TAYSHA GENE THERAPIES

Bringing New Cures to Life

Corporate Presentation

October 2021

Legal disclosure

FORWARD LOOKING STATEMENTS

This presentation contains forward-looking statements that involve substantial risks and uncertainties. All statements, other than statements of historical facts, contained in this presentation, including statements regarding our strategy, future operations, future financial position, future revenues, projected costs, prospects, plans and objectives of management, are forward-looking statements. The words “anticipate,” “believe,” “estimate,” “expect,” “intend,” “may,” “might,” “plan,” “predict,” “project,” “target,” “potential,” “will,” “would,” “could,” “should,” “continue,” and similar expressions are intended to identify forward-looking statements, although not all forward-looking statements contain these identifying words. These forward-looking statements are subject to a number of risks, uncertainties and assumptions. Risks regarding our business are described in detail in our Securities and Exchange Commission filings, including in our Annual Report on Form 10-K for the year ended December 31, 2020, and our Quarterly Report on Form 10-Q for the quarter ended June 30, 2021. We may not actually achieve the plans, intentions or expectations disclosed in our forward-looking statements, and you should not place undue reliance on our forward-looking statements. Actual results or events could differ materially from the plans, intentions and expectations disclosed in the forward-looking statements we make. The forward-looking statements contained in this presentation reflect our current views with respect to future events, and we assume no obligation to update any forward-looking statements except as required by applicable law.

This presentation includes statistical and other industry and market data that we obtained from industry publications and research, surveys and studies conducted by third parties as well as our own estimates of potential market opportunities. All of the market data used in this prospectus involves a number of assumptions and limitations, and you are cautioned not to give undue weight to such data. Industry publications and third-party research, surveys and studies generally indicate that their information has been obtained from sources believed to be reliable, although they do not guarantee the accuracy or completeness of such information. Our estimates of the potential market opportunities for our product candidates include several key assumptions based on our industry knowledge, industry publications, third-party research and other surveys, which may be based on a small sample size and may fail to accurately reflect market opportunities. While we believe that our internal assumptions are reasonable, no independent source has verified such assumptions.

Driven by a relentless focus on

discovering, developing, and

commercializing novel AAV-based gene therapies

for devastating disorders of the central nervous system

Taysha summary overview

Multiple product candidates with anticipated near-term catalysts to enhance value	• High dose cohort data for TSHA-120 in GAN in 2H 2021 • First-in-human clinical data for TSHA-101 in GM2 gangliosidosis in 2H 2021 • Preliminary Phase 1 clinical data in CLN7 disease expected by YE 2021 • Initiation of Phase 1/2 trial for TSHA-118 in CLN1 in 2H 2021 • Submit four IND/CTA filings, including Rett syndrome, in 2021 • Advancement of four product candidates in IND-enabling studies, four in discovery in 2021
Portfolio of 27 CNS gene therapy programs across 3 distinct franchises	• Current pipeline of 27 AAV gene therapy programs • Portfolio addressing over 500,000 patients (US+EU) across monogenic CNS diseases, including neurodegenerative diseases, neurodevelopmental disorders, and genetic epilepsies
UT Southwestern Gene Therapy Program strategic alliance	• Led by Drs. Steven Gray and Berge Minassian; established to accelerate R&D, with integration of translational research, clinical development and GMP manufacturing • Exclusive access to resources, expertise, and novel technology platforms for delivery and dosing of gene therapies
Validated capsid, manufacturing system and route of delivery	• Clinically and commercially proven AAV9 vector platform • Highly scalable suspension HEK293 manufacturing process with excellent yield • Intrathecal delivery enables direct targeting to the CNS with validated biodistribution and safety
Proven management team and investor syndicate	• Deep expertise in the development of gene therapies for rare diseases • Key leadership team members and investors previously led the development and commercialization of Zolgensma®, the first FDA-approved gene therapy for CNS disease

Leadership team uniquely positioned to deliver on corporate mission

Leadership

RA Session II *Founder, President & CEO*	
Suyash Prasad, MBBS, MSc, MRCP, MRCPCH, FFPM *Chief Medical Officer and Head of R&D*	
Kamran Alam, CPA, MBA *Chief Financial Officer*	
Fred Porter, PhD *Chief Technical Officer*	
Mishima Gerhart *Chief Regulatory Officer and Head of Quality*	
Sean McAuliffe *Chief Commercial Officer*	
Mary Newman *Chief Development Officer*	
Jim Rouse *Chief Information Officer*	
Emily McGinnis *Chief Patient Officer & Head of Government Affairs*	
Tim Douros, JD *Chief Legal Officer and Corporate Secretary*	
Tracy Porter, M.Ed., SPHR *Chief People Officer*	

Advisors

Steven Gray, PhD *Chief Scientific Advisor*	
Berge Minassian, MD *Chief Medical Advisor*	

Board of Directors

Sean Nolan *Chairman*	
Paul Manning	
Phillip Donenberg	
Sukumar Nagendran, MD	
Laura Sepp-Lorenzino, PhD	
Kathleen Reape, MD	
RA Session II	

Scientific Advisory Board of preeminent international scientific and clinical thought leaders in gene therapy, CNS diseases and drug discovery and development

Scientific Advisory Board

Deborah Bilder, MD	University of Utah Registry of Autism and Developmental Disabilities (URADD); Utah Regional Education; BioMarin Pharmaceutical
Alan Boyd, BCc, MB, ChB, FRSB, FFLM, FRCP, FFPM	Boyd Consultants; Royal Colleges of Physicians; University of Birmingham Medical School; AstraZeneca; Ark Therapeutics Ltd
Wendy K. Chung, MD, PhD	Columbia University; Simons Foundation Autism Research Initiative (SFARI)
David P. Dimmock, MD	Rady Children's Institute for Genomic Medicine; FDA; CDC
Michael W. Lawlor, MD, PhD	The Neuroscience Research Center at the Medical College of Wisconsin; Solid Biosciences
Gerald S. Lipshutz, MD, MS	David Geffen School of Medicine at University of California, Los Angeles; Wellcome Trust, UK; NIH

Diverse pipeline focused exclusively on monogenic disorders of the central nervous system

Neurodegenerative Diseases

Diseases characterized by the progressive degeneration of the structures and function of the CNS and PNS

Neurodevelopmental Disorders

Multi-faceted conditions characterized by impairments in cognition, behavior, and motor function

Genetic Epilepsies

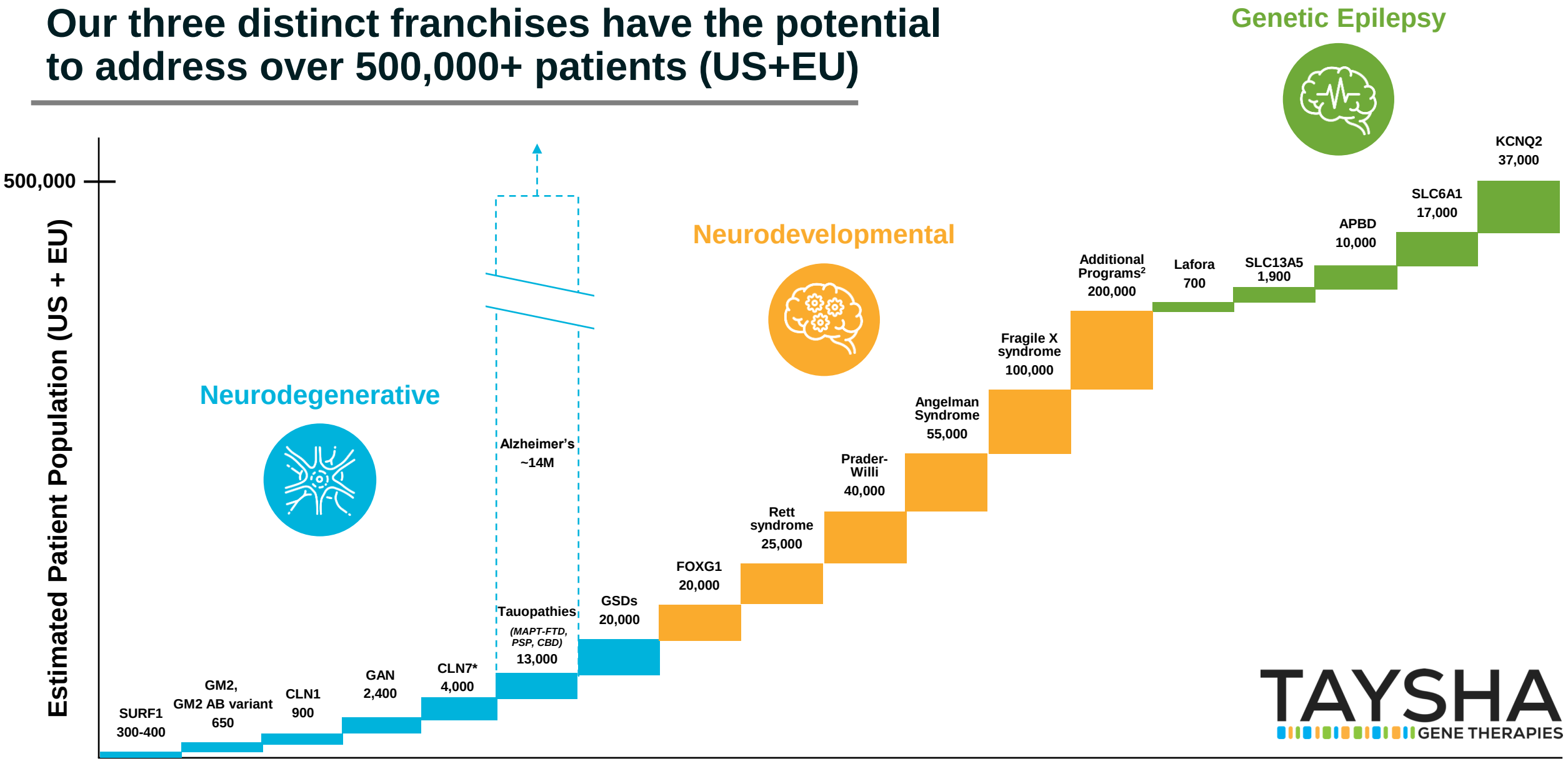
Disorders characterized by recurrent seizures often leading to abnormal development of the brain

Unparalleled gene therapy pipeline focused exclusively on monogenic CNS disorders

PROGRAM		INDICATION	DISCOVERY	PRECLINICAL	PHASE 1/2	Pivotal	GLOBAL COMM. RIGHTS
NEURODEGENERATIVE DISEASES							
TSHA-120	*GRT*	Giant Axonal Neuropathy				Regulatory guidance YE 2021	TAYSHA GENE THERAPIES
TSHA-101	*GRT*	GM2 Gangliosidosis				Currently open CTA	
TSHA-118	*GRT*	CLN1 Disease				Currently open IND	
TSHA-119	*GRT*	GM2 AB Variant					
TSHA-104	*GRT*	SURF1-Associated Leigh Syndrome				IND/CTA submission 2H 2021	
TSHA-113	*miRNA*	Tauopathies					
TSHA-115	*miRNA*	GSDs					
Undisclosed	GRT/*shRNA*	Undisclosed					
Undisclosed	GRT	Undisclosed					
NEURODEVELOPMENTAL DISORDERS							
TSHA-102	*Regulated GRT*	Rett Syndrome				IND/CTA submission 2H 2021	TAYSHA GENE THERAPIES
TSHA-106	*shRNA*	Angelman Syndrome					
TSHA-114	*GRT*	Fragile X Syndrome					
TSHA-116	*shRNA*	Prader-Willi Syndrome					
TSHA-117	*Regulated GRT*	FOXG1 Syndrome					
TSHA-107	*GRT*	Autism Spectrum Disorder					
TSHA-108	*GRT*	Inborn Error of Metabolism					
TSHA-109	*GRT*	Inherited Metabolism Disorder					
Undisclosed	*GRT*	Undisclosed					
Undisclosed	mini-gene	Undisclosed					
GENETIC EPILEPSY							
TSHA-103	*GRT*	SLC6A1 Haploinsufficiency Disorder					TAYSHA GENE THERAPIES
TSHA-105	*GRT*	SLC13A5 Deficiency					
TSHA-112	*miRNA*	APBD					
TSHA-111-LAFORIN	*miRNA*	Lafora Disease					
TSHA-111-MALIN	*miRNA*	Lafora Disease					
TSHA-110	mini-gene	KCNQ2					
Undisclosed	mini-gene	Undisclosed					

GRT: Gene replacement therapy **miRNA**: microRNA **shRNA**: short hairpin RNA

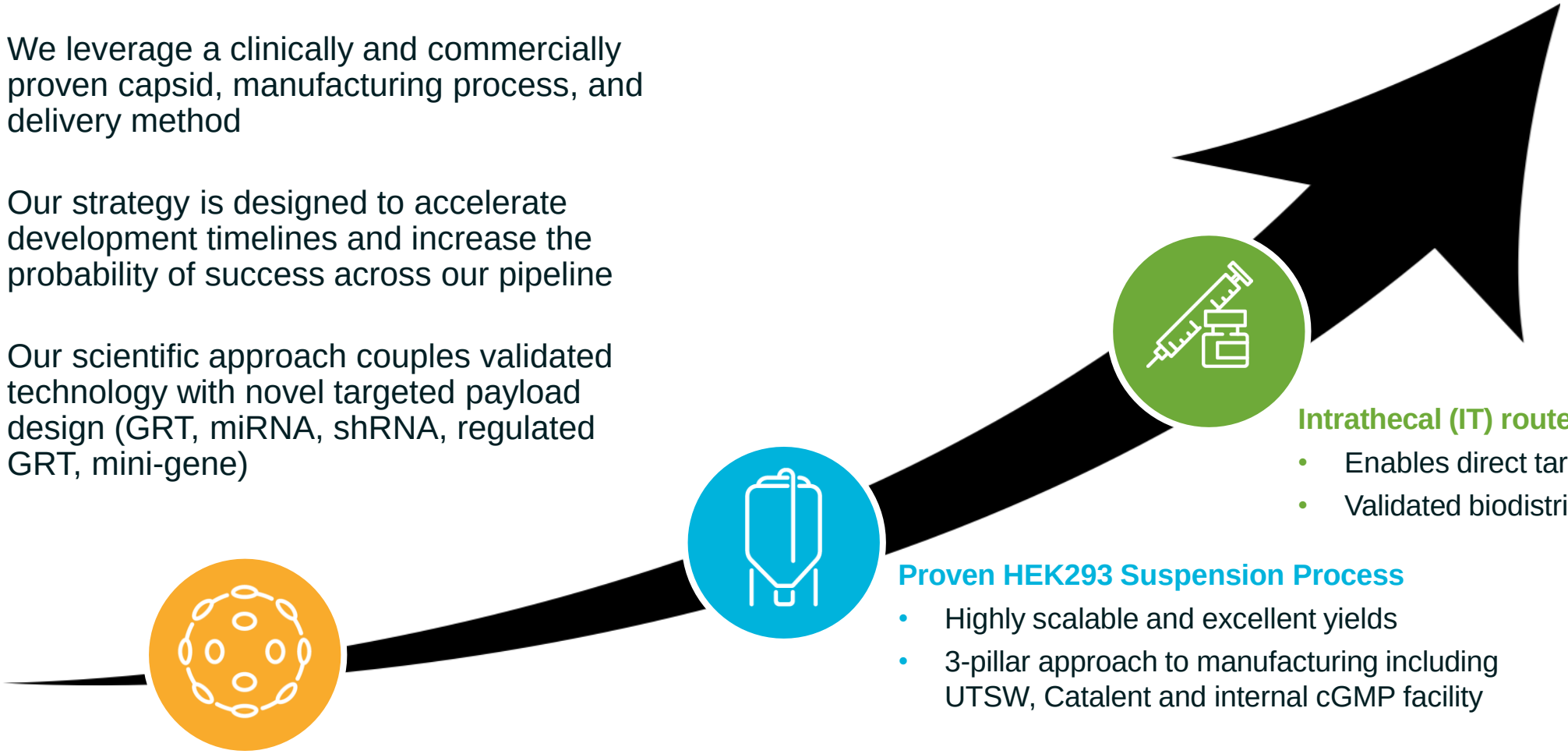
Our three distinct franchises have the potential to address over 500,000+ patients (US+EU)



*Worldwide
[1]Tauopathies only include MAPT-FTD, PSP, CBD
[2]Additional programs include TSHA-107, TSHA-108, and TSHA-109

Our strategy is focused on rapid clinical and commercial development

- We leverage a clinically and commercially proven capsid, manufacturing process, and delivery method
- Our strategy is designed to accelerate development timelines and increase the probability of success across our pipeline
- Our scientific approach couples validated technology with novel targeted payload design (GRT, miRNA, shRNA, regulated GRT, mini-gene)



Intrathecal (IT) route of administration

- Enables direct targeting to CNS
- Validated biodistribution and safety profile

Proven HEK293 Suspension Process

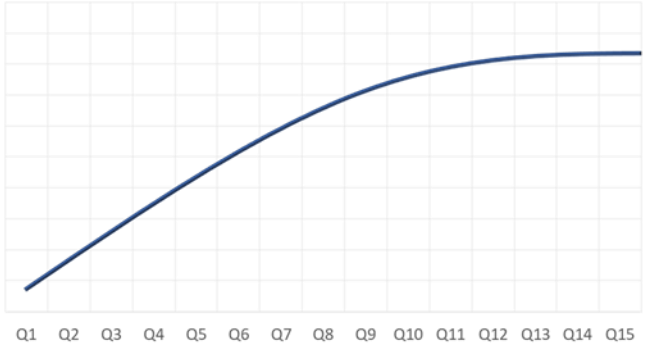
- Highly scalable and excellent yields
- 3-pillar approach to manufacturing including UTSW, Catalent and internal cGMP facility

AAV9 vector for delivery of therapeutic transgene

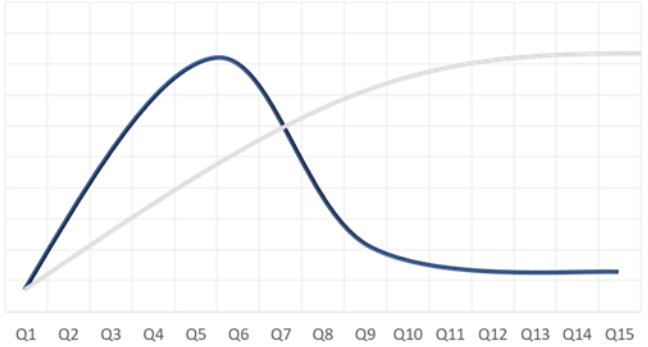
- Demonstrated safety and efficacy across multiple CNS indications

Creating a sustainable business model for gene therapy

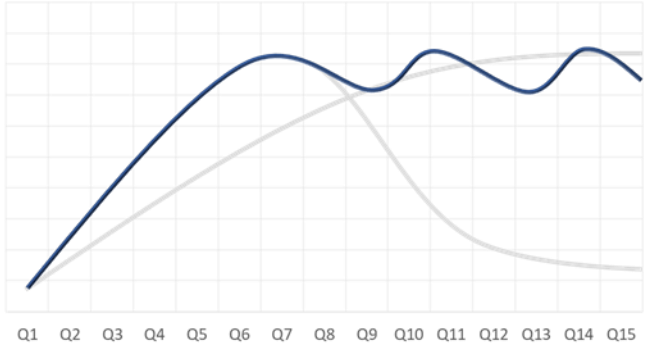
Traditional chronic dosing business model



One-time dosing business model



Taysha's sustainable gene therapy platform business model



Approach and ability to deliver various payloads

Gene Replacement

- Replace gene of interest to treat diseases or disorders with limited gene expression
- Comprised of a codon-optimized DNA transgene that encodes the wild type gene of interest
- Transgene (or mini-gene) coupled with a promoter selected to ensure expression in the cell or tissue-type of interest

Regulated Gene Replacement

- Regulate expression of a therapeutic transgene
- Built-in regulation system to replace dose-sensitive genes safely and at therapeutic levels
- Uses miRARE, our novel miRNA target panel

Vectorized RNA

- Transgenes designed to express miRNA (small, non-coding sequences of RNA that result in silencing of gene expression)
- Transgenes designed to express short-hairpin RNA (shRNA), which reactivate a silenced gene upon binding to the target of interest

Mini-Gene Payloads

- Many genes are too large to fit in AAV capsids
- Mini-genes designed to overcome limited AAV packaging capacity
- Collaboration with Cleveland Clinic to advance next-generation mini-gene payloads initially for genetic epilepsies and neurodevelopmental disorders

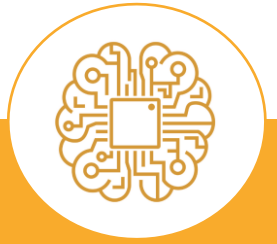
Novel platform technology that powers our research engine

Novel AAV Dosing Platform

- Potential to facilitate redosing via vagus nerve
- Efficient targeting of vagal neurons demonstrated in adult rats, with potential to improve autonomic nervous system symptoms in humans
- Normal vagal nerve fibers and neurons post AAV delivery to the vagus nerve in dogs

miRARE Platform

- Novel miRNA target panel derived from high-throughput miRNA profiling and genome mining
- Designed for safely regulated transgene expression levels in the brain
- Needed in disorders like Rett syndrome where high doses of transgene-expressing vectors may be harmful while low doses may avoid toxicity but be subtherapeutic
- Built-in regulation system harnesses endogenous systems



Novel Capsid Identification

- Improves targeted delivery through use of machine learning, capsid shuffling and directed evolution
- Allows rapid identification of capsids with improved properties in mice and Non- Human Primates (NHPs) to maximize translational relevance
- Potential to drive new product candidates with novel biodistribution and transduction profiles into pipeline

Our strategic partnership with UTSW

UT Southwestern Medical Center

We have access to a world-class team of scientists and cutting-edge technology through an exclusive, worldwide royalty-free license to discover, develop, and commercialize gene therapies led by:

- **Berge Minassian, MD**, Division Chief of Child Neurology
  - Pediatric neurologist with expertise in neurodegenerative diseases, neurodevelopmental disorders, and genetic forms of epilepsy
  - Discovered *MECP2* CNS isoform (Rett syndrome)
- **Steven Gray, PhD**, Director of Viral Vector Core, Associate Professor Dept of Peds
  - AAV-based vector engineering expertise and optimizing CNS delivery of transgenes
  - Administered the first AAV9-based therapy to patients via intrathecal route
- Exclusive access to a flexible, scalable, and well-characterized GMP manufacturing suite that utilizes a suspension HEK293 process
- Exclusive access to next generation platform technologies, including novel redosing platform, transgene regulation (miRARE), and capsid development

Manufacturing strategy allows flexibility and scalability to support broad pipeline

UT Southwestern Medical Center®

- Support the UTSW viral vector core to supply early-phase clinical material
  - Active technical collaboration and knowledge sharing for process information and analytical methods
  - First program is ongoing
- Capabilities
  - 50L tox production
    - 200L available by EOY
  - 500L GMP manufacturing
    - GMP operations began in December 2020
  - In-house support for critical release and stability testing

Catalent®

- Establish collaborations with leading CDMO to provide additional capacity for early-phase and pivotal supply
  - Strategic partnership in place with Catalent Gene Therapies
    - Two programs ongoing
    - Able to leverage process, methods and materials across programs
- Current Capabilities
  - 200/400L tox production
  - 800L GMP manufacturing
  - Full support for release and stability testing

