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

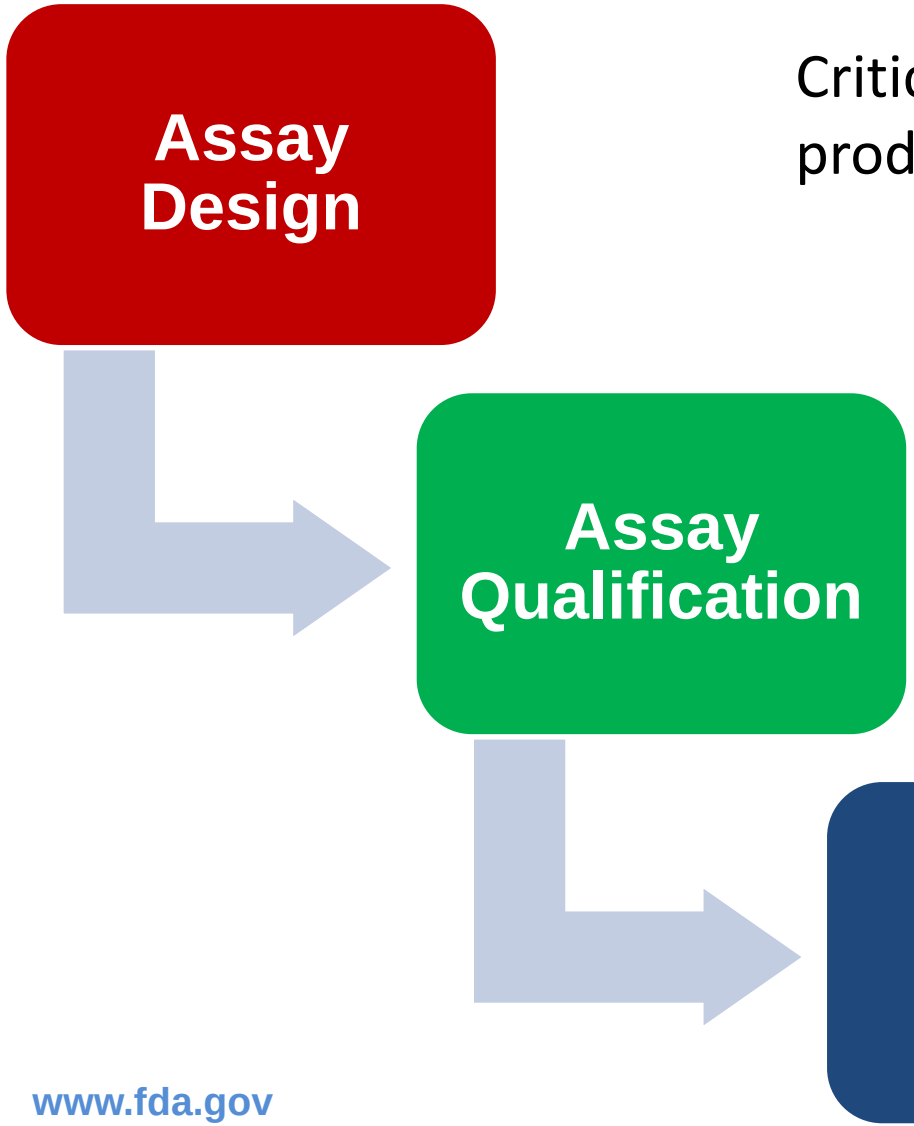
- Disease state is associated with protein levels above or below a certain range
- Certain protein levels are associated with disease absence or minimal disease

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

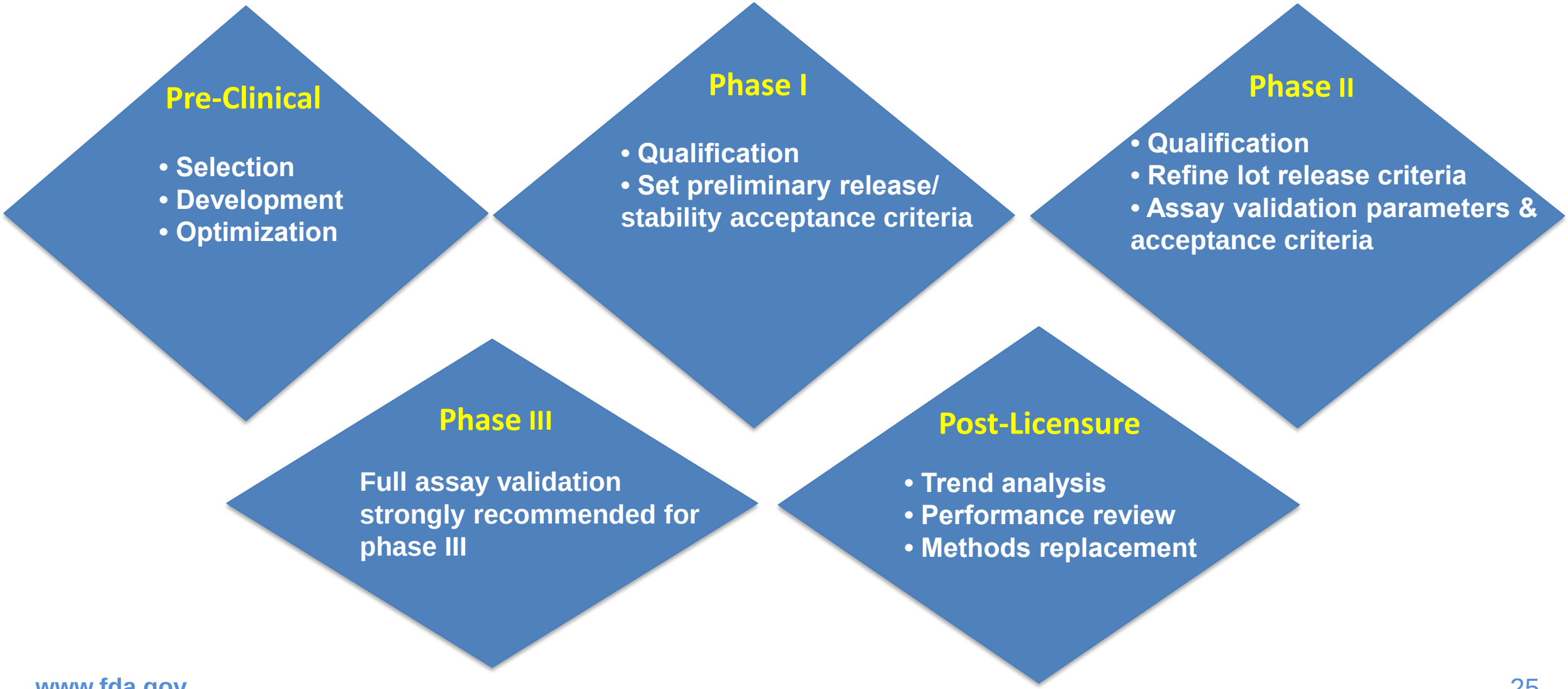
- **Limit of Detection (LOD):** is the ability of the assay to distinguish signal from background

- **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

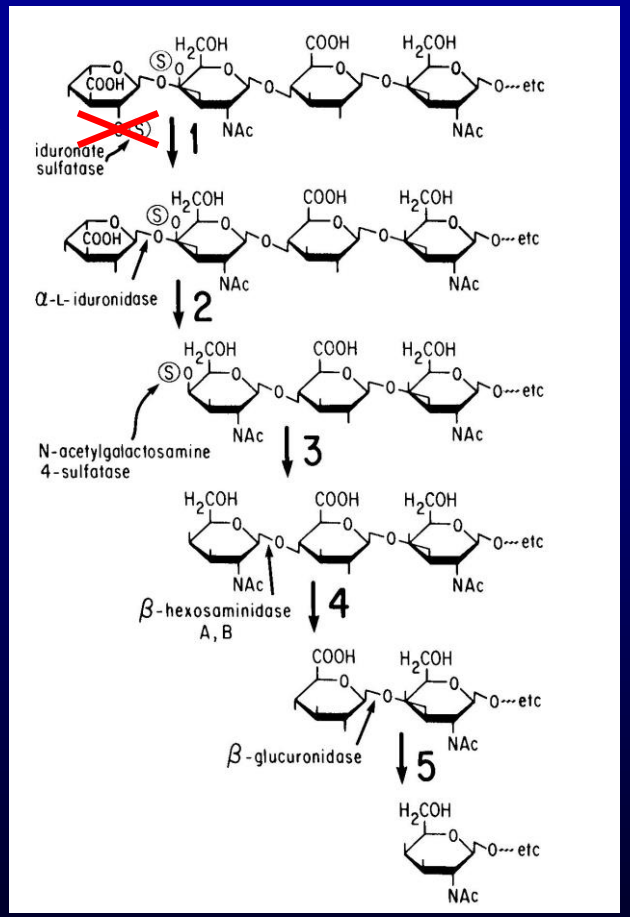
Muenzer J. Rheumatology (Oxford). 2011; 50 Suppl 5:v4-12 *Verheyen S et al. J Med Genet (2022); 59:957-964

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

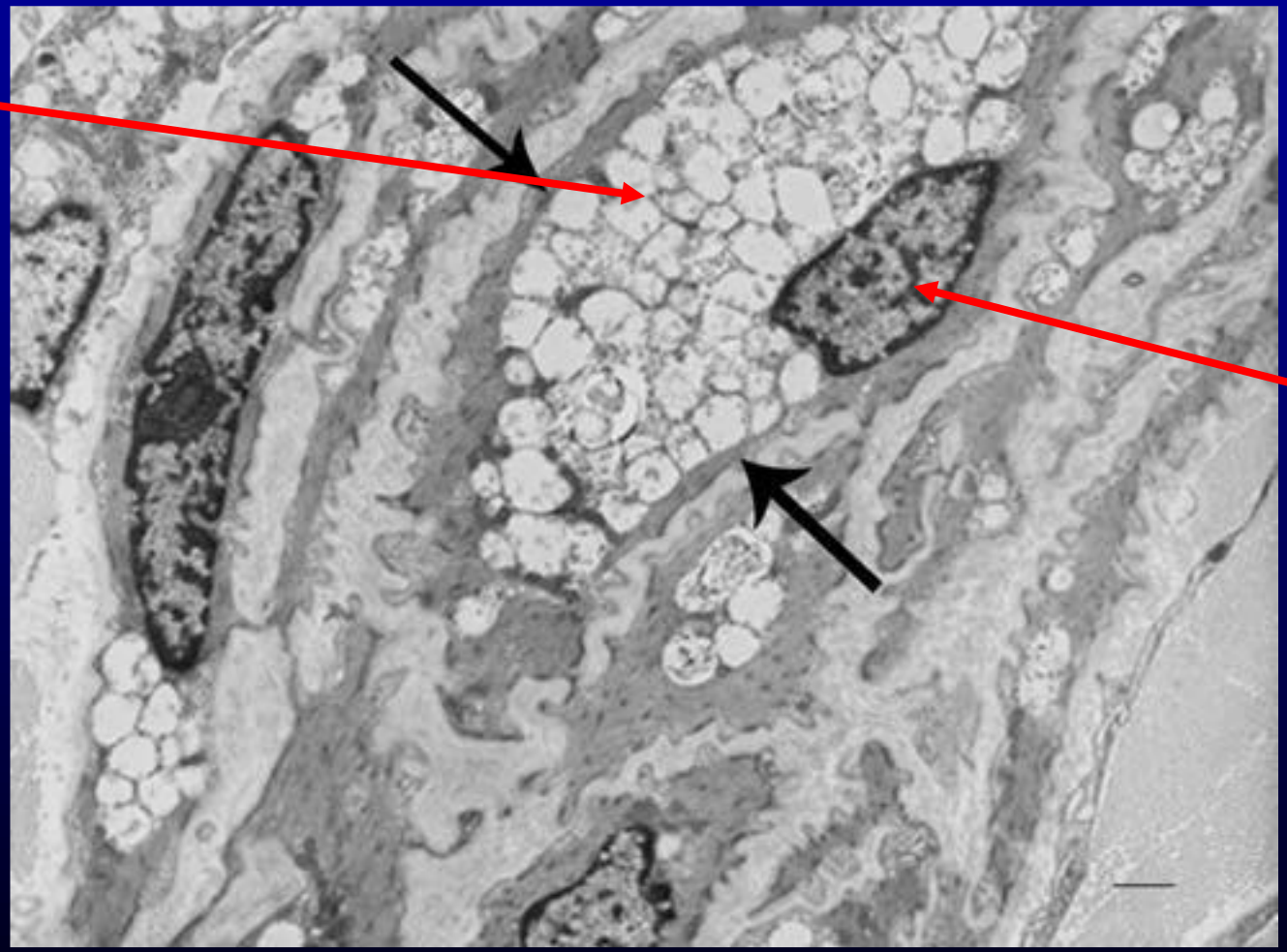
Muenzer J et al. Pediatrics (2009) 124:1228-39

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.

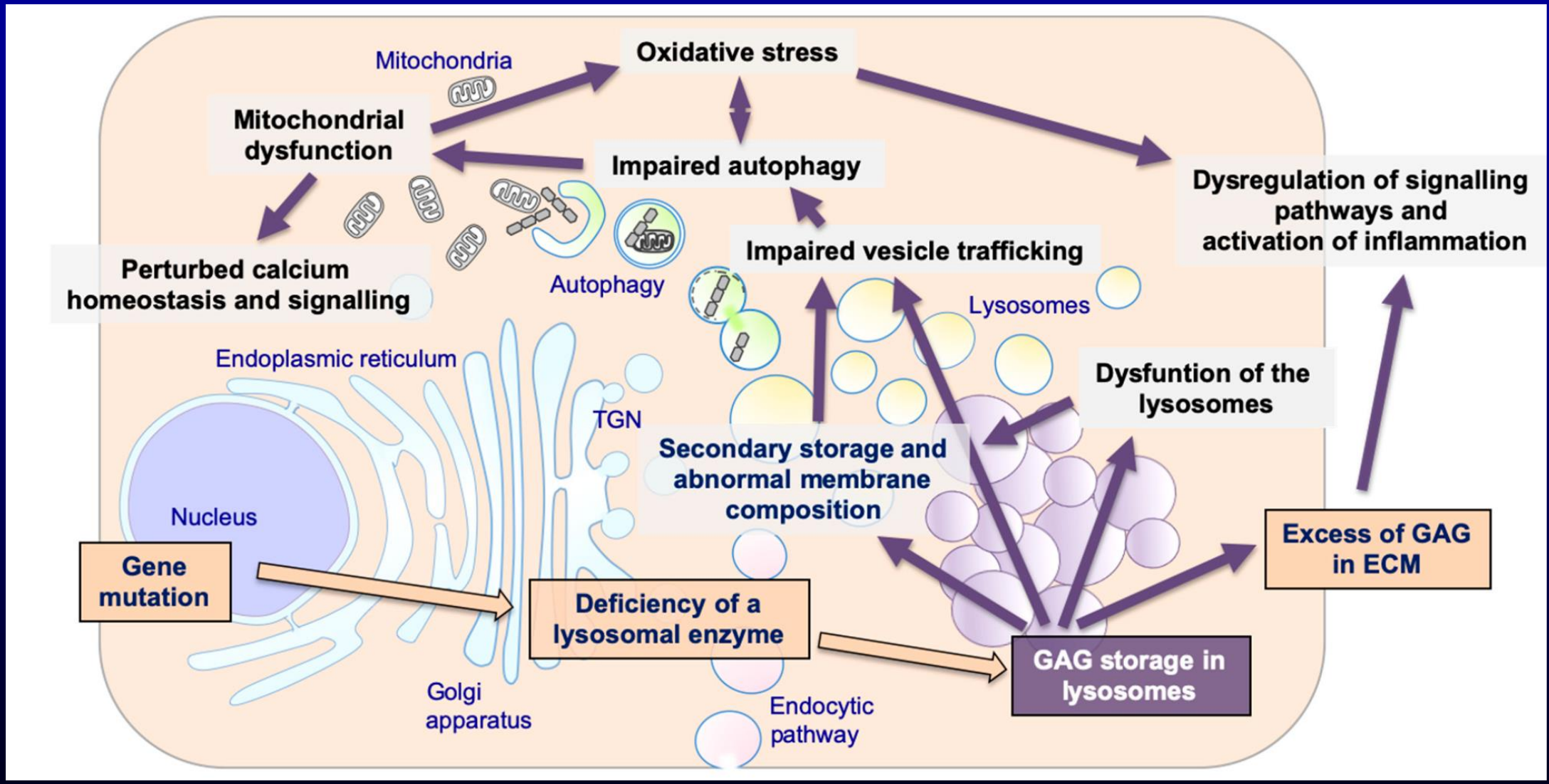
Presenter's own opinion

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)

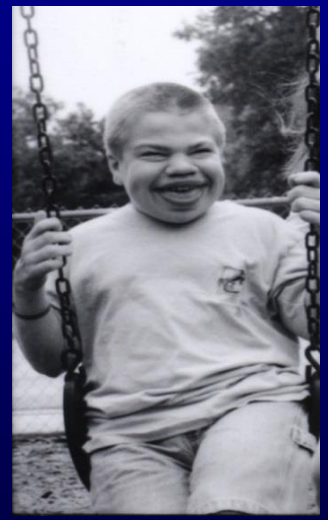
- MPS II is a rare X-linked recessive disorder (est. incidence 1:100,000)[1] due to the deficiency of lysosomal enzyme iduronate-2-sulfatase.
- MPS II is an ultra-orphan disorder with an estimated US prevalence of 450 to 500 patients.[2]
- About 2/3 of MPS II patients develop cognitive impairment with 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

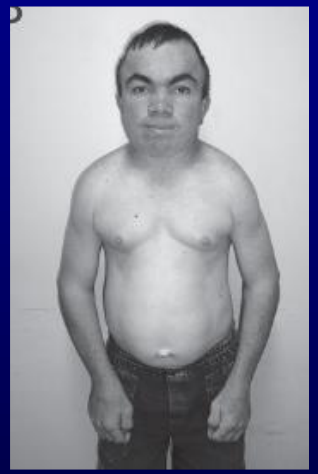
Image used with permission

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.

