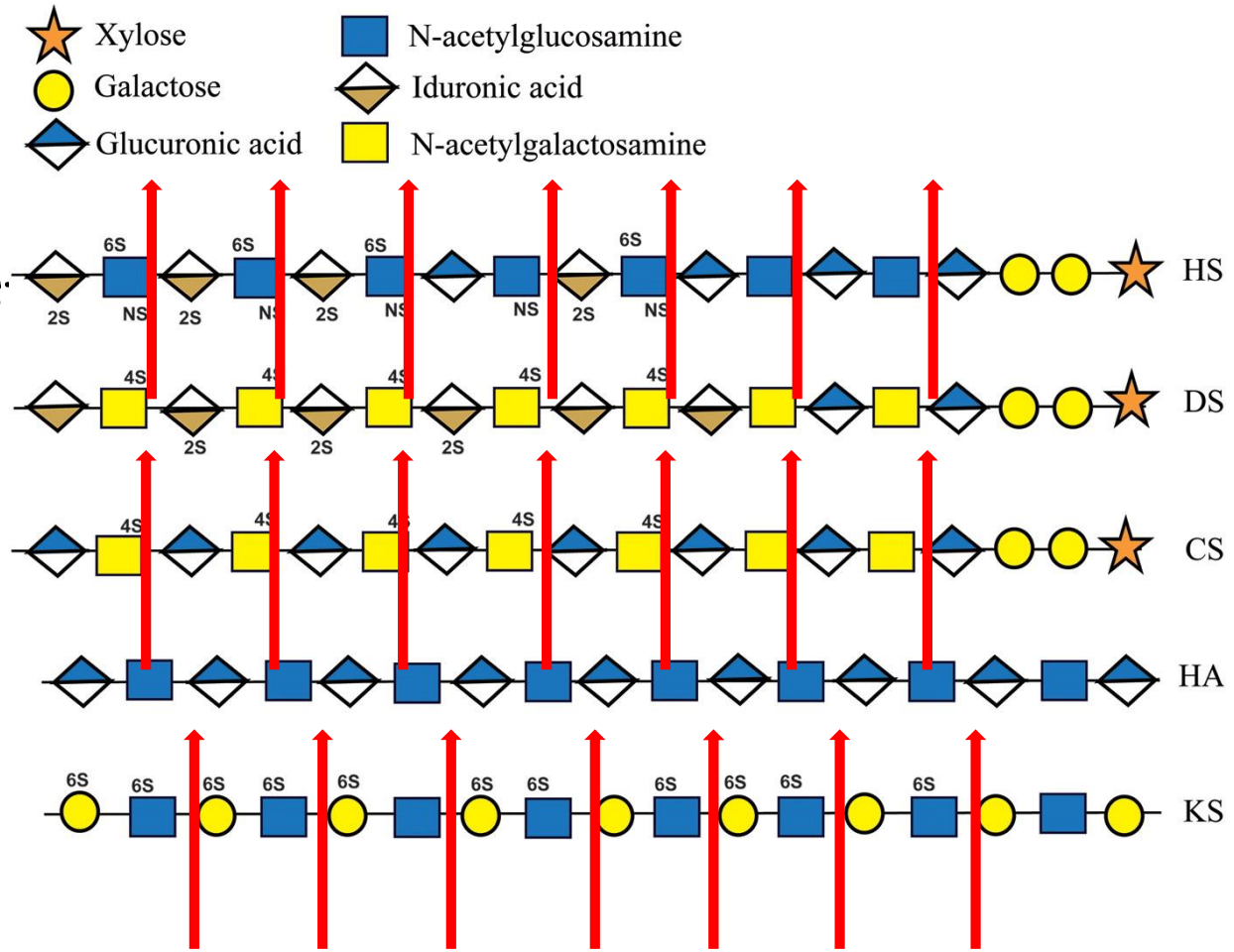
(nitrous acid, HCl methanolysis/butanolysis)

- molar enrichment
- disadvantage is that the structural detail is lost
- artifacts produced[1]

2 Enzymatic depolymerisation

(heparanase, chondroitinase, keratanase)

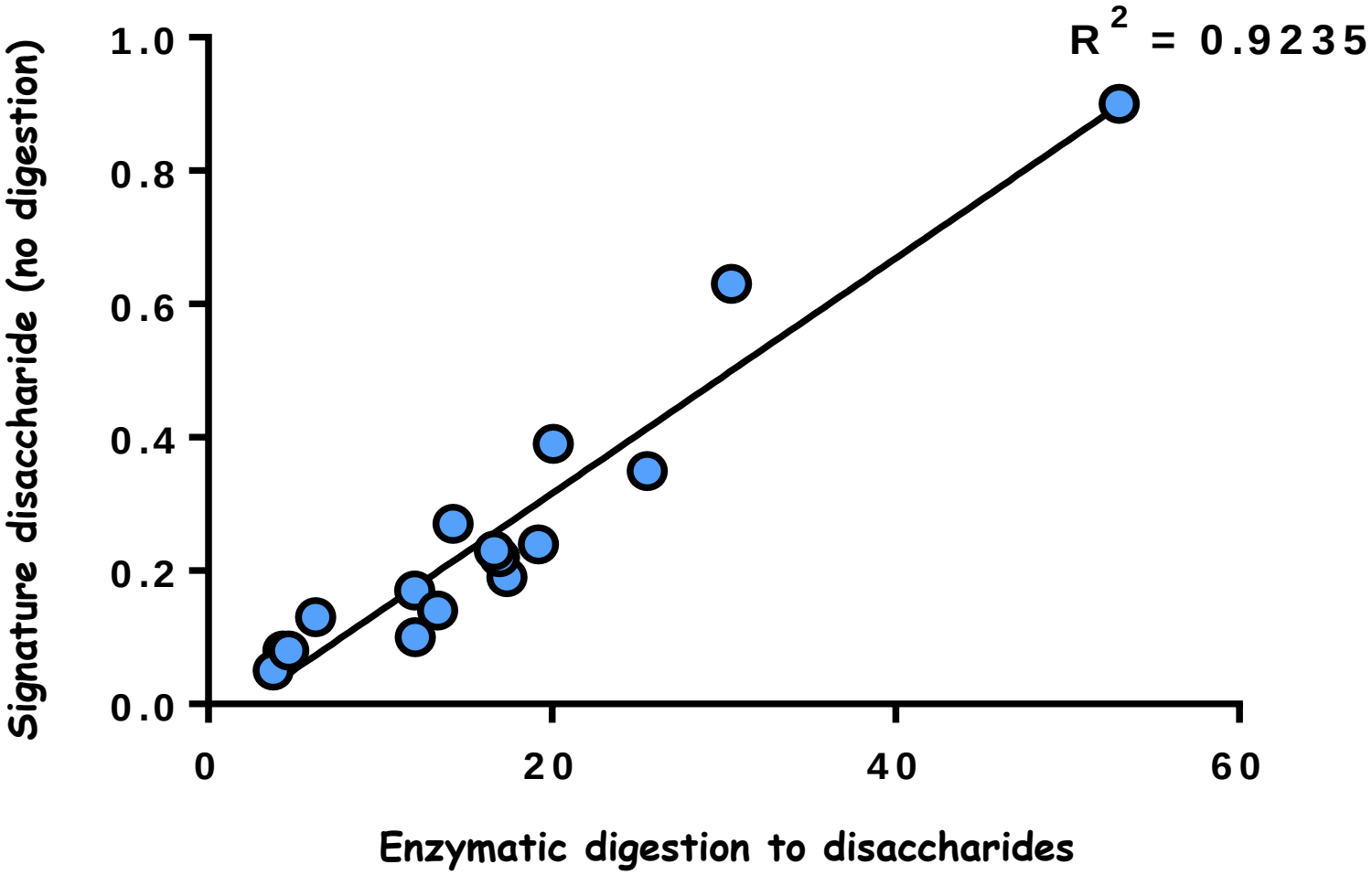
- molar enrichment
- structural detail preserved



[1] Wang et al. Int J Mol Sc 2020;21:5449

HCl, hydrochloride

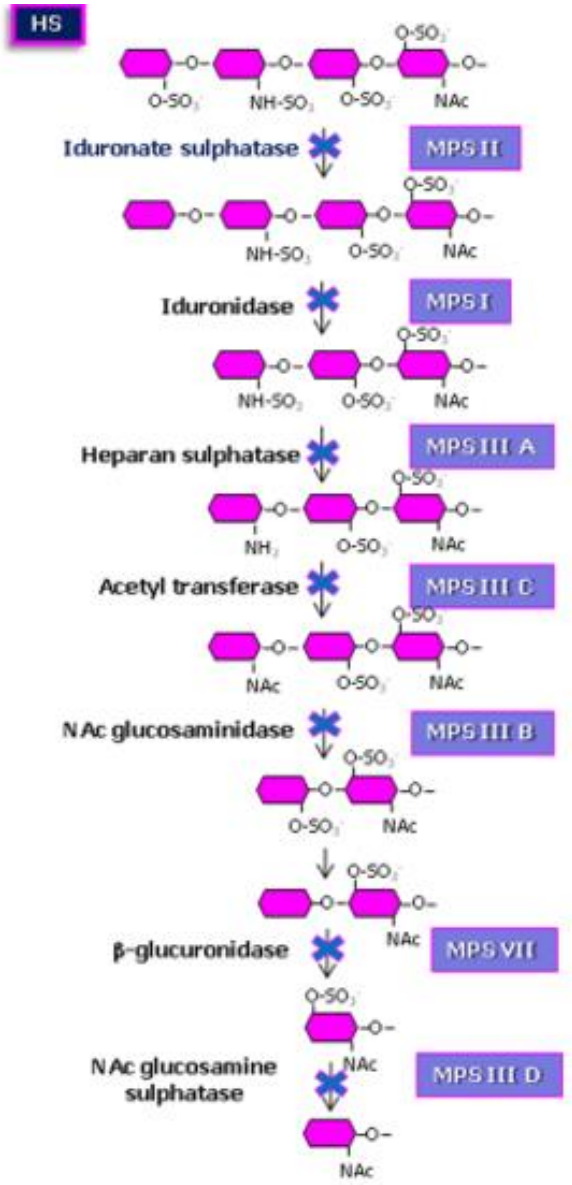
comparing mass spectrometry methods



Chin S et al., JIMD Rep 2020;55:68-74

heparan sulphate (HS)

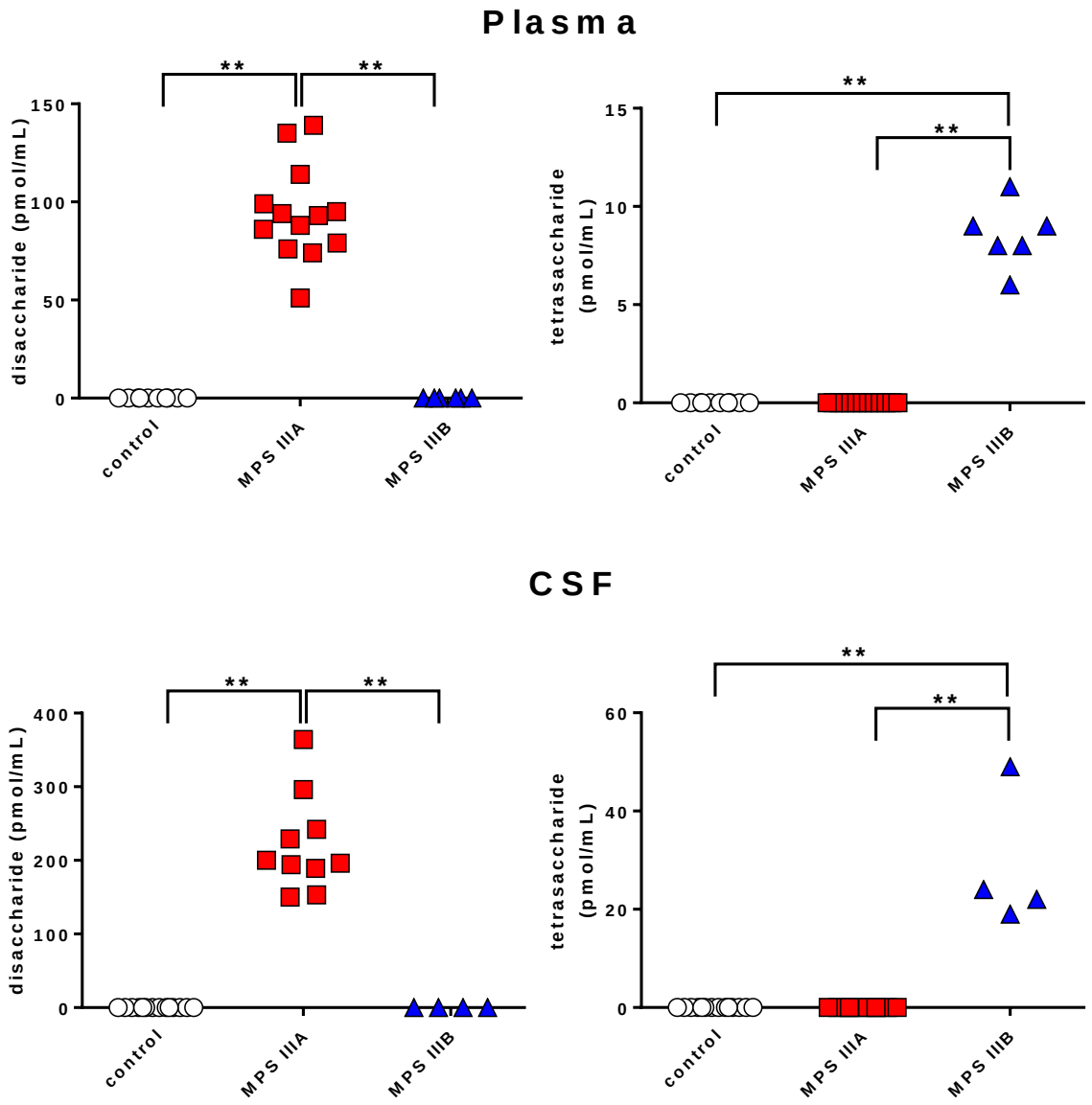
- uronic acid (HexA) and N-acetylglucosamine (GlcNAc)
- sulphated and acetylated domains
- important for function: co-receptors for key signaling path
- critical for proper neuronal development and function
- partially degraded HS is the primary pathological insult in r III and VII)
- HS storage present at birth



Li et al. Int Rev Cell Mol Biol 2016;325:215-273; De Risi et al. Nat Commun 2021;12:3495; Saville et al. Hum Gene Ther 2021;32:420-430; Saville et al. Mol Genet Metab 2019;128:68-74.

MPS, mucopolysaccharidosis; HS, heparan sulphate

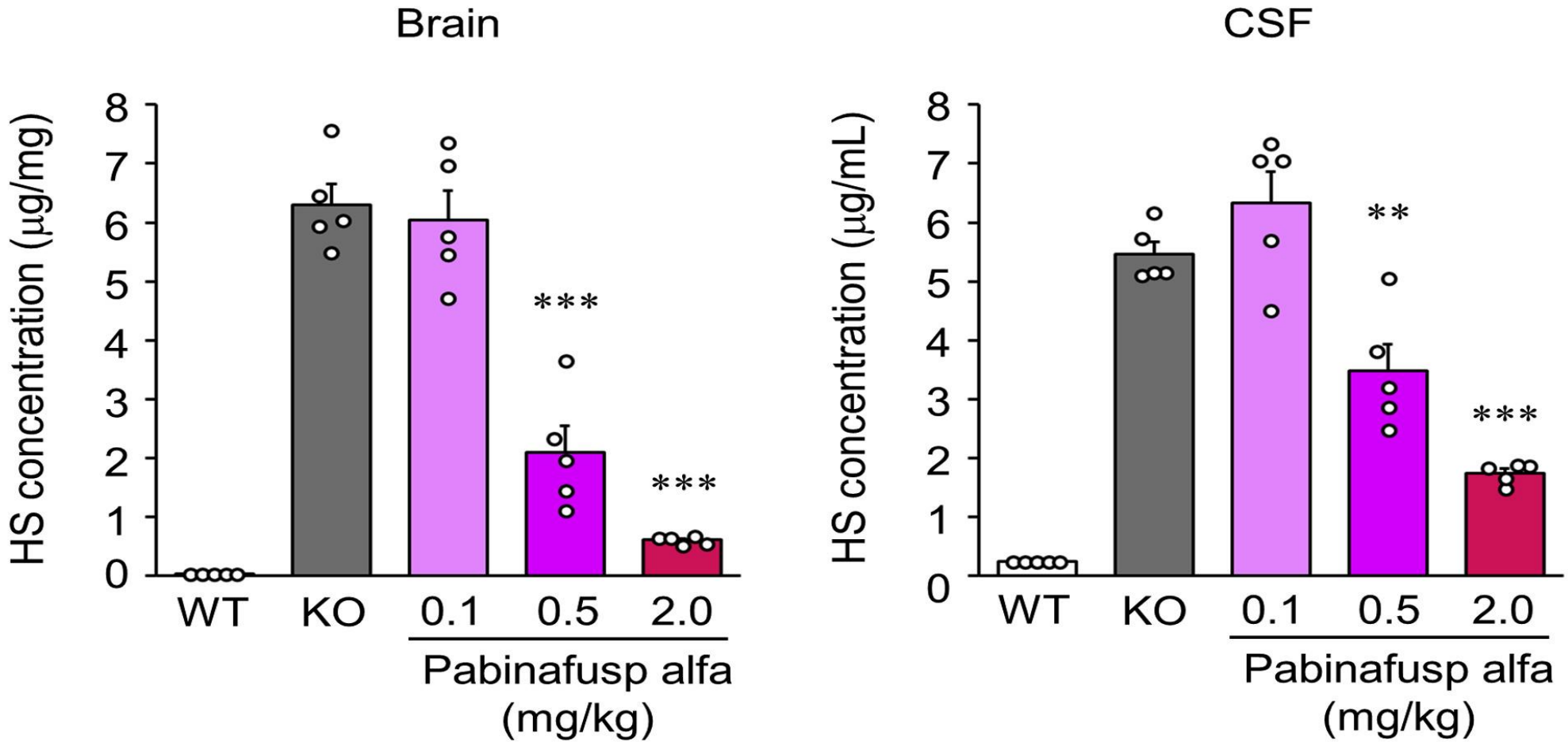
plasma and cerebrospinal fluid (CSF) in MPS III



Saville et al. Mol Genet Metab 2019;128:68-74

MPS, mucopolysaccharidosis

heparan sulphate (HS) in the brain is reflected in the CSF

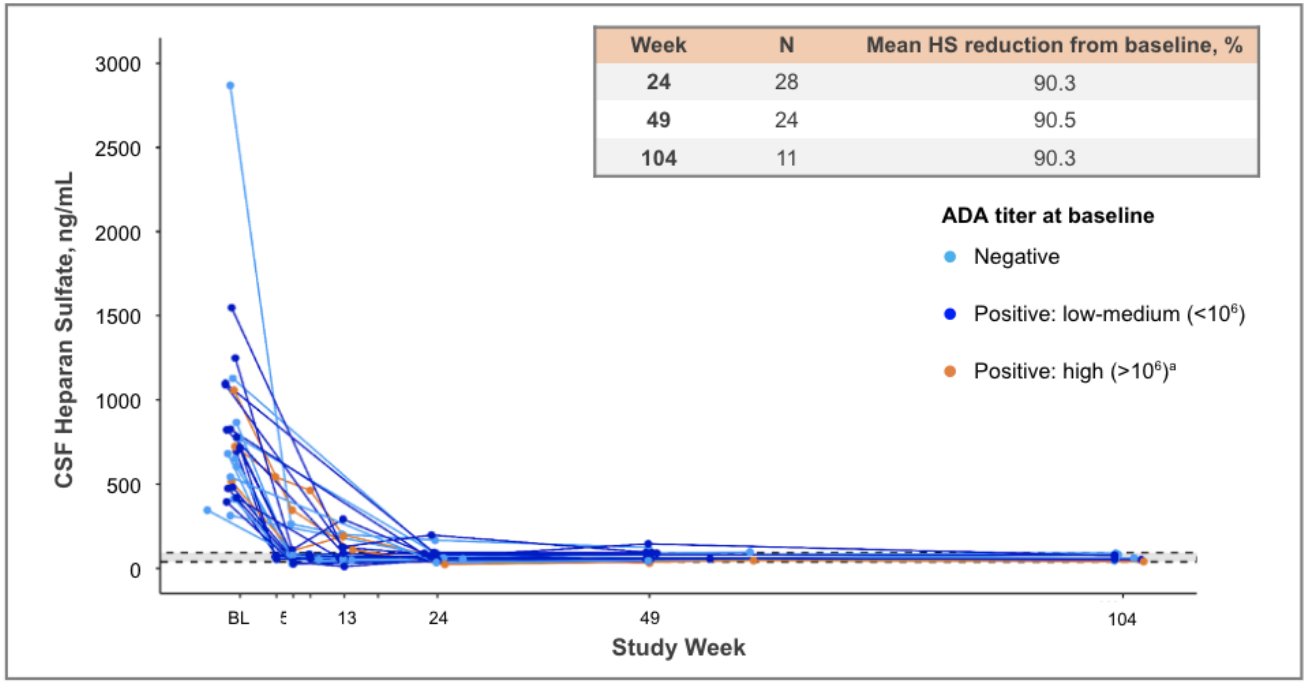


Morimoto et al. Mol Ther Methods Clin Dev 2022;534-544

CSF, cerebrospinal fluid; HS, heparan sulphate; WT, wild type; KO, knockout

heparan sulphate reduces with therapeutic intervention

cerebrospinal fluid (CSF) heparan sulphate



Normal levels of CSF HS[b] were achieved and sustained over time, including in those with pre-existing high ADA

Data cutoff: 2 Mar 2023. ADA, anti-drug antibody; BL, baseline; CSF, cerebrospinal fluid; HS, heparan sulfate.
[a]3 participants had high baseline ADA titer; [b]CSF HS was measured as a sum of the disaccharides D0A0, D0A6, D0S0, and D2S6 by mass spectrometry after enzymatic digestion. Preliminary normal range (10th-90th percentile) was based on analysis of CSF from healthy adults (n=30; median [range] age, 52 [18-80] years); 39.1-92.51 ng/mL. Total CSF GAG levels are similar in adults and children.[1]

1. Hendriksz CJ, et al. *Mol Genet Metab Rep.* 2015;5:103-106.

Interim analysis of a phase 1/2 study of weekly intravenous DNL310 (brain-penetrant enzyme replacement therapy) in MPS II

Data courtesy of Denali Therapeutics

heparan sulphate oligosaccharides are biomarkers

"A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention"

NIH Biomarkers Definitions Working Group

NIH, National Institutes of Health Biomarkers definitions working group. Clin Pharmacol Ther 2001;69:89-95

case presentation exemplifies the clinical utility

- amniotic fluid tested in a case of fetal hepatomegaly
- NGS on 151 genes associated with fetal hydrops and lysosomal disease
- identified a hemizygous VUS: D532G in exon 9 of the IDS gene causing MPS type II
  - ACMG guidelines predicted likely pathogenic
- enzyme activity in cultured amniocytes: 11 nmol/4 h/mg (reference range: 90–170)
- no signature oligosaccharide in the amniotic fluid
- baby was unremarkable at birth and no signature oligosaccharide in the urine
- older brother (8 years of age) with same genotype, phenotypically normal and no signature oligosaccharide in the urine
- 3 years since the birth, both boys have no signs/symptoms of disease

Fuller and Ketteridge, JIMD Rep 2021;60:10-14

NGS, next generation sequencing; VUS, variant of uncertain significance;
ACMG, American College of Medical Genetics and Genomics; GAG, glycosaminoglycan

concluding statements

- oligosaccharides – not GAG - are reliable biomarkers
- heparan sulphate oligosaccharides in the CSF do reflect the brain
- disease specific
- highly precise and highly sensitive
- validated methods correlate
- driver of pathology – the oligosaccharide is the metabolite – not the enzyme
- highly likely to translate clinical outcomes

thank you

Q & A SESSION

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

REAGAN-UDALL FOUNDATION FOR THE FDA

Lunch

The meeting will resume at 12:40 pm ET

REAGAN-UDALL FOUNDATION FOR THE FDA

Case Study: Animal Model Translation to Human Application

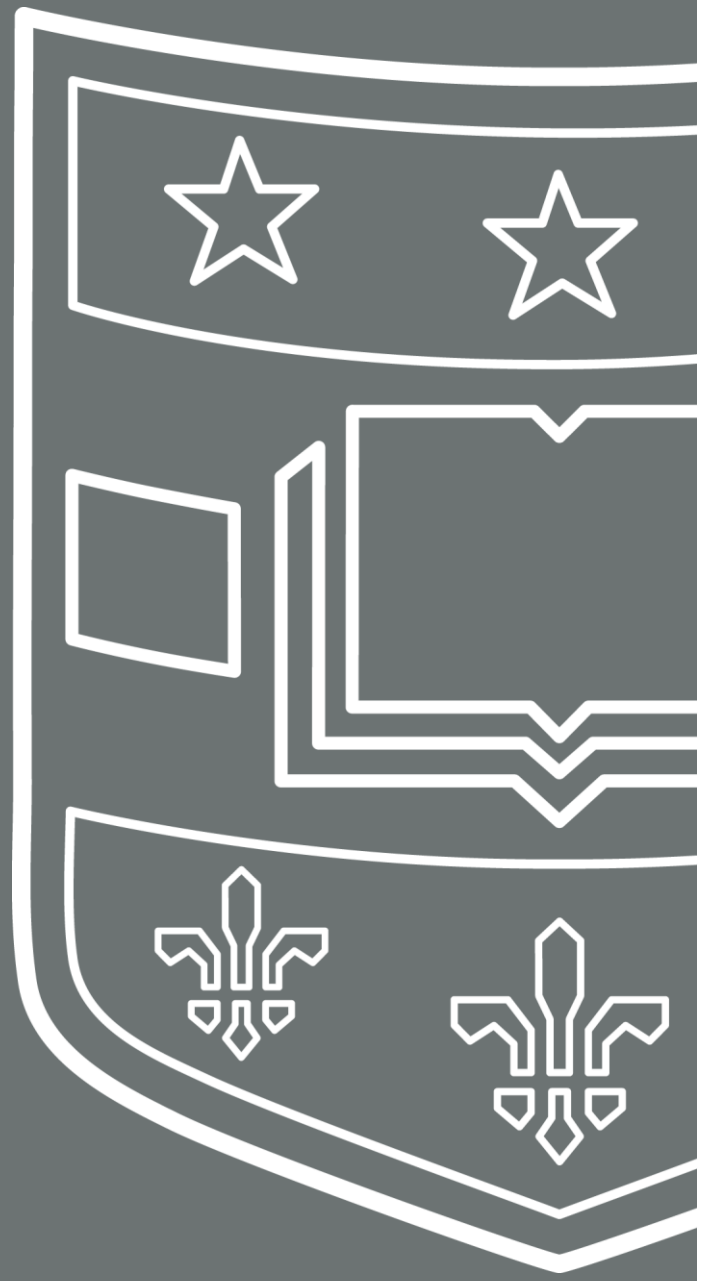
- **Nidal Boulos, PhD**, REGENXBIO, Inc.
- **Patricia Dickson, MD**, Washington Univ. School of Medicine, St. Louis
- **Matthew Ellinwood, DVM, PhD**, National MPS Society

REAGAN-UDALL FOUNDATION FOR THE FDA

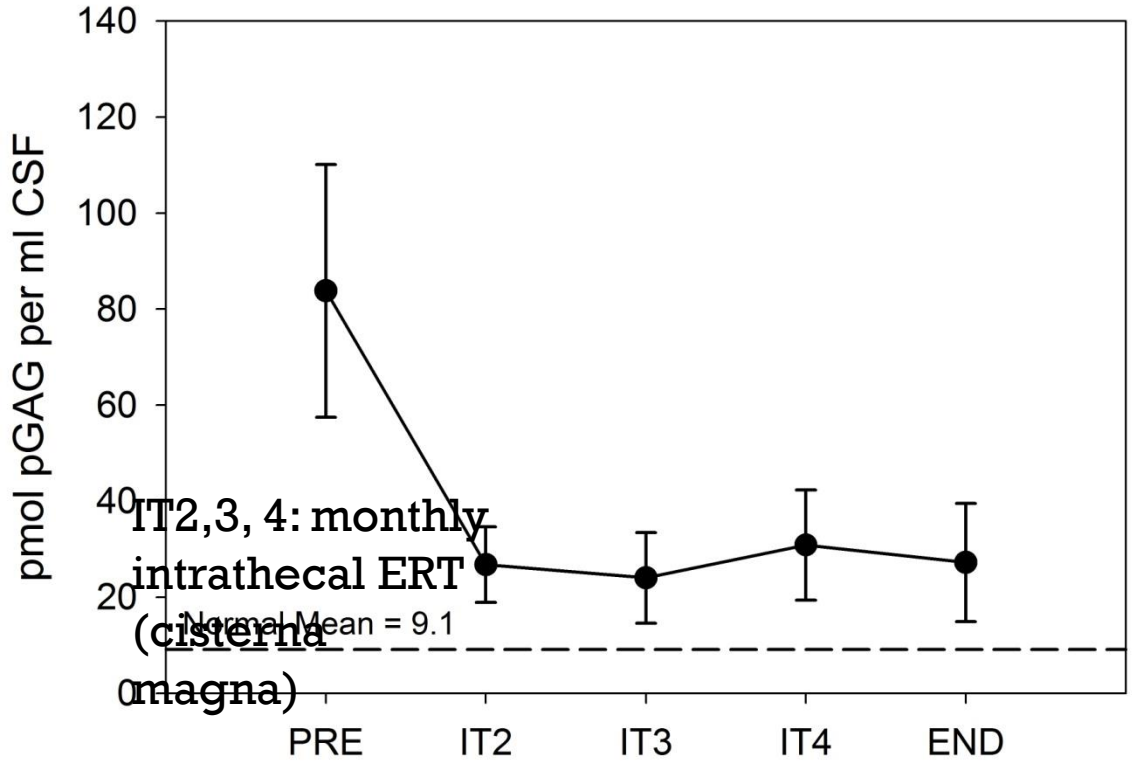
Membrane-tethered NAGLU to explore origins of CSF heparan sulfate