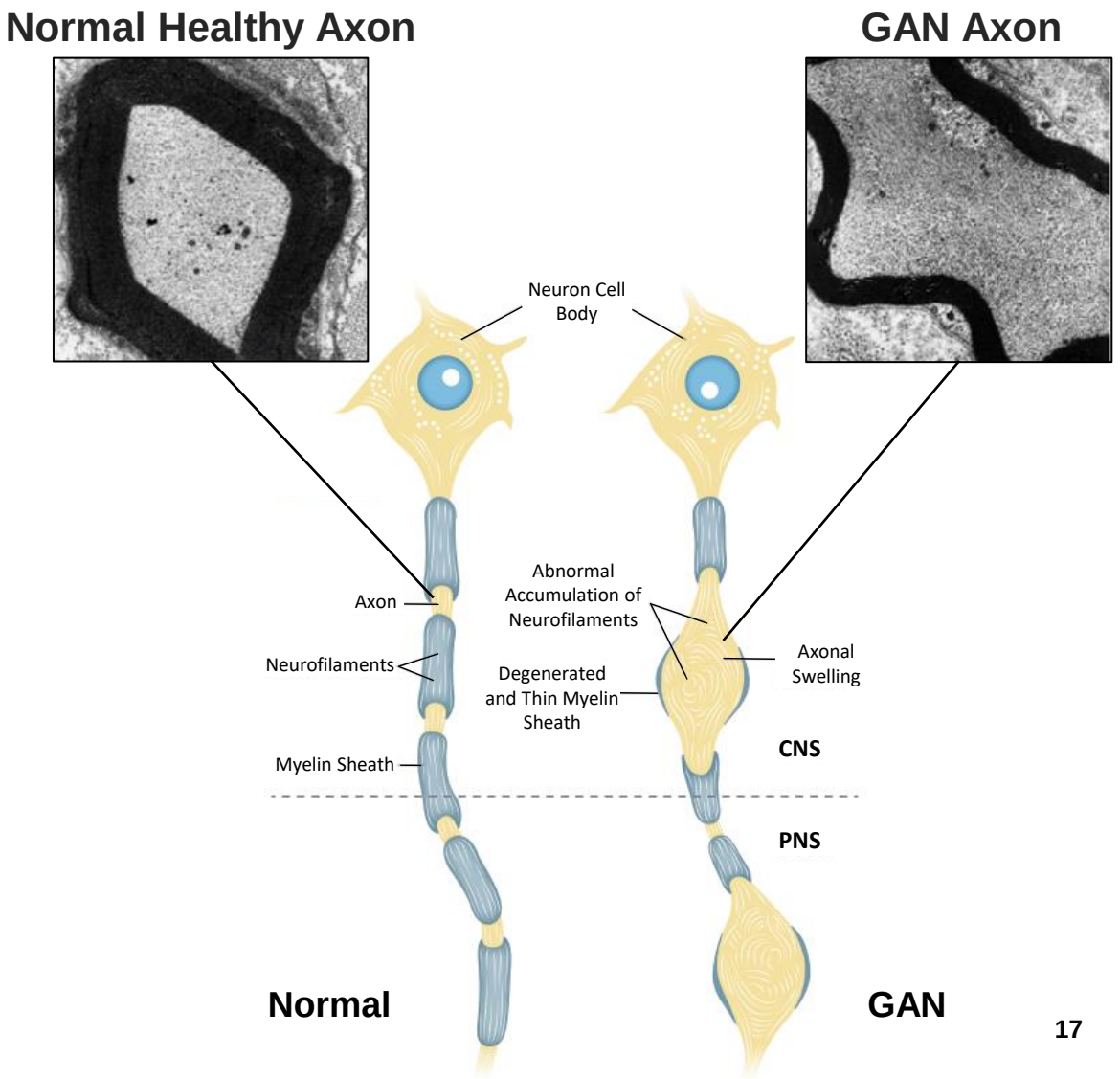
TAYSHA GENE THERAPIES

- Build internal manufacturing facility to support clinical and commercial manufacturing
  - Initial build includes two vector manufacturing trains, one fill/ finish suite, QC and technical development labs
  - Building secured in Durham, NC
    - Growing hub for gene therapy manufacturing
- Facility timing
  - Kicked off 1Q 2021
  - Office and development labs operational in 1Q 2022
  - GMP ready in 2023

Neurodegenerative Disease Franchise

Rationale for targeting the *GAN* gene

- Mutations affect production of the protein gigaxonin
  - Leads to accumulation of neurofilaments in giant axons causing signal interruption and neurodegeneration
- Genetic changes in the *GAN* gene have been shown to cause Giant Axonal Neuropathy
- Good candidate for gene transfer approach
  - Small gene that is easy to package into AAV9 capsid
  - High transduction to target organ
  - Low-level expression may restore function
  - A clear model for other disorders with similar mechanism such as GM2 gangliosidosis, CLN1 disease, SURF1-associated Leigh syndrome and amyotrophic lateral sclerosis (ALS)

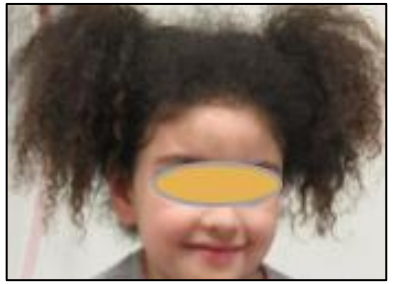


Giant axonal neuropathy (GAN) is a rare inherited genetic disorder that affects both the central and peripheral nervous systems

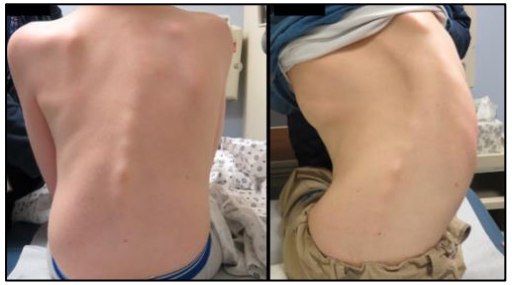
TSHA-120
GAN

- Rare autosomal recessive disease of the central and peripheral nervous systems caused by loss-of-function gigaxonin gene mutations
- Majority of children with GAN show symptoms and features before age 5
  - Dull, tightly curled hair
  - Progressive scoliosis
  - Contractures
  - Giant axons
  - Spinal cord atrophy
  - White matter abnormality
- No approved disease-modifying treatments available
- Symptomatic treatments attempt to maximize physical development and minimize deterioration
- Early- and late-onset phenotypes – shared physiology
  - Late-onset often categorized as Charcot-Marie-Tooth Type 2 (CMT2), with lack of tightly curled hair and CNS symptoms, and relatively slow progression
  - Represents 1% to 6% of all CMT2 diagnosis
  - Late-onset poor quality of life but not life-limiting
- Estimated prevalence of GAN is 2,400 patients (US+EU)

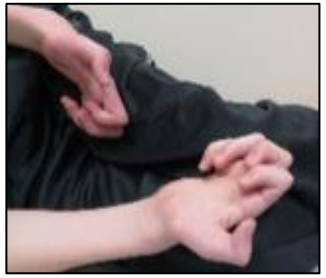
Tightly Curled Hair



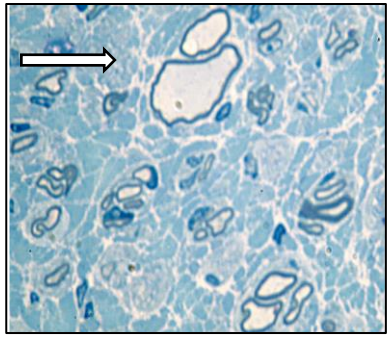
Progressive Scoliosis



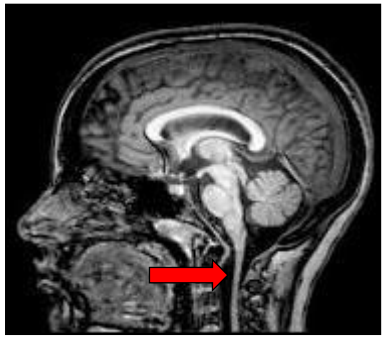
Contractures



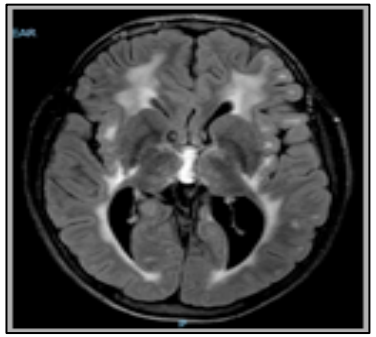
Giant Axons



Spinal Cord Atrophy

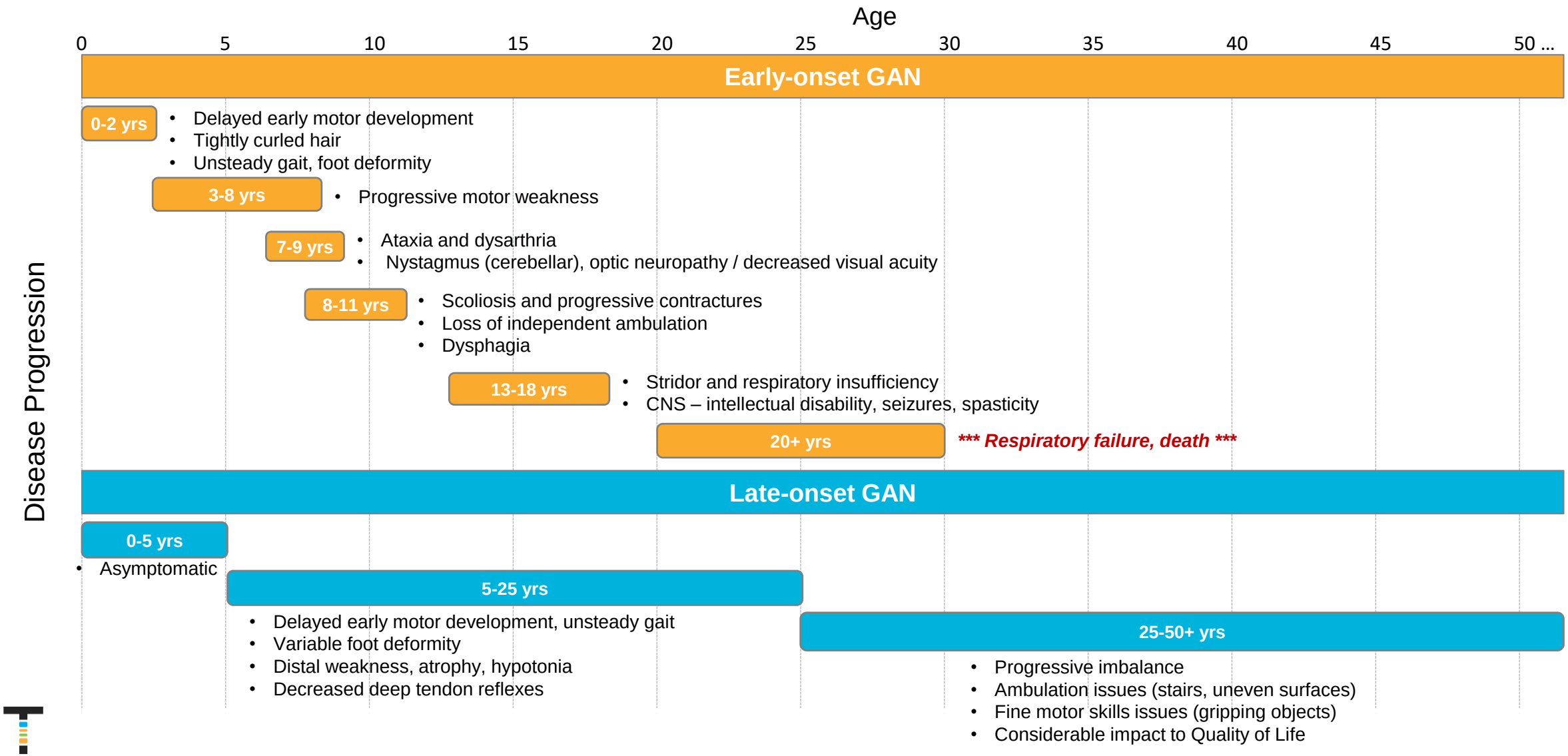


White Matter Abnormality



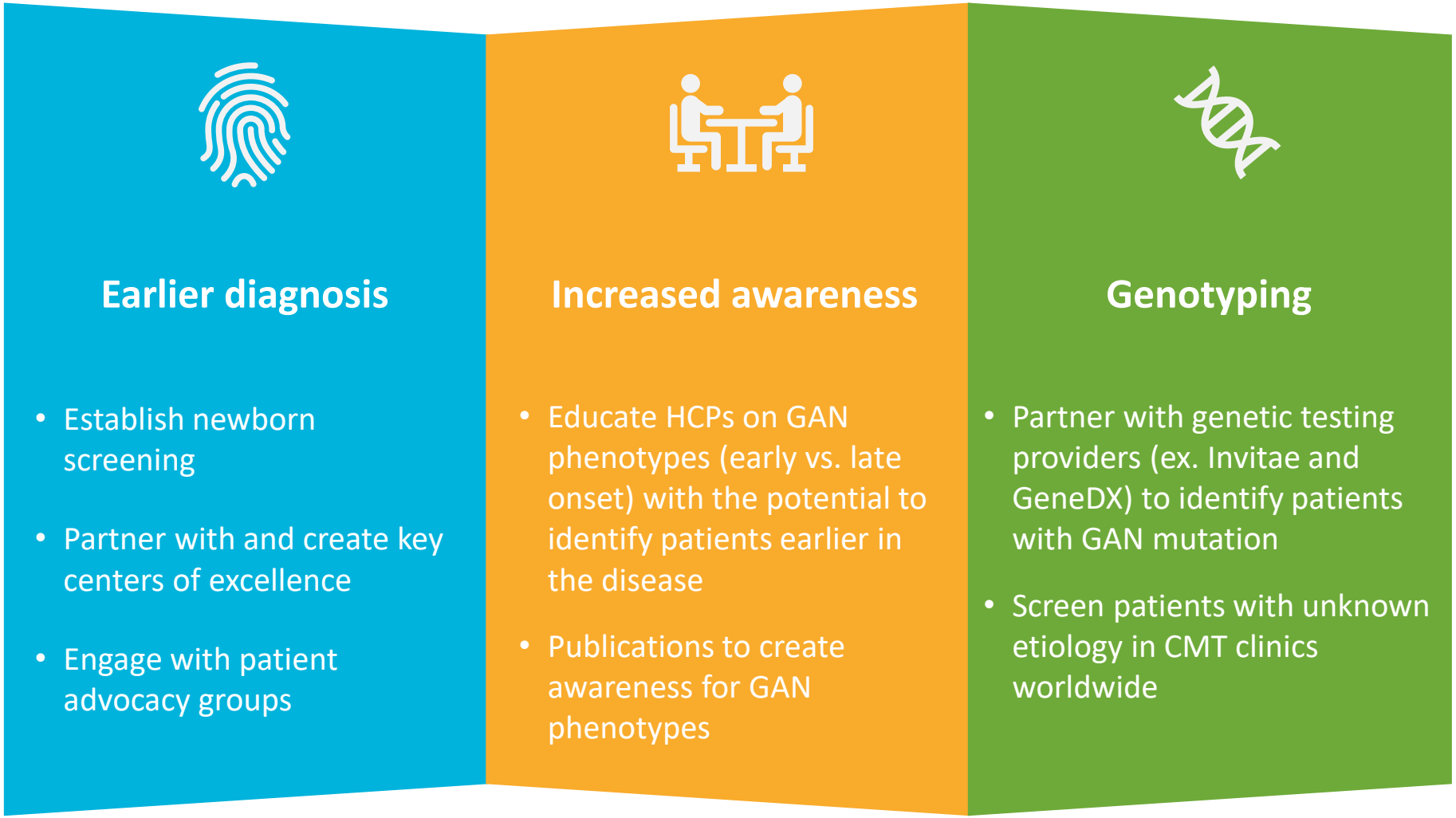
Murphy SM et al. *J Neurol Neurosurg Psychiatry* 2012; Gess B et al. *Neuromuscul Disord* 2013; Bacquet J et al. *BMJ Open* 2018; Antoniadi T et al. *BMC Med Genet* 2015

GAN natural history and disease progression



Maximizing patient access and identification to address the estimated 2,400 patients in US and EU

TSHA-120
GAN



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Primary efficacy endpoint is the Motor Function Measure (MFM32) – A validated quantitative scale

- Validated instrument used in multiple regulatory approvals
- A 32-item scale for motor function measurement developed for neuromuscular diseases
- Assesses severity and progression of motor function across a broad spectrum and in 3 functional domains
  - Standing, transfers and ambulation
  - Proximal and axial function
  - Distal function
- 32 items scored between 0 and 3 for a maximum score of 96
  - A higher score means that an individual was able to complete the task
  - Sometimes, the score is converted to a percentage
- A 4-point change is considered clinically meaningful in the following indications:
  - DMD
  - SMA
  - LAMA2-related muscular dystrophy
  - Cerebral palsy

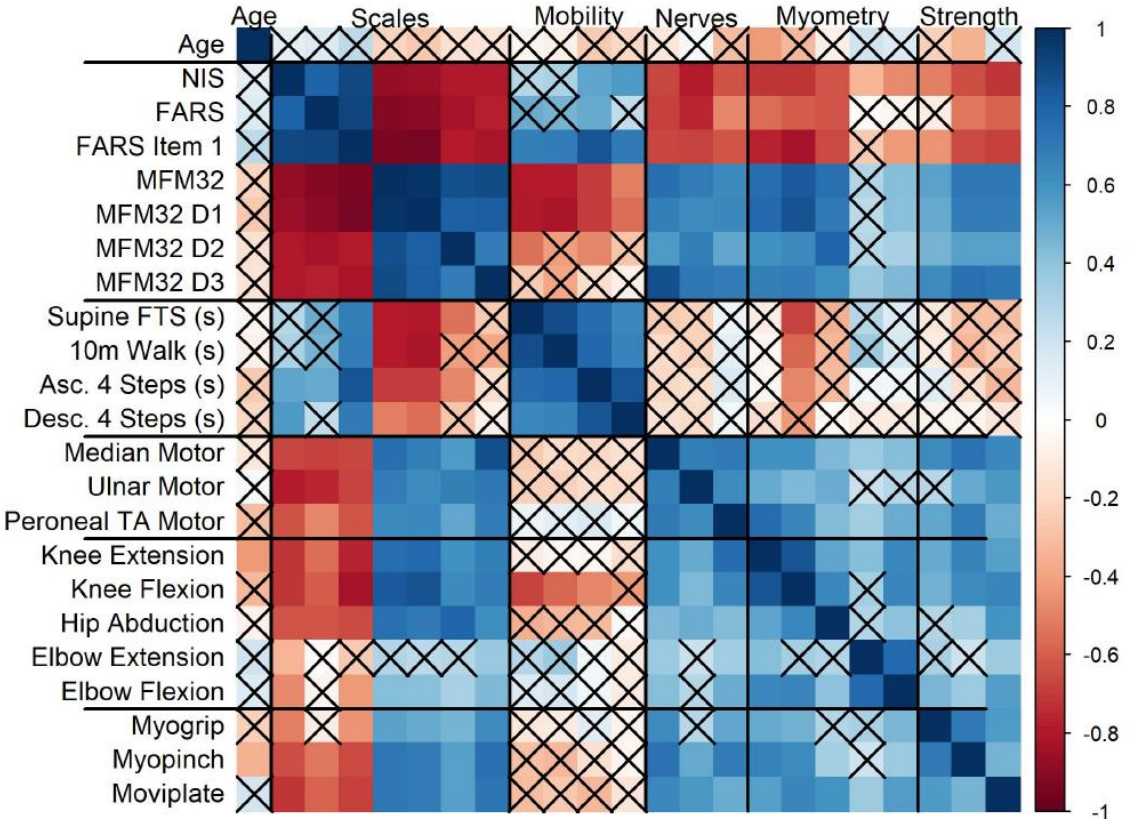
Examples of tasks

No.	Domain	Starting Position	Exercise Requested
1	D1	Supine, lower limbs half-flexed, kneecaps at zenith, and feet resting on mat	Raise the pelvis; the lumbar spine, the pelvis and the thighs are aligned and the feet slightly apart
2	D1	Supine	Without upper limb support, sits up
3	D1	Seated on the mat	Stands up without upper limb support
4	D1	Standing	Without upper limb support, sits down on the chair with the feet slightly apart
5	D1	Seated on chair	Stands up without upper limb support and with the feet slightly apart
6	D1	Standing with upper limb supported	Releases the support and maintains a standing position for 5s with the feet slightly apart, the head, trunk, and limbs in the midline position
7	D1	Standing with upper limb supported on equipment	Without upper limb support, raises the foot for 10s
8	D1	Standing	Without support, touches the floor with 1 hand and stands up again
9	D1	Standing without support	Takes 10 steps forward on both heels
10	D1	Standing without support	Takes 10 steps forward on a line
11	D1	Standing without support	Runs for 10m
12	D1	Standing on 1 foot without support	Hops 10 times in place

MFM32 correlations across various motor and demographic assessments

- Multiple measures of disease severity were evaluated, with MFM32 identified as having the highest correlation between all tested measures of mobility, neurophysiologic measures, force (by myometry measures), distal grip and pinch strength
- MFM32 correlated with:
  - LE strength ($p<0.001$ & $p=0.005$)
  - Median motor CMAP amplitude ($p=0.005$)
  - Grip strength ($p=0.003$)
- **NIS, FARS, MFM32** scores correlated most strongly with one another and with measures of strength and with motor CMAP amplitudes (NCS)

Correlation Matrix Measuring Strength and Frequency of Correlations Across Various Motor and Demographic Assessments



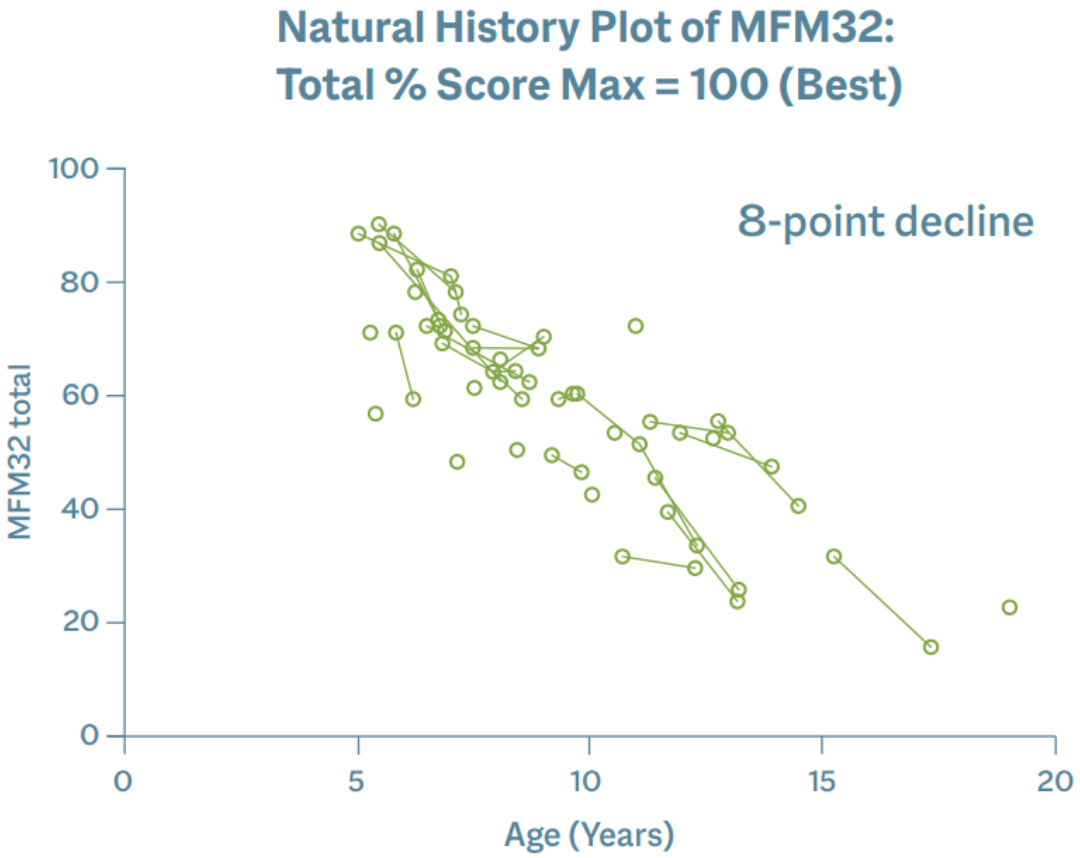
Bharucha-Goebel D et al. *Brain* 2021

GAN natural history study data as a dependable comparator for future studies

TSHA-120
GAN

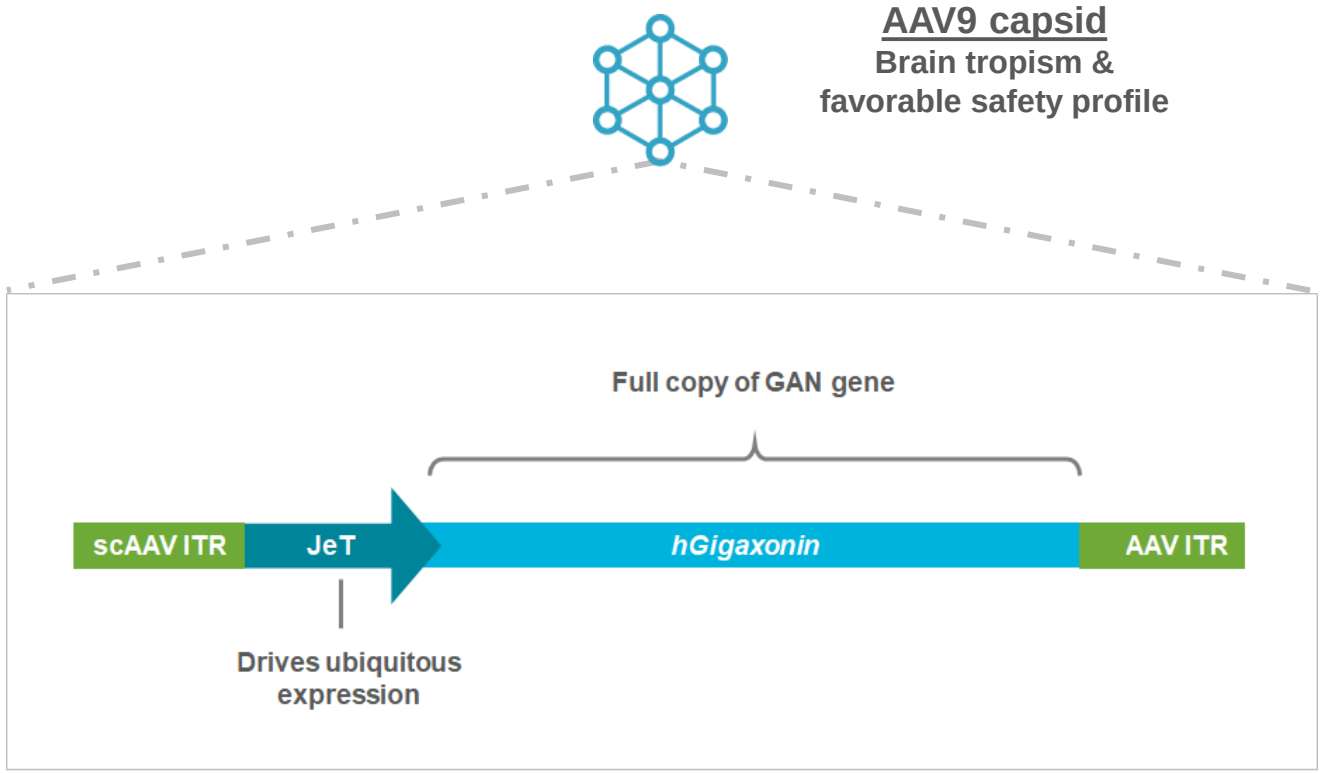
- 45 GAN patients (2013-present) ages 3-21 years
  - Can be accessed for treatment study
  - Will be used as comparator for treatment study
- MFM32
  - MFM32 total score shows uniform decline between patients of all age groups over time
  - Average decline is ~8 points per year
  - 4-point change is considered clinically meaningful
- MFM32 selected as primary endpoint due to least variability and its use in confirmatory trials

