AAV Gene therapy and clinical trial for MPS IVA

GRIDS2025: NOVEMBER 16 – 17, 2025

Capital One Hall

Shunji Tomatsu, MD, PhD

Professor and Head, Nemours Children's Health, DE, USA

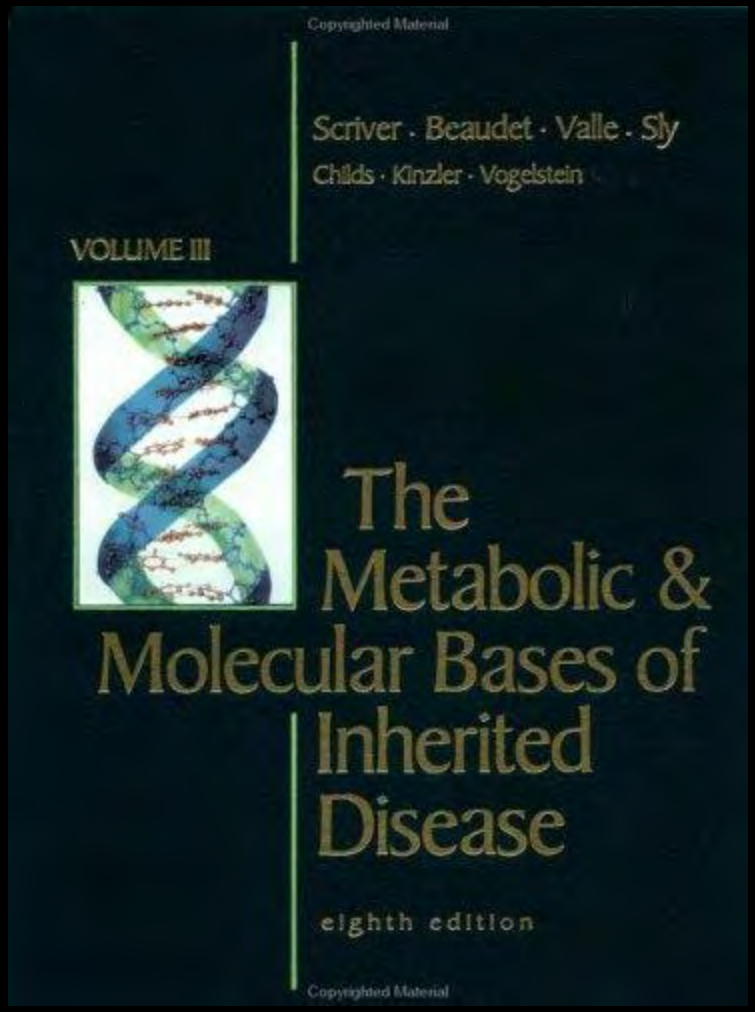
Disclosures

- Shunji Tomatsu has received the following grants.
- Morquio Foundations and families: Scarlett Grifith, Bennett, A Cure for Roberts, and Morquio Conference.
- MPS Societies: Japanese, National, and Austrian.
- NIH grants: 1-R01-HD102545, NIH, NICHD, Tomatsu (PI), 1R01HD104814-01A1, NIH, NICHD, Langan, T.J. (PI), Role: Site-PI, R43HD114328-01, NIH, ACOSTA, WALTER (PI), Role: site PI, 1R43AR084638-01, NIH, MOUNZIH, KHALID (PI).
- Foundation of NIH: FNIH RFP NUMBER: 2022-BGTC-005 Tomatsu (PI).

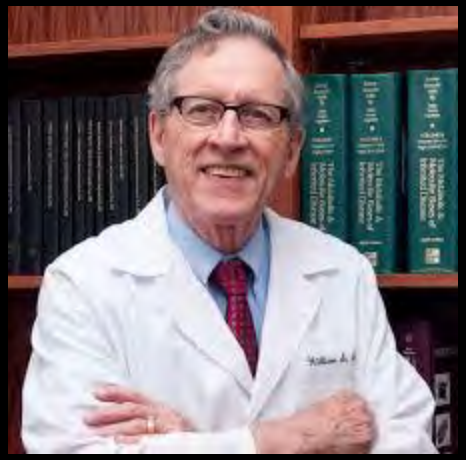
Learning OBJECTIVES

- At the conclusion of this activity, participants will be able to:

1. Define the clinical features of Morquio A (MPS IVA)
2. Describe the unmet challenge
3. Review AAV gene therapy for MPS IVA in mouse models
4. Design the clinical trial for MPS IVA



1973: Sly described the first case of mucopolysaccharidosis VII, a rare genetic condition that now bears his name.
1990: The Overexpressed Human 46-kDa Mannose 6-Phosphate Receptor Mediates Endocytosis and Sorting of β-Glucuronidase
1991: He was a member of the National Academy of Sciences.



William S. Sly, MD
October 19, 1932 - May 31, 2025

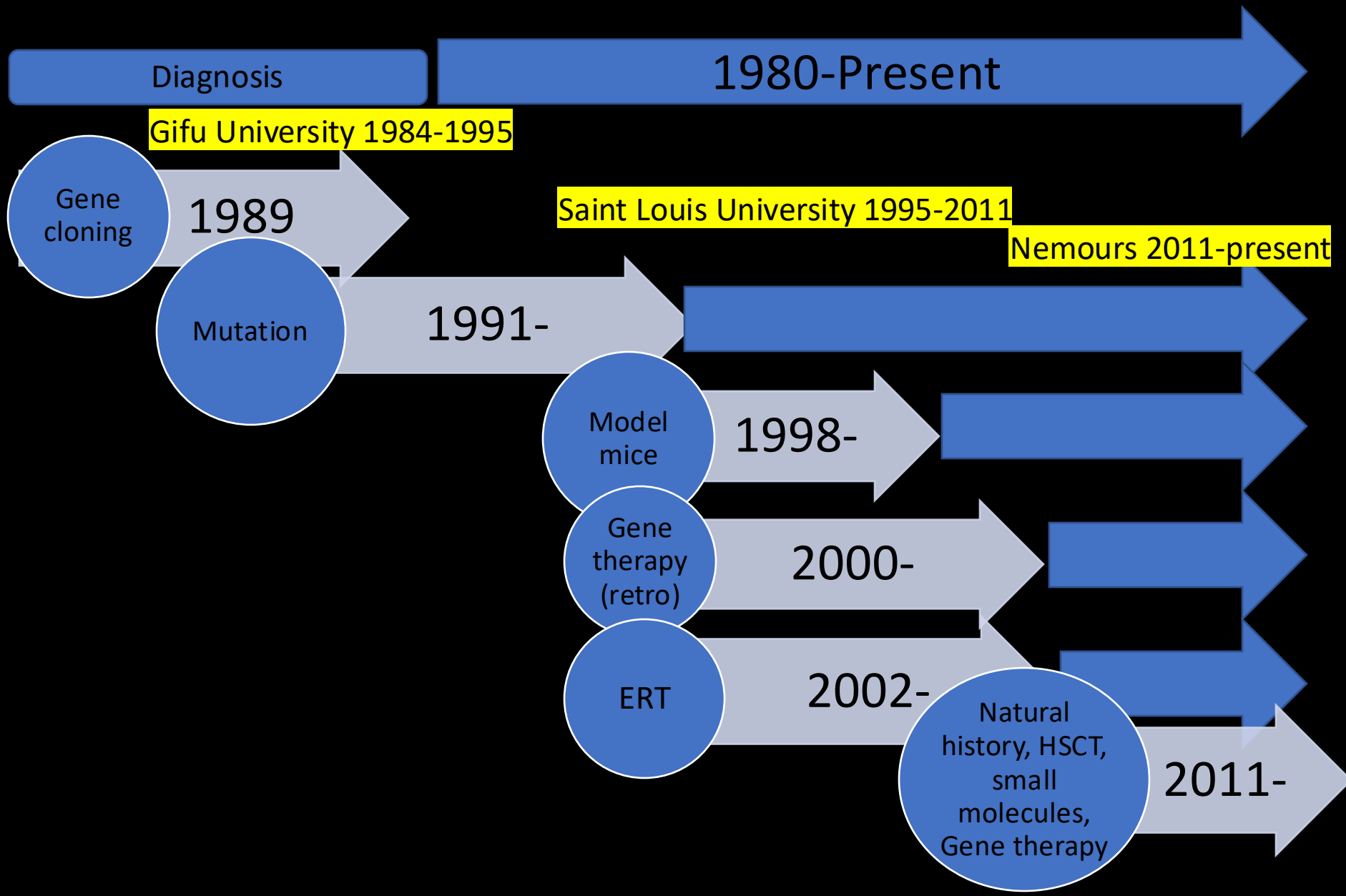
27,379	394	87
Citations by ***14,681 documents***	Documents	*h*-index View *h*-graph

Research field

- Mucopolysaccharidoses; 1 out of 25,000 births
- Especially, Mucopolysaccharidosis IVA; 1 out of 250,000 births
- Accumulation of undegraded metabolites in lysosomes.
- Systemic skeletal deformities and/or early death if untreated.
- Available therapy: enzyme replacement therapy or hematopoietic stem cell transplantation.
- For maximum benefit of available therapies, early detection and intervention.

Goal: Establishment of diagnosis, biomarkers, screening, and treatment

Research History for Morquio A

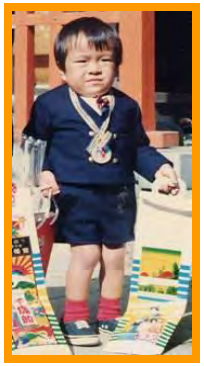


Disease progression: MPS IVA

1-2 months

1 year

2 years



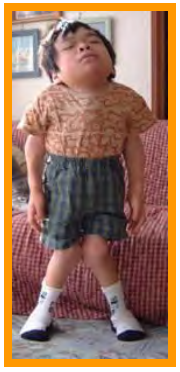
3 years

6 years

7 years

8 years

11 years

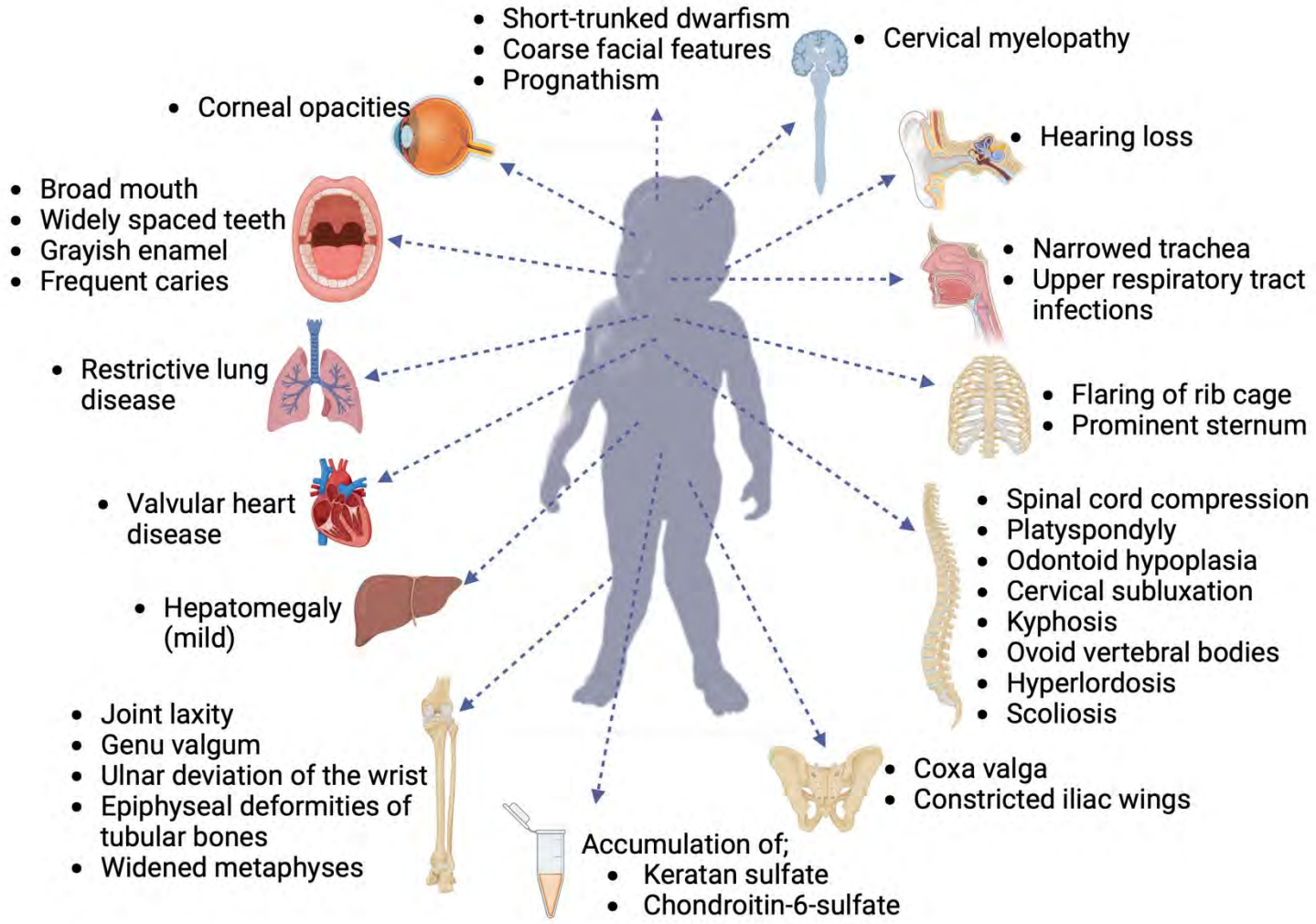


16 years

16 years

Yasuda et al. Mol. Genet. Metab. 2013

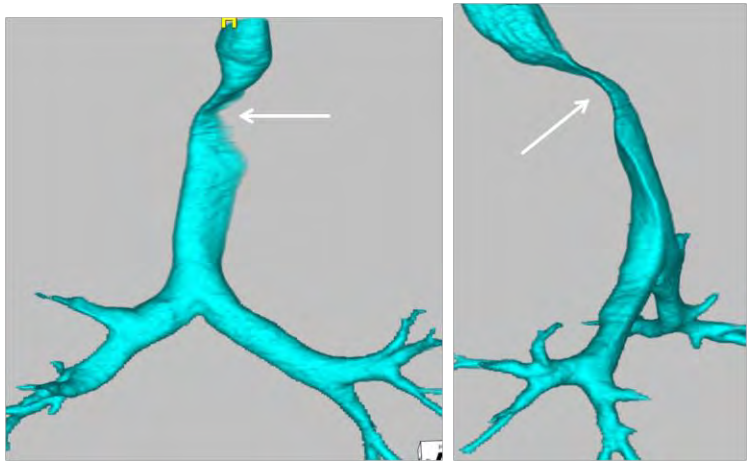
Clinical Manifestations: Imbalance of growth



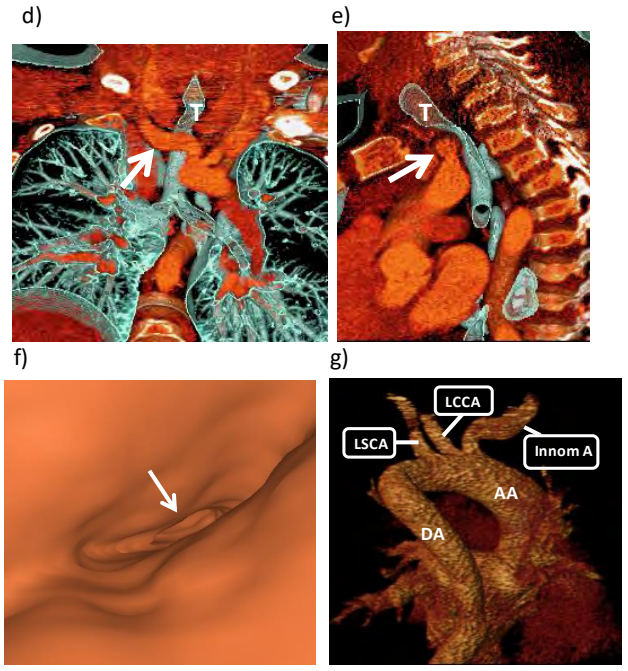
Tracheal Obstruction: Imbalance of growth

Life Threatening: under 120 cm
ERT does not impact the growth!

Compression of trachea



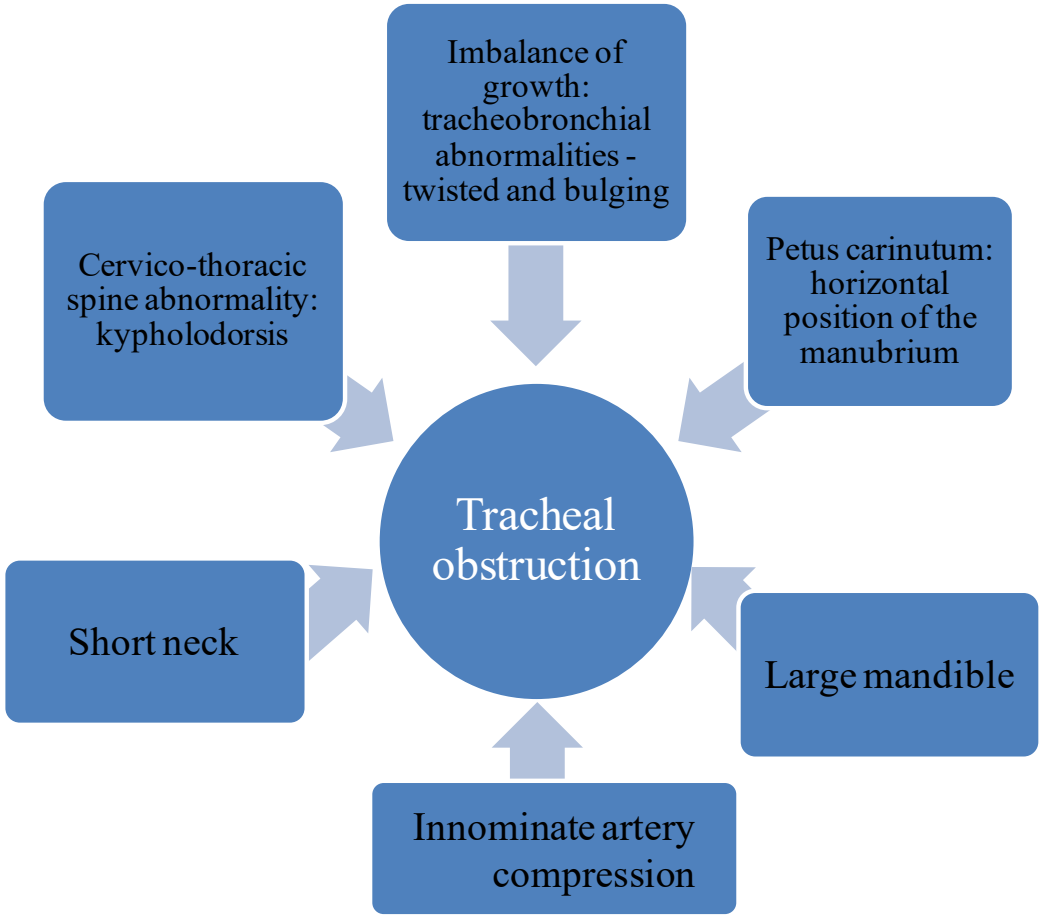
Thoracic inlet is crowded



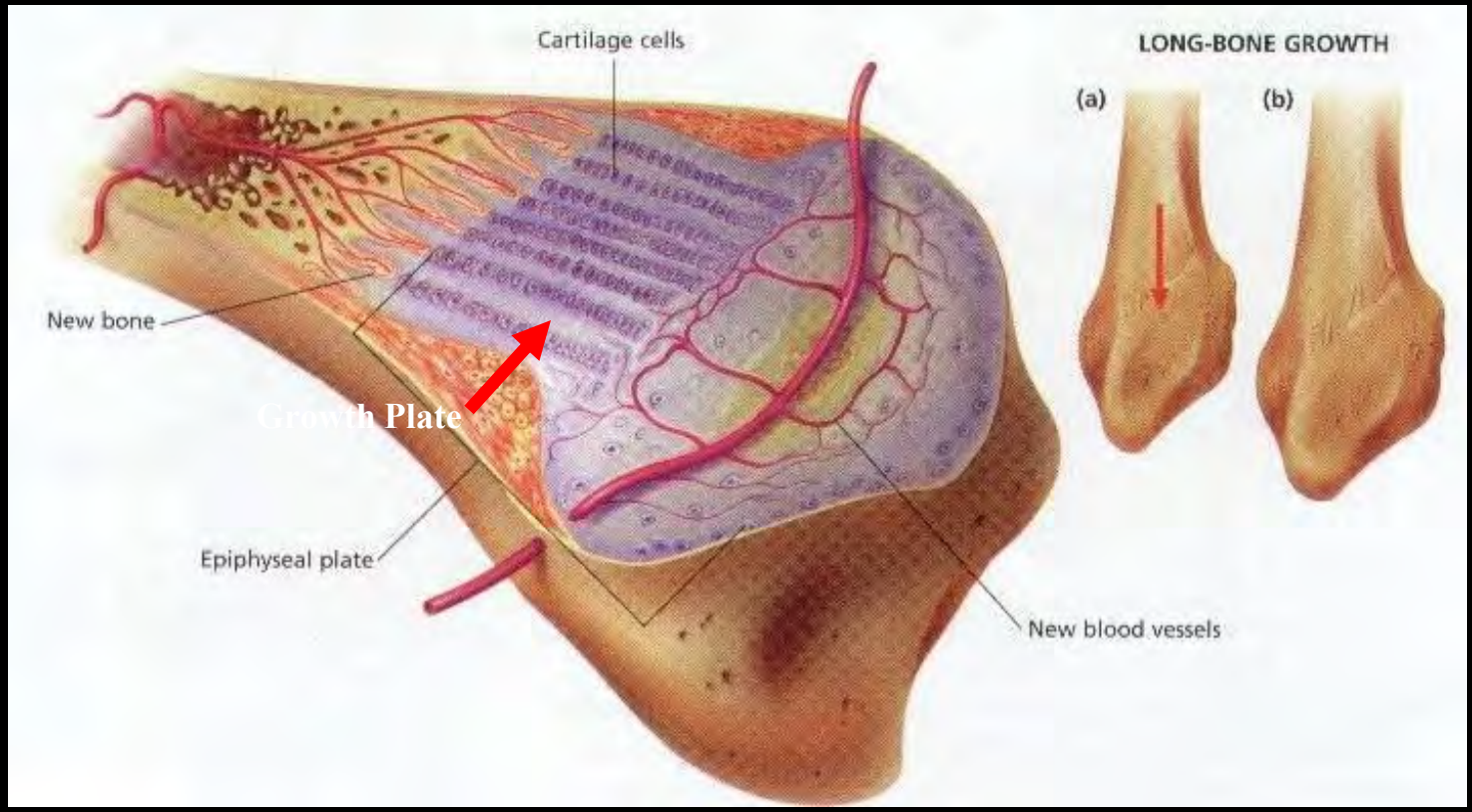
Over 25 cases underwent tracheal surgery.

Pizarro et al Ann Thorac Surg. 2016

Mechanism of tracheal obstruction



How can we reach the bone? Why is it difficult?



No Vessels in Cartilage Layers!

Therapies for bone in MPS IVA

1. Enzyme Replacement Therapy (ERT)
2. Hematopoietic stem cell replacement (HSCT)
3. Gene therapy (AAV, lentivirus, CRISPR/Cas9 gene editing)
4. Small molecule
5. Anti-inflammatory drug
6. Orthopedic surgical procedure
7. Tracheal reconstructive surgery

Discussion: Timing of therapy, biomarker for bone disease

1st Morquio Conference

(July 19-21, 2012: Nemours at Wilmington, DE)

Aim

No treatment is effective for MPS IVA patients with bone abnormalities.

To improve bone pathology in MPS IVA, we propose the various AAV vectors expressing the GALNS enzyme.

AAV Gene Therapy In Mucopolysaccharidosis IVA Murine Models

Dr. Khan

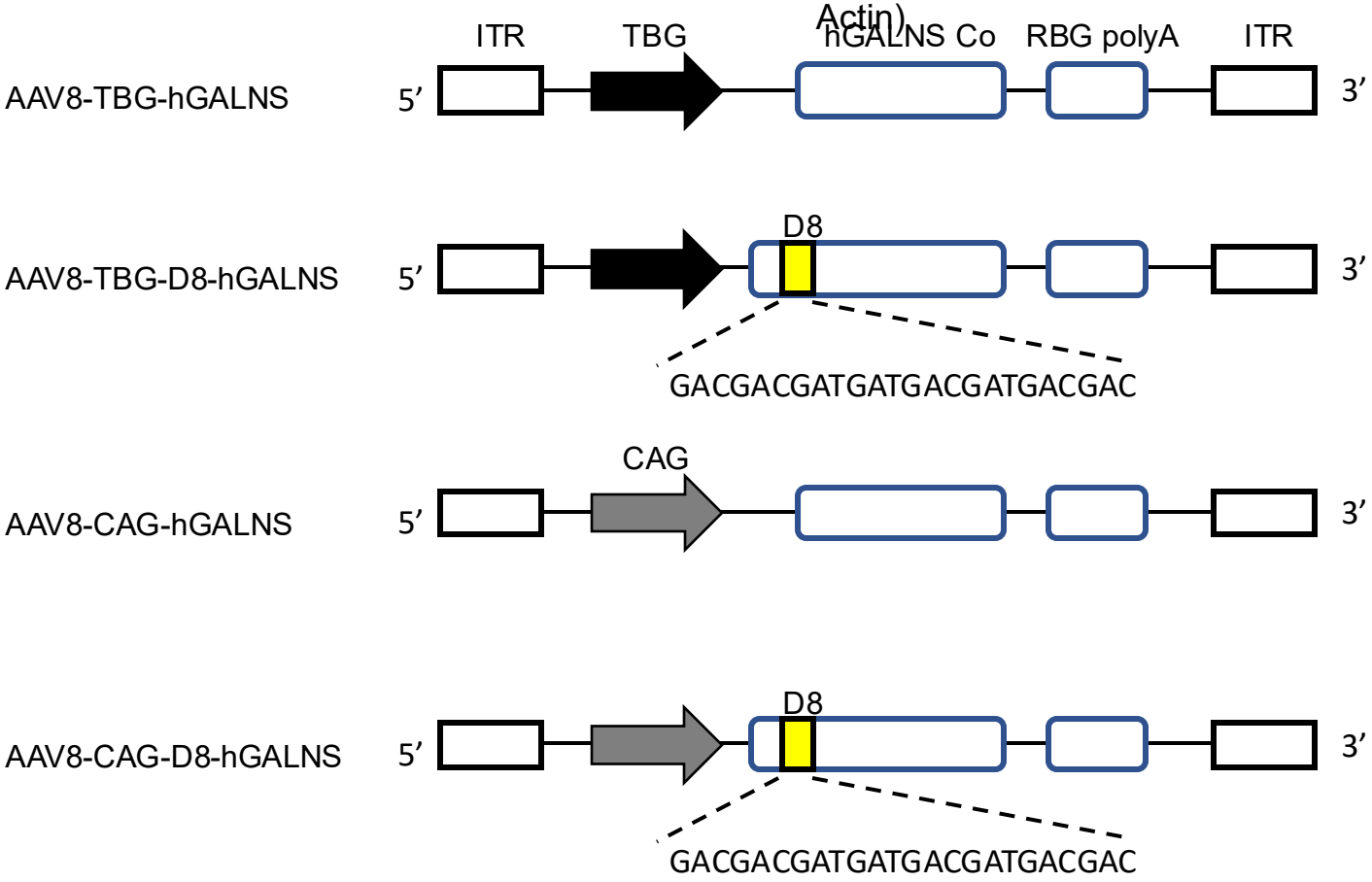
Sawamoto et al., Mol Ther: Methods & Clin Dev (2020);

Herreño-Pachón et al., Hum Gene Ther (2024);

Khan et al., Mol Ther: Methods & Clin Dev (2025)

Vector comparison

Promoter: Liver-specific TBG (thyroxine-binding globulin protein) or CAG (chicken beta-Actin)



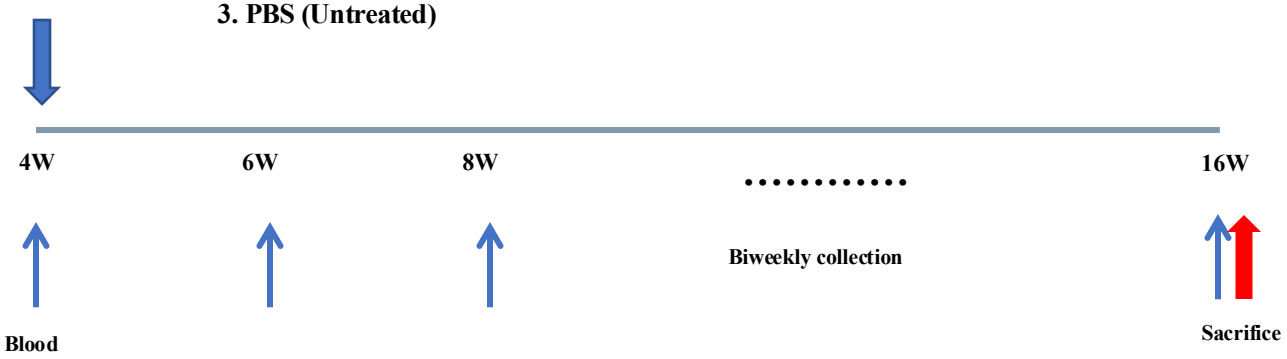
Sawamoto et al., Mol Ther: Meth & Clin Dev (2020); Herreño-Pachón et al., Hum Gene Ther (2024).