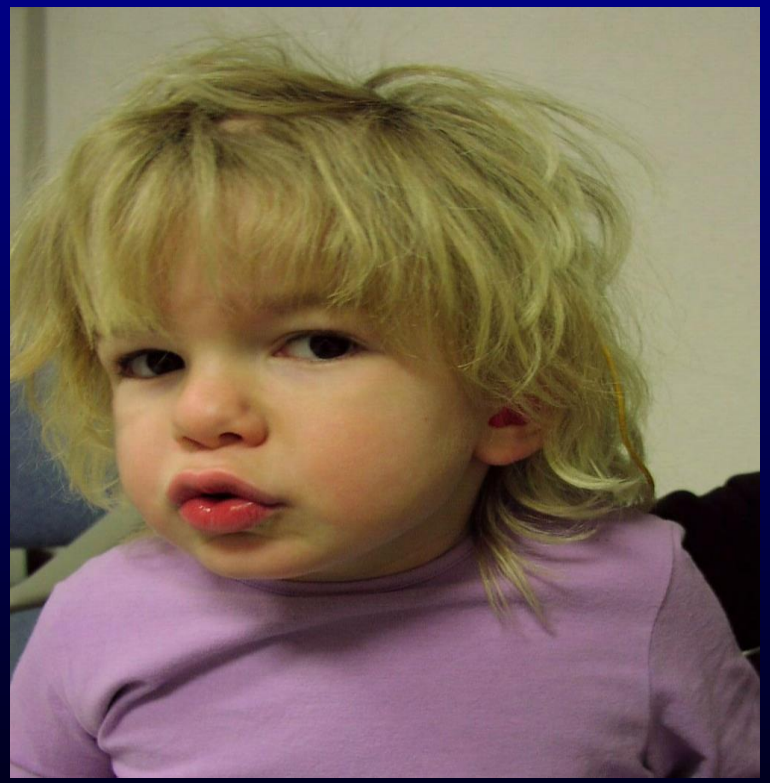
[1]Nijmeijer SCM et al. Orphanet J Rare D (2019) 14:249

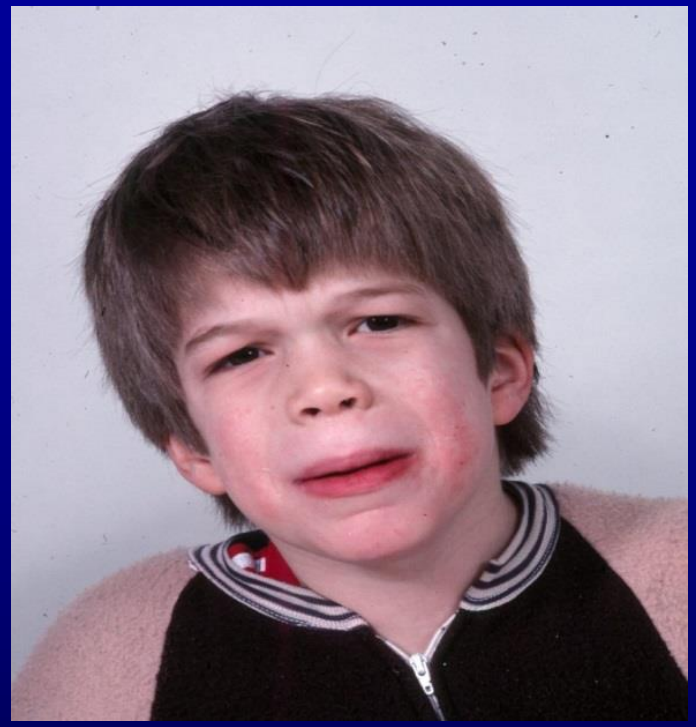
Sanfilippo Syndrome (MPS III)

- **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

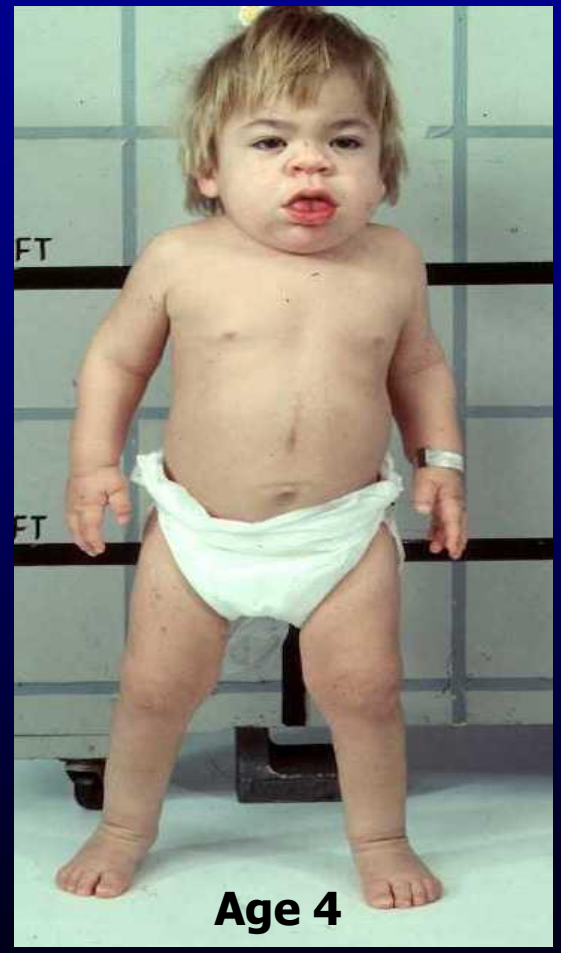
MPS III





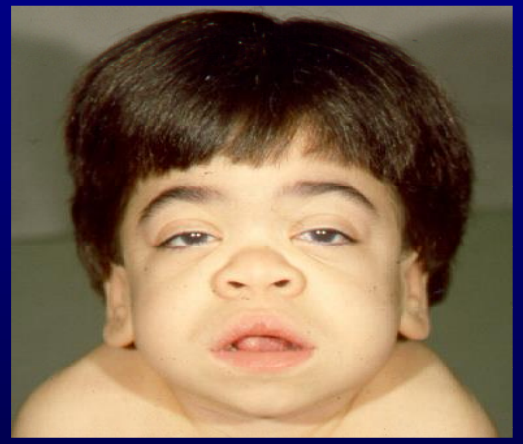
Mucopolysaccharidosis I (MPS I)

- 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



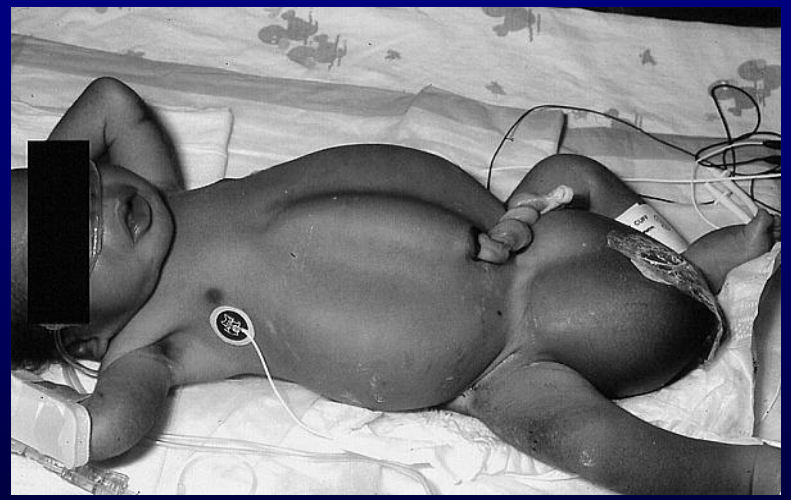
Hurler-Scheie

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)