- **Natural history data: 8-point decline annually in MFM32**
- **4-point change in MFM32 considered clinically meaningful**



TSHA-120 program overview and construct

- Construct invented in the Gray Lab
- AAV9 viral vector with engineered transgene encoding the human gigaxonin protein
- Self-complementary AAV capsid (scAAV) for rapid activation and stable expression
- JeT promoter drives ubiquitous expression
- Designed to deliver a functional copy of the GAN gene with optimal tropism and rapid expression
- Received orphan drug and rare pediatric disease designations
- Clinical study ongoing at NIH, led by Carsten Bönnemann, MD

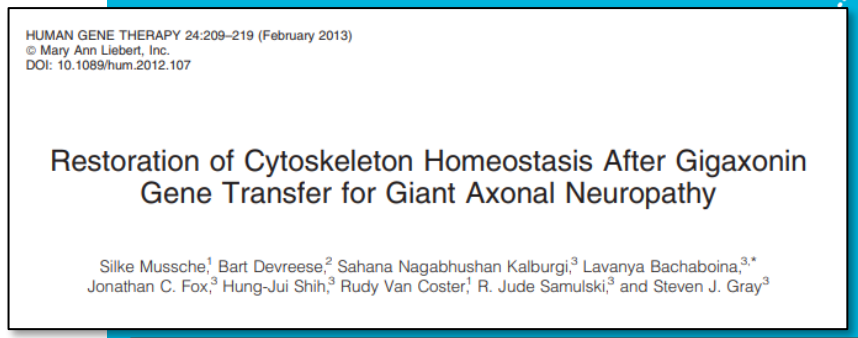


Preclinical data supported intrathecal dosing of TSHA-120

TSHA-120
GAN

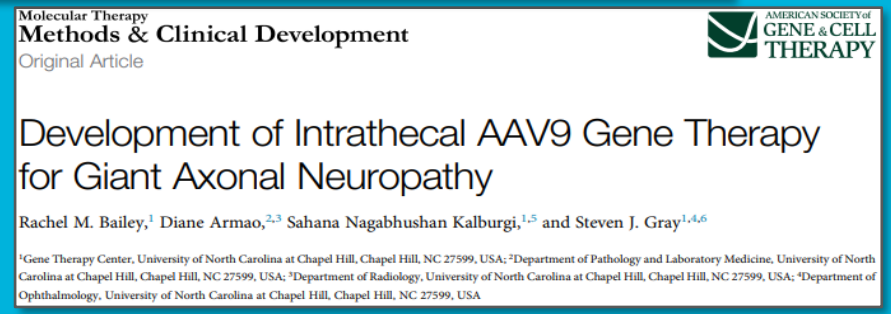
Comprehensive preclinical results demonstrated:

- Efficacy of gigaxonin gene replacement demonstrated *in vitro* and *in vivo*
- Resolution of intermediate filaments and improved disease pathology in GAN mice, including DRG and peripheral nerve
- Phenotypic rescue in GAN mice and GAN rats after intrathecal injection, improving motor function
- No toxicities in mice or non-human primates (NHPs) at up to a 4-fold overdose up to 1 year post injection
- No toxicities observed in rats at a 10-fold overdose up to 6 months post injection


HUMAN GENE THERAPY 24:209–219 (February 2013)
© Mary Ann Liebert, Inc.
DOI: 10.1089/hum.2012.107

Restoration of Cytoskeleton Homeostasis After Gigaxonin Gene Transfer for Giant Axonal Neuropathy

Silke Mussche,[1] Bart Devreese,[2] Sahana Nagabhushan Kalburgi,[3] Lavanya Bachaboina,[3,*] Jonathan C. Fox,[3] Hung-Jui Shih,[3] Rudy Van Coster,[1] R. Jude Samulski,[3] and Steven J. Gray[3]


Molecular Therapy
Methods & Clinical Development
Original Article

AMERICAN SOCIETY of GENE & CELL THERAPY

Development of Intrathecal AAV9 Gene Therapy for Giant Axonal Neuropathy

Rachel M. Bailey,[1] Diane Armao,[2,3] Sahana Nagabhushan Kalburgi,[1,5] and Steven J. Gray[1,4,6]

[1]Gene Therapy Center, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; [2]Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; [3]Department of Radiology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA; [4]Department of Ophthalmology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599, USA


Gene Therapy (2013), 1–10
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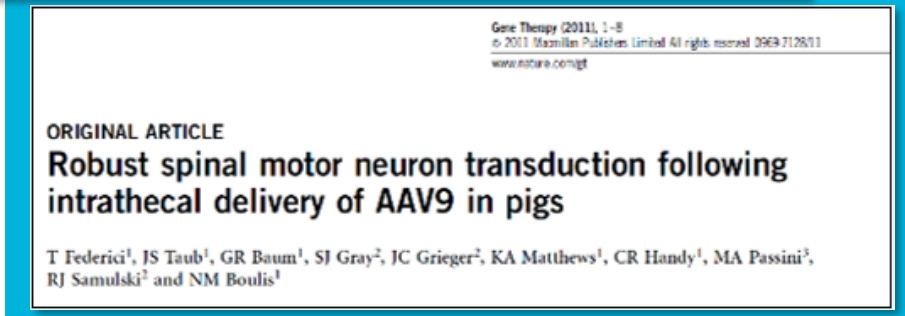
www.nature.com/gt

ORIGINAL ARTICLE

Global CNS gene delivery and evasion of anti-AAV-neutralizing antibodies by intrathecal AAV administration in non-human primates

SJ Gray, S Nagabhushan Kalburgi, TJ McCown and R Jude Samulski


Gene Therapy (2011), 1–8
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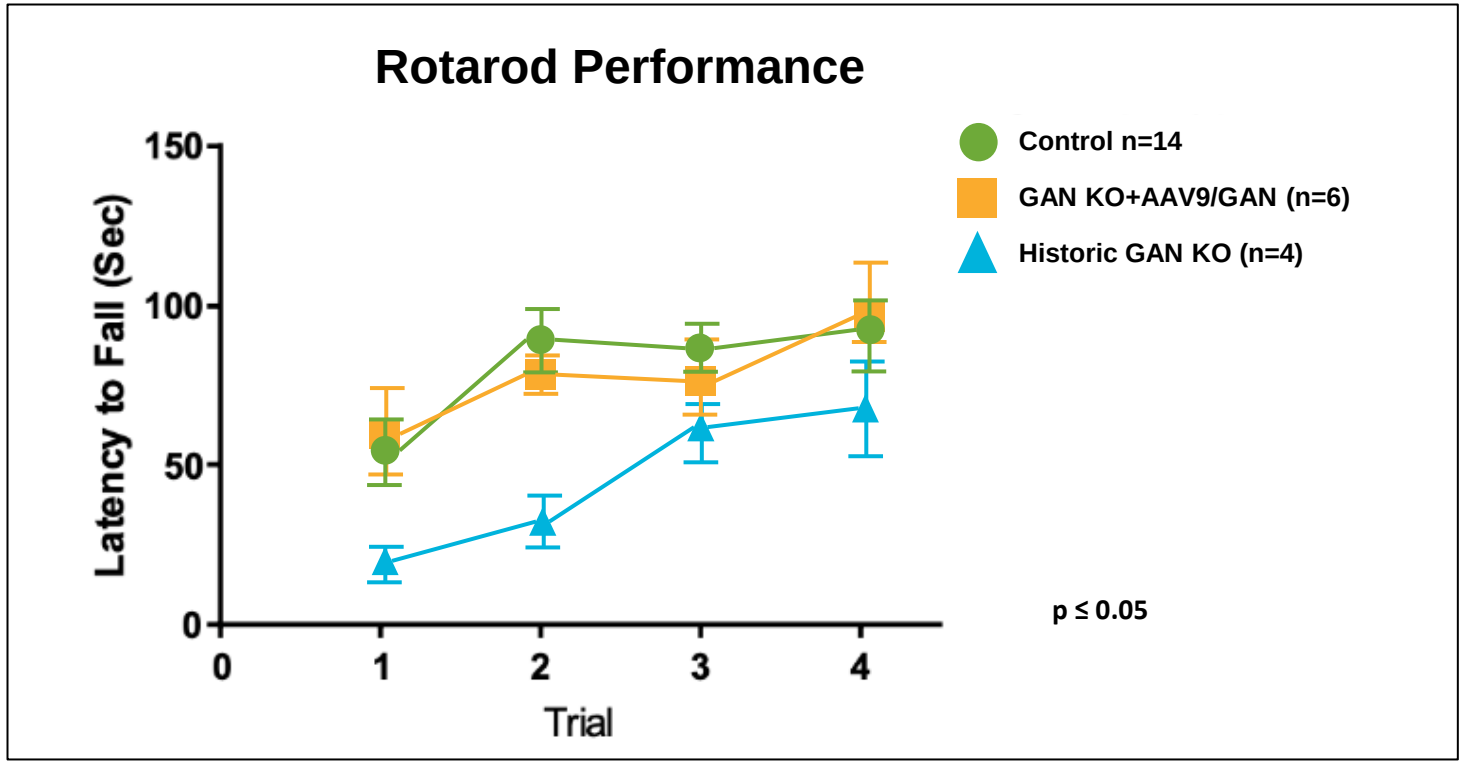
ORIGINAL ARTICLE

Robust spinal motor neuron transduction following intrathecal delivery of AAV9 in pigs

T Federici[1], JS Taub[1], GR Baum[1], SJ Gray[2], JC Grieger[2], KA Matthews[1], CR Handy[1], MA Passini[3], RJ Samulski[2] and NM Boulis[1]

TSHA-120 normalized performance of 18-month-old GAN rodent knockout model

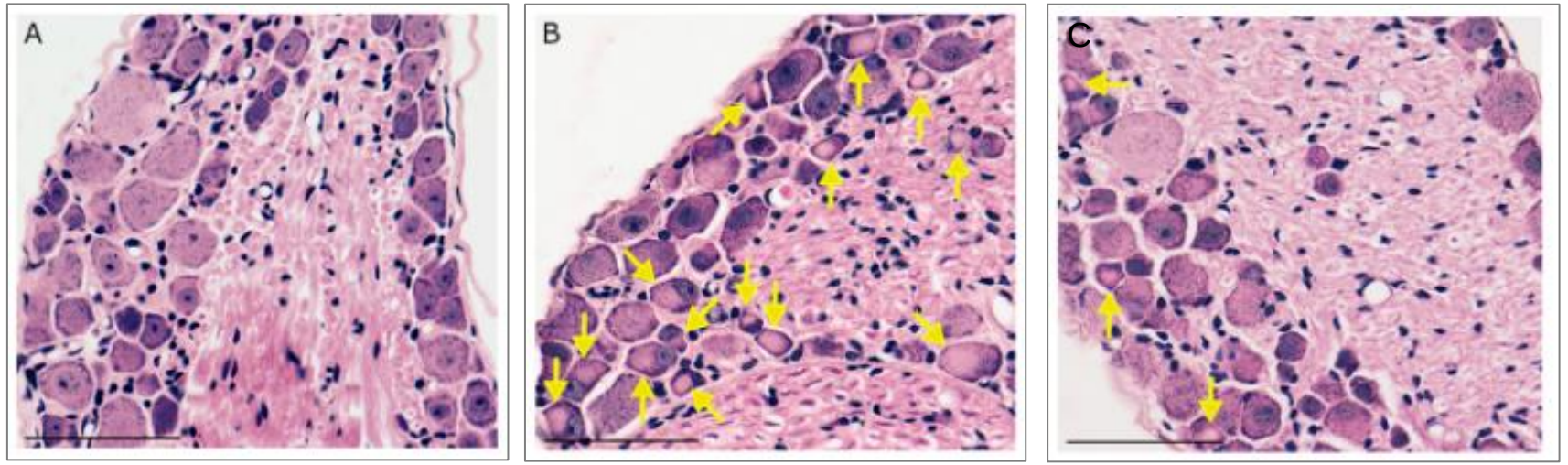
- Untreated GAN rodents performed significantly worse than heterozygous controls
- GAN rodents treated at 16 months old performed significantly better than untreated GAN rodents at 18 months old
- GAN rodents treated at 16 months old performed equivalently to heterozygous controls



Gray, S.J., *unpublished*

TSHA-120 improved pathology of the DRG in the GAN KO mice

TSHA-120
GAN



Normal control **GAN KO – vehicle injected** **GAN KO – AAV9-GAN**

Representative images of light microscopic evaluation of lumbar DRG in 24-month-old normal control (A), vehicle IT-injected GAN /Y KO mice (B), and AAV9/JeT-GAN IT-injected GAN/Y KO mice (C). H&E staining shows unremarkable DRG neurons in control mice (A) versus abundant, brightly eosinophilic inclusion-bearing neurons of vehicle-treated GAN/Y KO mice (B). Neuronal inclusions in GAN-treated mice were significantly reduced compared to vehicle-treated GAN KO mice (C and D). Scale bar represents 61 mm. Arrows indicate neuronal inclusions

Significant reduction in % neuronal inclusions

Bailey, R. et al, *MTMCD* 2018

Groundbreaking, historic dose escalation clinical trial – First intrathecally-dosed gene therapy

TSHA-120
GAN

Goals and Targets of Trial

Goals

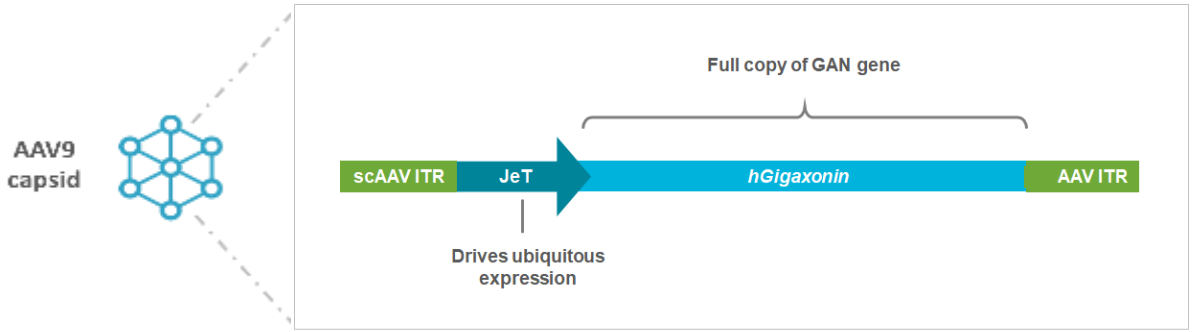
- **Primary** – Safety: clinical and laboratory assessments
- **Secondary** – Efficacy: pathologic, physiologic, functional and clinical markers

Target Recruitment

- 14 subjects injected
- > 5 years old

Target Areas to Transduce

Product Details and Dose Cohorts



Dose Cohorts*

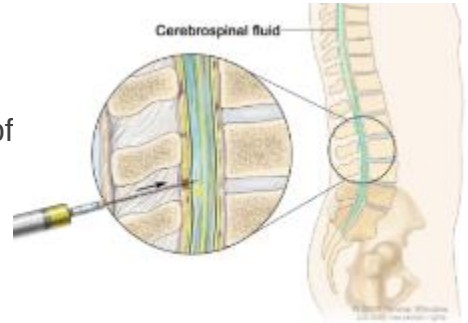
- 1x: 3.5 x 10^{13} total vg (n=2)
- 3.3x: 1.2 x 10^{14} total vg (n=4)
- 5x: 1.8 x 10^{14} total vg (n=5)
- 10x: 3.5 x 10^{14} total vg (n=3)

*Doses calculated by qPCR

Route and Method of Administration

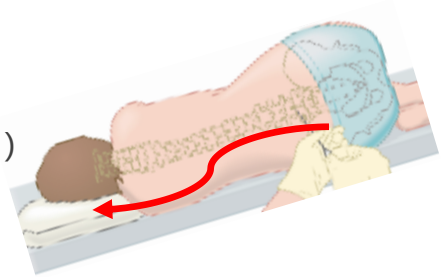
Administration

- Lumbar Intrathecal Infusion (IT)
- Amount and rate: 1 mL/min for total of 10-12 mL
- Immunosuppression regimen of prednisolone and sirolimus



Technique to Improve Transduction

- Trendelenburg position (15-30°)
- During infusion & 1 hour post infusion



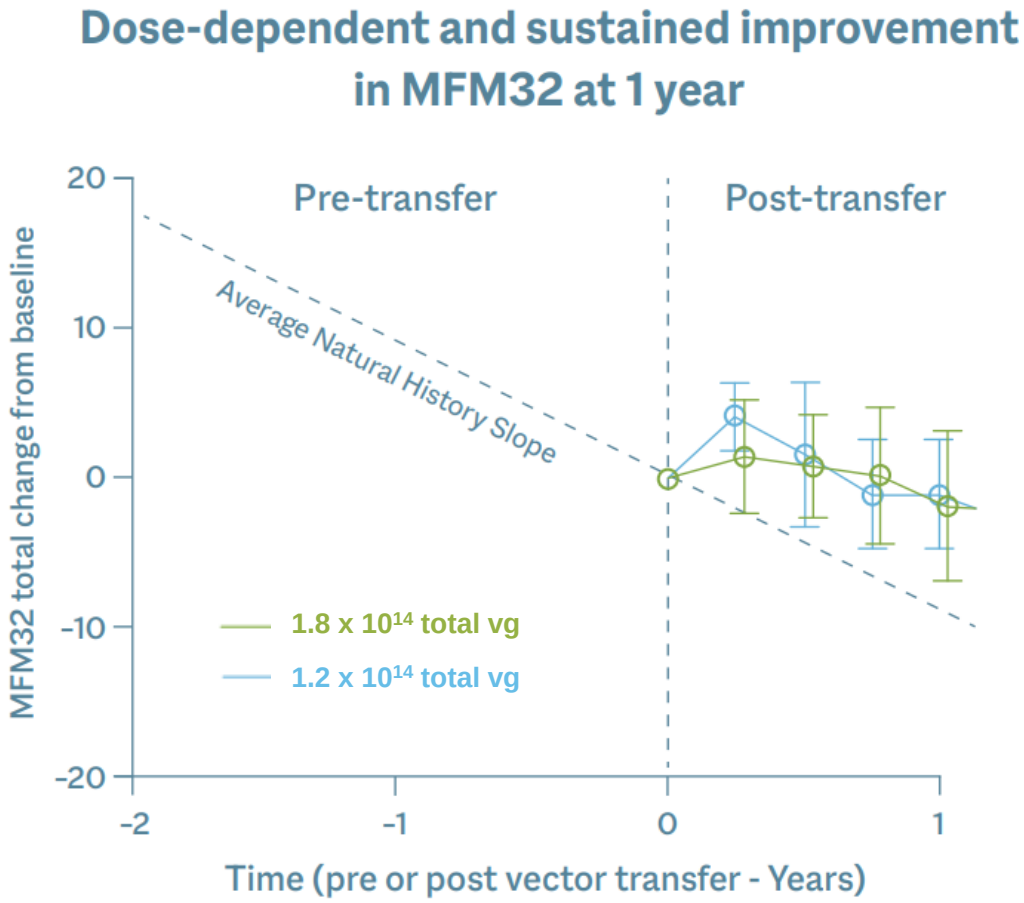
NOTE: Subsequent slides only show data from 1.2 x 10^{14} vg and 1.8 x 10^{14} vg doses

Clinical Trial: NCT02362438

TSHA-120 achieved sustained improvement in primary efficacy endpoint and was well tolerated at multiple doses

TSHA-120
GAN

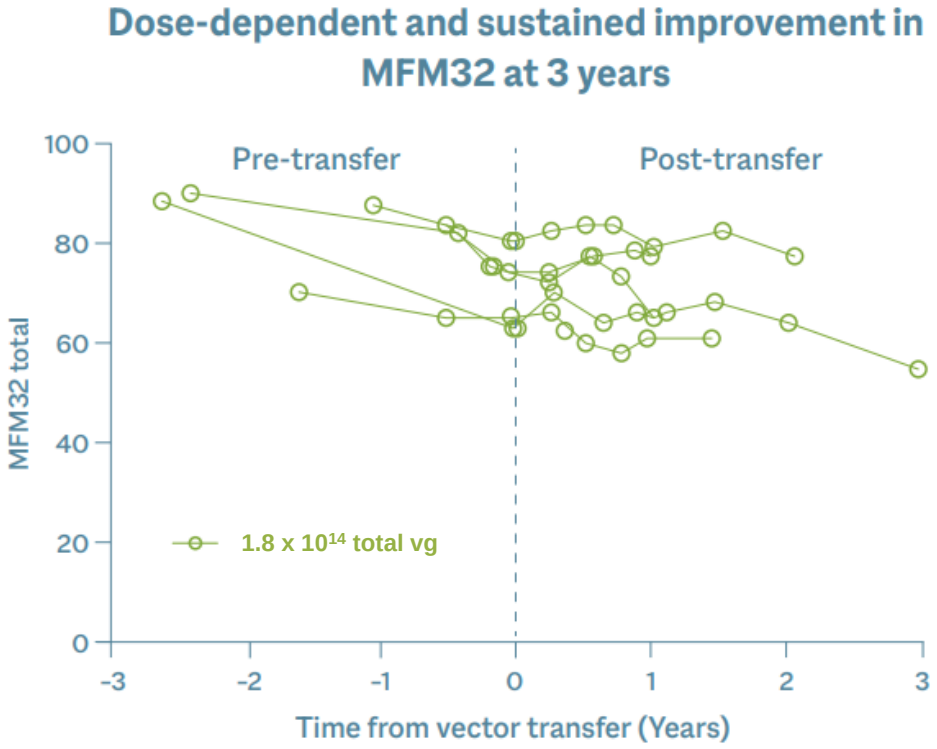
- First successful in-human intrathecal gene transfer
- 14 patients dosed
- Positive efficacy results support a dose-response relationship with TSHA-120
  - $1.8x10^{14}$ total vg dose and $1.2x10^{14}$ total vg dose cohorts demonstrated statistically significantly slowing of disease progression
  - Data only recently publicly presented
- Treatment with TSHA-120 was well tolerated
  - No signs of significant acute or subacute inflammation
  - No sudden sensory changes
  - No drug-related or persistent elevation of transaminases
- 6 patients beyond 3+ years initial treatment

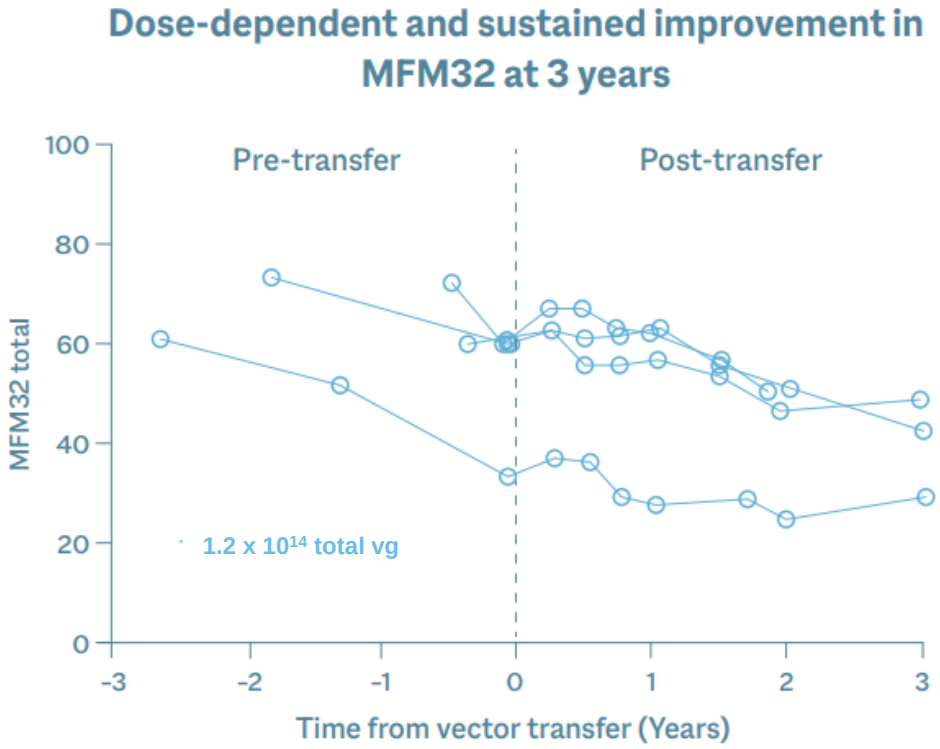


Bönnemann, C. et al; 2020

Treatment with TSHA-120 resulted in a clear arrest of disease progression at therapeutic doses and long-term durability