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

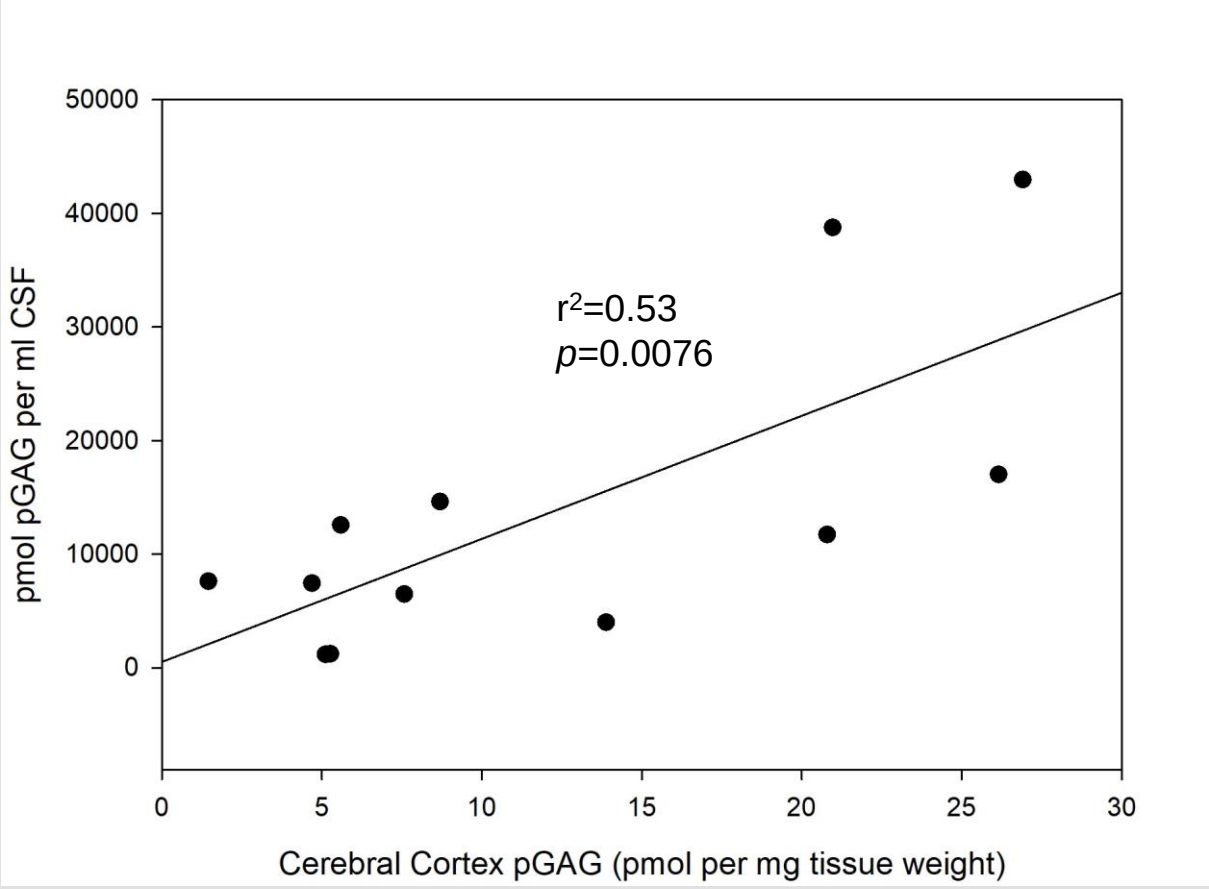


IT2,3, 4: monthly intrathecal ERT (cisterna magna)

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



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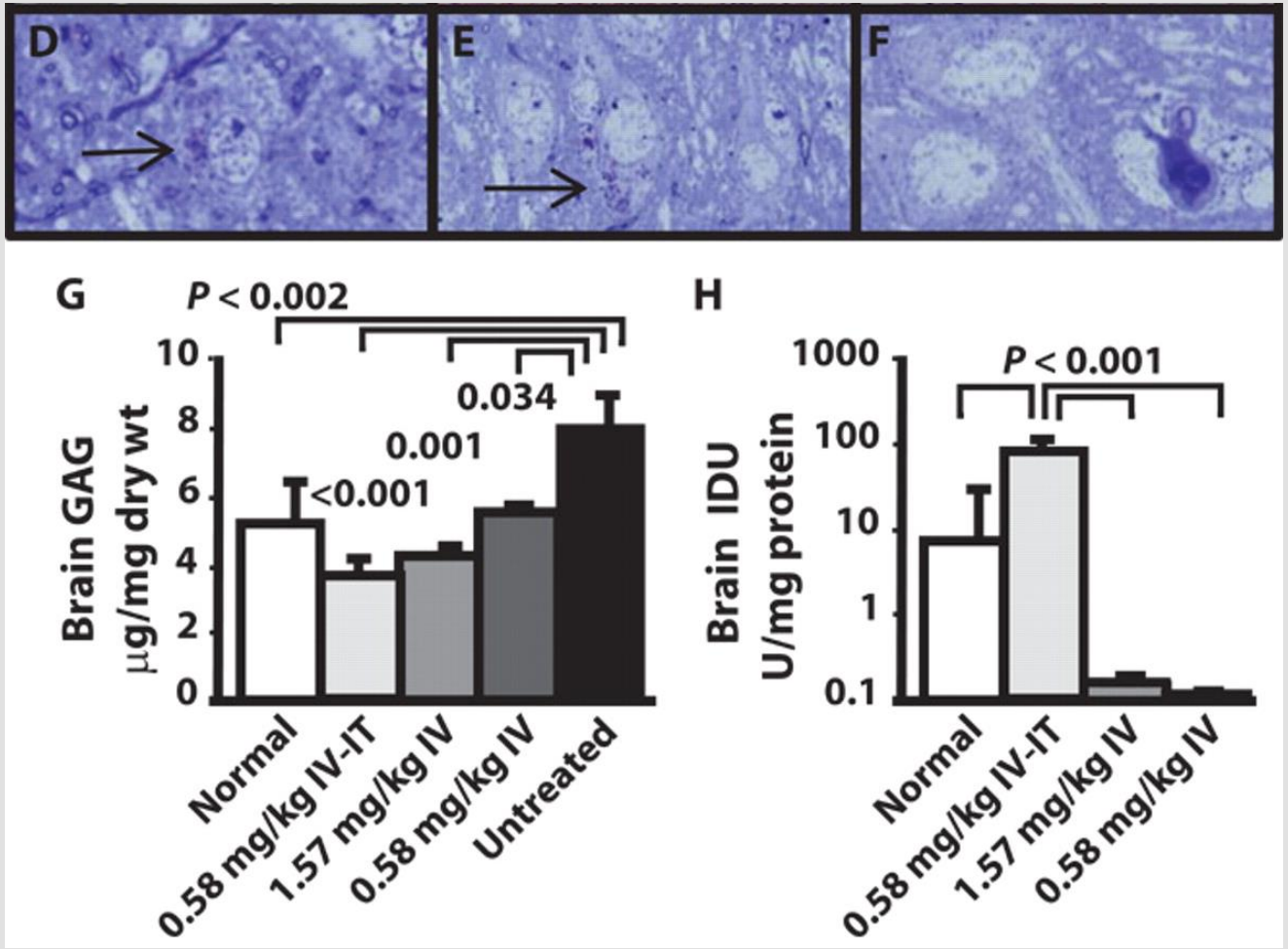
Unpublished. Data are from Dickson et al Mol Genet Metab 2012

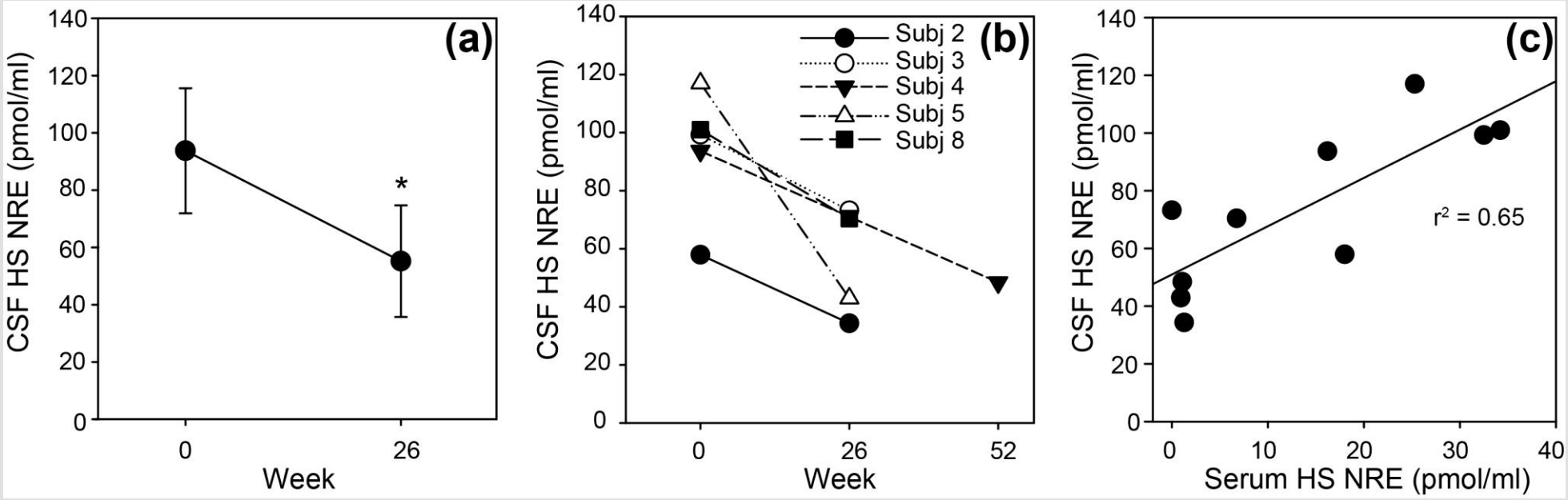
Intravenous enzyme replacement therapy reduces GAG and storage in brain of MPS I dogs



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.

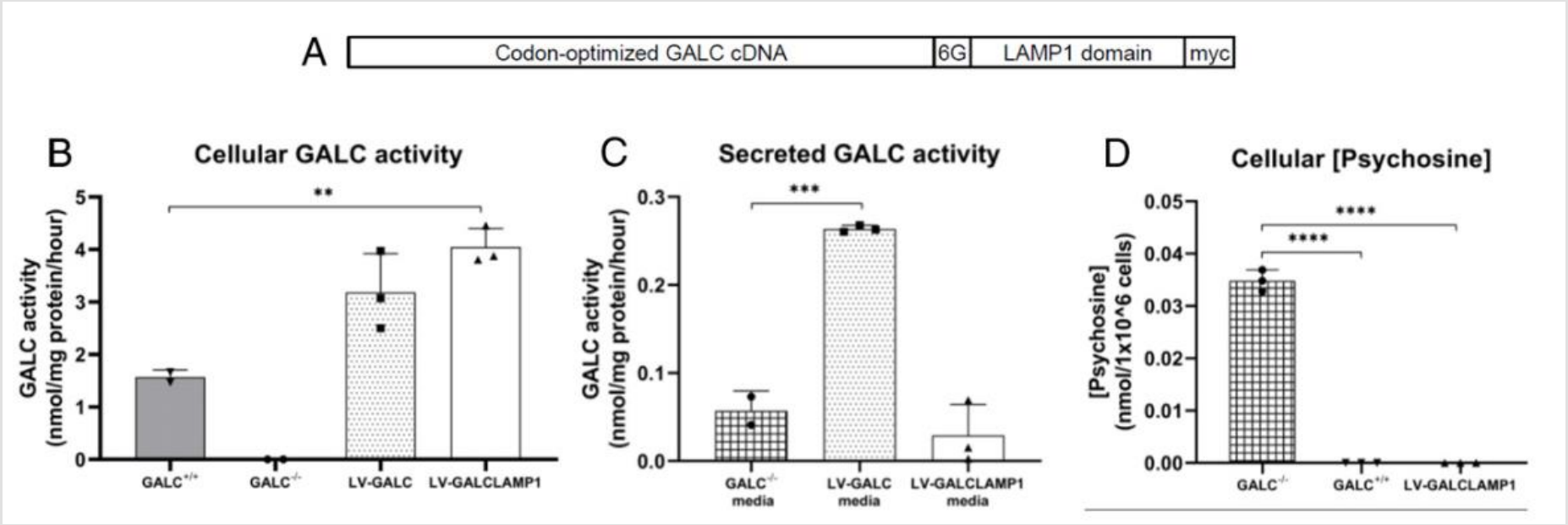
Hypothesis 2: CSF heparan sulfate does not reflect brain.
Could CSF heparan sulfate originate in the bloodstream?

Problem: How do we test these hypotheses?

Cell-autonomous expression of the acid hydrolase galactocerebrosidase

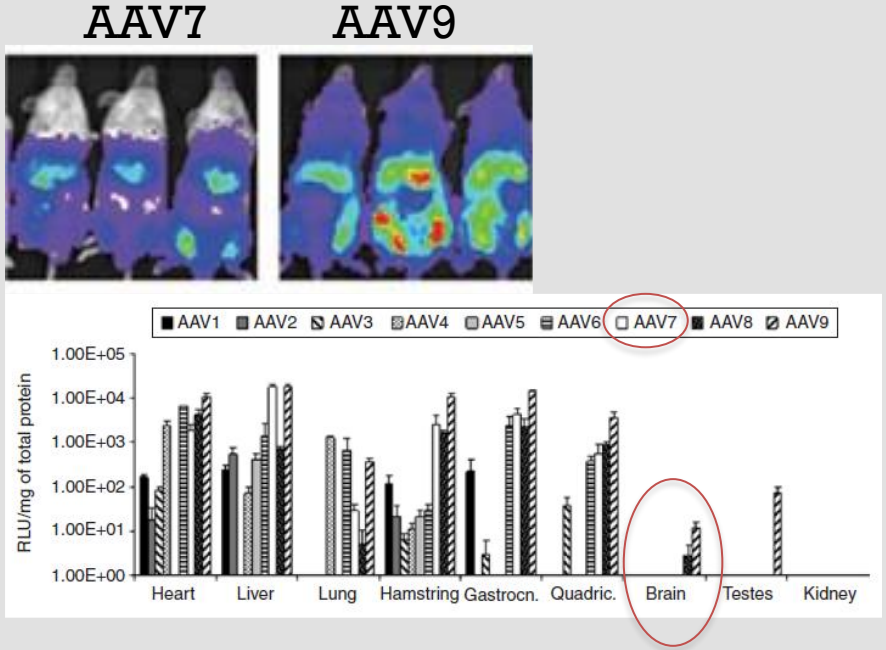
Christina R. Mikulka[a], Joshua T. Dearborn[a], Bruno A. Benitez[b], Amy Strickland[c], Lin Liu[a,1], Jeffrey Milbrandt[c], and Mark S. Sands[a,c,2]

[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

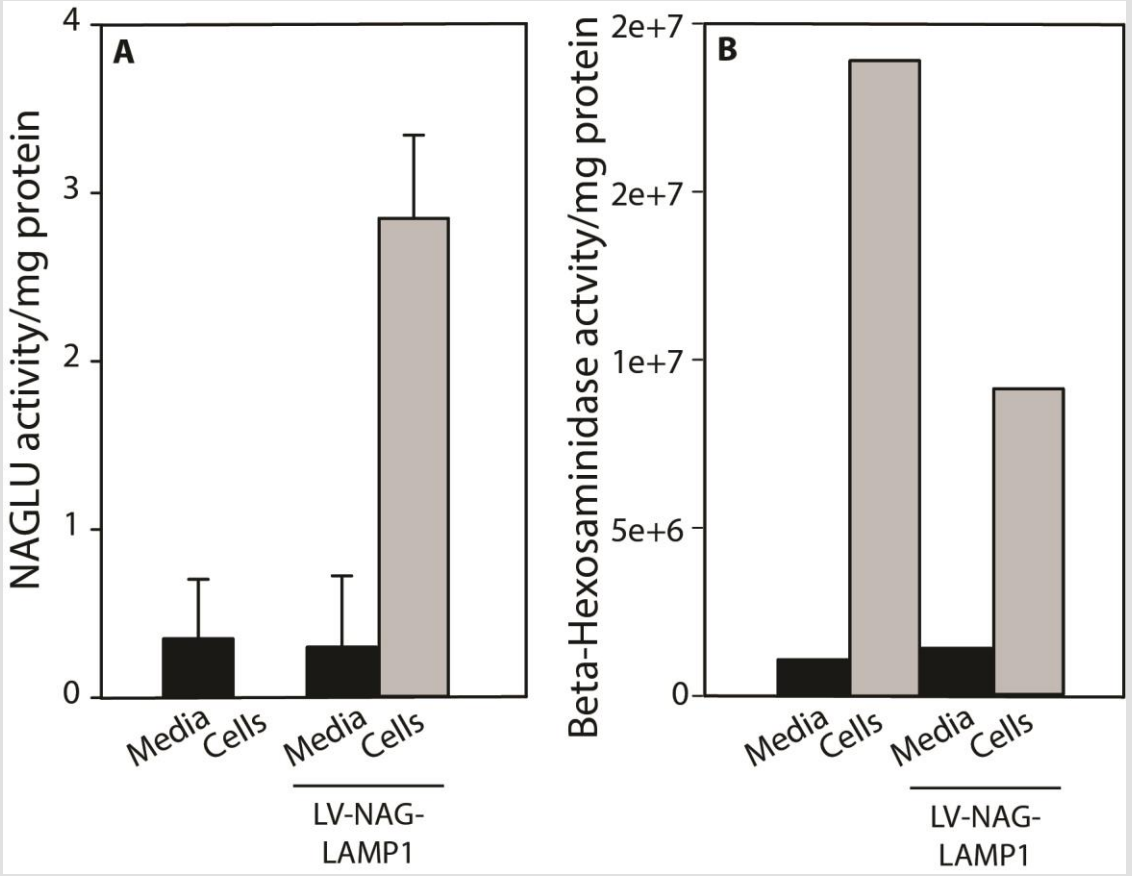


Mikulka et al, PNAS 2020

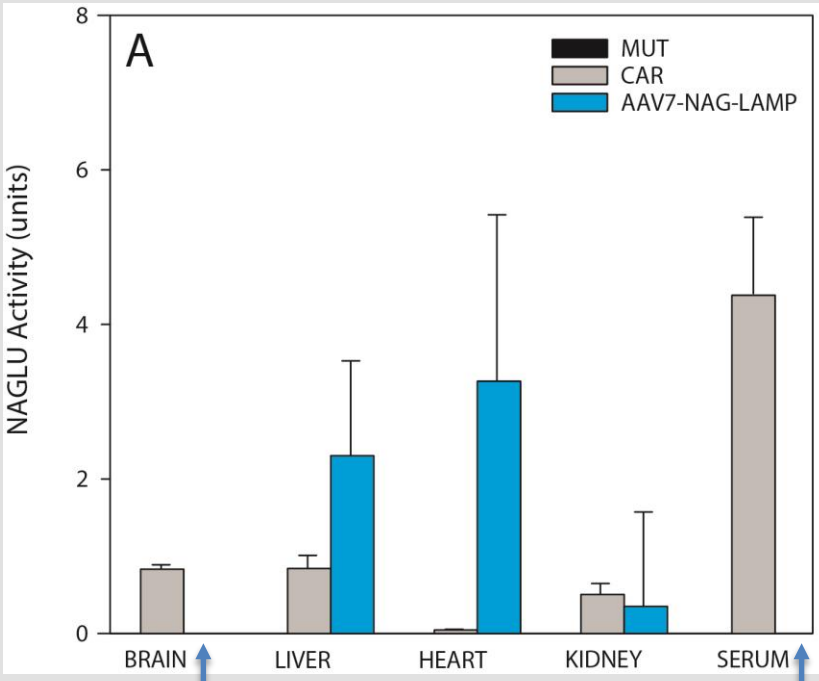
Membrane-tethered NAGLU

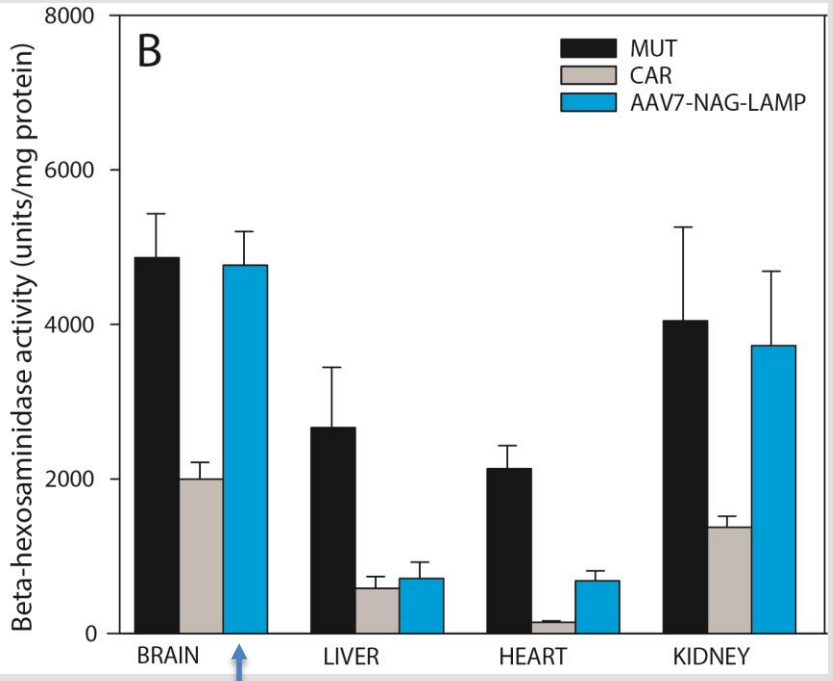


From Zincarelli et al, Mol Ther 2008 (not our work)



Intravenous AAV7 NAGLU-LAMP1: isolated systemic treatment





No NAGLU activity in brain

No NAGLU activity in serum

No treatment effect in brain

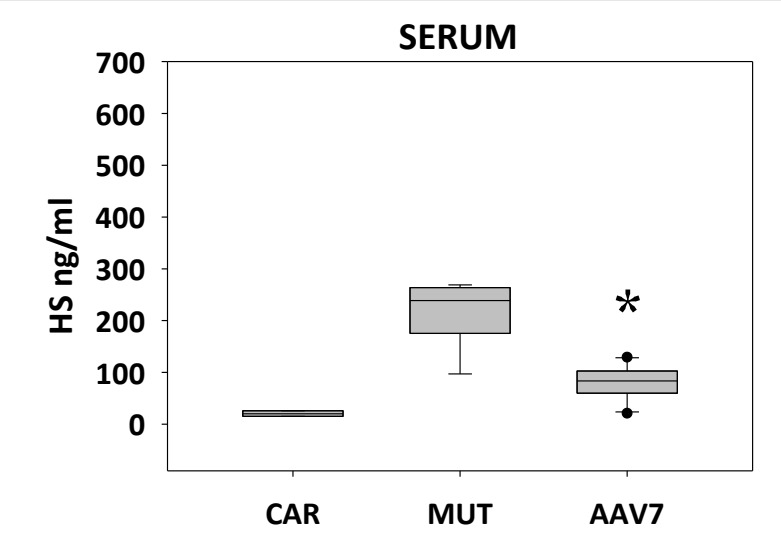
Sanfilippo B (Naglu-/-) mice received $1.5x10^{11}$ vg/mouse IV AAV7 NAGLU-LAMP1 (CBA promoter) and were compared to untreated affected and carrier mice (n=10-12 per group).

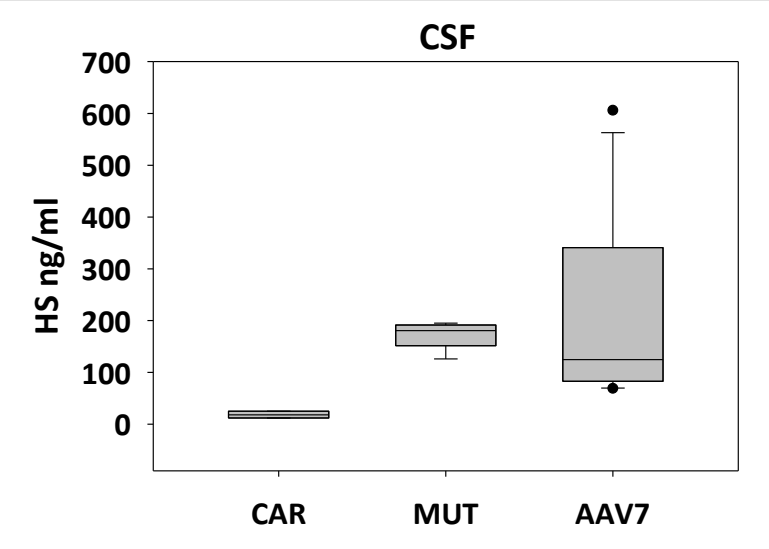
Treated at age 4 weeks
Studied at age 8 weeks

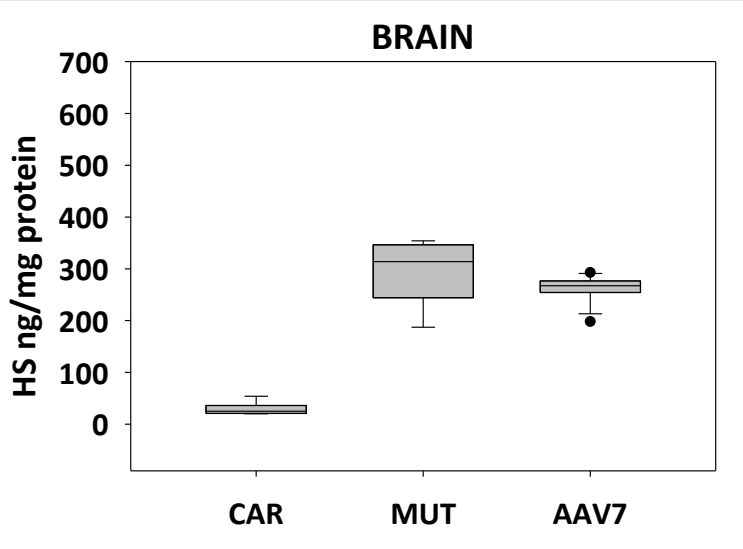
Steven Le, abstract WORLD 2023

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

in Sanfilippo B mice treated with IV AAV7 NAGLU-LAMP1





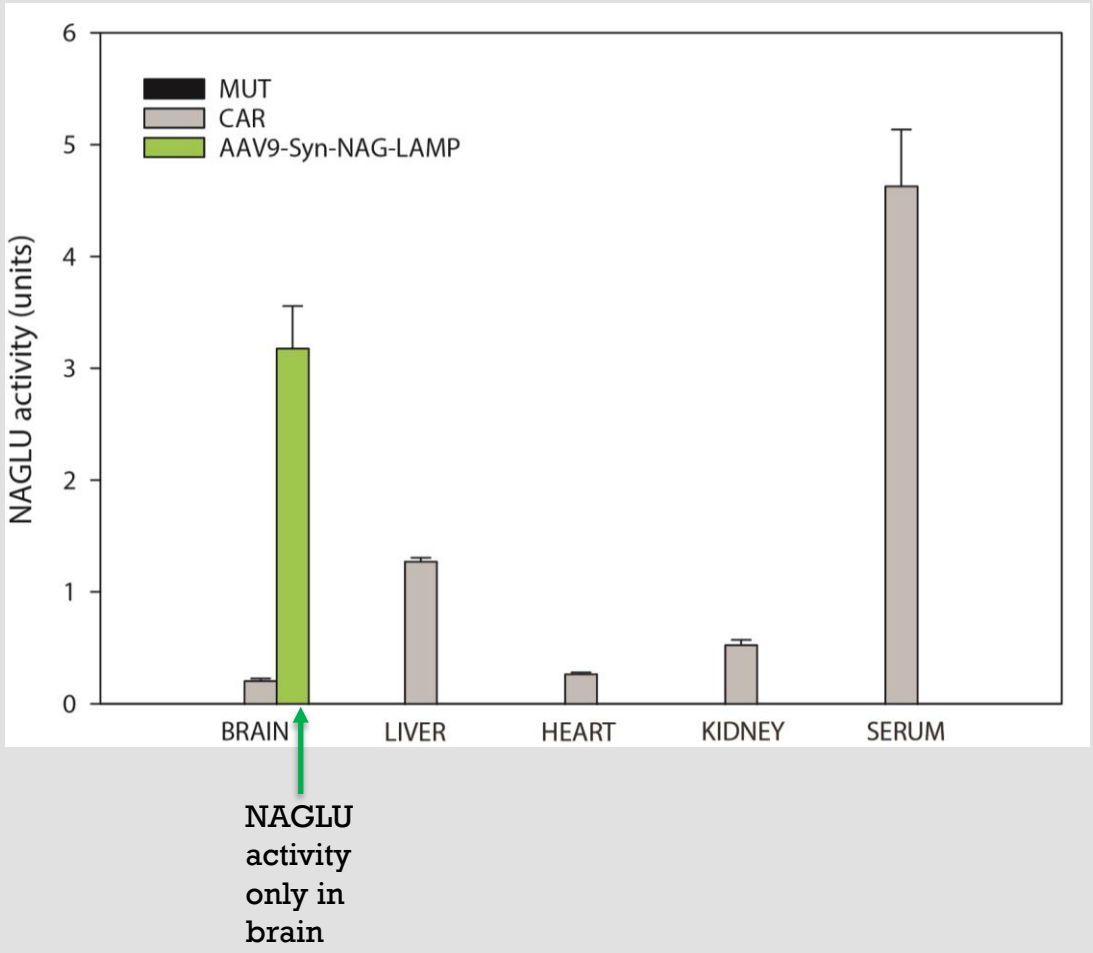


	Female	Male
CAR	5	5
MUT	5	5
AAV7	8	4

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

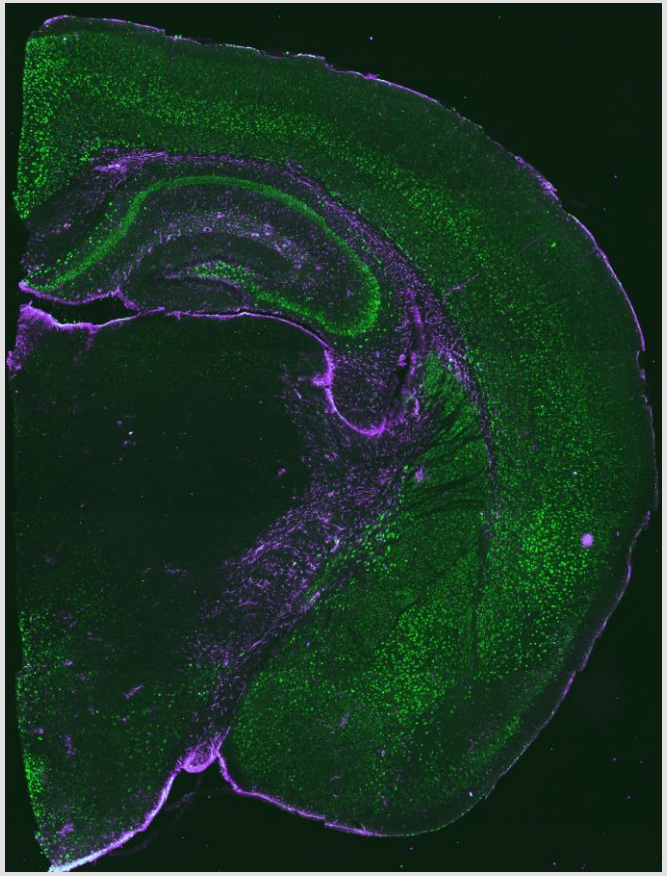


We designed an AAV9 viral vector with NAGLU-LAMP1 under a Synapsin-1 promotor to express NAGLU in neurons and delivered 6.5E+10 vg/mouse ICV to Sanfilippo B (Naglu-/-) mice in order to confine NAGLU restoration to the brain.