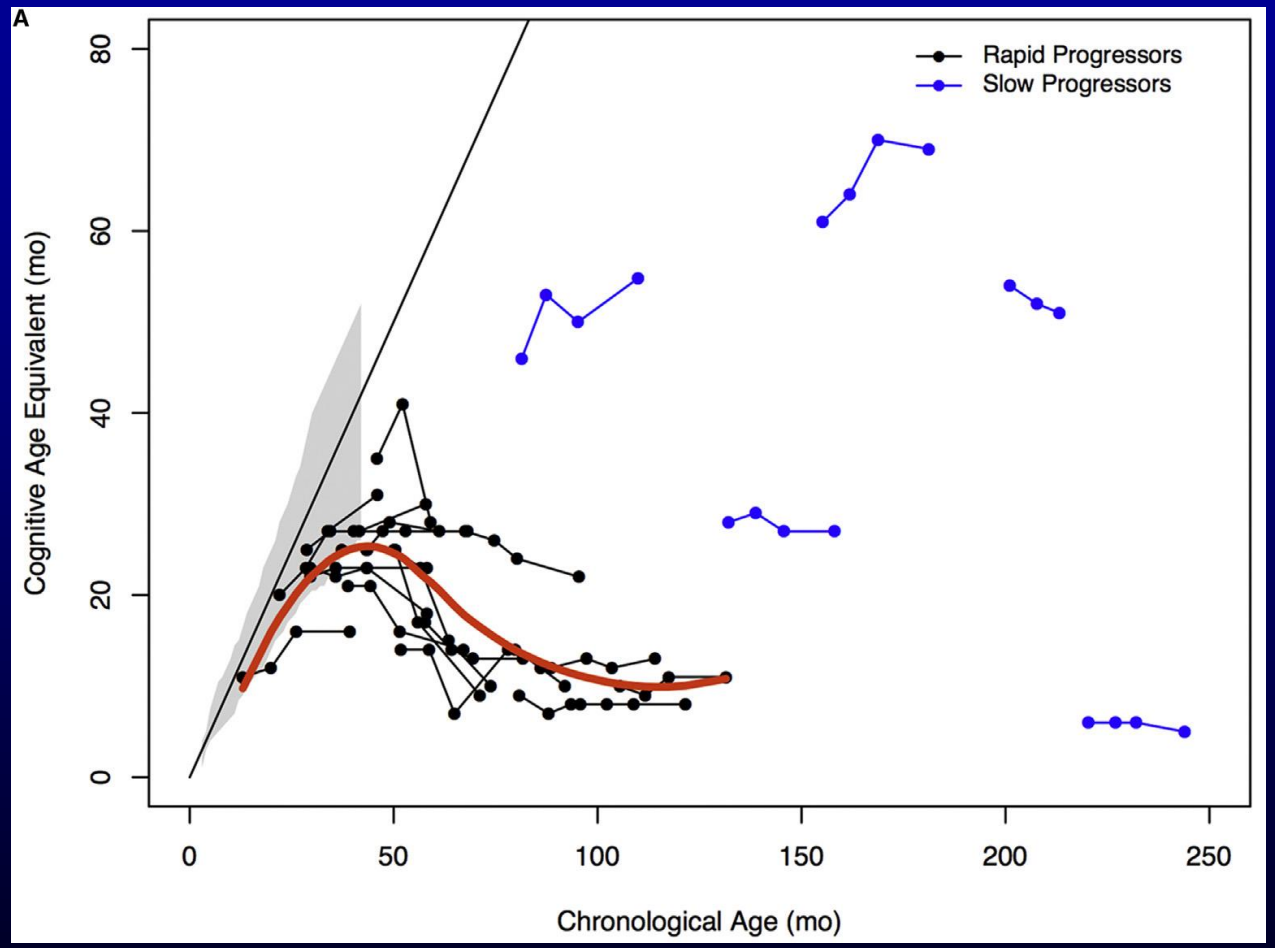
Presenter's own opinion

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



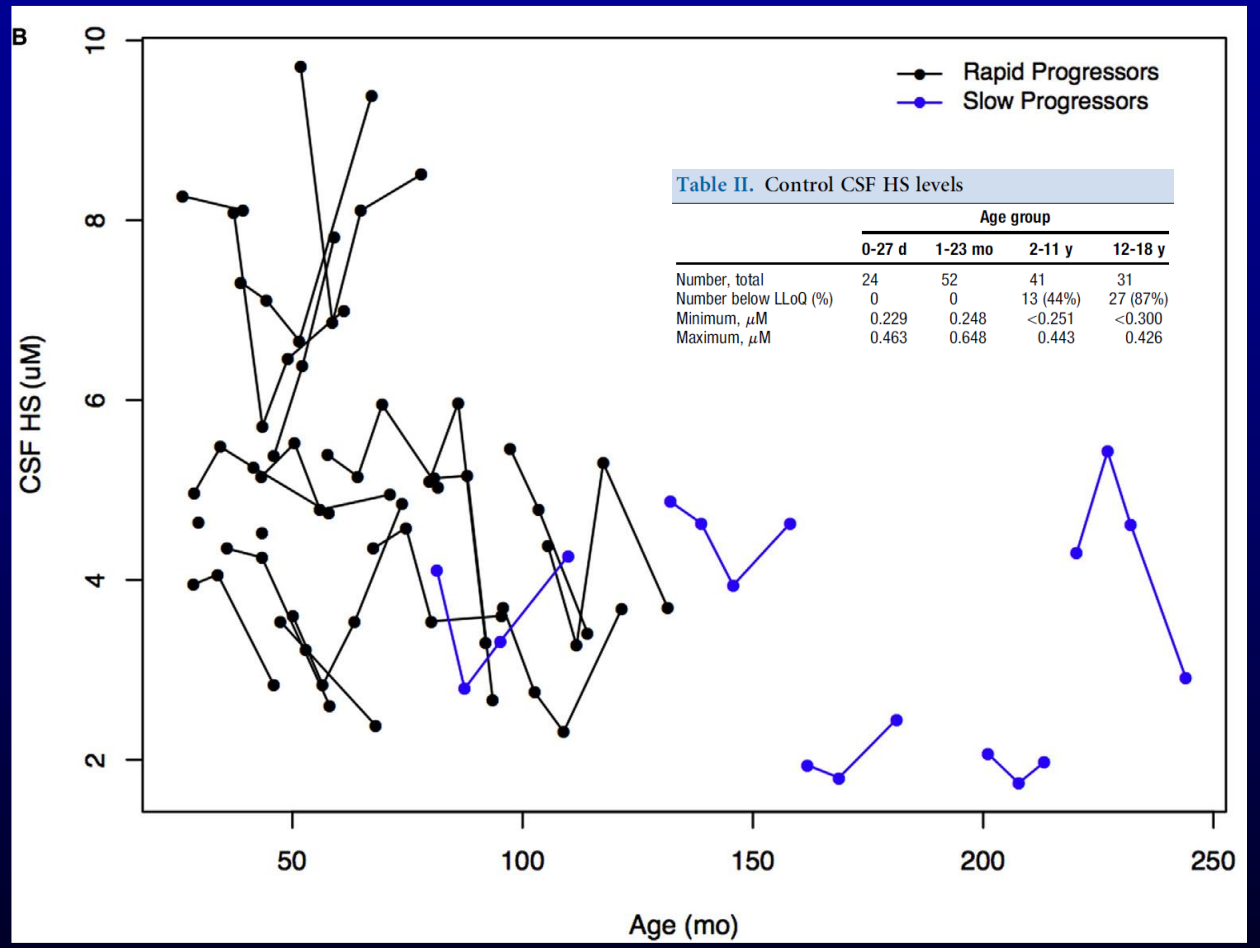


Table II. Control CSF HS levels

	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

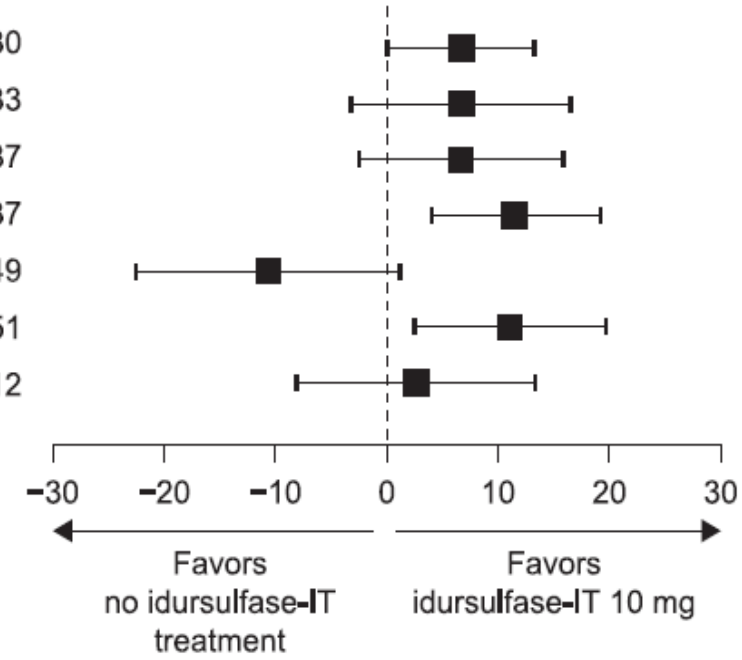
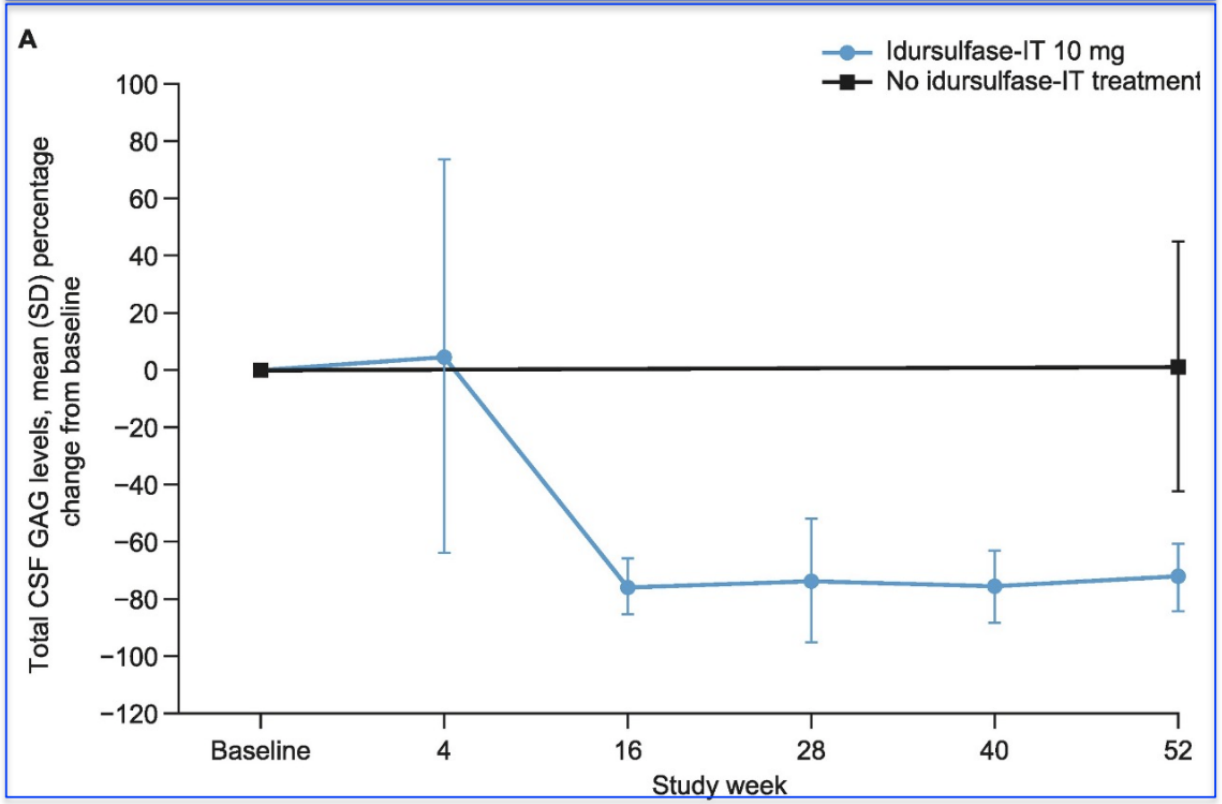


Fig. 4. Rate of change (weighted slope) in DAS-II GCA scores by prespecified subgroup. CI, confidence interval; DAS-II, Differential Ability Scales-II; GCA, General Conceptual Ability; IT, intrathecal; ITT, intention-to-treat; SE, standard error.

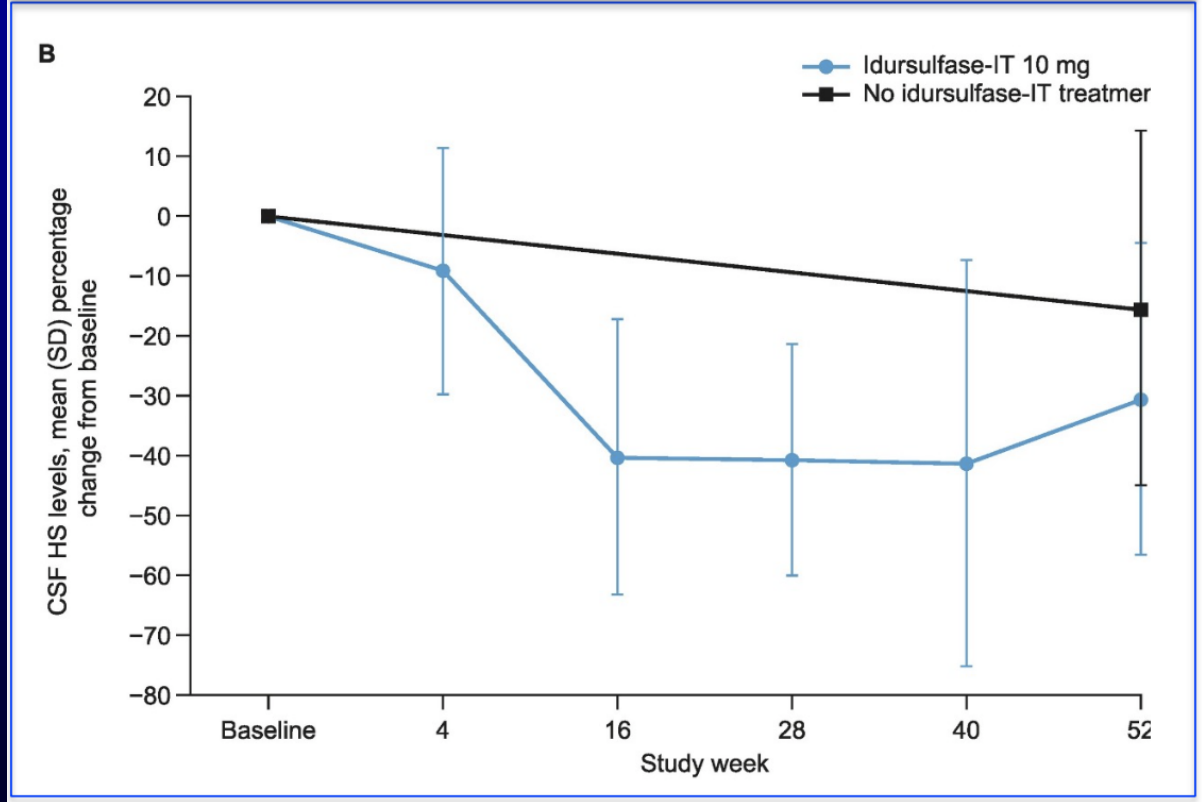
Muenzer et al. Mol Genet Metab (2022) 137:127-139

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



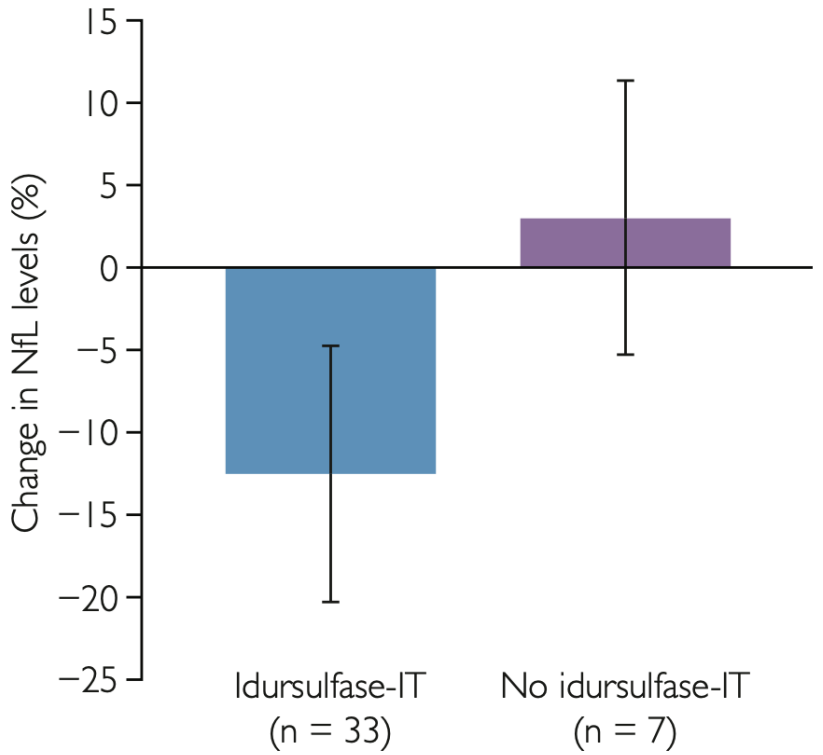
GAG measured by thrombin activity assay

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.*

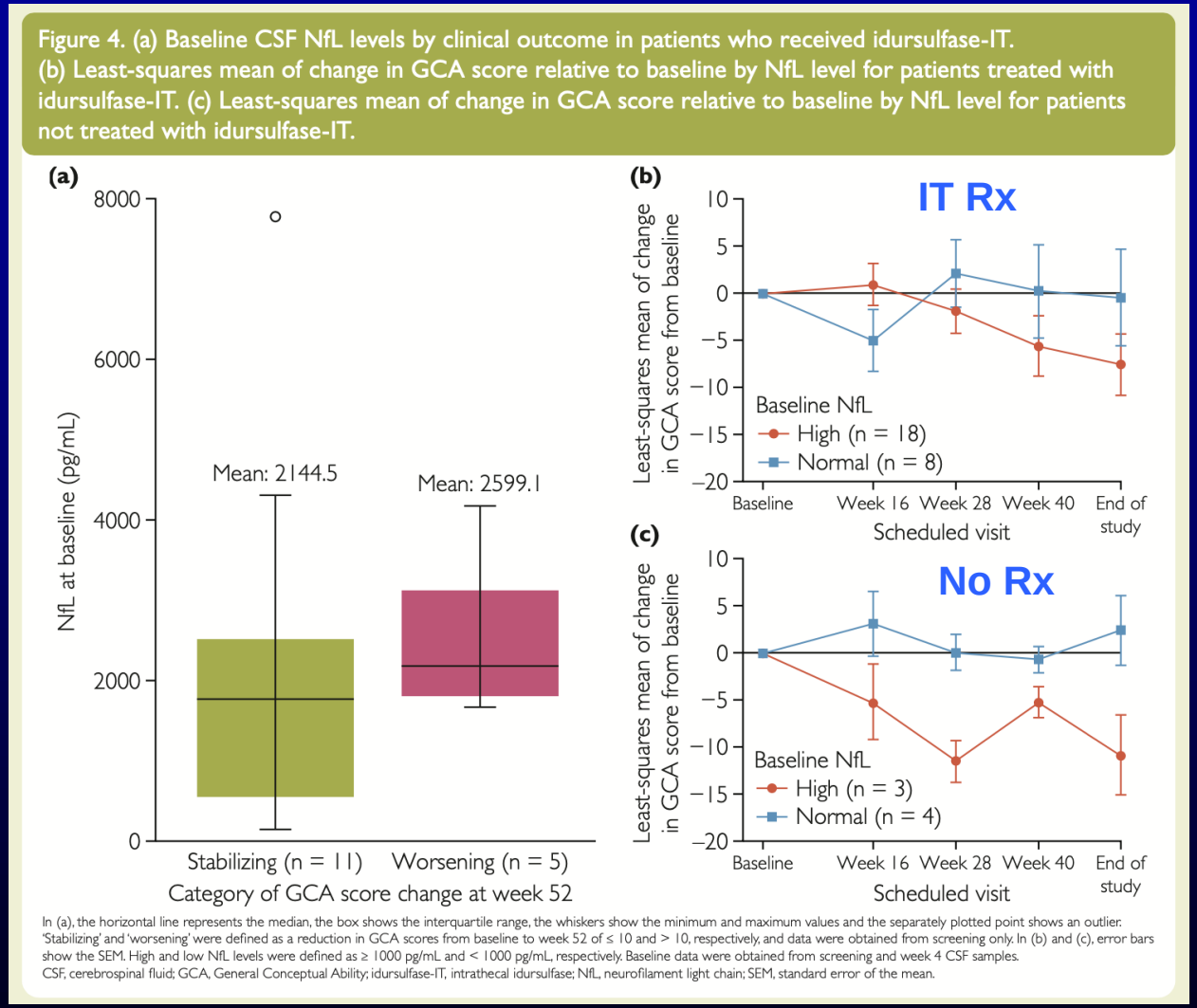


Figure 4. (a) Baseline CSF NfL levels by clinical outcome in patients who received idursulfase-IT. (b) Least-squares mean of change in GCA score relative to baseline by NfL level for patients treated with idursulfase-IT. (c) Least-squares mean of change in GCA score relative to baseline by NfL level 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

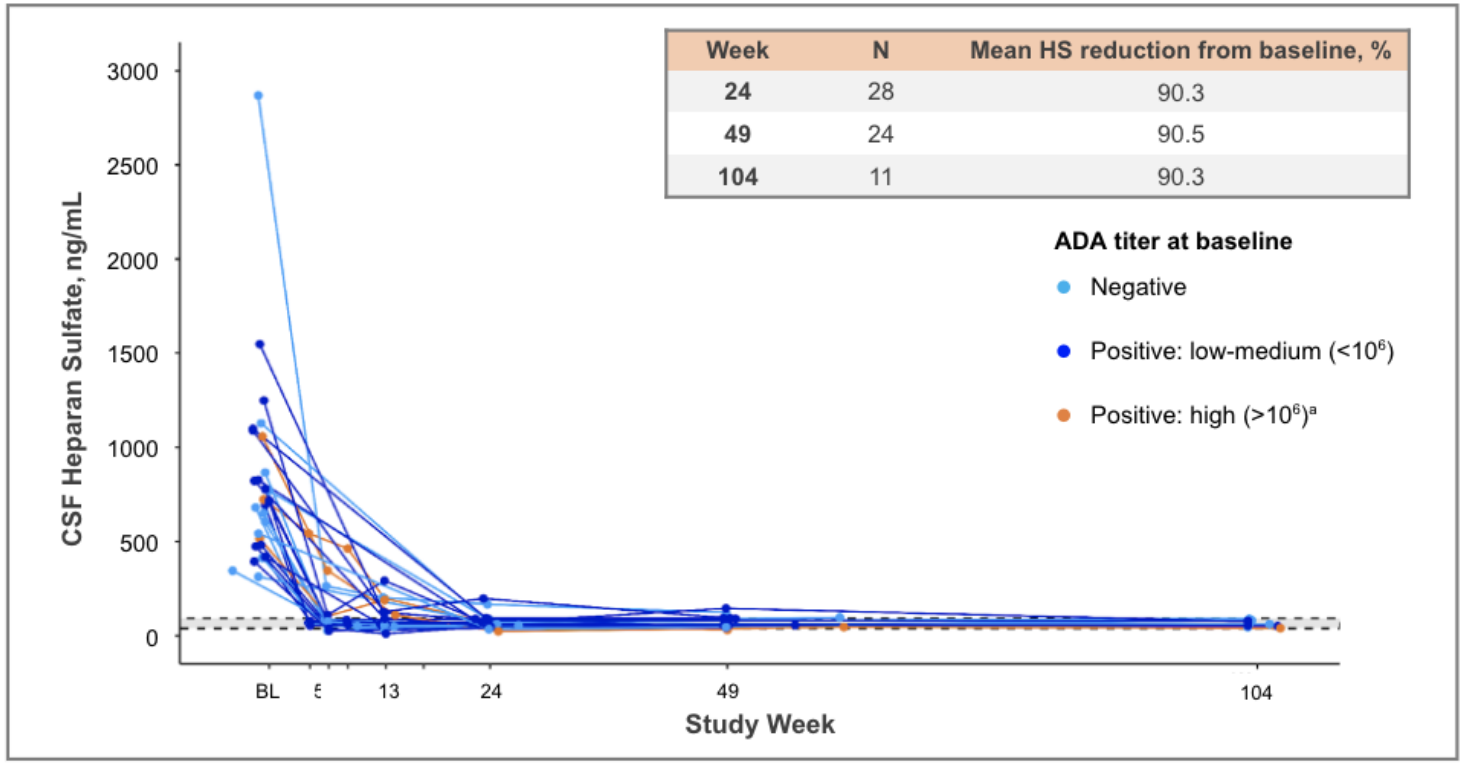
Presented at the WORLD Symposium, San Diego, CA (Feb 2024) by C. Argueta

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.