TSHA-120
GAN





- Arrest of disease progression at therapeutic doses
- TSHA-120 was well tolerated at multiple doses

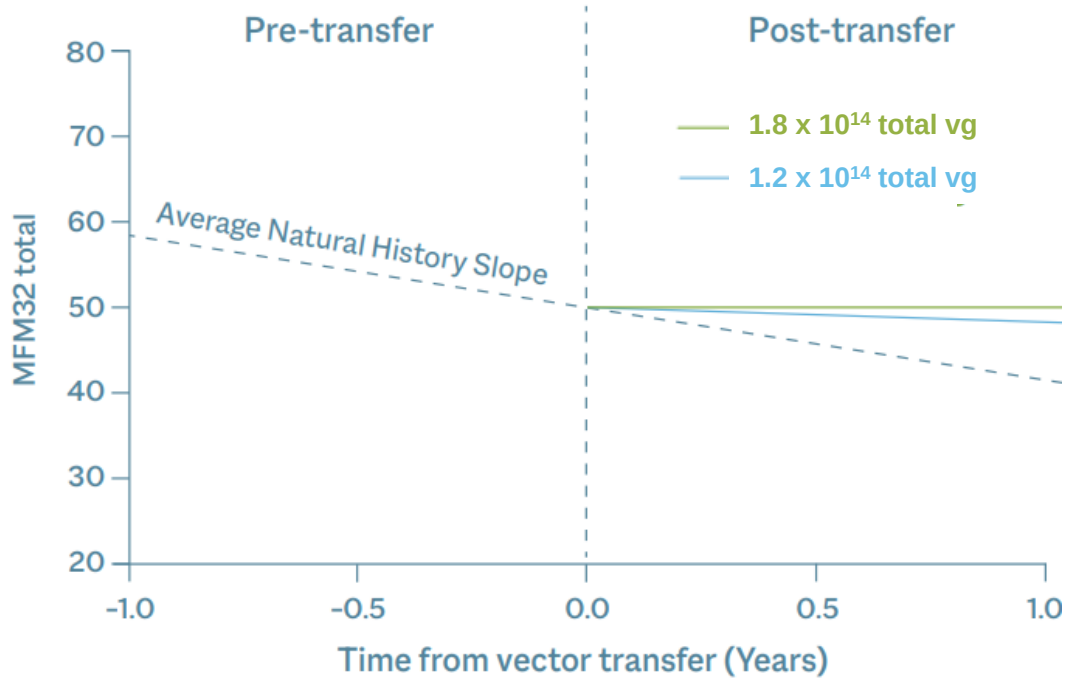
- 6 patients treated for 3+ years supporting long-term durability
- Plan to engage with regulatory agencies to discuss regulatory pathway as soon as possible

Bönnemann, C. et al; 2020

Additional analysis using Bayesian methodology confirmed arrest of disease progression

TSHA-120
GAN

- Bayesian analysis
  - Enables direct probability statements about any unknown quantity of interest
  - Enables immediate incorporation of data gathered as the trial progresses
  - Useful and accepted by regulatory agencies when treating rare diseases and small patient populations
  - Can be used as a sensitivity analysis to support the more commonly accepted frequentist approach
  - Can be used as a way of statistically increasing the power of a clinical trial in a small patient population when used to incorporate auxiliary information
- Confirmed documented natural history data of an 8-point decline in the MFM32 total % score per year
  - 4-point decline in the MFM32 is clinically meaningful
- TSHA-120 dose of 1.8x10^{14} total vg resulted in an arrest of disease progression that was statistically significant



	Bayesian Analysis		Frequentist Analysis		
	Mean	Std Dev	Estimate	Std Error	p-Value
Post infusion: 1.8x10^{14} total vg	7.78	1.94	7.78	1.89	<0.001
Post infusion: 1.2x10^{14} total vg	6.09	2.11	6.07	2.05	0.004
Natural history decline	-8.19	0.74	-8.18	0.72	<0.001

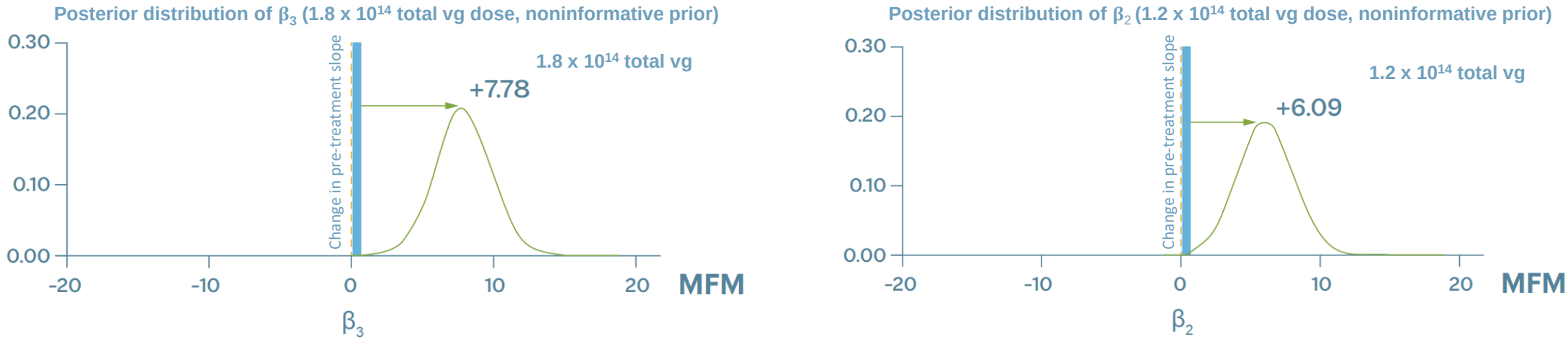
Bönnemann, C. et al; 2020

TSHA-120 halted patient pre-treatment rate of decline at 1.8x10^{14} total vg dose

TSHA-120
GAN

Bayesian Efficacy Analysis

Compared to individual historical data



X-axis = change in slope compared to pre-gene transfer
Blue line = pre-treatment change in slope = 0

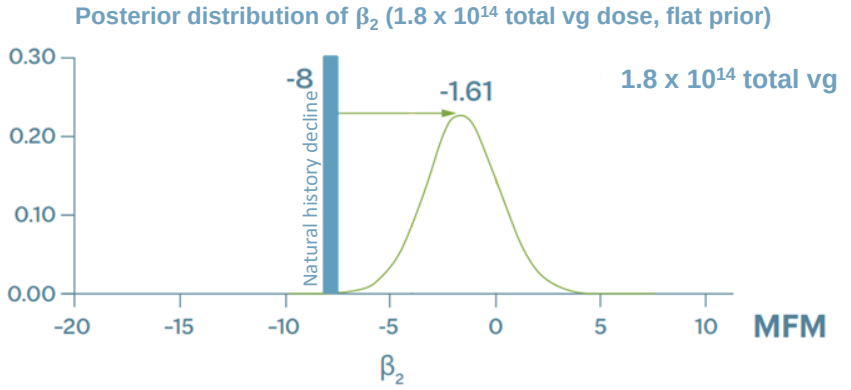
- Treated population average annual post-treatment decline for both the 1.8x10^{14} total vg cohort and 1.2x10^{14} total vg cohort
- 1.8x10^{14} vg halted patient pre-treatment rate of decline, avg annual slope improvement of 7.78 points
- 1.2x10^{14} vg resulted in clinically meaningful slowing of disease progression confirming dose response, avg annual slope improvement of 6.09 points
- Both doses showed superior results compared to natural decline of GAN patients

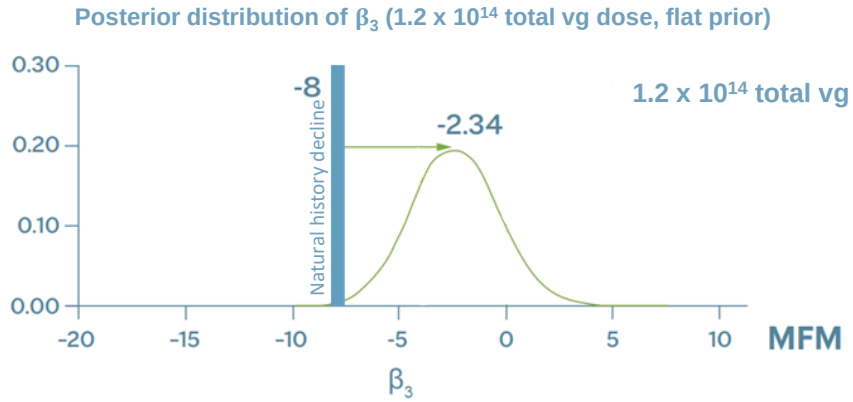
Bönnemann, C. et al; 2020

Further analyses confirmed nearly 100% probability of clinically meaningful slowing of disease compared to natural history

TSHA-120
GAN

- Further analyses were conducted to assess the probability of clinically meaningful slowing of disease as compared to natural history
- A 4-point decline in MFM32 is considered clinically meaningful
- Graphs depict treated population annual decline for both the 1.8x10^{14} total vg cohort and the 1.2x10^{14} total vg cohort as compared to natural history
  - 1.8x10^{14} total vg dose confirmed nearly 100% probability of clinically meaningful slowing of disease compared to natural history decline of GAN patients
  - 1.2x10^{14} total vg dose confirmed approximately 85% probability of clinically meaningful slowing of disease and 100% probability of any slowing of disease





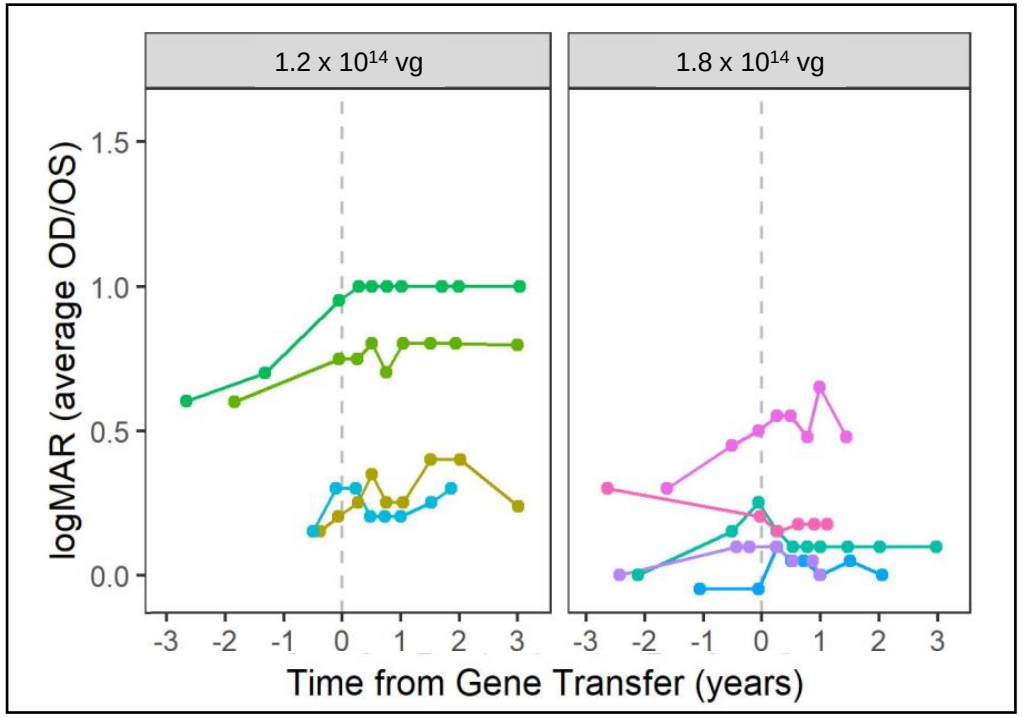
X-axis = annual decline in MFM32 total % score
Blue line = natural history decline (-8 points per year)

	Values = % Probability	
Change in disease progression	**1.8x10^{14} total vg**	**1.2x10^{14} total vg**
Any Slowing	99.9	99.8
Clinically meaningful slowing 50% or more	98.3	84.9

Bönnemann, C. et al; 2020

Newly obtained GAN exploratory endpoint showed improvement in visual acuity

- Data from 11 patients were analyzed for visual acuity via the Logarithm of the Minimum Angle of Resolution (LogMar)
  - Dose-dependent trend towards stabilization of visual acuity, i.e., a slowed increase in LogMAR values, observed and appeared to be independent of visual acuity at the time of treatment
- Over the natural history of disease, individuals with GAN experienced a decrease in visual acuity and therefore an increase in their LogMAR score



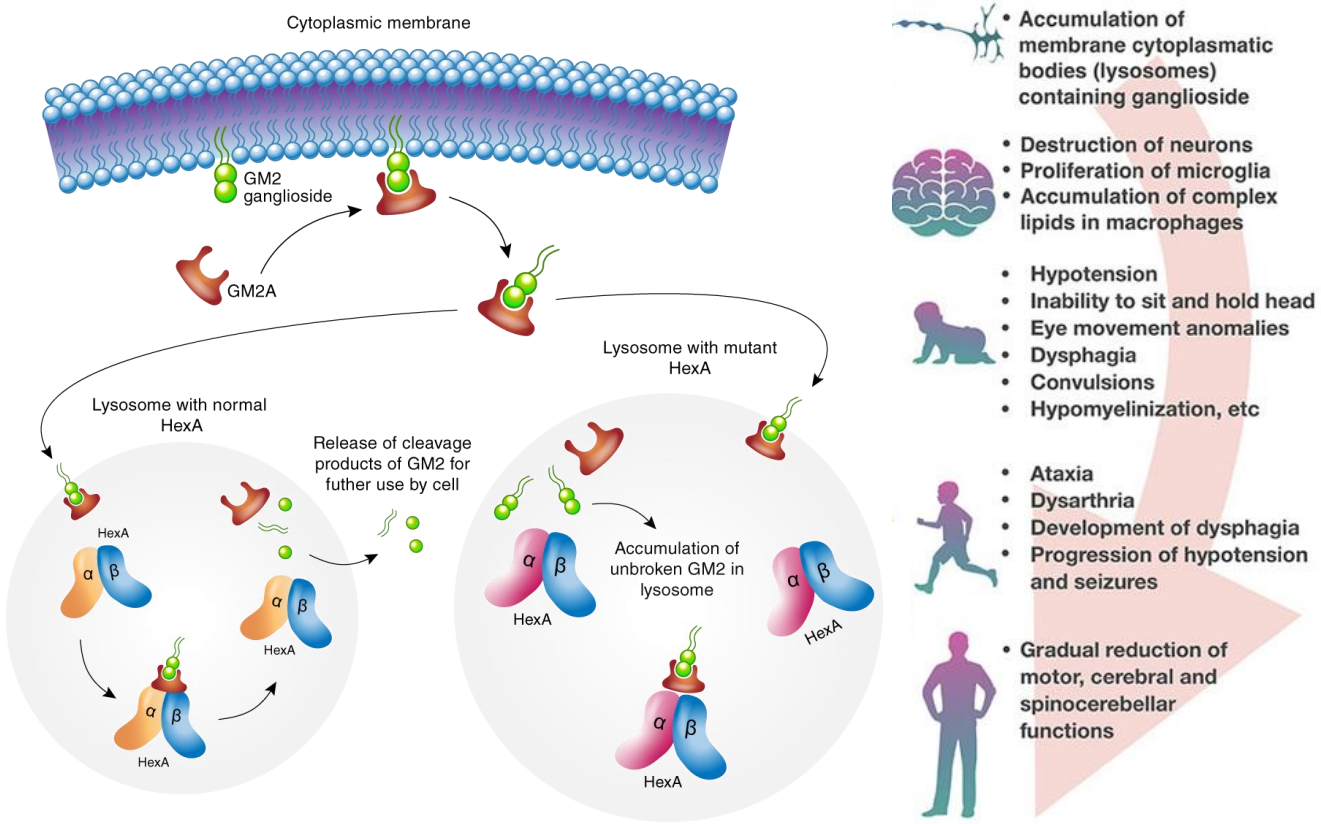
D. Saade et al. *Neuromuscular Disorders* 2020

Anticipated next steps for TSHA-120 by the end of 2021

- Complete transfer data from the NIH
- Initiate manufacturing of commercial-grade GMP material
- Discuss regulatory pathway for TSHA-120
- Request regulatory guidance from EMA and MHRA
- Initiate new clinical sites in US and EU
- Update on regulatory interactions and current clinical program, including $3.5x10^{14}$ total vg cohort

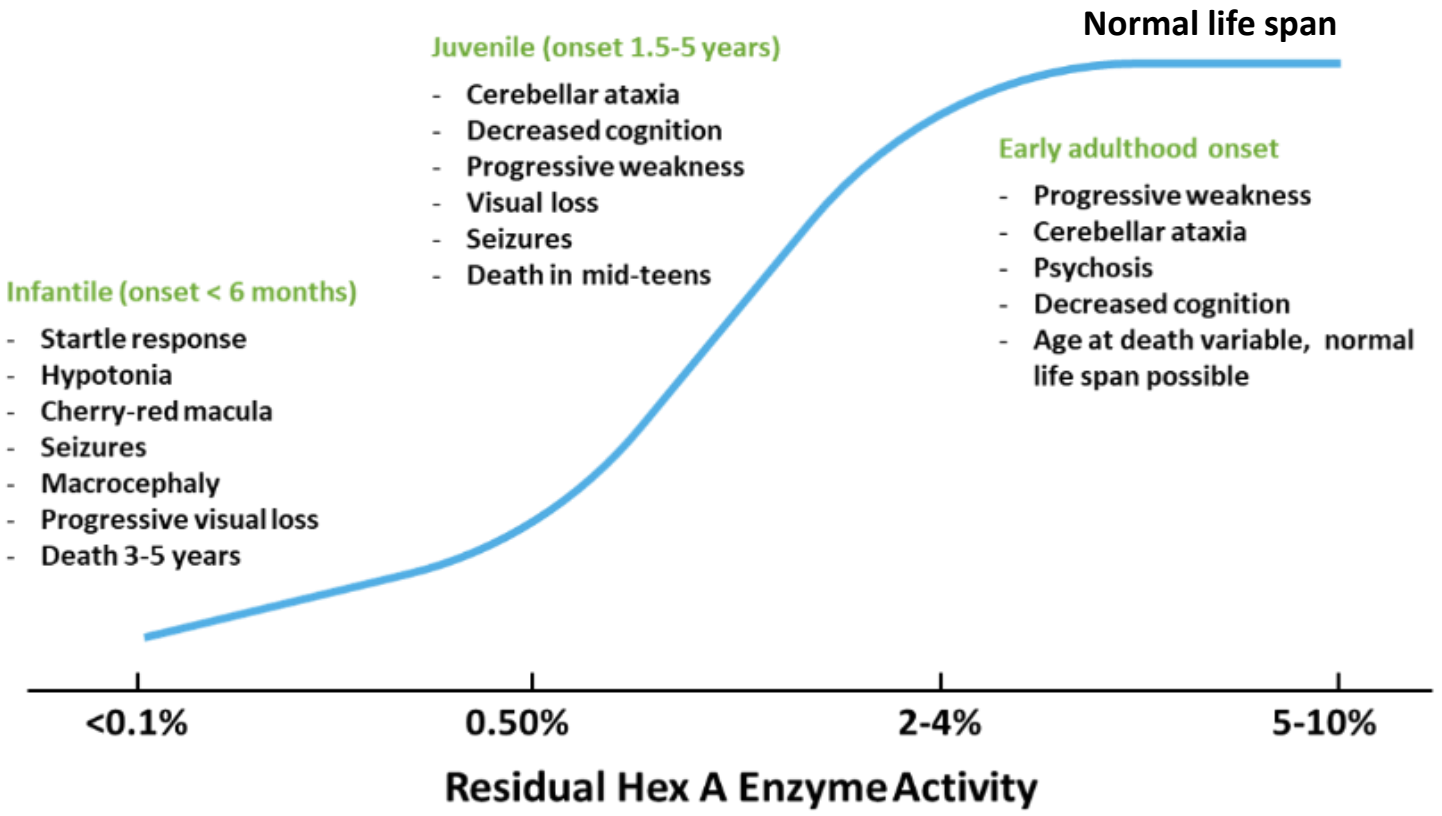
GM2 gangliosidosis is a severe neurodegenerative disease

- GM2 gangliosidosis results from a deficiency in the β-hexosaminidase A (Hex A) enzyme
- Hex A is comprised of 2 subunits encoded by the alpha-subunit, HEXA, coded for by the *HEXA* gene, and the beta-subunit, HEXB, coded for the *HEXB* gene
- Mutations of the HEXA gene cause Tay-Sachs disease (TSD) while mutations of the HEXB gene cause Sandhoff disease (SD)
- Estimated prevalence is 500 patients (US+EU)



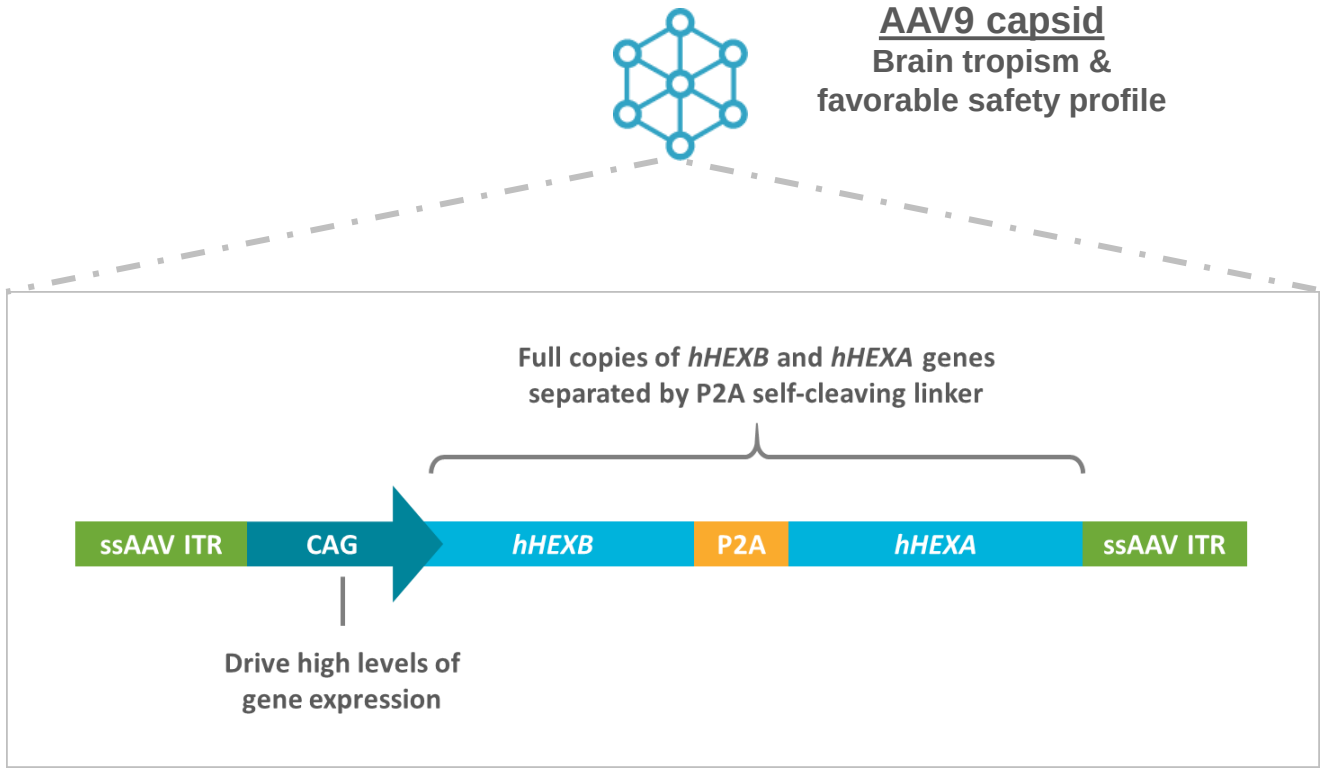
Residual Hex A activity determines the severity of GM2

- Small increases in Hex A activity may lead to significant improvements in clinical outcomes and quality of life
- Infantile onset is the most severe form of GM2
- Infantile forms may die within the first 4 years of life, and juvenile onset patients rarely survive beyond mid-teens