Treated at PND 1 or 2
Studied at age 4 weeks

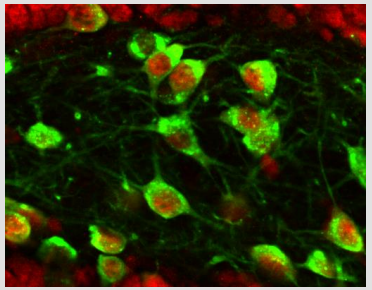
Steven Le, abstract WORLD 2024

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



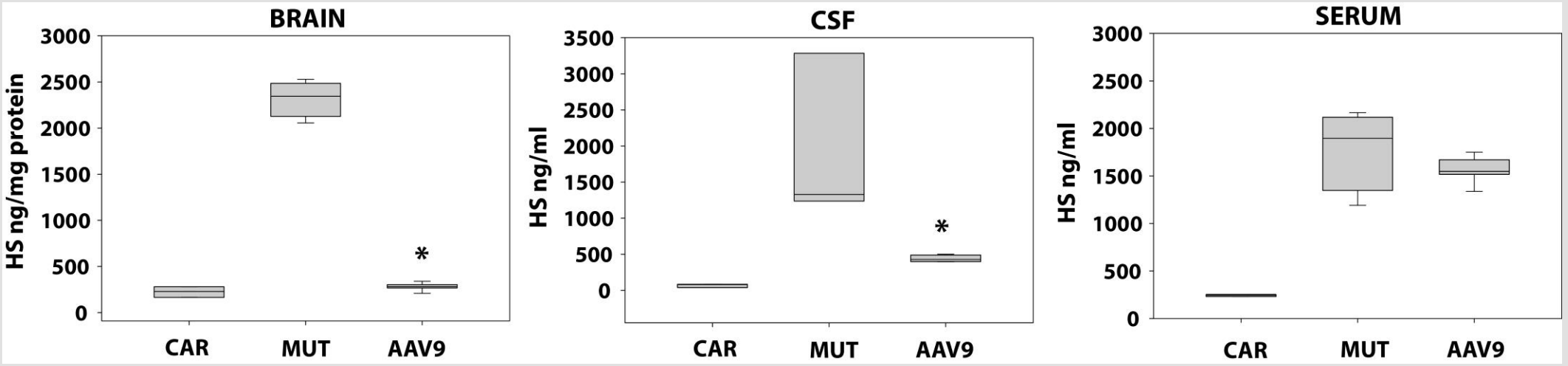
Green: NAGLU
Red: NeuN

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

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- 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

National MPS Society

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

- A full-time employee as the Chief Scientific Officer of the National MPS Society, Inc., a 501(c)(3) non-profit
- No personal conflict of interests to disclose
- Honoraria, travel, conference registration, and/or consultancy paid to or received by the Society from:
  - American College of Medical Genetics
  - 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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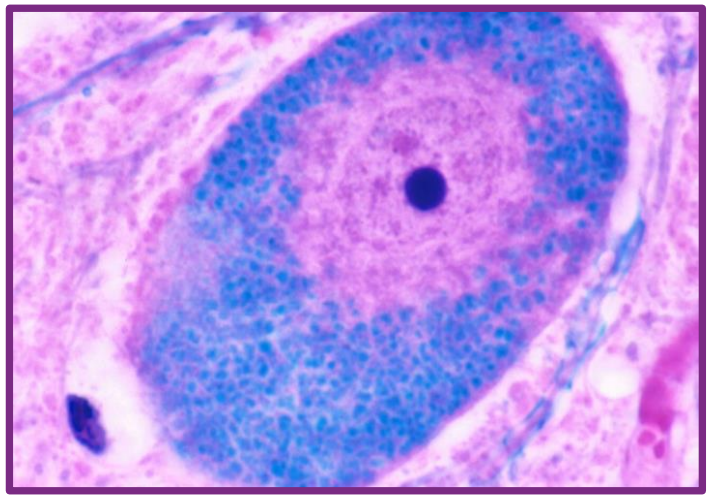
Clinical Manifestations of Canine MPS IIIB

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

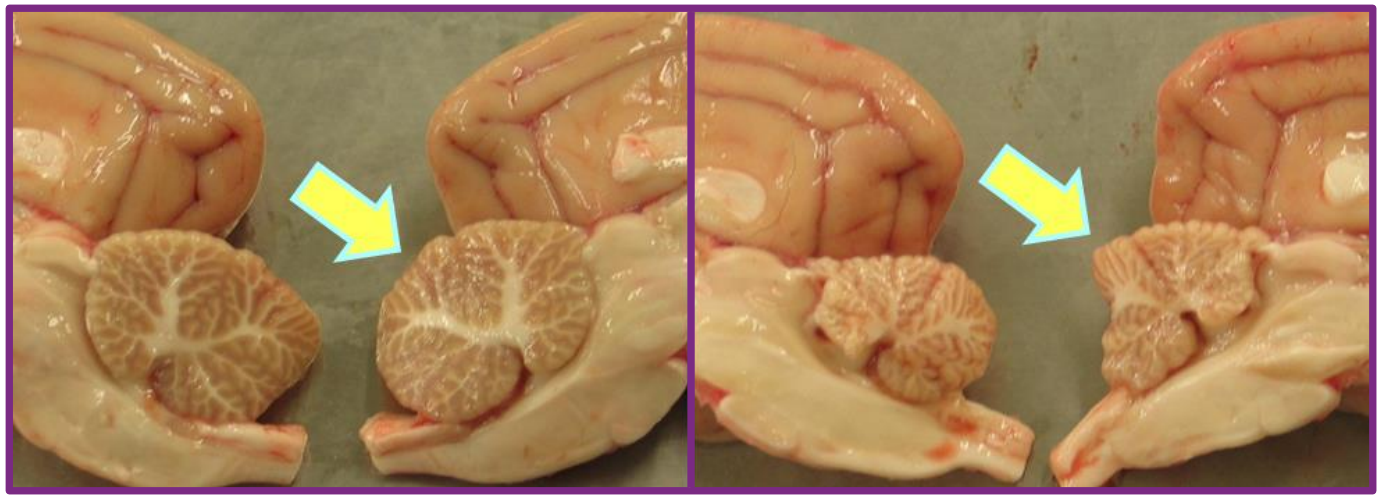
Clinical Progression in Canine MPS IIIB

- 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

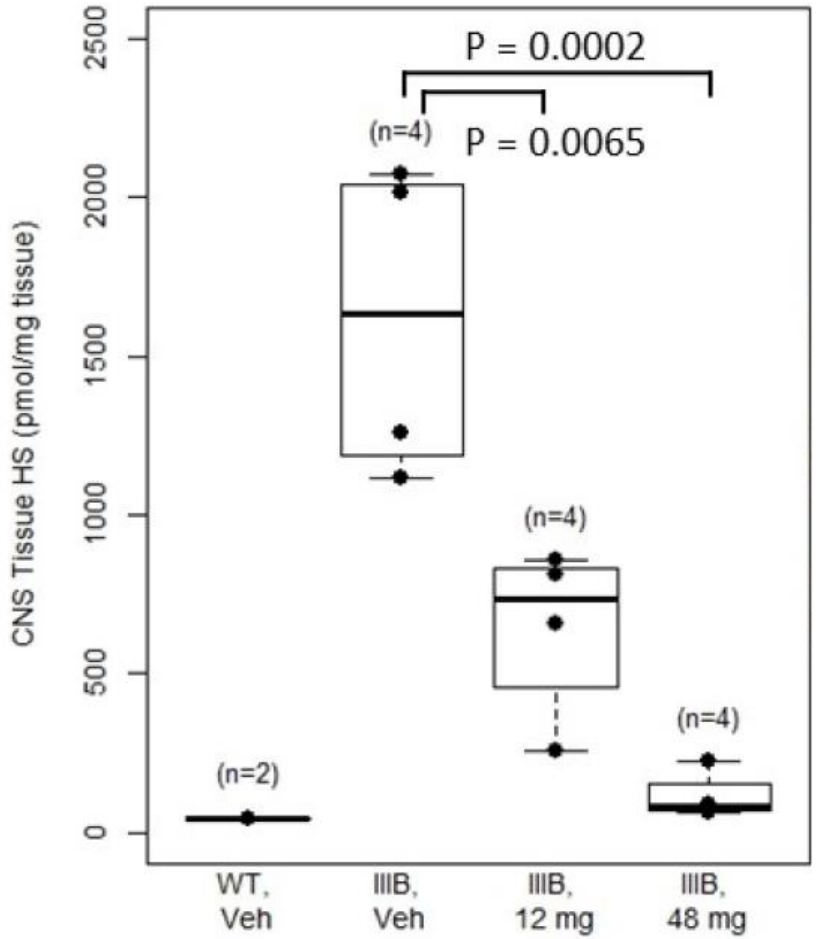
Ellinwood, Unpublished data

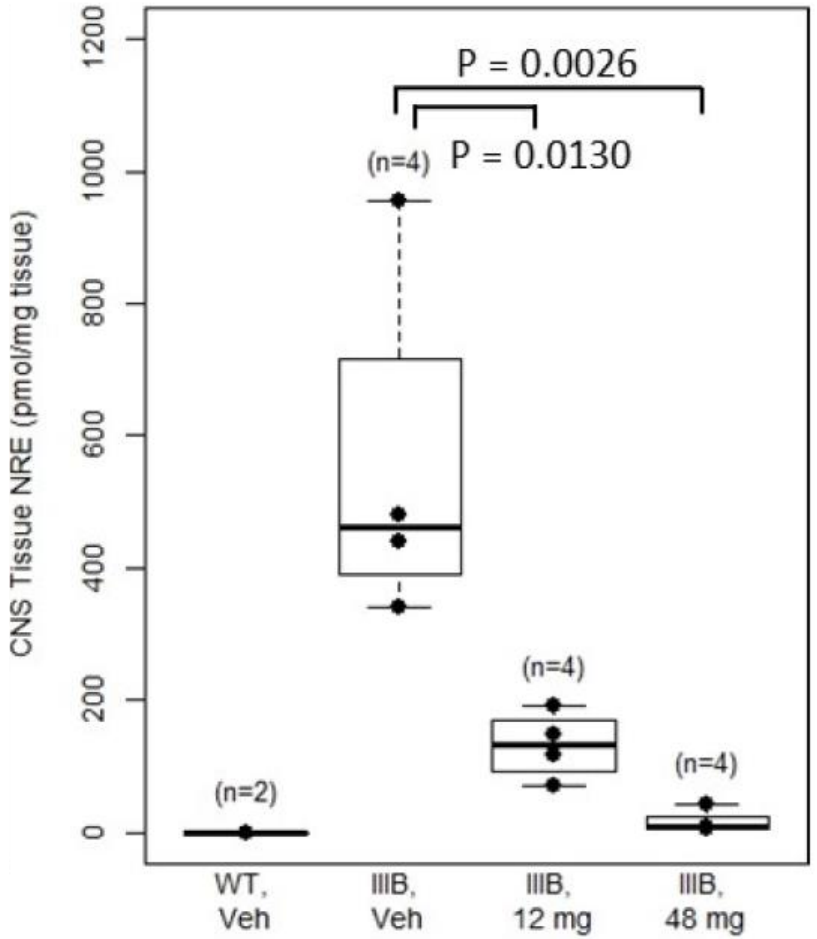
End Stage MPS IIIB

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

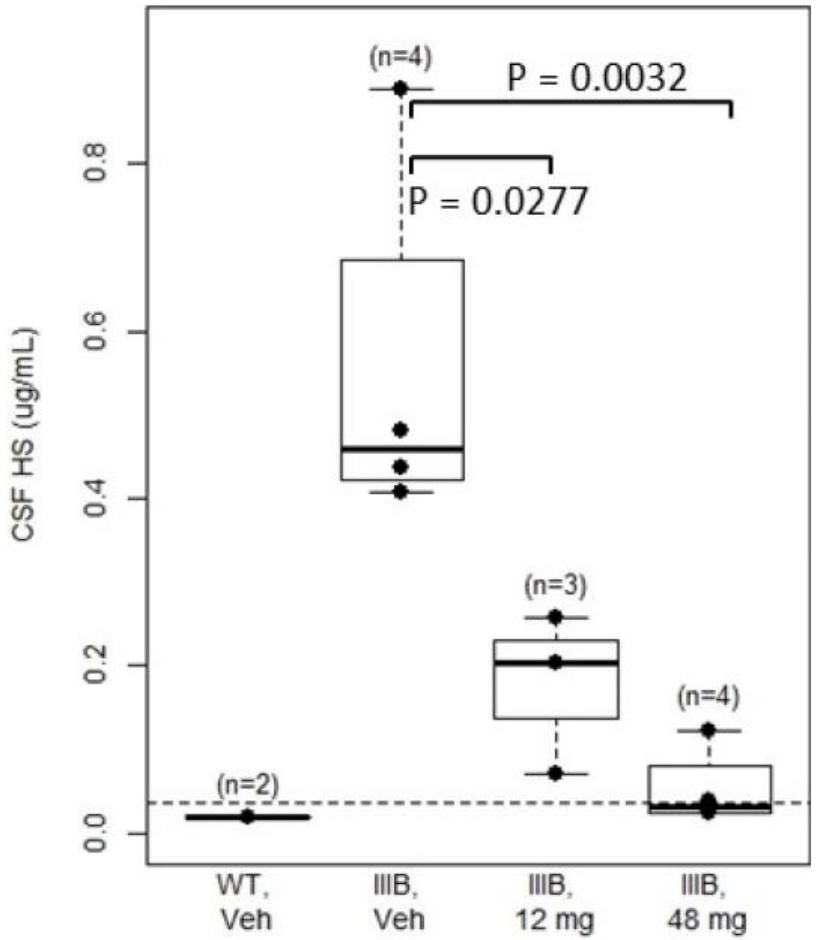
Tissue HS Derived Disaccharides (HS) and MPS IIIB Non-reducing End Oligos (NREs)

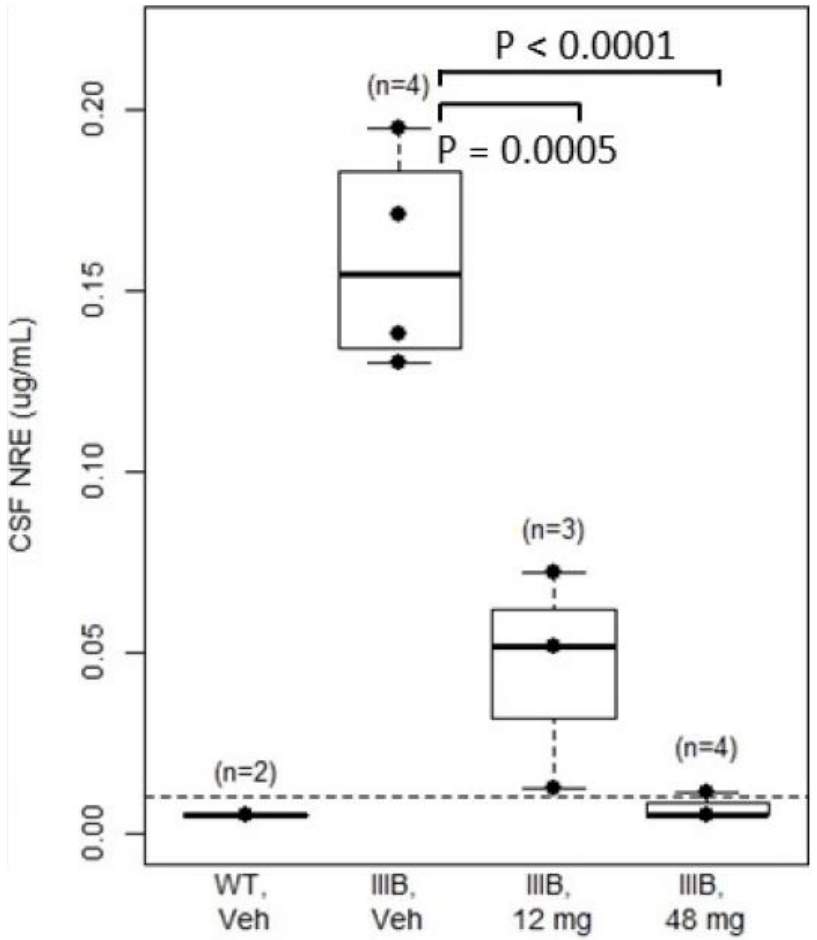




Ellinwood et al., J Pharmacol Exp Ther. 2022. DOI: 10.1124/jpet.122.001119

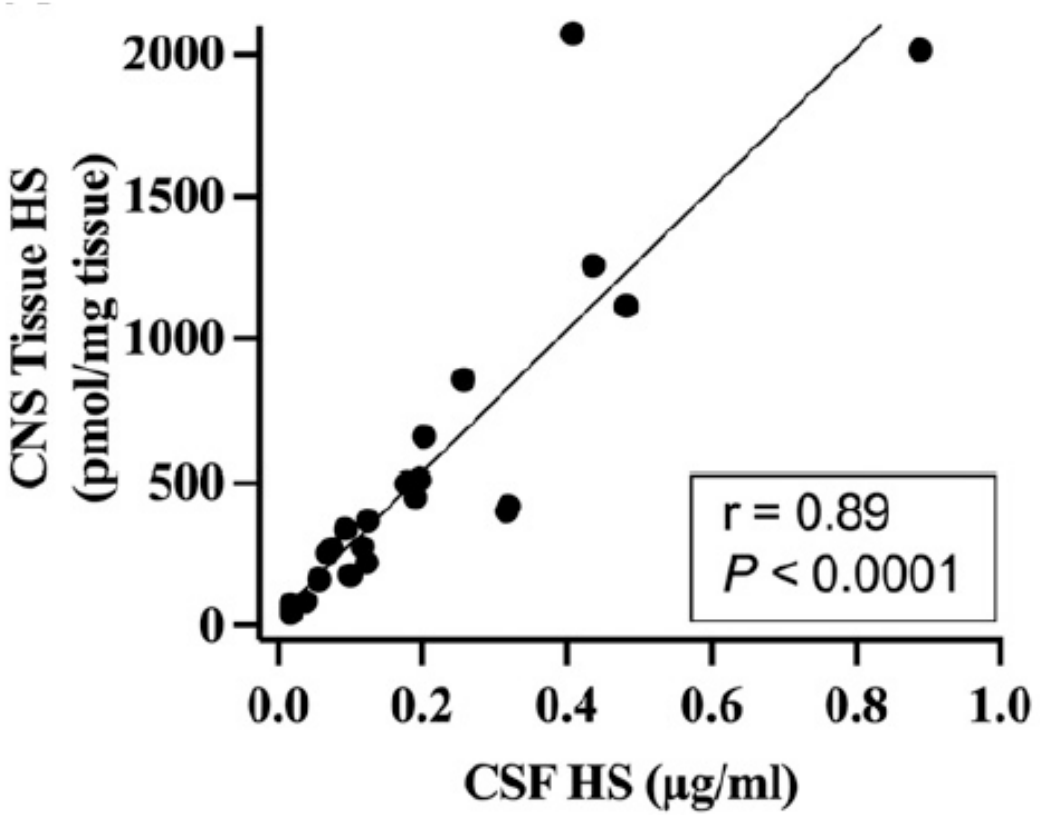
CSF HS and MPS IIIB NREs

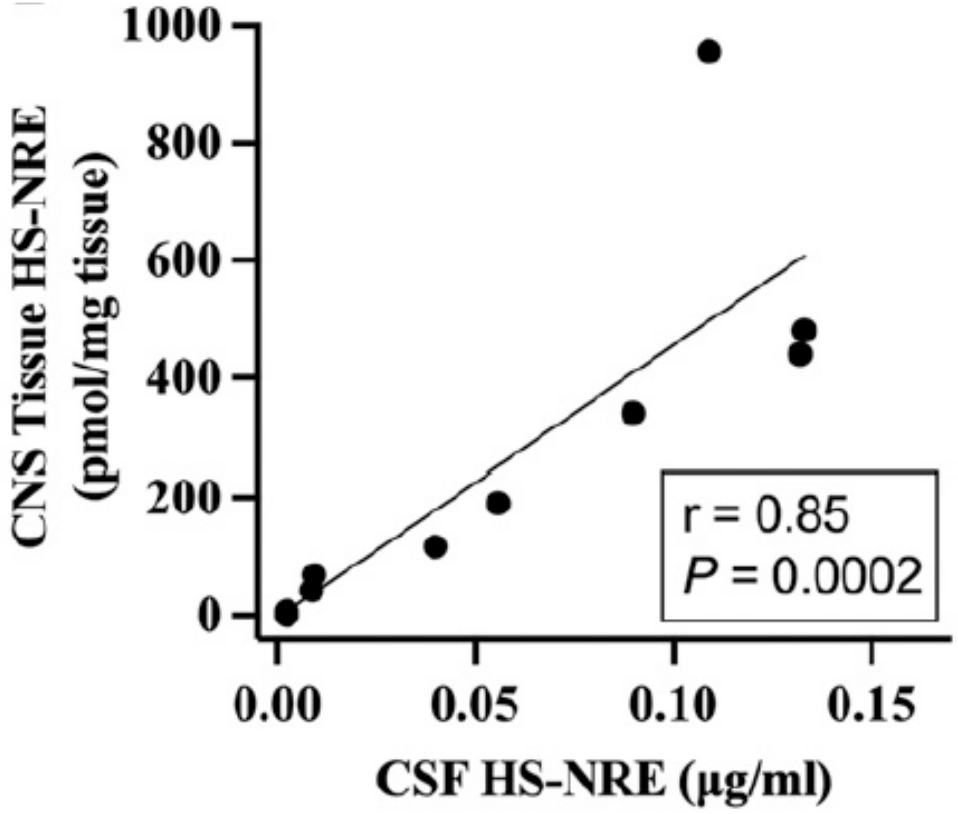




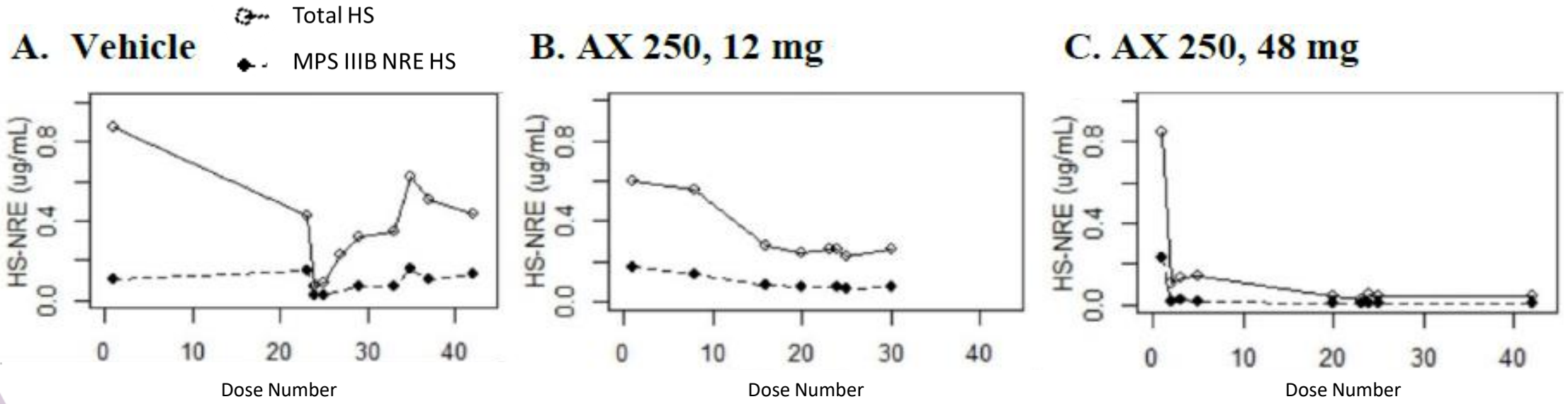
Ellinwood et al., J Pharmacol Exp Ther. 2022. DOI: 10.1124/jpet.122.001119

Tissue and CSF Correlations of HS and MPS IIIB NREs





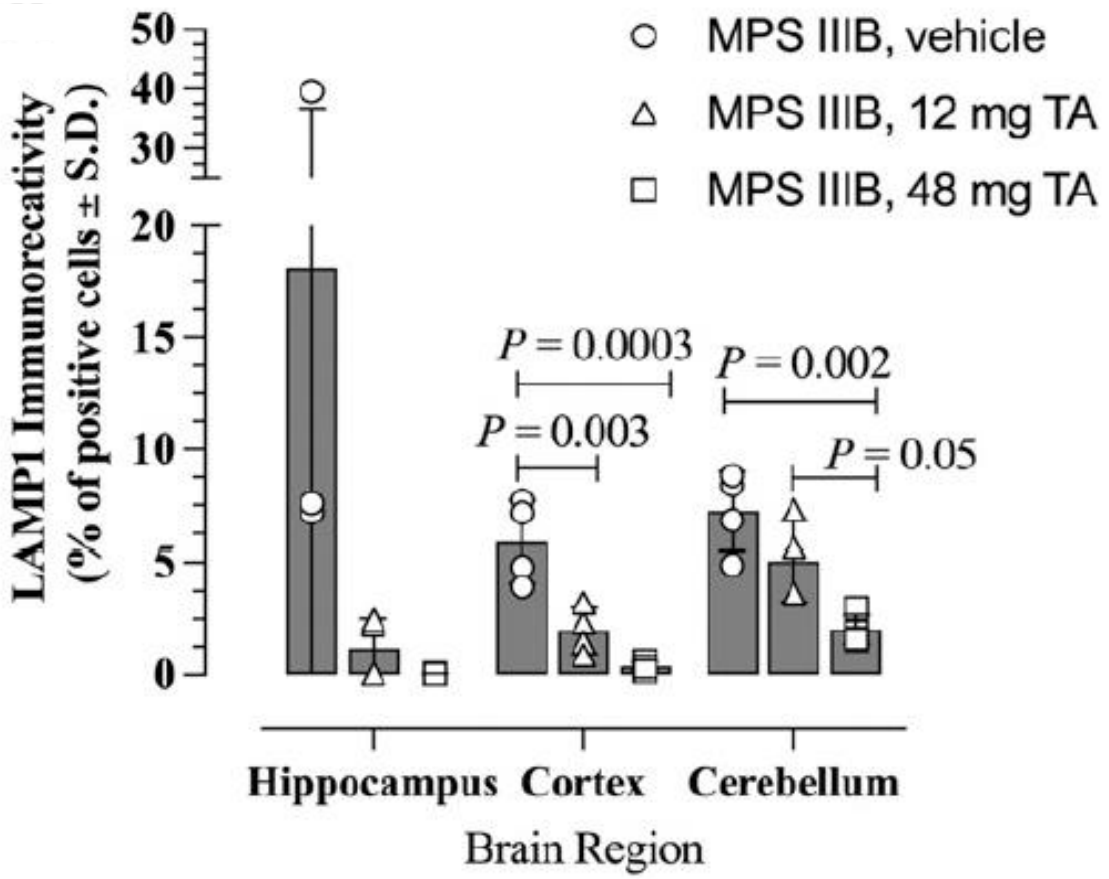
Dose Dependent Decrease of CSF HS and MPS IIIB NREs Over Time



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

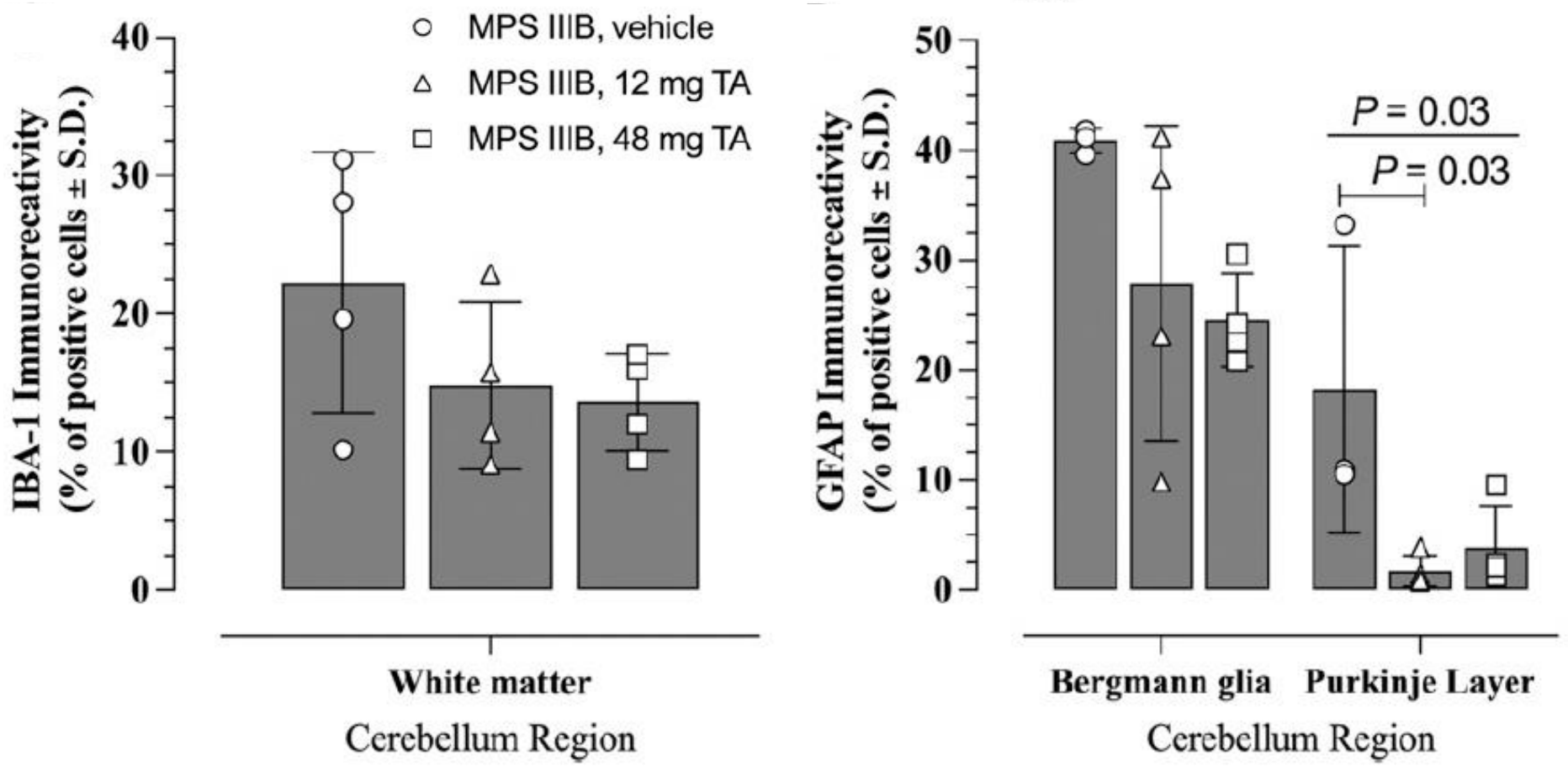
Ellinwood et al., unpublished data

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

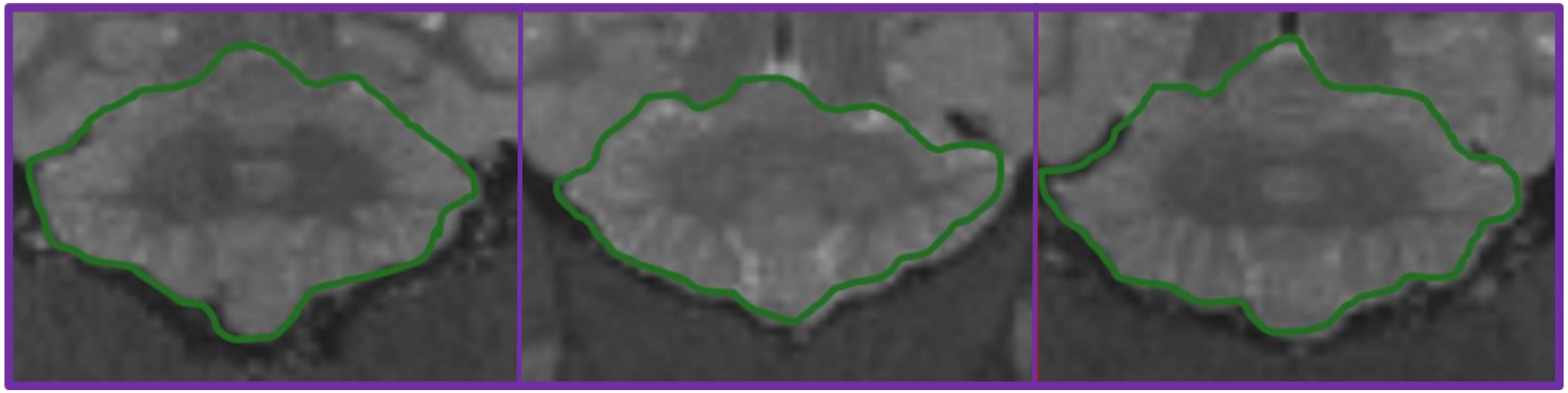
Decrease in Cerebellar Microglial Activation and Astrocytosis