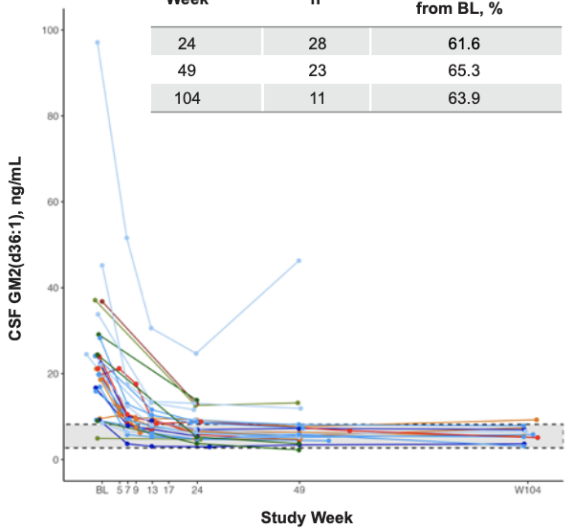
Data presented at the WORLD Symposium, San Diego, CA (Feb 2024)

CSF Lipid Reduction with Weekly IV DNL310

Figure 4. Lysosomal Lipids: Gangliosides and Glucosylsphingosine

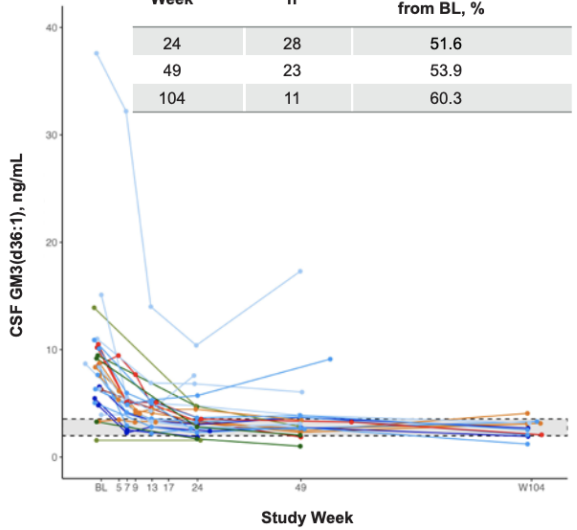
A GM2[a]

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



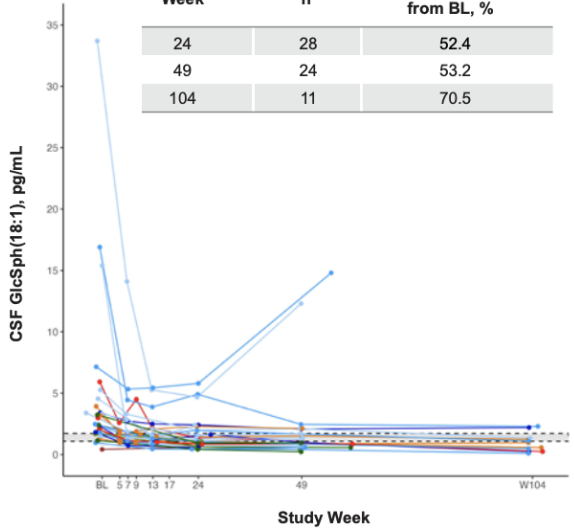
B GM3[a]

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

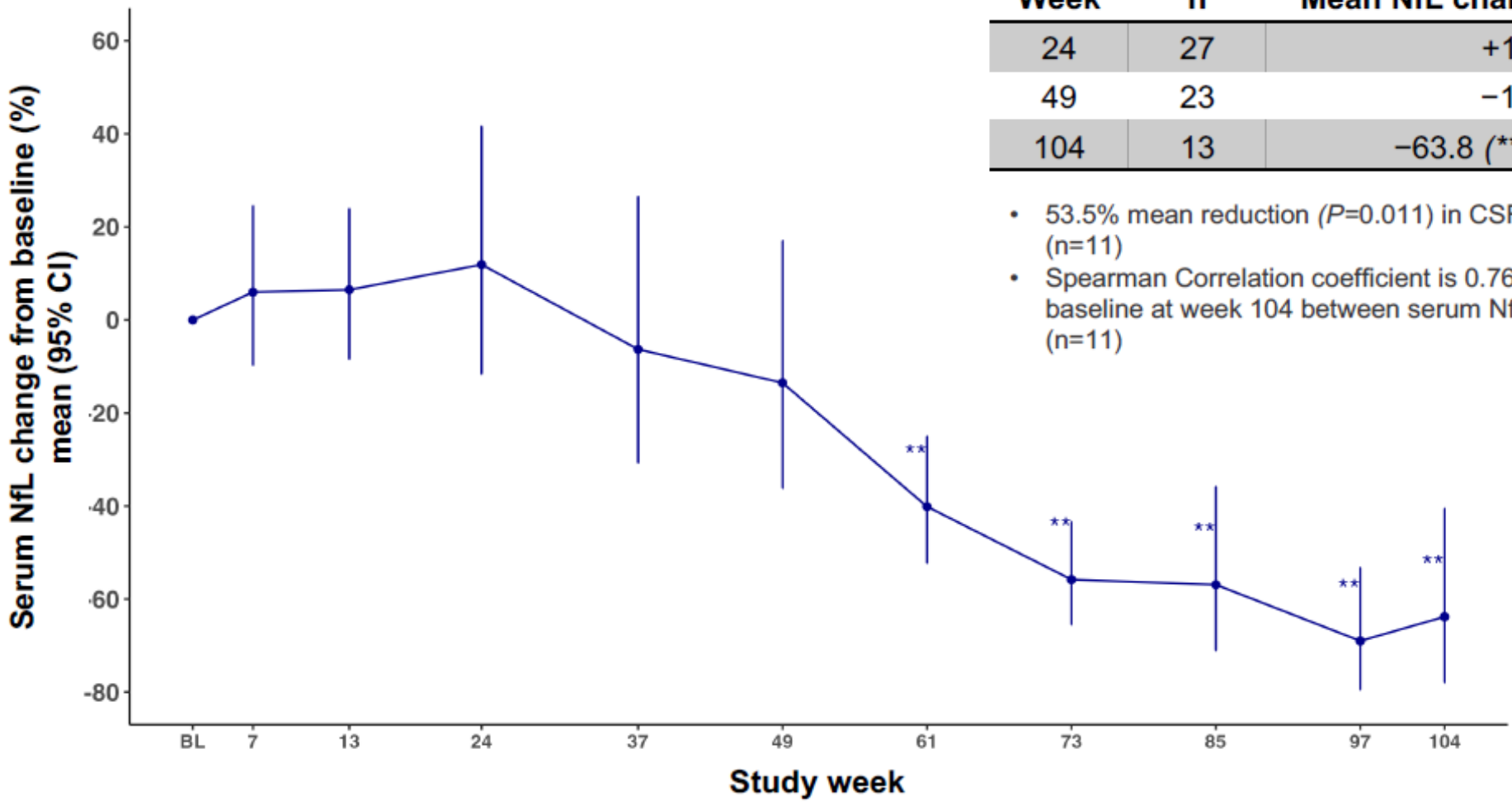


Cohort	Dose	Cohort	Dose	Cohort	Dose	Cohort	Dose
A	3 to 30 mg/kg	B2	7.5 to 15 mg/kg	C	15 mg/kg	E	15 mg/kg
B1	3 to 15 mg/kg	B3	15 mg/kg	D	15 mg/kg	High baseline ADA titer[b]	

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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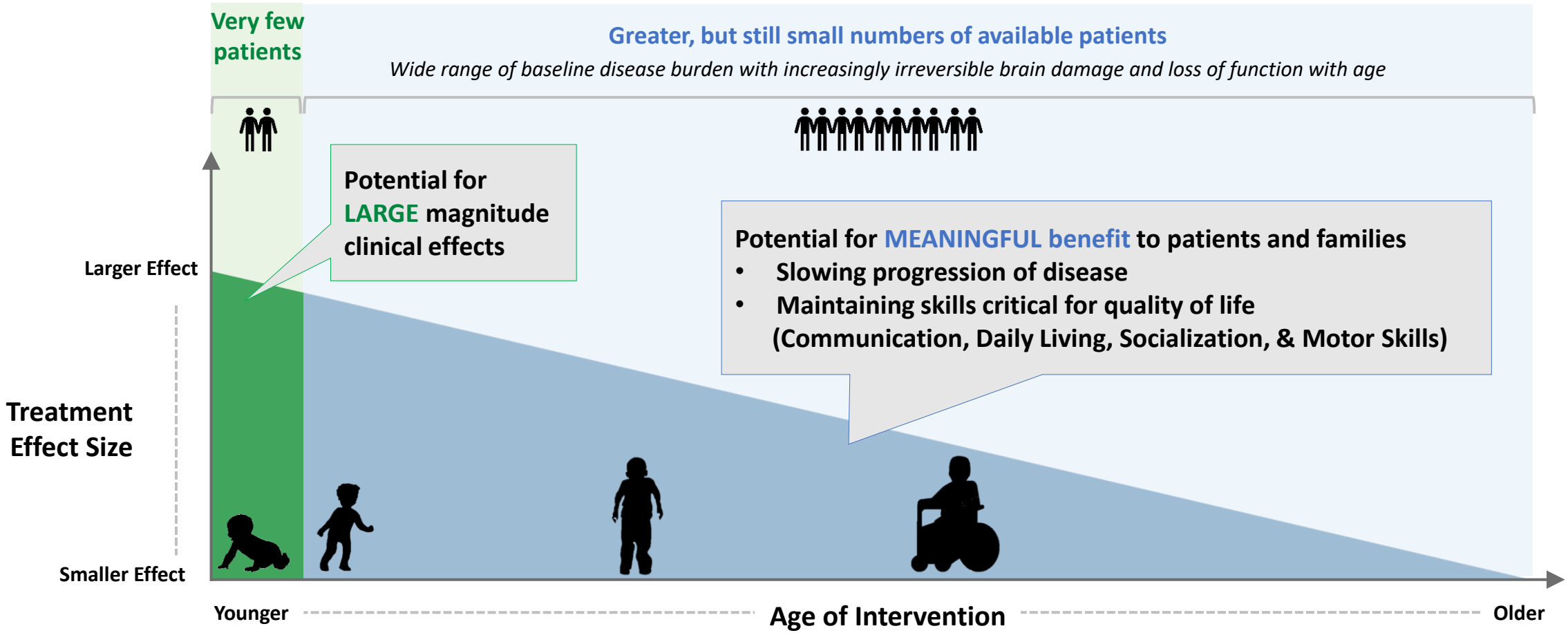
10. Regulatory flexible is needed <u>now</u> to bring treatments to individuals with nMPS using the FDA 2020 industry guidelines.

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

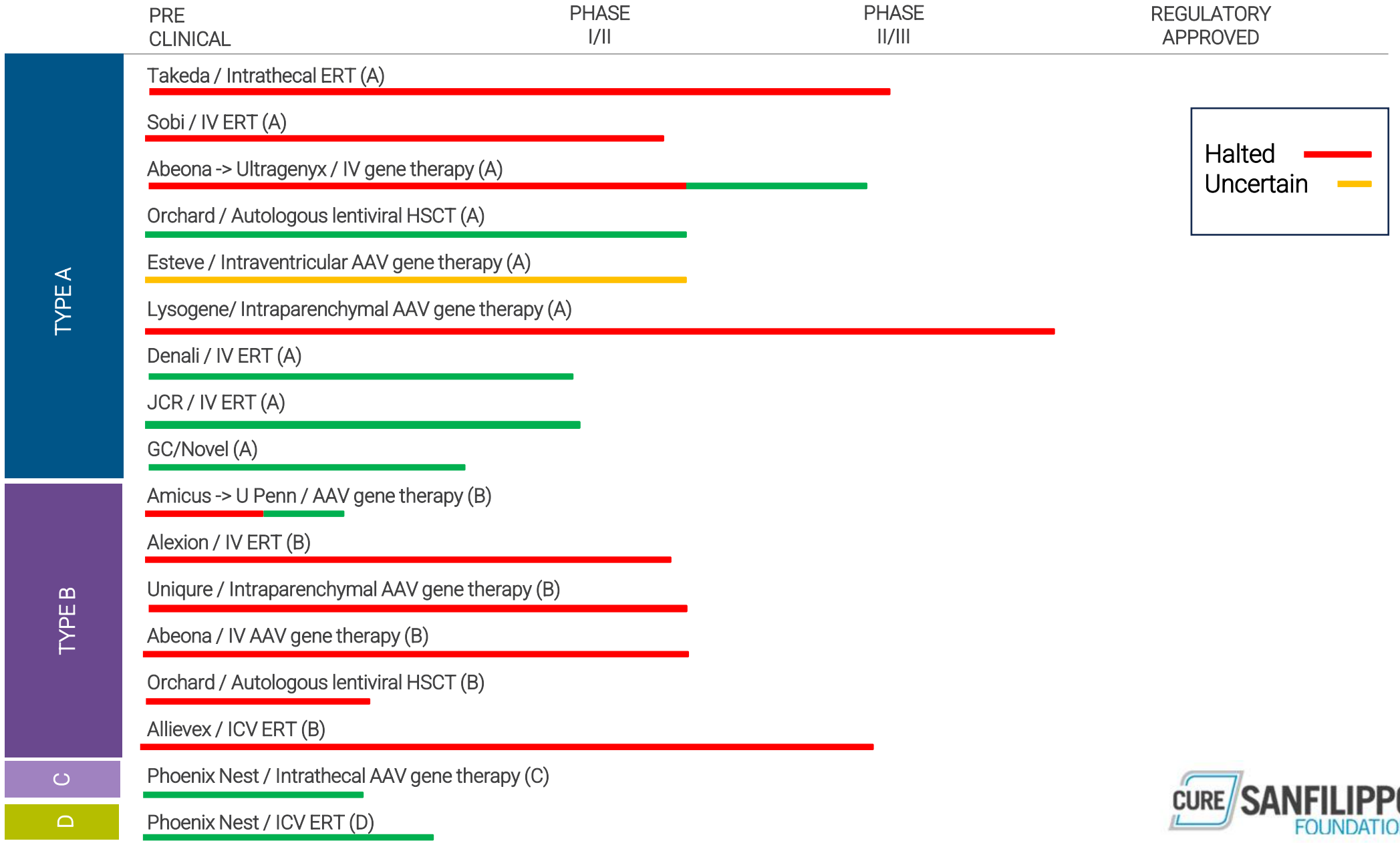
"I'll take that [my child] can sit and enjoy doing something for three more minutes than before. **I'll even take an intensive invasive medical procedure to get me six more months.**"

Porter KA, et al. ***Parent Experiences of Sanfilippo Syndrome Impact and Unmet Treatment Needs: A Qualitative Assessment****. Neurol Ther. 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