Study Design (*in vivo*)

Dose: 5x10^{13} GC/kg body weight (n = 4-8 per experiment)

i.v. injection:

1. **AAV8-hGALNS (TBG or CAG)**
2. **AAV8-D8-hGALNS (TBG or CAG)**
3. **PBS (Untreated)**

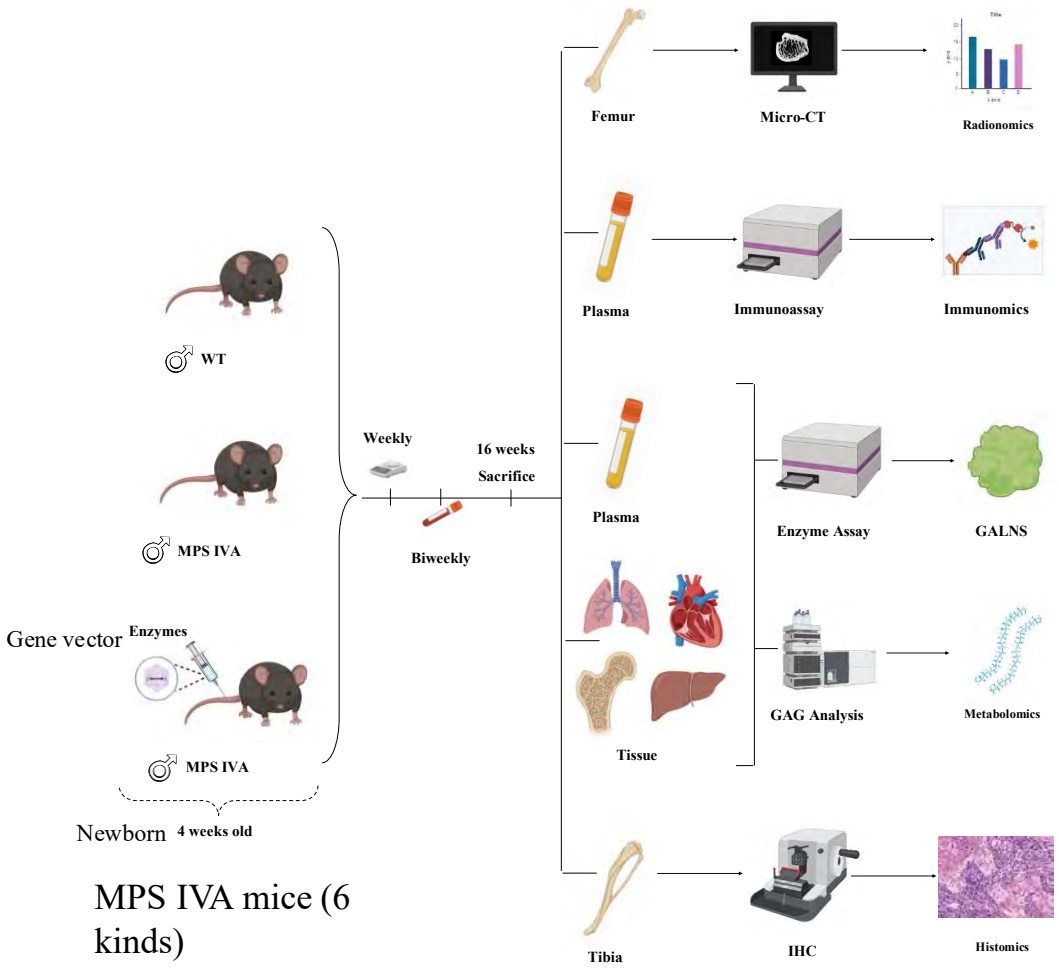


MPS IVA Mouse Models:

1. **GALNS$^{-/-}$ (KO)**
2. **GALNS$^{tm(hC79S.mC76S)slu}$ (Tolerant: MTOL)**

Sawamoto et al., Mol Ther: Methods & Clinical Development 2020

Standard therapeutic protocol for mice



MPS IVA mice (6 kinds)

Nidhi, Sai, Baya

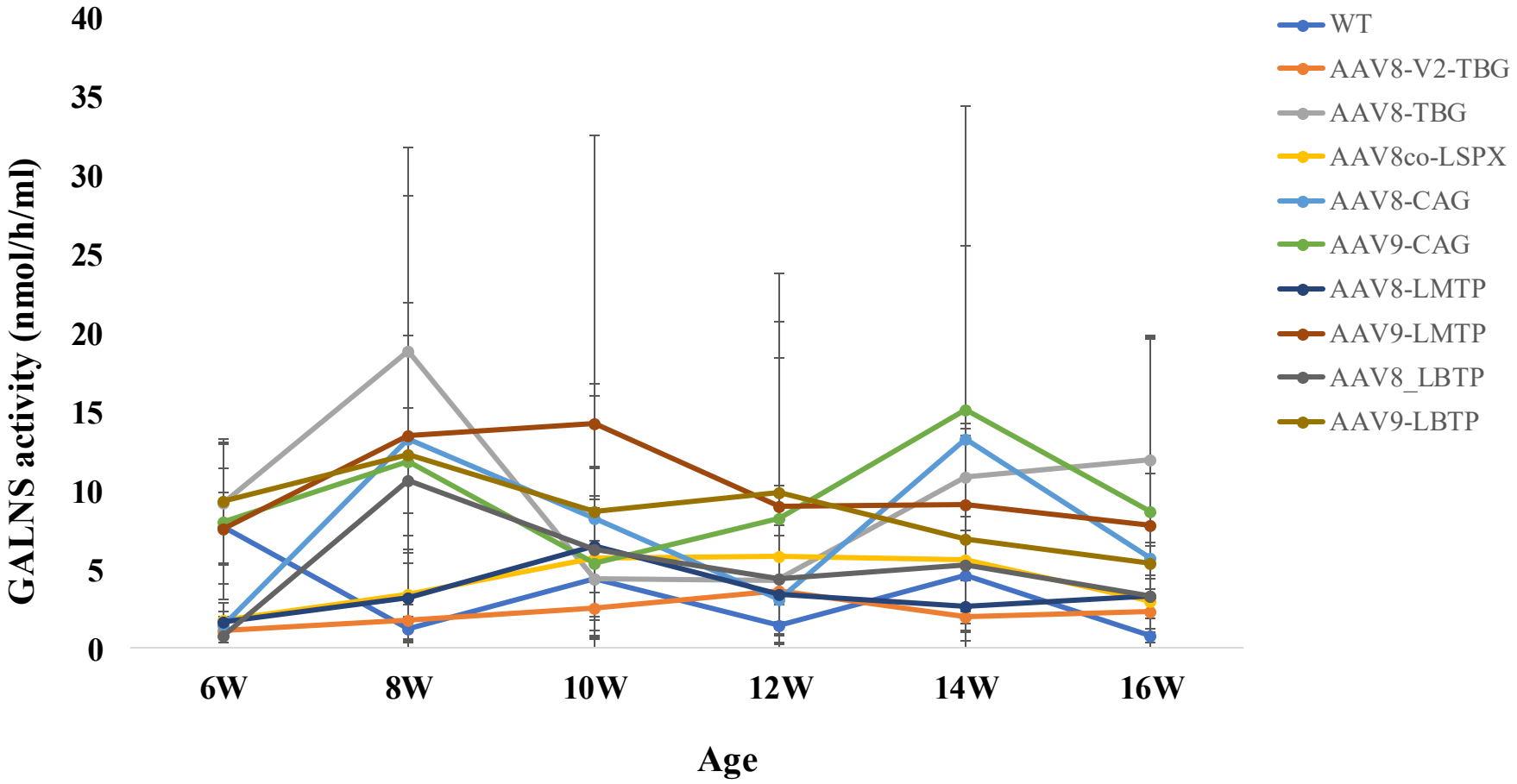
Each researcher

Each researcher

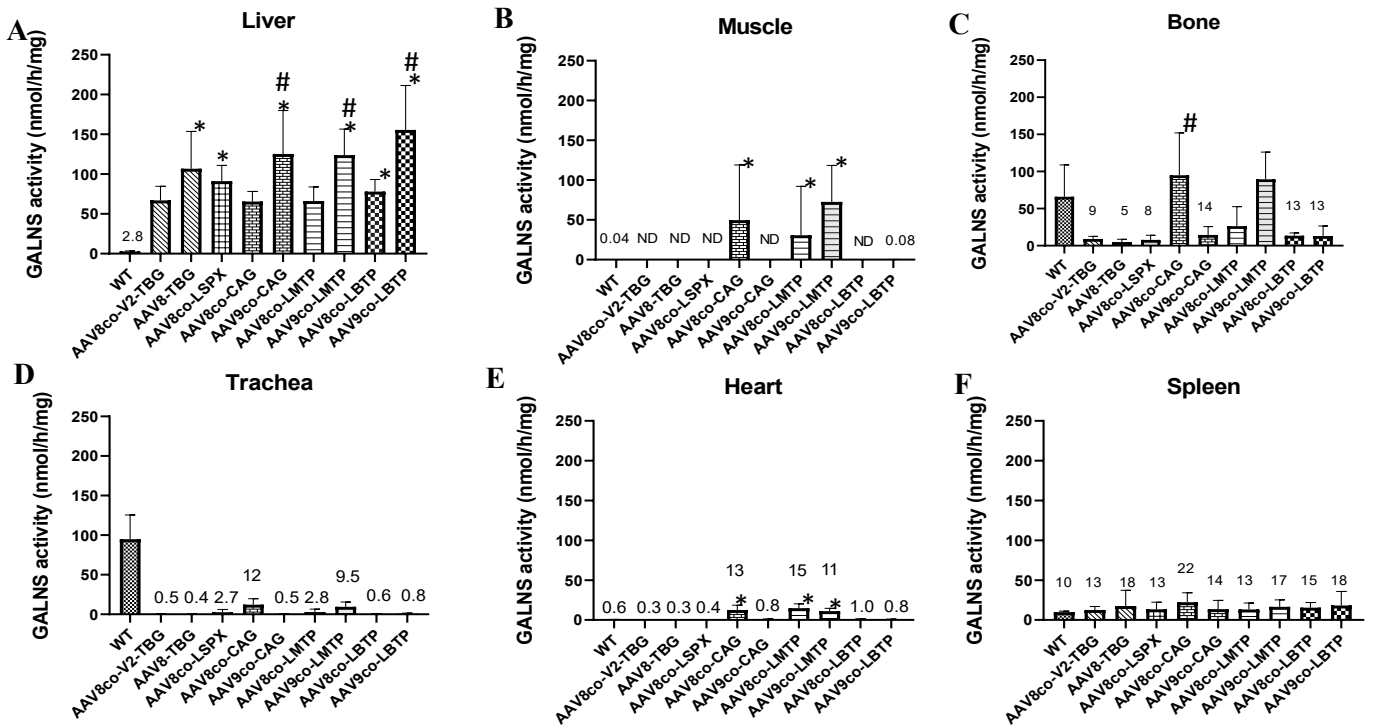
Khan, Benincore
Florez

Nidhi, Histology lab (Heather Hardy)

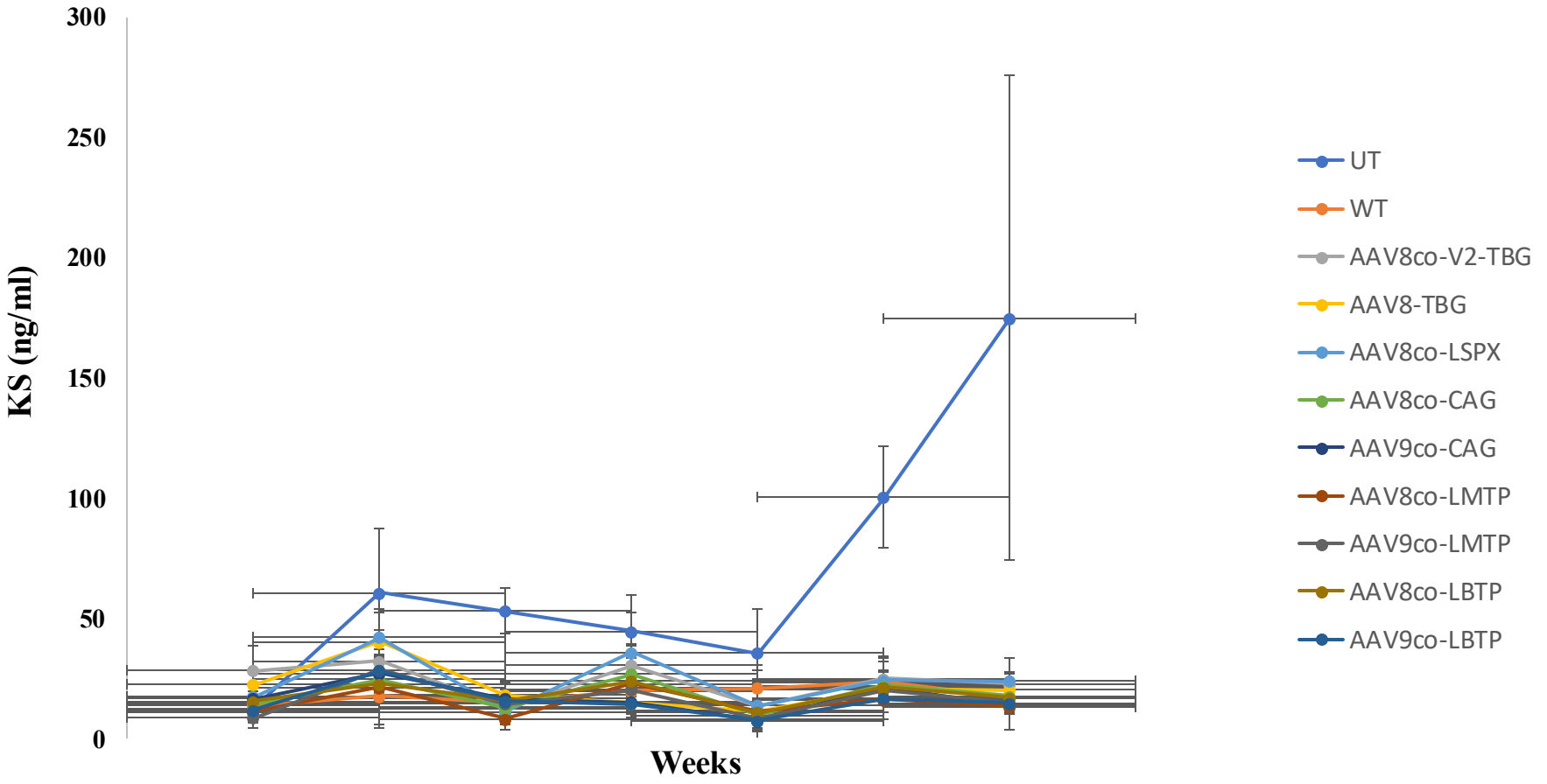
GALNS enzyme activity in plasma



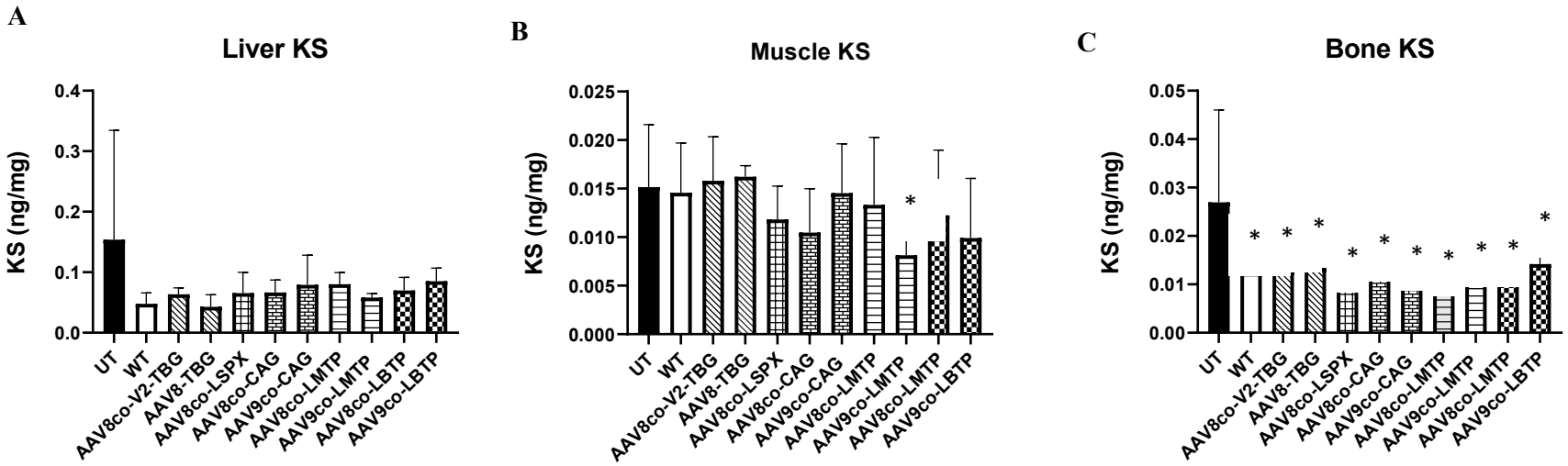
GALNS enzyme activity in tissues (16 weeks)



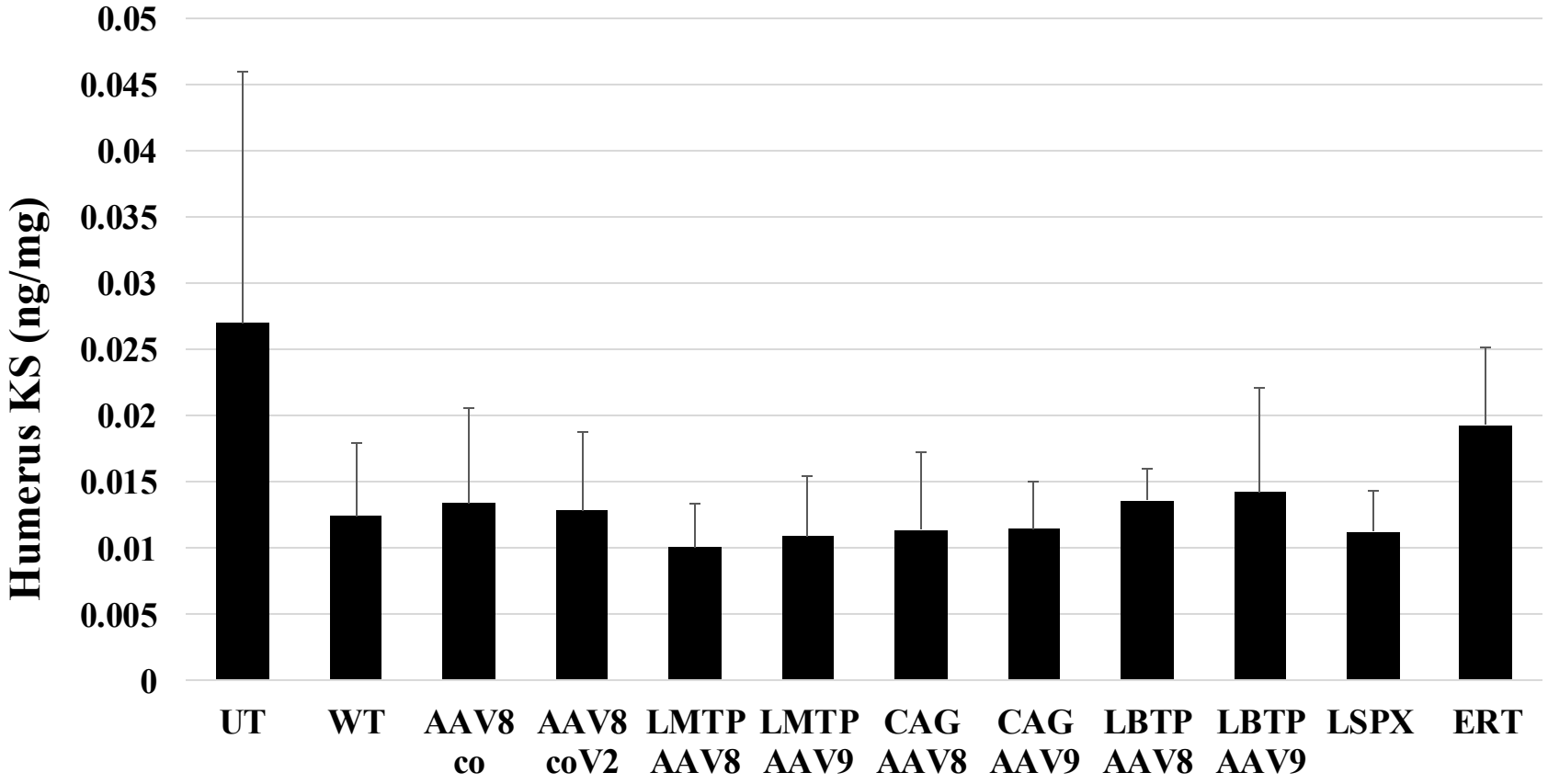
KS level in plasma (16 weeks)



KS level in tissues

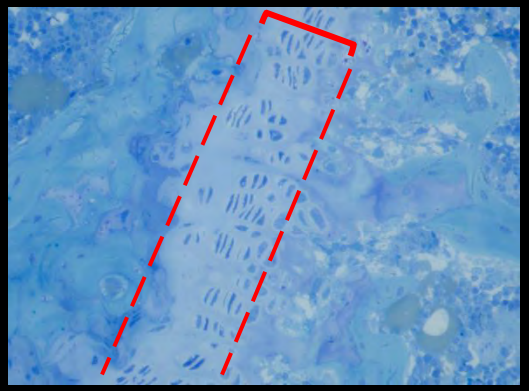


Humerus KS in AAV vectors

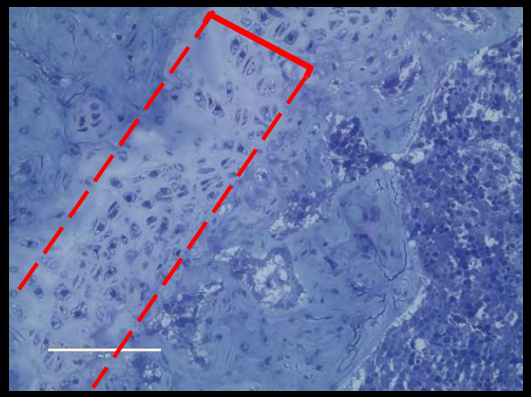


KO Femur Growth Plate

Images at 40X magnification



WT (16W)



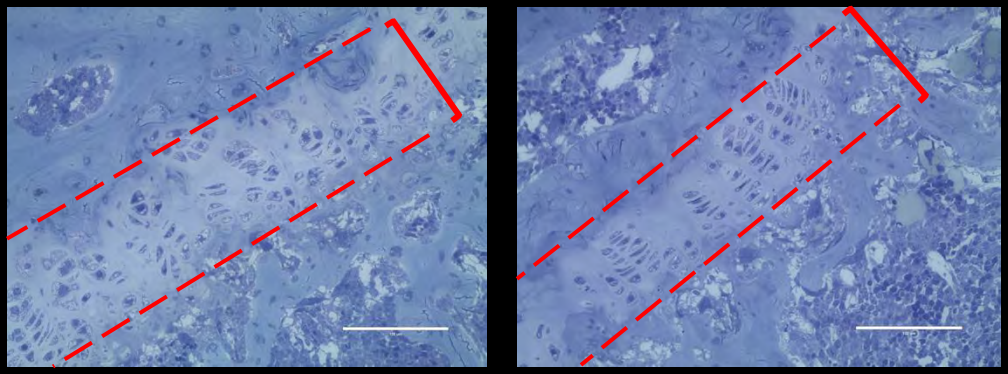
Untreated (16W)

WT: all Chondrocytes are non-vacuolated, and the column structure is well organized

Untreated: all Chondrocytes are vacuolated, and the column structure is largely disorganized and distorted

KO Femur Growth Plate

Images at 40X magnification

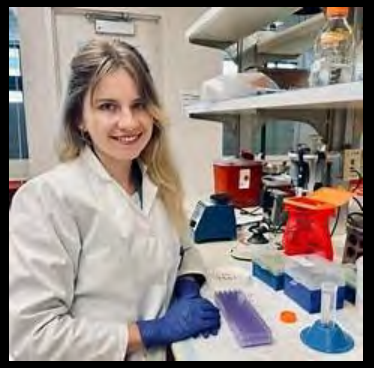


AAV8-hGALNS (16W) **AAV8-D8-hGALNS (16W)**

hGALNS: Chondrocytes are vacuolated and the column structure is moderately distorted

D8: Chondrocytes are moderately vacuolated, but the column structure shows greater recovery

Adeno-Associated Virus-Based Gene Therapy Delivering Combinations of Two Growth-Associated Genes



Dr. Rintz

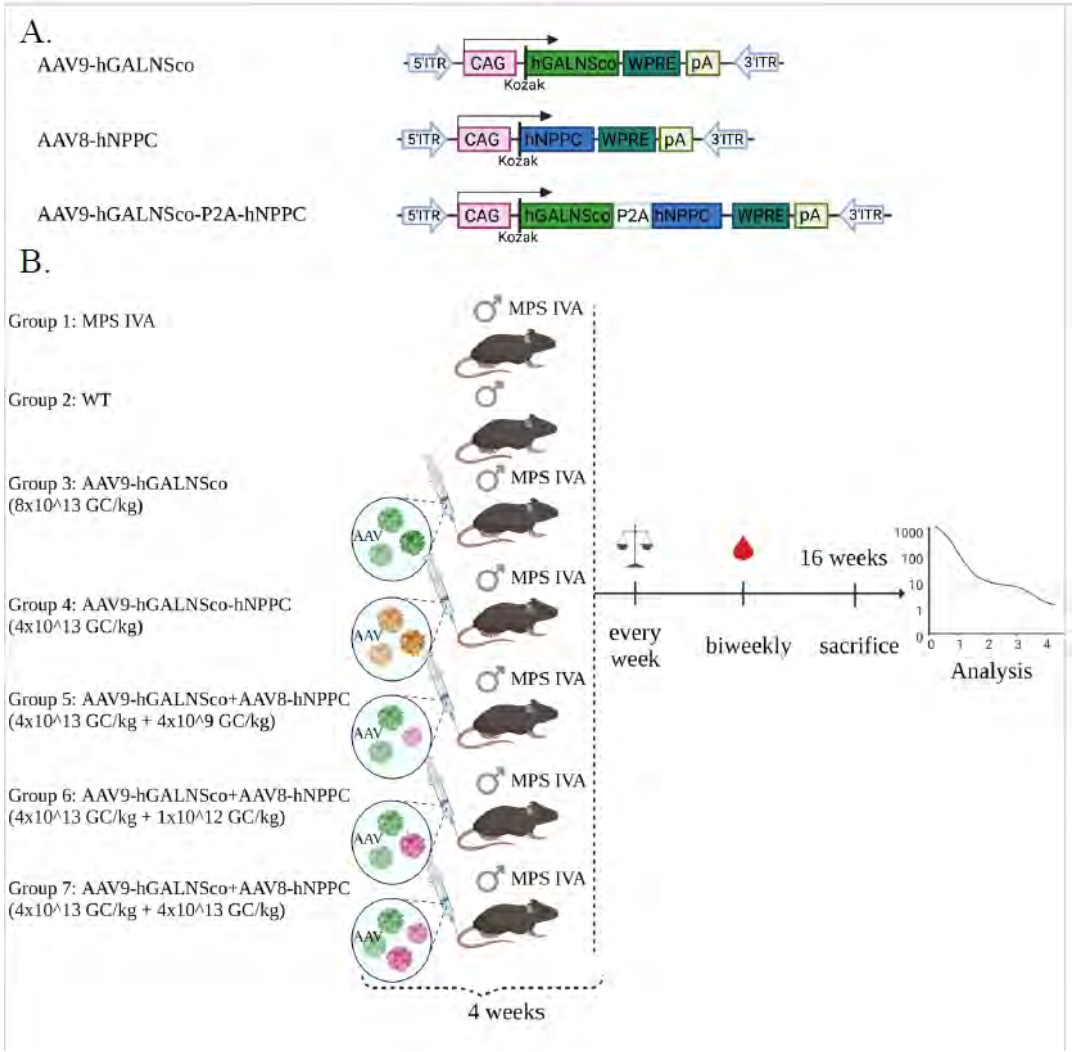
Rintz E et al. Mol Ther Nuc Acid 2024
Rintz E et al. Int J Mol Sci. 2023

Aim

• Improve bone pathology in MPS IVA, we propose a novel combination treatment with the AAV vectors expressing GALNS enzyme and a C-type natriuretic peptide (CNP; NPPC gene) as a growth-promoting agent.