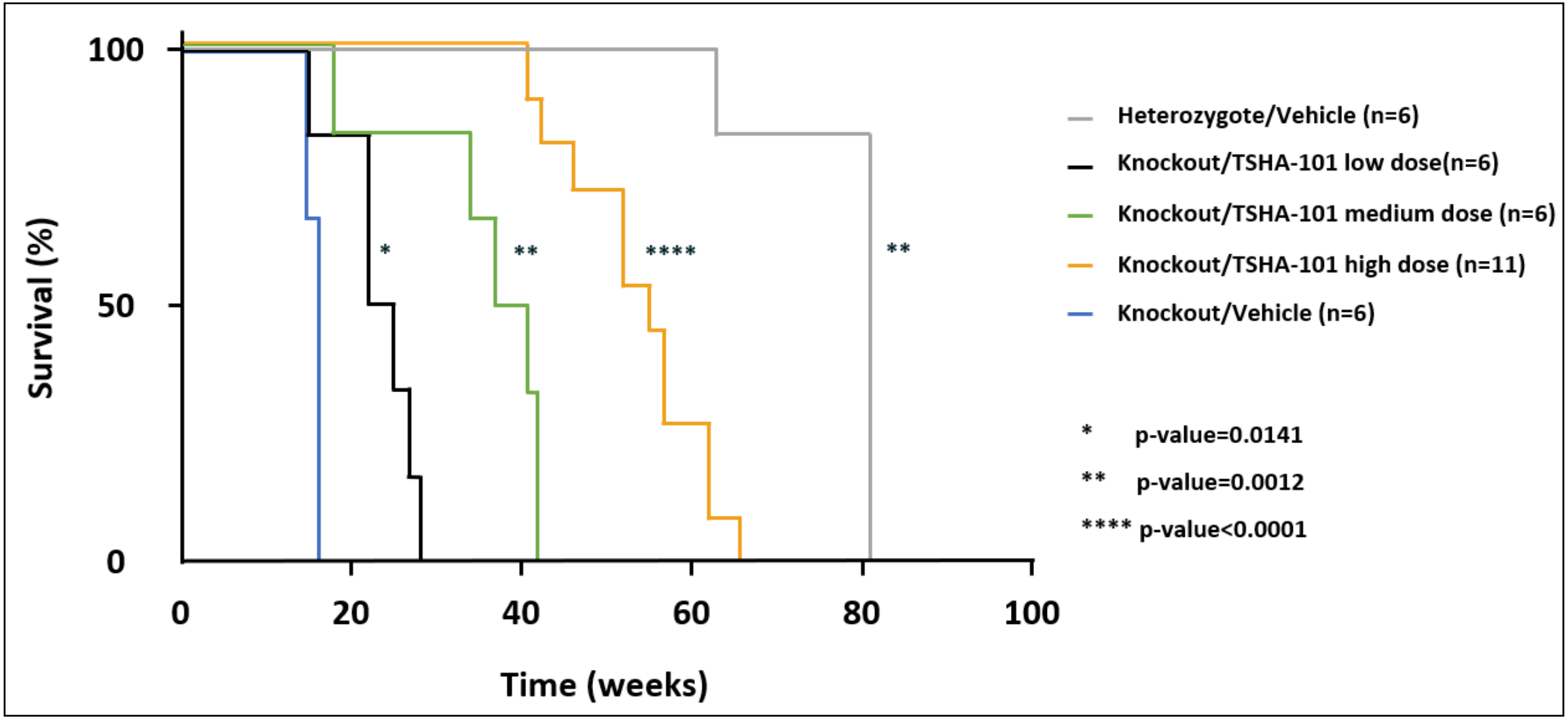


Novel bicistronic vector design allows consistent expression of *HEXA* and *HEXB* genes

- *HEXA* and *HEXB* genes are required to produce the subunits of the beta-hexosaminidase A enzyme
- The novel bicistronic vector design enables 1:1 expression of the alpha-subunit, *HEXA,* and the beta-subunit, *HEXB,* under the control of a single promoter with a P2A-self-cleaving linker
- SD mice received vehicle or varying doses of TSHA-101 after 6 weeks:
  - High dose ($2.5x10^{11}$ vg/mouse)
  - Medium dose ($1.25x10^{11}$ vg/mouse)
  - Low dose ($0.625x10^{11}$ vg/mouse)
  - Vehicle controls

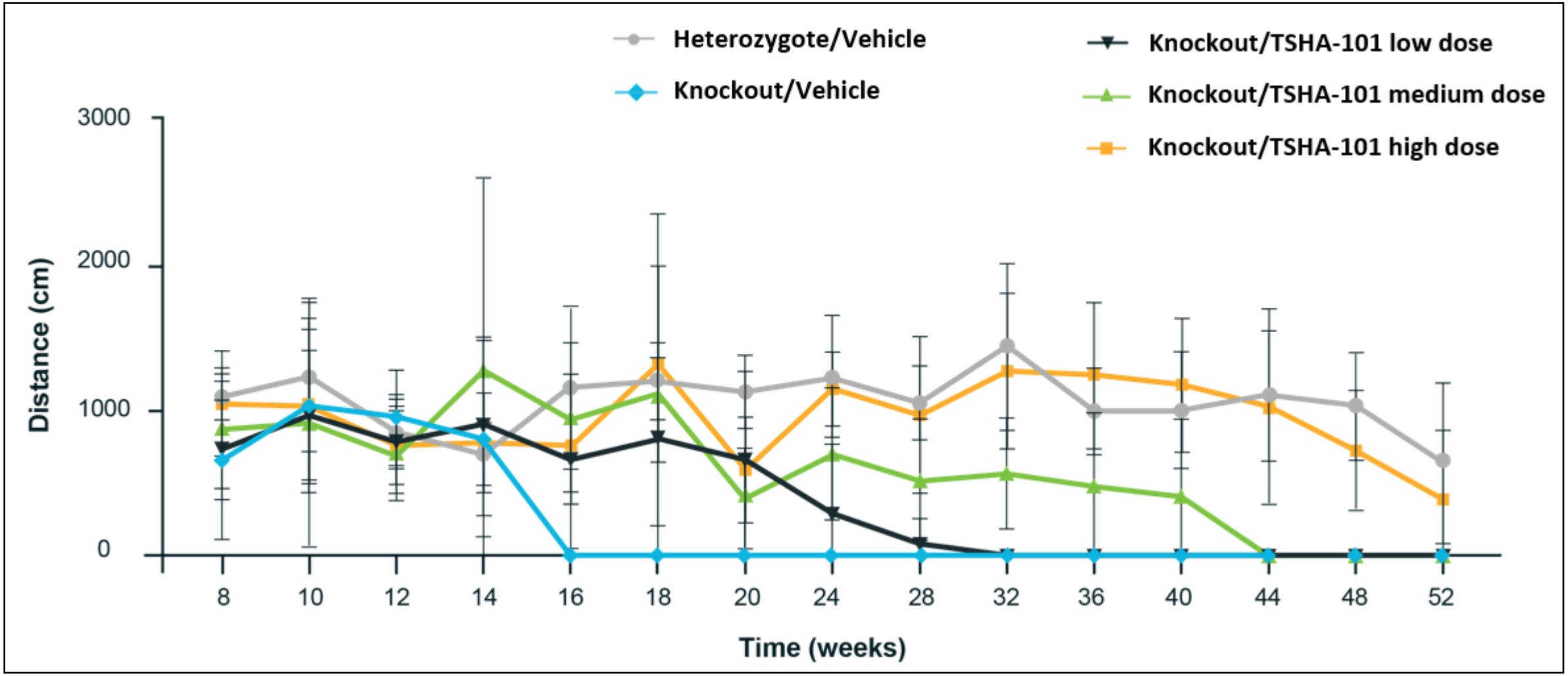


Preclinical pharmacology – Significant, dose-dependent improvement in survival observed in mice treated with TSHA-101

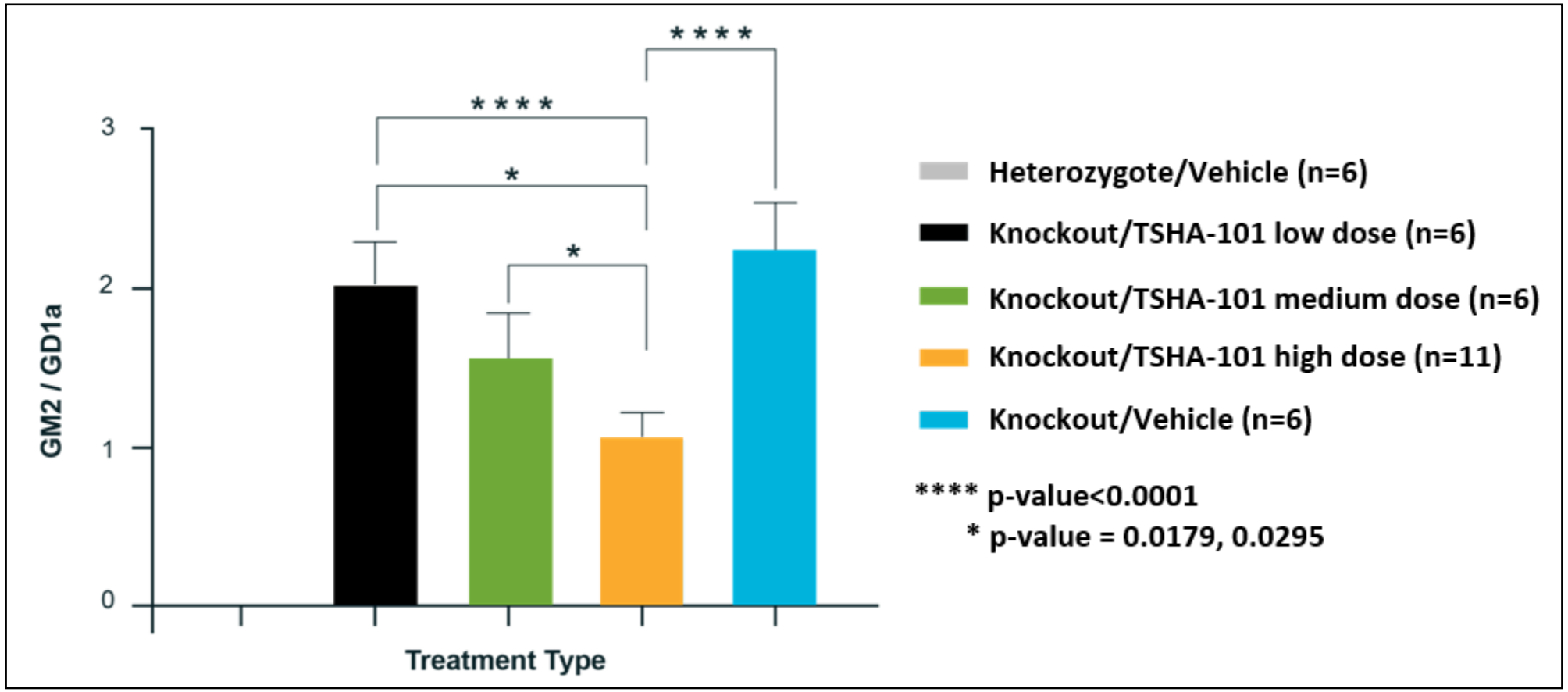


Preclinical pharmacology – Dose-dependent improvements observed in rotarod assessments in mice treated with TSHA-101

TSHA-101
GM2



Preclinical pharmacology – GM2 accumulation significantly reduced in mid-section of brain following treatment with TSHA-101 after 16 weeks



Phase 1/2 adaptive trial for TSHA-101 in GM2 gangliosidosis

TSHA-101
GM2

Goals and Targets of Trial

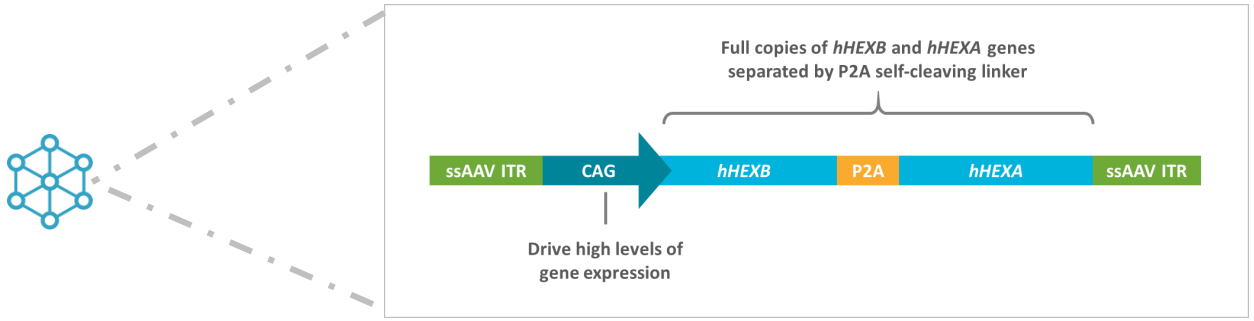
Goals

- **Primary** – Safety: clinical and laboratory assessments
- **Secondary** – Efficacy: pathologic, physiologic, functional and clinical markers

Target Recruitment

- Up to 6 subjects
- Age younger than or equal to 12 months at time of enrollment

Product Details and Dose Cohorts



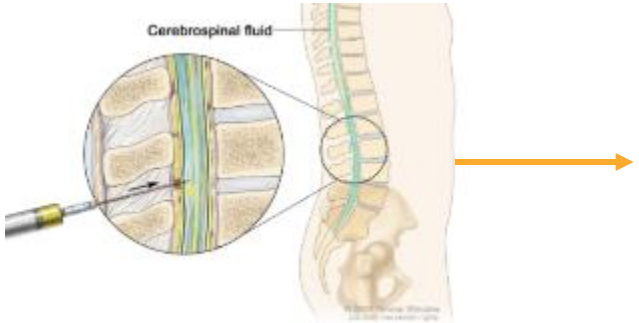
Dose Cohorts

- 5 x 10^{14} total vg (n=4)

Route and Method of Administration

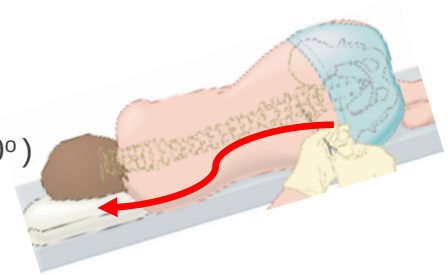
Administration

- Lumbar Intrathecal Infusion (IT)
- Amount and rate: 1 mL/min for total of 10-12 mL
- Immunosuppression regimen of prednisolone and sirolimus



Technique to Improve Transduction

- Trendelenburg position (15-30º)
- Following IT injection, for 15 minutes post infusion



TSHA-101 Canadian IST endpoints

Disease-Specific / Global Assessments
- Hypotonia
- Dysphagia
- Head Control Scale
- CHOP INTEND
- Modified Ashworth scale
- Vineland-3
- Bayley-III / WPPSI-IV

Quality of Life/Other Assessment
- PedsQL Infant Scales
- PedsQL Family Impact Module
- CGI – Improvement (CGI-I)

Imaging
- Echocardiography
- MRI / MRS

Biomarkers
- Hex A enzyme activity in serum and CSF
- Aspartate aminotransferase (AST)
- Lactate dehydrogenase
- Neuron specific enolase
- Myelin basic protein
- Sphingolipids (GM1, GM2, GM3)

Seizures and Electrophysiological Monitoring
- Seizure diary
- Electroencephalogram (EEG)

Communication Assessments
- Observer-Reported Communication Ability (ORCA)

Auditory & Ophthalmic
- Brainstem auditory evoked response (BAER)
- Fundus photography and Visual Evoked Potential

Anticipated next steps for TSHA-101 by the end of 2021

Preliminary Phase 1/2 safety and biomarker data (Queen's University study) in 2H 2021

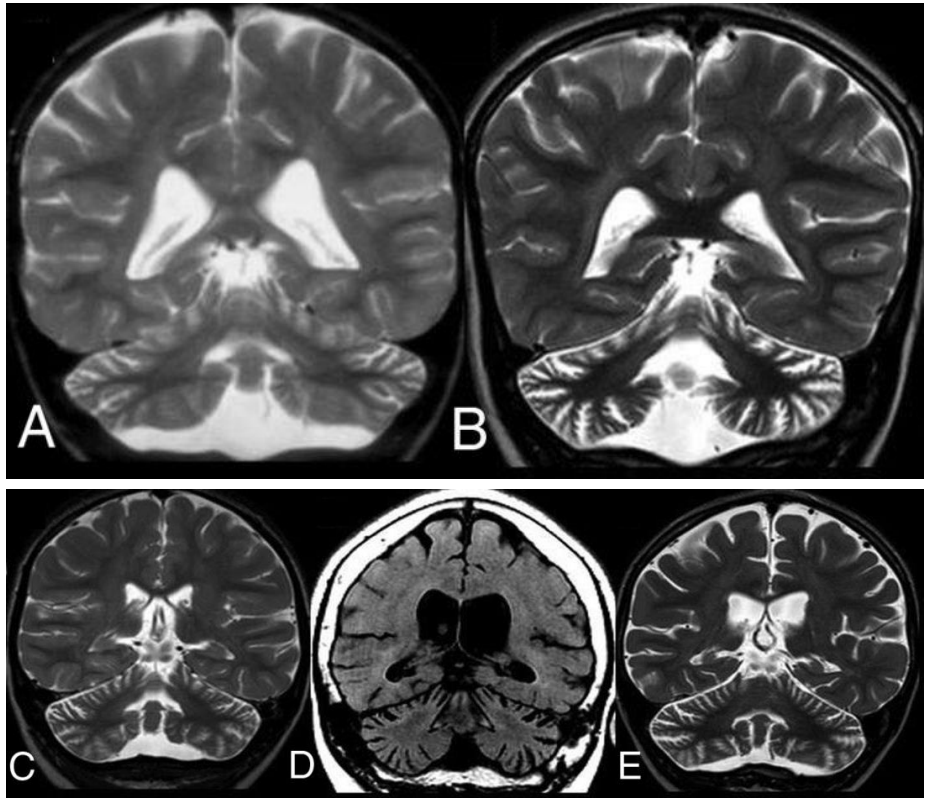
US study utilizing material from commercial process

Submit IND in 2H 2021

Initiate US Phase 1/2 study in 2H 2021

CLN7 disease is a severe neurodegenerative lysosomal storage disease

- Batten disease is the common name for rare, fatal, inherited disorders of the nervous system called neuronal ceroid lipofuscinoses (NCLs)
- CLN7 disease, a form of Batten disease, is caused by a mutation in the gene, Major Facilitator Superfamily Domain Containing 8 (*MFSD8*), resulting in a lysosomal storage disease (LSD)
- CLN7 presents as a “late infantile” or “variant late infantile” disease, with almost all patients experiencing symptom onset (>95% of reported cases) between ages 2-7 years
- Inherited autosomal recessive pattern - both copies of the *CLN7* gene variant (one from each parent, or carrier) must be present for diagnosis
- Survival rarely beyond teenage years
- Symptomatic intervention to treat seizures, dystonia, anxiety, sleep disorders, and spasms
- Estimated prevalence is 4,000 patients worldwide – numbers of diagnosed patients have significantly increased over the last 3 years with newly genetic testing techniques such as next generation sequencing panels

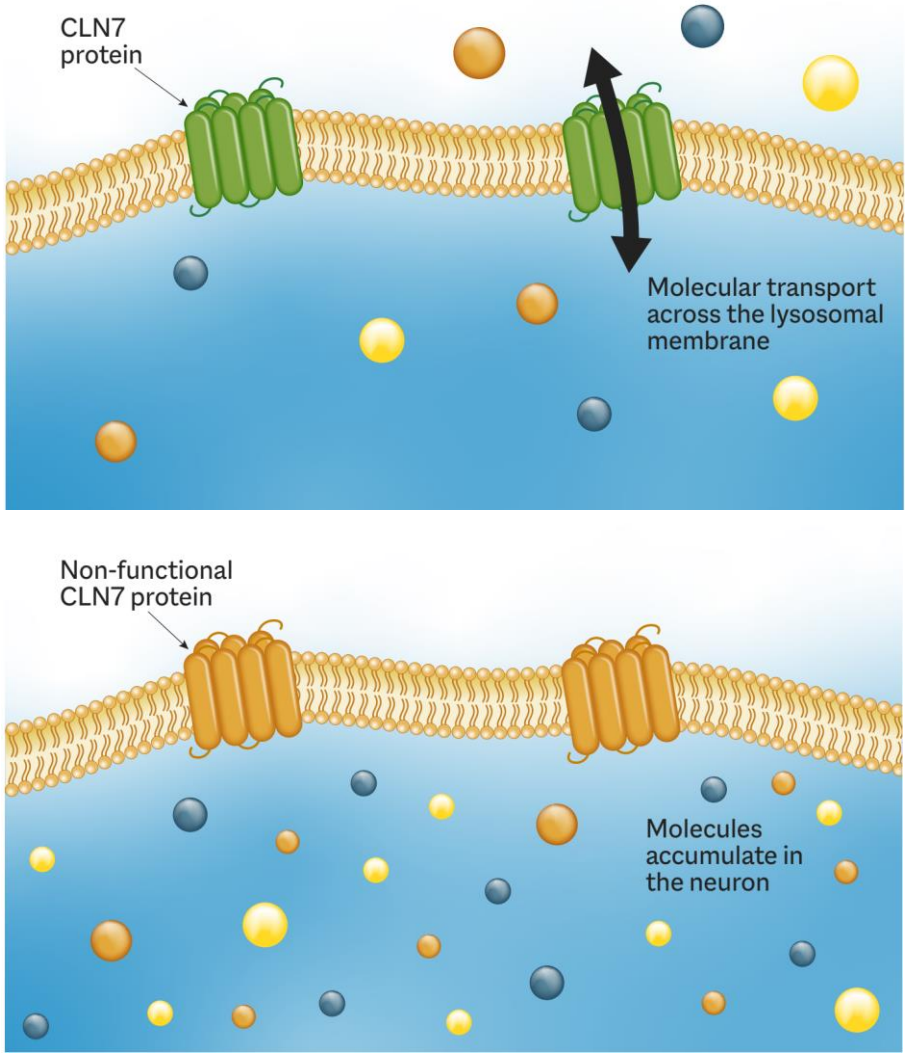


Coronal T2-weighted MR imaging of 4 different patients (*A*, *B*, *C*, and *E*) and coronal reformatted CT of 1 patient (*D*). Note predominant cerebellar-over-cerebral atrophy.