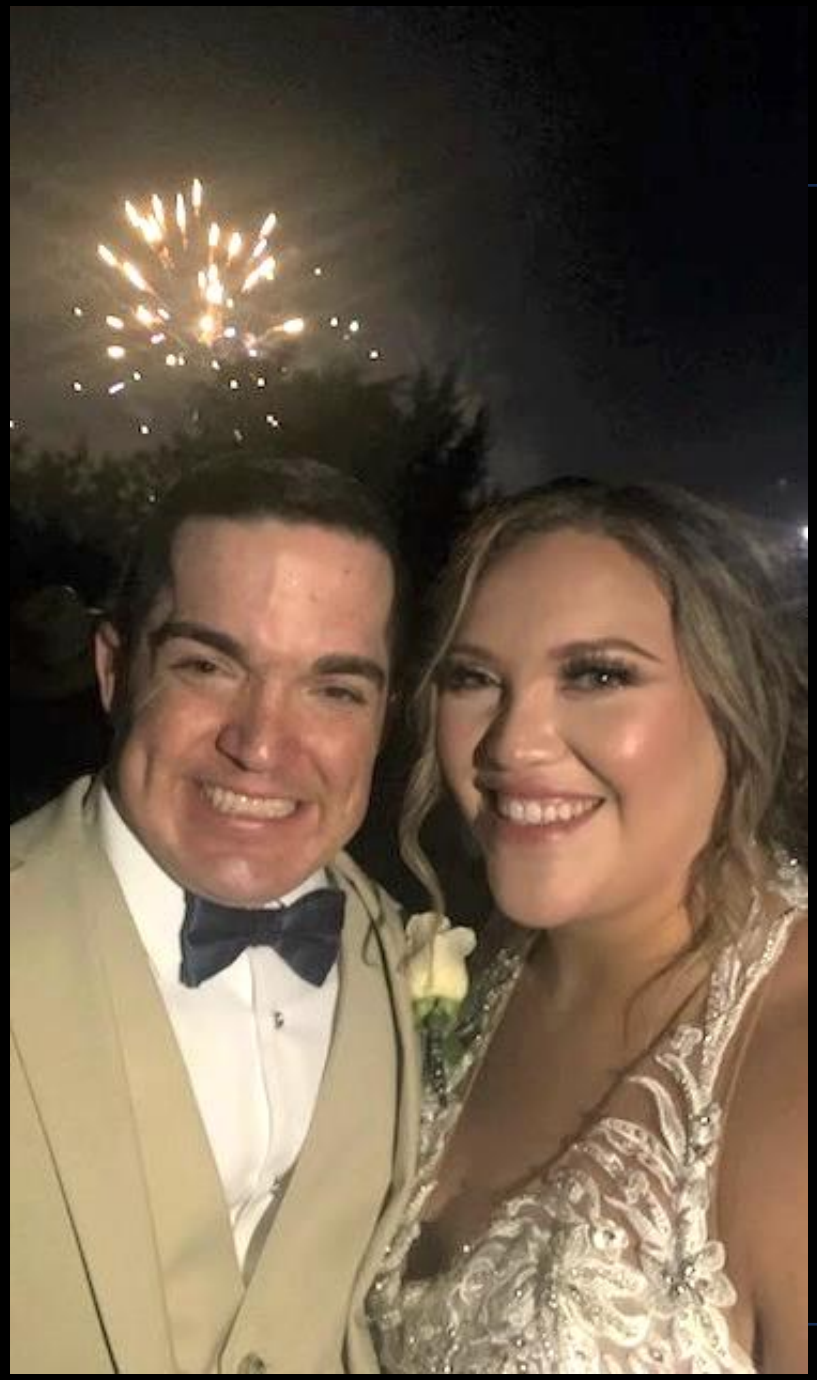
CURE SANFILIPPO FOUNDATION

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

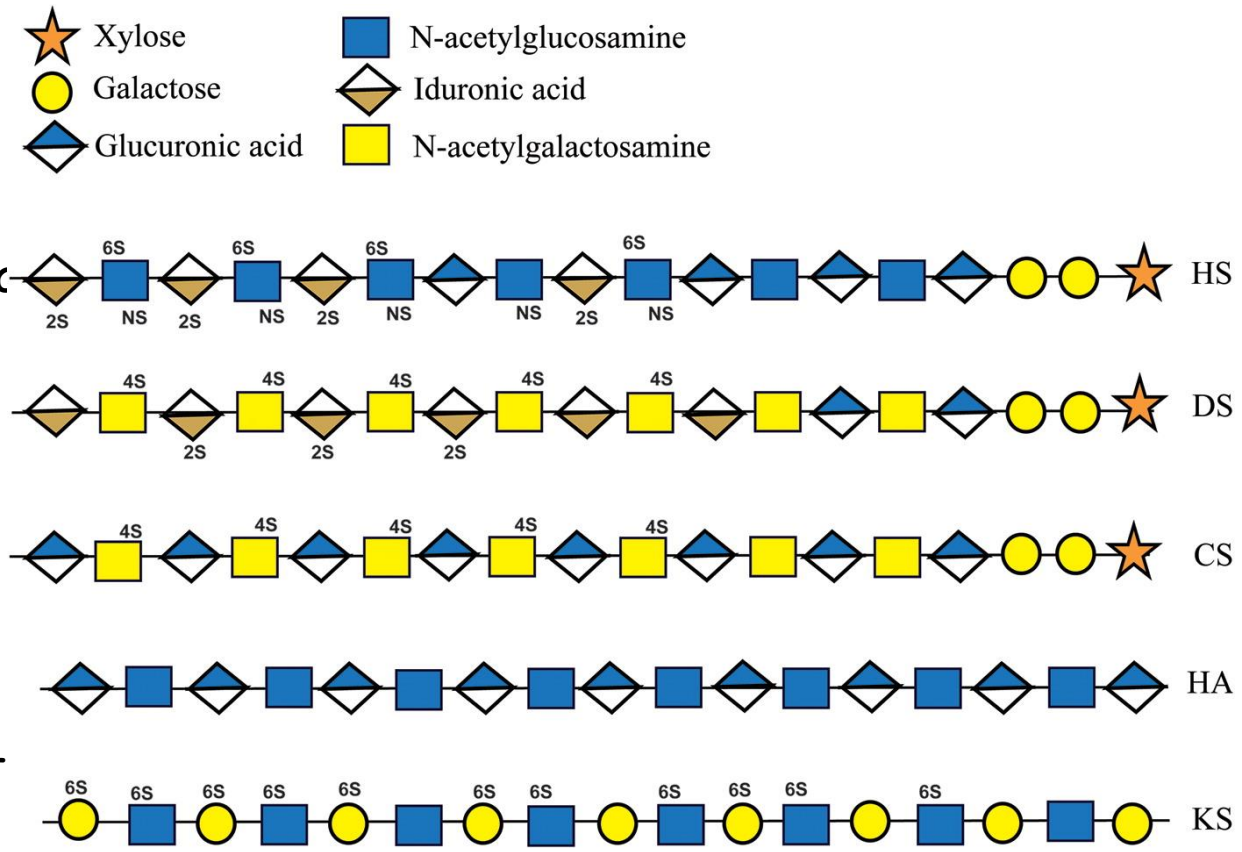
at Women's and Children's Hospital; Adelaide Medical School and School of Biological Sciences, University of Adelaide,

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

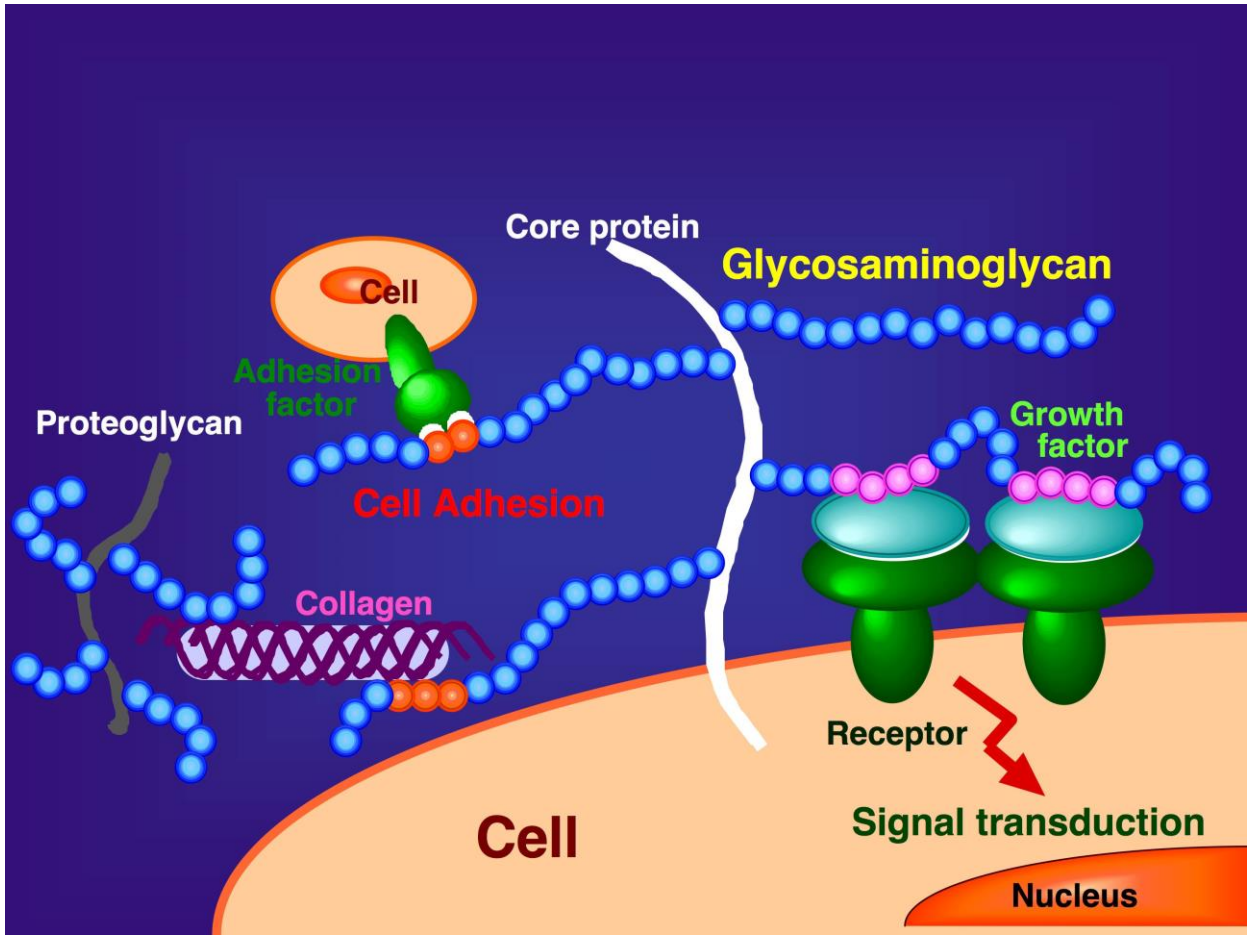


HS, heparan sulphate; DS, dermatan sulphate; CS, chondroitin sulphate
HA, hyaluronic acid; KS, keratan sulphate

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
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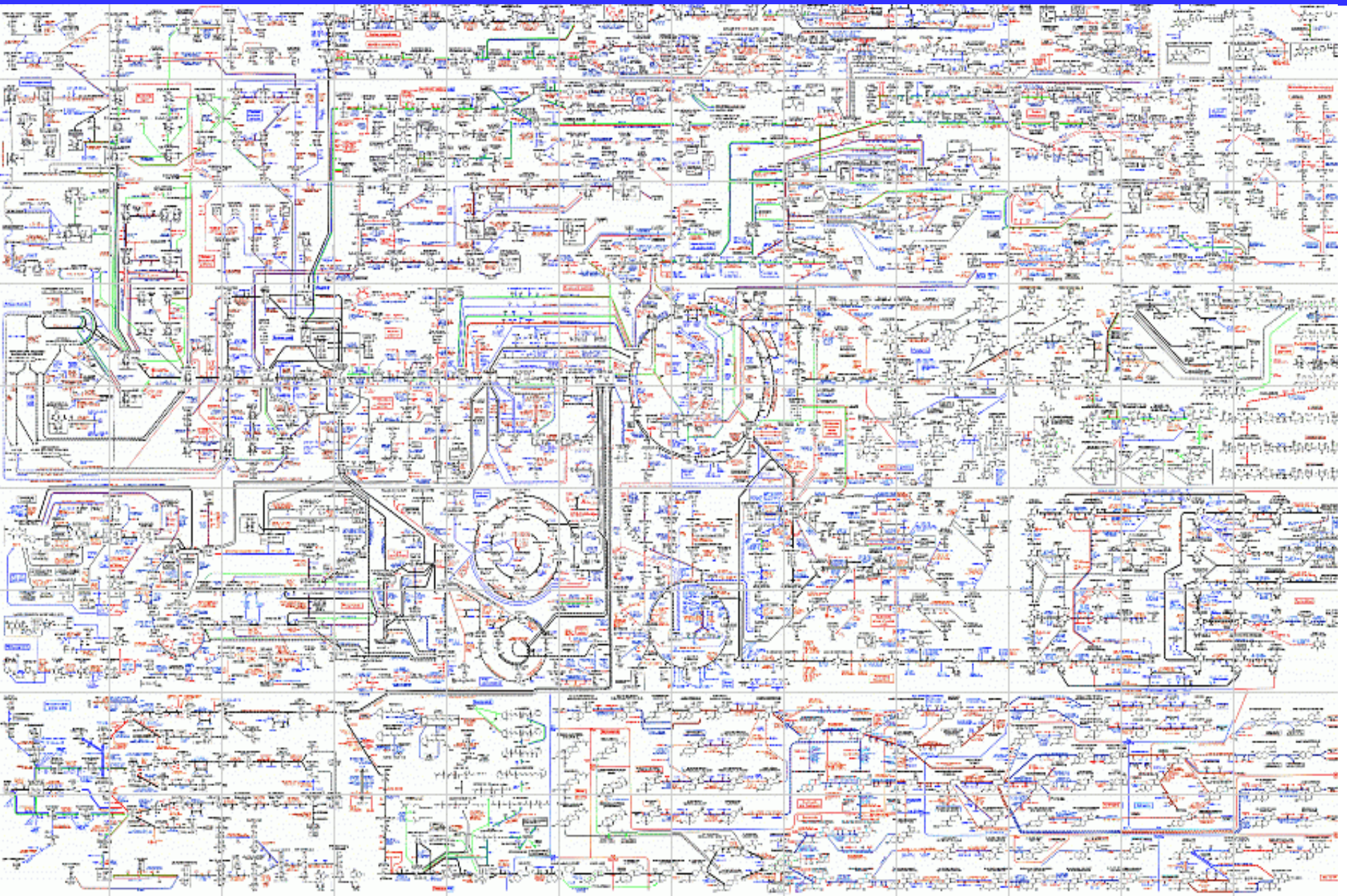
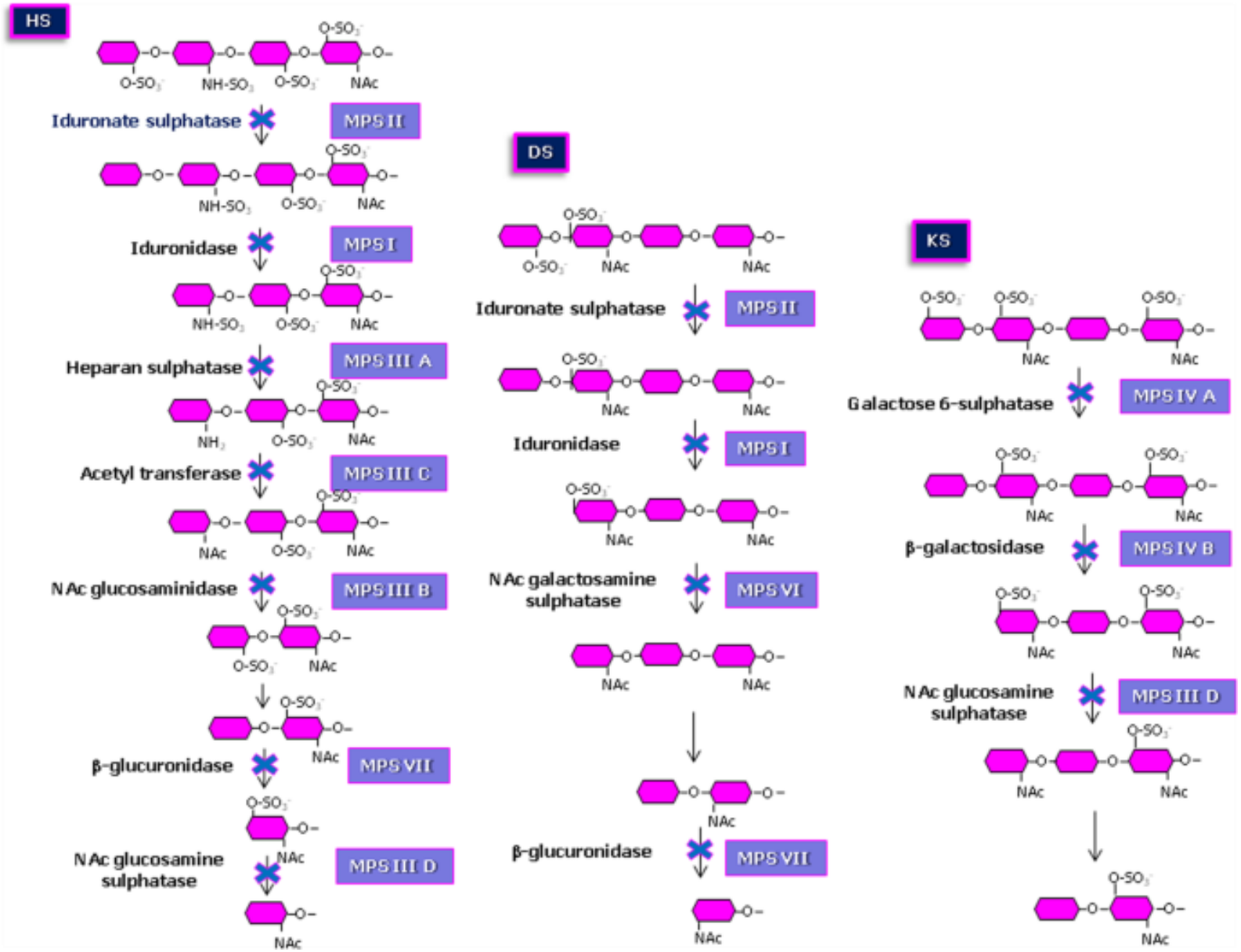
the quantity of glycosaminoglycans (GAG) is critical