- Rintz et al., Mol Ther Nucleic Acids (2024)

Experimental design

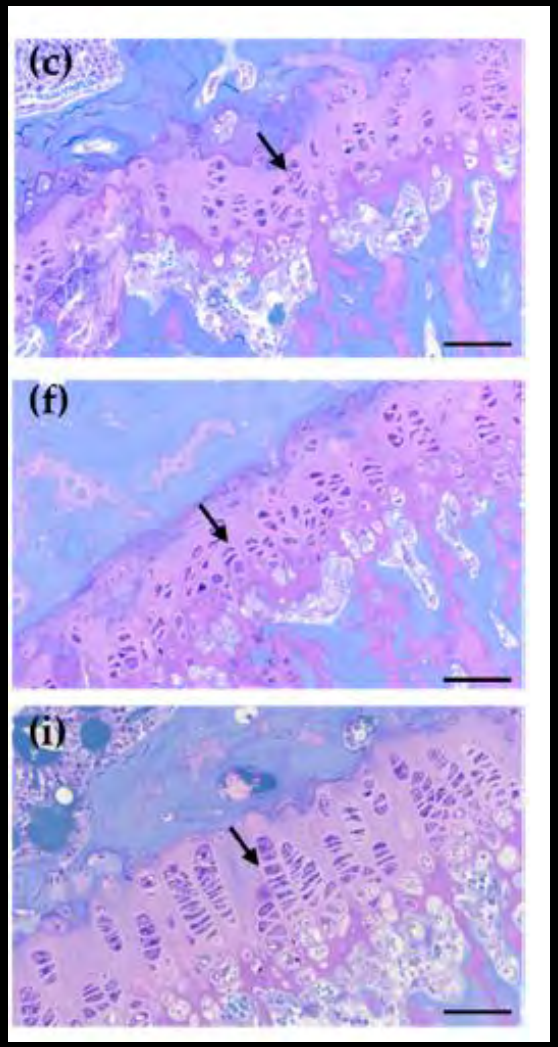


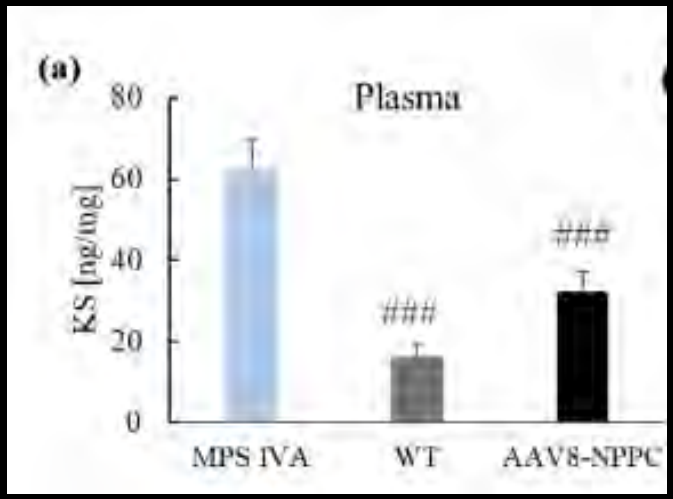
Correction of bone pathology (Tibia growth plate)

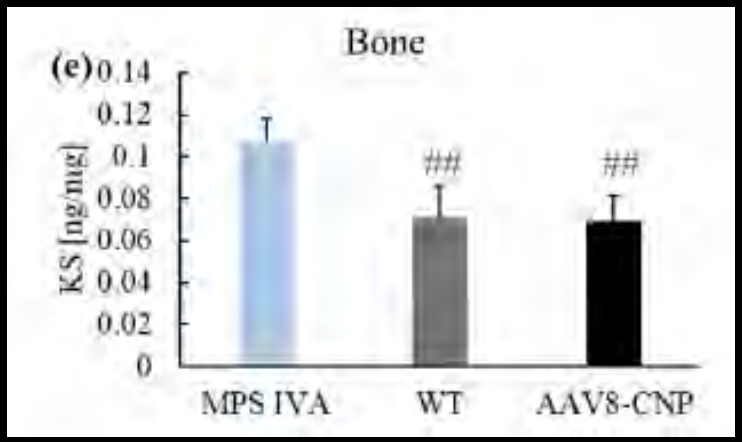
MPS IVA

WT

AAV8-CNP

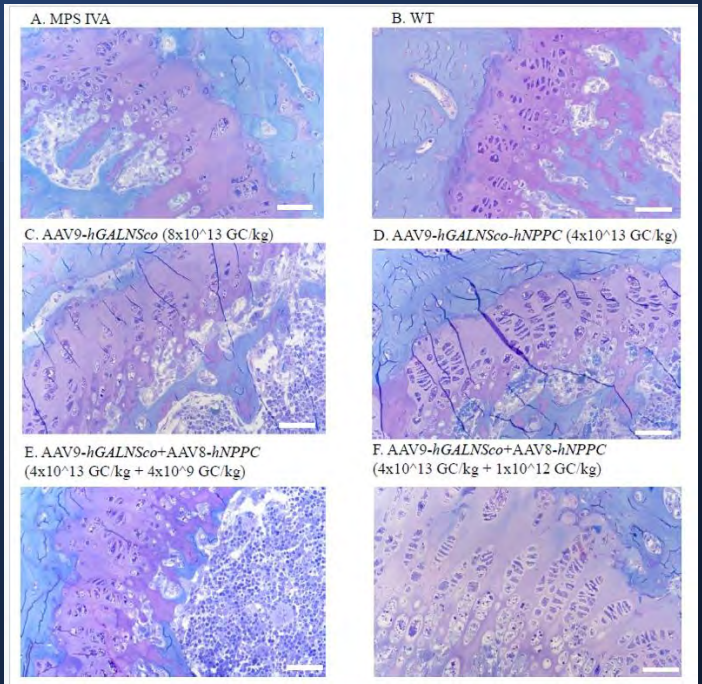


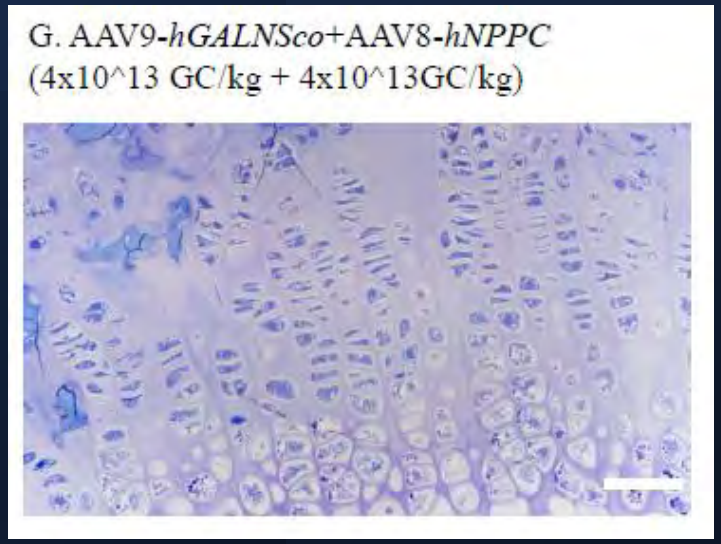




Rintz E et al. Int J Mol Sci. 2023

Correction of bone pathology (Femur growth plate)





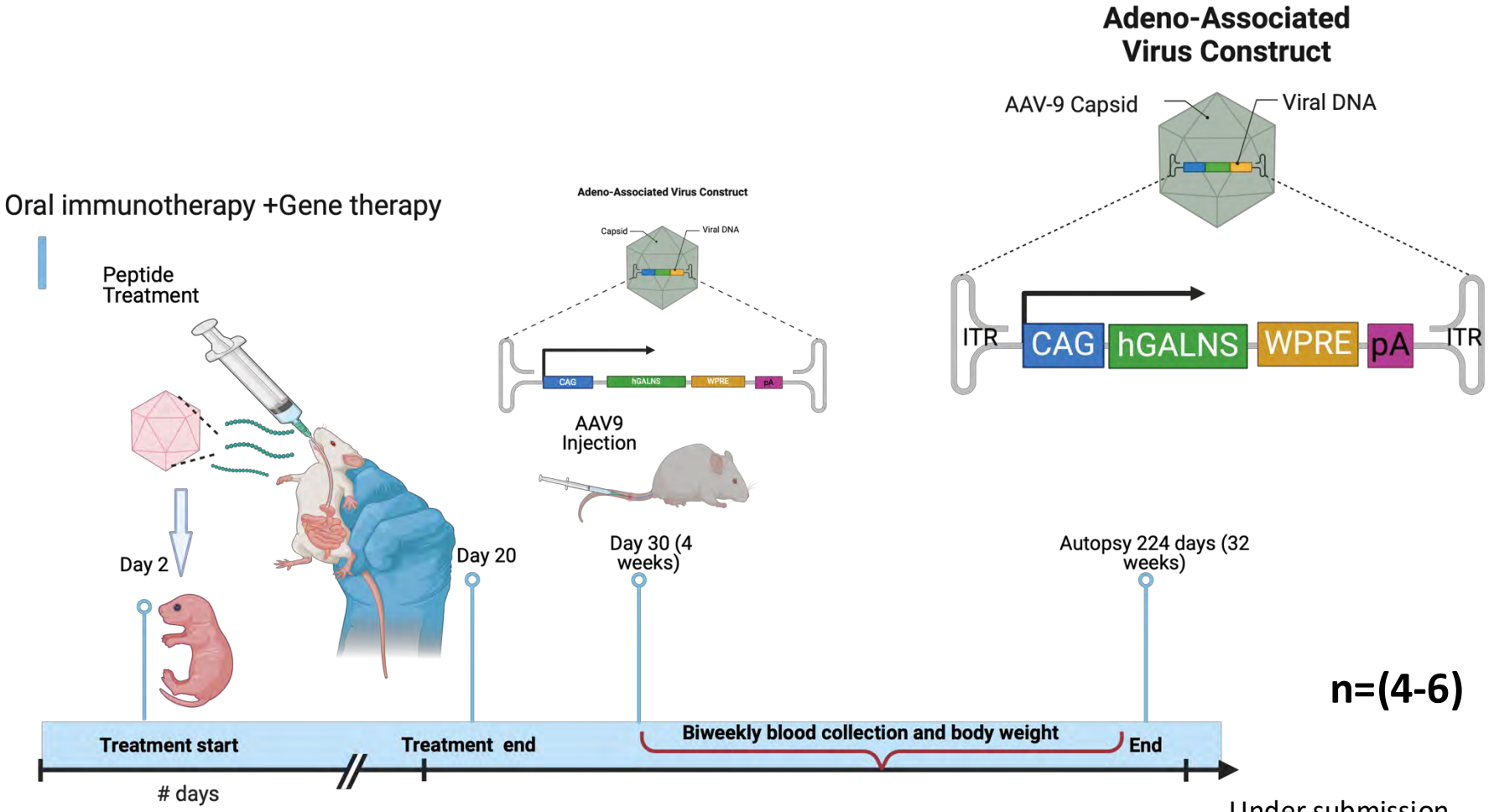
Rintz et al., Mol Ther Nucleic Acids (2024)

Immune modulation for AAV9 gene therapy by oral administration of peptides or GALNS for MPS IV A

Sampurna Saikia

PhD student

Schematic Illustration of Research



n=(4-6)

Under submission

Peptide groups showed the highest enzyme activity in plasma correlated with null anti-GALNS IgG

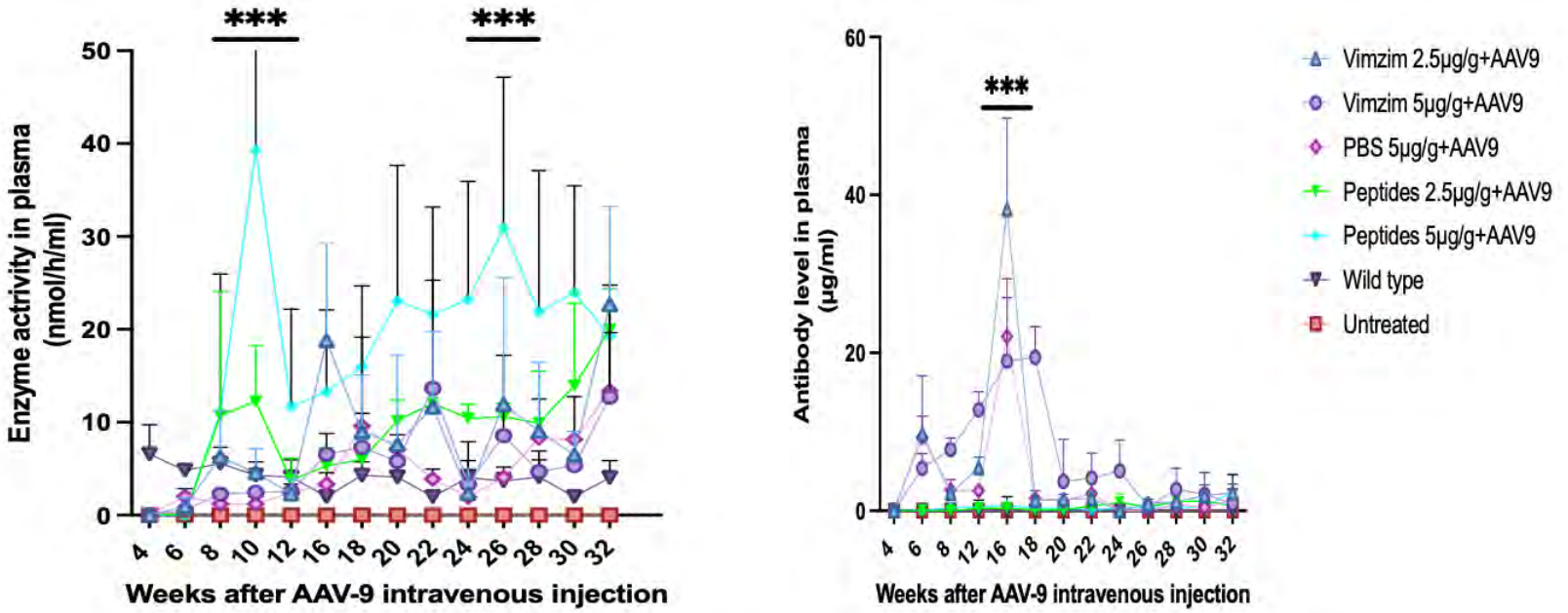


Figure 1: A) Enzyme activity in plasma over time; B) Anti-GALNS IgG in Plasma

Under submission

Aim

- Fetal therapy via AAV vector delivered Fc-fusion proteins

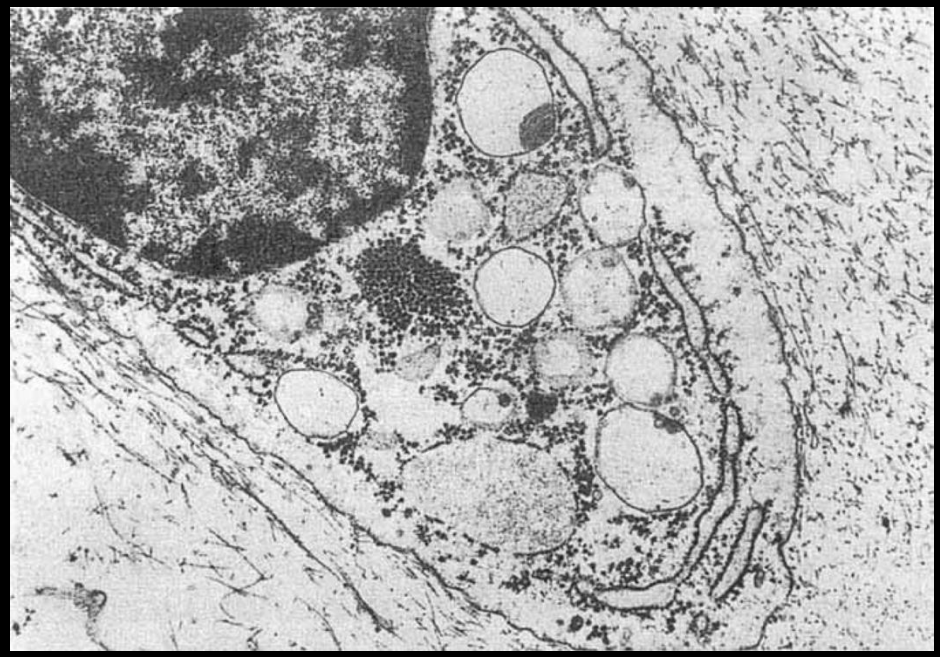
Amali Karunathilaka, PhD student

Fetal Therapy

The small size of the fetus allows one to maximize the dose per recipient weight

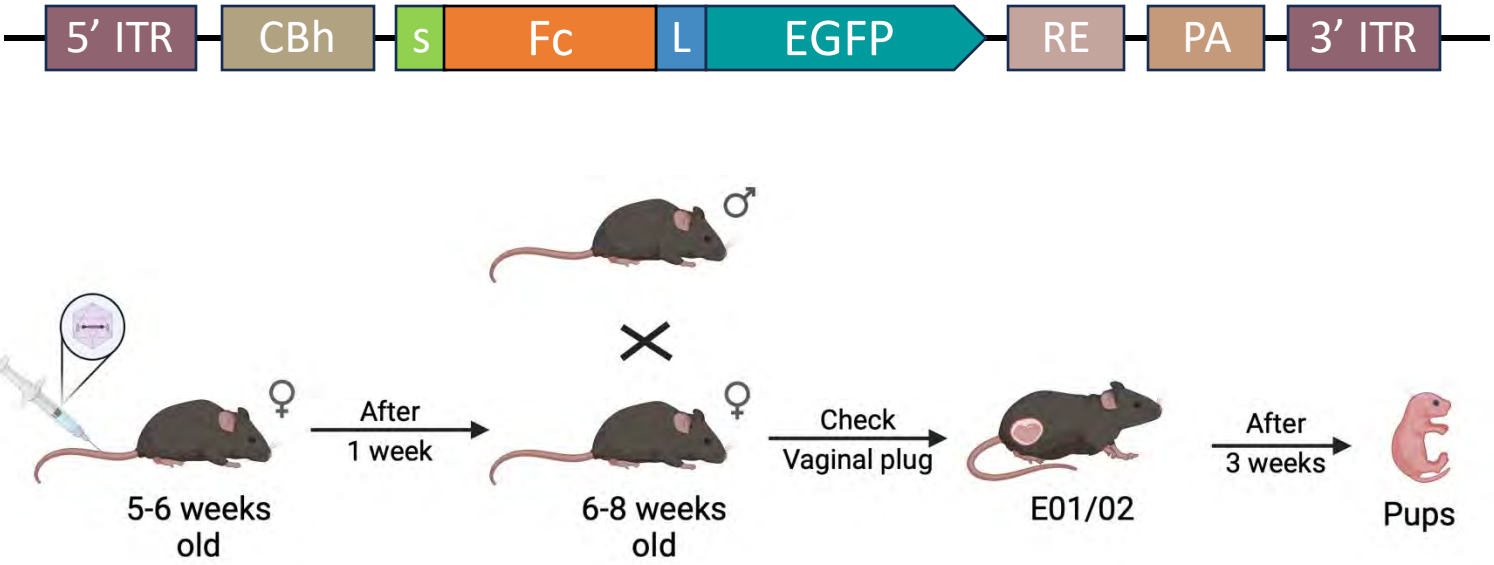
The immune system of the fetus is prone to a tolerogenic phenotype

The accessibility of different organs/cells



Beck et al. 1992

Transplacental ability of Fc-fusion proteins



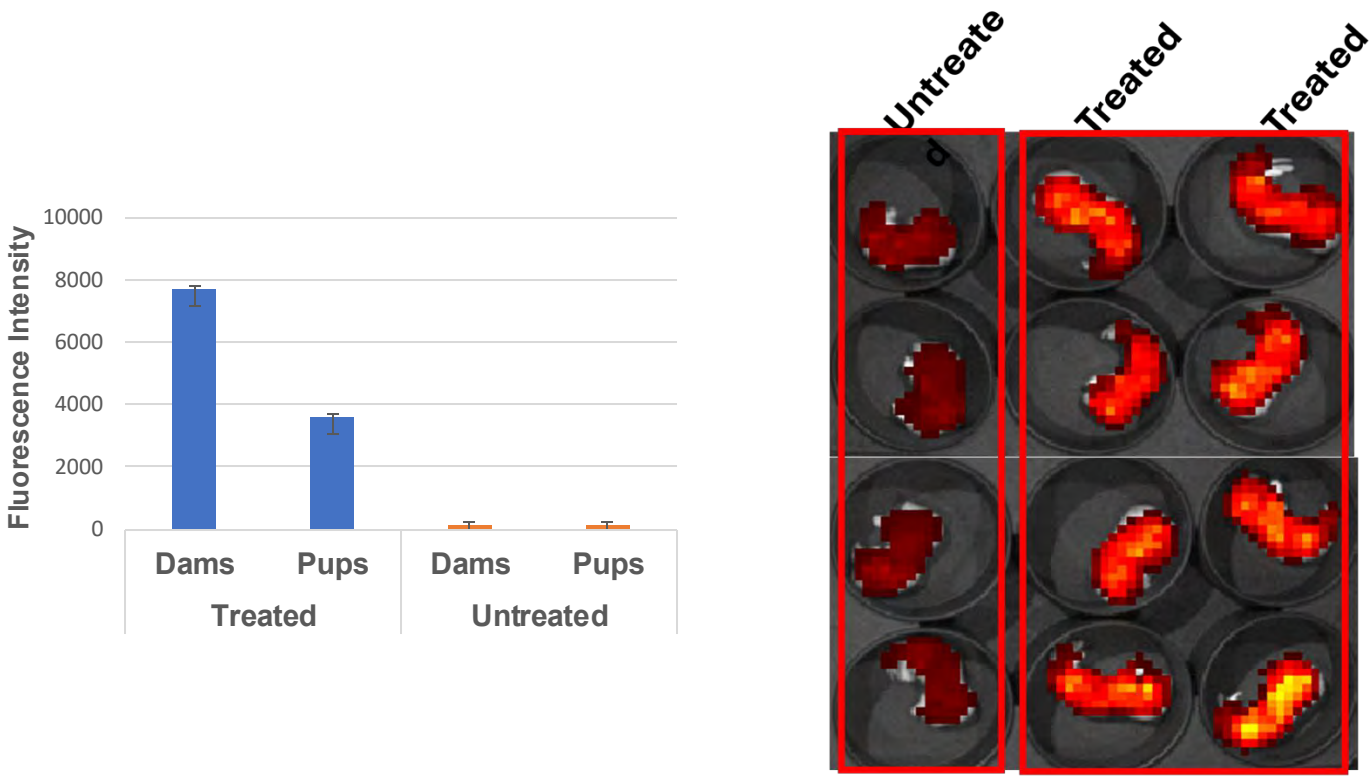


Figure 9: GFP detection in treated pups. A) Fluorescence intensity in plasma, B) IVIS imaging of treated and untreated pups.

AAV gene therapy Summary

- **Greater efficacy in reducing bone lesions of MPS IVA mouse models**
  - GALNS enzyme activity level in blood was maintained at high levels during this study period
  - KS levels in the blood were immediately normalized
  - Bone and heart pathology were improved
- **Reduction of blood KS and improvement of bone pathology were more significant in AAV gene therapy than in weekly ERT**
- **Move to a clinical trial funded by the Foundation of NIH.**

Wait for new therapies

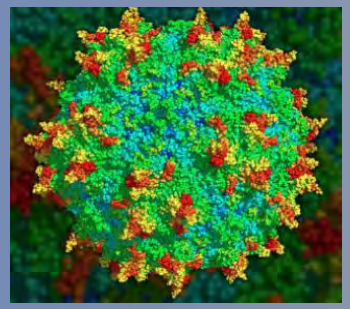
- Scarlett Griffith Fundraising Party (Woodbridge, NJ: 2025)

Clinical trial

- FNIH RFP NUMBER: 2022-BGTC-005
- AAV Gene Therapy for Mucopolysaccharidosis IVA
- Protocol NEM101
- PIs; Dr. S. Mackenzie, Dr. S. Tomatsu
- Announced on May 16, 2023 at American Society of Gene and Cell Therapy

Accelerating Medicines Partnership®
Bespoke Gene Therapy Consortium (BGTC)



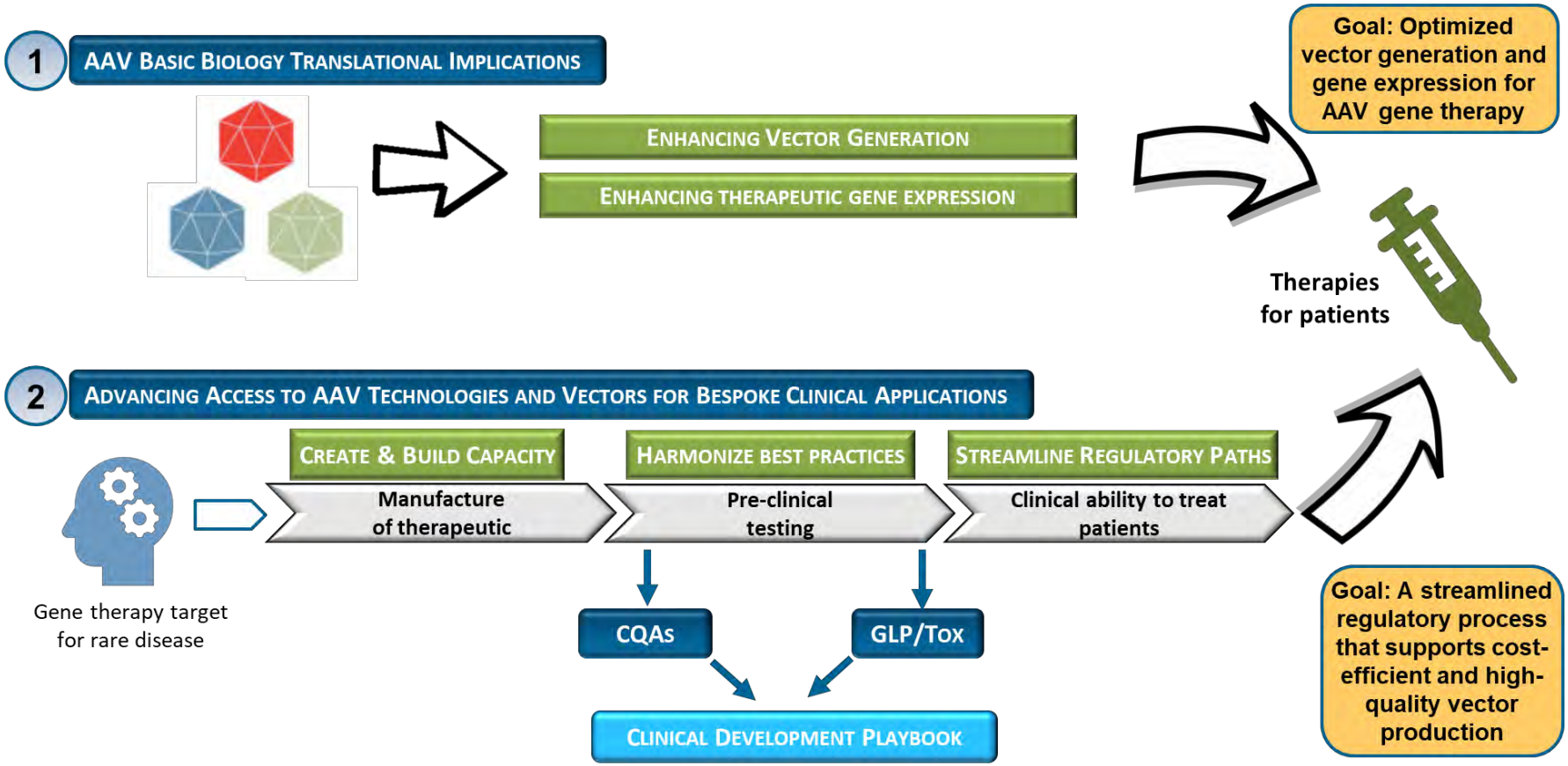



Clinical Subteam co-chairs
Susana Serrate-Sztein, MD (NIAMS/NIH)
Ed Neilan, MD, PhD (NORD)

Program Management
Courtney Silverthorn, PhD (FNIH)
Brad Garrison, MBA (FNIH)

FNIH
Foundation for the
National Institutes of Health

Objectives


1
AAV Basic Biology Translational Implications
Enhancing Vector Generation
Enhancing therapeutic gene expression
Goal: Optimized vector generation and gene expression for AAV gene therapy
Therapies for patients
2
Advancing Access to AAV Technologies and Vectors for Bespoke Clinical Applications
Create & Build Capacity
Harmonize best practices
Streamline Regulatory Paths
Manufacture of therapeutic
Pre-clinical testing
Clinical ability to treat patients
Gene therapy target for rare disease
CQAs
GLP/Tox
Clinical Development Playbook
Goal: A streamlined regulatory process that supports cost-efficient and high-quality vector production

BGTC combines resources from a broad set of public and private partners

- National Center for Advancing Translational Sciences
- *Eunice Kennedy Shriver* National Institute of Child Health and Human Development
- National Eye Institute — Research Today...Vision Tomorrow
- National Heart, Lung, and Blood Institute
- National Human Genome Research Institute
- National Institute of Arthritis and Musculoskeletal and Skin Diseases
- National Institute of Dental and Craniofacial Research
- National Institute of Mental Health
- National Institute of Neurological Disorders and Stroke
- National Institute on Deafness and Other Communication Disorders