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

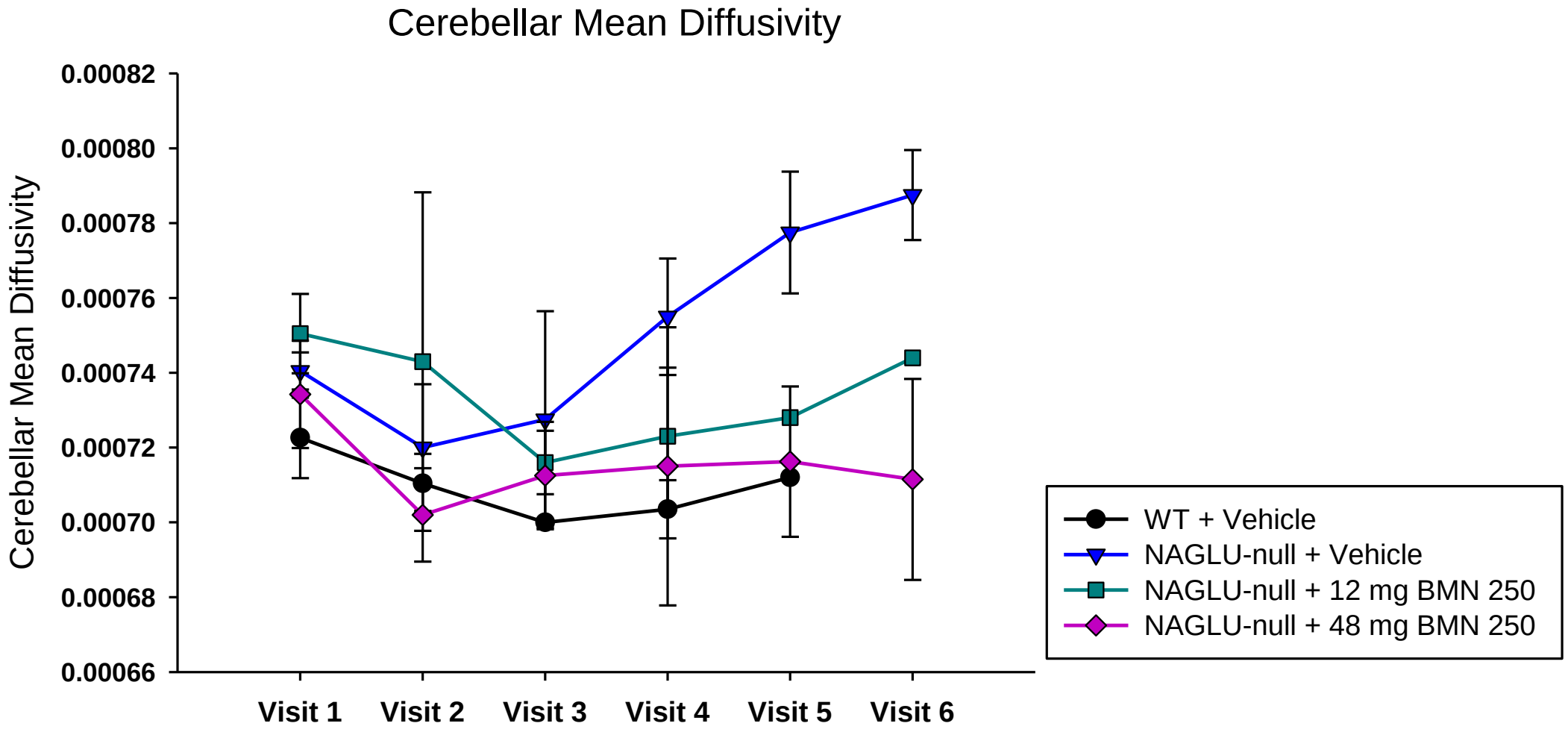
Biochemical and Histopathological Findings Support AX 250 Role in Prevention of Cerebellar Atrophy

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



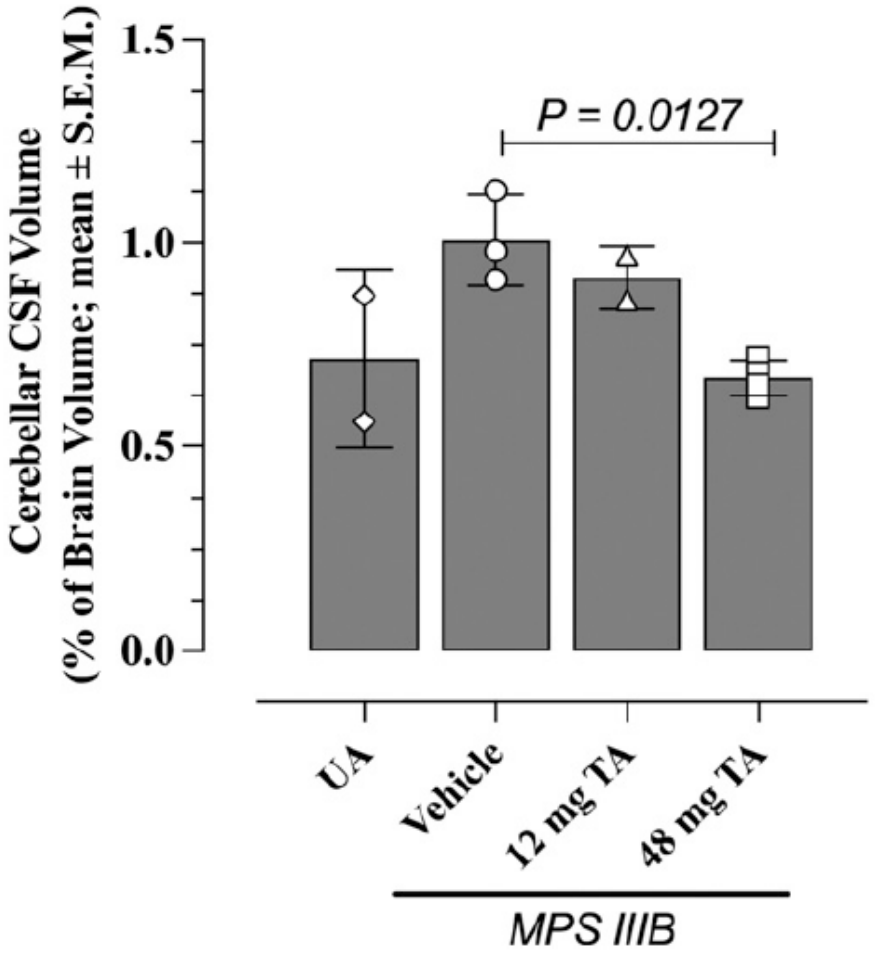
Ellinwood et al., unpublished data

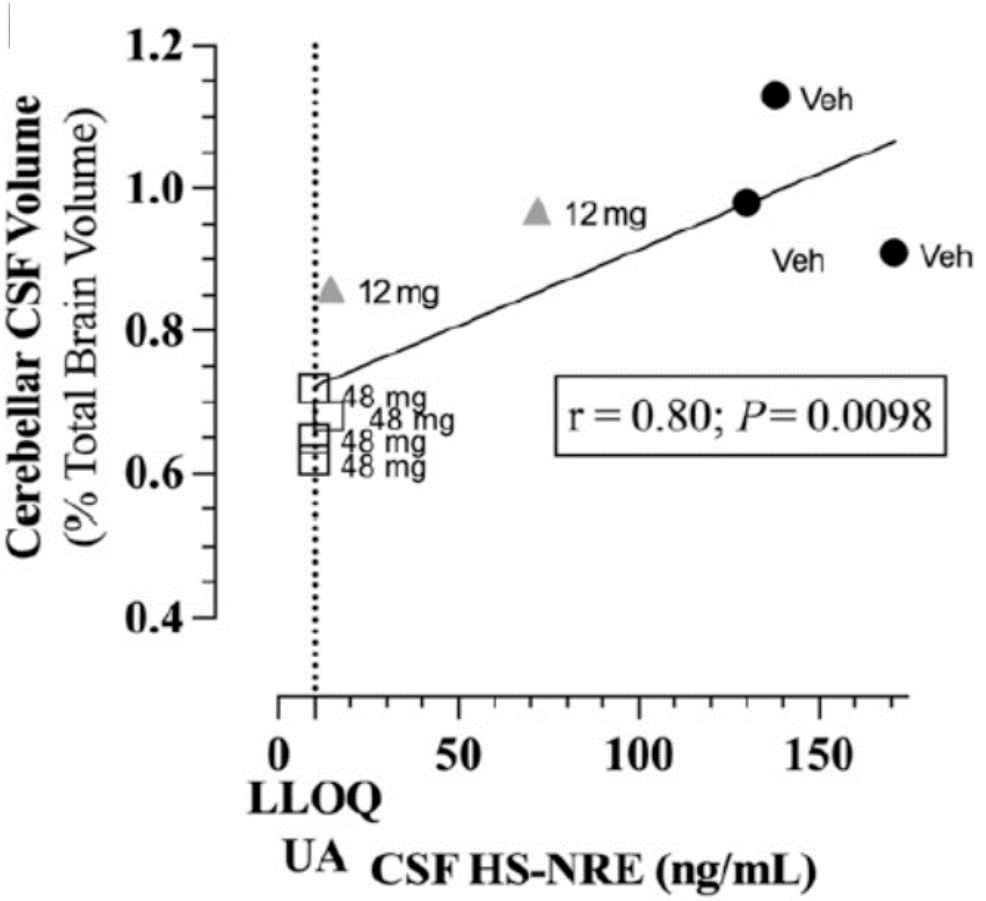
Dose-Dependent Preservation of Cerebellar Volume: CSF volume and mean diffusivity in cerebellum



Ellinwood et al., unpublished data

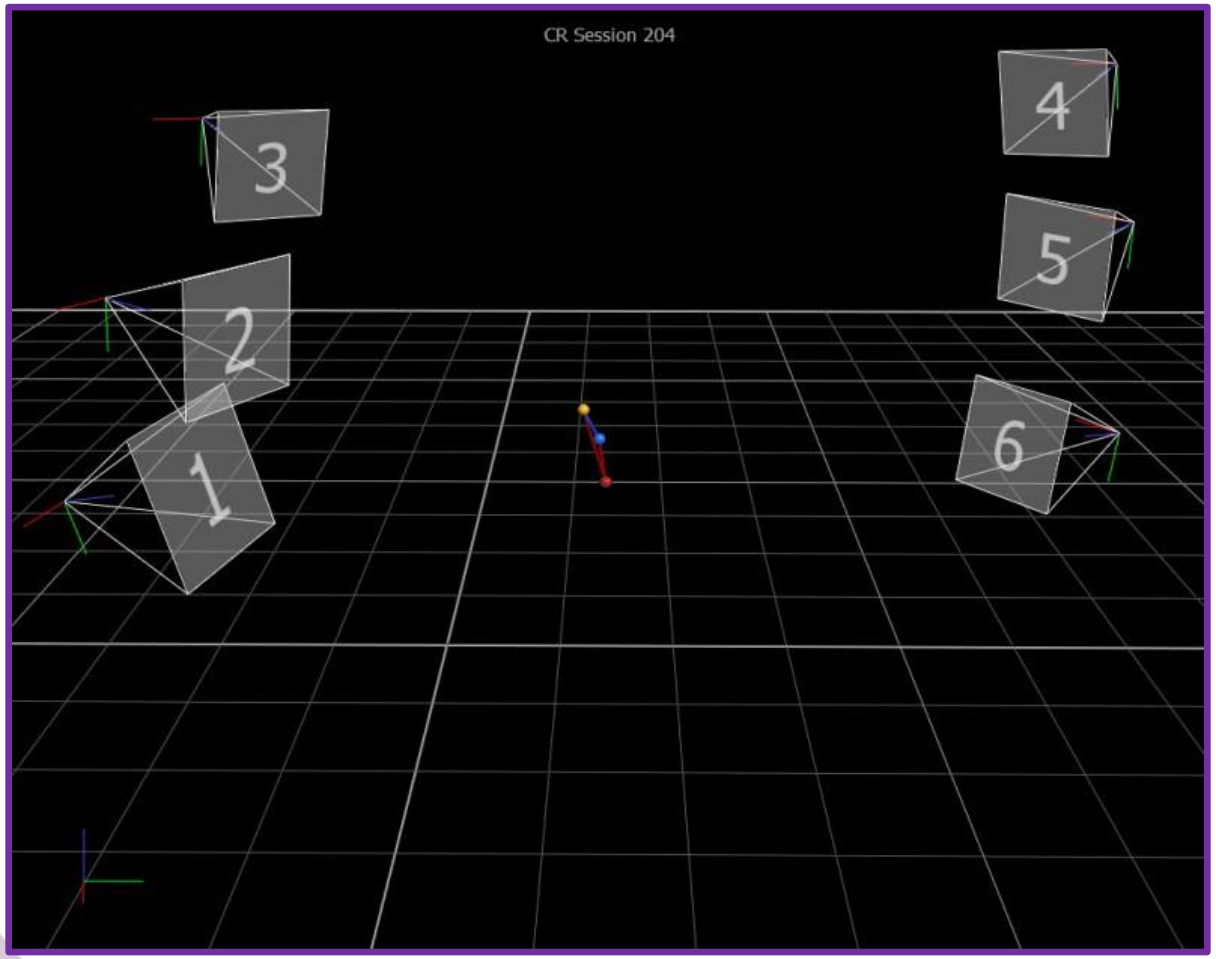
Pharmacokinetic Dose-Dependent Preservation of Cerebellar Volume: CSF volume and mean diffusivity in cerebellum





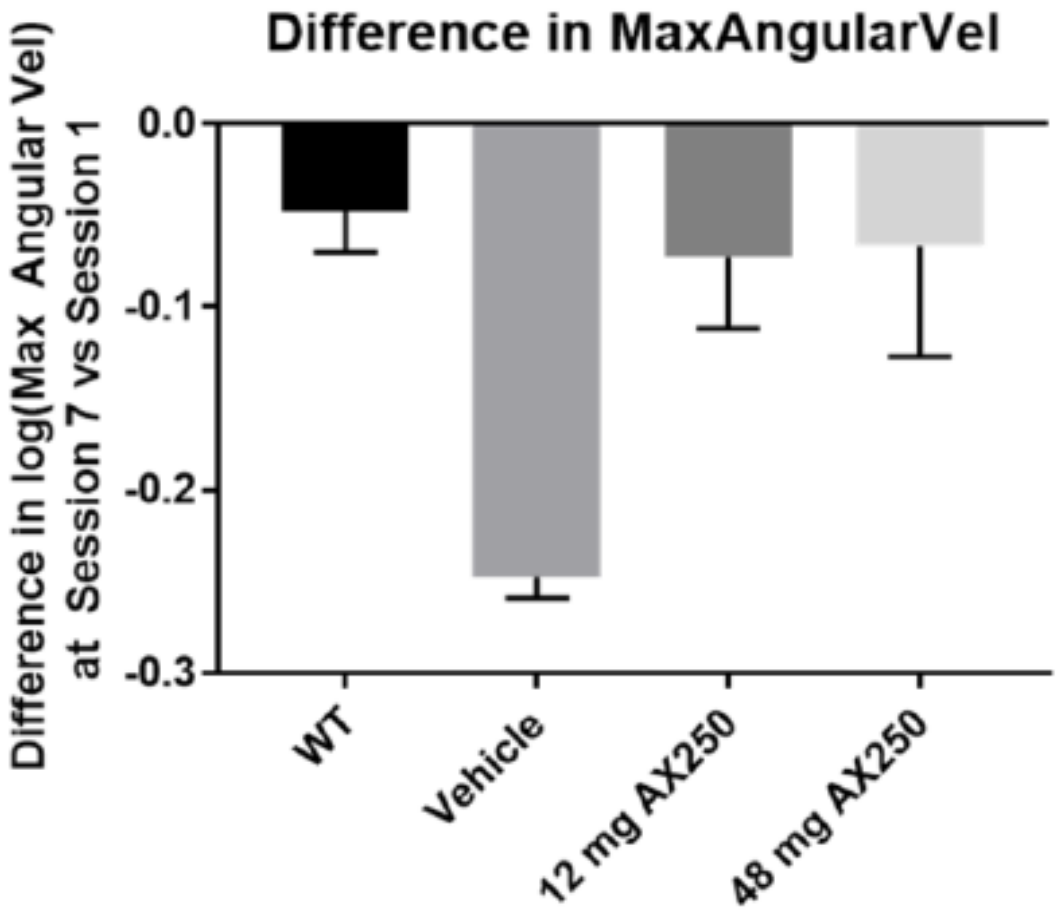
Ellinwood et al., J Pharmacol Exp Ther. 2022. DOI: 10.1124/jpet.122.001119

Functional Response in Cerebellar Performance



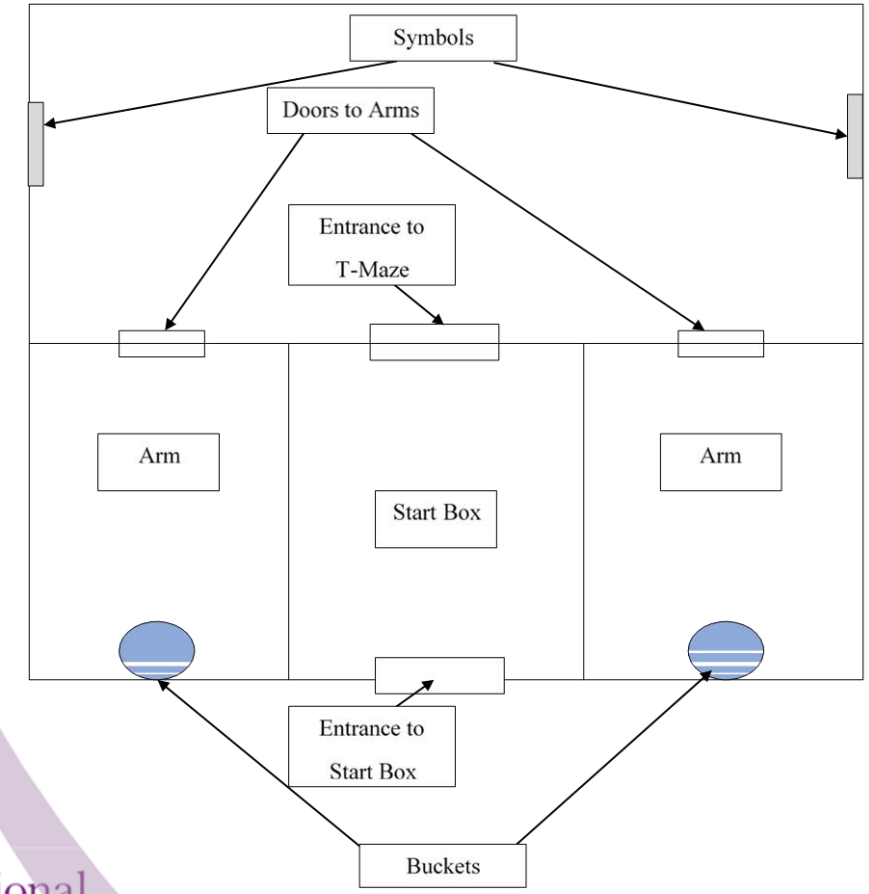
Ellinwood et al. Unpublished Data

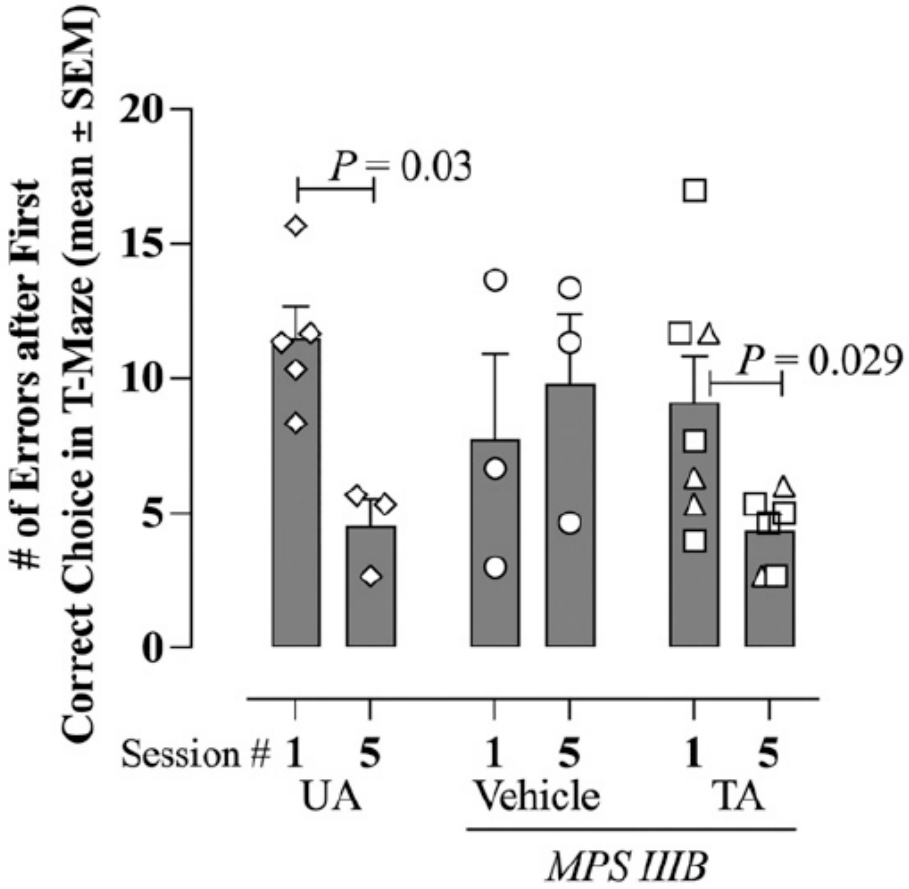
Functional Response in Cerebellar Performance



Ellinwood et al. Unpublished Data

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



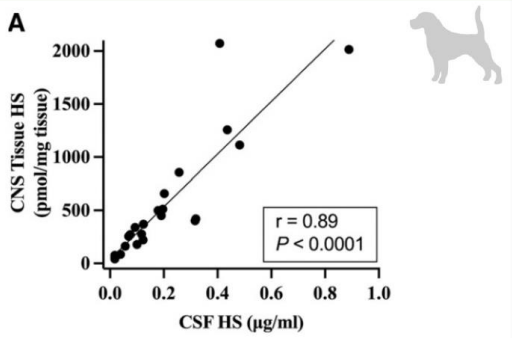


Ellinwood et al., J Pharmacol Exp Ther. 2022. DOI: 10.1124/jpet.122.001119

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

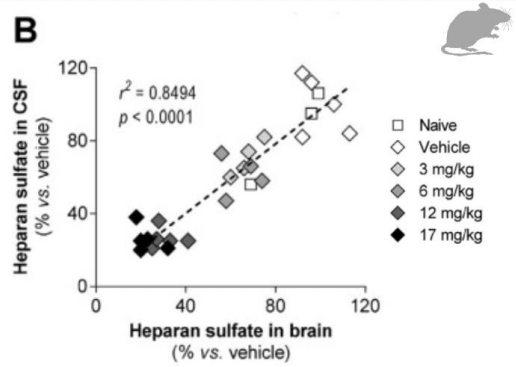


Notes: Dogs were administered vehicle, 12mg or 48mg test article ICV every other week for 83 weeks (n=12) or 15 or 30 mg ICV weekly for 8 weeks (n=11) with tralesinidase alfa, a fusion protein of recombinant human NAGLU and a modified human insulin-like growth factor 2.

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

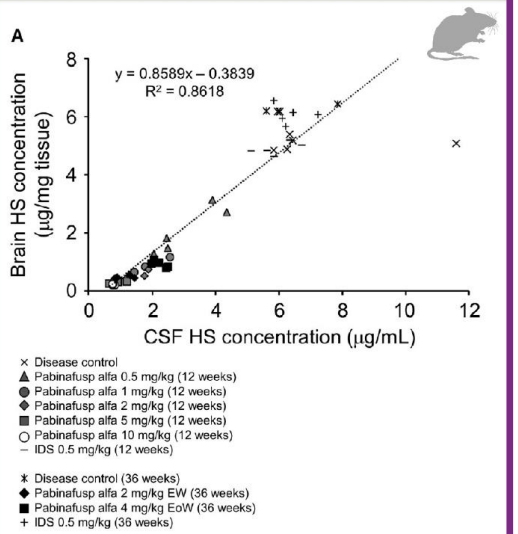
Brain and CSF HS correlation in MPS IIIA murine model



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.

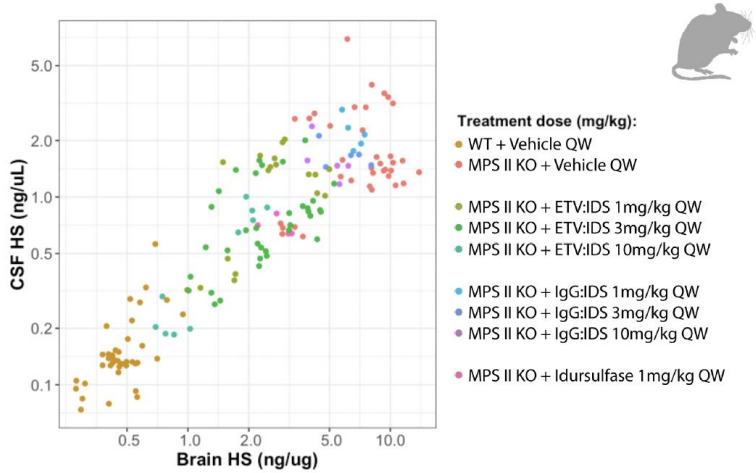
Brain and CSF HS correlation in MPS II murine model



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.

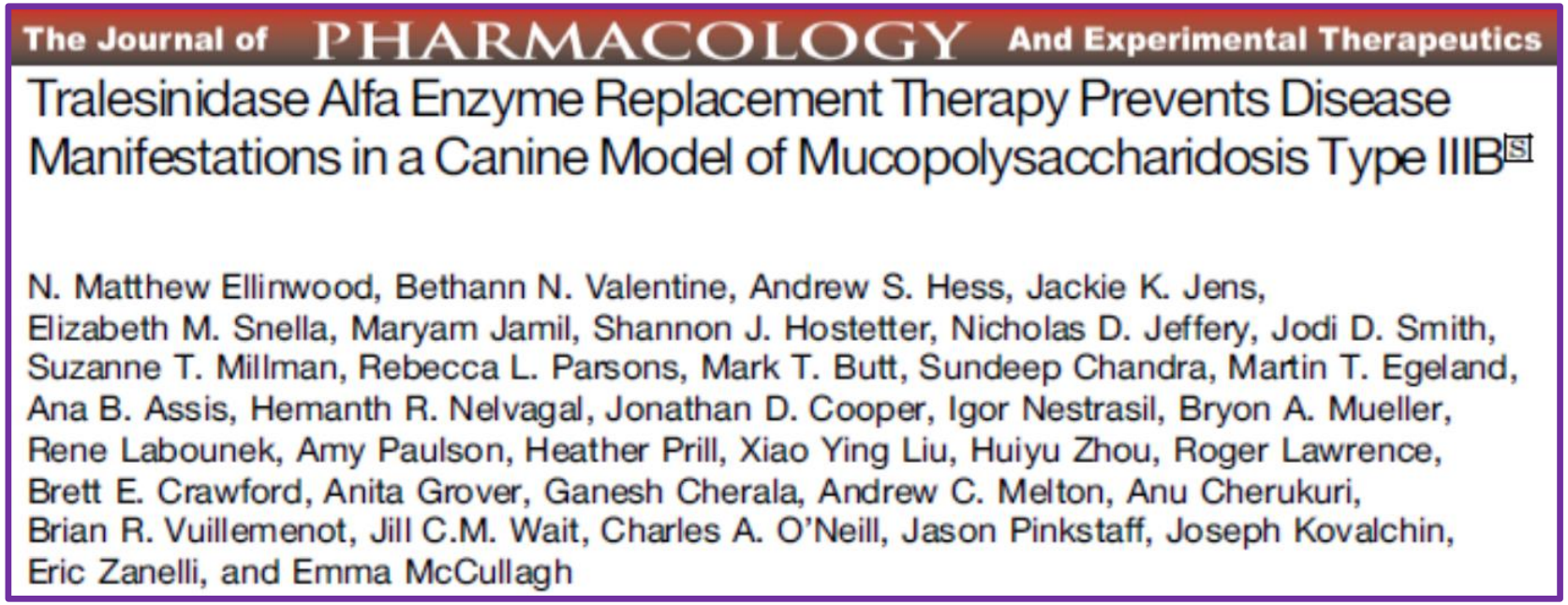
Conclusions

- 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

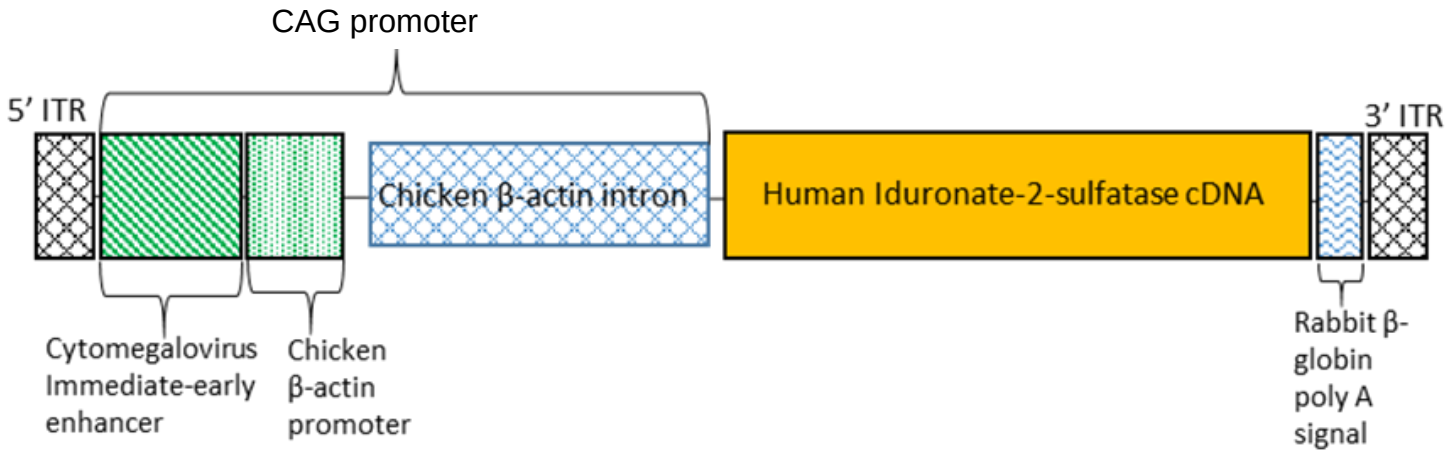
Case Study: Animal Model Translation to Human Application