1. Anderson GW, Goebel HH, Simonati A. Human pathology in NCL. *Biochim Biophys Acta*. 2013;1832(11):1807-1826. doi:10.1016/j.bbadis.2012.11.014
2. Mink JW, Augustine EF, Adams HR, Marshall FJ, Kwon JM. Classification and natural history of the NCLs. *J Child Neurol*. 2013;28(9):1101-1105. doi:10.1177/0883073813494268
3. Biswas A, Krishnan P, Amirabadi A, Blaser S, Mercimek-Andrews S, Shroff M. Expanding the Neuroimaging Phenotype of NCLs. *AJNR*. 2020;41(10):1930-1936. doi:10.3174/ajnr.A6726
4. https://www.ninds.nih.gov/Disorders/Patient-Caregiver-Education/Fact-Sheets/Batten-Disease-Fact-Sheet

Molecular underpinnings of CLN7

- More than 45 different *CLN7* mutations have been characterized
- Disruption of the *MFSD8/CLN7* gene identified as the cause of the LINCL form of the NCLs
- Although the exact function of the protein is unknown, it is thought to transport molecules across the lysosomal membrane
- Dysfunction of the MFSD8 protein results in accumulation of lysosomal storage material or autofluorescent ceroid lipopigments in neuronal and peripheral tissues
- Accumulations can cause cell damage leading to cell death
- Individuals with CLN7 disease have gradual nerve cell loss in certain parts of the brain, which likely leads to the signs and symptoms of this condition

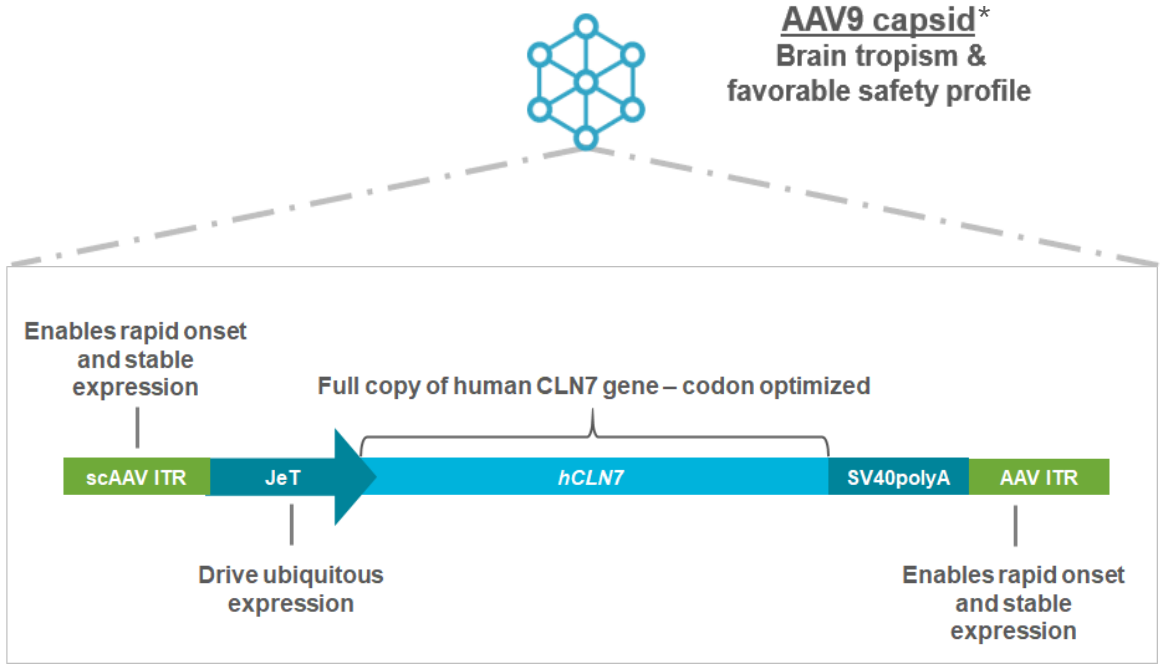


1. Connolly KJ, O'Hare MB, Mohammed A, et al. The neuronal ceroid lipofuscinosis protein Cln7 functions in the postsynaptic cell to regulate synapse development. *Sci Rep*. 2019;9(1):15592. Published 2019 Oct 30. doi:10.1038/s41598-019-51588-w
2. Kousi M, Siintola E, Dvorakova L, et al. Mutations in CLN7/MFSD8 are a common cause of variant late-infantile neuronal ceroid lipofuscinosis. *Brain*. 2009;132(Pt 3):810-819. doi:10.1093/brain/awn366

CLN7 is a neurodegenerative disease caused by autosomal recessive mutations in the *MFSD8* gene[1]

- MFSD8 is a 518 amino acid transmembrane protein thought to be a lysosomal protein[1]
- *MFSD8* mutations are associated with a uniform late-infantile manifestation, suggesting mutations cause a complete loss of gene function[2]
  - One exception of a mutation causing a juvenile onset form of the disease has been described[3]
  - Specific compound heterozygous alleles are associated with non-syndromic localized maculopathy in adults[4]
- Disease onset typically occurs at 2-7 years of age
  - Presenting symptoms include developmental regression and seizures[2]
- Disease progression is rapid with further mental and motor regression and profound speech impairment[2]
- Mean age of death is 11.5 years of age (range 6.5-18 years) [2]
- First-generation construct in clinical development with next-generation construct to be used in planned pivotal study

* First-generation construct



- Aims to deliver a full-length copy of hCLN7 using the AAV9 capsid
- Engineered for rapid and sustained gene expression

1. Siintola et al. Am J Hum Genet 2007, 2. Kousi et al. Hum Mutation 2012, 3. Kousi et al. Brain 2009,, 4. Khan et al. Inve Opthal Vis Sci. 2017

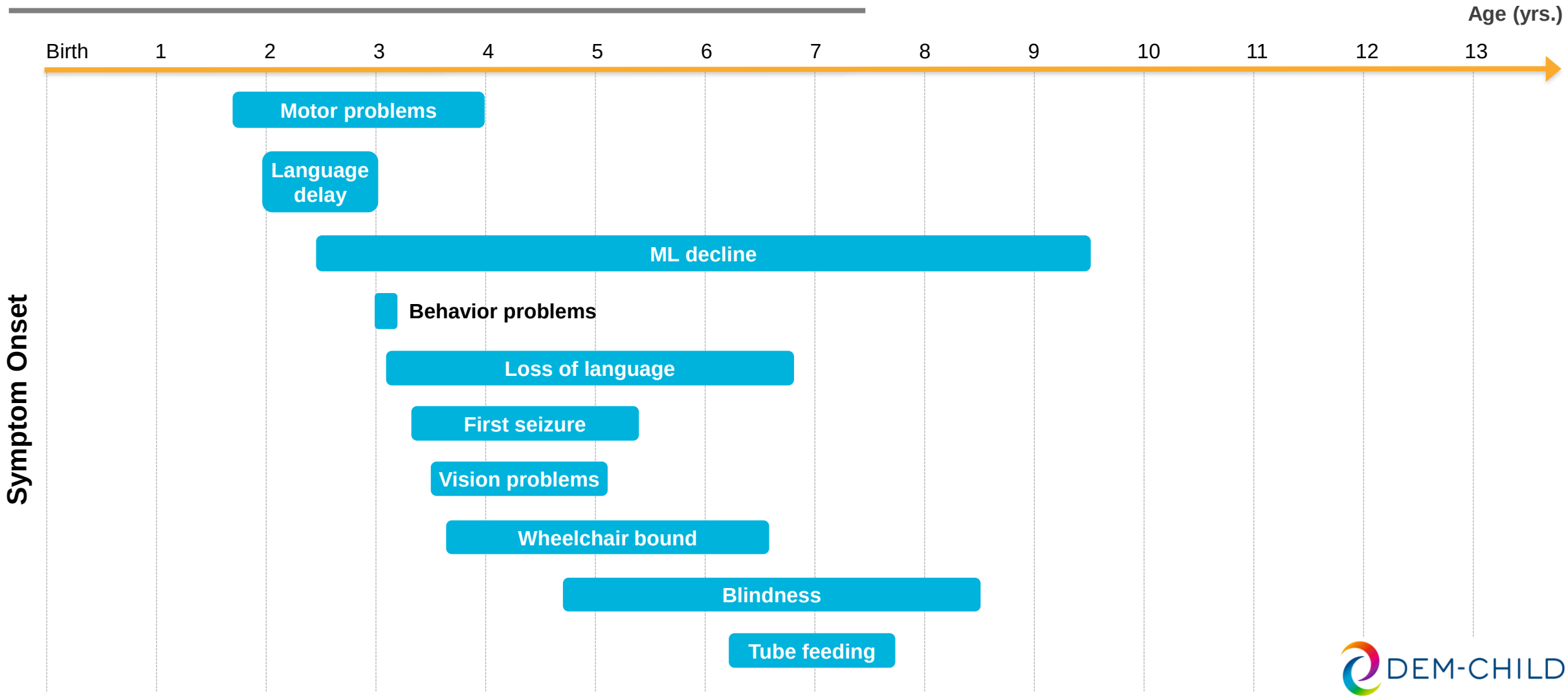
Batten disease natural history data

Ongoing natural history studies in the EU and US underway that encompass all NCLs, including CLN7:

- Coordination of international DEM-CHILD Patient Database for all NCLs (data on >250 NCL patients)
  - To collect precise natural history data of all NCL types
  - To improve early diagnosis of NCLs
  - To optimize standard of care for patients
  - To establish evaluation tools for experimental therapies
  - To make these data available to third parties (scientists and industry) in a transparently regulated and time-effective manner
- Publication anticipated in the near future by UTSW

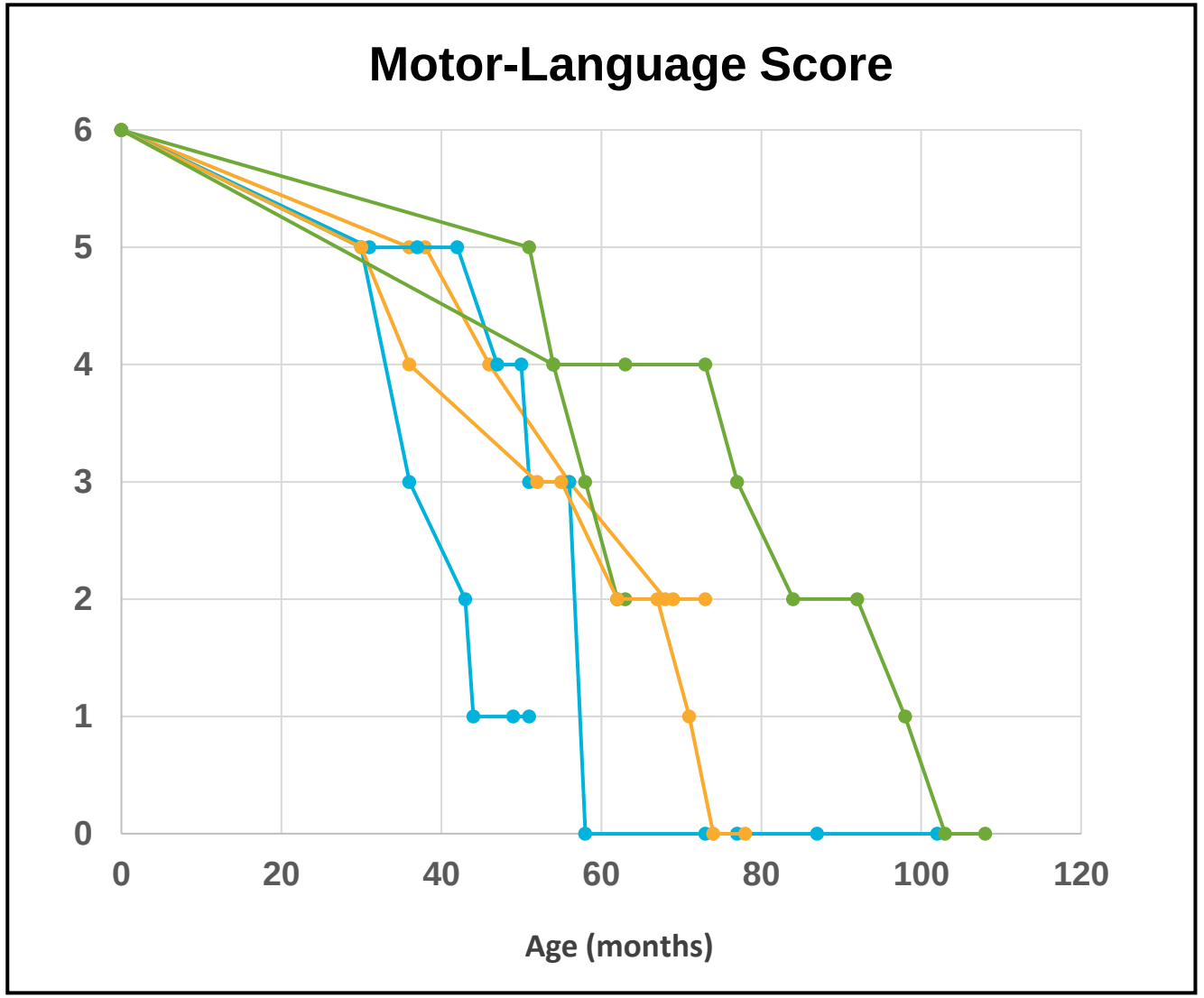
1. https://clinicaltrials.gov/ct2/show/NCT04613089

CLN7 disease – Age ranges at first symptom onset



DEM-CHILD

Hamburg LINCL Motor-Language Scale in CLN7 patients

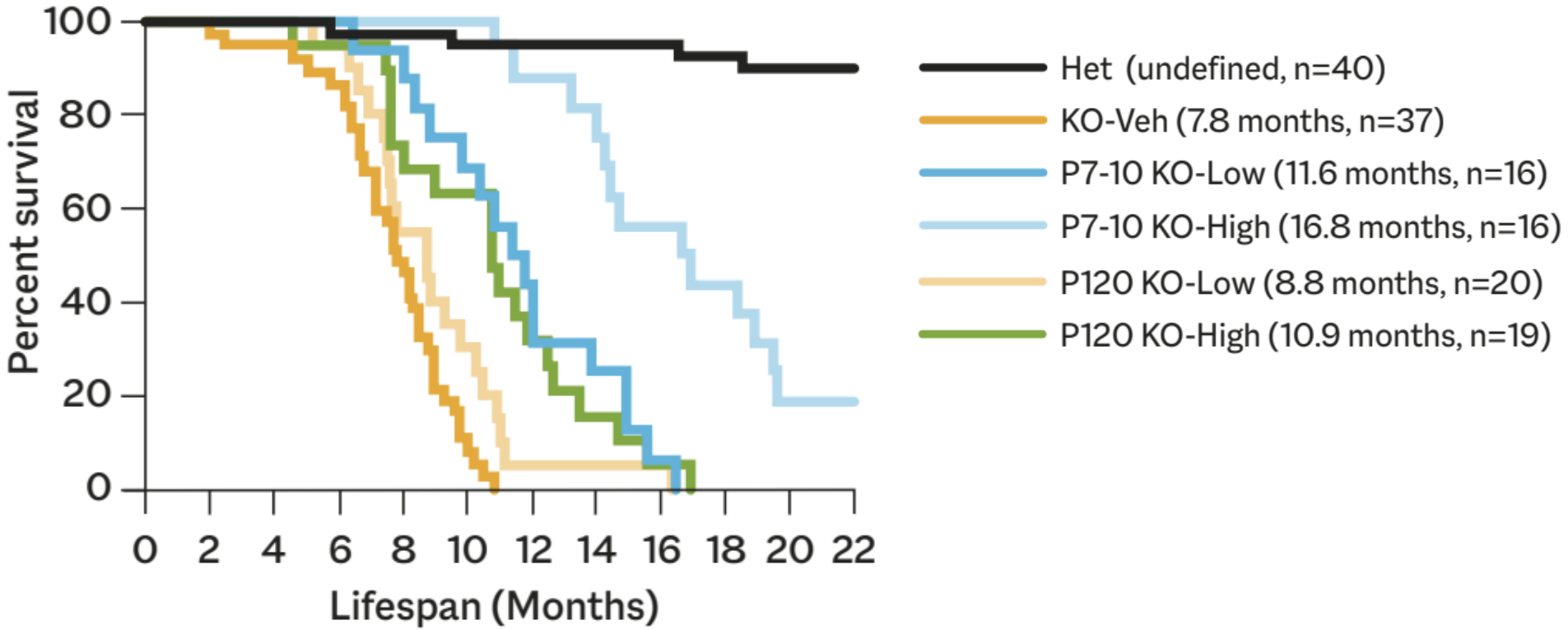


First-generation CLN7 construct – Key preclinical studies to date

TSHA-121
CLN7 disease

#	Study Scope (ID)	Model System	Route of Administration & Dose (vg/animal)	Major Findings
1	In vitro PoC Assay (NSR-004.2-PHAR-1)	Patient fibroblasts (CLN7 -/-)	Titers tested were 1×10^{3}, 1×10^{4}, 1×10^{5}, and 5×10^{5}	• Mutations in CLN7 reduce lysosomal function and restoring the expression of wild-type CLN7 in patient fibroblast lines with AAV vectors can restore function in living cultures
2	PoC In vivo efficacy study (NSR-004.2-PHAR-2) (NSR-004.2-PHAR-3)	CLN71d mice	IT KO Low: 1.25×10^{11} IT KO High: 5×10^{11}	• Accumulation of SCMAS and astrogliosis are major hallmarks of the underlying disease pathology, and CLN7 gene transfer into the mouse model reduces this pathology • Survival was greatly improved in a dose and age of treatment dependent manner, evident that high dose at earlier age provides the largest benefit for survival and quality of life • Behavioral rescue in Rotarod performance, a measure of motor function, was also observed in treated mice up to 9 months following injection, providing evidence for durable therapeutic benefit beyond survival
3	Non-GLP immunotoxicity study (NSR-004.2-TOXI-1)	First-generation construct in CLN71d mice	IT KO Low: 1.25×10^{11} IT KO High: 5×10^{11}	• An Interferon γ (INFγ) response is unlikely to confound the preclinical safety or efficacy studies conducted for first-generation construct • The codon-optimized human CLN7 transgene has a low risk of generating novel immune epitopes that would stimulate an INFγ response
4	Dose-ranging non-GLP chronic toxicology study (NSR-004.2-TOXI-2)	Juvenile WT CH57BL/6J mice	IT 8WK: 9.5×10^{11} IT 6WK: • 4.47×10^{11} • 1.48×10^{11} • 4.47×10^{10}	• First-generation construct vector does not affect weight or body condition over a 1-year period of longitudinal monitoring • Histopathology did not find any signs of toxicity due to first-generation construct at 1-year post-injection • No significant difference of survival rates between male or female mice • IT doses up to 9.5×10^{11} vg/mouse are safe and well tolerated in WT mice. Highest dose injected in the mice is a 3.8-fold higher titer than the highest dose proposed in humans, and twice the volume proposed in humans. Thus, the maximum tolerated dose in mice up to one-year post-injection provides a wide safety margin above what is proposed in humans
5	Dose-ranging GLP toxicology study (NSR-004.2-TOXI-3)	Sprague Dawley juvenile rats	IT Low: 5×10^{11} IT Mid: 2×10^{12} IT High: 6×10^{12}	• Following out to 91 days post-injection, found no significant test article-related effects on study parameters, with the exception of increased thymus weights in males at high dose, suggesting first-generation construct was overall well tolerated • No test article-related clinical observations, body weight, food consumption, or accelerating Rotarod values, therefore, the NOAEL was 6×10^{12} vg/animal

CLN7 knockout mice treated with first-generation construct had improved survival rates

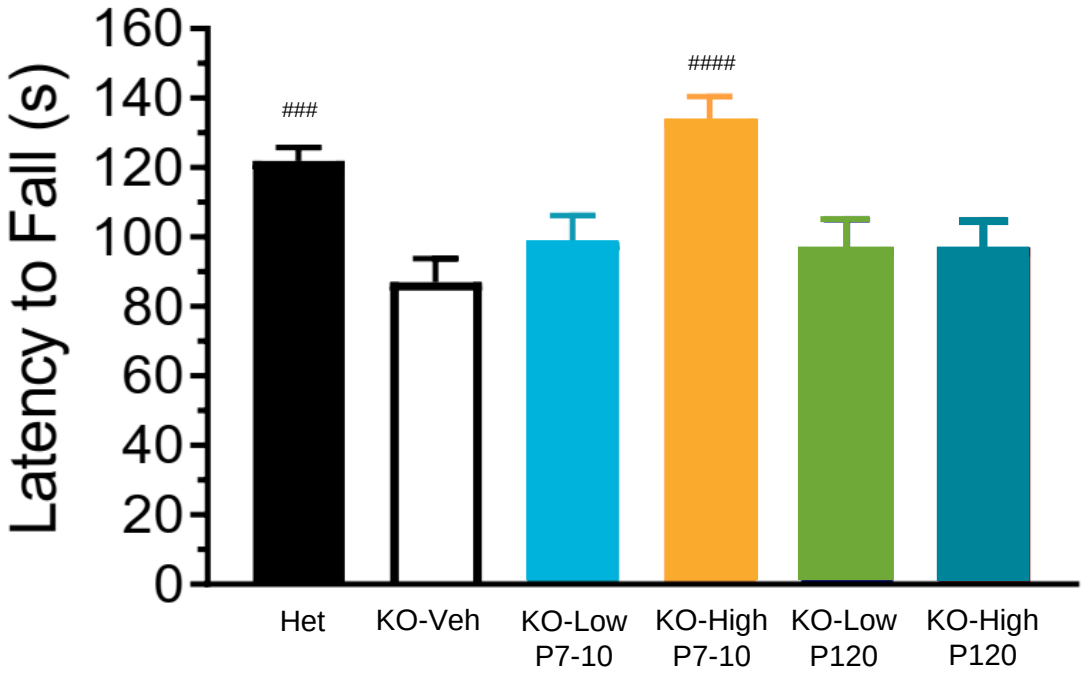


- No behavioral deficits observed between Het control animals and KO-Veh or KO-first-generation construct dosed animals at 2 months or 4 months of age
- Survival of KO-Veh animals drastically decreased between 6 and 9 months of age
- High-dose/early treatment group showed the greatest survival benefit (doubled lifespan), and low-dose/late treatment groups showed moderately increased survival

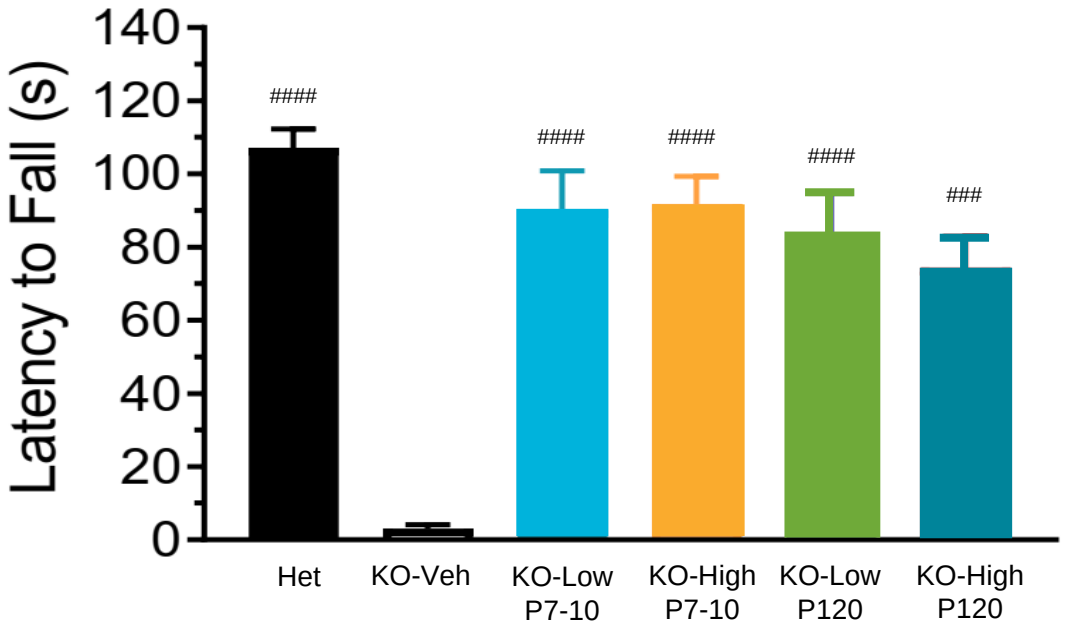
Low dose: 1.25 $\times 10^{11}$ vg/animal
High dose: 5 $\times 10^{11}$ vg/animal

CLN7 knockout mice treated with first-generation construct had sustained preservation of motor function on rotarod

6 Month Data*



9 Month Data*



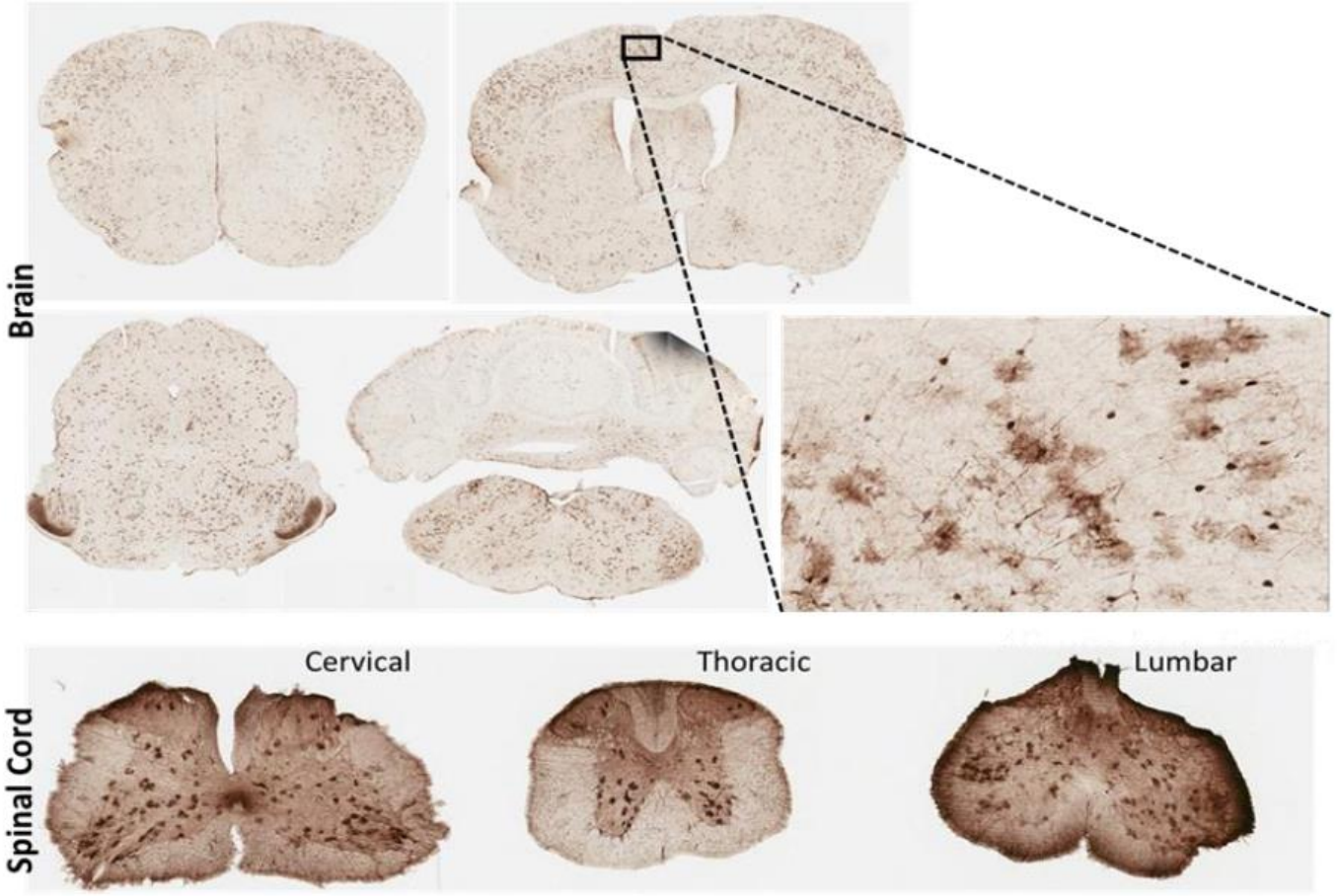
* Rotarod: Maximum

###p<0.001, ####p<0.0001 compared to KO-Veh

Low dose: 1.25 ×10^{11} vg/animal
High dose: 5 ×10^{11} vg/animal

Widespread GFP expression throughout the CNS and peripheral tissues with first-generation construct