BRAIN INITIATIVE

FDA

$39.5M *Public commitments*

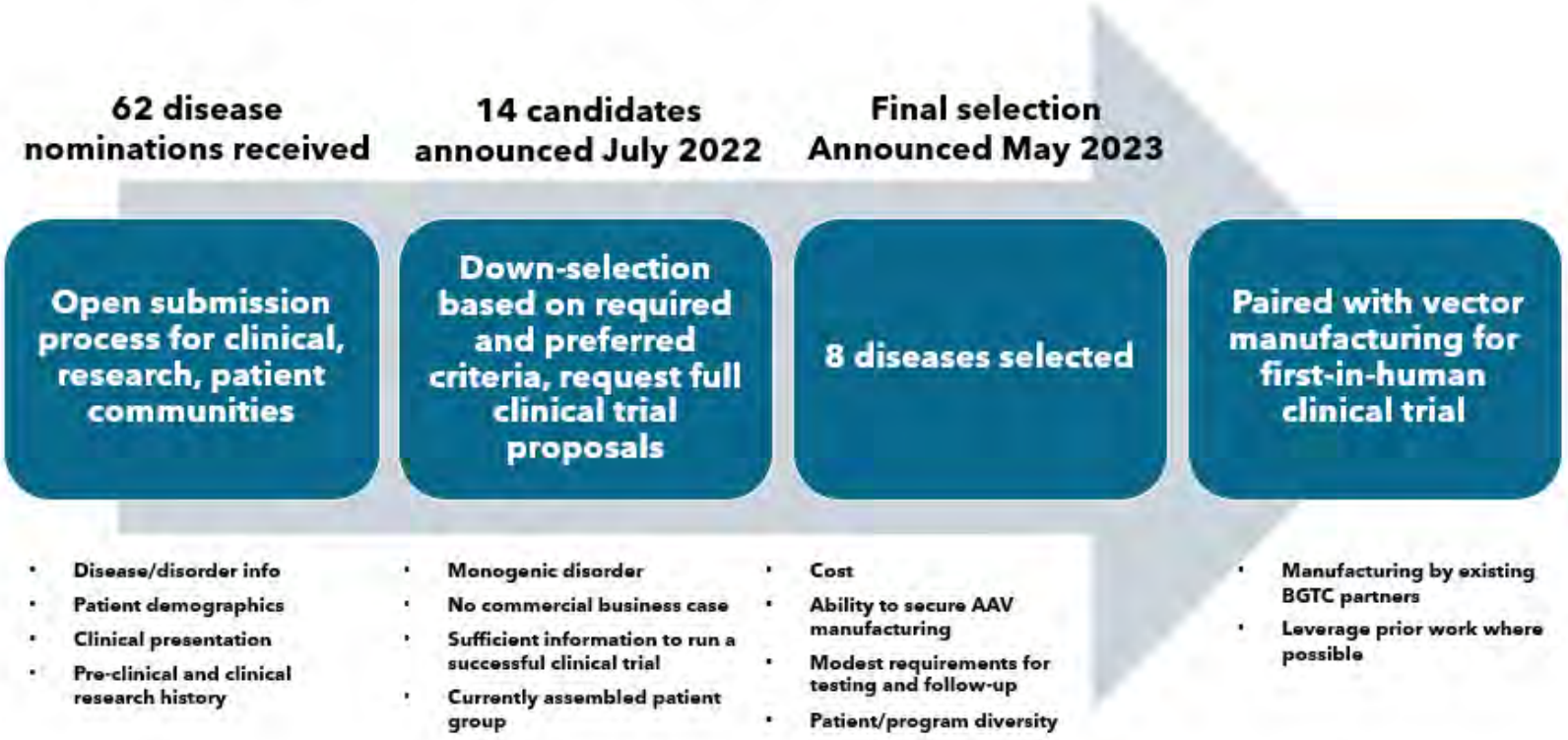
$35.7M *Private donations*

$26.2M+ *Private in-kind contributions*

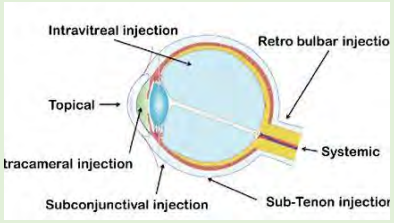
FNIH

FNIH
Foundation for the National Institutes of Health

Disease Selection Process



Clinical portfolio approved by the Steering Committee



Ocular

- Congenital Hereditary Endothelial Dystrophy (CHED)
- Retinal Degeneration (NPHP5)
- Retinitis pigmentosa 45 (CNGB1)

Neurological

- Multiple Sulfatase Deficiency
- Charcot Marie Tooth disease type 4J
- Spastic Paraplegia type 50

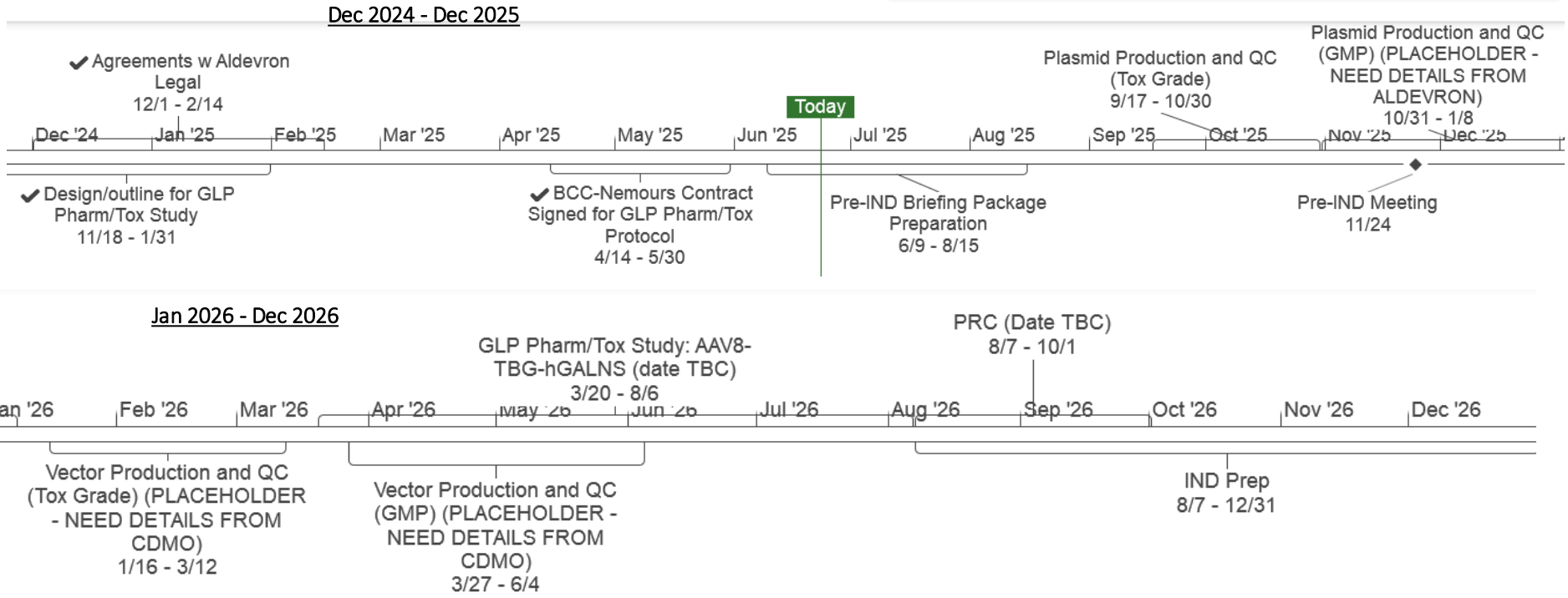
Systemic

- Propionic Acidemia
- Mucopolysaccharidosis IVA

Morquio A Current Timeline

Critical Tasks:

- Tox-grade material manufacturing for GLP pharm/tox study
- Initial draft GLP pharm/tox study protocol



Note: Pre-IND and IND timeline may be accelerated by foregoing the dose-range finding study – to be reevaluated upon manufacturing and GLP study start

Lab members

- Shaukat Khan (Assistant Professor)
- Yasuhiro Ago (Post doc)
- Andrés Felipe Leal Bohórquez (Post doc)
- Betul Celik (PhD student)
- Nidhi (PhD student)
- Sampurna Saikia (PhD student)
- Amali Karunathilaka (PhD student)
- Angelica Maria Herreño Pachon (PhD student)
- Georgina Neema Baya (PhD student)
- Dione (PhD student)
- Krishna Sai Musini (master student)
- Eliana Patricia Benincore Flórez (research assistant)
- Allison Bradford (research assistant)
- Lan He (Lab manager)

Rooting For Robert

- Support the stipends for the graduate students
- Establish new mouse and rat models.
- Develop Novel therapies including Stem Cell Therapy and Gene Therapy (AAV, lentivirus, CRISPR/Cas9; gene editing)
- Connect with pharmaceuticals
- Meet families together
- Has been one of the most supportive organizations

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Acknowledgement

- Morquio clinical and translational experts; Mackenzie, Stuart; Pizarro, Christian; Averill, Lauren; Kecskemethy, Heidi; Theroux, Mary; Shrader, M.; Rahman, Tariq; Nagao, Kyoko; Shaffer, Thomas; Alderfer, Melissa; Bober, Michael B; Hossain, Jobayer; Khan, Shaukat; Klipner, Kimberly; Church, Chris
- Administration team; Boyce, Jasmine; Wilder, Shawkita K.; Aleman, James; Funanage, Vicky L; Hostetter, Margaret
- Collaborators; Gifu University, Shimane University, Udel, Javeriana Uni, Universitario de Santiago de Compostela, ……

Sialidosis: Mechanisms of Pathogenesis and Therapy

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Disclosures

Alessandra d'Azzo has no relevant financial relationships with ineligible companies to disclose.

Learning OBJECTIVES

1. Recognize and define the main clinical features of sialidosis

2. Discuss the importance of faithful in vivo models for studying disease pathogenesis and implementing therapeutic modalities

3. Illustrate the role of lysosomal enzymes, i.e. NEU1, in controlling basic physiological processes, like lysosomal exocytosis

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ldrtc.cds.affinityced.com

Sialidosis, a prototypical LSD

Primary genetic defect

Lysosomal sialidase, **neuraminidase 1** or **NEU1**

Mode of inheritance

- Autosomal recessive – *NEU1* gene on chromosome 6p21.3
- Often the affected child is compound heterozygous for two allelic variants.
- Parents are typically unaffected carriers.

Incidence in the population

- Sialidosis is very rare.
- Precise incidence among newborns is not well established, but it's estimated at <1 in 4,000,000 live births worldwide.
- Fewer than a few hundred cases have been reported in the literature.

Ethnicity

It has been described across different ethnic groups, but some clustering of cases occurs due to founder mutations (e.g., in certain populations in Italy and Japan).

Sialidosis : Clinical Forms

Type I

- Macular cherry red spot- myoclonus syndrome
- Late onset of symptoms, but age of onset is variable
- Milder course of the disease
- Often difficult to diagnose
- Poor correlation between type of mutations and clinical severity
- Genetic background and lifestyle may influence the clinical course
- 1-5% residual NEU1 activity
- Sialylated compounds in the urine

Type II

- Macular cherry red spot
- Early onset of symptoms, (birth to early childhood)
- Severe course of the disease
- Poor correlation between type of mutations and clinical severity
- <1% residual NEU1 activity
- Sialylated compounds in the urine

Sialidosis:
Main clinical symptoms

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Type I

- Cherry-Red Spot Myoclonus Syndrome
- Onset: **Adolescence to adulthood**.

Key Symptoms:

- Visual impairment, poor color vision, night blindness.
- Myoclonus: Sudden, involuntary muscle jerking or twitching that may worsen over time.
- Ataxia: Difficulty with coordination.
- Seizures: Convulsive episodes.
- Gait disturbances: Problems with walking.
- Eye findings: Cherry-red spots on the retina, and sometimes other abnormalities like nystagmus.

- Generally mild or no cognitive impairment and long survival

Type II

- Onset: **Congenital (hydropic)**
  Infantile/juvenile

Key Symptoms:

- Coarse facial features, skeletal deformities.
- Short stature, growth retardation
- Organomegaly: Enlargement of the liver and spleen.
- Muscle weakness
- Hearing loss
- Gingival hyperplasia
- Flat nasal ridge. Widely spaced teeth

- Intellectual disability and myoclonus epilepsy
- Ataxia, tremor

- Congenital form: May present as hydrops fetalis (generalized swelling) in newborns and can lead to stillbirth or survival for only a few months.
- Later-onset Type II: May develop myoclonus and cherry-red spots in childhood or adolescence

Diagnosis of sialidosis

- Primary Diagnostic method
  - **Enzyme assay and molecular analysis**: Abnormally low NEU1 activity; *NEU1 mutation(s)*
    - Can be measured in chorionic villi (prenatally), peripheral blood leukocytes, or skin fibroblasts
- Key Diagnostic Hallmarks
  - **Oligosaccharidiuria**
    - Increased excretion of sialylated oligosaccharides in urine and other body fluid
  - **Macular cherry-red spot**
    - Central reddish area surrounded by retinal opacification (from ganglion cell storage material)
    - May be accompanied by progressive loss of visual acuity
  - **Skeletal abnormalities**
    - Dysostosis multiplex or other skeletal changes can support diagnosis
  - **Neurological findings**
    - Myoclonic epilepsy in juveniles or young adults, sometimes occurs without visual impairment

Genetic mutations in sialidosis

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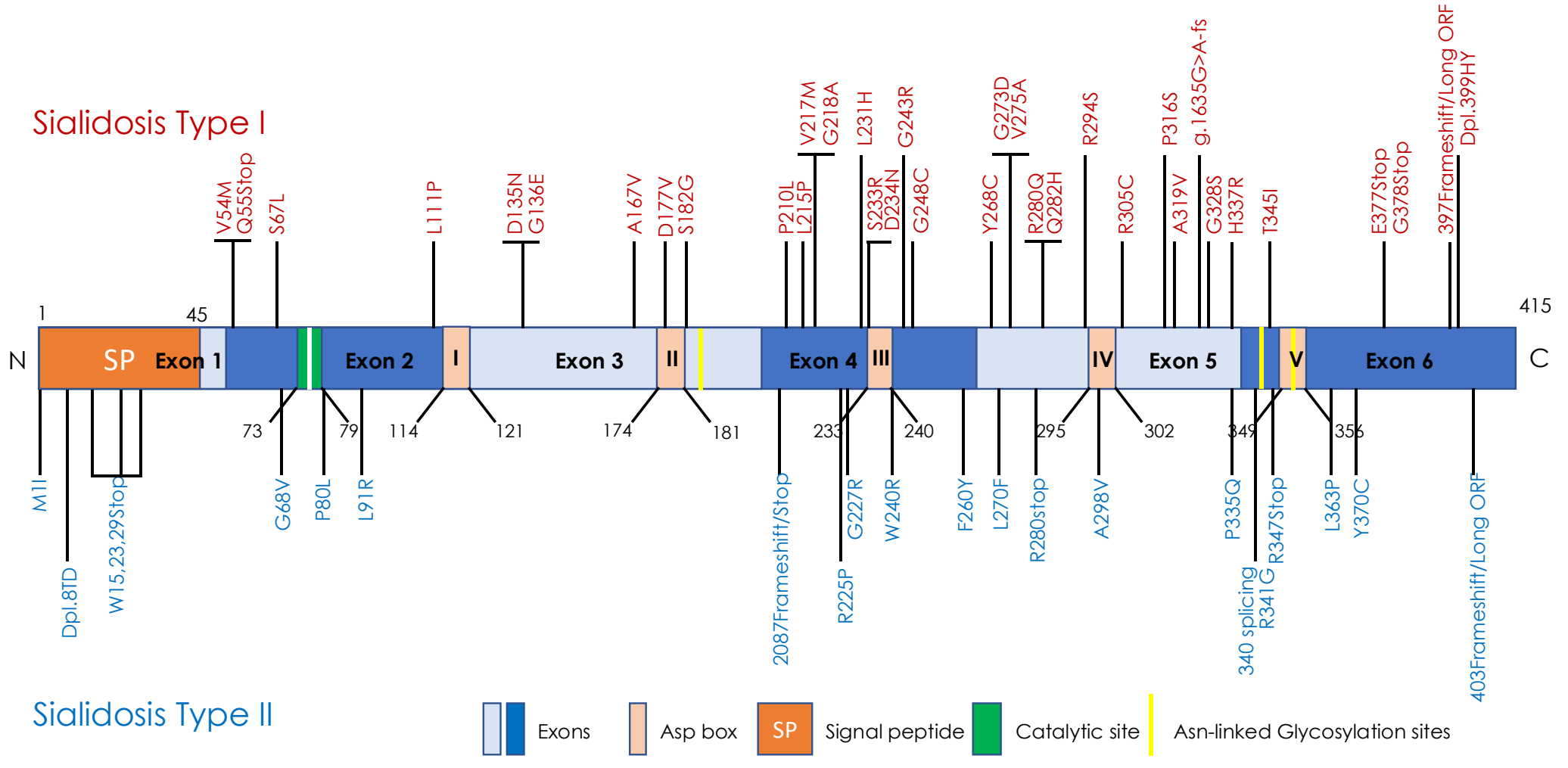
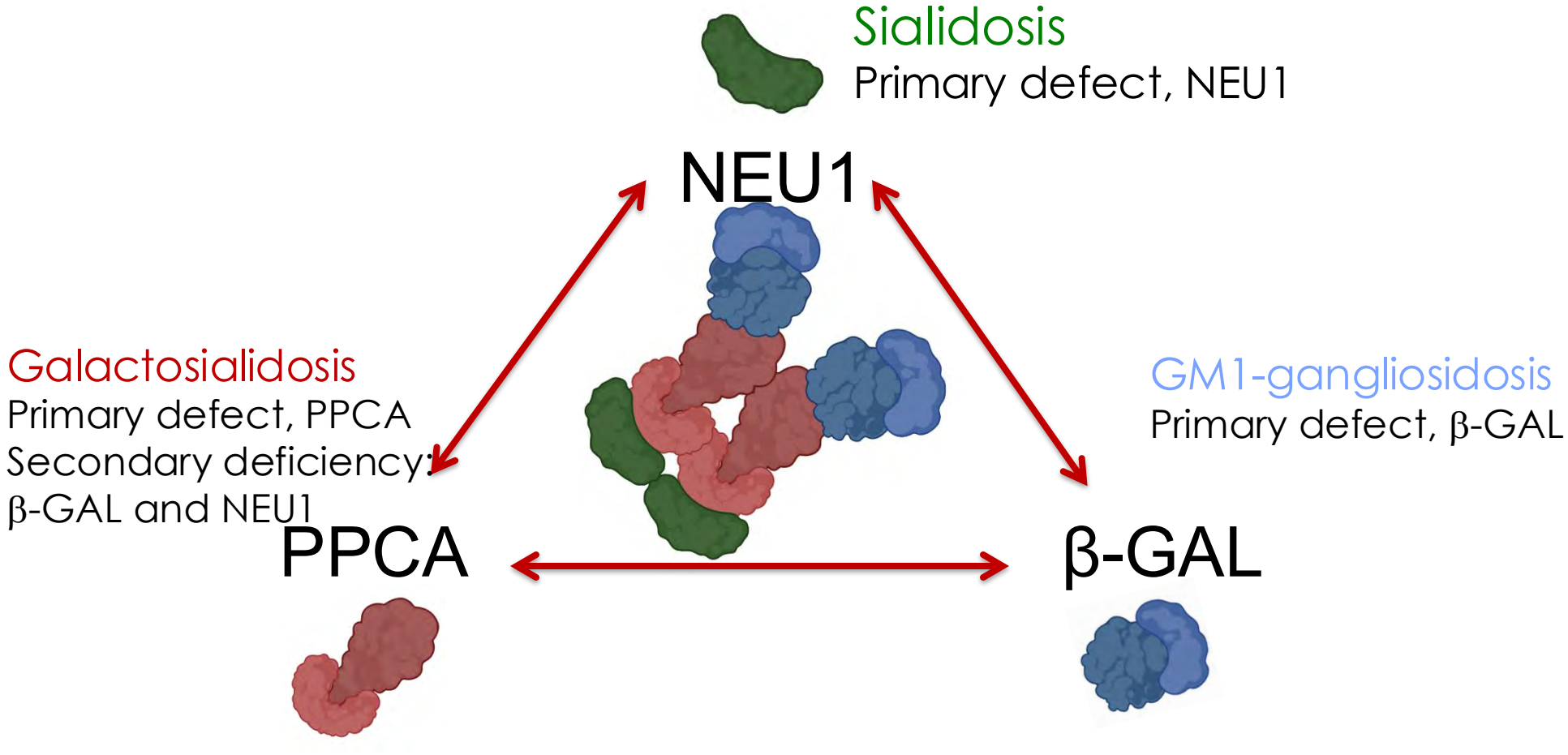


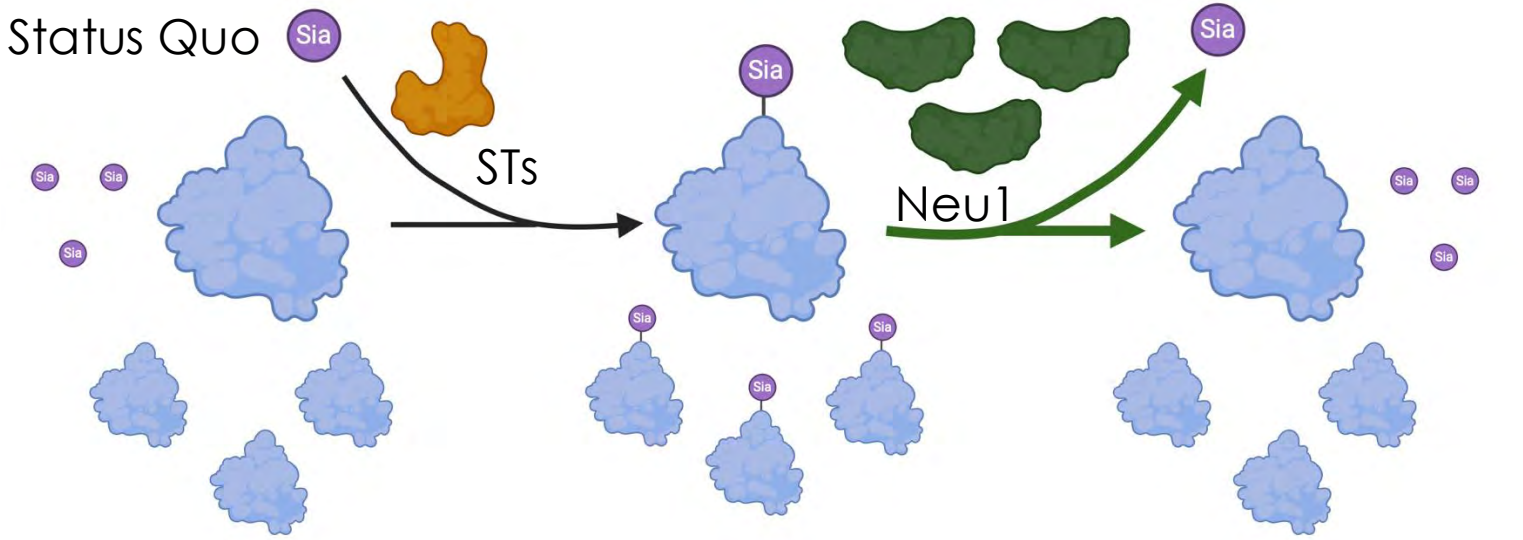
Mostly missense mutations , a few splice variants and nonsense mutations

NEU1 protein functions in a complex (LMC)



Tessitore et al, *Mol. Cell*, 2004; Cuervo et al., *EMBO J*, 2004; Sano et al, *Blood,* 2005 ; Yogalingam et al, *Dev Cell*, 2008; Sano, Annunziata et al, *Mol Cell* 2009; Annunziata et al, *Nature Commun* 2013; Machado et al, *Science Adv*, 2015 ; Annunziata et al., *Nature Commun*, 2019; van de Vlekkert et al., *Science Adv,* 2019; Annunziata et al., *Frontiers Cell Dev Biol*, 2021; Machado et al, *Commun. Biol,* 2022; Weesner et al *Cell Rep*, 2024; van de Vlekkert et al., *Mol. Ther* 2024; Fremuth et al., *Cell Rep*. 2025

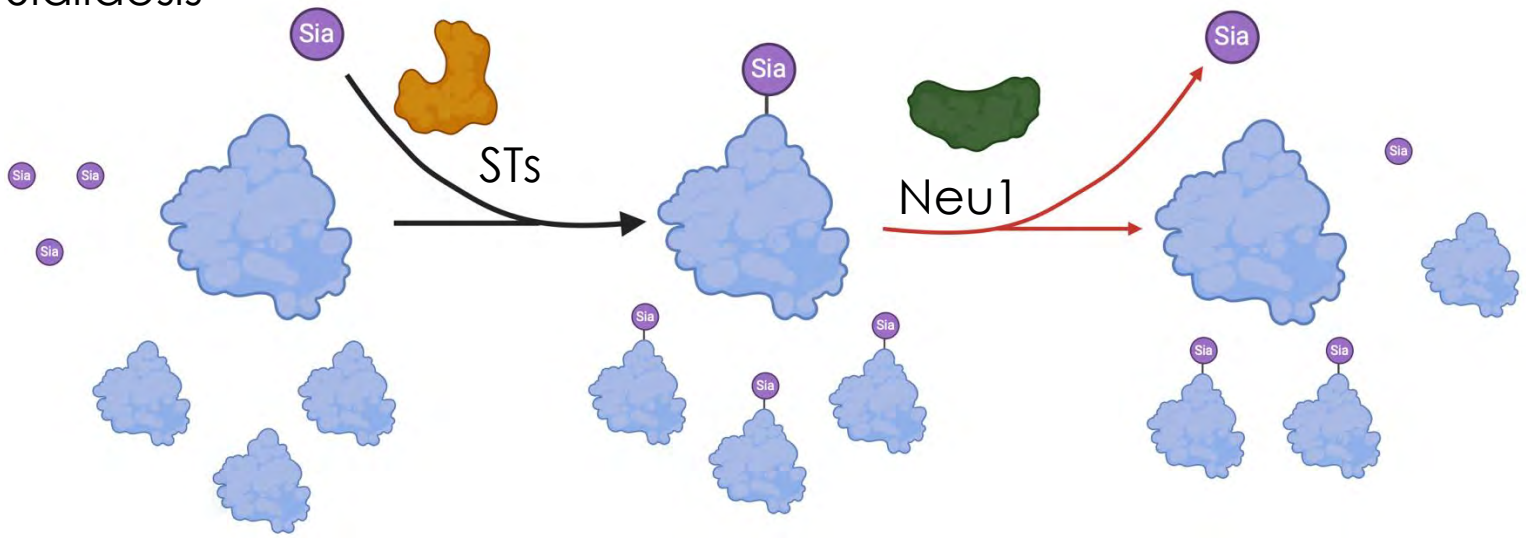
NEU1 physiological function



Target substrates of NEU1 in vivo:

Sialic acid-containing glycoproteins or glycolipids: **BOUND** sialic acids

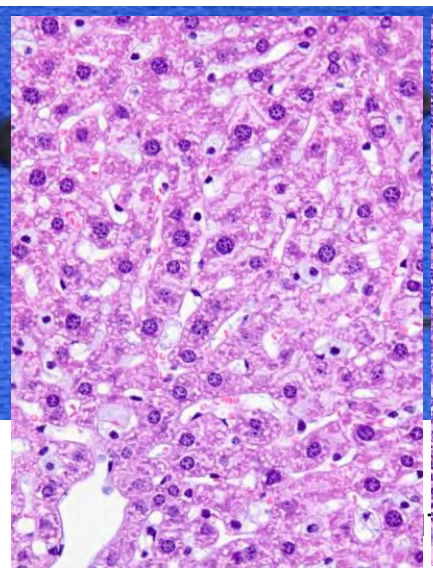
NEU1 cleaves sialic acids from sialylated substrates, initiating their degradation and creating a pool of **FREE** sialic acids

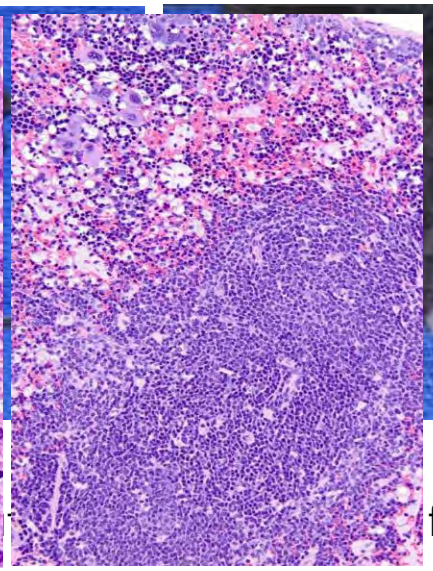


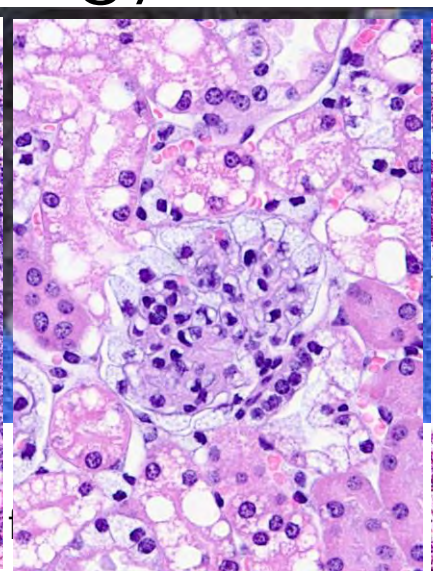
Deficiency of NEU1 causes **accumulation** of substrates that retain their sialic acids, consequently impacting their biochemical properties and function