Nidal Boulos, Ph.D.
Director, Clinical Science

February 21st, 2024

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

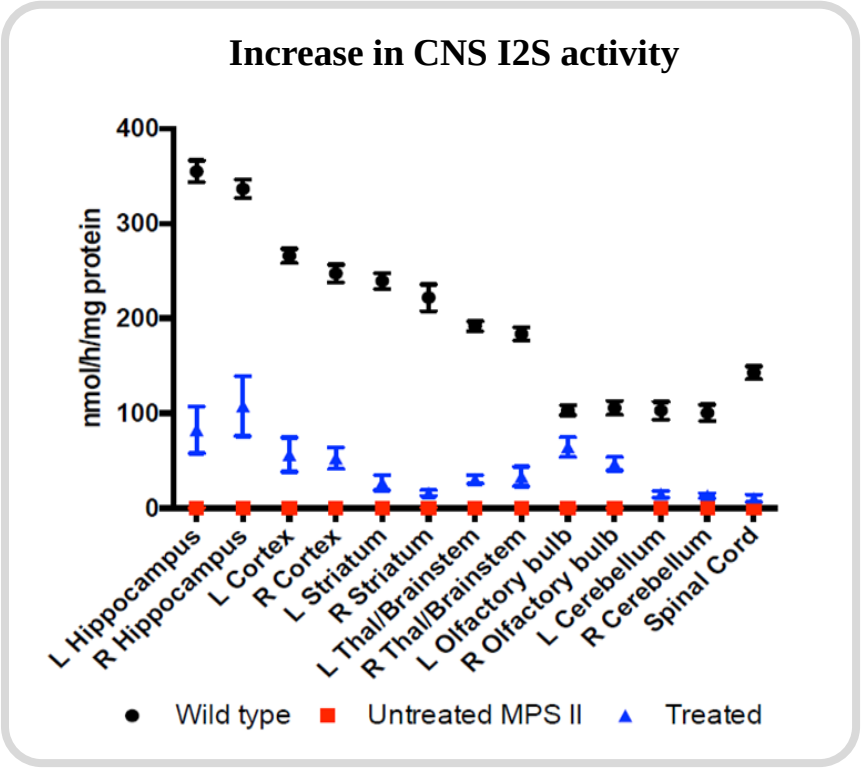
- RGX-121 is a non-replicating recombinant AAV9 containing human iduronate-2-sulfatase expression cassette.
- RGX-121 is designed for efficient expression of iduronate-2-sulfatase enzyme (I2S) in the CNS.
- RGX-121 is being investigated as a potential treatment for MPS II in a phase I/II/III clinical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

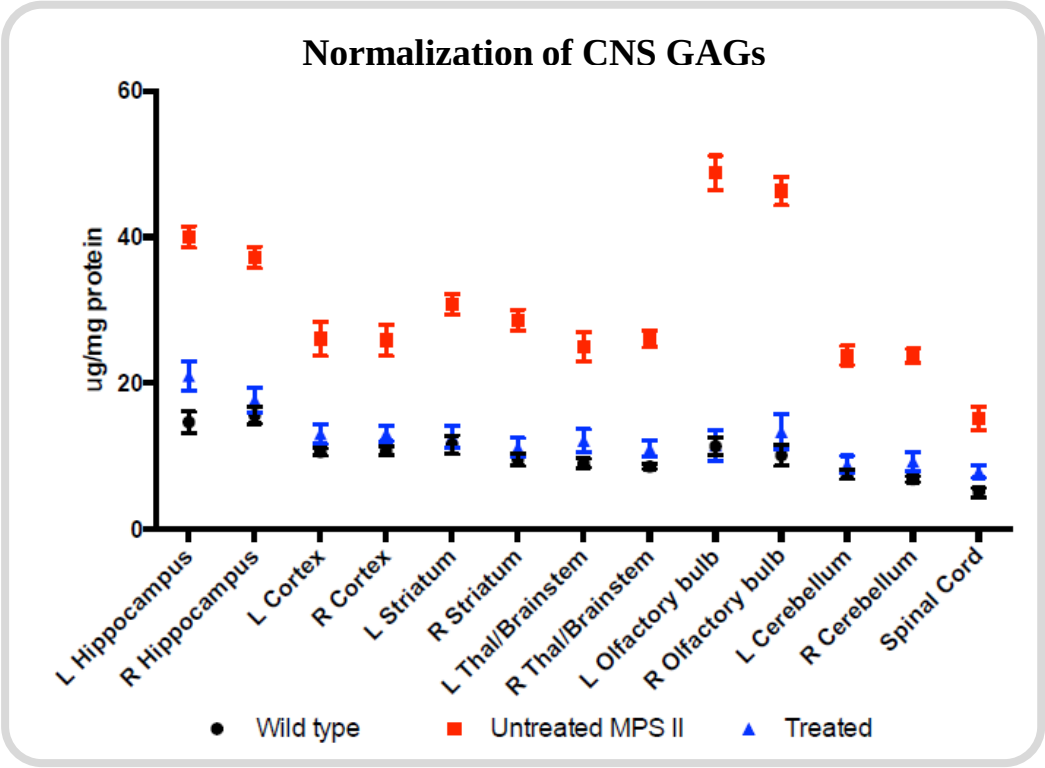


* RGX-121 is an investigational therapy and has not been approved by any regulatory authority.

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)

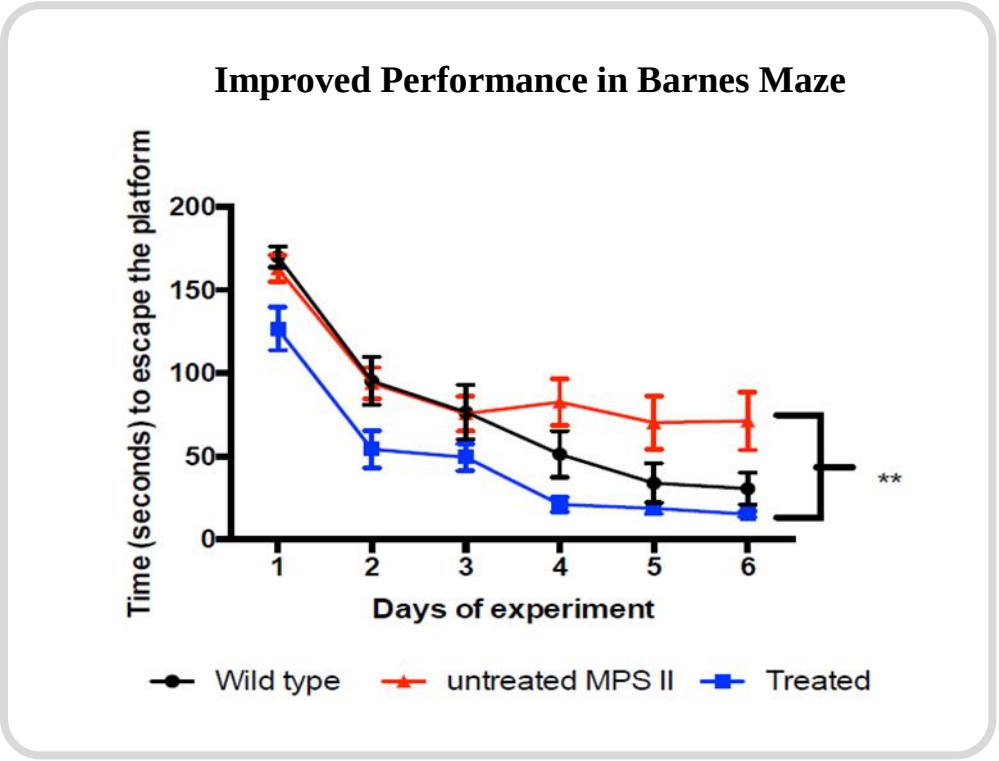




Laoharawee *et al*. Human Gene Ther 2017 28(8):626-638

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

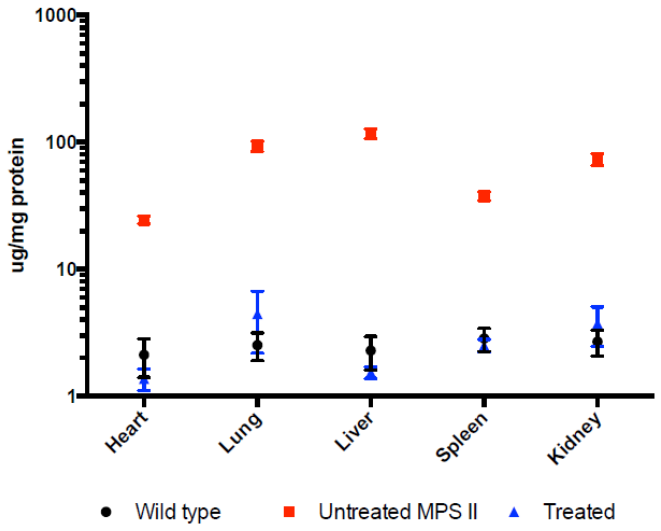
Neurobehavioral assessment of mice: spatial learning and memory

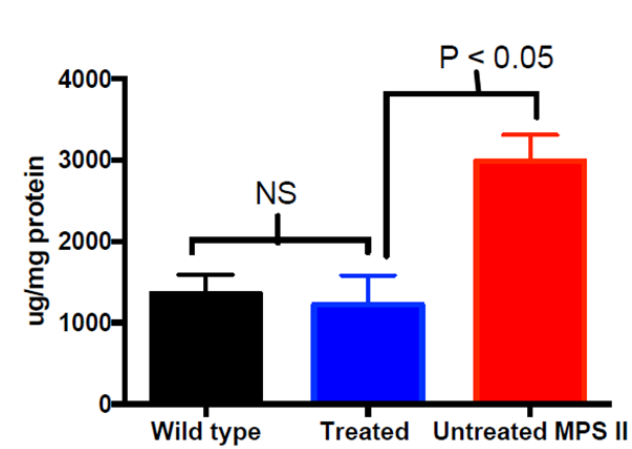


Laoharawee *et al*. Human Gene Ther 2017 28(8):626-638

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.

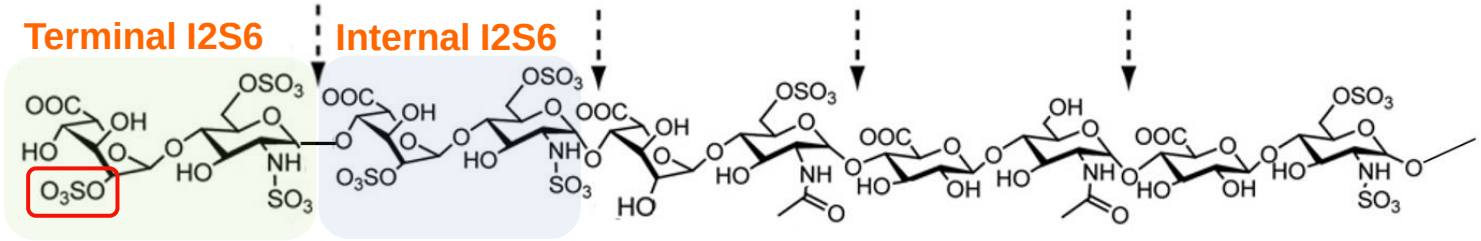
MPS Type	Main GAG stored	Neurologic Symptoms
MPS I	HS, DS	Hurler: Severe Hurler-Scheie and Scheie: mild to absent
MPS II	HS, DS	Severe (fast progressing phenotype) to mild or none (slow progressing phenotype)
MPS IIIA, B, C, D	HS	Severe
MPS IVA, B	KS	None
MPS VI	DS	None
MPS VII	HS, DS	Severe or mild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

Heparan Sulfate is a key biomarker in neuronopathic MPS types

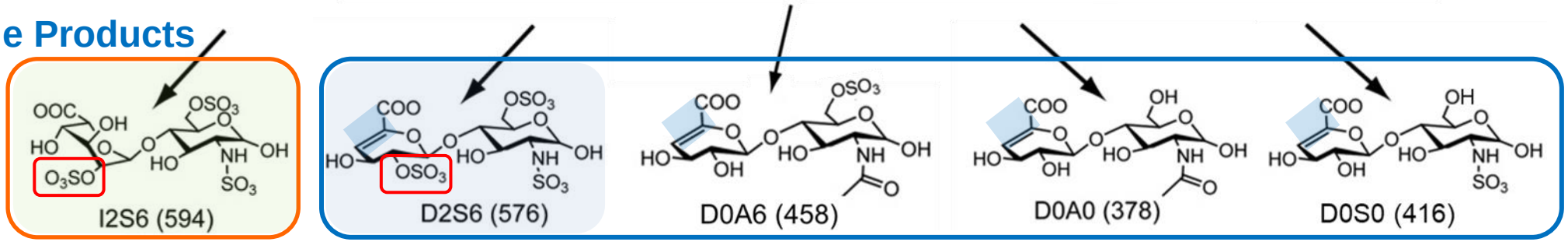
Heparan Sulfate digestion into disaccharides

Heparinase Sites for Cleavage

HS Chain



HS Disaccharide Products



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

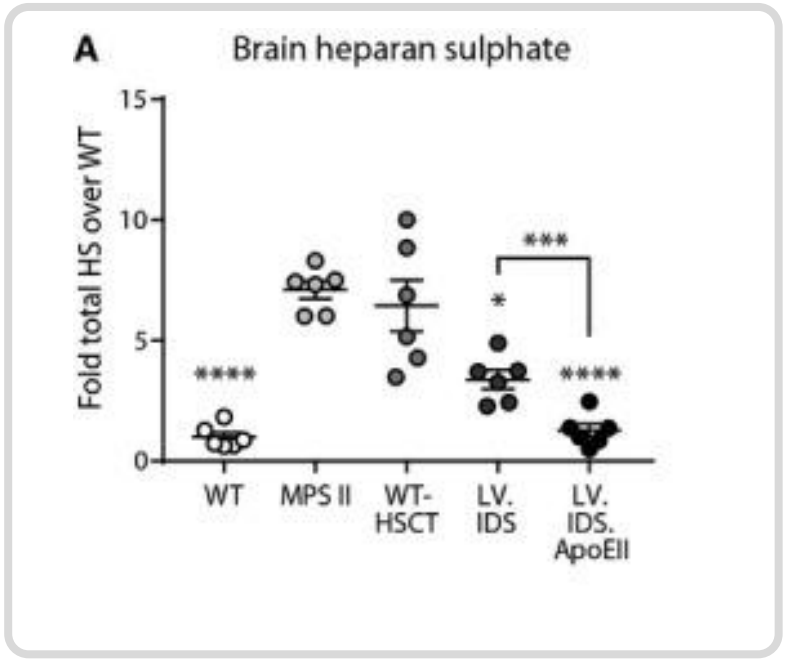
D2S6 contains 2-O-sulfated ends which are cleaved by I2S enzyme.

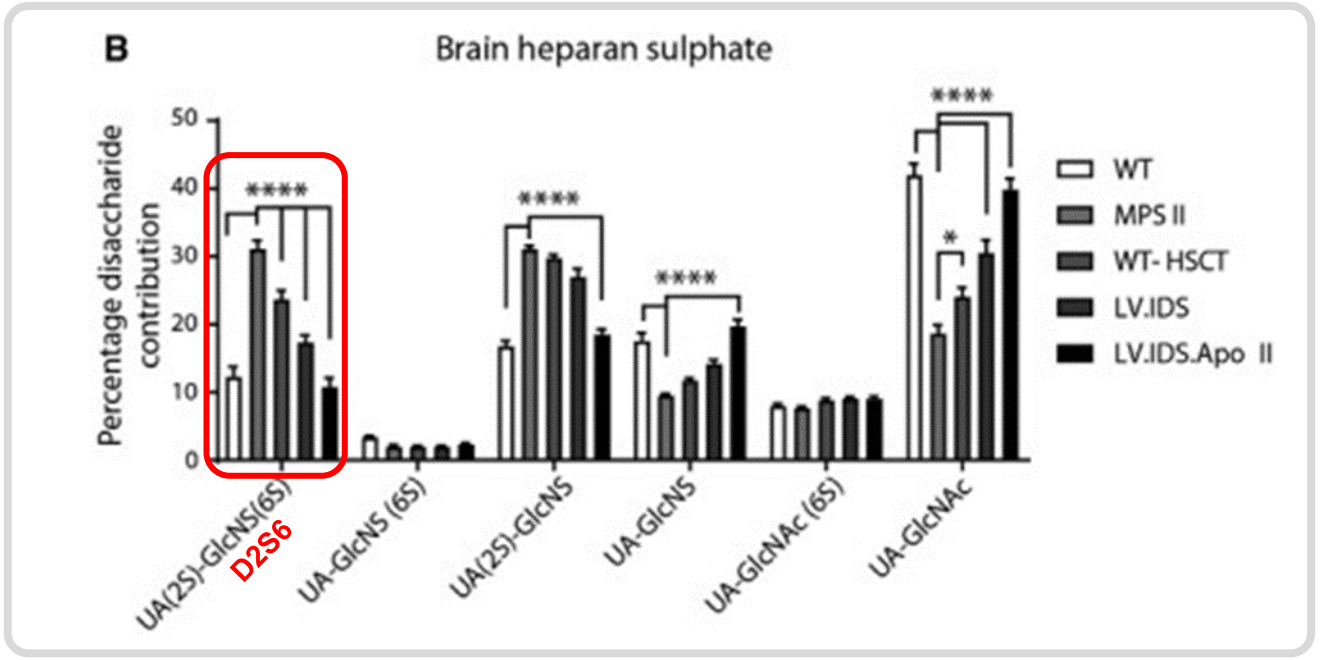
HS D2S6 disaccharide contains the 2-sulfate on non-reducing end = substrate for I2S enzyme (complicit with IDS gene deficiency)

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

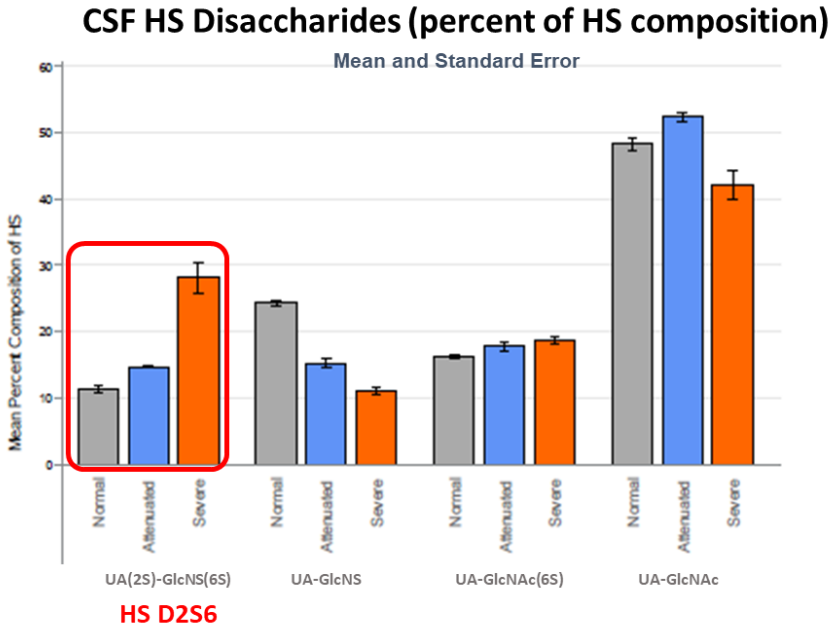
- **31% of total HS in the MPS II brain tissue shown to be HS D2S6**
- **HS D2S6 was the disaccharide most responsive to treatment**
- **Reductions in HS D2S6 associated with corrections in other disease markers, e.g., neuroinflammation, astrocytosis (GFAP, MCP-1, MIP-1α and IL-1α)**
- **Normalization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

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

Translation to Human Application

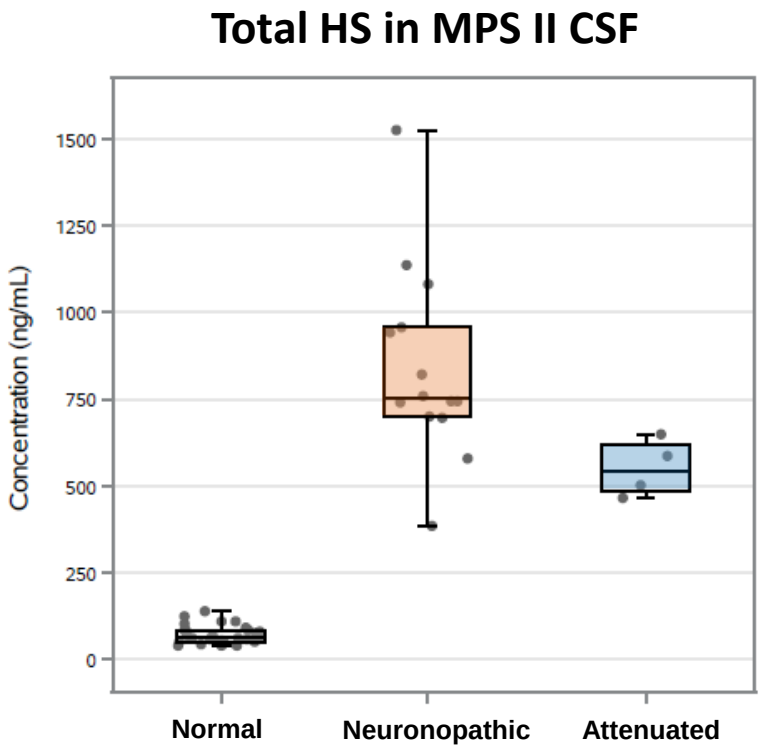
HS D2S6 is Increased in human CSF of neuronopathic MPS II compared to attenuated MPS II and Normal CSF (REGENXBIO generated data)

- 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

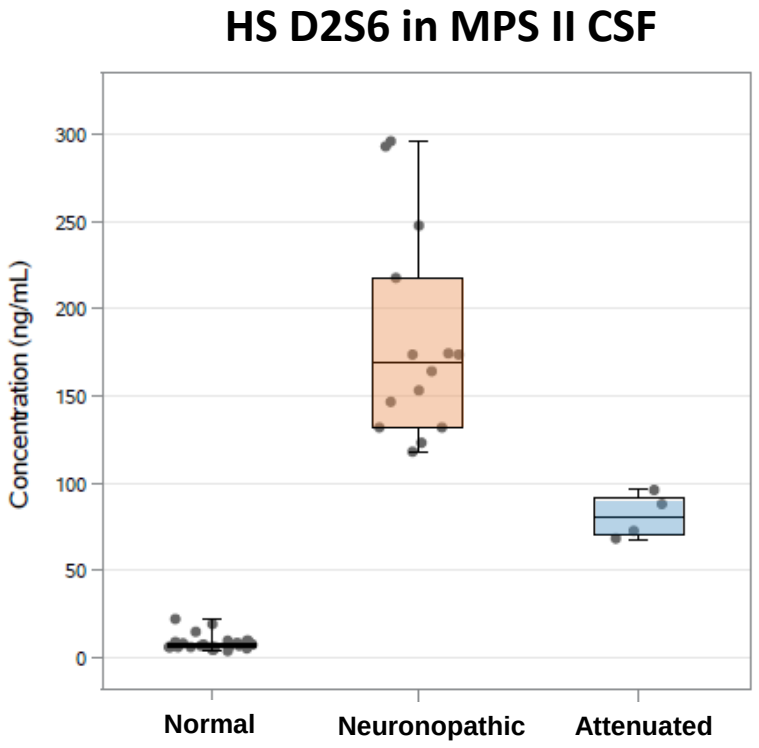


Boulos, WORLD*Symposium,* San Diego, CA 2020

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

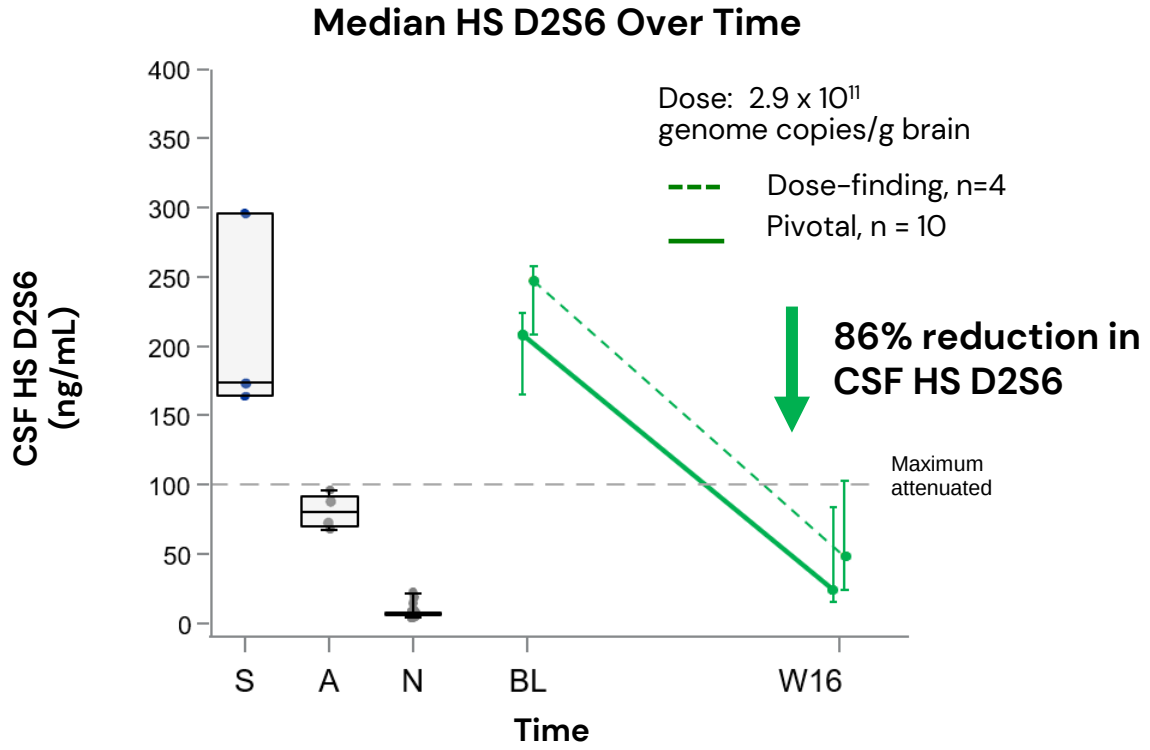
29 Normal CSF samples were purchased from BioIVT (n=20) and Discovery Life Sciences (n=6) or courtesy of Dr. Giugliani (n=3)
3 neuronopathic MPS II, 4 non-neuronopathic MPS II and all MPS I samples are courtesy of Dr. Giugliani
11 neuronopathic MPS II samples are from RGX-121-101

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?