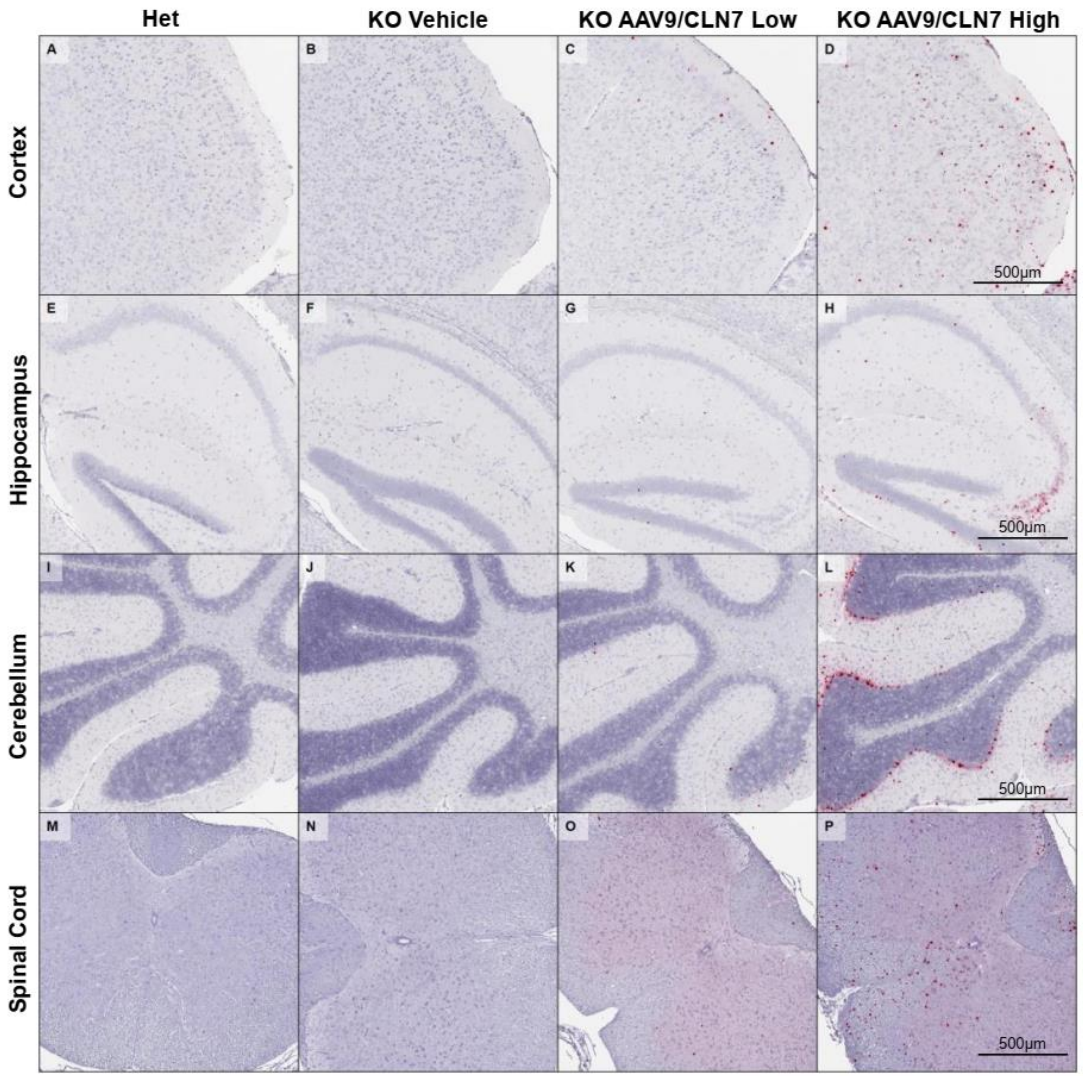
- A high-dose IT AAV9 study was conducted in eight-week-old wild type mice, using a self-complementary AAV9/CBh-GFP reporter vector at a dose of $4.15x10^{11}$ vg per mouse
- This vector construct is identical to the first-generation construct, except that the GFP expression cassette is inserted in place of the CLN7 expression cassette
- The dose is 1.8-fold higher than the high IT dose of the first-generation construct vector that was tested. This resulted in widespread GFP expression throughout the CNS and peripheral tissues
- At 4 weeks post-injection, immunohistochemistry of the brain and spinal cord was conducted to visualize the spatial distribution of GFP expression (n=4)
  - GFP expression is indicated by brown staining



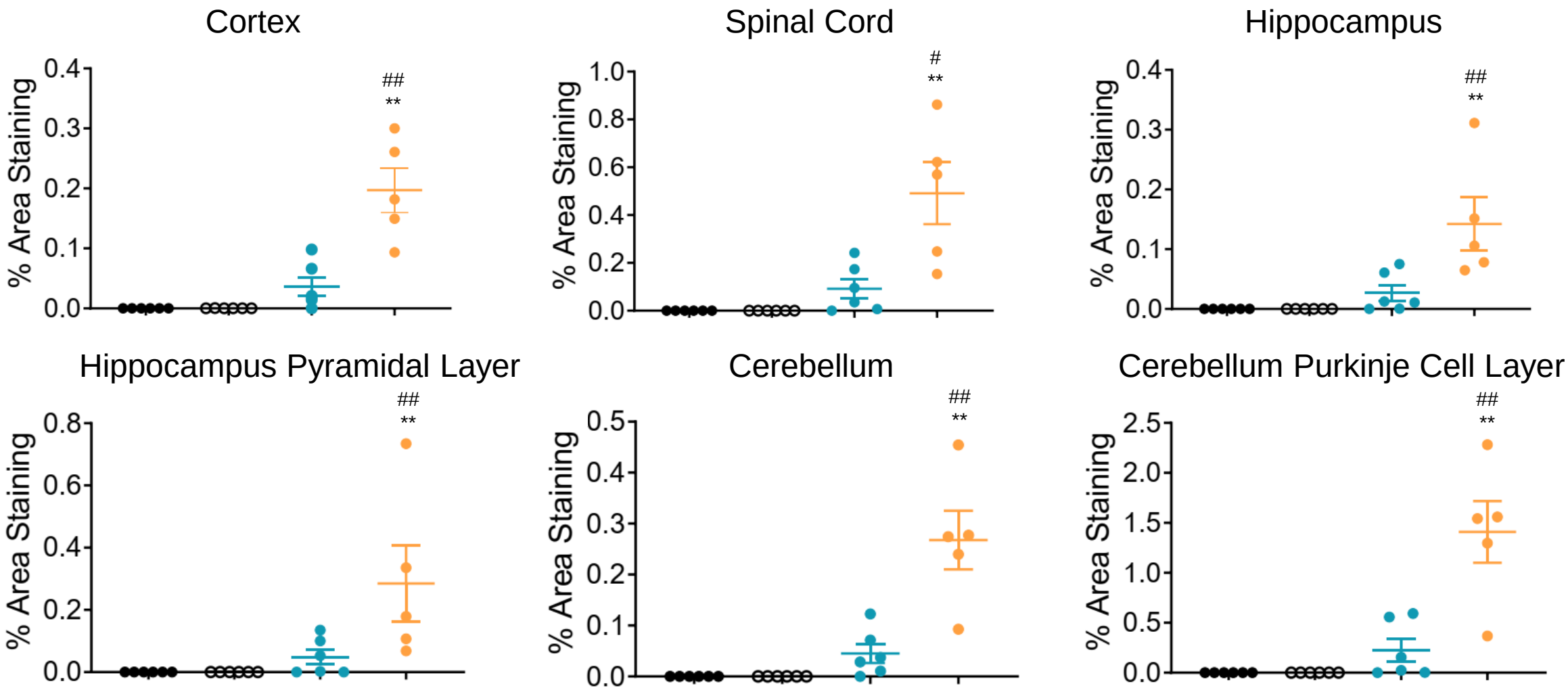
Bailey RM, Rozenberg A, and Gray SJ. Brain Research, 2020

mRNA staining in CLN7 knockout mice demonstrated target tissue expression

- RNAscope staining was used in a semi-quantitative analysis of hCLN7 mRNA expression in the brain and spinal cord of first-generation construct-dosed CLN71d KO mice
- CLN71d mice treated with first-generation construct at PND 7-10 also had dose-dependent detectable levels of hCLN7 mRNA in all tissues and brain regions assessed up to 4.5 months post-injection
- Positive hCLN7 mRNA staining is observable as a deep red color
- These results provide evidence for target tissue expression in animal models of CLN7 disease, and further highlight the need of a high dose to achieve desirable CLN7 expression levels across the CNS
- Results are consistent with previous published studies using a GFP reporter



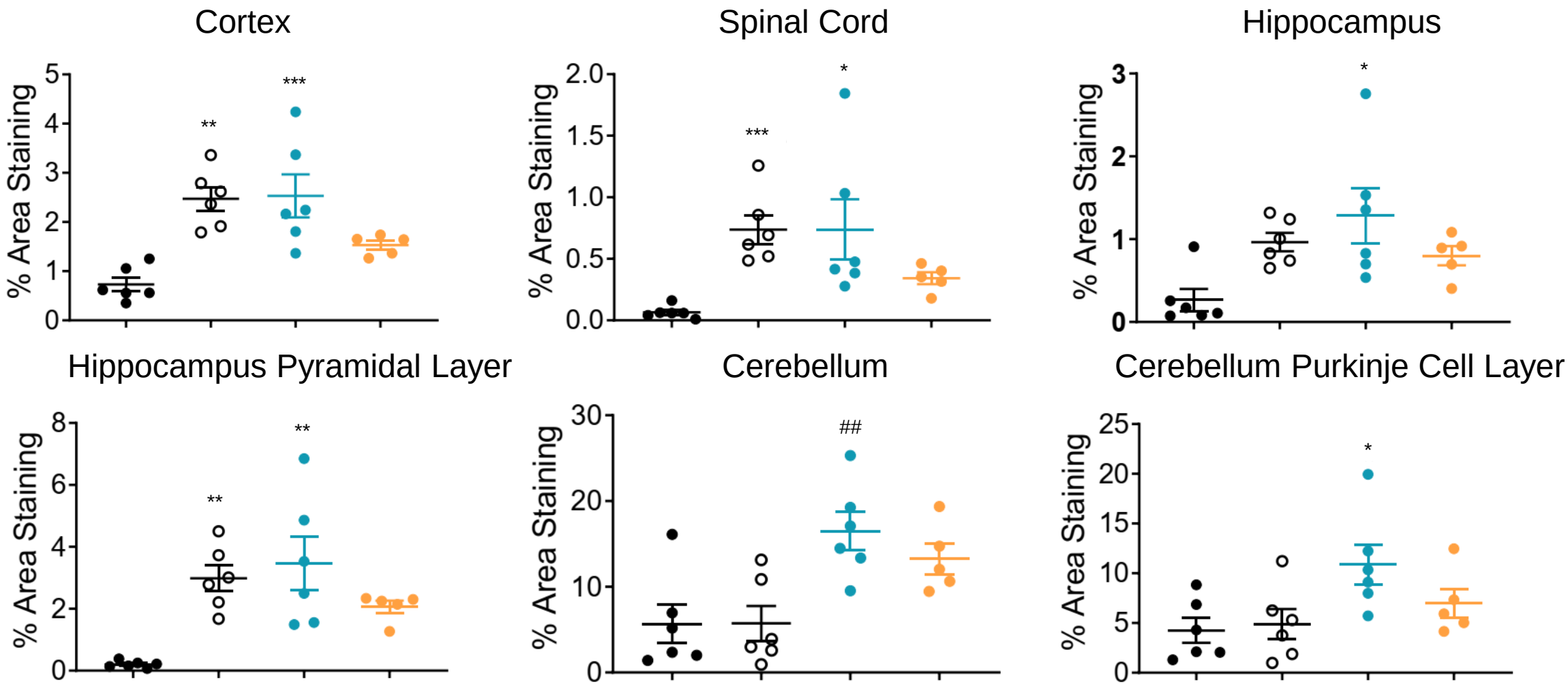
Percent area staining positive for hCLN7 mRNA by tissue region from first-generation construct



**p<0.01 compared to Het
##p<0.01, #p<0.1 compared to KO-Veh

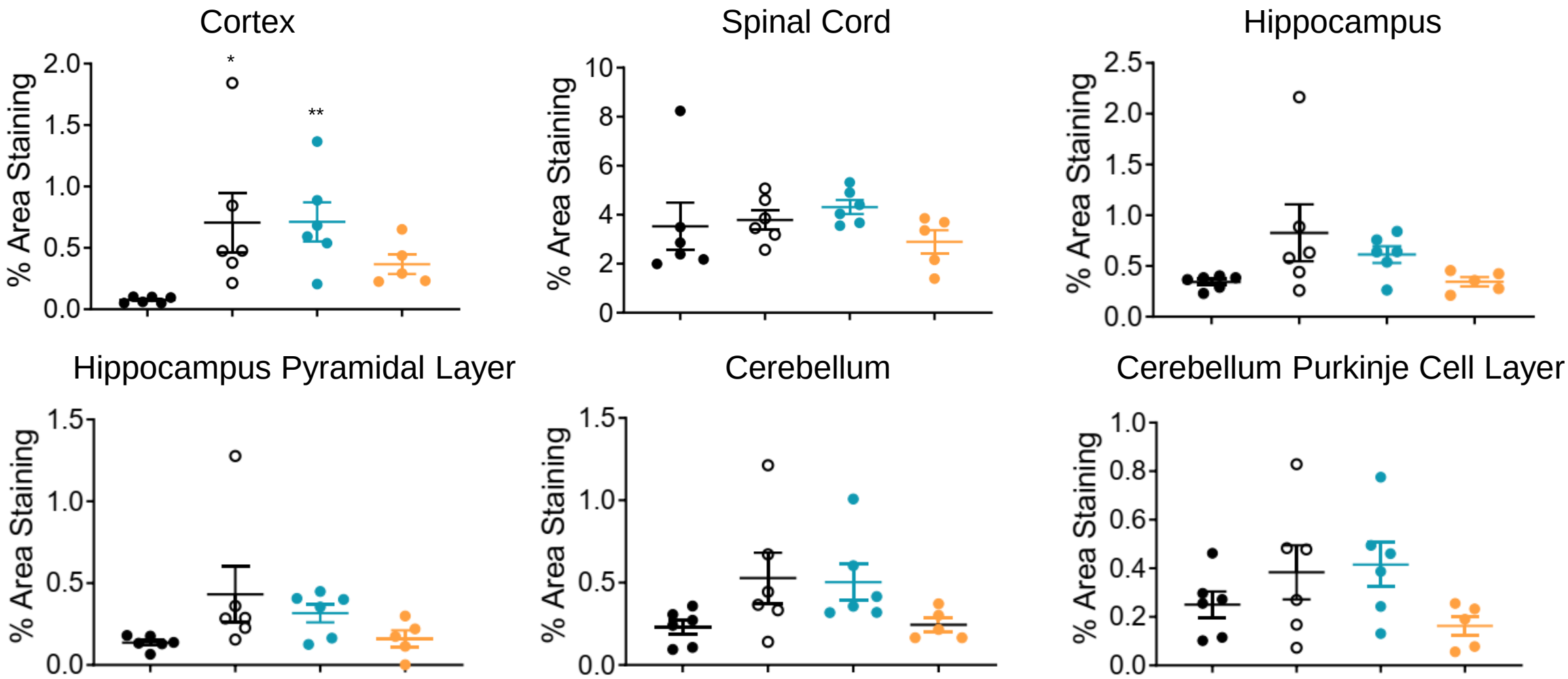
Dose-responsive reduction in SCMAS (lysosome storage material) across the CNS with first-generation construct

TSHA-121
CLN7 disease



*p<0.1, **p<0.01, ***p<0.001 compared to Het
##p<0.01 compared to KO-Veh

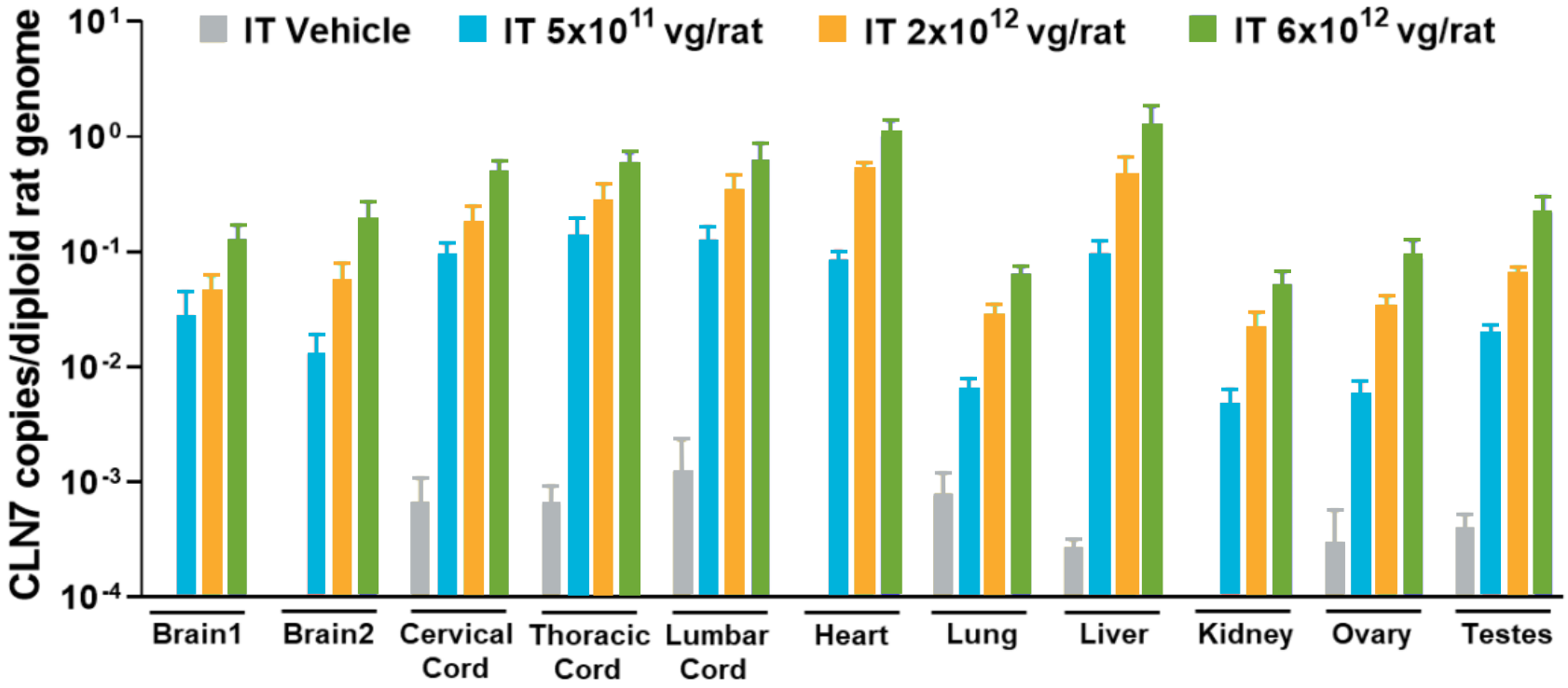
Dose-responsive reduction of GFAP with first-generation construct demonstrated reduced inflammation across the CNS



*p<0.1, **p<0.01 compared to Het

Safety and biodistribution of first-generation construct in wild type rats

- IT delivery of first-generation construct resulted in dose dependent increase of CLN7 vector DNA across the CNS (brain and spinal cord) and peripheral organs (heart, lung, liver, kidney, ovary, and testes)
- Biodistribution patterns matched expected results based on previous published and unpublished studies
- Overall, first-generation construct was well-tolerated across all dose levels and time points



Translating nonclinical data into human setting

- Disrupting the *CLN7*/*MFSD8* gene in mice by targeted deletion of exon 2 generating a novel knockout (KO) mouse model for CLN7 disease recapitulates key features of human CLN7 disease pathology[1]
  - The ultrastructure of the storage material in neurons of CLN7 KO mice resembled the storage material found in brains of CLN7 patients
  - MRI revealed brain atrophy in the olfactory bulb, cerebellum and cerebral cortex of CLN7 KO mice at the end stage of the disease. CLN7 KO mice recapitulated key neuropathological features of human CLN7 disease, in which neuroinflammation and neurodegeneration in the cerebellar and cerebral cortex were observed
- In CLN71 mice, there was age and dose-dependent rescue of multiple disease phenotypes, providing strong evidence for benefit of the first-generation construct for CLN7 disease[2]
- Dose selection of 5.0 $\times 10^{14}$ total vg for the first patient followed by 1.0 $\times 10^{15}$ total vg for subsequent patients in the human clinical trial is based on the minimum effective dose (MED) in the mouse model and the NOAEL from the toxicology study

1. Brandenstein L, Schweizer M, Sedlacik J, Fiehler J, Storch S. Lysosomal dysfunction and impaired autophagy in a novel mouse model deficient for the lysosomal membrane protein Cln7. *Hum Mol Genet*. 2016;25(4):777-791. doi:10.1093/hmg/ddv615
2. *In vivo Efficacy: AAV9/CLN7 Ameliorates Behavioral Deficits and Improves Survival in CLN71d Mouse Model of Batten Disease (NSR-004.2-PHAR-3)*

Current Phase 1 open-label, clinical proof-of-concept CLN7 trial with first-generation construct

Goals and Targets of Trial

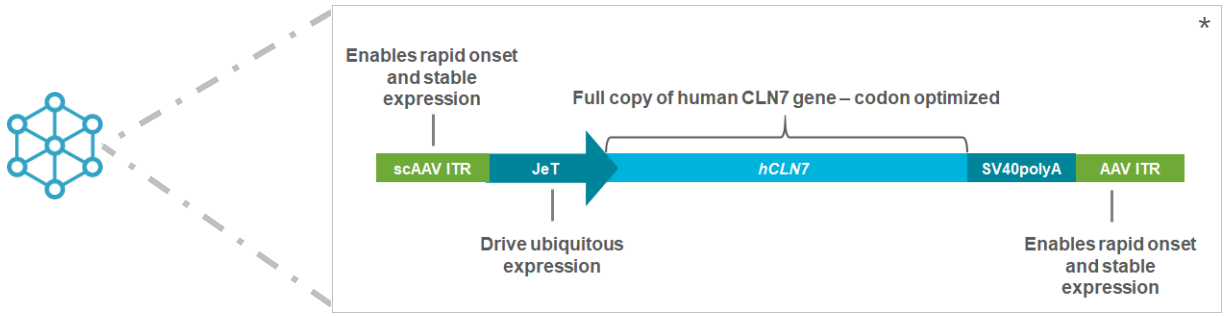
Goals

- **Primary** – Safety and tolerability by incidence and severity of treatment related SAEs
- **Secondary** – Efficacy: motor, cognition, and intelligence assessments

Target Recruitment

- 4 subjects 1-18 years of age, may expand number of patients based on DSMB discussions
- First patient has received initial dose of 5.0 x 10^{14} total vg
- Second patient has received higher dose of 1.0 x 10^{15} total vg
- Subsequent participants to receive higher dose of 1.0 x 10^{15} total vg

Product Details and Dose Cohorts



* First-generation construct

Dose Cohorts

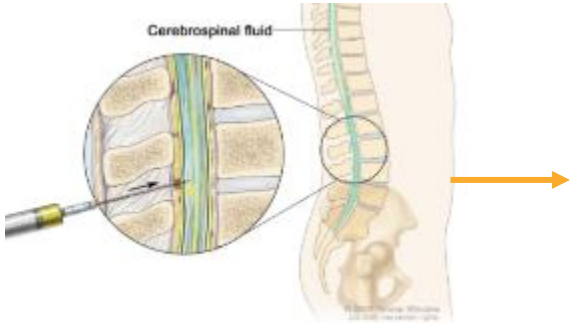
- 5.0 x 10^{14} total vg (n=1)
- 1.0 x 10^{15} total vg (n=3)

Doses are for participants ≥4 years of age and will be scaled down for younger participants as appropriate

Route and Method of Administration

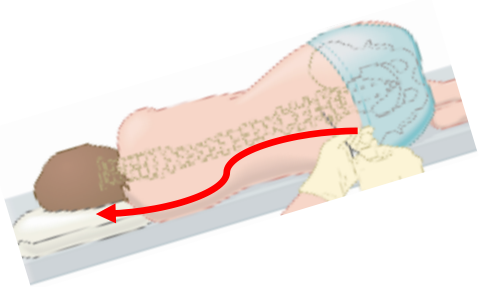
Administration

- Lumbar Intrathecal Infusion (IT)
- Amount and rate: 1 mL/min for total of 10-12 mL
- Immunosuppression regimen of prednisolone and sirolimus



Technique to Improve Transduction

- Trendelenburg position (15-30º)
- During infusion & 1 hour post infusion



Efficacy outcome measures

Disease burden assessments
- Clinical Global Impression Scale (CGI)
- Seizure diary
- Swallow function