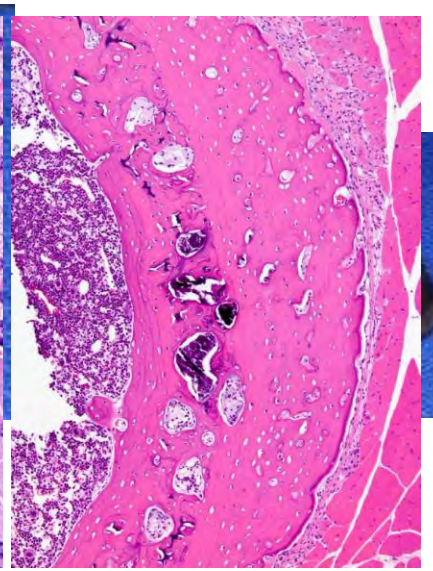
Sialidosis animal model

Pathology

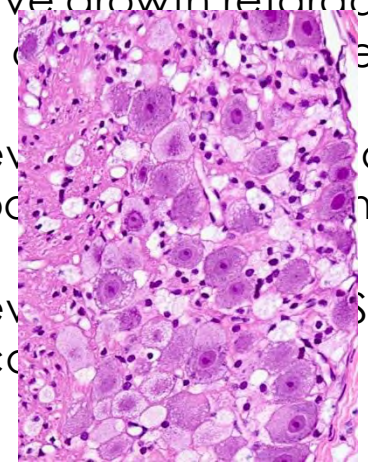

Liver

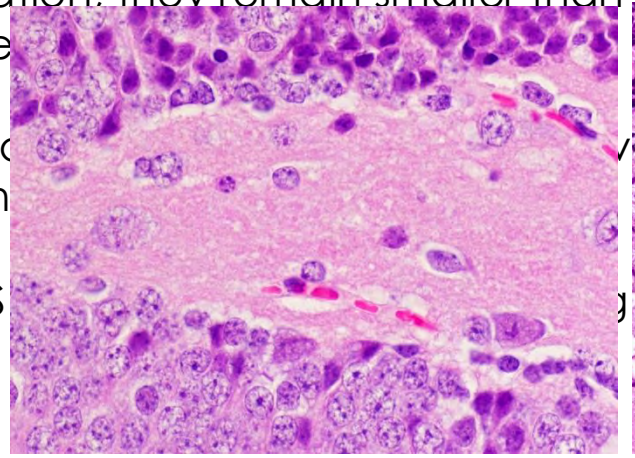

Spleen

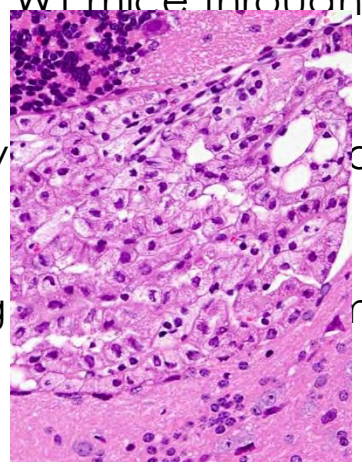

Kidney


Bone

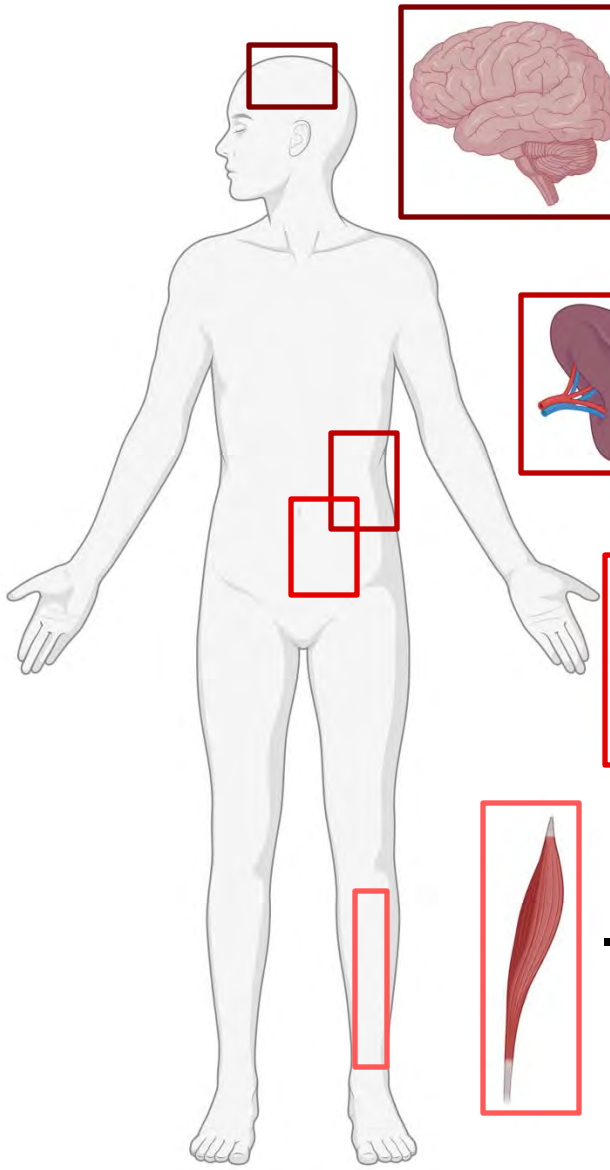
- They have growth retardation, they remain smaller than WT mice throughout their lifespan
- They dev onnective tissue, bo
- They dev n (hippoc


Ganglion


Dentate Gyrus


Choroid Plexus

Mechanisms of Disease Pathogenesis



Neurological symptoms

Annunziata, et al. 2013

Fremuth, et al. 2024

Splenomegaly

De Geest et al., 2000

Yogalingam, et al. 2008

Oligosaccharidiuria

De Geest et al., 2000

Myoclonus

Ataxia

Zanoteli, et al. 2010

Van de Vlekkert, et al. 2019

Furthers understanding of disease pathogenesis

Informs on possible successful therapeutic modalities

Used as readouts of therapeutic efficacy in pre-clinical work

Lysosomal Exocytosis

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Excessive release of active enzymes and exosomes

PM

Ca^{2+}

Ca^{2+}

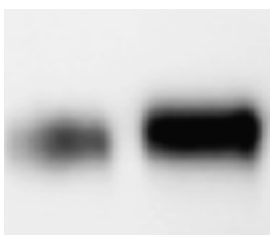
Ca^{2+}

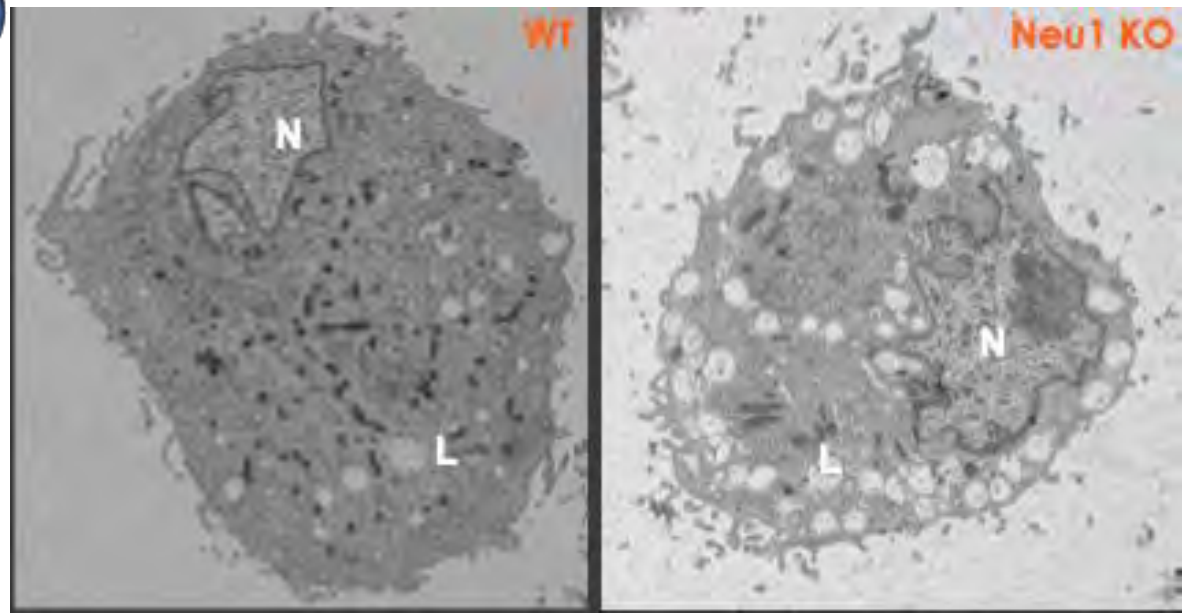
Ca^{2+}

Ca^{2+}

PM-LAMP1^{SiA}

WT KO

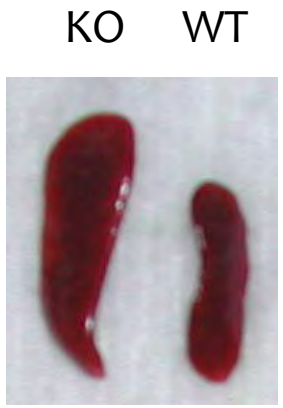


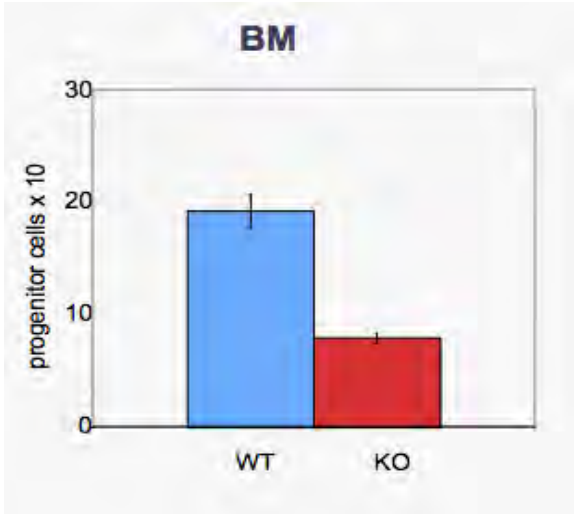


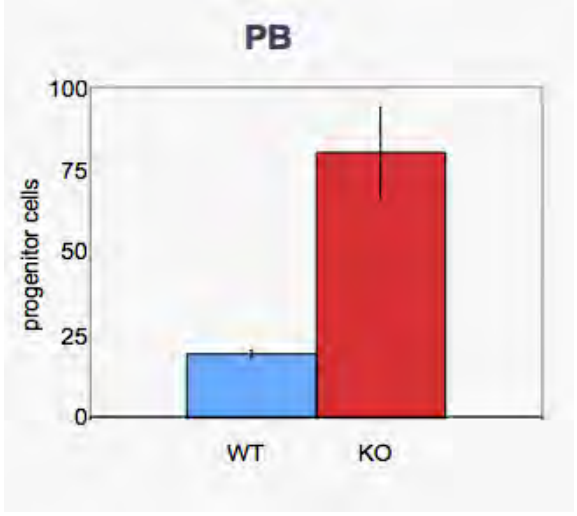
Docking:
LAMP1

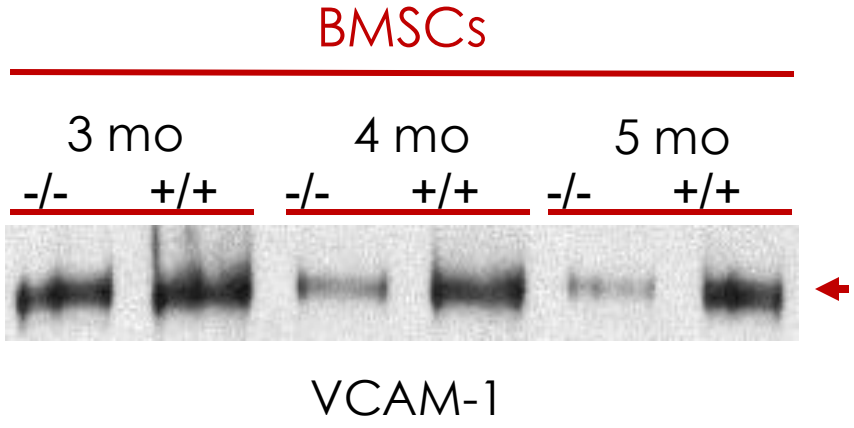
Yogalingam et al., Dev Cell 2008

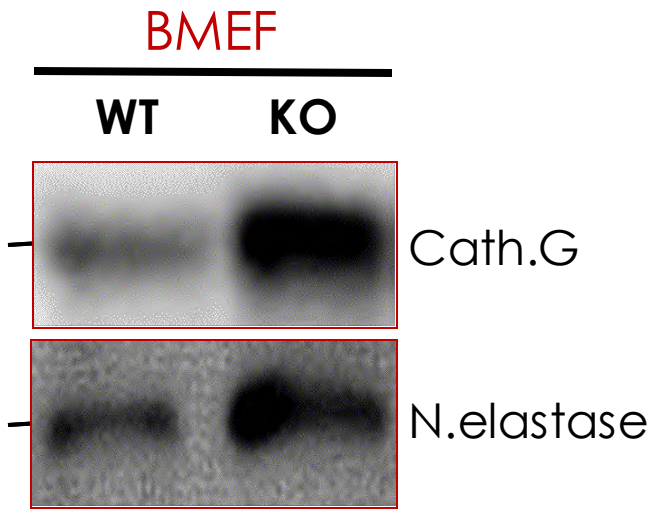
Splenomegaly results from EMH due to loss of HPCs' retention

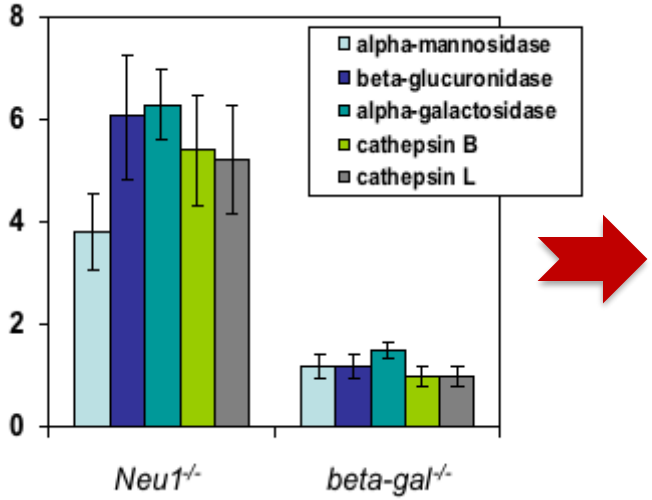








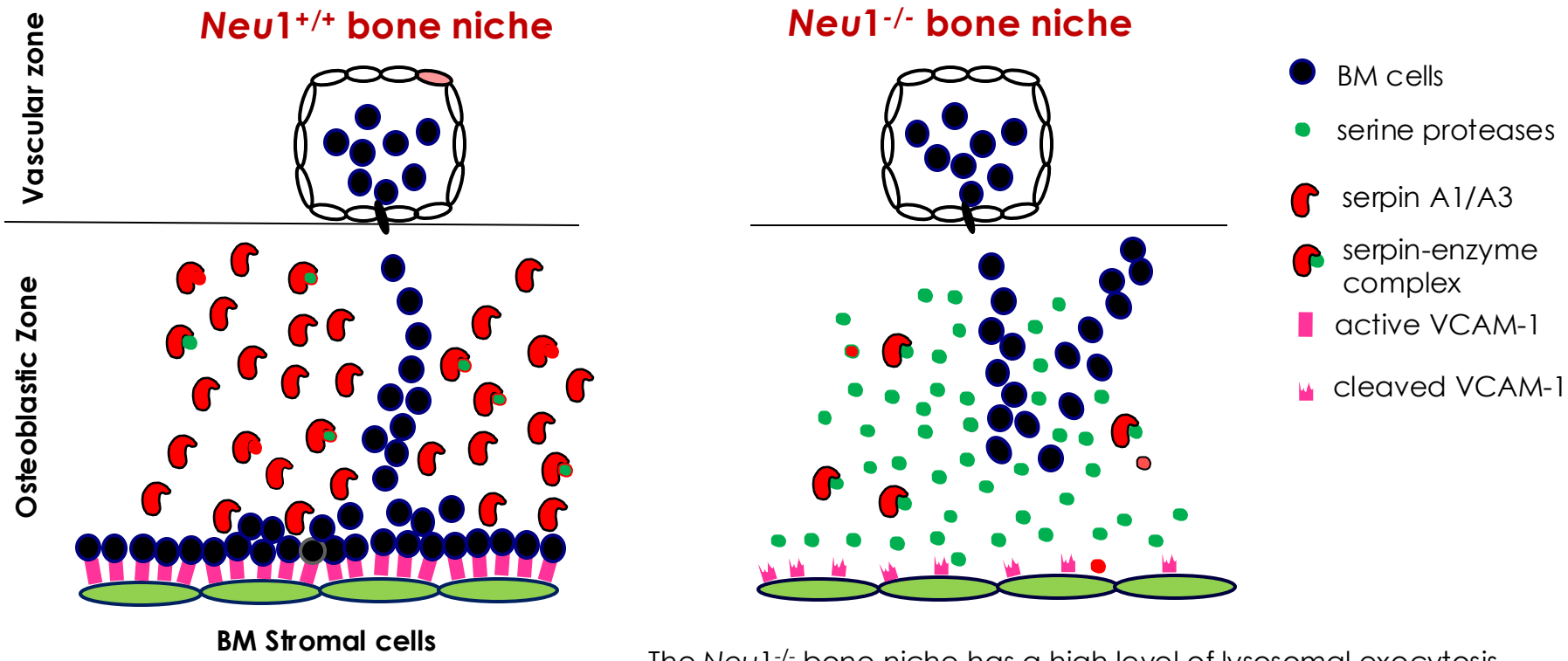




Excessive lysosomal exocytosis

Yogalingam et al., *Dev. Cell* 2008

How Neu1 deficiency affects the bone marrow niche > EMH



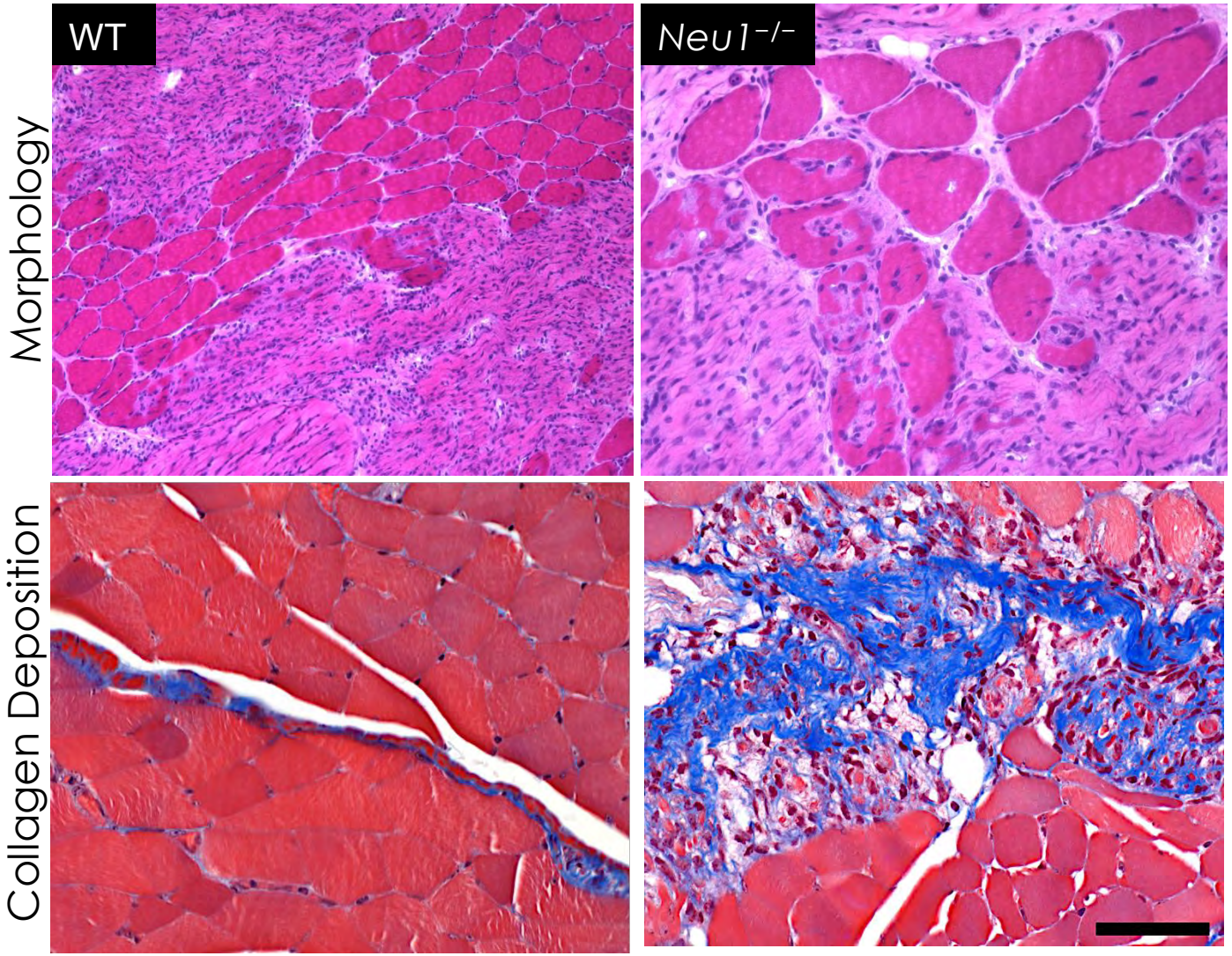
The *Neu*1+/+ bone niche has a low level of basal lysosomal exocytosis and an excess of extracellular clade A serpins.

Proteolytic activity towards VCAM-1 is therefore tightly controlled and BM cells are retained in the bone niche.

The *Neu*1$^{-/-}$ bone niche has a high level of lysosomal exocytosis resulting in increased serine proteases being present extracellularly, leading to increased extracellular proteolytic load and inactivation of serpins.

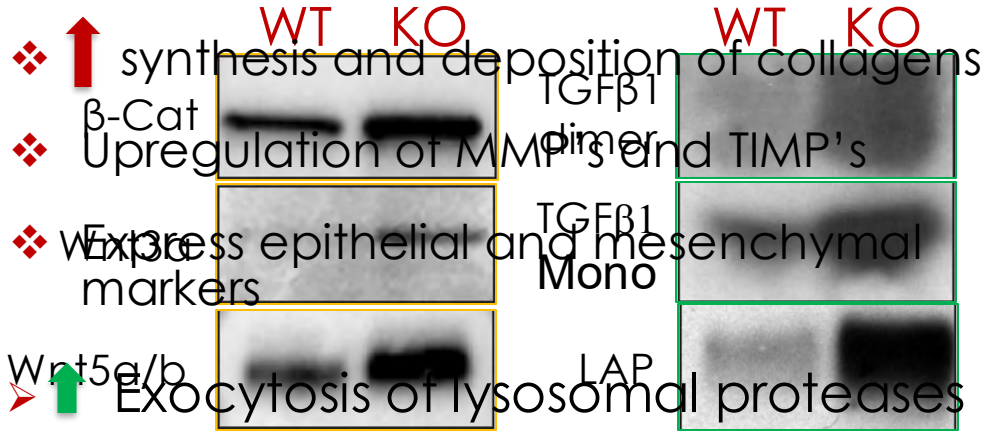
In the absence of serpins, the *Neu*1$^{-/-}$ bone niche is a hostile microenvironment with high levels of serine protease activities towards VCAM-1 that contribute to the loss of BM retention and subsequent EMH and impaired BMT.

Muscle atrophy and fibrosis is due to myofibroblasts stuck in an EMT state



Neu1$^{-/-}$ myofibroblasts

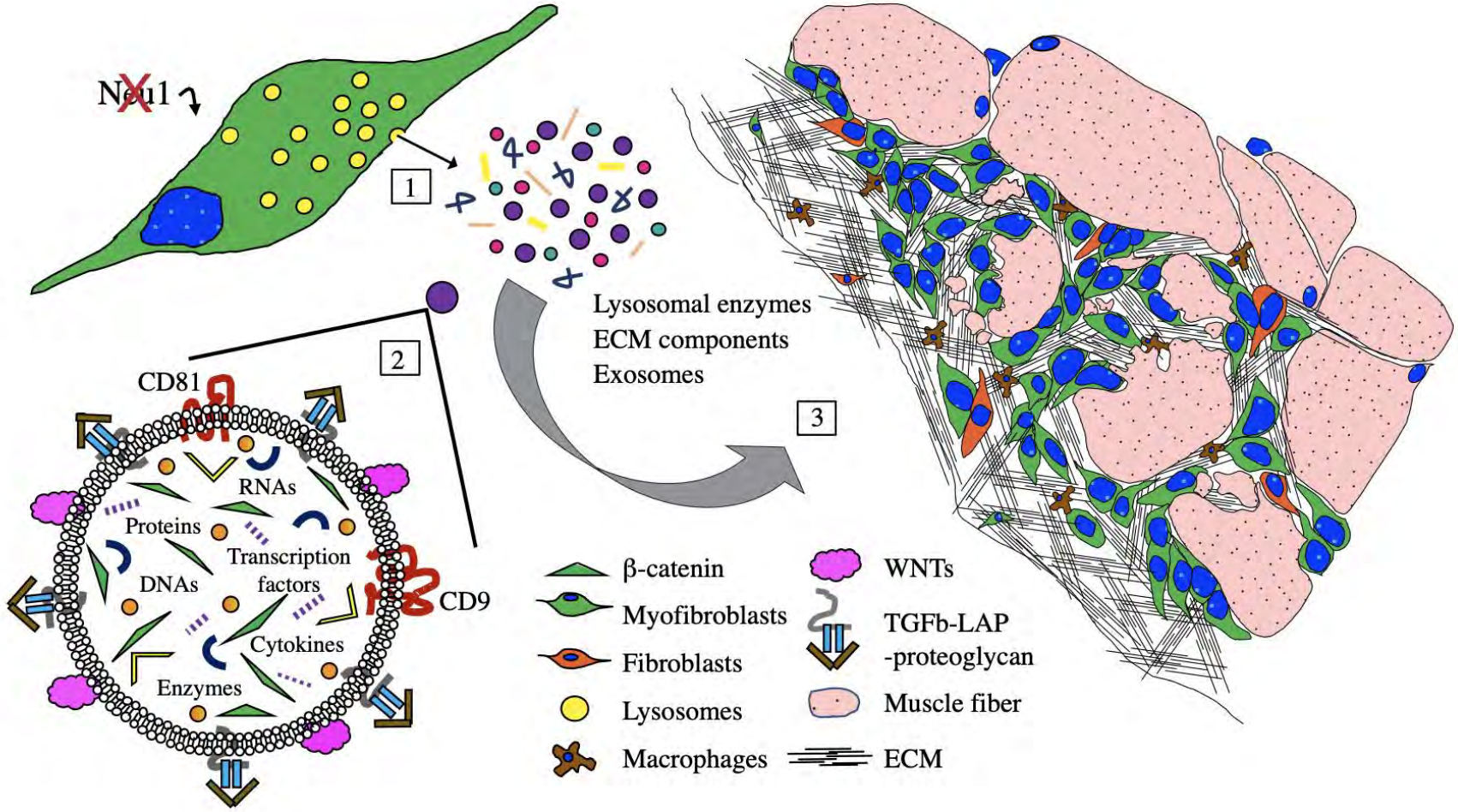
- Exosomes from *Neu1*$^{-/-}$ myofibroblasts contain migratory and fibrotic signals
- Proliferative
- Migratory/invasive
- ↑ synthesis and deposition of collagens
- Upregulation of MMP's and TIMP's
- Express epithelial and mesenchymal markers



- ↑ Exocytosis of lysosomal proteases
- ↑ Exocytosis of EXOSOMES

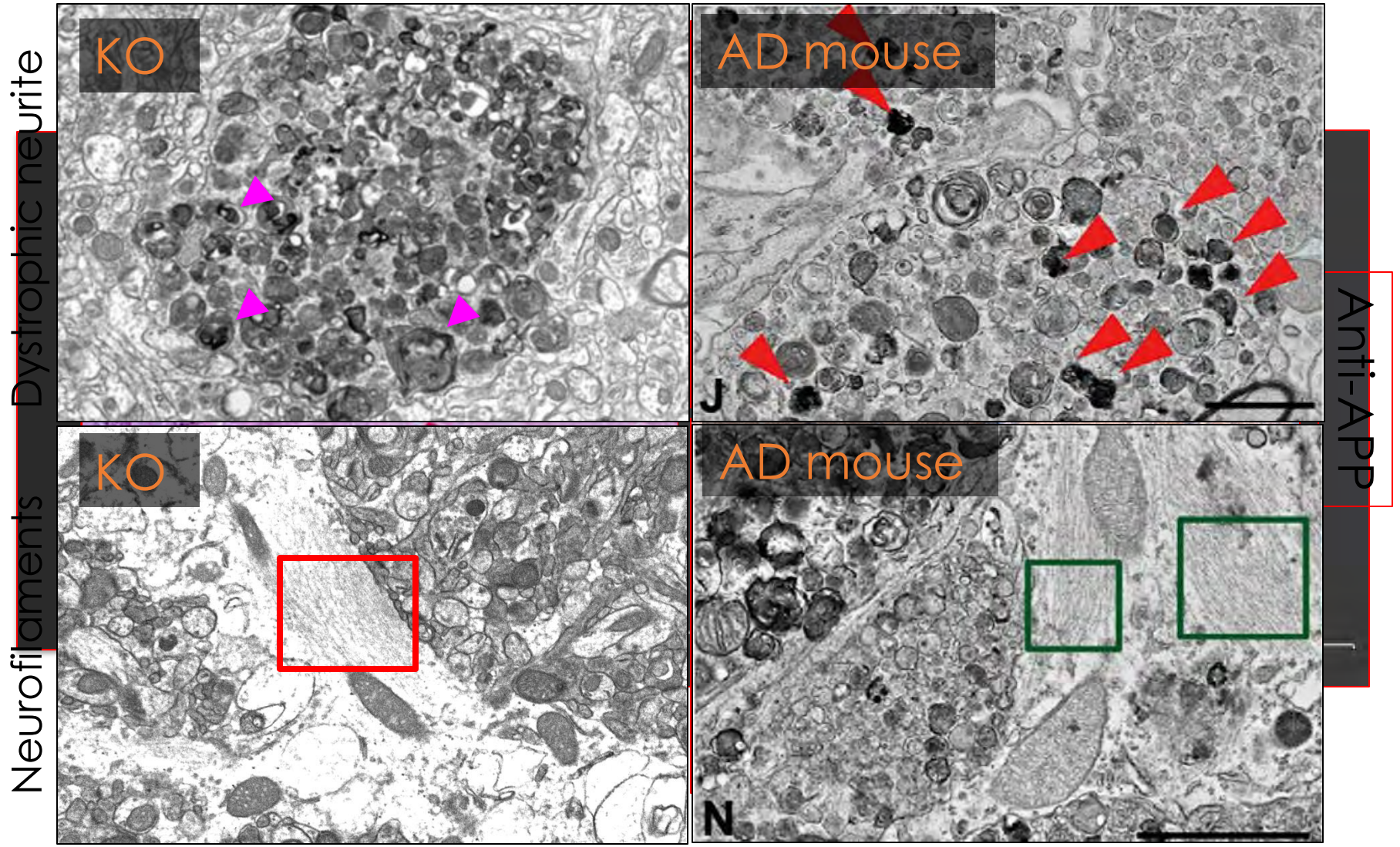
Van de Vlekkert et al., Science Adv. 2019