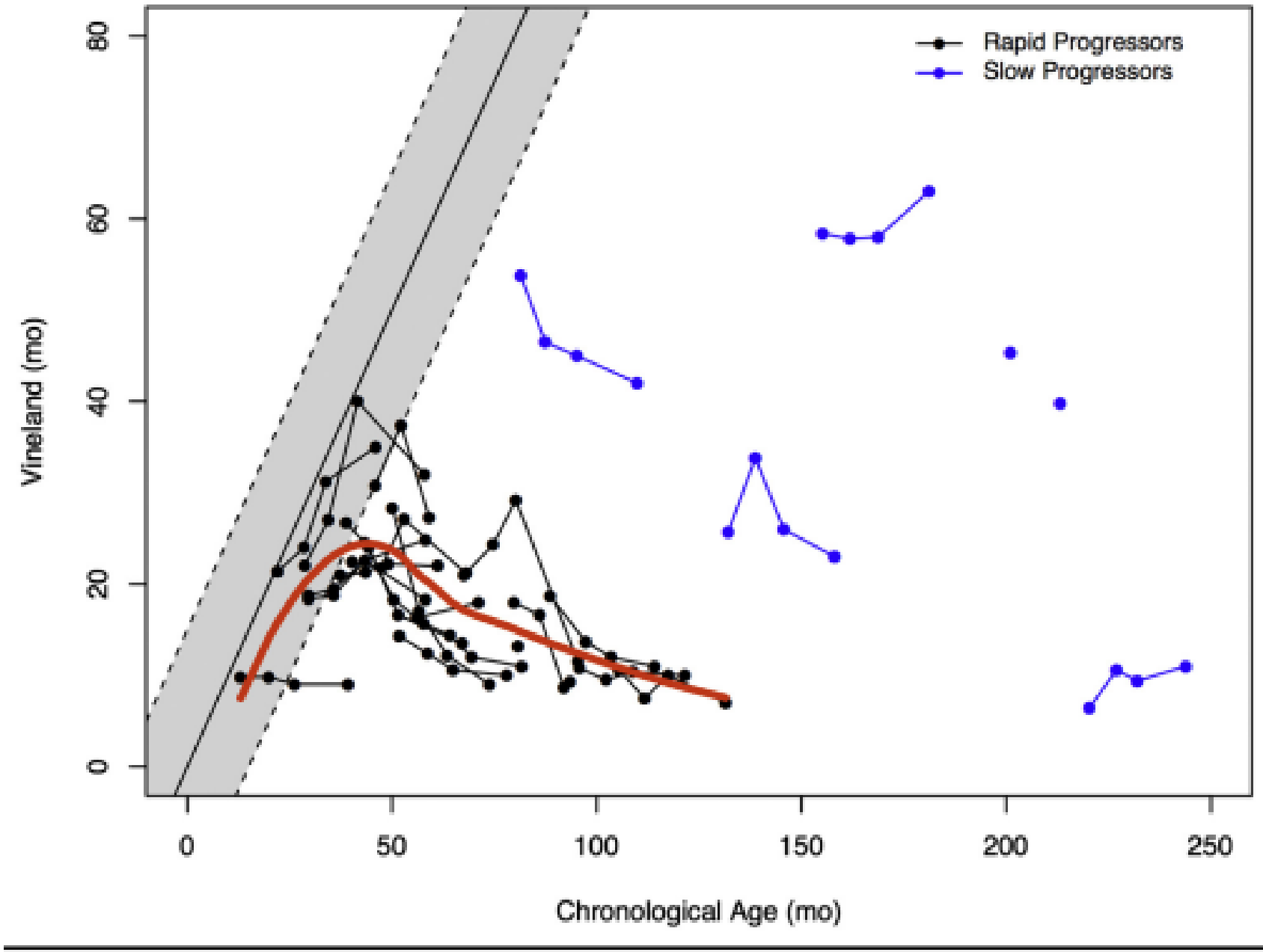
Simon Jones

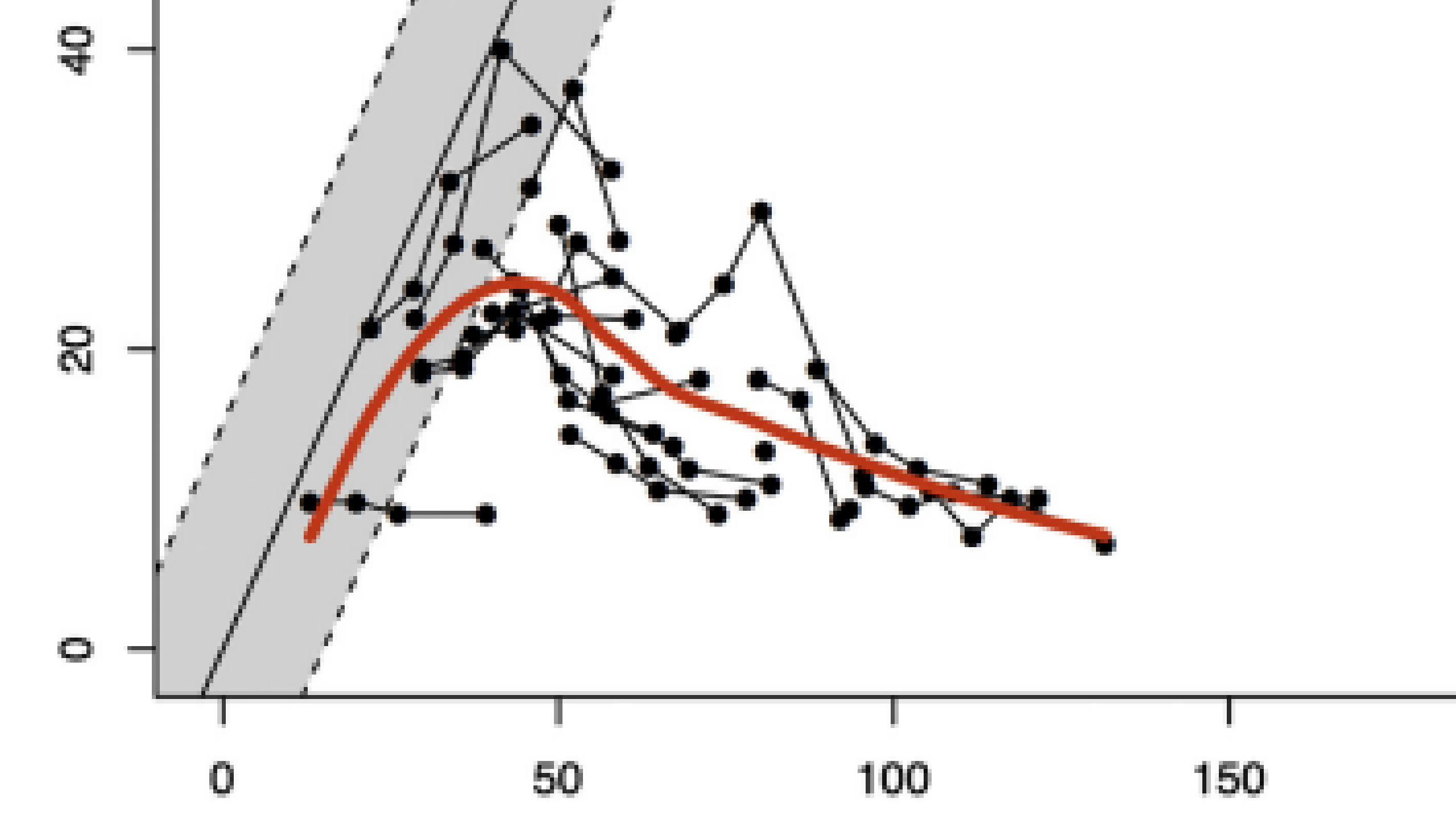
Consultant Paediatric Inherited Metabolic Disease, Manchester

Honorary Professor of Paediatrics and translational medicine

Medical Director, NIHR children's clinical research facility

Shapiro et al 2016

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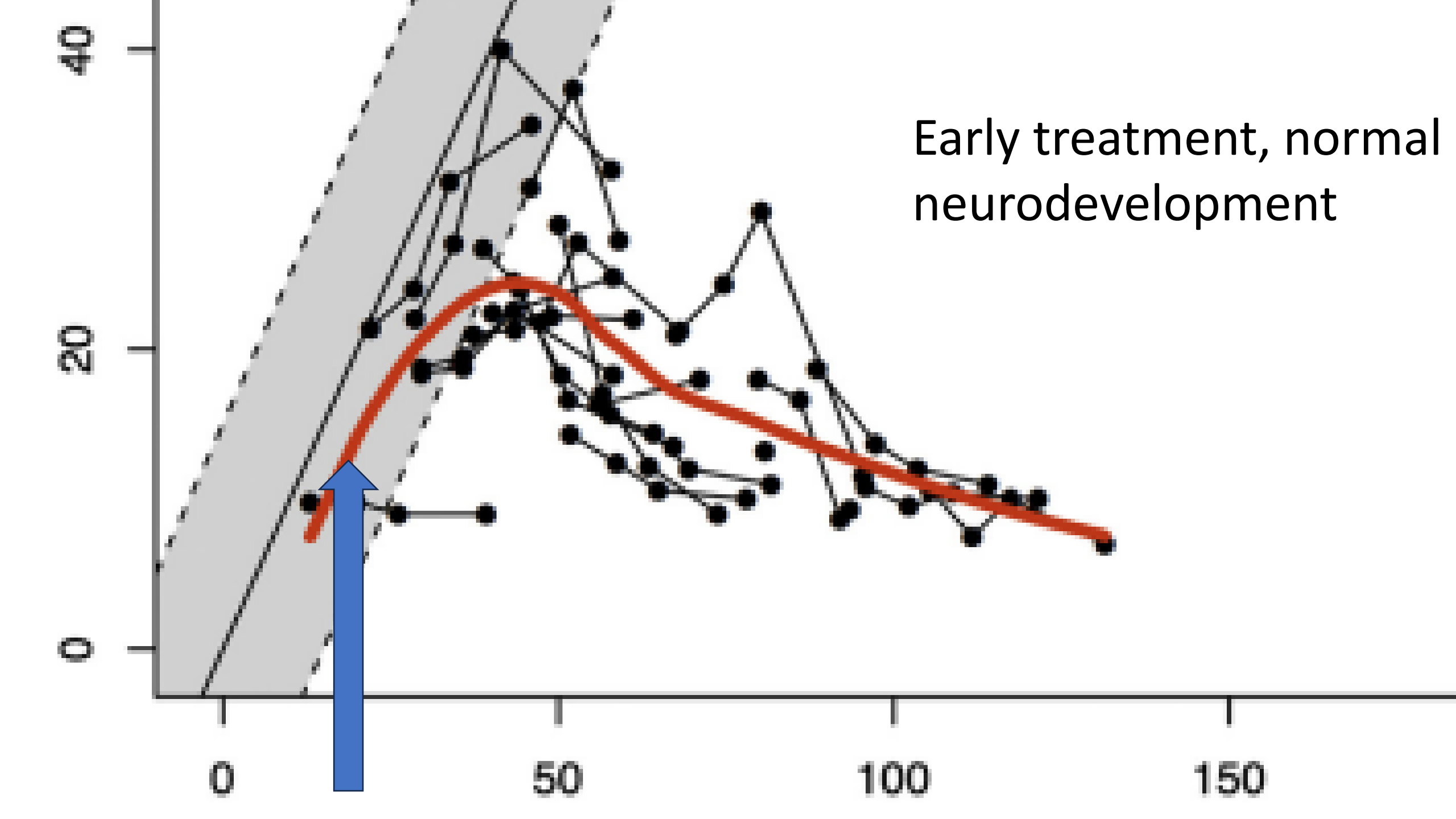
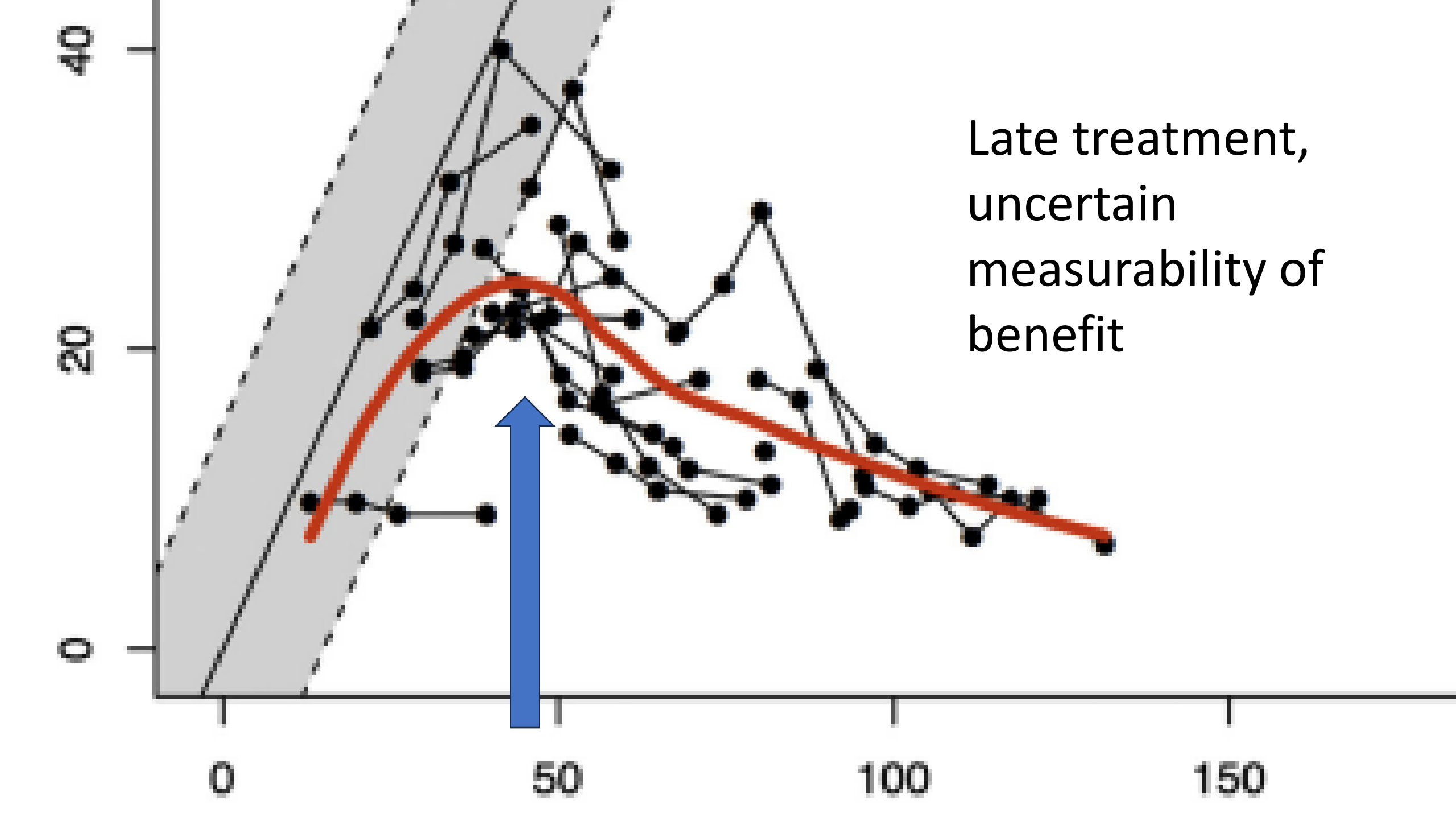


Early treatment, normal neurodevelopment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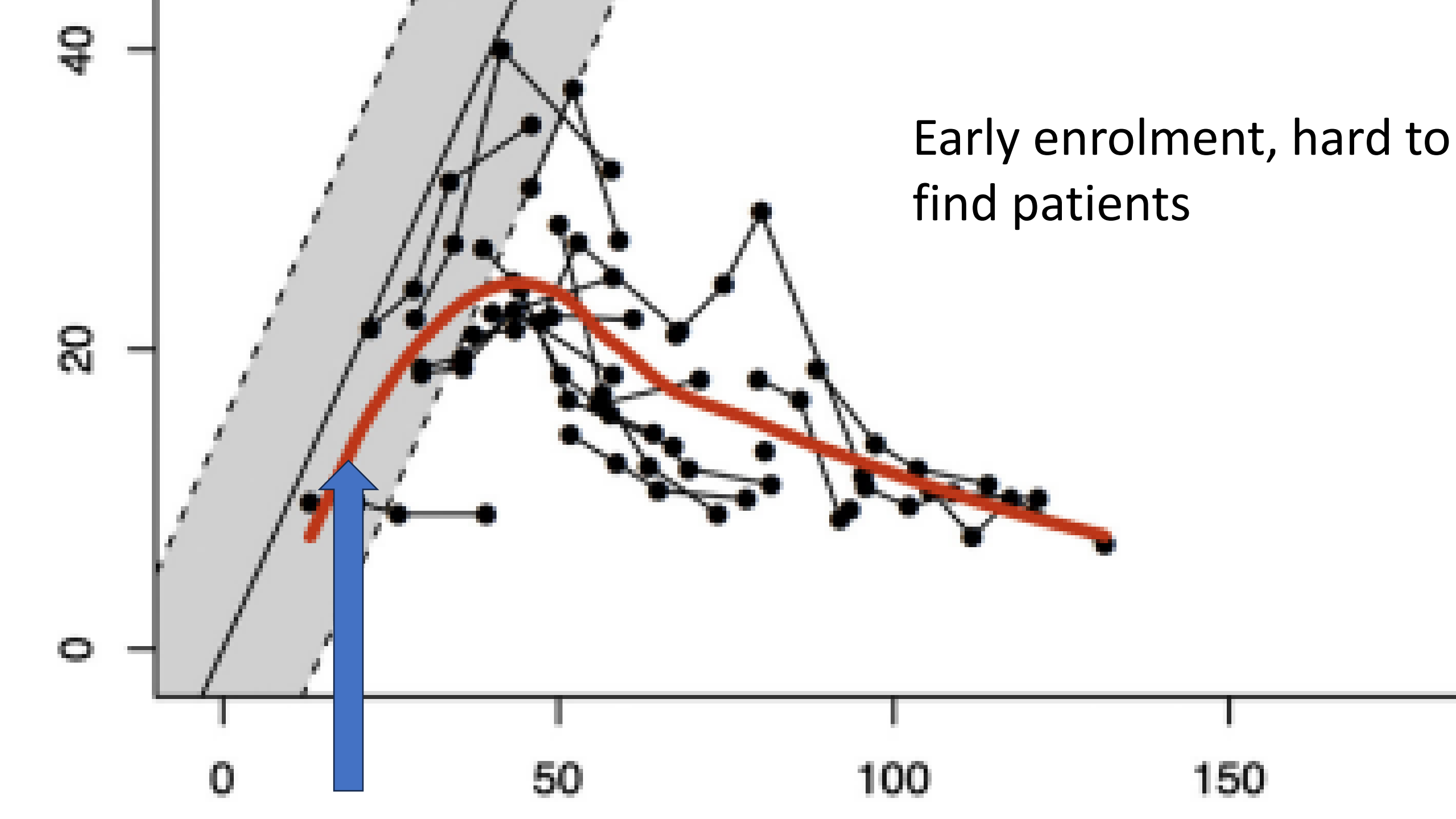
Late treatment, uncertain measurability of benefit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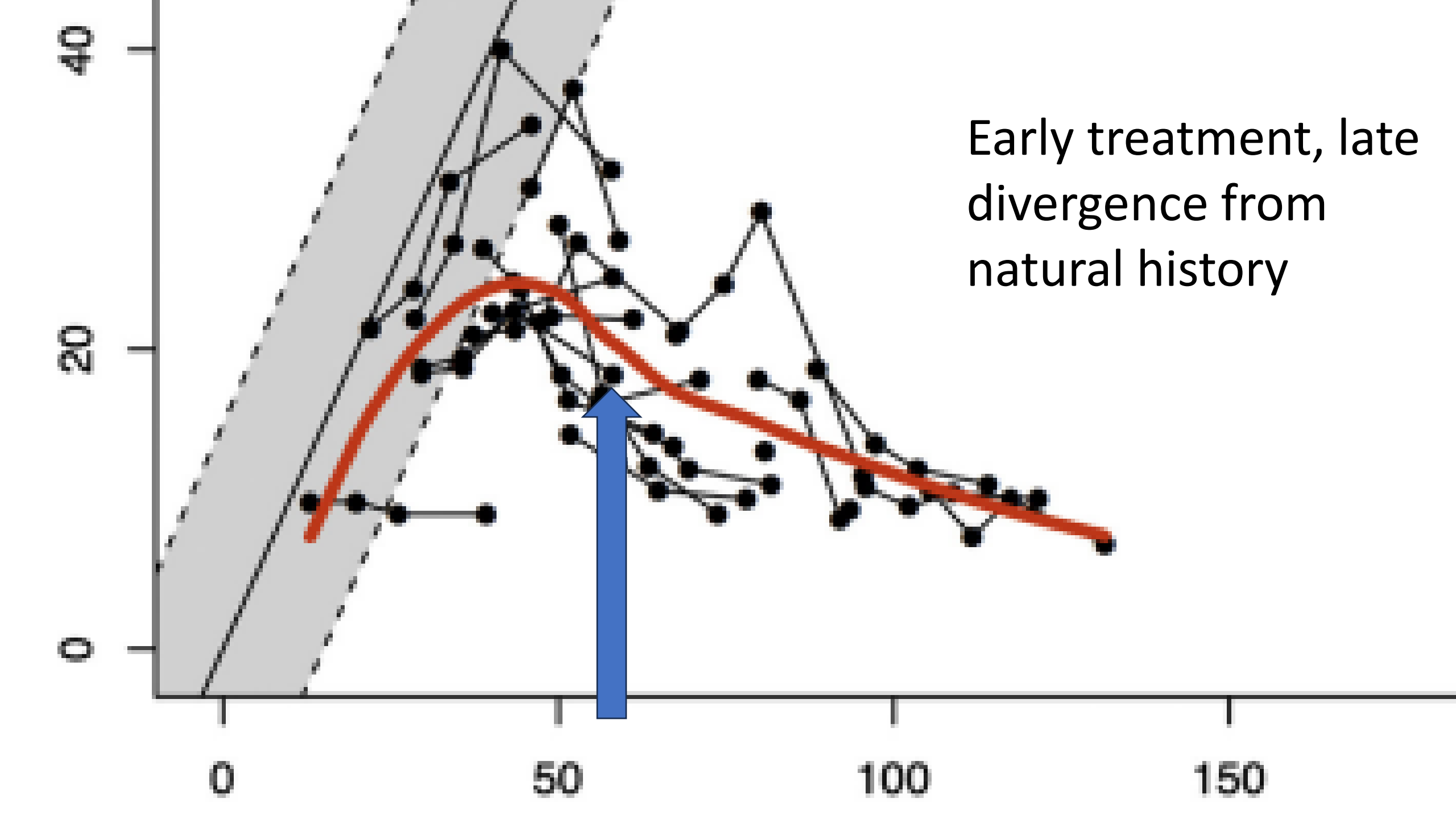
Early enrolment, hard to find patients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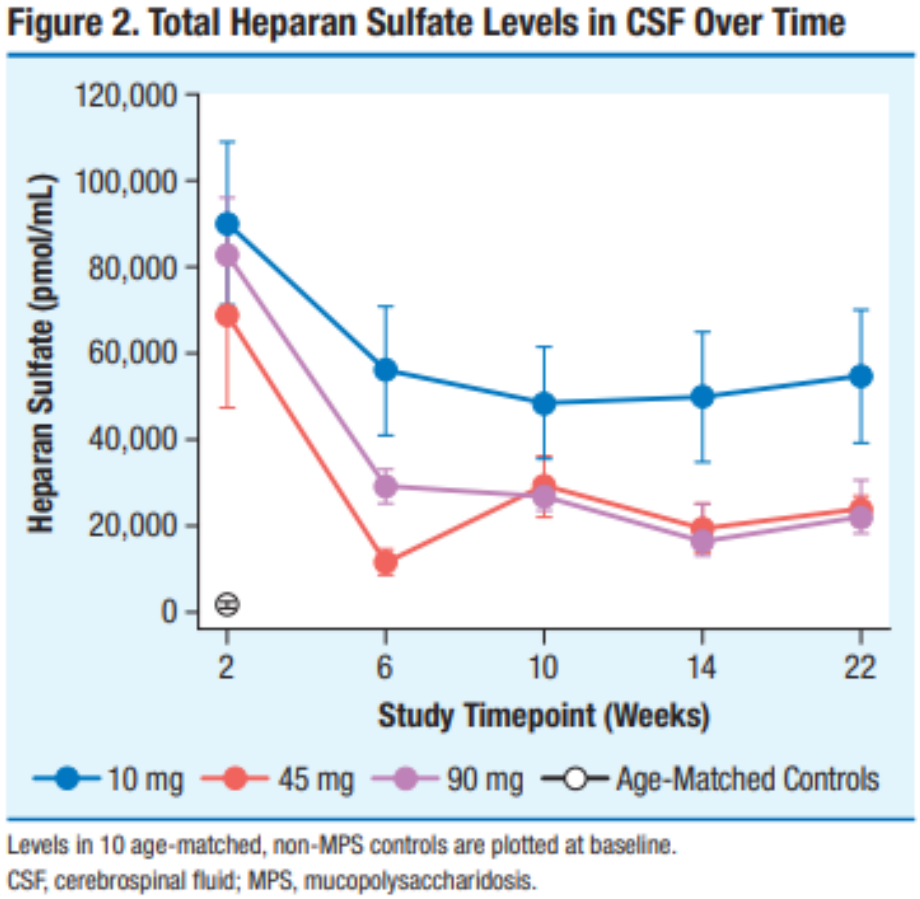
Early treatment, late divergence from natural history

A tale of three trials…..

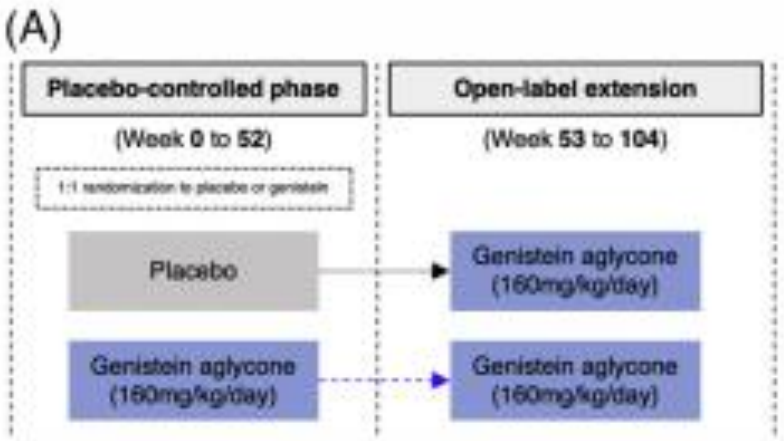
- Intra-thecal enzyme replacement therapy in MPSIIIA (Shire HGT/Takeda) phase I/II trial. Commenced 2010
- Genistein (isoflavone nutraceutical) in MPSIII phase III trial (academic), commenced 2015
- Lentiviral ex vivo stem cell gene therapy (academic but funded by Orchard Therapeutics) in MPSIIIA, commenced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



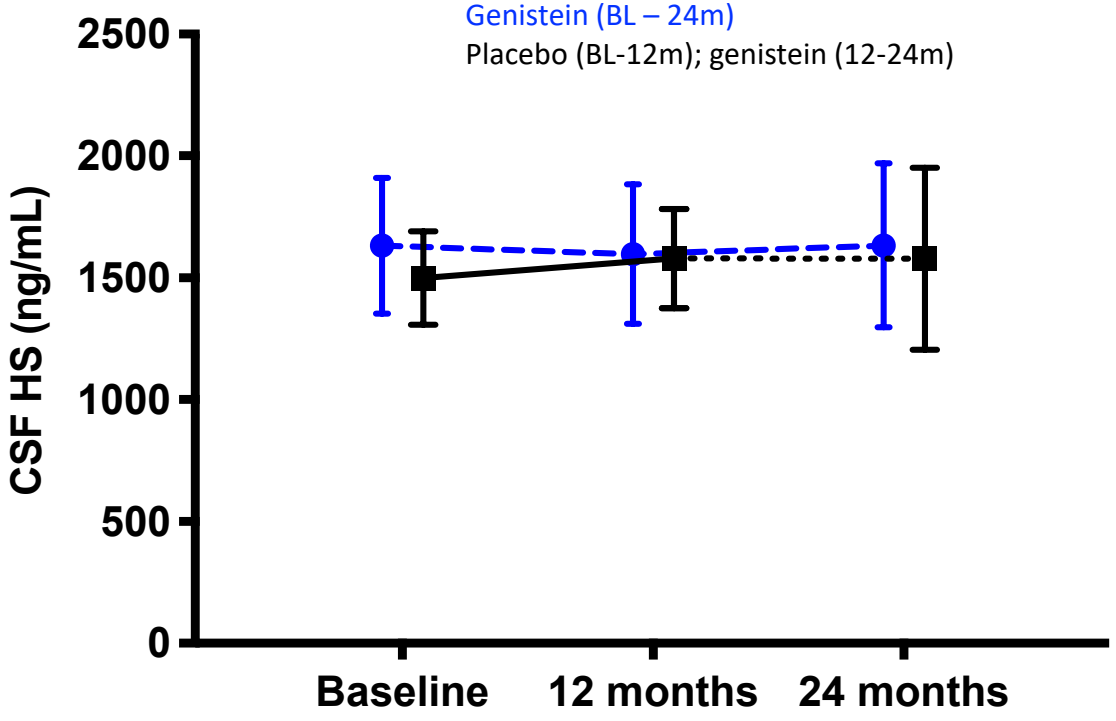
Received: 9 February 2021 | Revised: 25 May 2021 | Accepted: 27 May 2021
DOI: 10.1002/jimd.12407

ORIGINAL ARTICLE

JIMD SSIEM WILEY

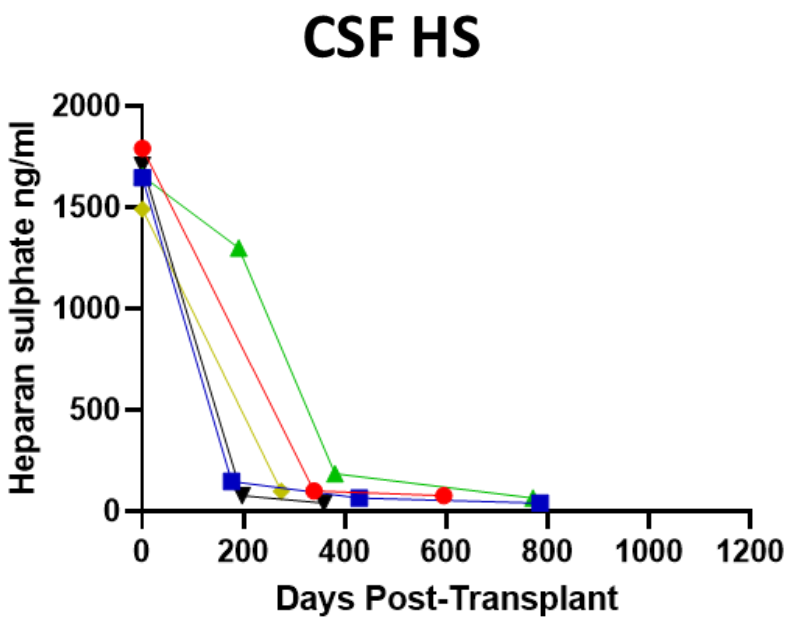
High dose genistein in Sanfilippo syndrome: A randomised controlled trial

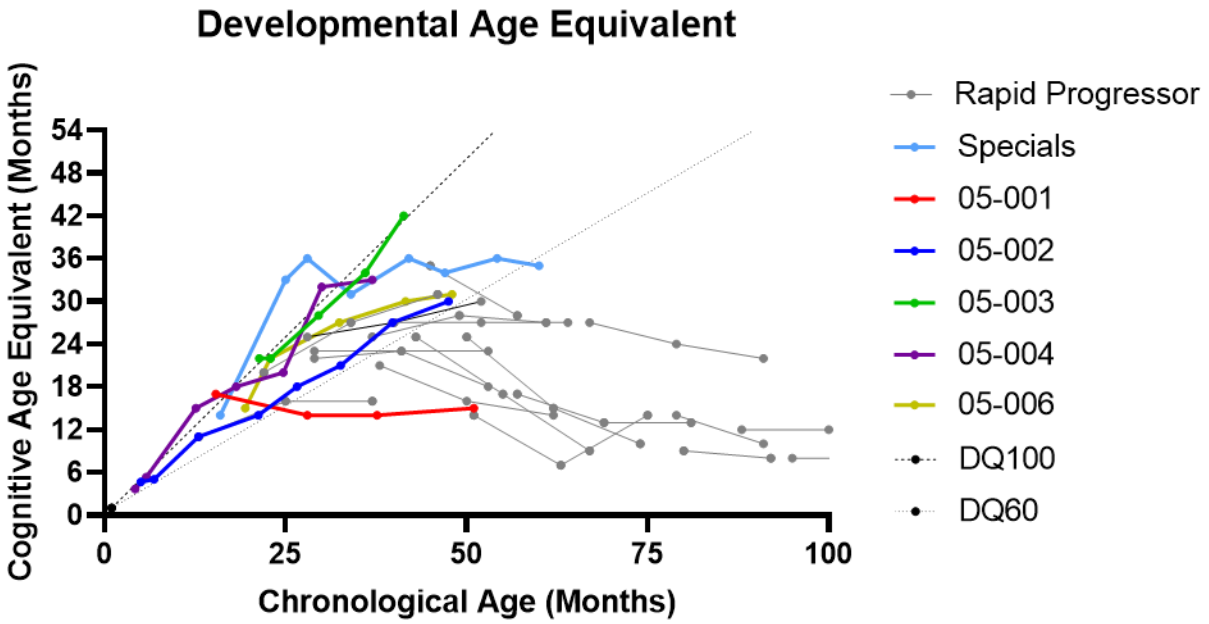
Arunabha Ghosh[1,2] | Stewart Rust[3] | Kia Langford-Smith[2] | Daniel Weisberg[3] | Maria Canal[4] | Catherine Breen[5] | Michelle Hepburn[6] | Karen Tylee[1] | Frédéric M. Vaz[7] | Andy Vail[8] | Frits Wijburg[9] | Claire O'Leary[2] | Helen Parker[2] | J. Ed Wraith[1†] | Brian W. Bigger[2] | Simon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