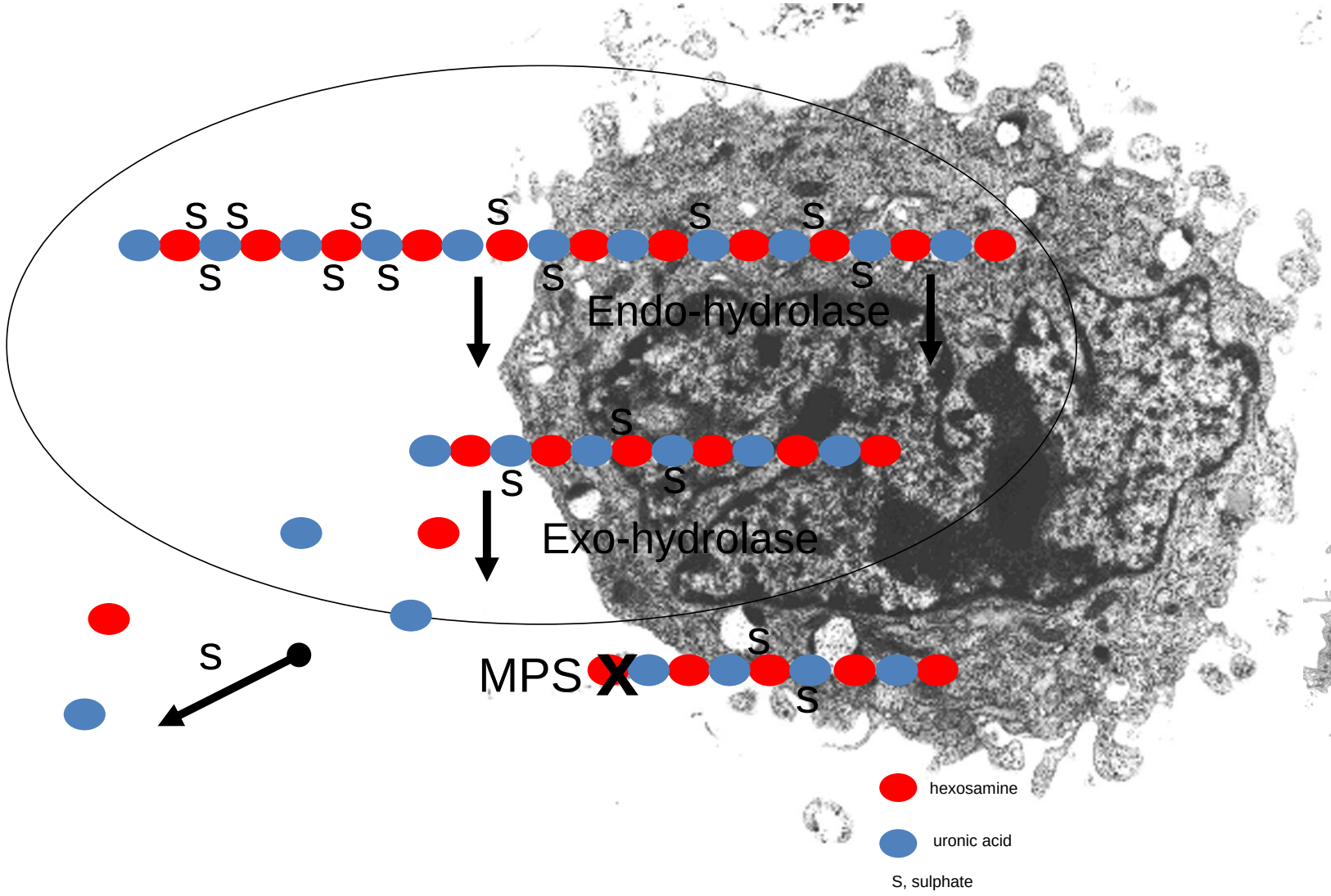


GAG degradation is sequential with no redundancy

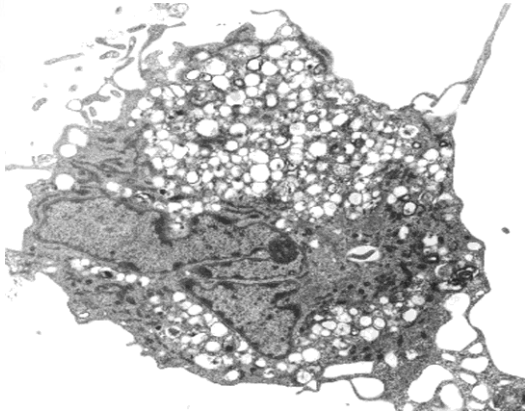


GAG, glycosaminoglycans
HS, heparan sulphate
DS, dermatan sulphate
KS, keratan sulphate
MPS, mucopolysaccharidoses

partially degraded GAG accumulate in lysosomes



unaffected cell



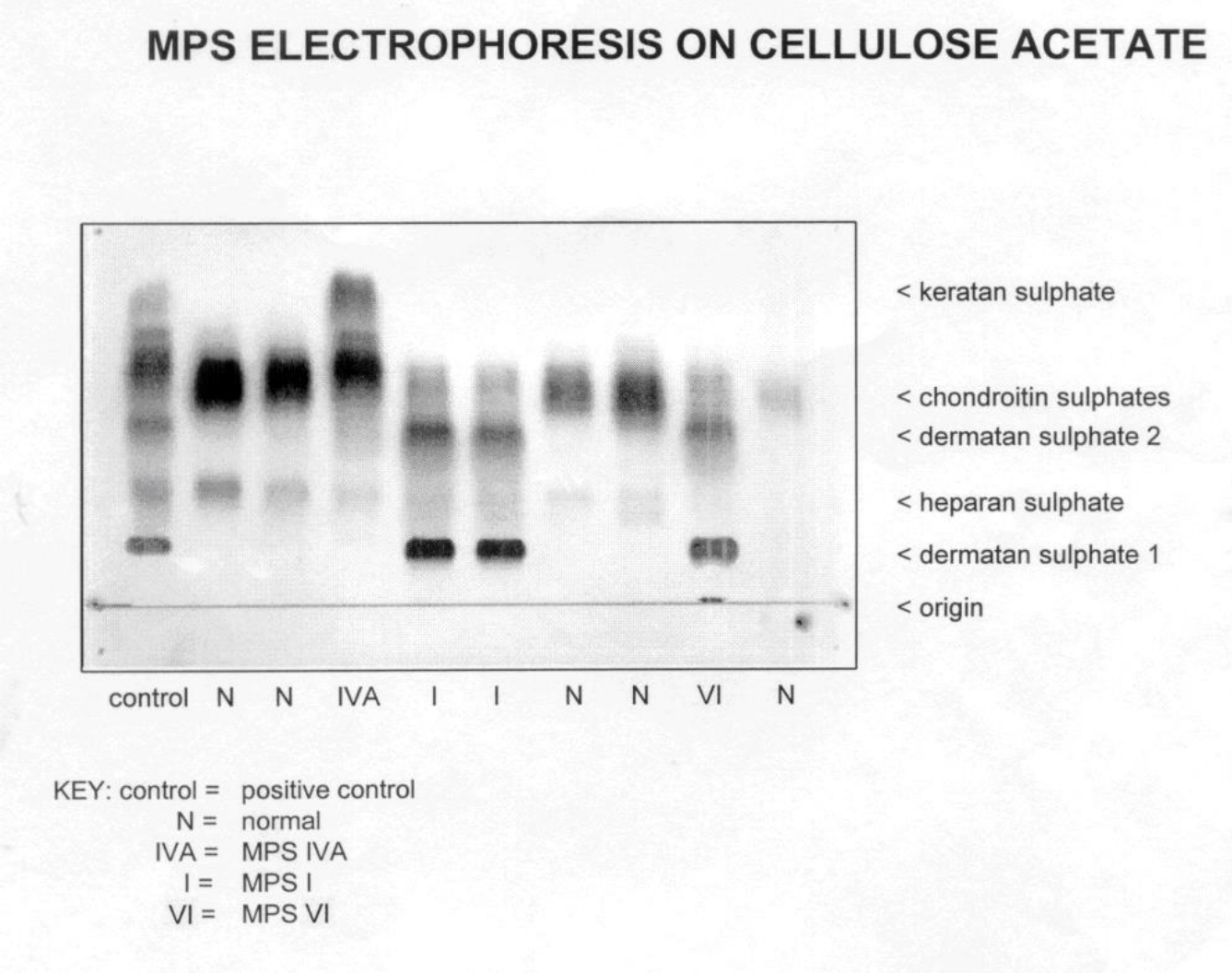
MPS cell

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

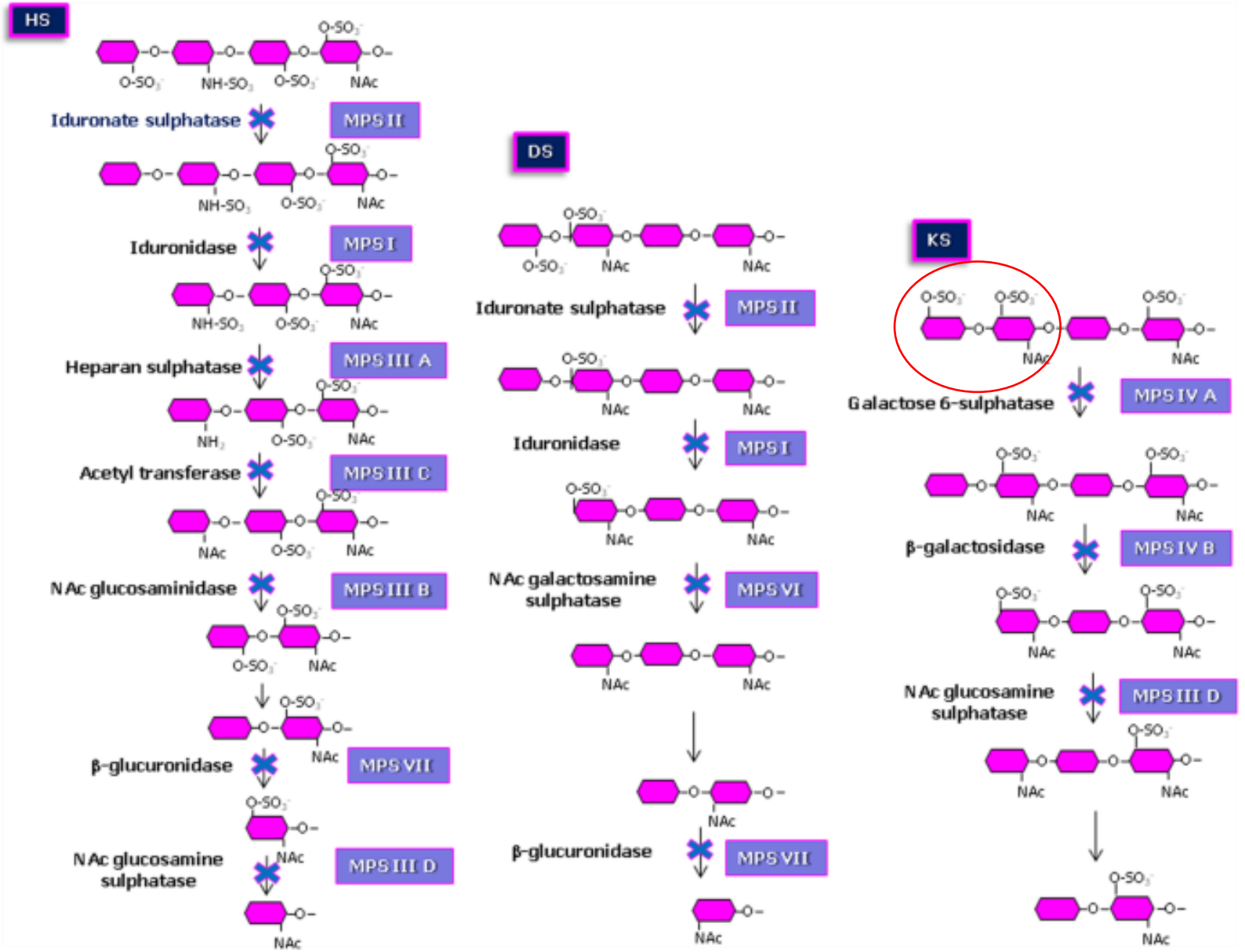
case presentation exemplifies the problem

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



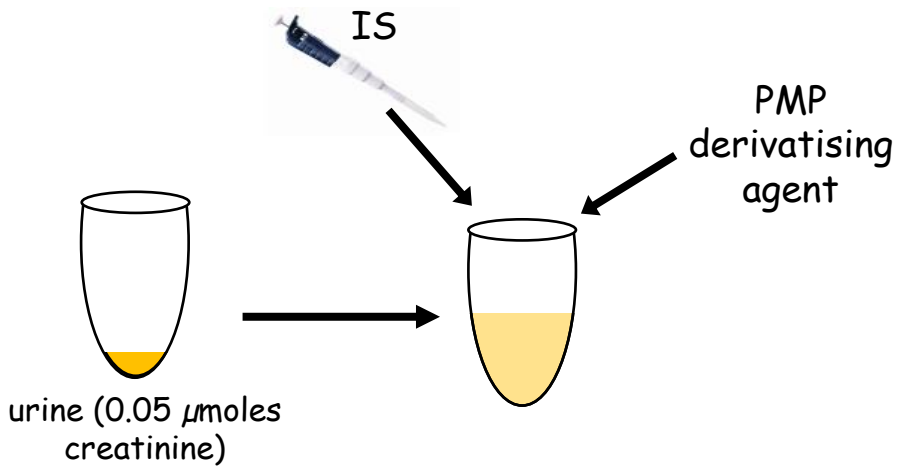
GAG, glycosaminoglycans
HS, heparan sulphate
DS, dermatan sulphate
KS, keratan sulphate
MPS, mucopolysaccharidoses

mass spectrometry: a game changing measuring tool

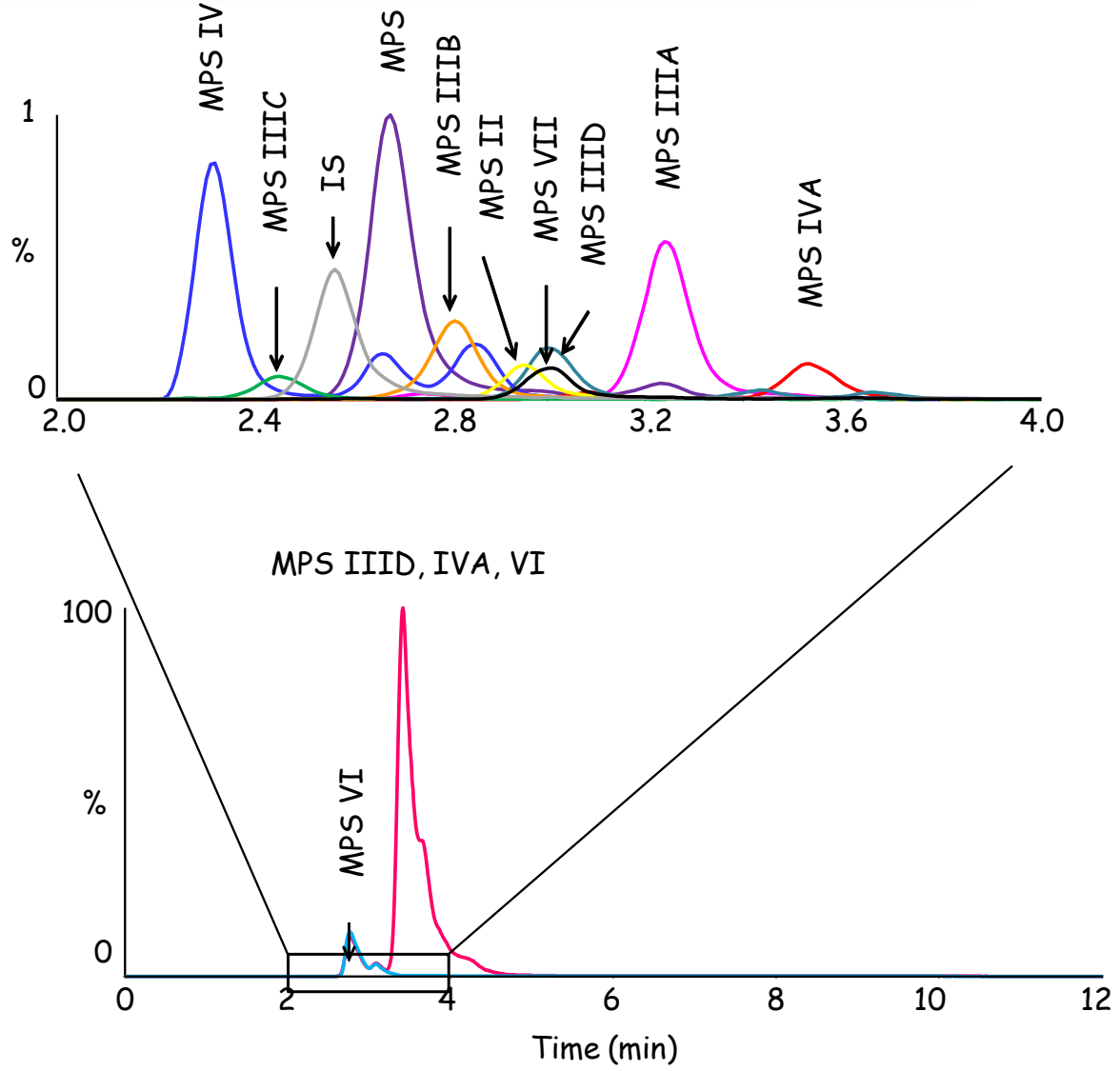
- measures compounds in the femtomole ra
- mass spectrometry has been a game chan
  affords partial structural elucidation – w
- internal standards have allowed absolute
- dye binding for total GAG and electropho

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.