How *Neu1*$^{-/-}$ myofibroblasts initiates and perpetuates fibrosis



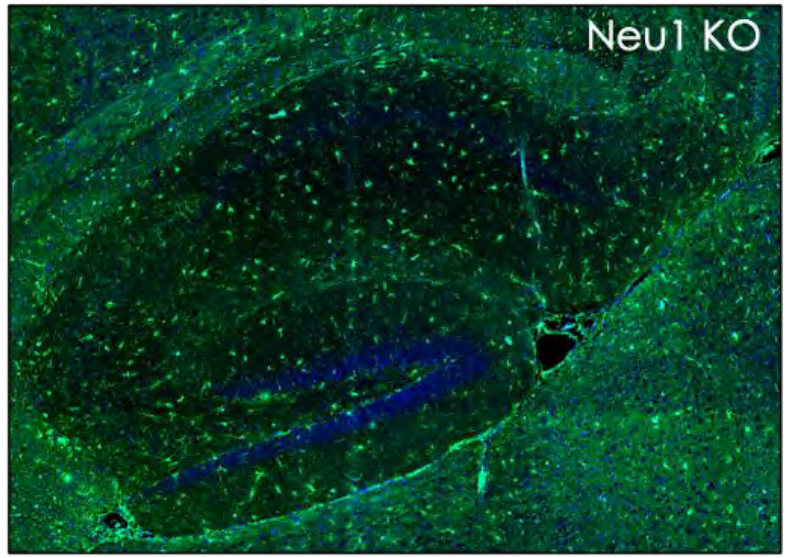
Van de Vlekkert et al., Science Adv. 2019

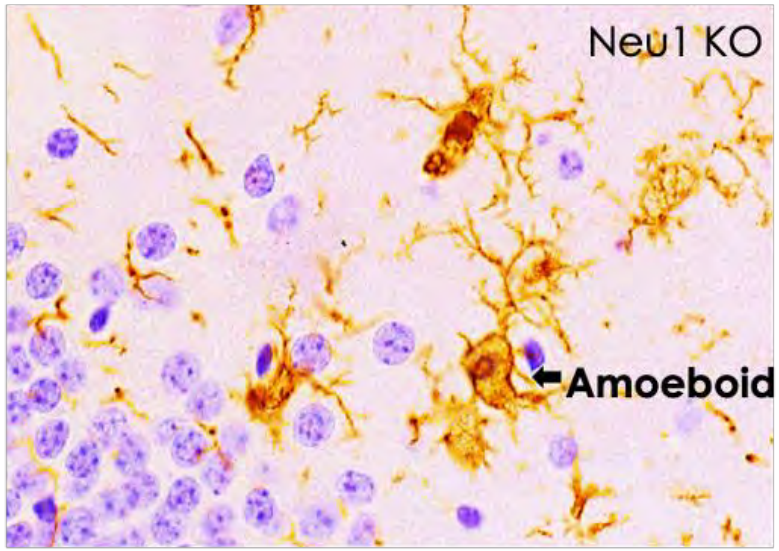
Nav1-/- hippocampus has features of an AD mouse model

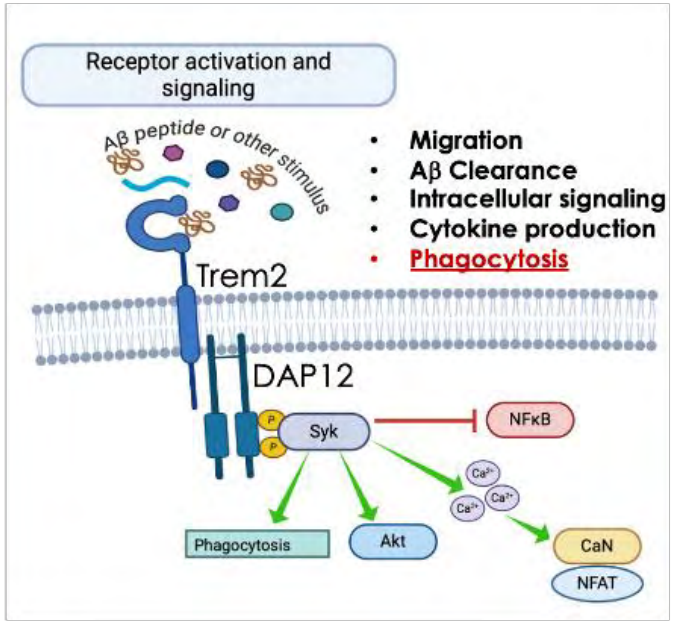


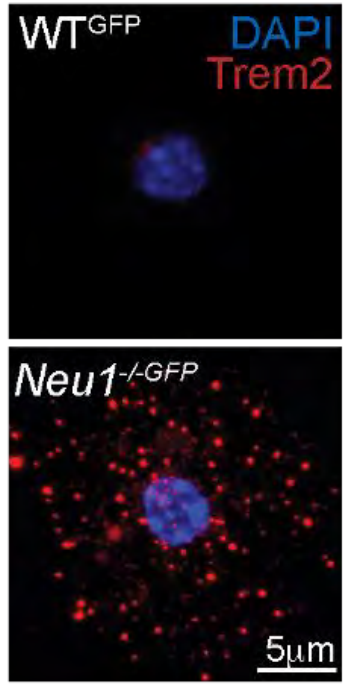
Adapted from Espuny-Camcho et al., Neuron 2017

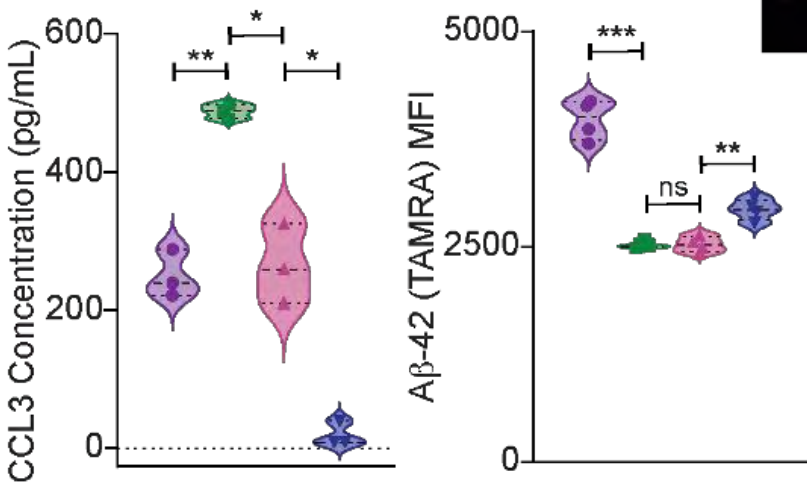
Loss of Neu1 leads to toxic, pro-inflammatory microglial responses

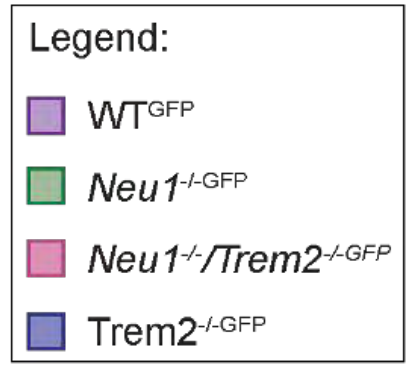






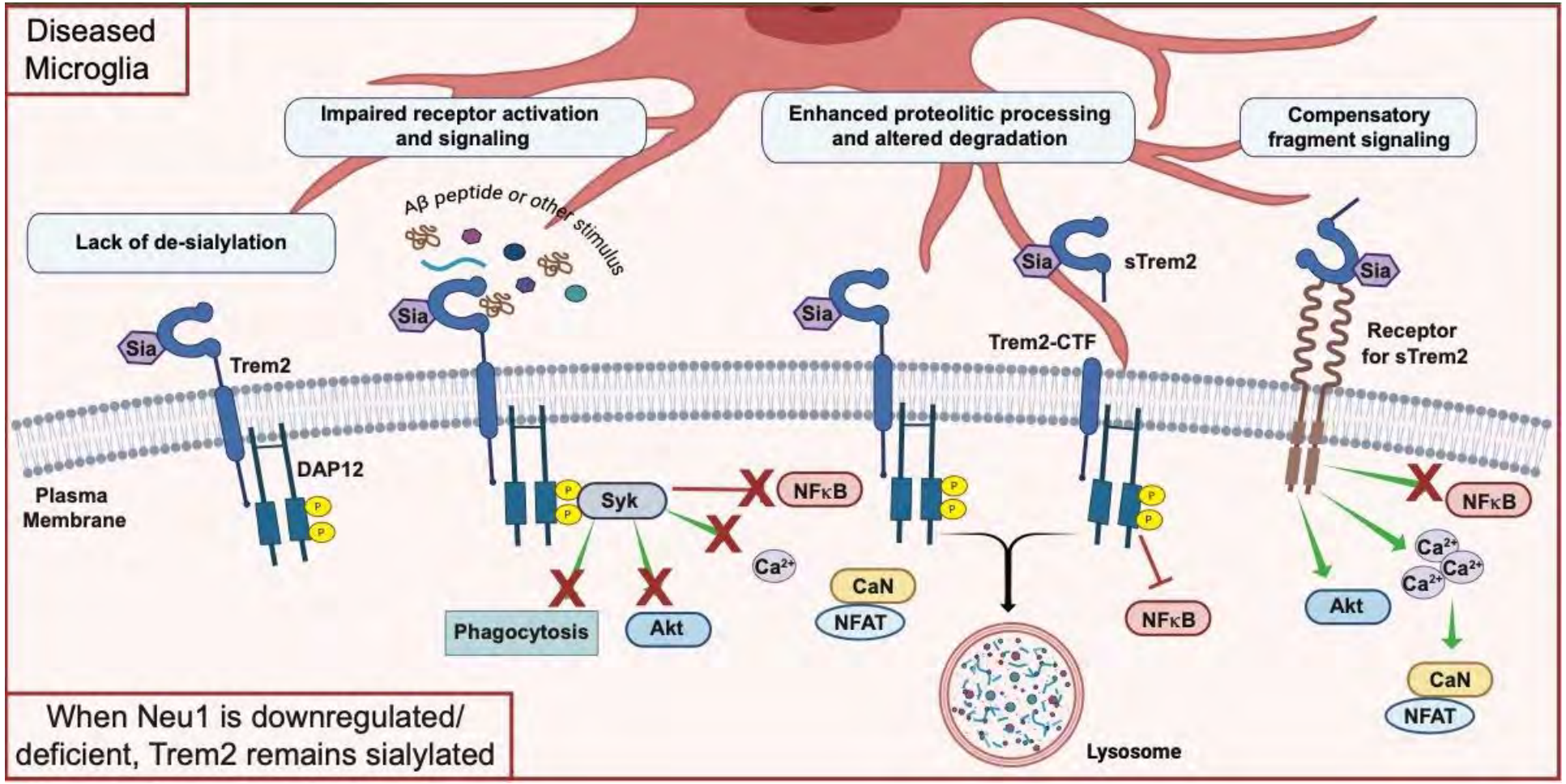




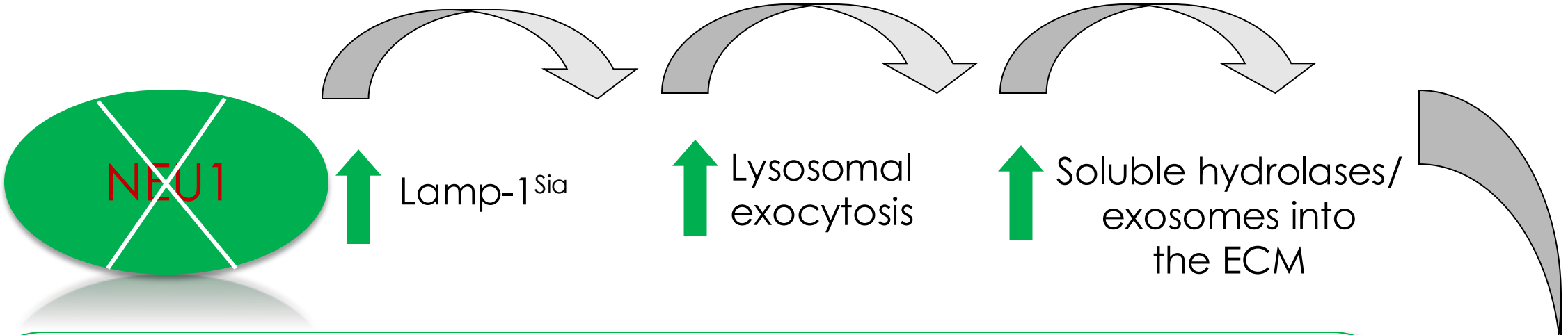


Fremuth et al., *Cell Reports* 2025

Neu1 regulates microglial states



Fremuth et al., *Cell Reports* 2025



Excessive lysosomal exocytosis is at the basis of pathogenesis in sialidosis but could also contribute to disease outcome in other adult conditions, including Alzheimer's disease, fibrosis, cancer

(Annunziata et al., Nature Comm 2013; van de Vlekkert et al., Sci. Adv. 2019; Machado et al., Sci Adv. 2015; Fremuth et al., Cell Rep. 2025)

This discovery offers new therapeutic options for the treatment of LSDs and also the possibility to exploit lysosomal enzymes therapeutically for more common adult diseases

Where we stand on therapy for sialidosis

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Sialidosis type I, the normosomatic form

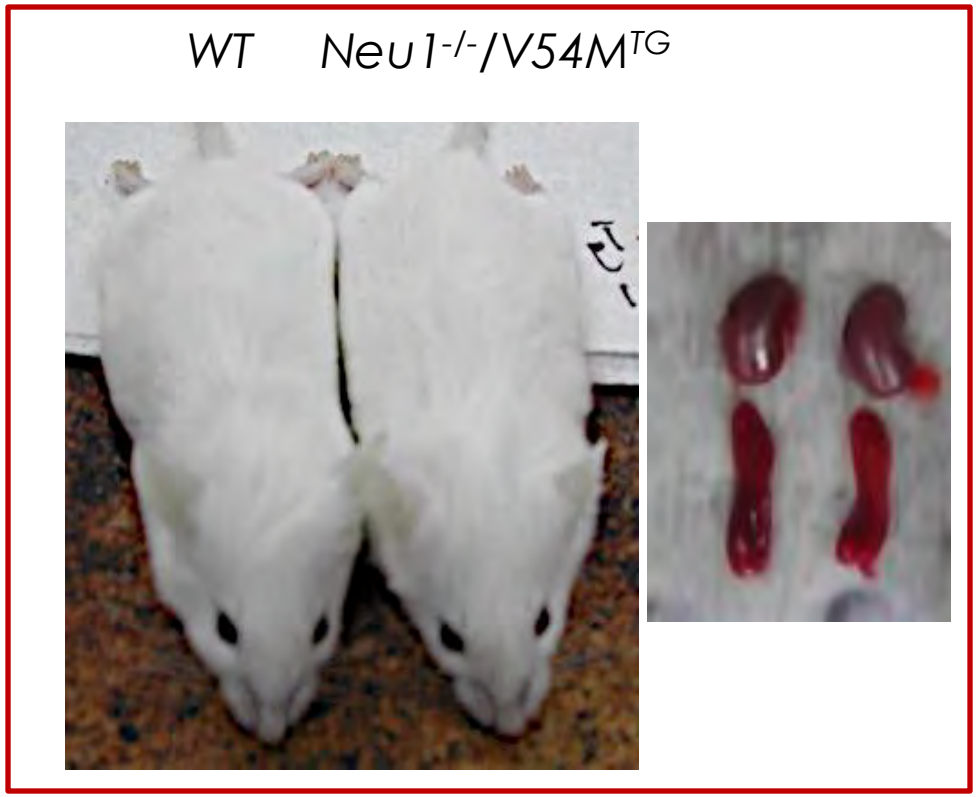
- Age of onset second decade of life
- Gait disturbance
- Bilateral cherry red macular spot
- Myoclonus
- Ataxia
- No neurological involvement
- Oligosaccharidiuria, 1-5% residual NEU1 activity

Patients live a normal life until finally diagnosed mostly by a skilled ophthalmologist or internist or by exome sequencing

>30 patients have been referred to my lab for confirmation diagnosis. They are from different ethnic groups and nationalities

Some of the patients have been participating to a study group at the National Human Genome Research Institute

Neu1$^{-/-}$/*NEU1*^{V54M} mice, a model of type I sialidosis



Neu1$^{-/-}$/*V54M*^{TG}

- Fertile, normal size
- Absence of splenomegaly and EMH
- Normal life span

Neu1$^{-/-}$/*NEU1*^{V54MTG} mice appear and behave normally

Chaperone-mediated gene therapy for sialidosis type I

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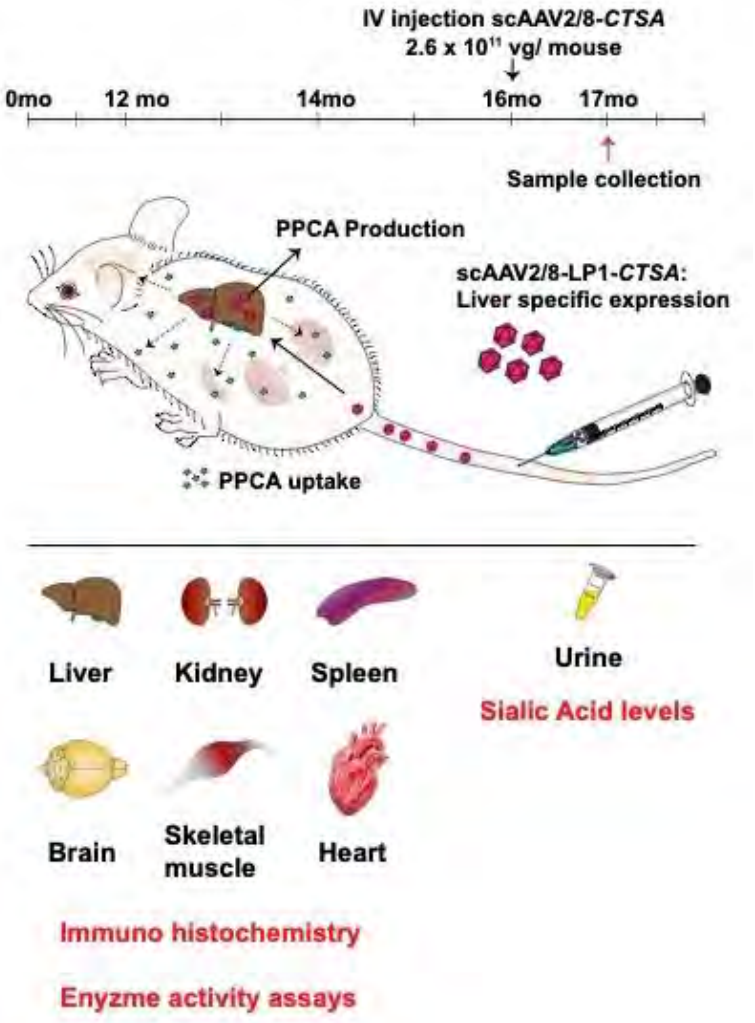
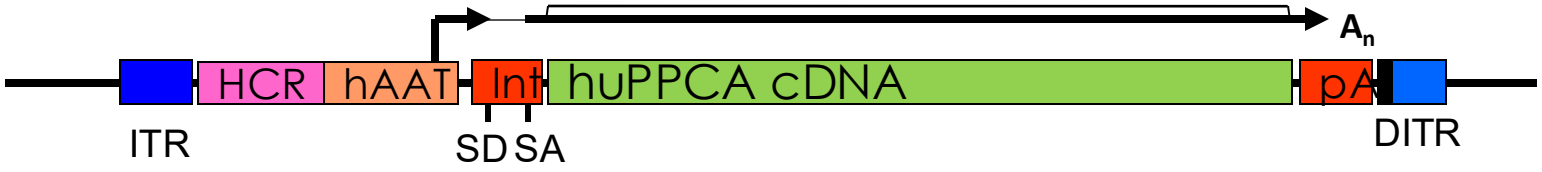
Therapeutic Approach: Intravenous injection of an AAV2/8 vector expressing PPCA





- Effective in treating systemic organs
  - Short term treatment
  - Increased Neu1 activity in visceral organs
  - Improved tissue morphology

Biochimica et Biophysica Acta 1832 (2013) 1784–1792

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

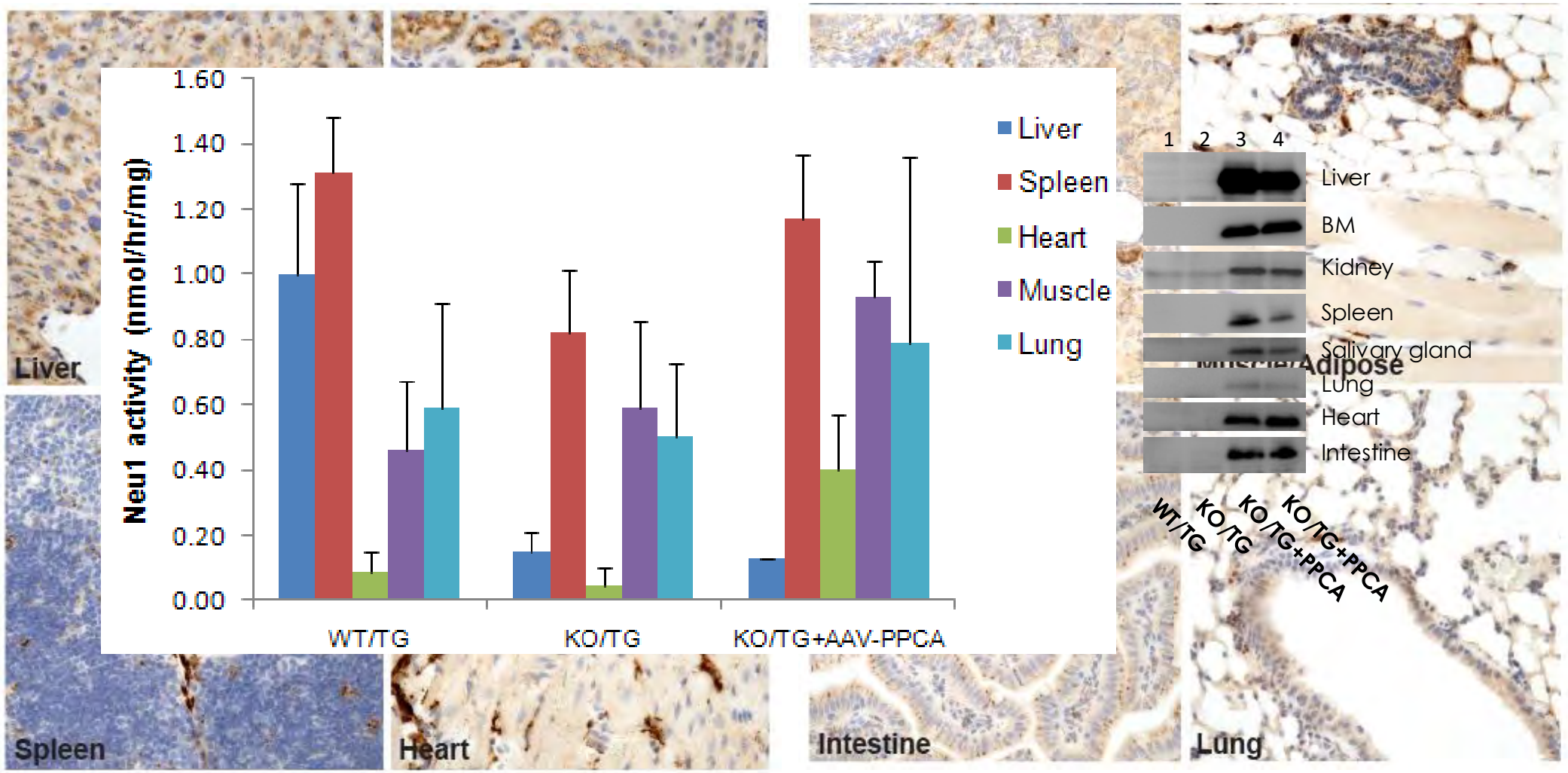
journal homepage: www.elsevier.com/locate/bbadis

Chaperone-mediated gene therapy with recombinant AAV-PPCA in a new mouse model of type I sialidosis

Erik J. Bonten [1], Gouri Yogalingam [2], Huimin Hu, Elida Gomero, Diantha van de Vlekkert, Alessandra d'Azzo *

Department of Genetics, St. Jude Children's Research Hospital, 262 Danny Thomas Place, Memphis, TN 38105, USA

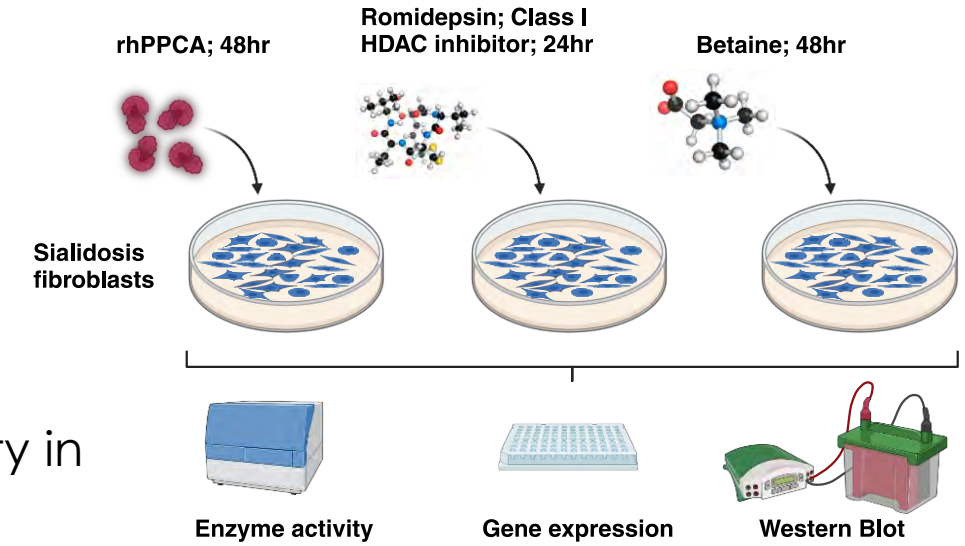
Neu1$^{-/-}$/*V54M*^{TG} mice treated with rAAV-PPCA



Conventional and unconventional therapy for sialidosis

Therapeutic Approach: recombinant human PPCA, pharmacological and dietary compound to increase NEU1 activity

- rhPPCA, romidepsin, tanganil and betaine increase NEU1 activity and gene expression
- Betaine treatment in mice increased Neu1 enzyme activity in PBMCs and kidneys and reduced sialic acid in urine



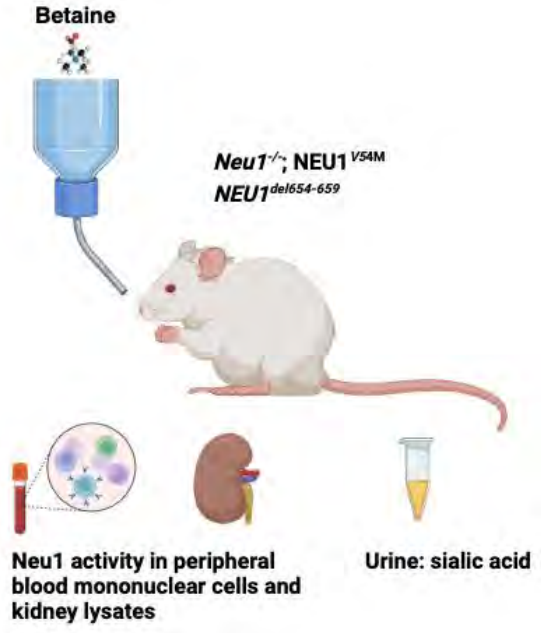
Journal of Clinical Medicine

MDPI

Article

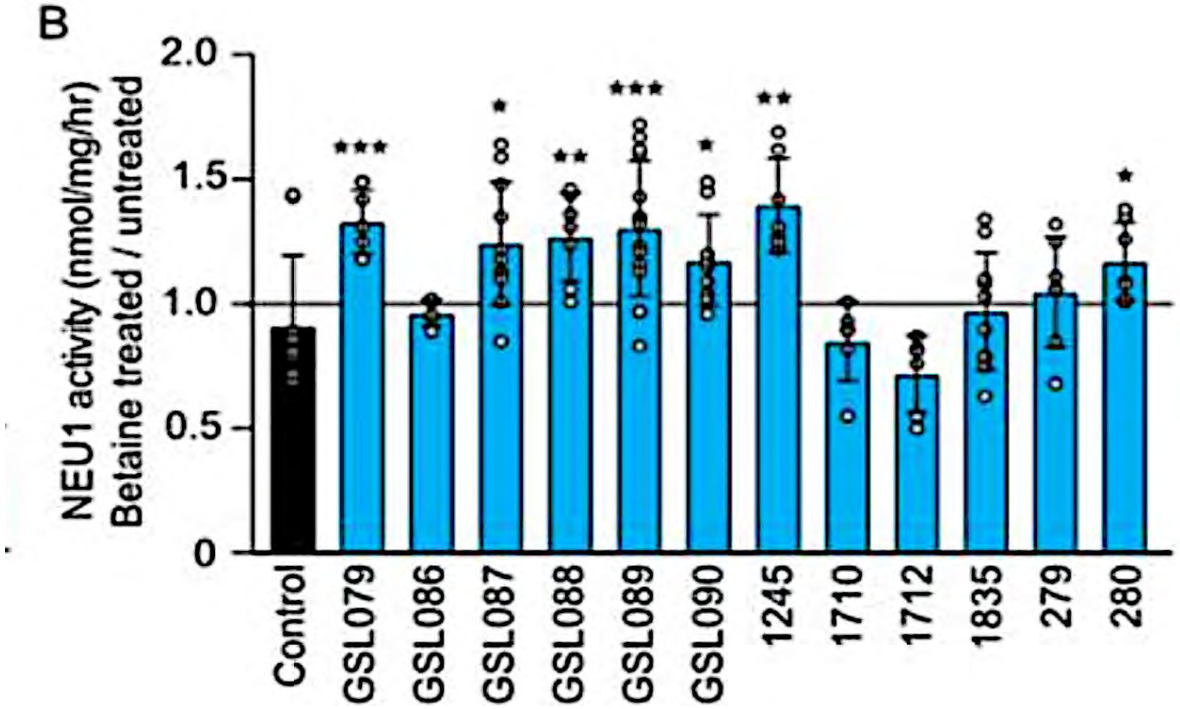
Conventional and Unconventional Therapeutic Strategies for Sialidosis Type I