Manchester University NHS Foundation Trust

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Amsterdam Medical Centre

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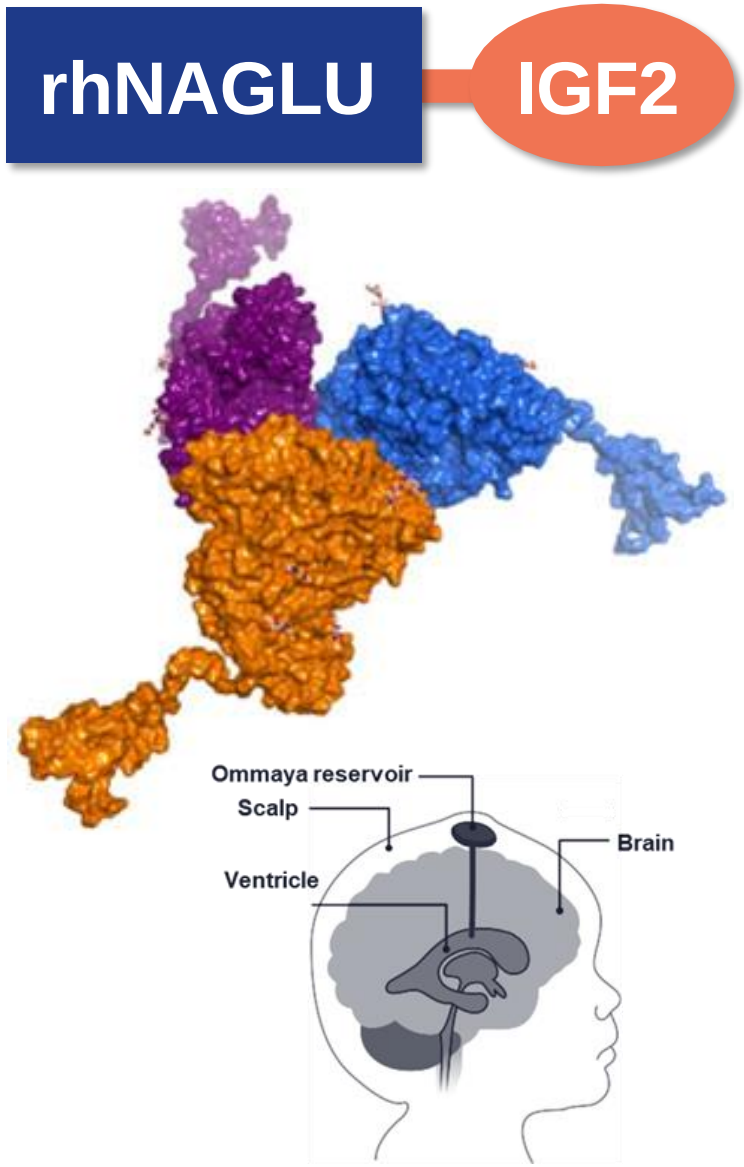
Tralesinidase Alfa protects Sanfilippo type B patients' cognitive functions by normalization heparan sulfate and preserving brain volumes

Eric Zanelli, PhD
Allievex Corporation

February 21, 2024

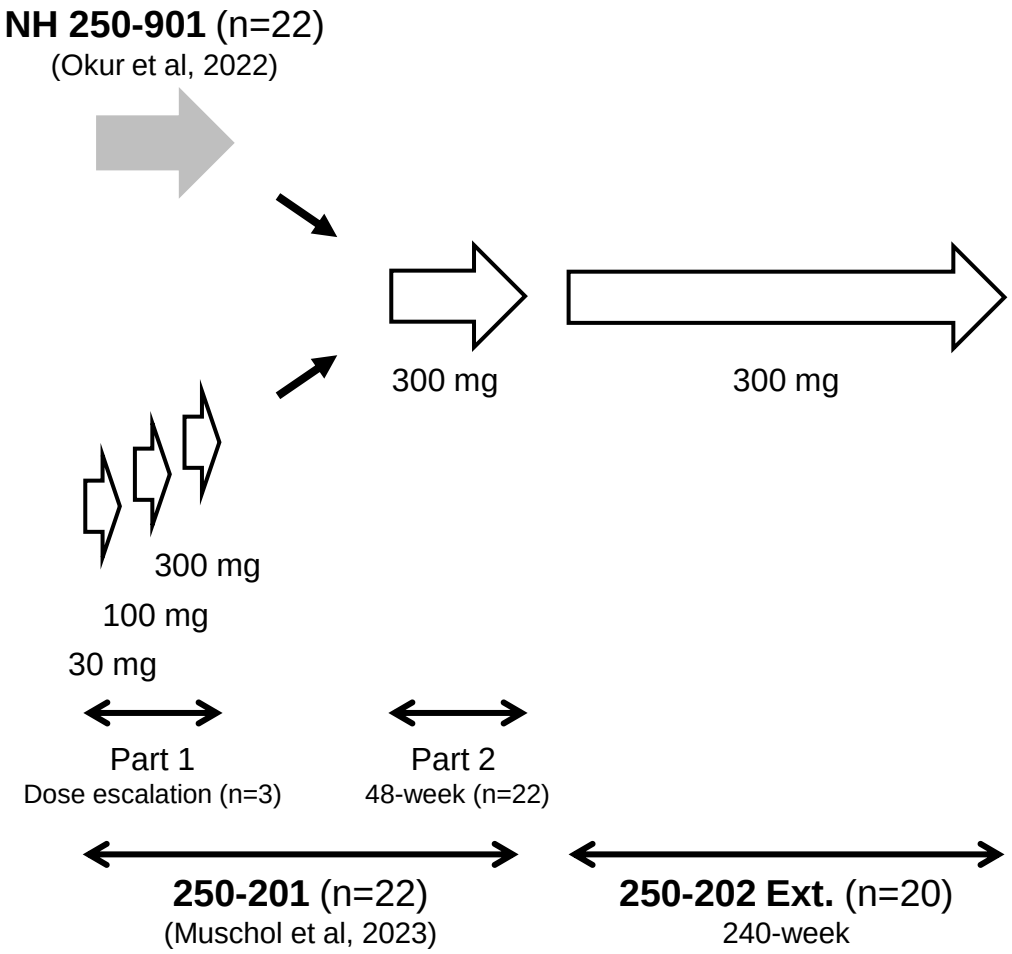
ALLIEVEX

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

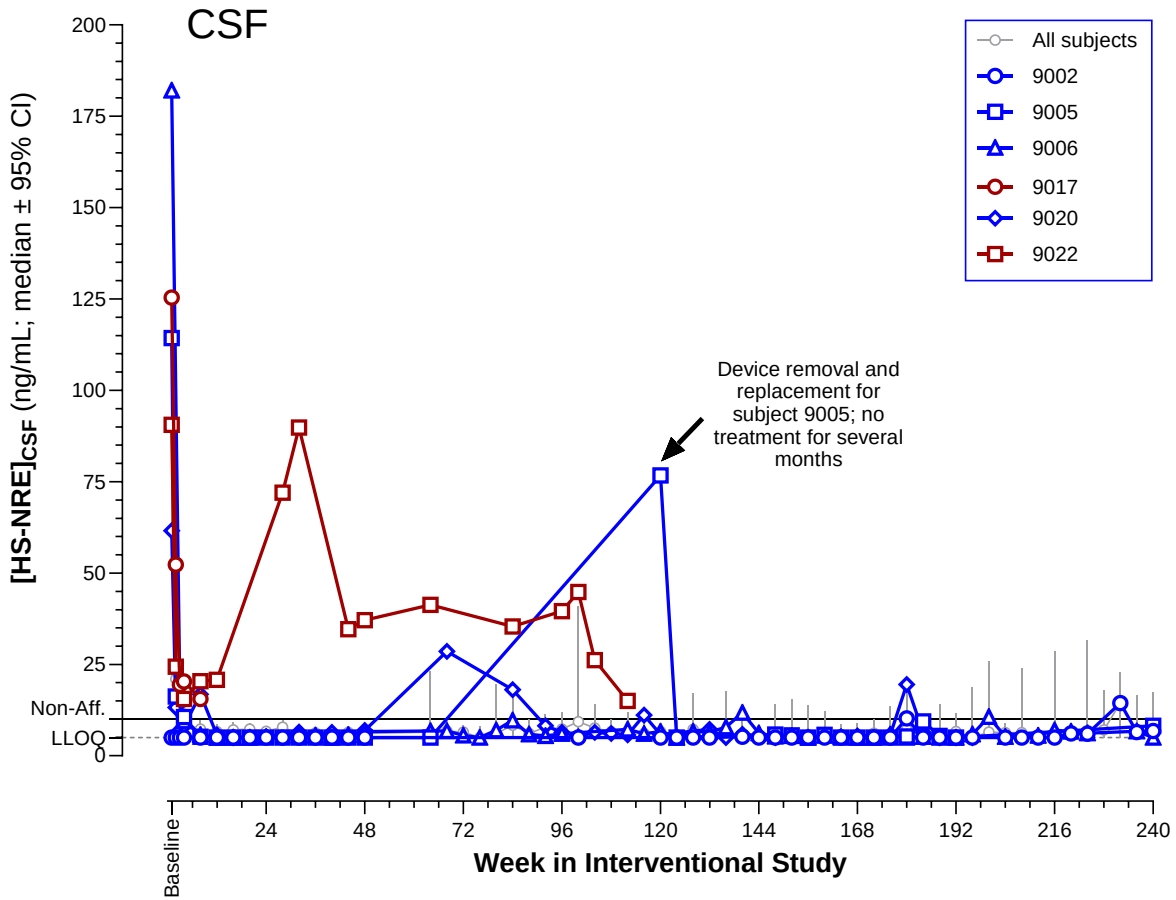
STUDY DESIGN AND ENDPOINTS

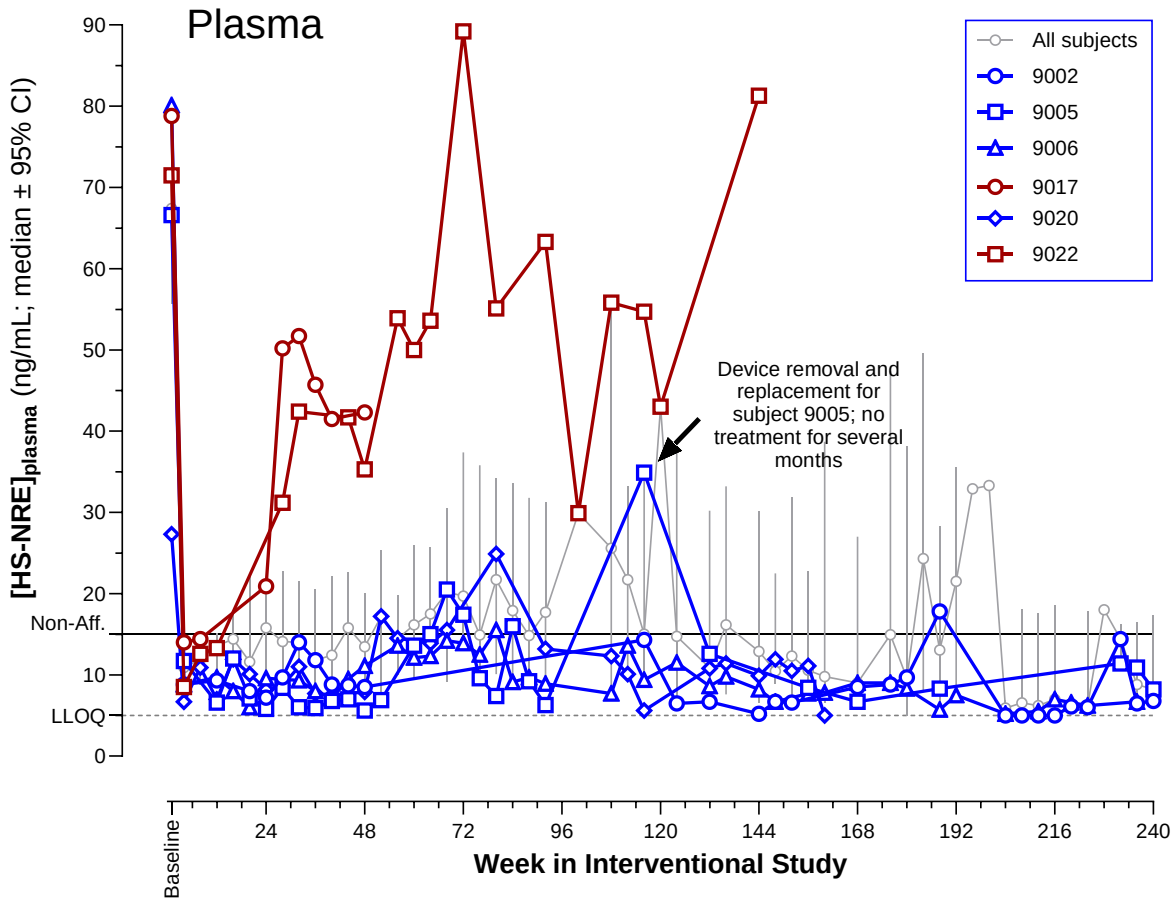


AX 250, 30, 100 or 300 mg weekly then bi-weekly

- **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

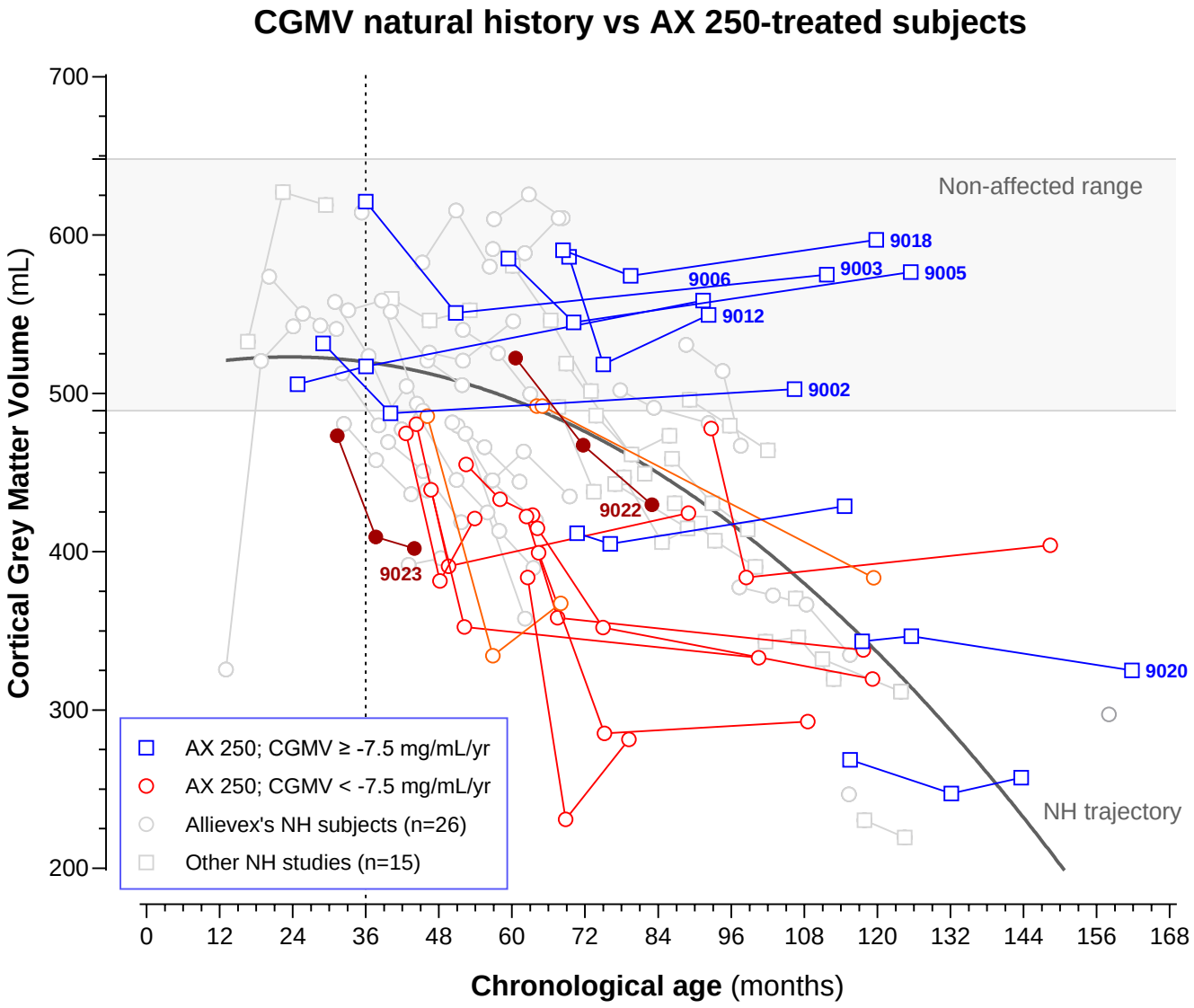
HS-NRE NORMALIZATION DEMONSTRATES TARGET ENGAGEMENT



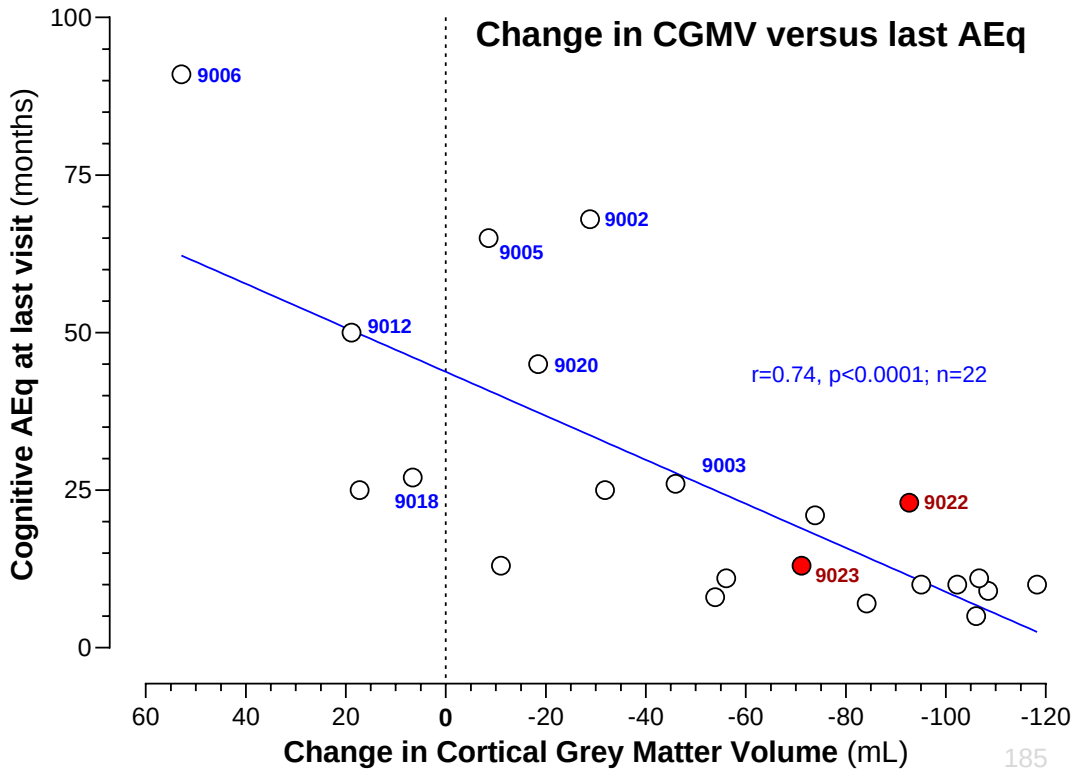


- Subjects with cognitive skills measured by KABC-II instrument, i.e., 9002, 9005, 9006 and 9020, sustain normal CSF and plasma HS-NRE levels. HS normalization is NOT sustained without treatment.
- Plasma HS-NRE has more dynamic range and can still be measured when subjects have treatment interruptions,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

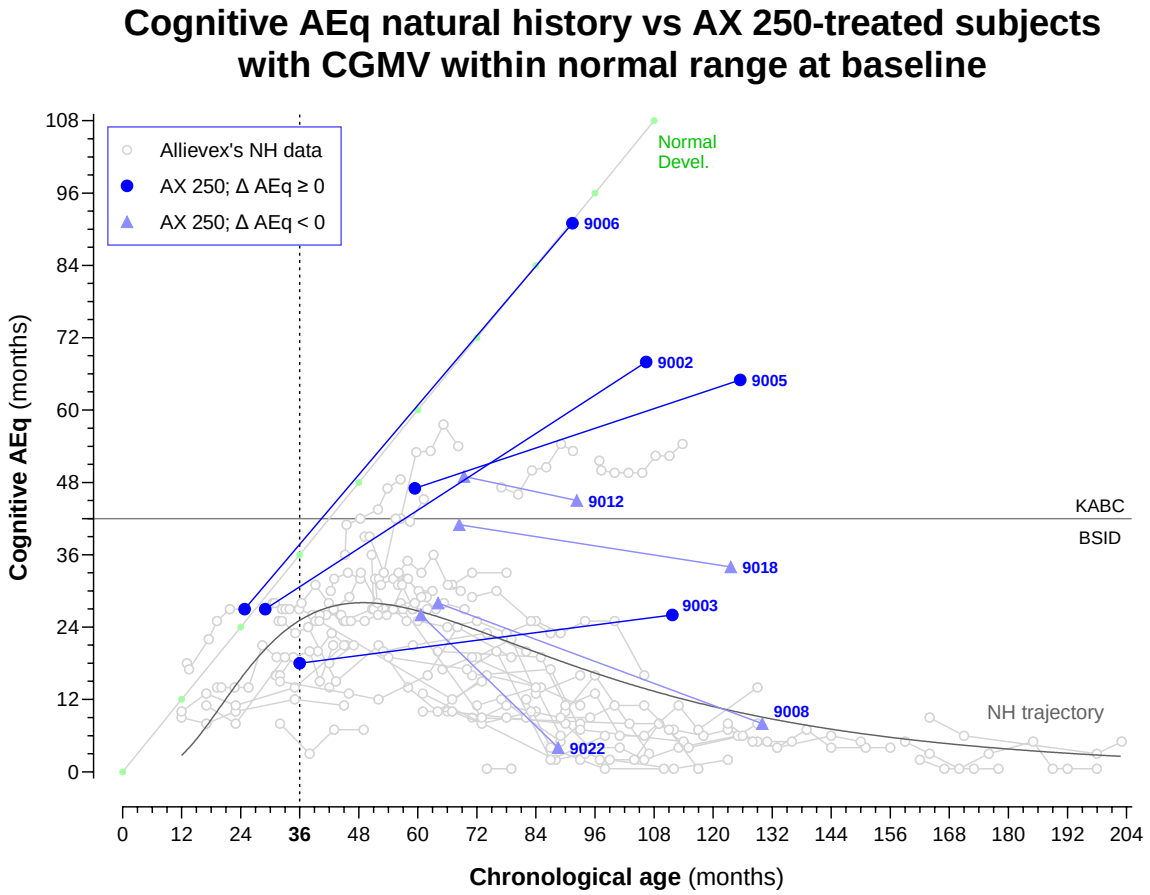
CGMV PRESERVATION CORRELATES WITH COGNITIVE OUTCOME

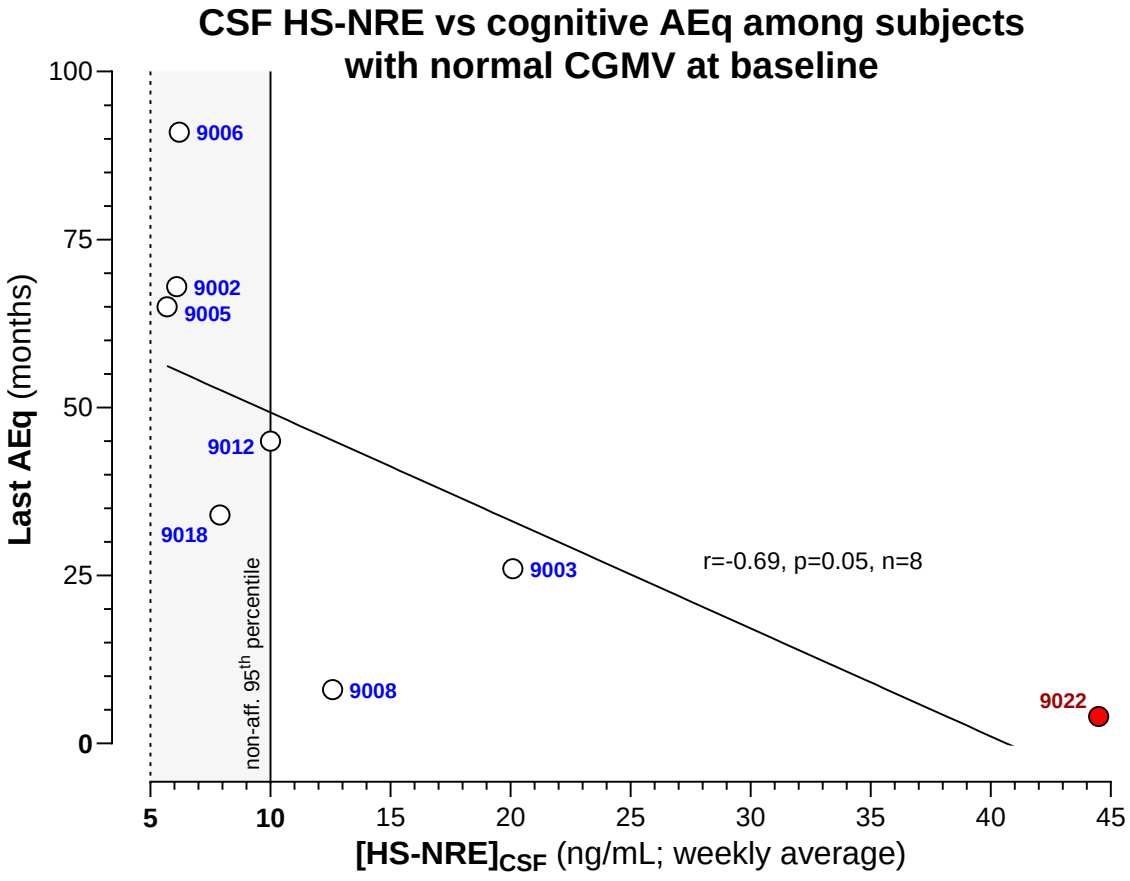


- 5 subjects have normal CGMV after > 3 years of treatment, sometimes after > 6 years.
- After an initial drop in CGMV due to acute elimination of HS from the brain tissue, tralesinidase alfa stops or limits brain atrophy in all subjects.
- There is a significant correlation between change in CGMV and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



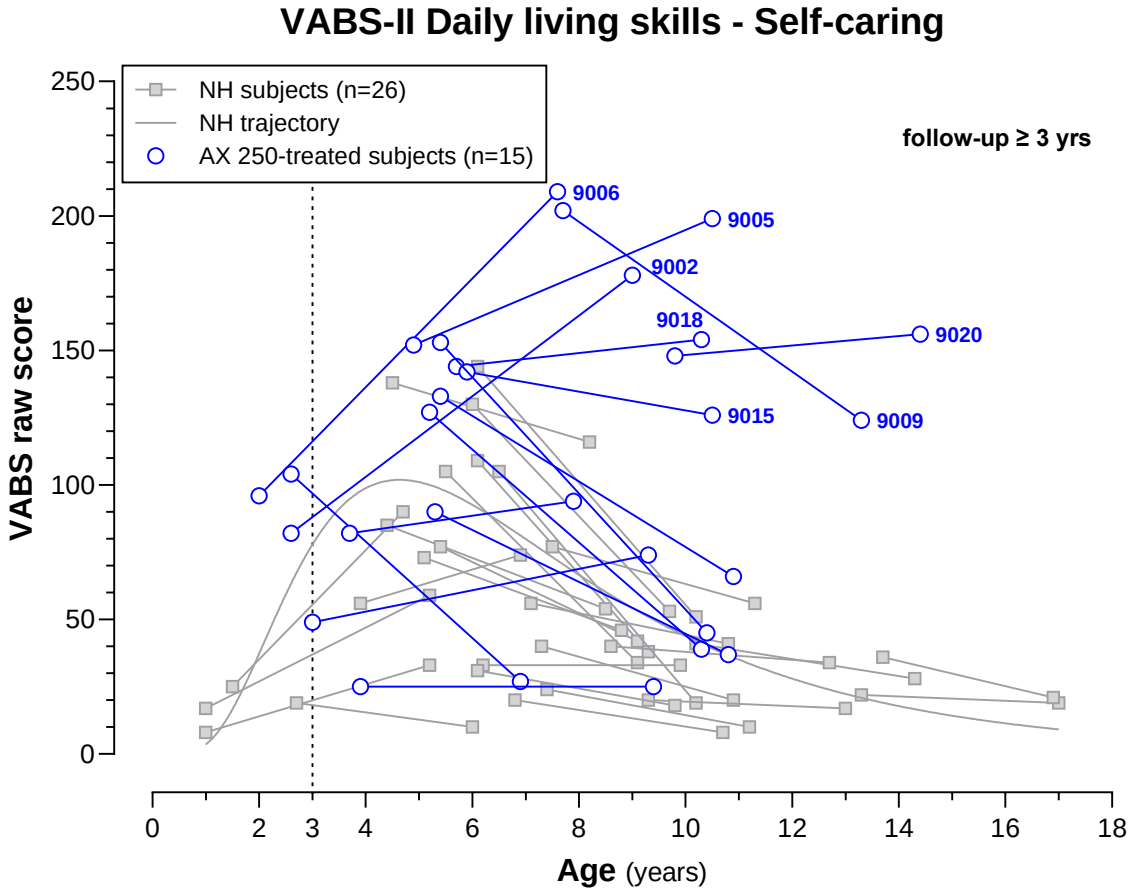
CSF HS-NRE NORMALIZATION ALONE PREDICTS GOOD COGNITION AMONG SUBJECTS WITH CGMV WITHIN NORMAL RANGE AT BASELINE

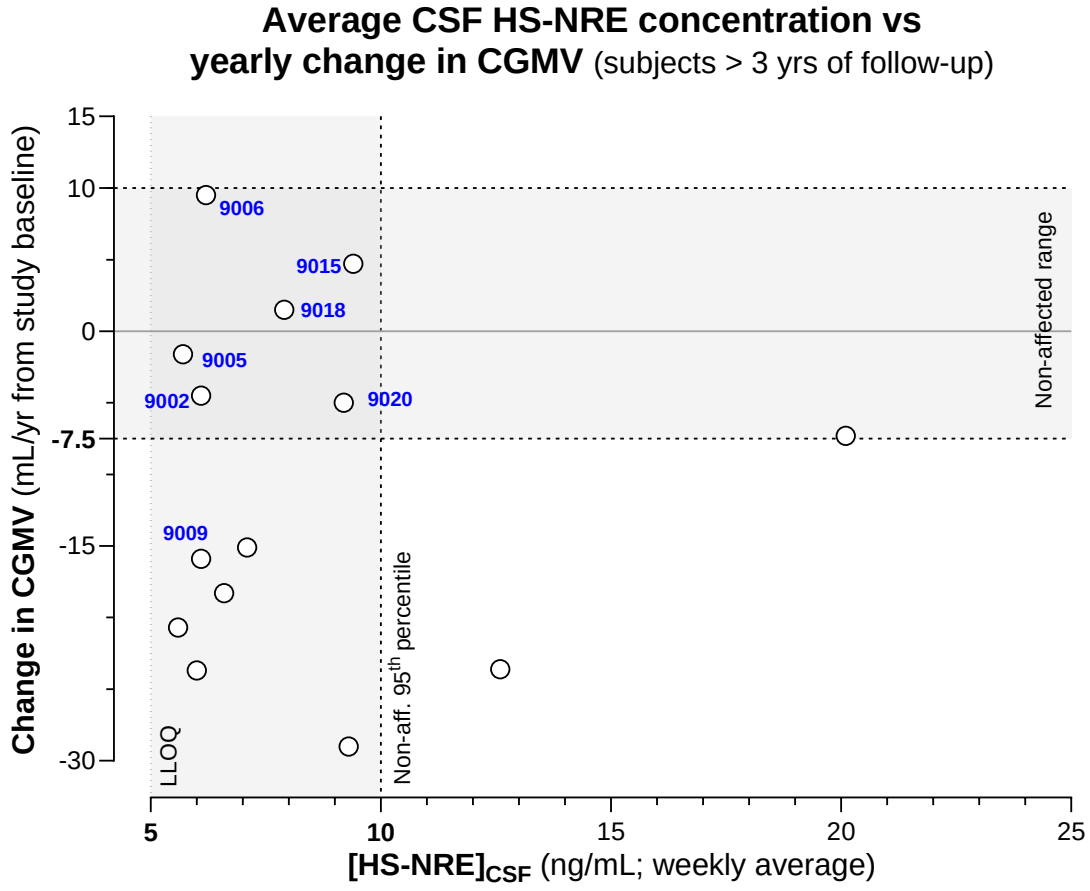




- 4 out of 8 subjects with normal CGMV at baseline have higher cognitive AEq after ≥ 6 years of treatment than at baseline; 3 of these 4 subjects were aged ≤ 3 at treatment initiation
- Subjects with highest AEq at last visit are subjects with sustained normalized HS-NRE in CSF

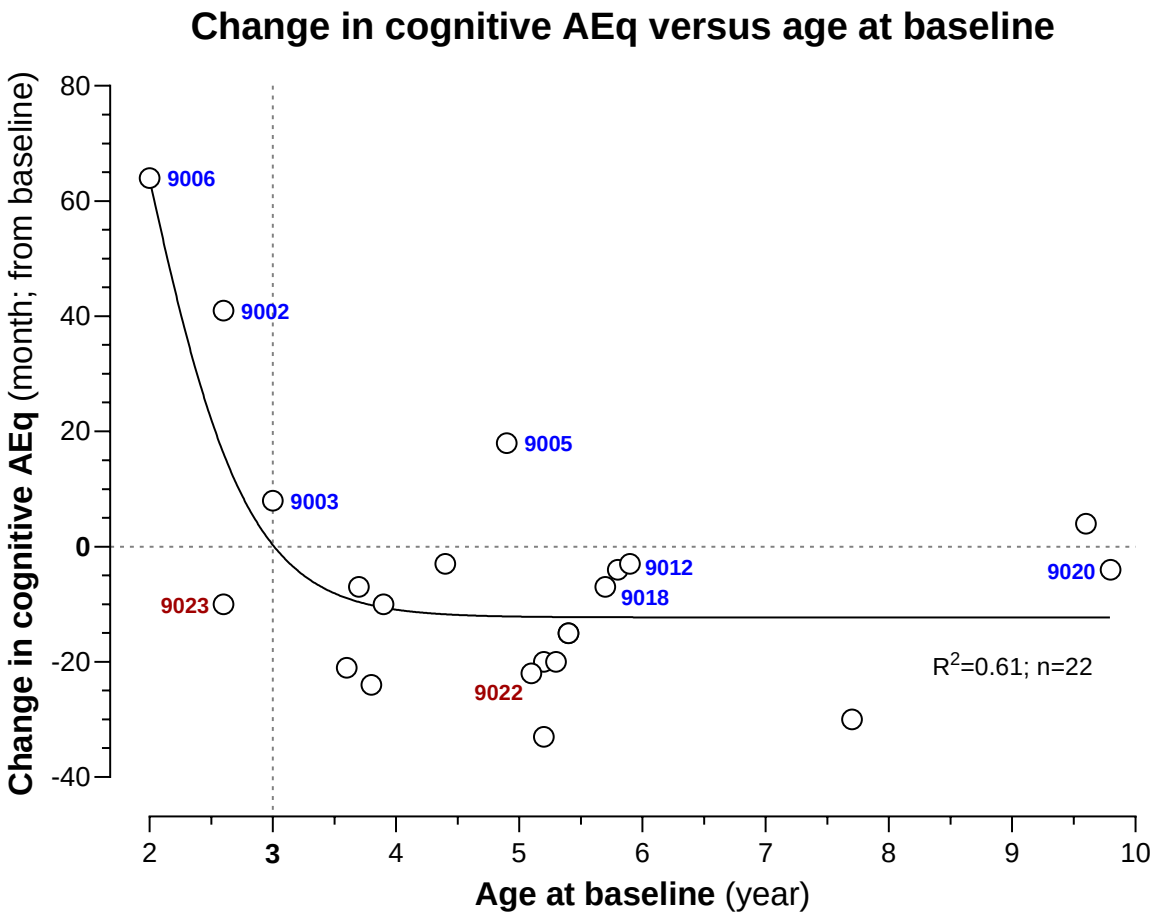
CSF HS-NRE NORMALIZATION COMBINED WITH LIMITED LOSS OF CGMV PREDICTS HIGH ADAPTIVE BEHAVIOR AMONG ALL SUBJECTS





- 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.