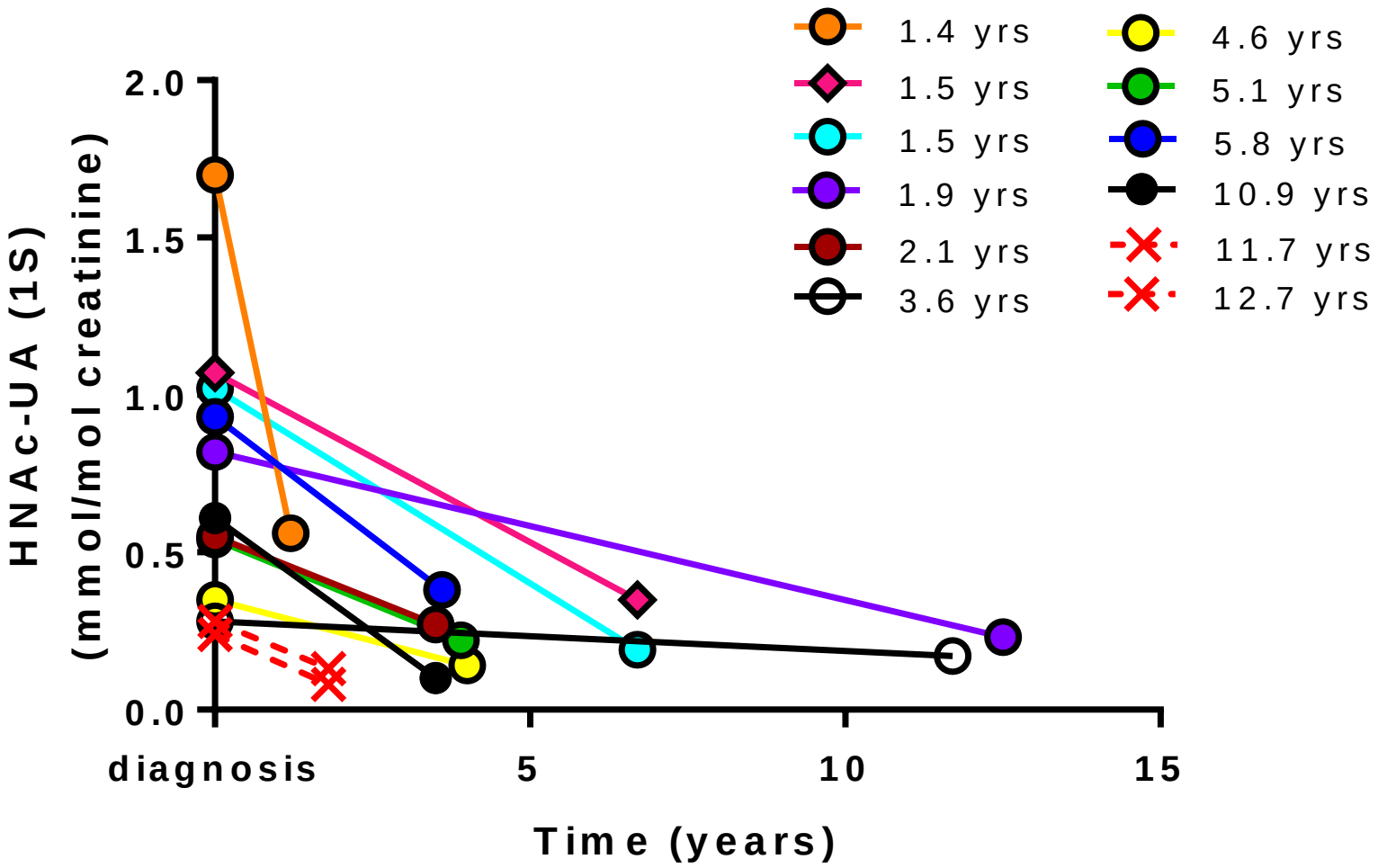
MPS, mucopolysaccharidosis; IS, internal standard; PMP, 1-phenyl 3-methyl 5-pyrazolone

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)

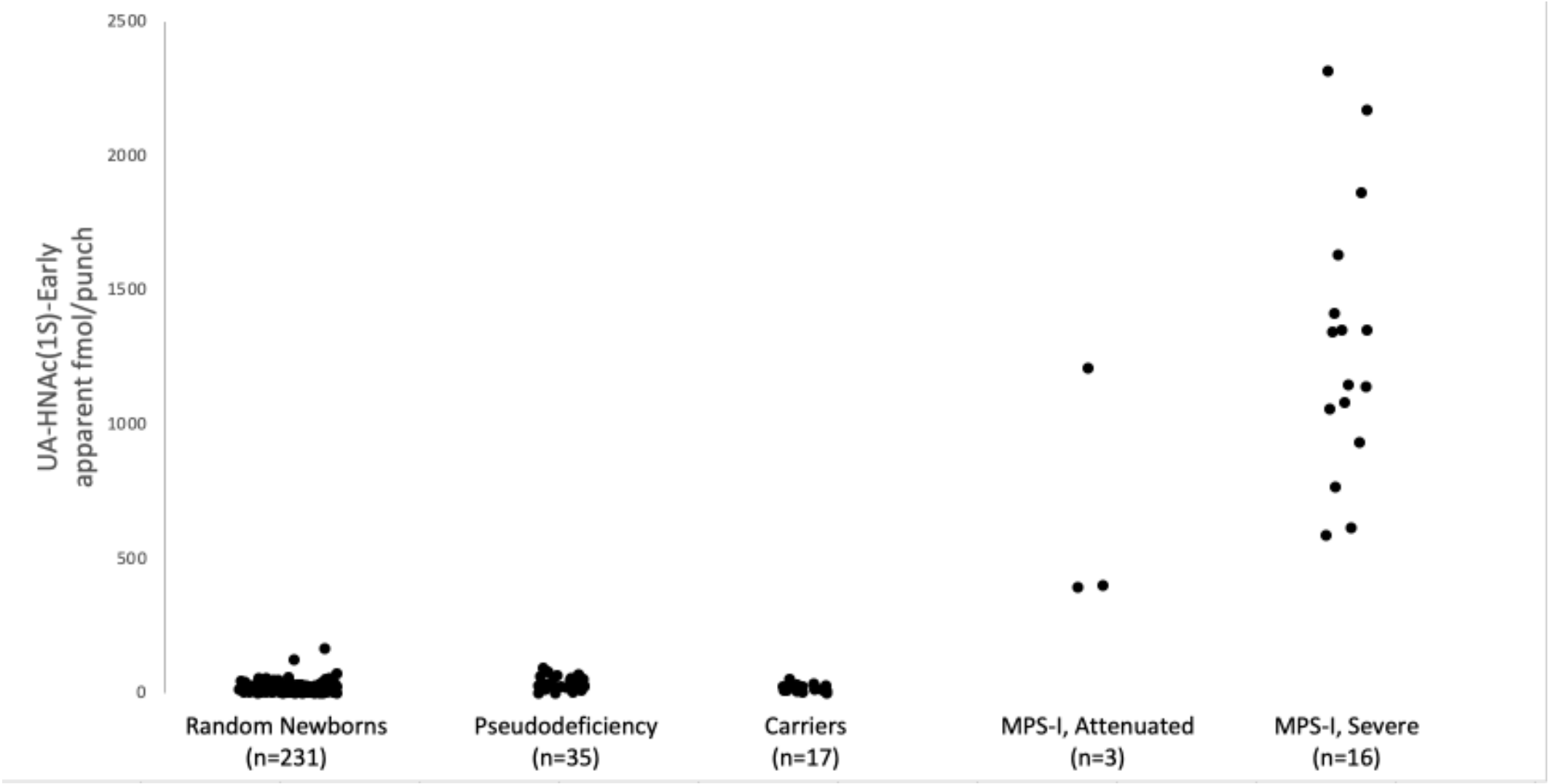
NPAAC, National Pathology Accreditation Advisory Council;
ERNDIM, European Research Network for evaluation and improvement of screening, Diagnosis and treatment of Inborn errors of Metabolism;
MPS, mucopolysaccharidosis

monitoring enzyme replacement therapy



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

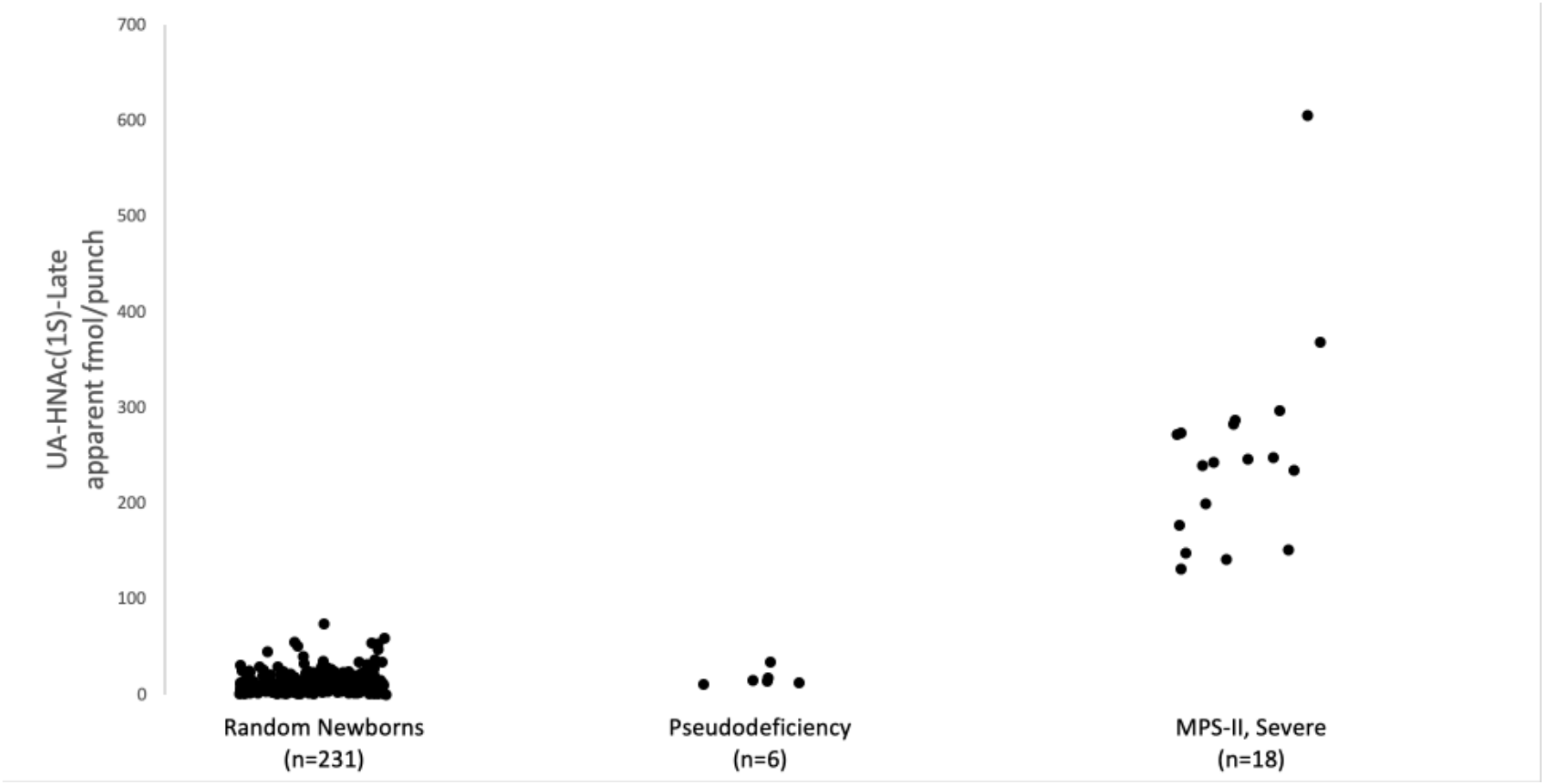
utility for newborn screening: MPS I



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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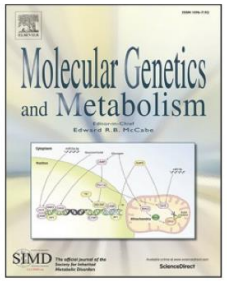

Check for updates

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

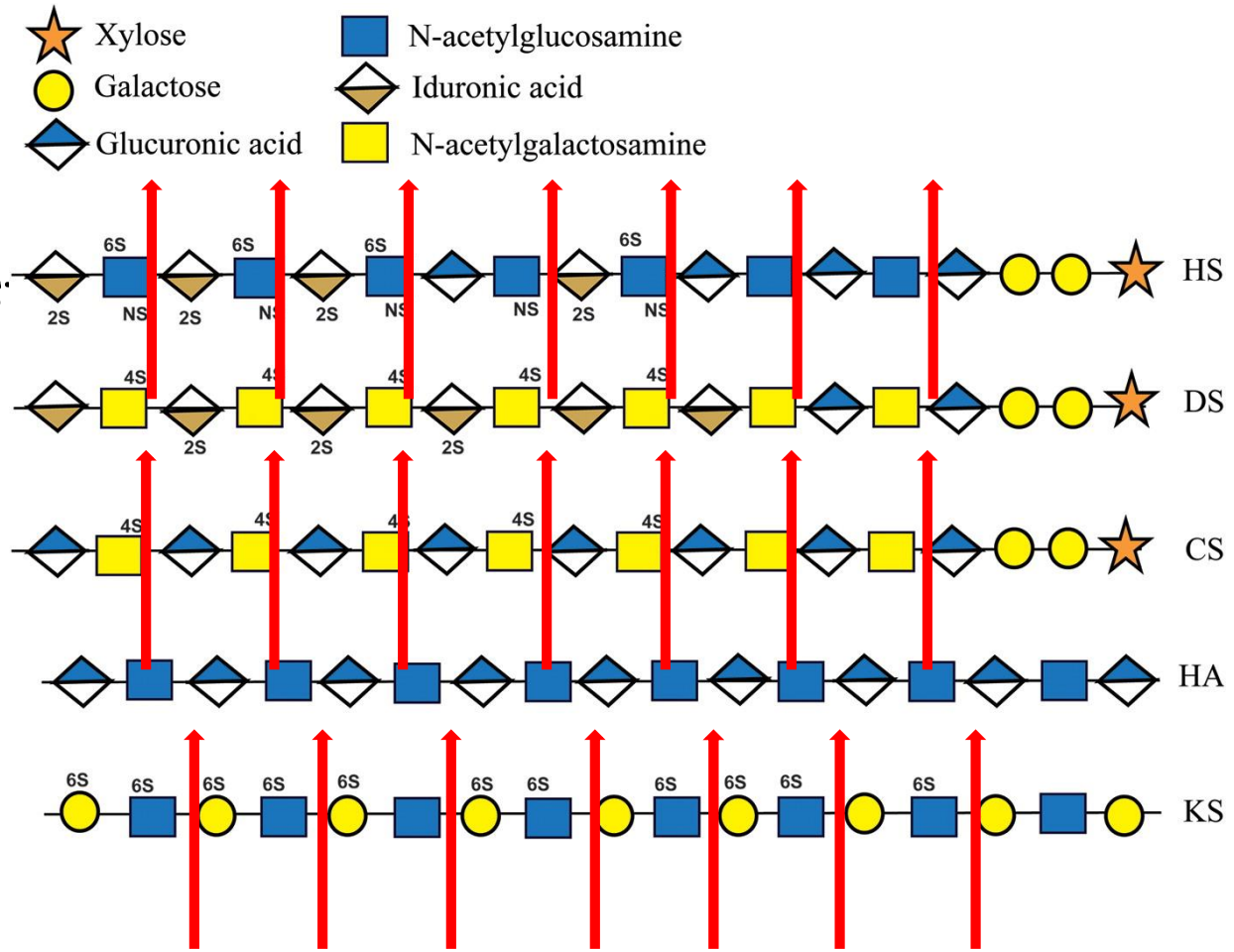
(nitrous acid, HCl methanolysis/butanolysis)

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

2 Enzymatic depolymerisation

(heparanase, chondroitinase, keratanase)