Rosario Mosca [1,†], Diantha van de Vlekkert [1,†], Yvan Campos [1], Leigh E. Fremuth [1,2], Jaclyn Cadaoas [3], Vish Koppaka [3], Emil Kakkis [3], Cynthia Tifft [4], Camilo Toro [5], Simona Allievi [6,7], Cinzia Gellera [6,7], Laura Canafoglia [7], Gepke Visser [8], Ida Annunziata [1] and Alessandra d'Azzo [1,*]

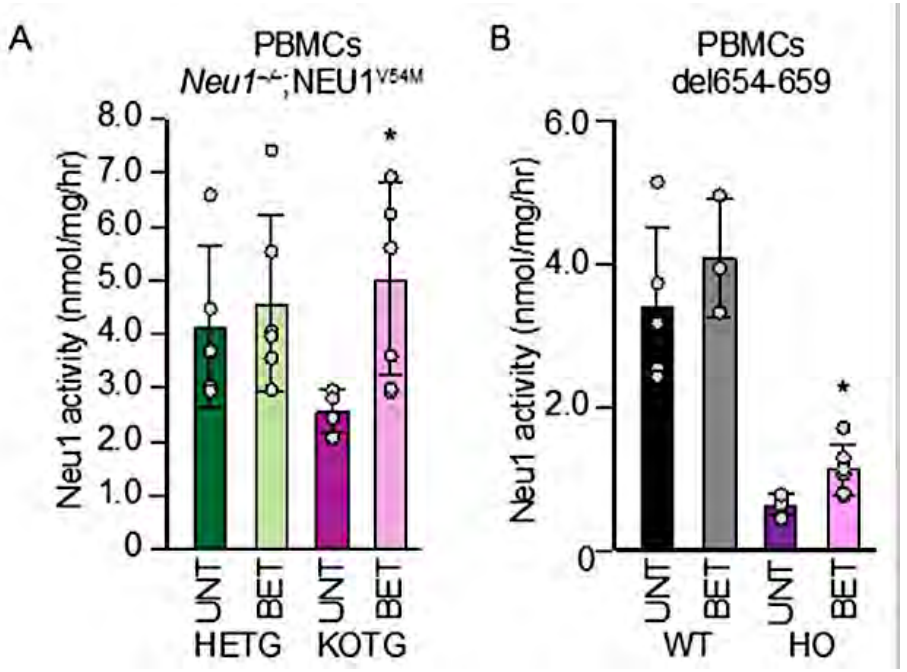


Betaine treatment in vitro and in vivo

Patients' fibroblasts



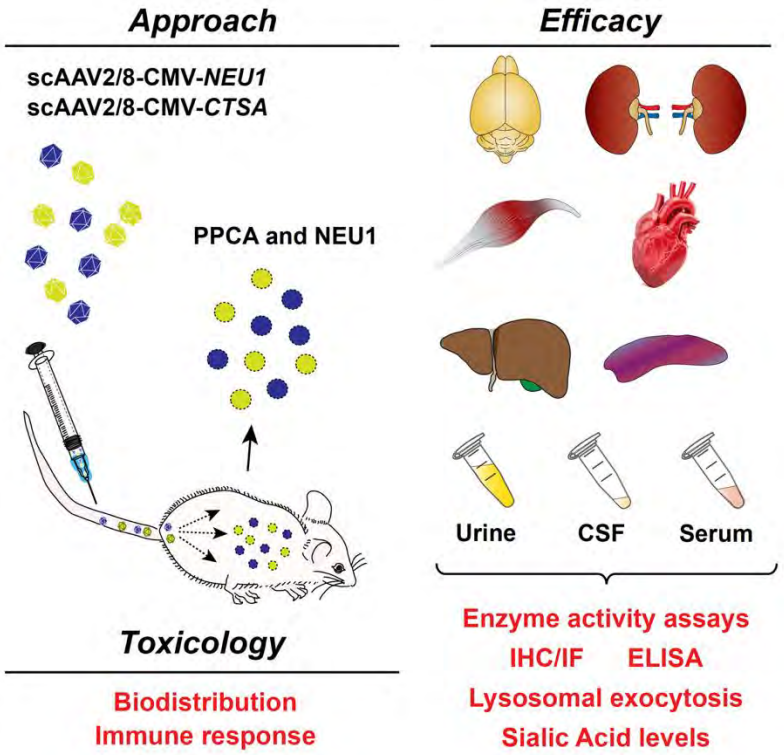
PBMCs



AAV-mediated gene therapy for sialidosis

Therapeutic approach: Intravenous injection of two AAV2/8 vectors expressing NEU1 and PPCA.

- 2x10E12 vg/mouse of scAAV2/8-CMV-NEU1
- 1x10E12 vg/mouse of scAAV2/8-CMV-PPCA



- Effective in treating systemic organs and neuropathology
  - Increased NEU1 activity
  - Improved tissue morphology
  - Reduction of inflammation

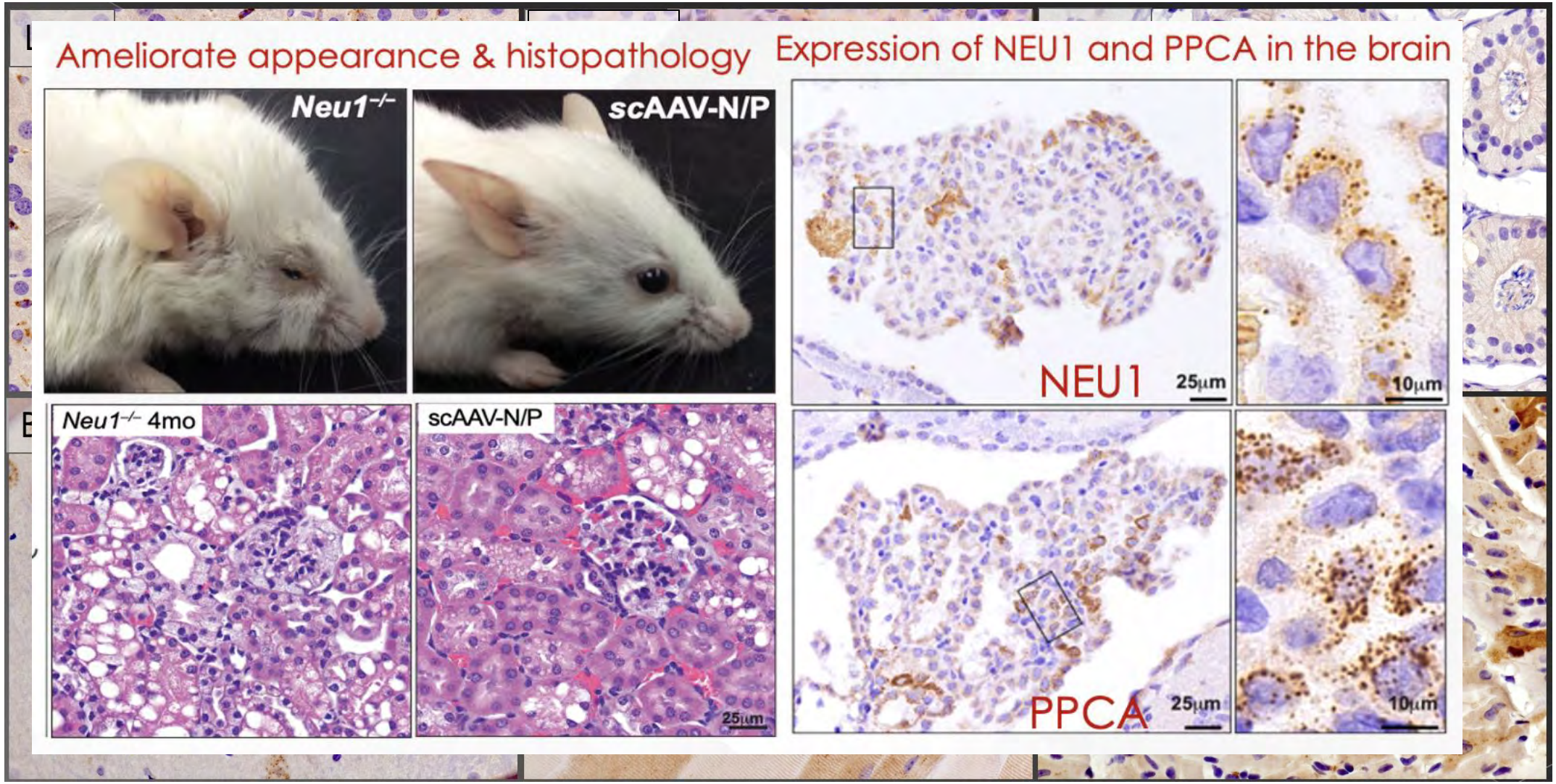
Molecular Therapy
Original Article

AAV-mediated gene therapy for sialidosis

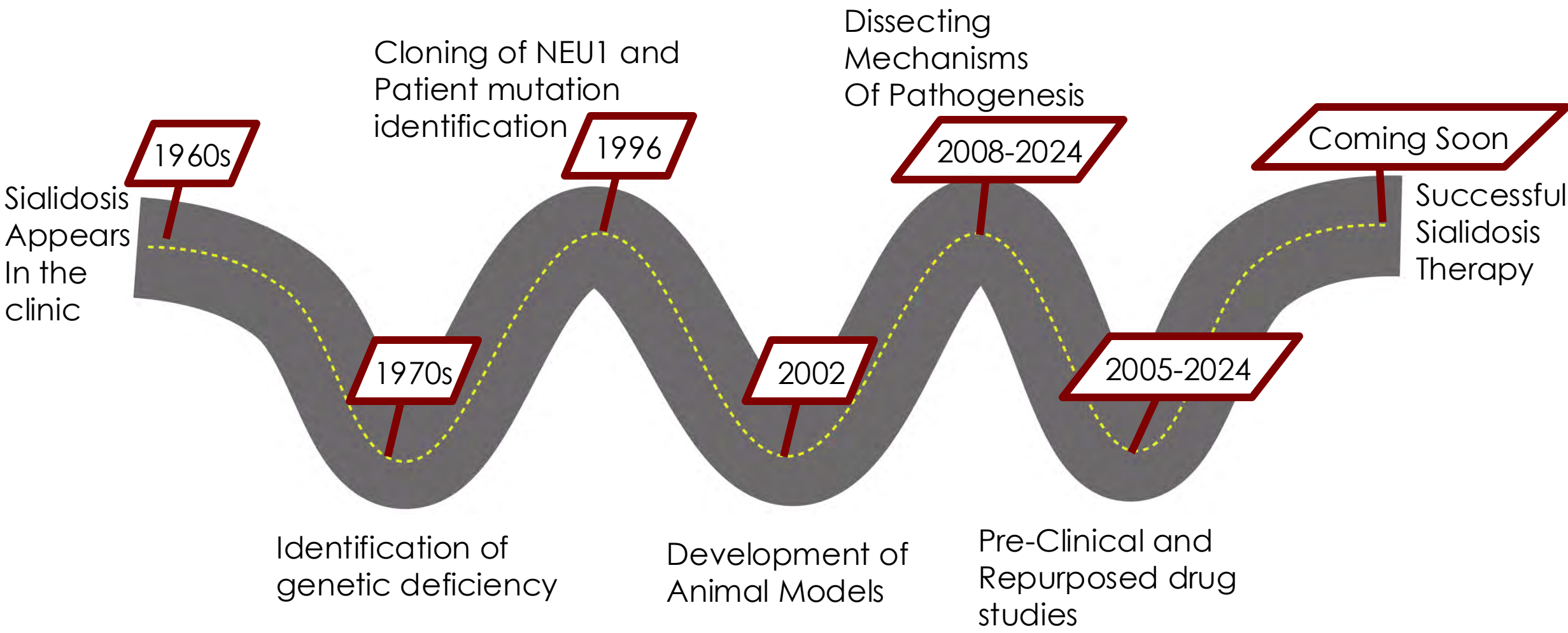
Diantha van de Vlekkert,[1,5] Huimin Hu,[1,5] Jason A. Weesner,[1] Leigh E. Fremuth,[1] Scott A. Brown,[2] Meifen Lu,[3] Elida Gomero,[1] Yvan Campos,[1] Heather Sheppard,[3] and Alessandra d'Azzo[1,4]

AAV-mediated gene therapy for sialidosis

St. Jude Children's Research Hospital
ALSAC • Danny Thomas, Founder

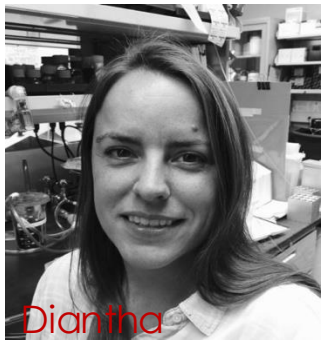


Milestones in a roadmap



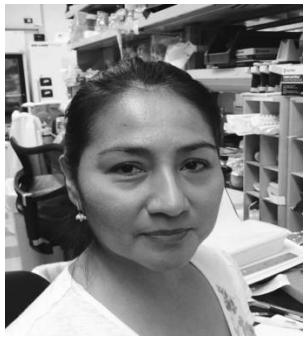
d'Azzo Lab

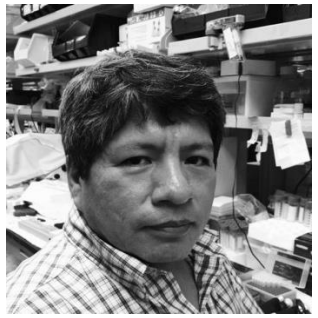

Ida Annunziata


Diantha van de Vlekkert


Eda Machado

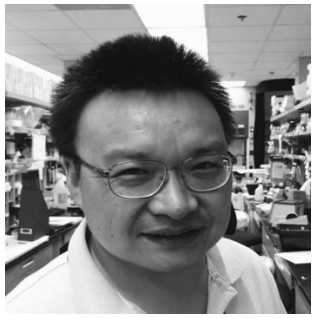

Noelia Escobedo


Elida Gomero


Yvan Campos


Huimin Hu


Rosario Mosca


Xiaohui Qiu


Jayce Weesner


Leigh Fremuth

Genetics
Gerard Grosveld
and lab members

Computational Biology
Geoff Neale
David Finkelstein

Veterinary Path
Laura Janke

Adv. Path Core
Heather Tillman

Center for Bioimage Informatis
Khaled Khairy
Mia Panlilio

Hematology
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Min-Joon Han

Cell. Imaging Shared Res..
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NHGRI, NIH
Cyndi Tifft
Camilo Toro
Their team of clinicians and nurses

BioStrategies
Carole Cramer
David Radin

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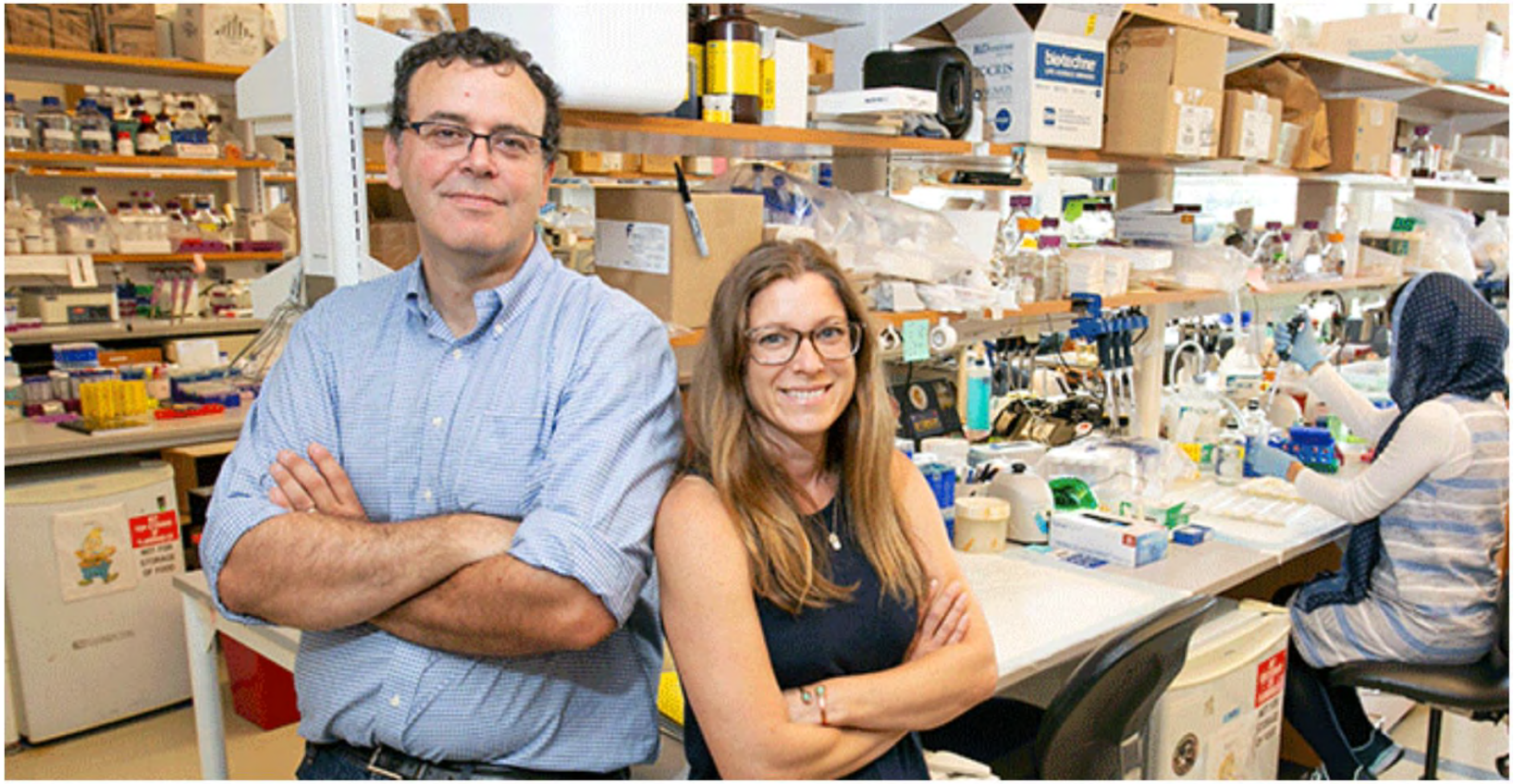
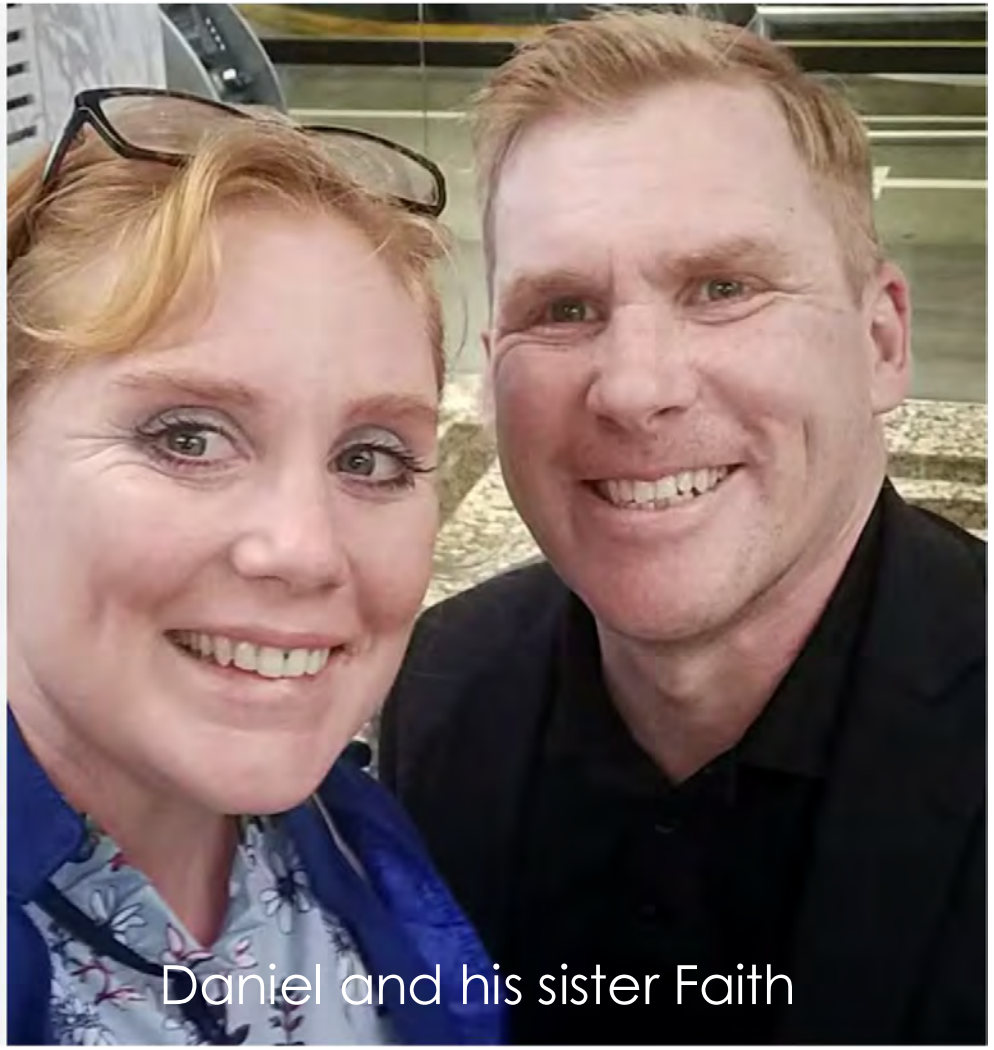
Funding: NIH GM60905, GM104981, DK52025 , CA021764, NS123174; Assisi Foundation of Memphis; Ultragenyx Pharmaceuticals, CCSJ, ALSAC

The next generation in sialidosis therapy research



Miguel Sena-Esteves and Heather Gray-Edwards
University of Massachusetts Medical School

Sialidosis patients



Daniel and his sister Faith

https://curemucolipidosis.org

MPSIIIC Gene Replacement Therapy with scAAV9/HGSNAT Vector

Merve Emecen Sanli, MD

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Key Contributors:
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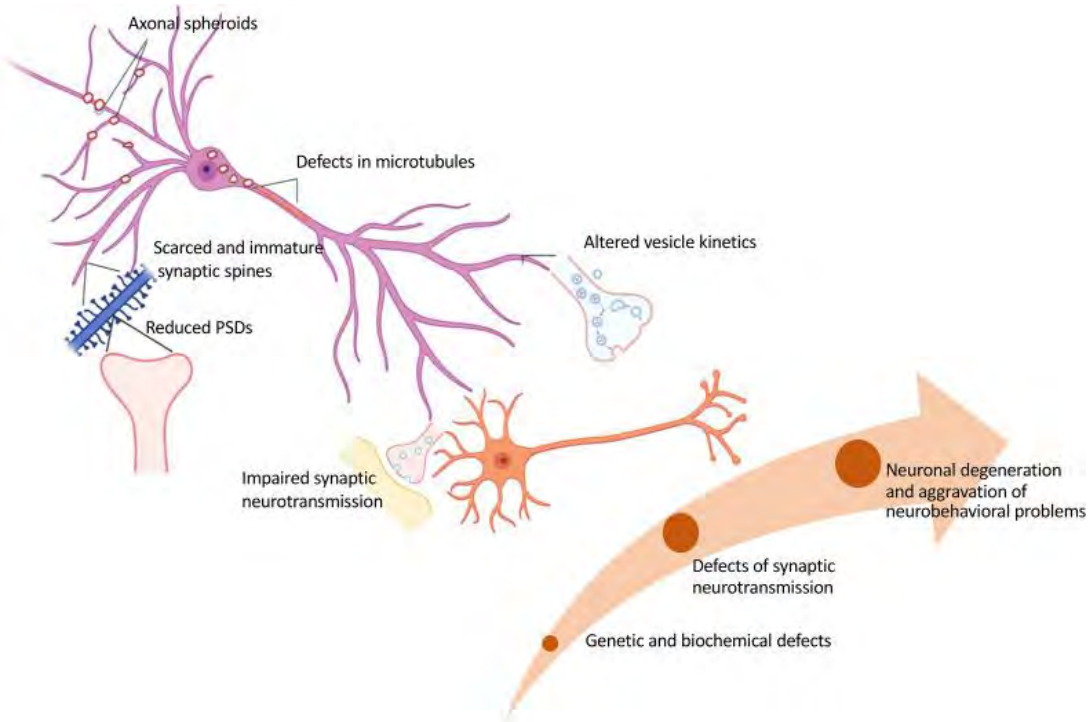
***Funding and Direction**: Phoenix Nest (Jill Wood and Sri Singamsetty)*

DISCLOSURES

- Dr. Sanli has no relevant financial relationships with ineligible companies to disclose.
- Disclosure will be made when a product is discussed for an unapproved use.
- This continuing education activity is provided by AffinityCE, The Lysosomal and Rare Disorders Research and Treatment Center (LDRTC), and CheckRare CE. AffinityCE adheres to the ACCME's Standards for Integrity and Independence in Accredited Continuing Education. All individuals in a position to control the content of a CME activity are required to disclose all relevant financial relationships with ineligible companies. All relevant financial relationships for anyone in control of content for this activity have been mitigated.
- Monetary commercial supported was received in the form of educational grants from ineligible companies. Please see the final program for a list of all supporters.

MPSIIIC (Sanfilippo syndrome type C)

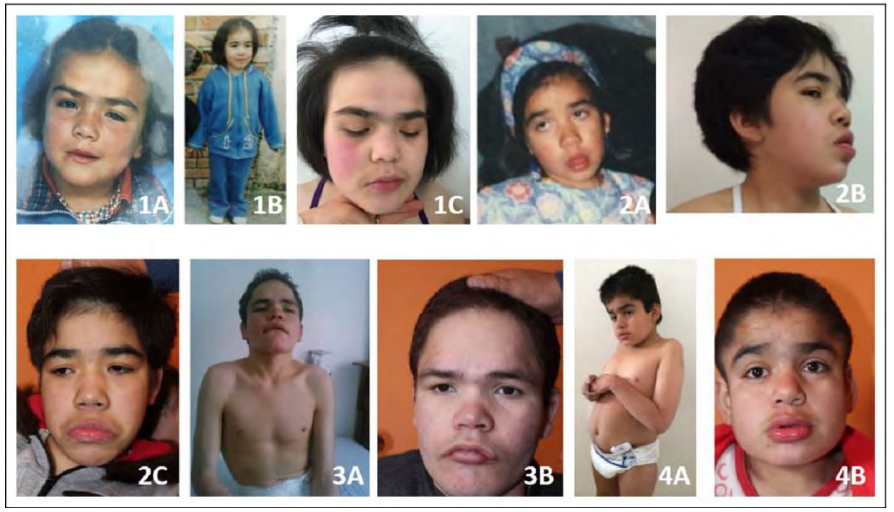
- Rare, autosomal recessive lysosomal disorder due to Heparan sulfate acetyl-CoA: alpha-glucosaminide N-acetyltransferase (HGSNAT) deficiency.
- Encoded by ***HGSNAT* gene.**
- Unable to break down a GAG called heparan sulfate.
- Incidence is estimated at 1 in 1,000,000 live births.