ACKNOWLEDGEMENTS

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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- Igor Nestrasil, University of Minnesota, Minneapolis, MN, USA
- 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

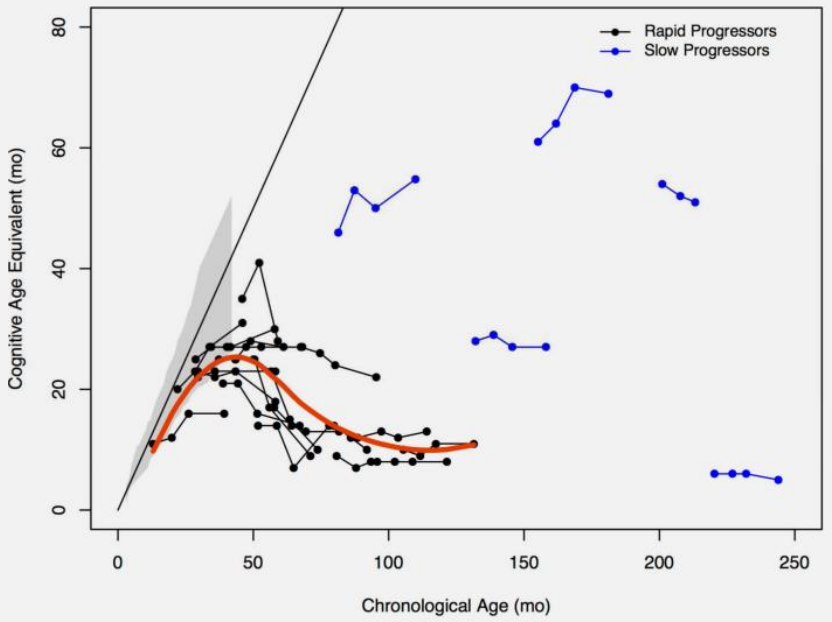
Ultragenyx Pharmaceutical Inc., Novato, CA

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

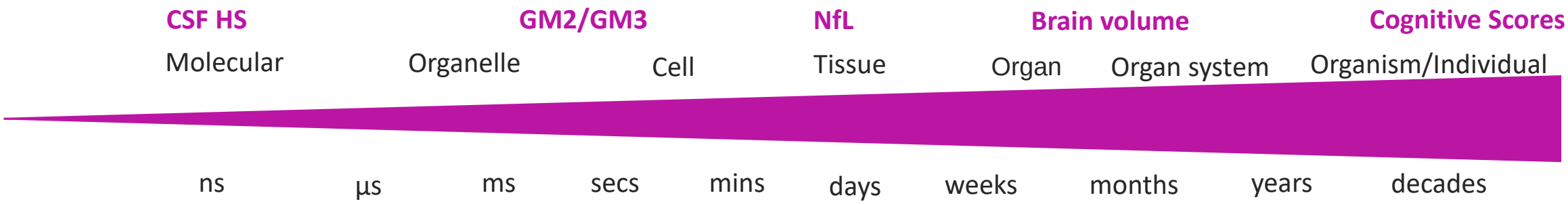
Reduction suggests recovery of organelle/cell function

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.

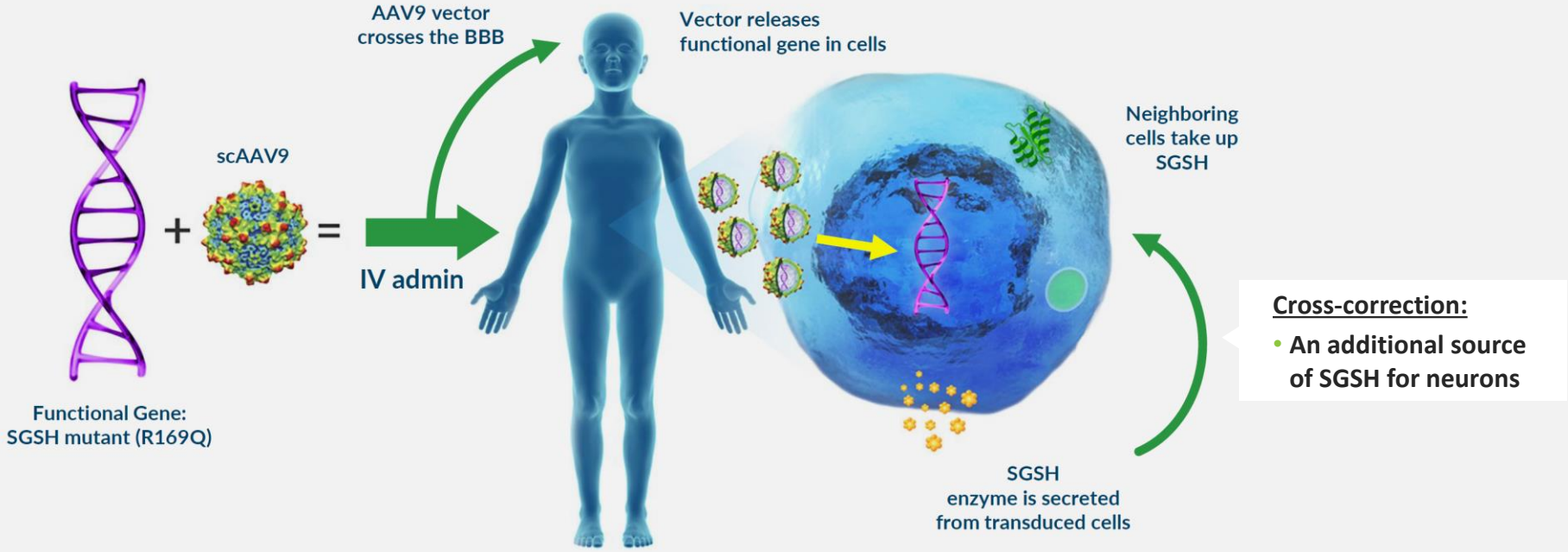
UX111 is designed to target underlying SGSH Enzyme Deficiency:

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

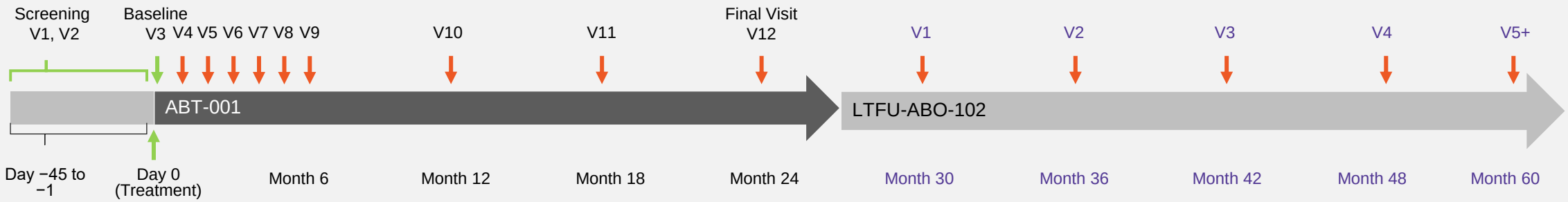
UX111 IV gene therapy results in reduction of the substrate, HS, based on both preclinical and clinical data

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

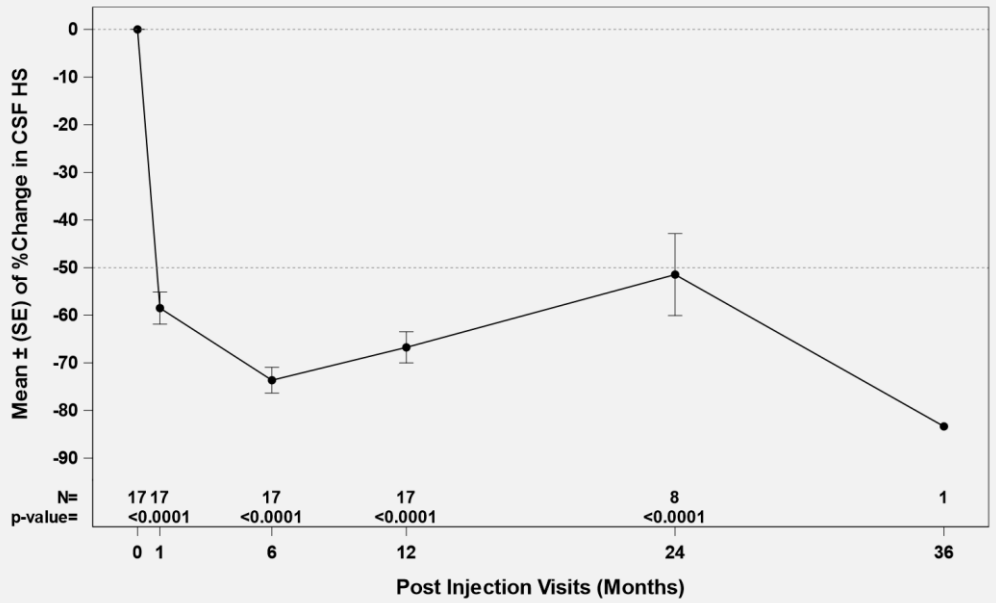
- The ABT-001 study designed as a 24-month Phase 1/2/3 study
  - Excluded attenuated genotypes
- Cohort 1 (0.5 x 1013 vg/kg): n = 3
- Cohort 2 (1 x 1013 vg/kg): n = 3
- **Cohort 3 (3 x 1013 vg/kg):** n = 22
- **Target population (mITT) defined as ≤2 years old or >2 years and cognitive DQ ≥ 60, n = 17**

As of 16 Aug 23 Data Snapshot:

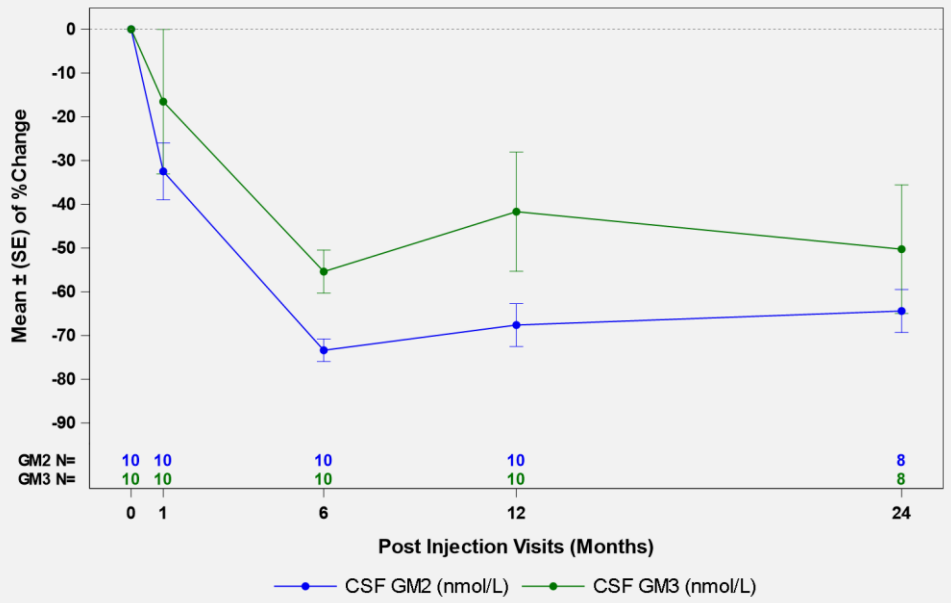
- 28 patients have been treated with UX111 in ABT-001
- 19 patients rolled into long term follow-up study (LTFU-ABO—102)
- 17 patients are in the mITT; at time of snapshot
  - 15 of the 17 mITT patients reached at least 30 months of age
  - 6 of 17 patients reached ≥ 60 months of age
  - Mean (SD) duration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

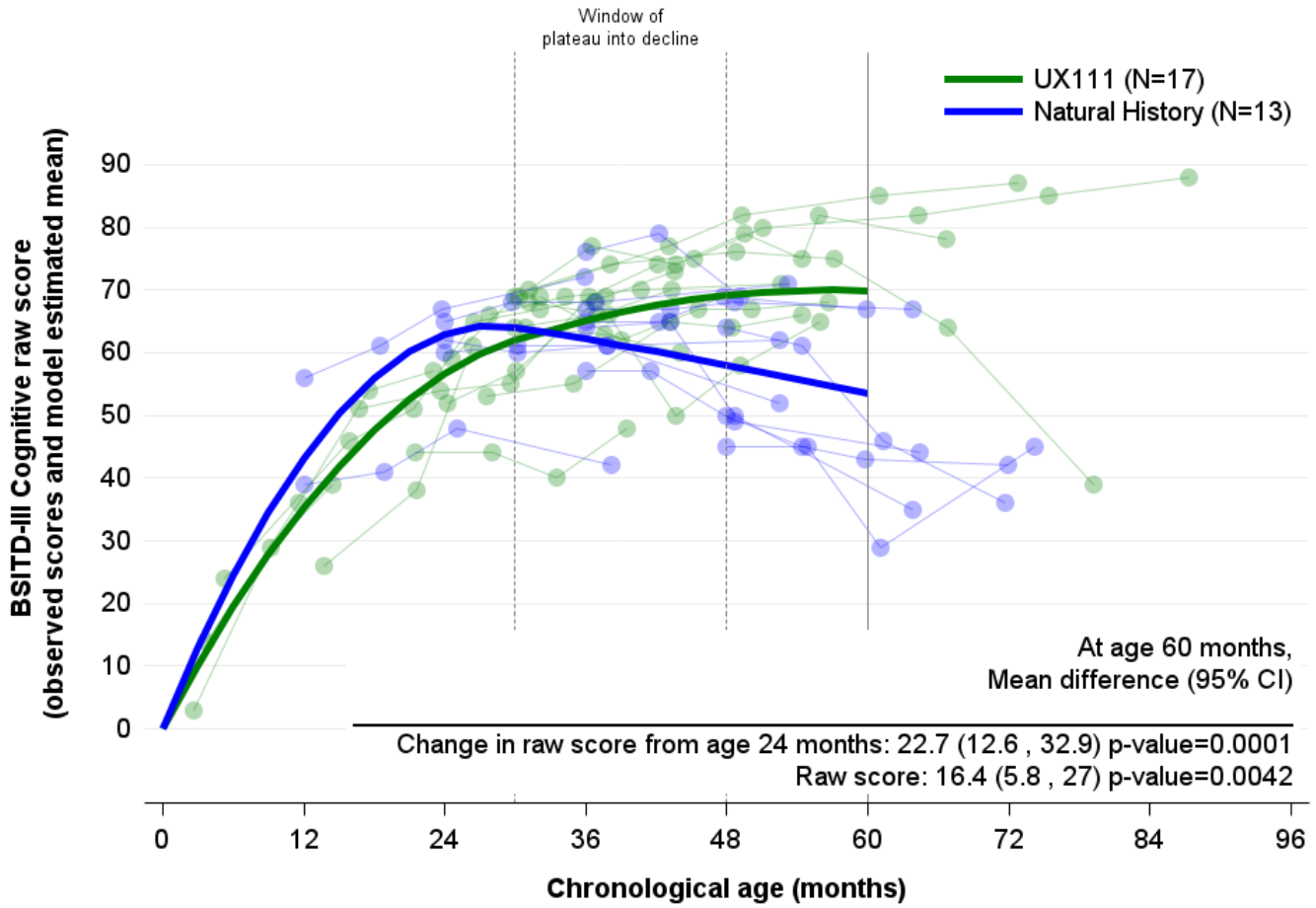
- Patient specific metric which accounts for fluctuations using all available CSF HS levels post-treatment
- May then be utilized to assess the relationship with cognitive outcomes
- **Mean reduction in CSF HS exposure was -63.3% (95% CI, -69.7%, -56.9%) in mITT**; over a median follow-up ~2 years

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)

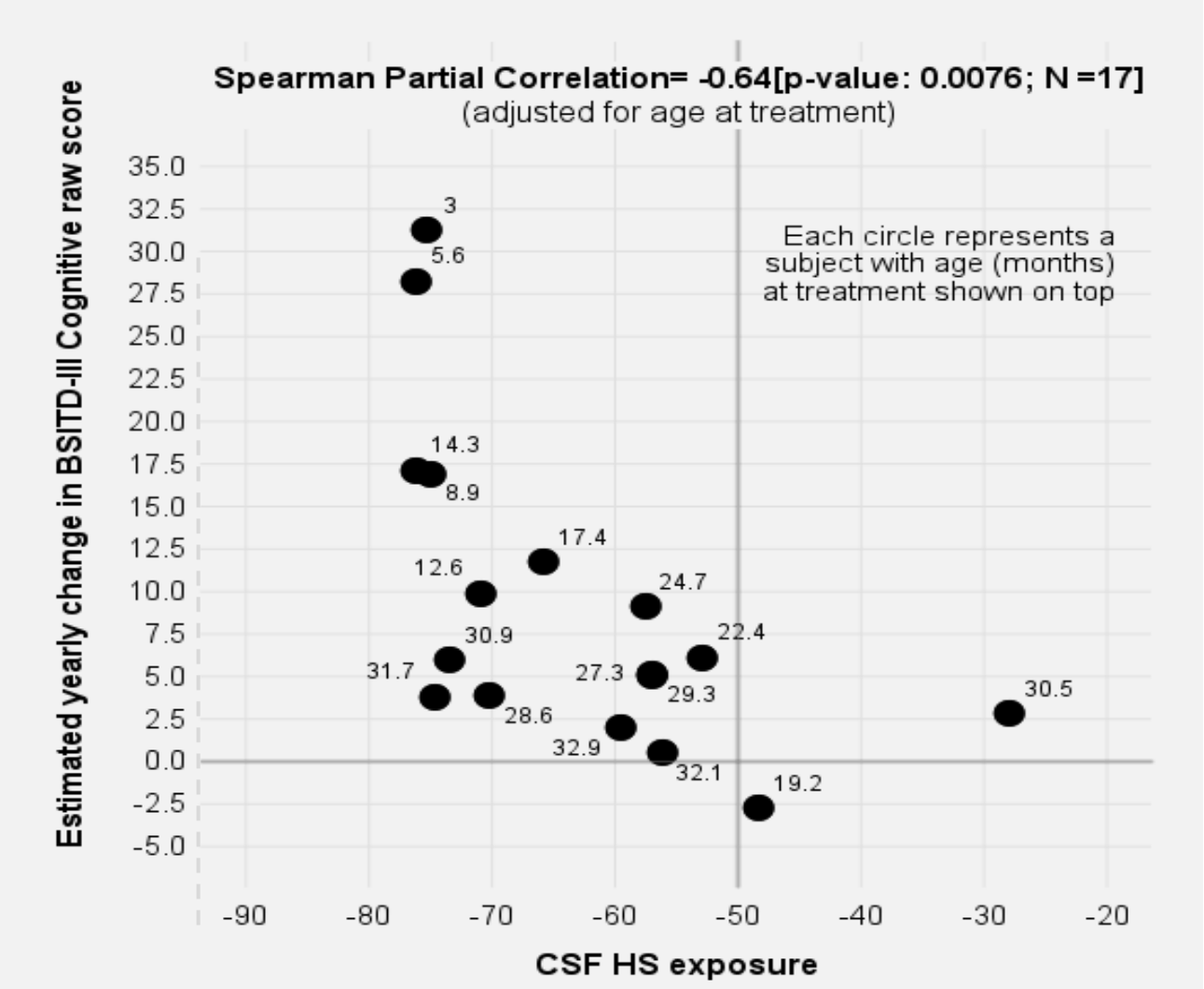
Natural history data reported in Shapiro EG, Nestrasil I, Delaney KA, et al. (2016) A Prospective Natural History Study of Mucopolysaccharidosis Type IIIA. J Pediatr 170: 278-287.e271-274 for which Sponsor has access to item level data.

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)

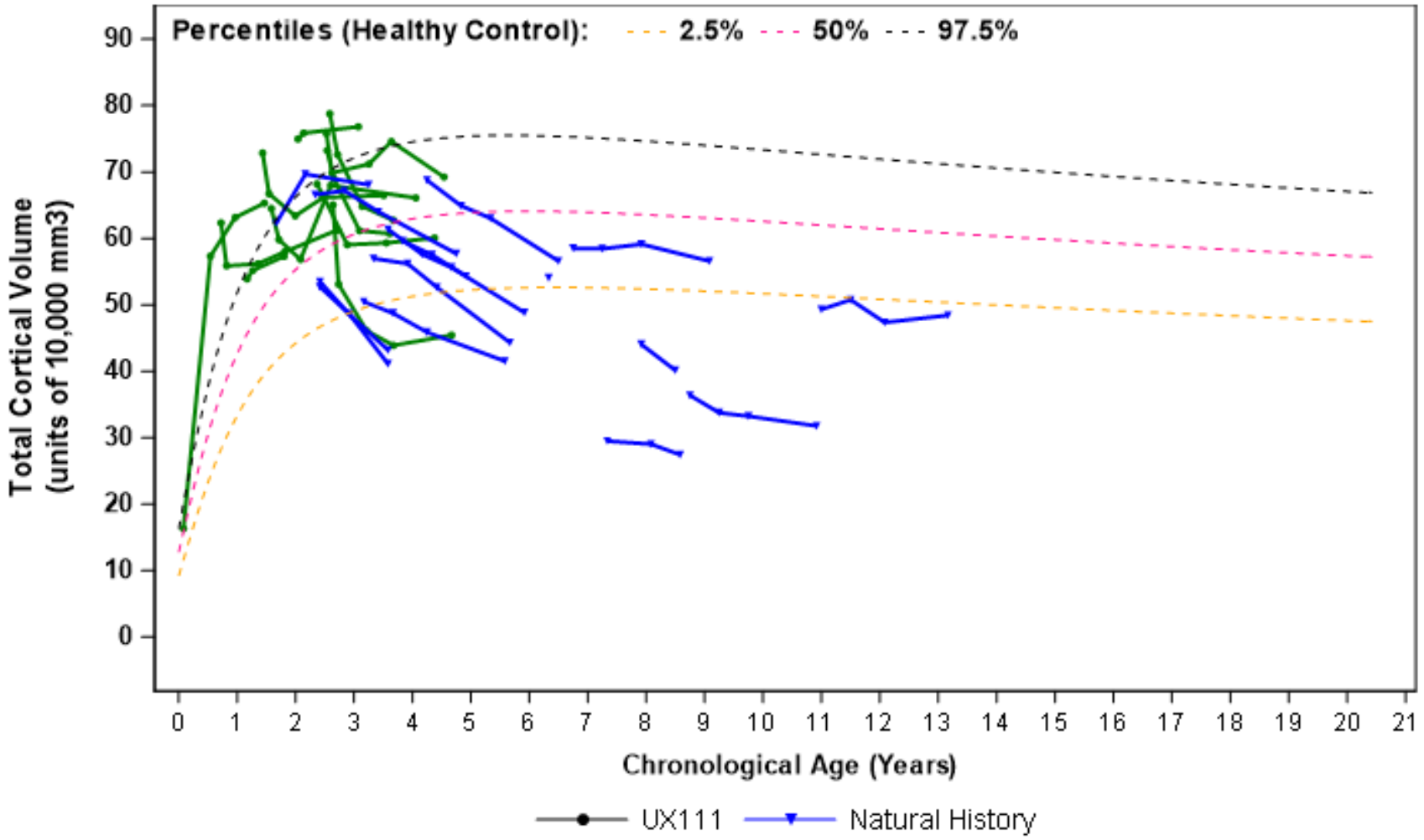


15 of 17 patients

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



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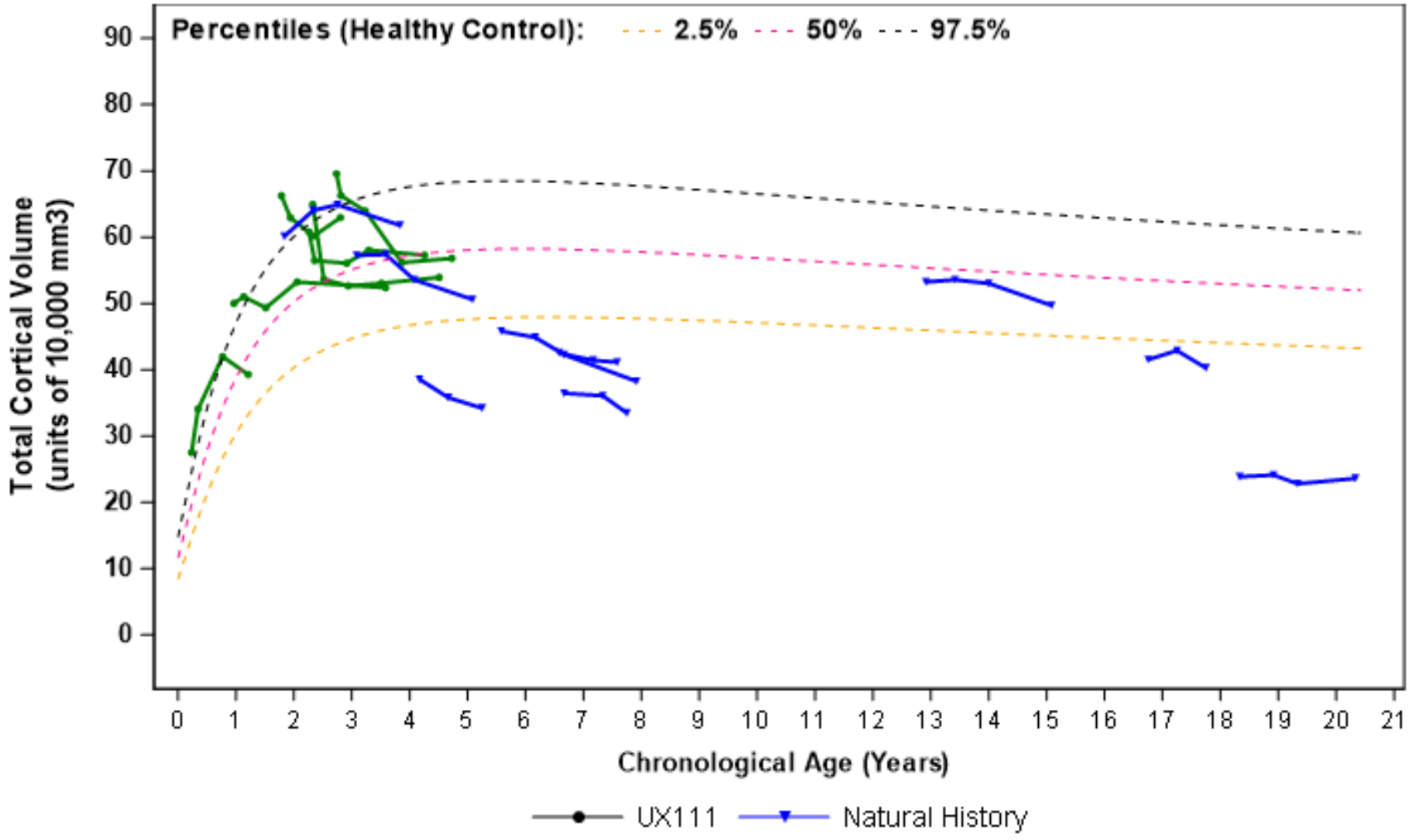
Late Biomarker: Total Cortical Brain Volumes normalize and are stable in UX111 Treated Compared To Untreated Female Patients with MPS IIIA



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

REAGAN-UDALL FOUNDATION FOR THE FDA

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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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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