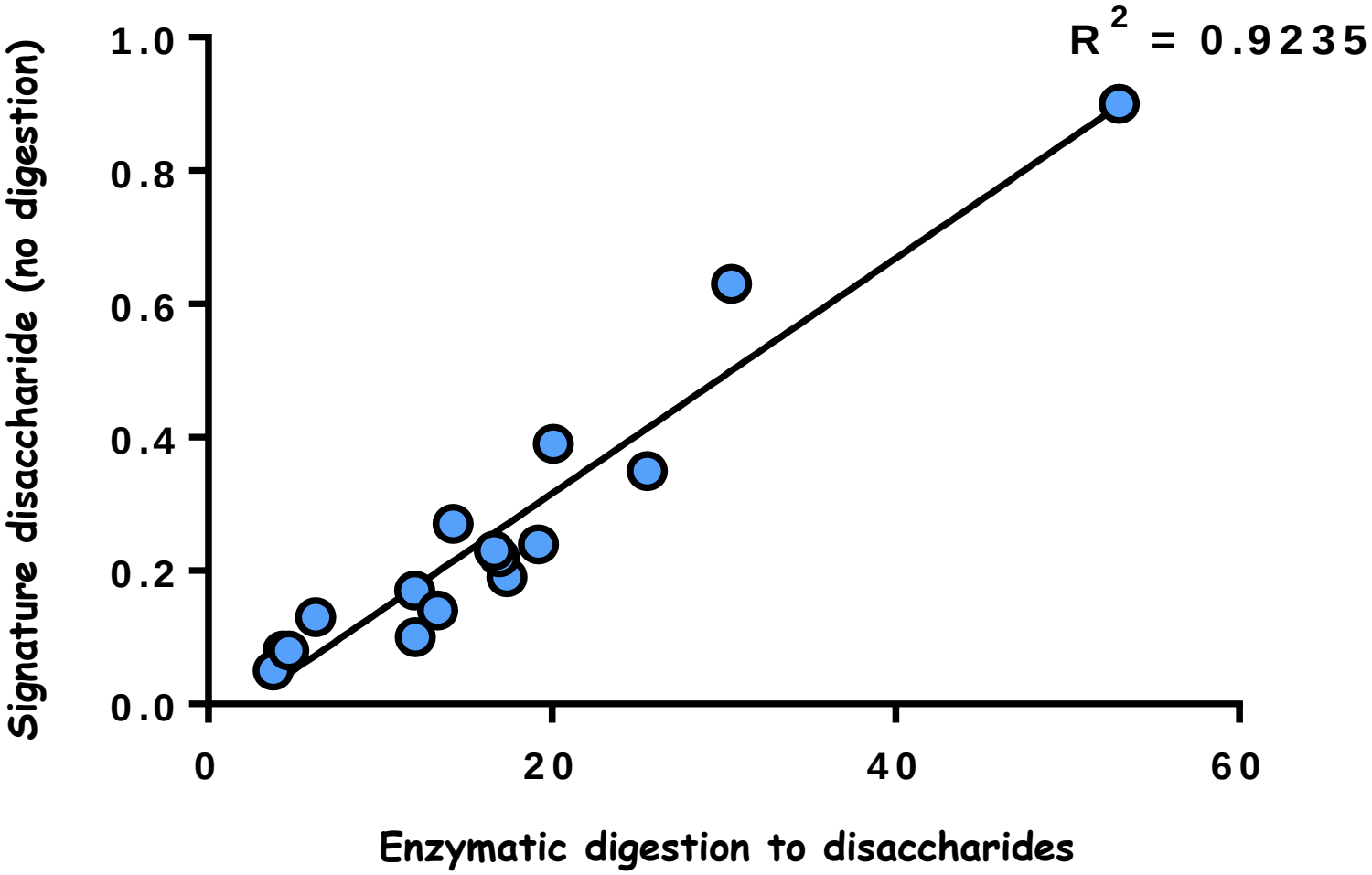
- molar enrichment
- structural detail preserved



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

HCl, hydrochloride

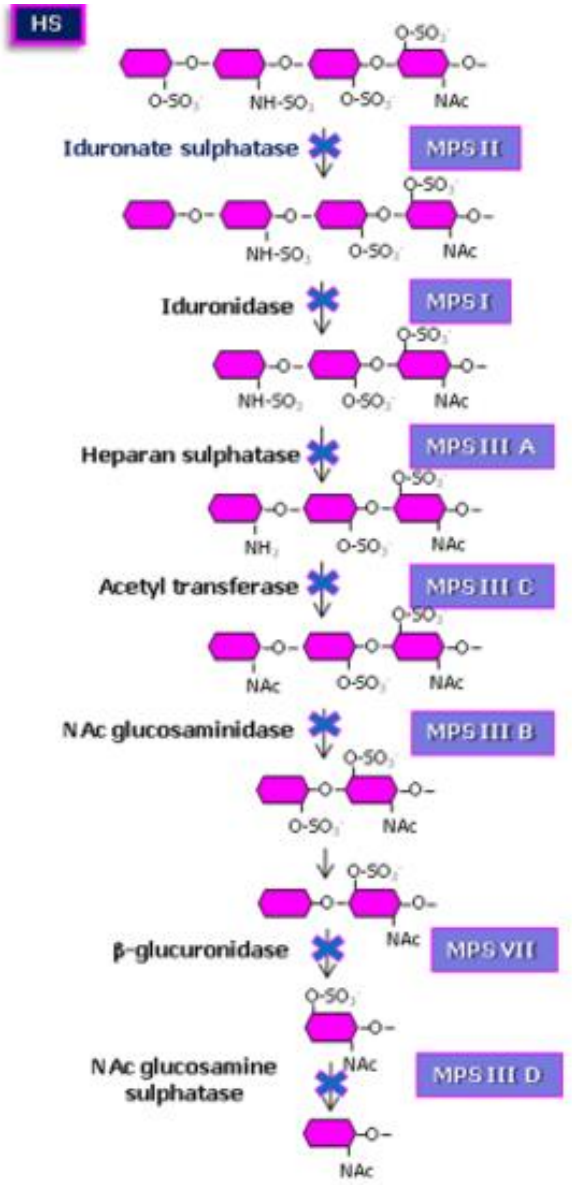
comparing mass spectrometry methods



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

heparan sulphate (HS)

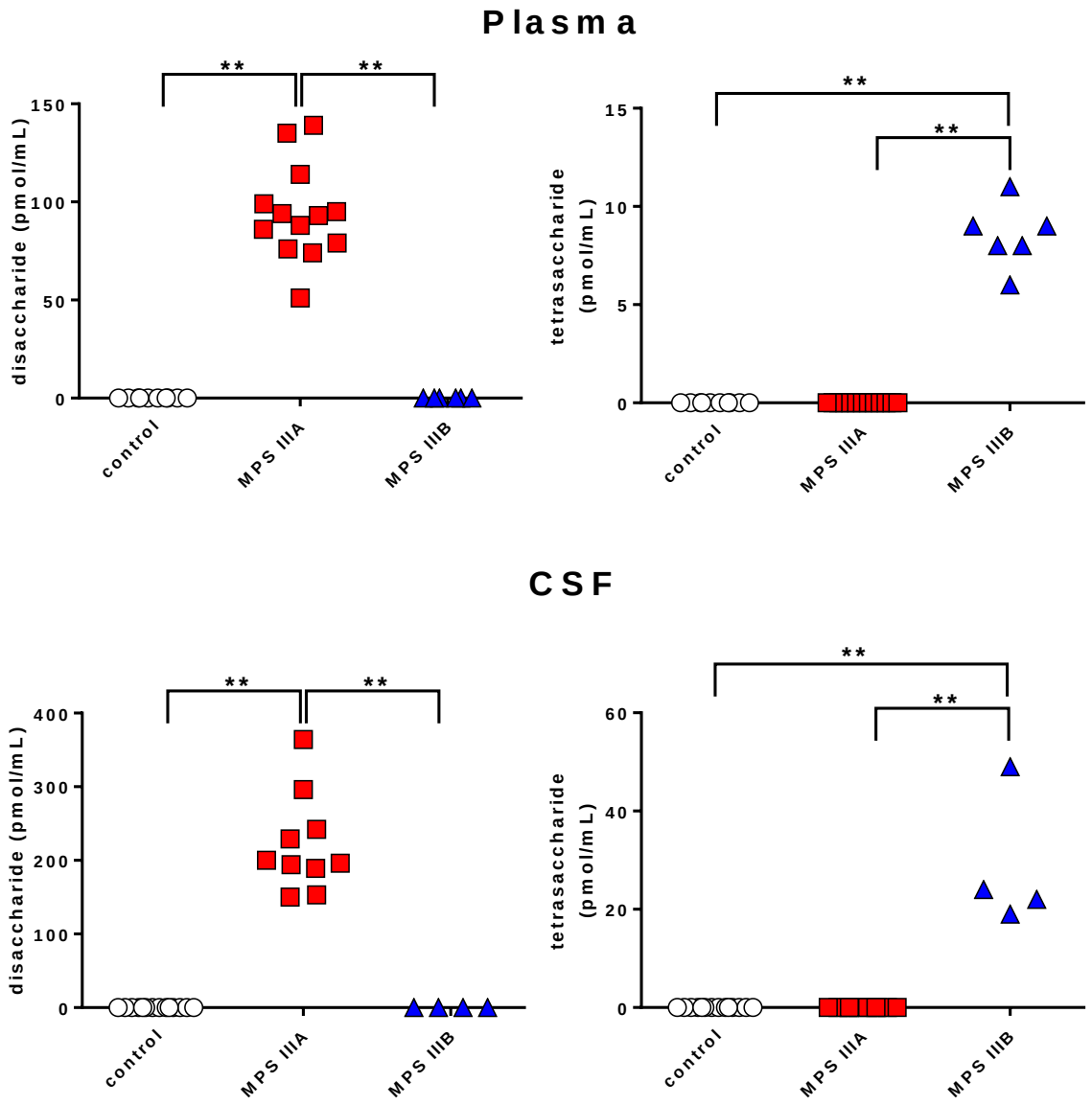
- uronic acid (HexA) and N-acetylglucosamine (GlcNAc)
- sulphated and acetylated domains
- important for function: co-receptors for key signaling path
- critical for proper neuronal development and function
- partially degraded HS is the primary pathological insult in r III and VII)
- HS storage present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

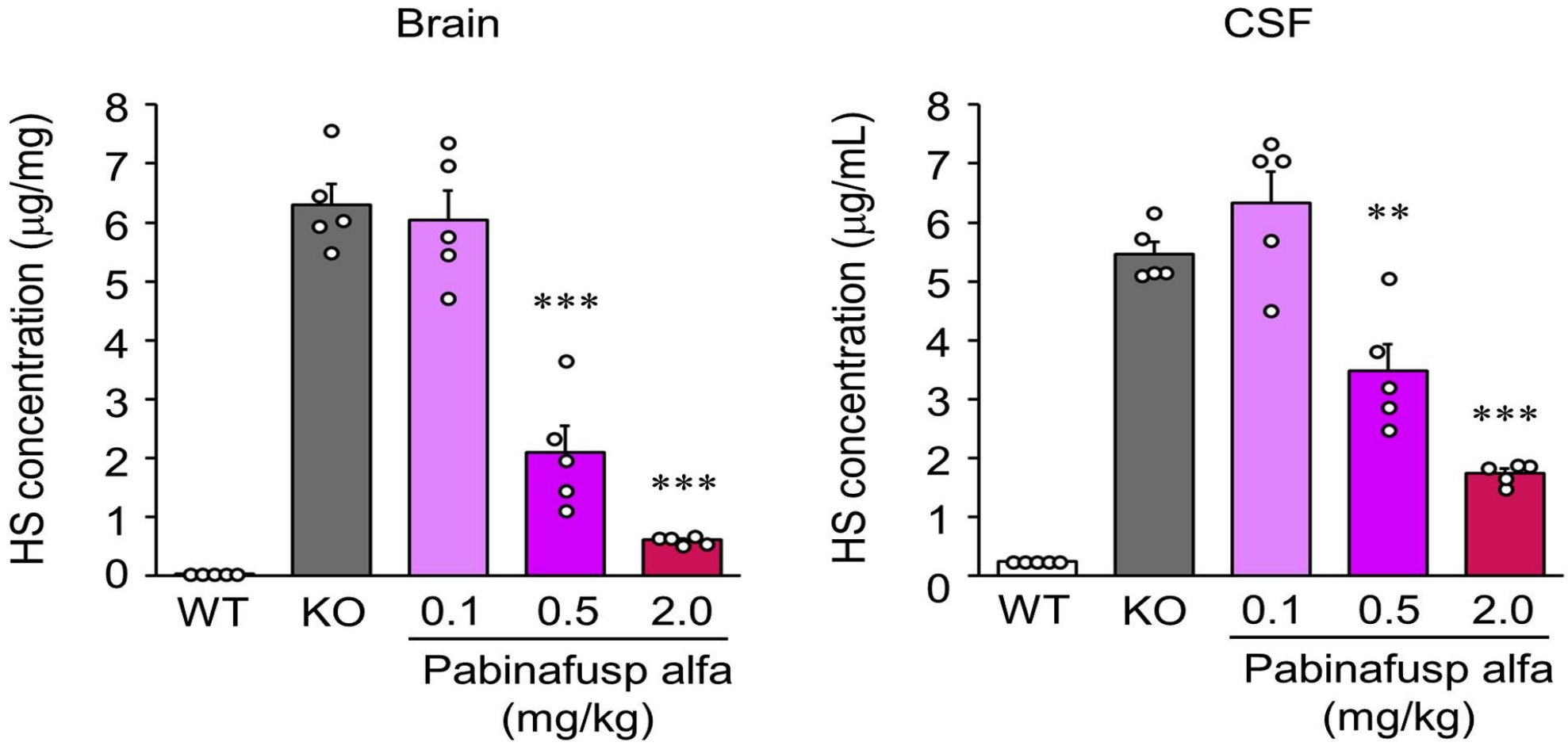
plasma and cerebrospinal fluid (CSF) in MPS III



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

MPS, mucopolysaccharidosis

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

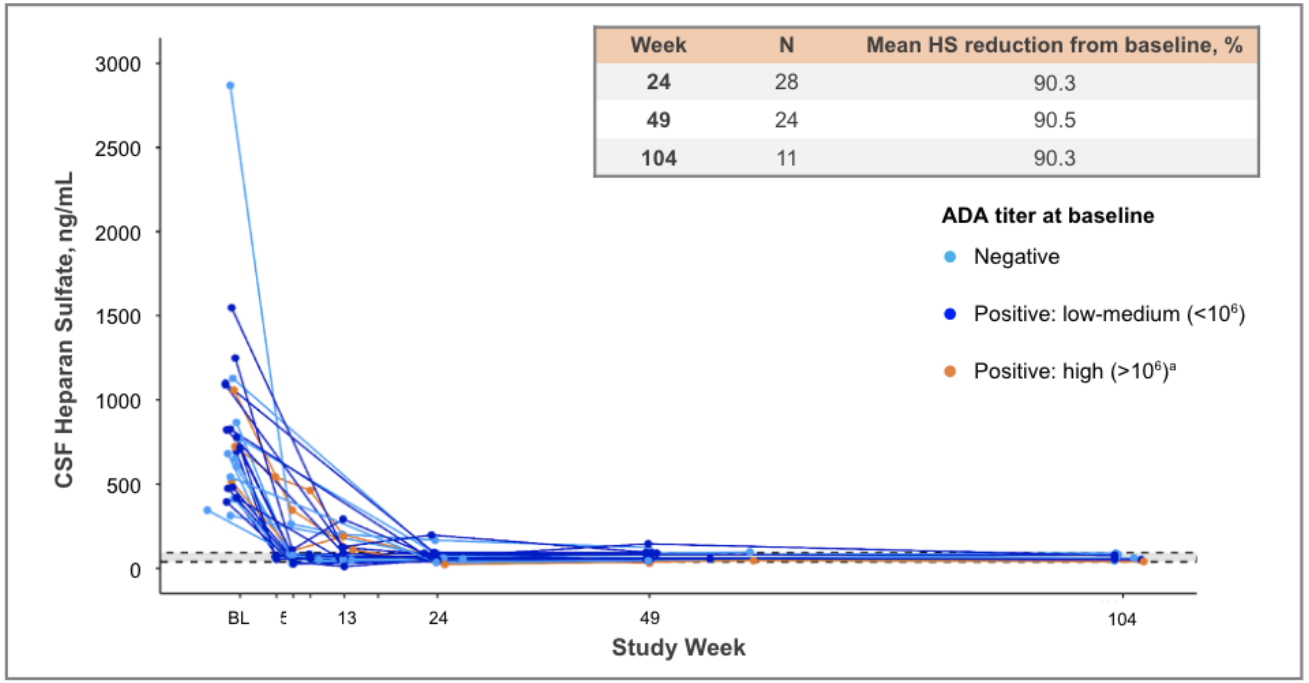


Morimoto et al. Mol Ther Methods Clin Dev 2022;534-544

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

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Lunch

The meeting will resume at 12:40 pm ET

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