Camila Pará et al, 2021

Clinical Features and Prognosis of MPSIIIC

•Onset: Typically between **2–6 years** of age



•**Neurodevelopmental:**
•Initial speech delay → progressive cognitive decline
•Loss of previously acquired skills

•**Behavioral and Psychiatric:**
•Hyperactivity, impulsivity, aggression, anxiety
•Sleep disturbances and autistic-like features

•**Neurological:**
•Seizures, spasticity, ataxia, loss of ambulation in advanced stages

DOI:10.1177/0883073816672391

•**Sensory:**
•Progressive **hearing and vision loss**

•**Systemic:**
•Mild hepatosplenomegaly, coarse facial features, joint stiffness

•**Prognosis:**
•Progressive neurodegeneration leading to loss of independence and early mortality (often in the 2nd–3rd decade)

Study Design

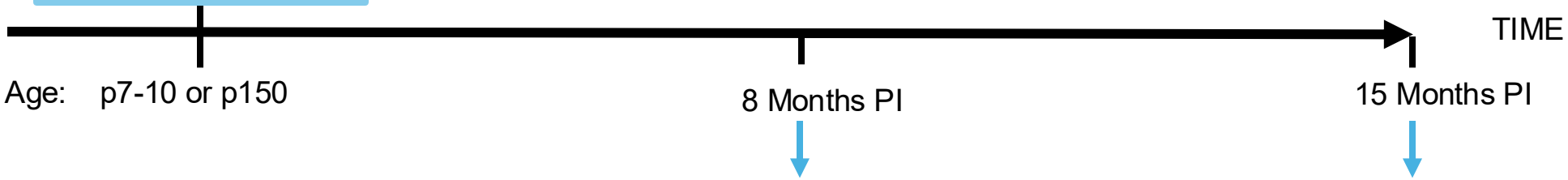
Dr. Alexey V. Pshezhetsky

Hgsnat^{P304L} mice

scAAV9/JeT-coHGSNAT-SpA

Safety study

Intrathecal vehicle or high dose to WT mice (n=20-22)



Efficacy study

Intrathecal vehicle, low dose, or high dose to HgsnatP304L mice (n=20-27)

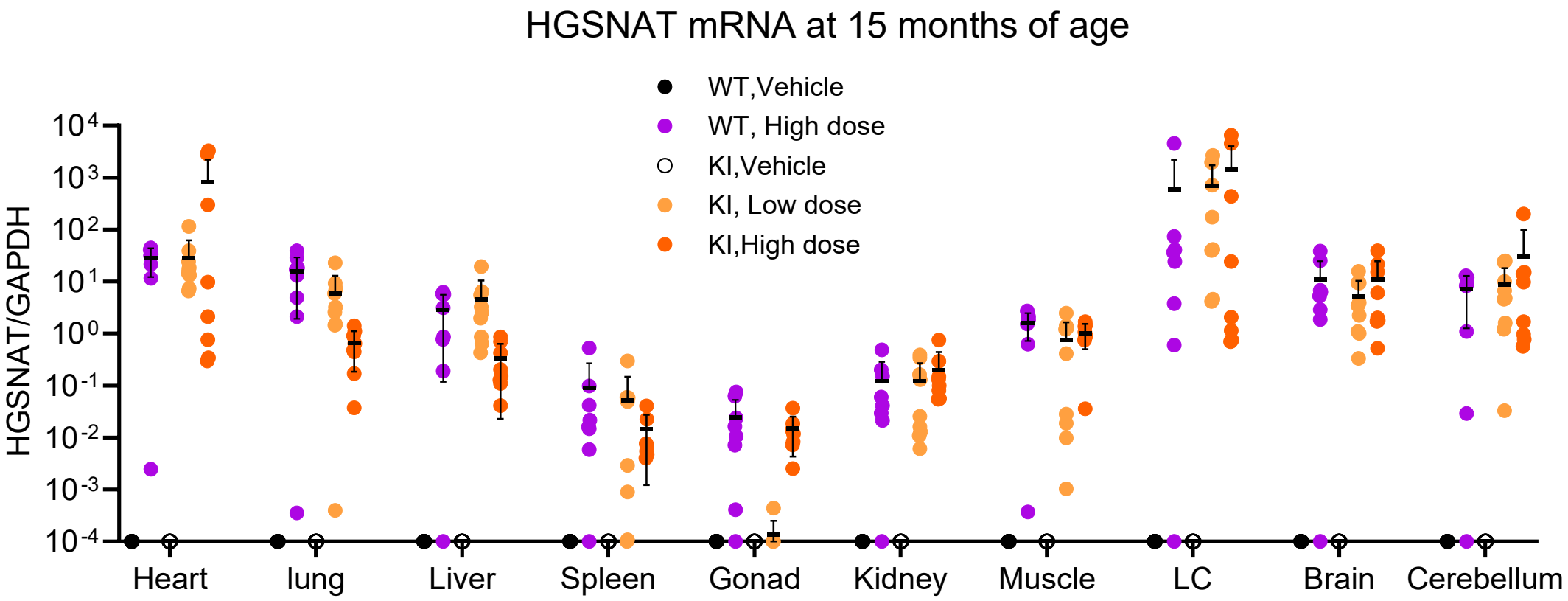
- Euthanize 5 mice / group
- Tissue enzyme activity and substrate
- Tissue toxicity evaluation

- Euthanize remaining mice
- HGSNAT mRNA expression
- Brain, liver, kidney, and spleen weight
- Tissue toxicity evaluation

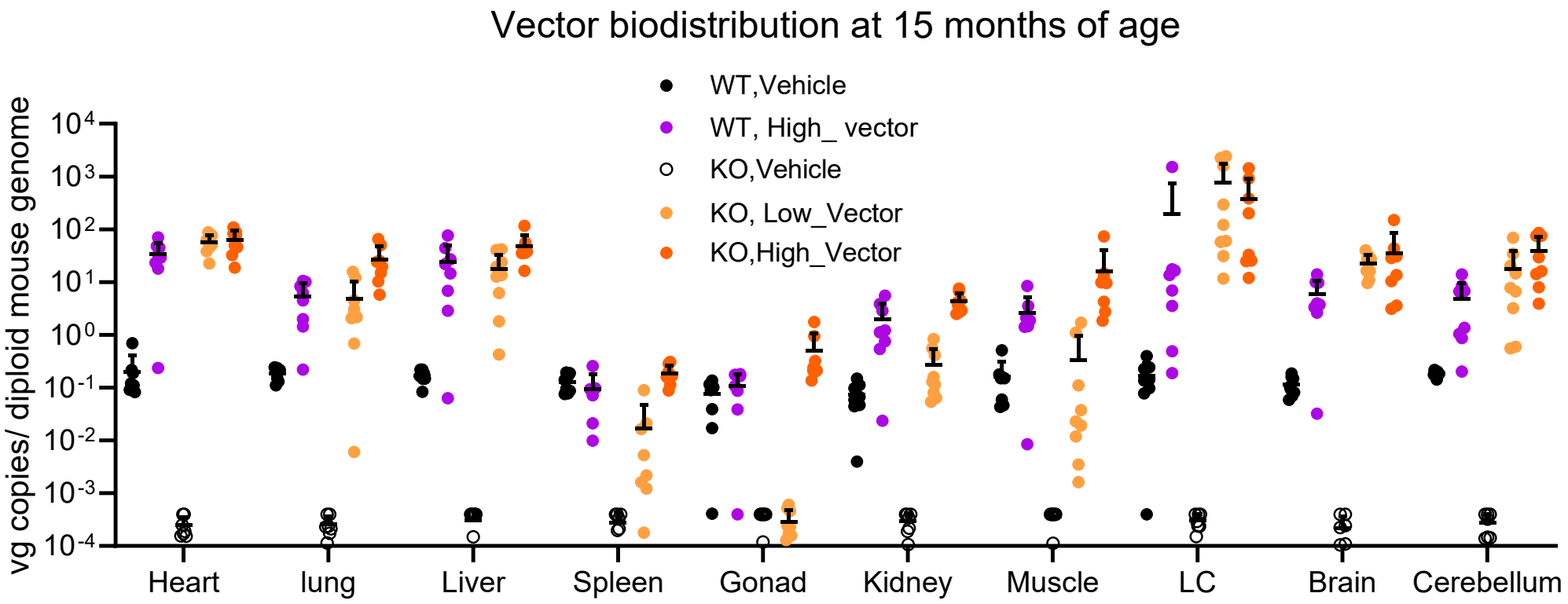
- Monitor long-term safety / abnormality /survival
- Weight BW weekly 1st month and then monthly

- Behavioral tests at 4, 6, 9, and 12 moa
  - OF, EPM, NOR, RR, Y-maze
- Urine collection: 4, 6, 9, 12, and 15 moa
- Serum toxicity panel: AST, BUN, CK

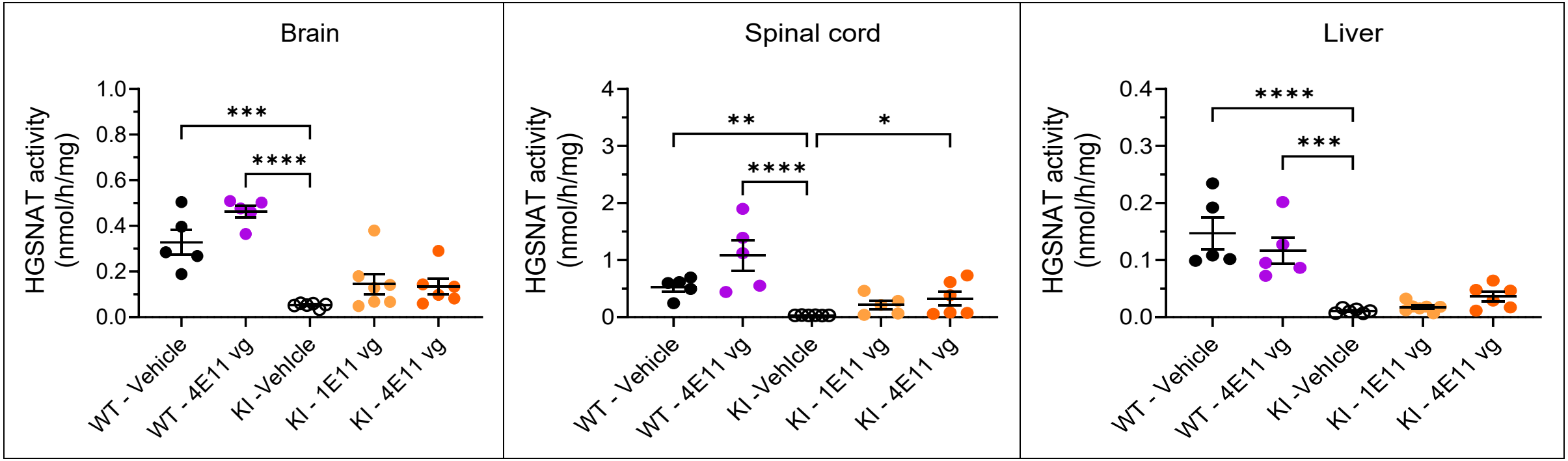
Dramatic and sustained increase in HGSNAT mRNA expression at 15 months in mice treated at P7–10



Marked and persistent increase in vector genome copies at 15 months in mice treated at P7–10

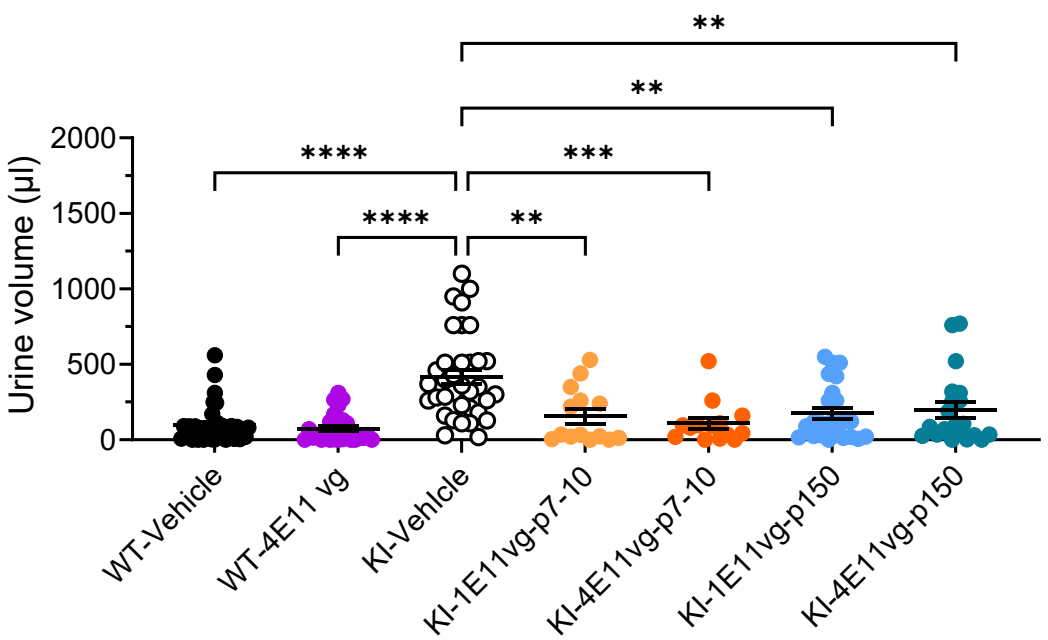


HGSNAT activity at 8 Months of age
is partially and dose responsively rescued with treatment at p7-10

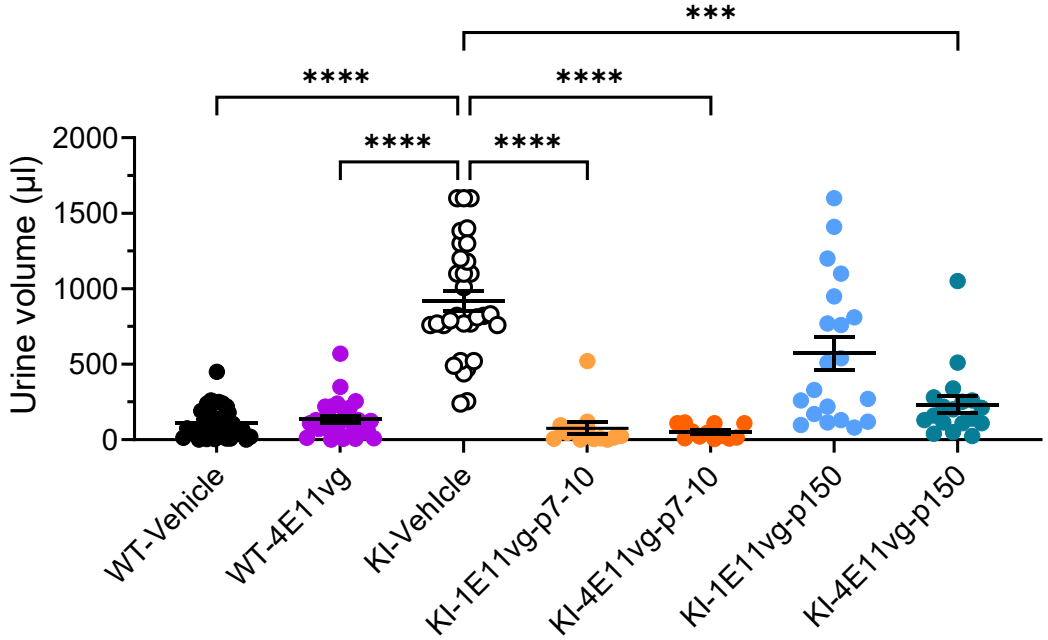


Urine retention at 9 and 12 months of age in MPSIIIC mice is rescued with the treatment at p7-10 or p150

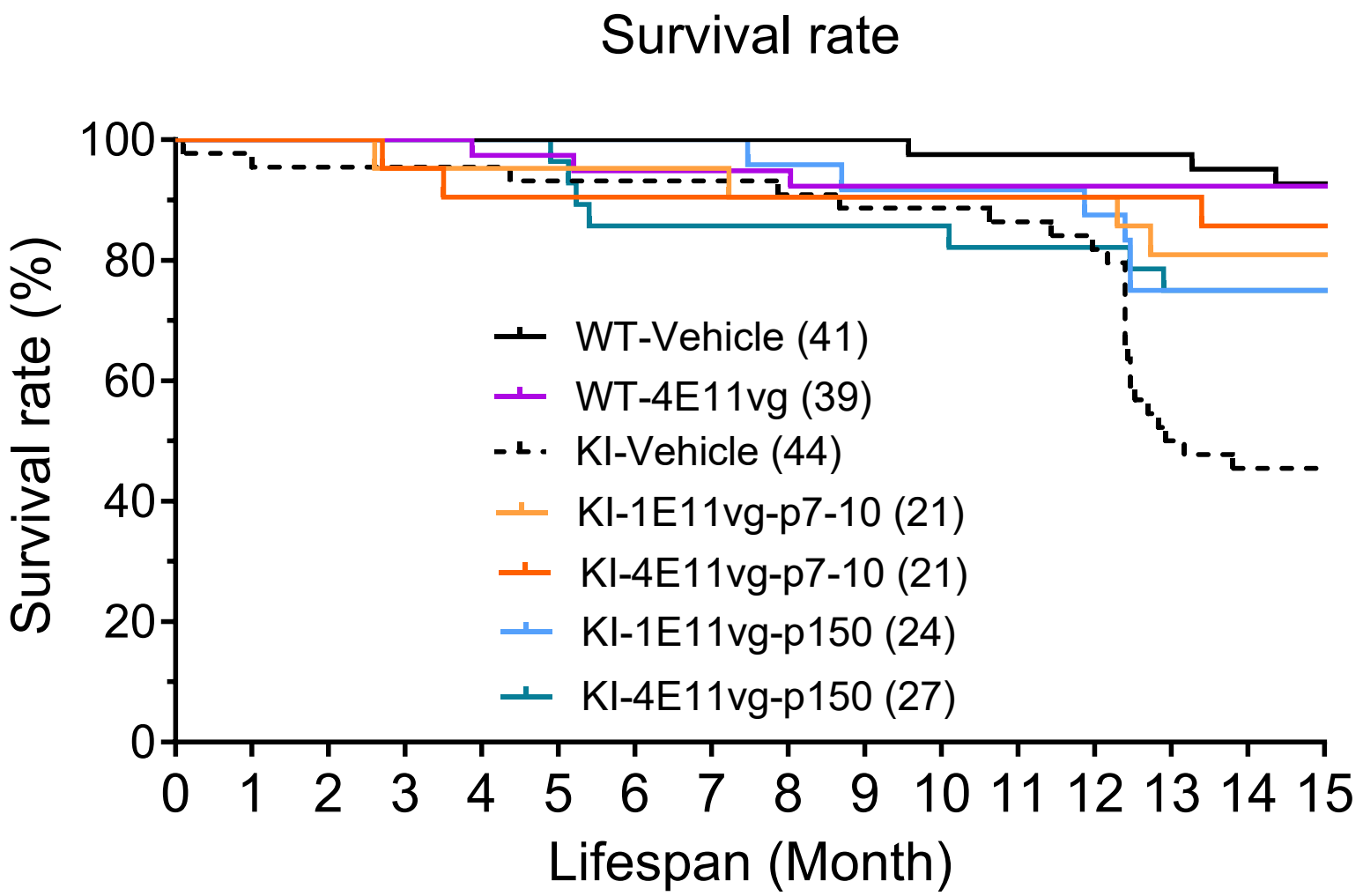
9 months old mice (n=14-33)



12 months old mice (n=12-34)

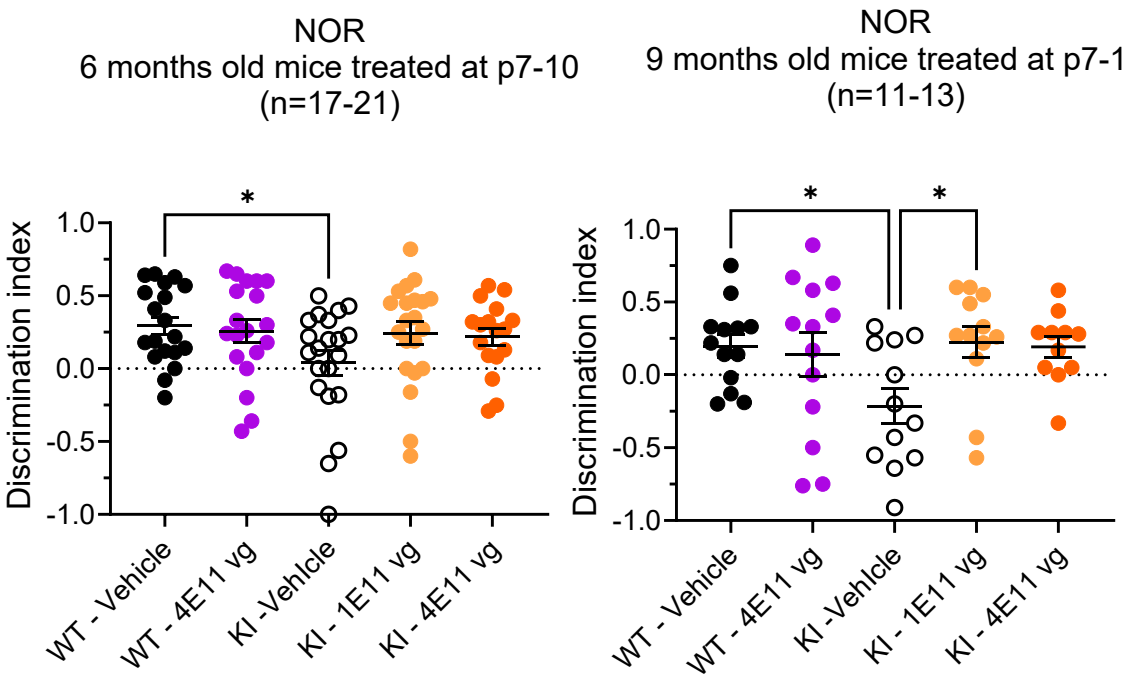


Treated MPSIIIC mice live longer than untreated mice (euthanasia due to extreme urinary retention)

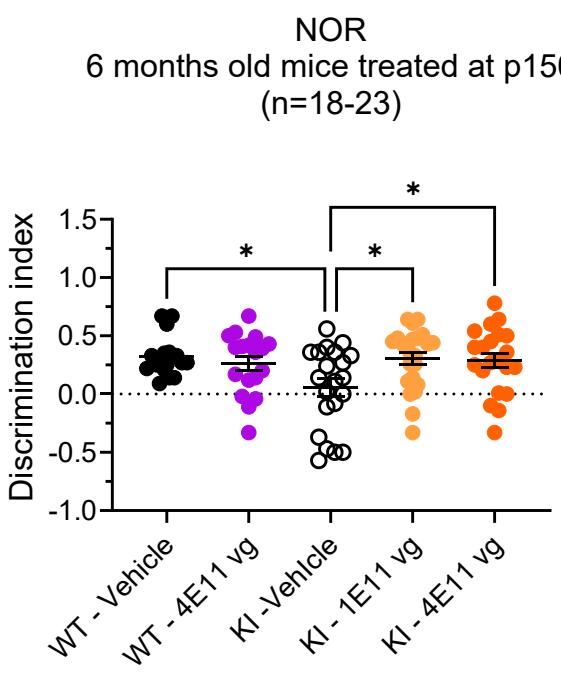


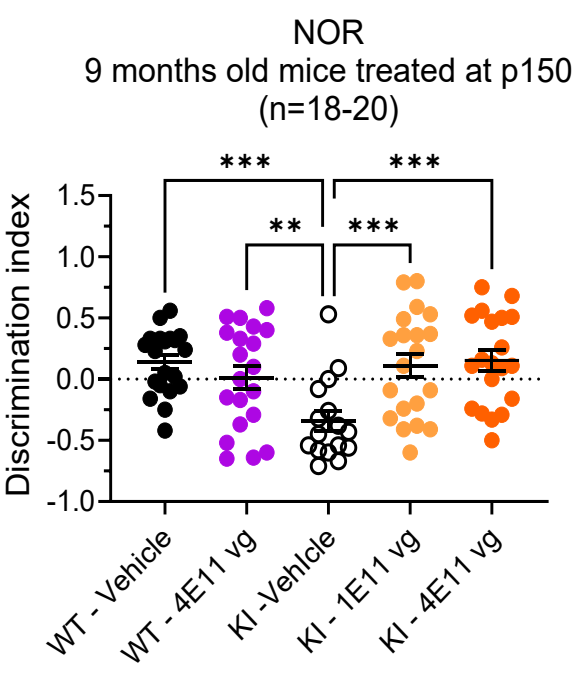
Reduced NOR performance in MPSIIIC mice is rescued with the treatment

Early Treatment

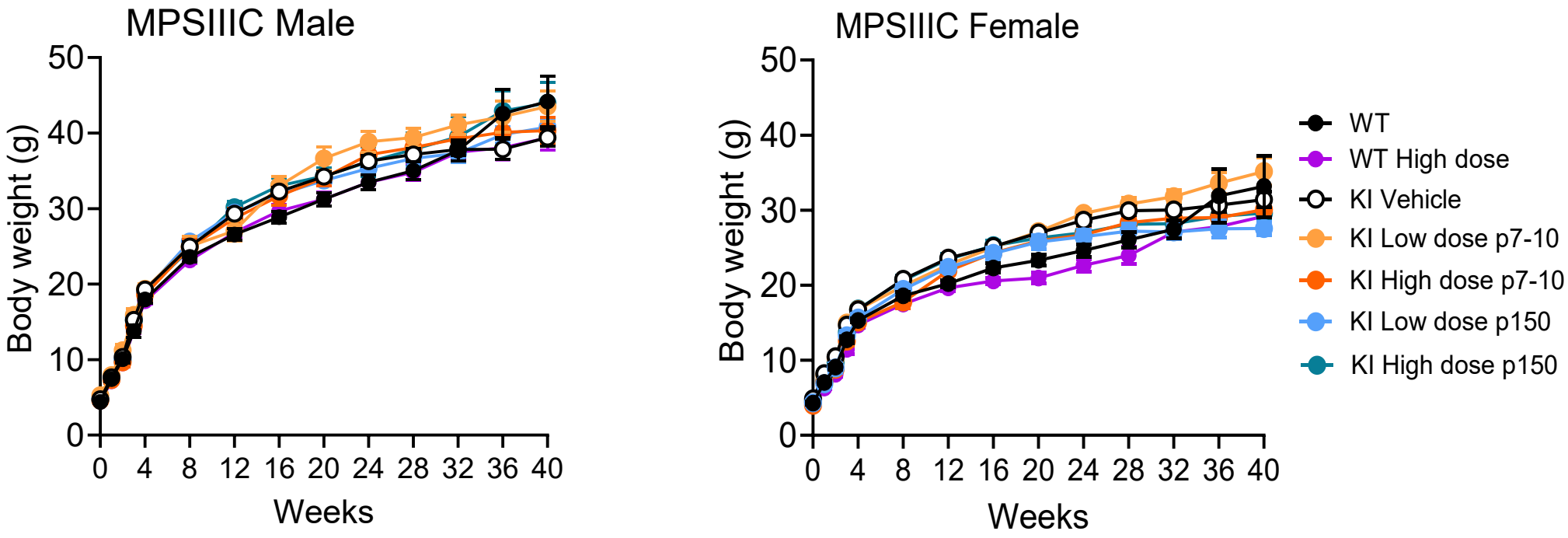


Late Treatment

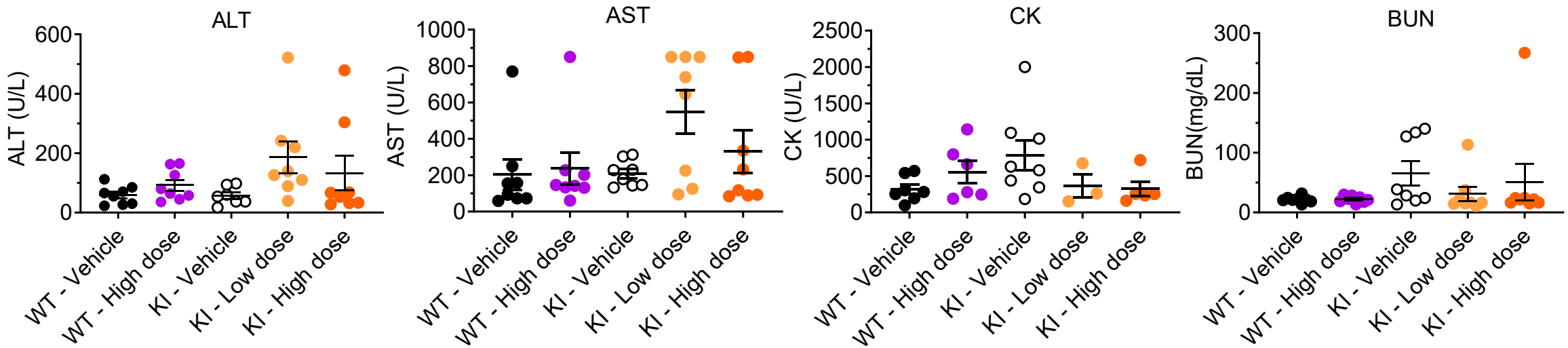




Body weight in MPSIIIC mice is similar across treated and non-treated groups



Serum toxicology panel is not affected with the treatment in MPSIIIC mice



Summary

Efficacy

- Dramatic and sustained increase in HGSNAT mRNA expression at 15 months in mice treated at P7–10.
- Reduced HGSNAT enzyme activity at 8 months in MPSIIIC mice is partially rescued with the treatment at p7-10.
- Urine retention at 9 and 12 months in MPSIIIC mice is rescued with the treatment at p7-10 or p150.
- Survival is improved in treated MPSIIIC mice.
- Reduced novel object recognition performance at 6 months in MPSIIIC mice is rescued with the treatment at p7-10 or p150.

Safety

- Body weight and serum toxicology panel are not affected with the treatment with scAAV9/HGSNAT (JLK-247) vector.

These findings highlight gene replacement therapy with JLK-247 as a promising strategy for treating MPSIIIC.

Ongoing studies

- Evaluate postmortem histology.
- Substrate accumulation.
- Potential biomarkers.
- GLP toxicology studies to further assess the safety profile of JLK-247 in rats.
- Advance this experimental therapy toward clinical application.

Acknowledgments

- Viralgen Vector Core, Spain.
- Dr. Shari Birnbaum, UTSW Rodent Behavior Core.
- Dr. Mary Wight-Carter. UTSW Animal Research Center.
- Funding sources