Motor function assessments
- Timed Walk tests (2-Minute or 6-Minute Walk Test)
- Pediatric balance scale
- Gross Motor Function Measure (GMFM)

Neuropsychological/Developmental
- Mullen Scales of Early Learning
- Vineland Adaptive Behavior Scales (Vineland-3)

Imaging and neurophysiology
- Brain MRI
- Standard awake 60-minutes electroencephalogram (EEG)

Ophthalmologic assessments
- Eye examination
  - Visual acuity (VA)
  - Electroretinography (ERG)
  - Optical Coherence Tomography (OCT)

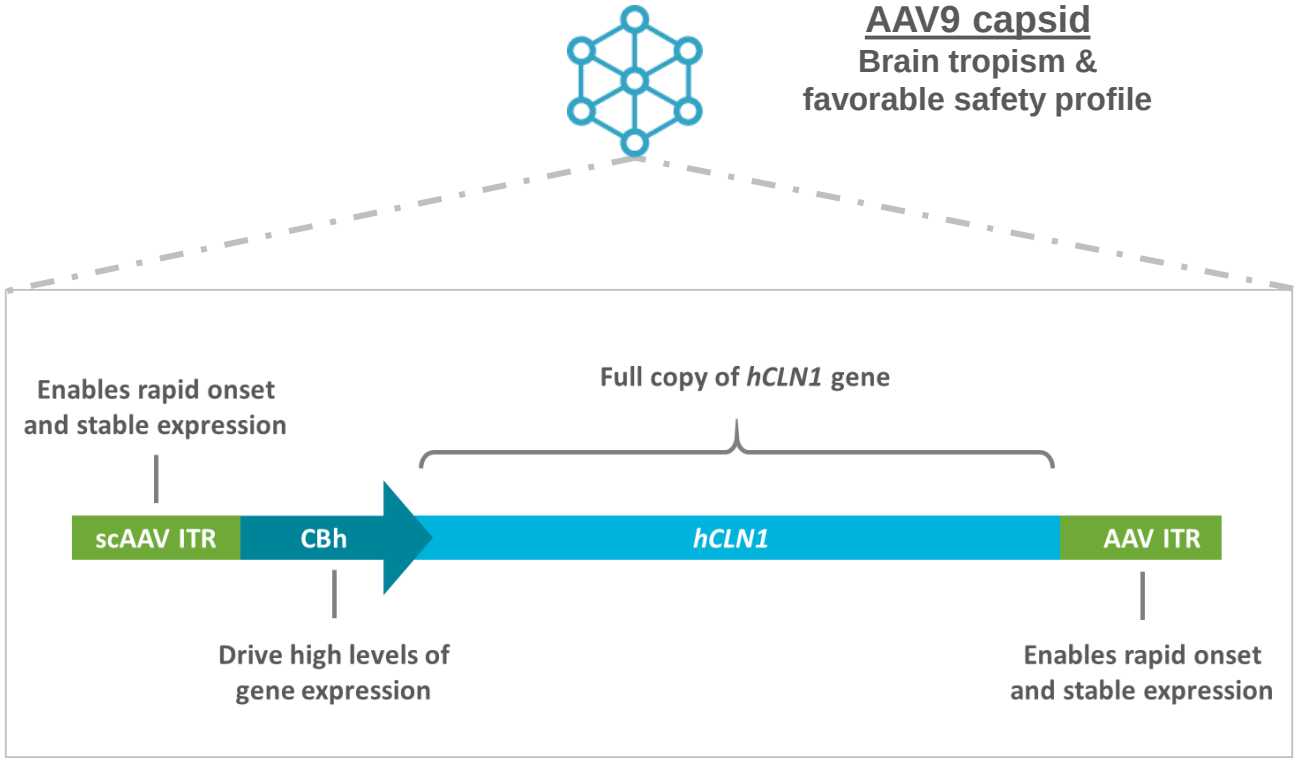
Quality of Life and Disease Burden measures
- Quality of Life Inventory-Disability (QI-Disability)
- Infant/Toddler Quality of Life Questionnaire (ITQOL)
- Healthcare resource utilization

Bridging from first-generation to next-generation construct for planned pivotal studies

- Next-generation construct design anticipated by year-end 2021
- Next-generation design enables opportunity to improve potency and potentially deliver lower doses
- Next-generation construct anticipated to improve safety profile over first-generation construct
- Significant improvements to packaging efficiency and manufacturability resulting in higher yields and lower cost of goods sold
- Commercial-grade GMP material for next-generation construct available in 2022
- Initiation of planned pivotal clinical trial with next-generation construct in 2022 with reference to clinical data from first-generation construct
- Oral presentation of preclinical CLN7 data from the first-generation construct at the 17th Annual International Congress on Neuronal Ceroid Lipofuscinosis (NCL) on October 8, 2021
- Poster on design rationale and discussion of outcome measures for ongoing Phase 1 clinical trial for the first-generation construct to be presented at the 17th Annual International Congress on NCL

CLN1 disease is a severe neurodegenerative lysosomal storage disease

- Severe, progressive, neurodegenerative lysosomal storage disease, with no approved treatment
- Caused by mutations in the *CLN1* gene, encoding the soluble lysosomal enzyme palmitoyl-protein thioesterase-1 (PPT1)
- The absence of PPT1 leads to the accumulation of palmitoylated substrate within the lysosome
- Disease onset is typically within 6-24 months, with progression visual failure, cognitive decline, loss of fine and gross motor skills, seizures, and death usually occurring by 7 years of age
- Estimated prevalence of CLN1 disease is 900 patients (US+EU)



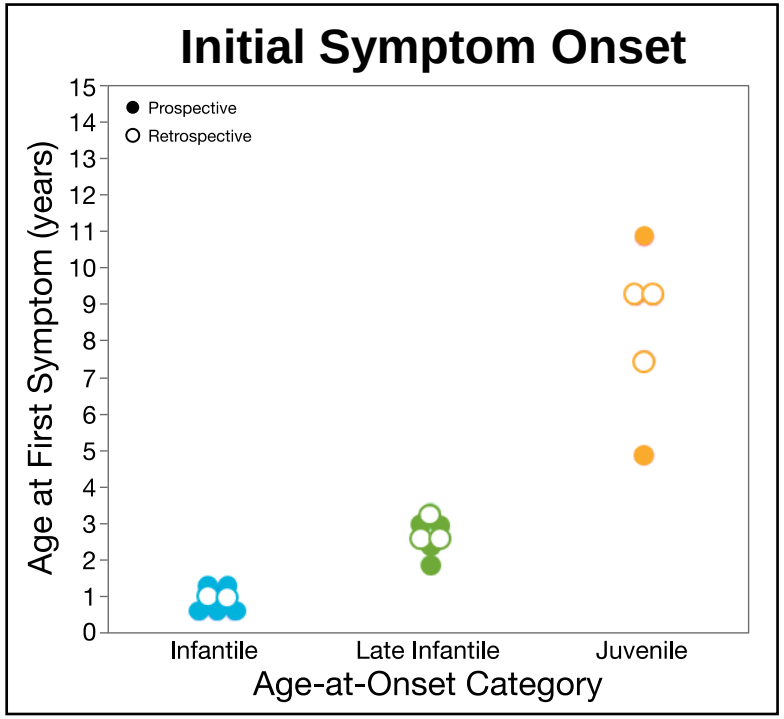
CLN1 disease natural history data

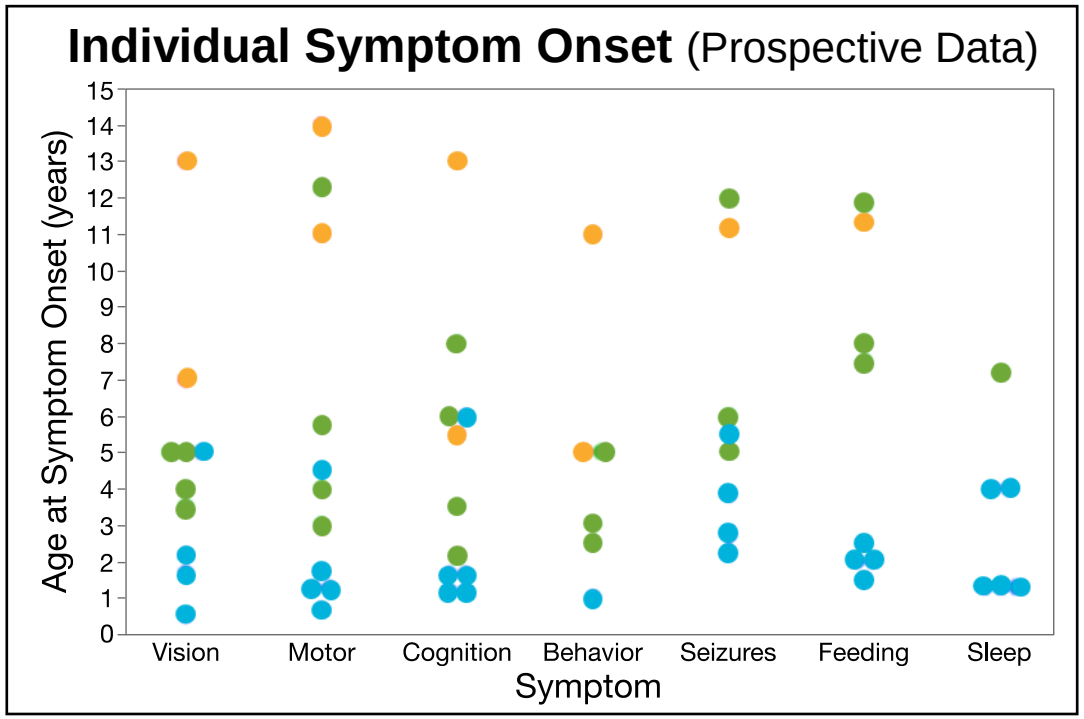
- Ongoing observational study to assess natural history of NCL diseases (including CLN1) as part of the international DEM-CHILD Database (Angela Schulz, Universitätsklinikum Hamburg-Eppendorf)
- University of Rochester NHS used a combined retrospective and prospective approach to characterize age-at-onset of major symptoms and relationship between age and severity
  - Medical records obtained for individuals with CLN1 disease for retrospective evaluations
  - Data obtained prospectively with the Unified Batten Disease Rating Scale (UBDRS) in an 18-year prospective natural history study of the NCLs

Prospective	Subjects identified through multiple methods; obtained relevant records and contacted providers • Batten Disease Support and Research Association (BDSRA) Annual Meeting • Facebook post • University of Rochester Batten Center (URBC) Website post • Newsletter sent to URBC contact registry participants
Retrospective	• Participants evaluated at annual BDSRA meeting for URBC • Data from the UBDRS physical subscale were used as a proxy for disease severity

Rochester CLN1 disease natural history data – Age and order of symptom onset

		Retrospective (n=8)	Prospective (n=12)
Sex	Female Male	7 (87.5%) 1 (12.5%)	6 (50%) 6 (50%)
Age-at-Onset	Infantile (0 - 1.5 years): Late Infantile (>1.5 - <5 years): Juvenile (≥5 years):	2 (mean 1.2 years) 3 (mean 2.9 years) 3 (mean 8.6 years)	5 (mean 1.0 years) 4 (mean 3.0 years) 3 (mean 8.0 years)



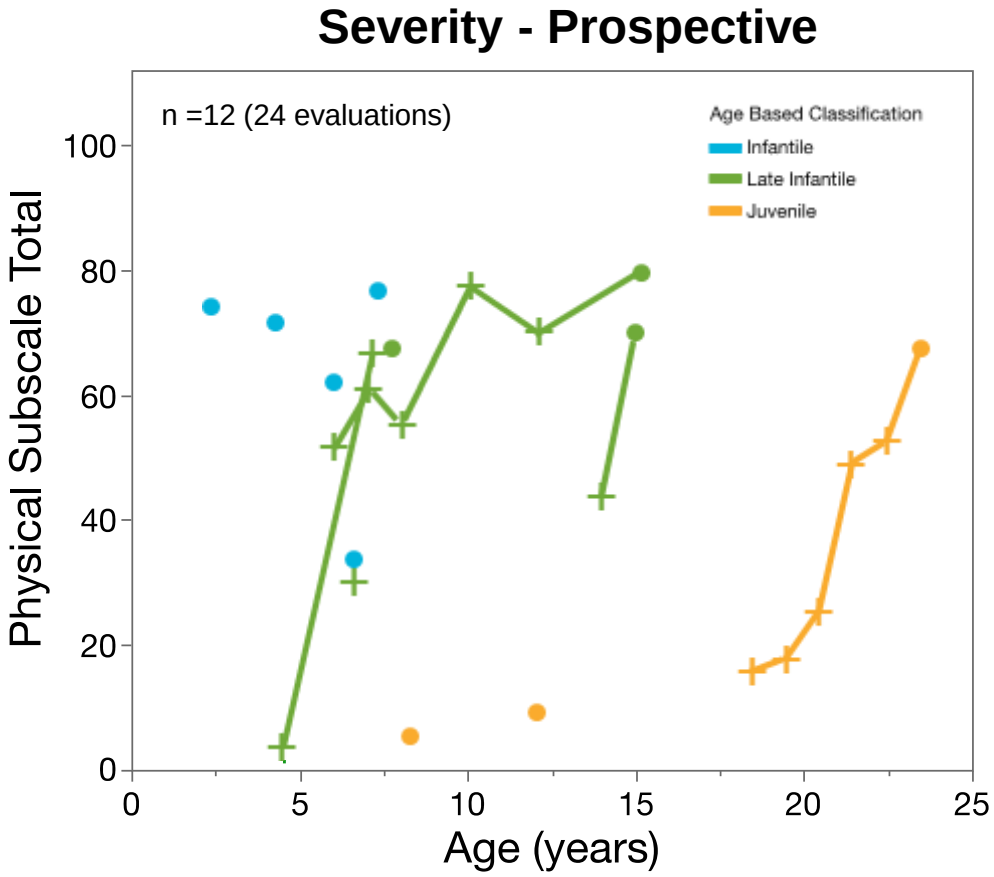


- Infantile: 0 - 1.5 yrs.
- Late Infantile: >1.5 - <5 yrs.
- Juvenile: ≥5 yrs.

Masten M et al. *Molecular Genetics and Metabolism* 2020

Rochester CLN1 disease natural history data – Change in disease severity over time

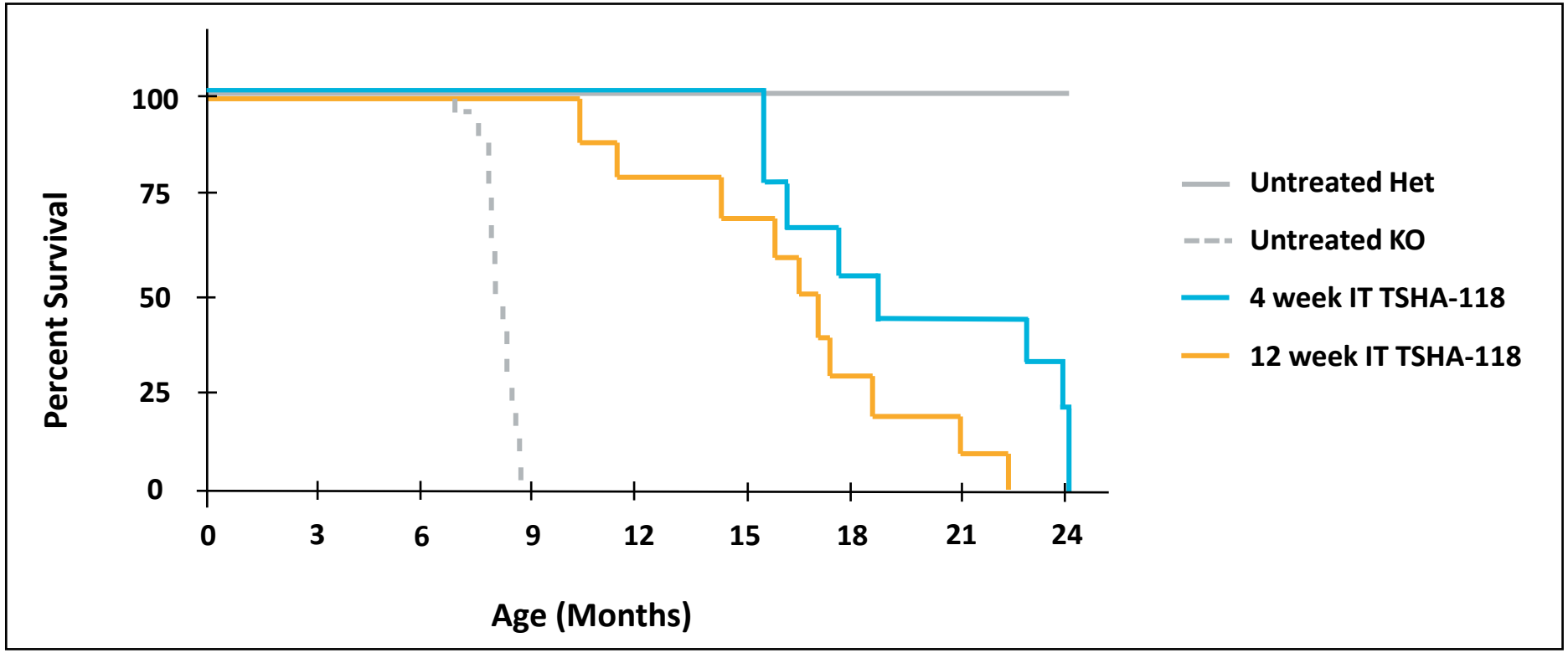
- Age-at-onset, initial symptom type, and order of symptom presentation variable and inconsistent across individuals with CLN1 disease
- Severity could be quantified for each individual in prospective arm
- Progression appeared to be relatively rapid, even in those with juvenile-onset
- Retrospective analysis limited by: small numbers, variability of information from medical records within and across patients, and medical records from individuals without genetic confirmation
- Current sample too small to conduct formal genotype-phenotype correlation



UBDRS physical subscale total score against age in years. Data from individuals with multiple data points connected by lines. Dots represent most recent evaluation. Colors represent age-based classification.

Masten M et al. *Molecular Genetics and Metabolism* 2020

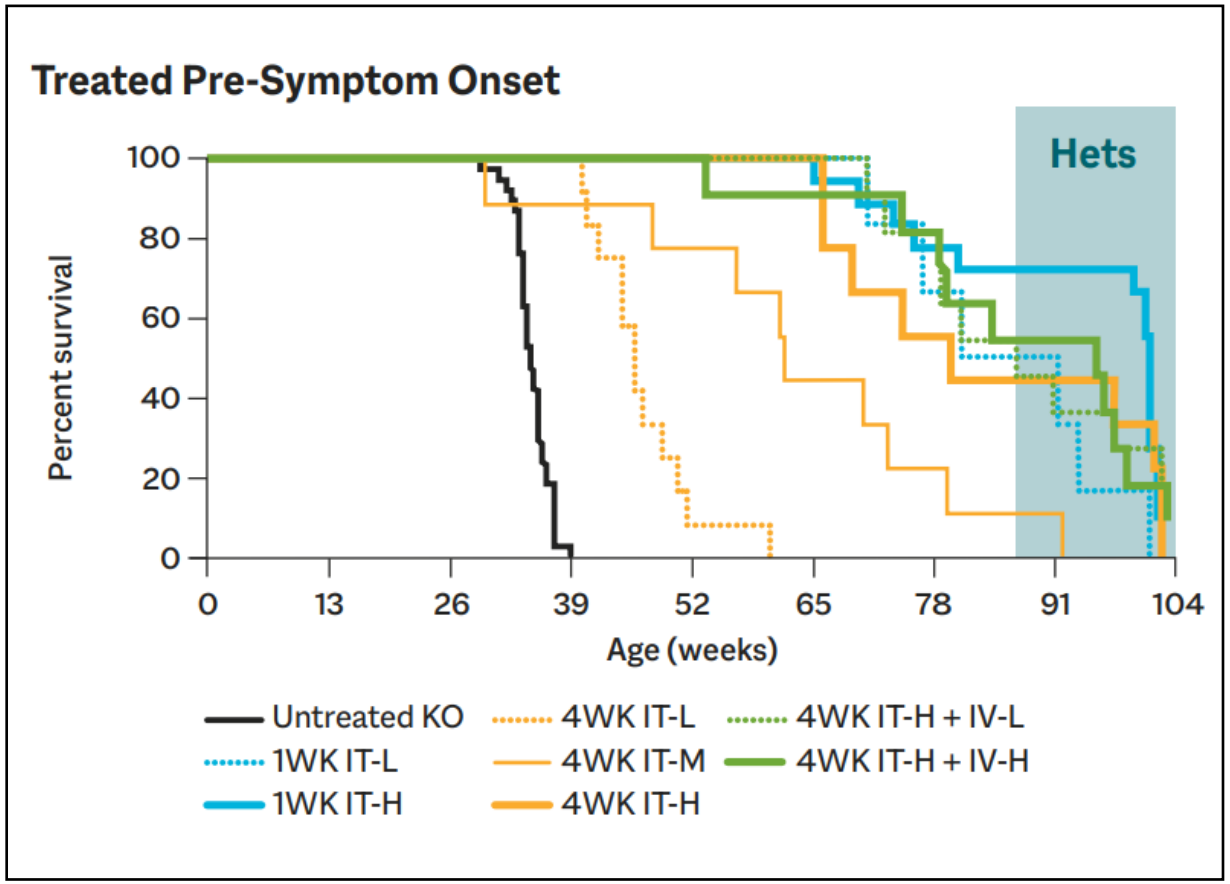
TSHA-118-treated CLN1 KO mice had improved survival rates

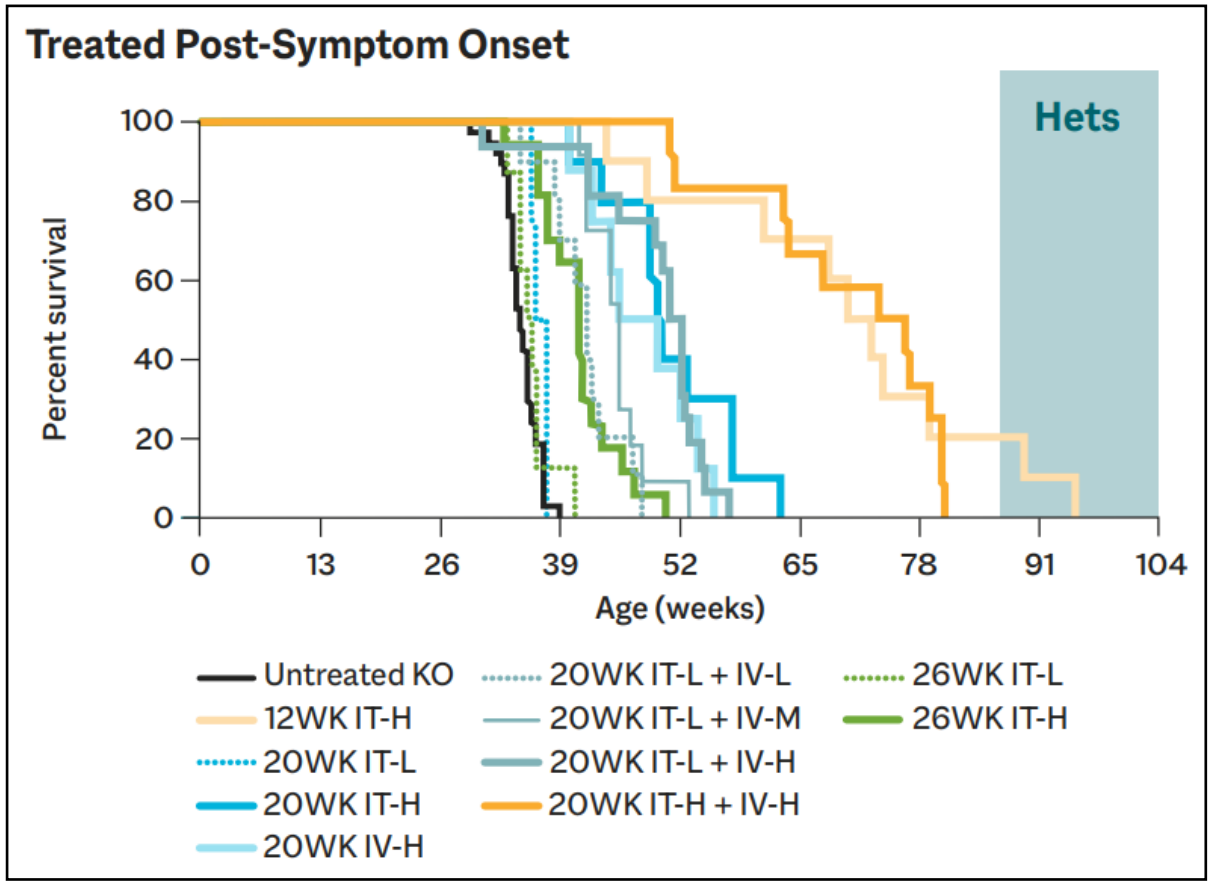


IT administration of TSHA-118 significantly extended survival of *PPT1* KO mice for all ages and at all dose levels

Higher doses of TSHA-118 and earlier intervention mediated stronger rescue of CLN1 KO mice

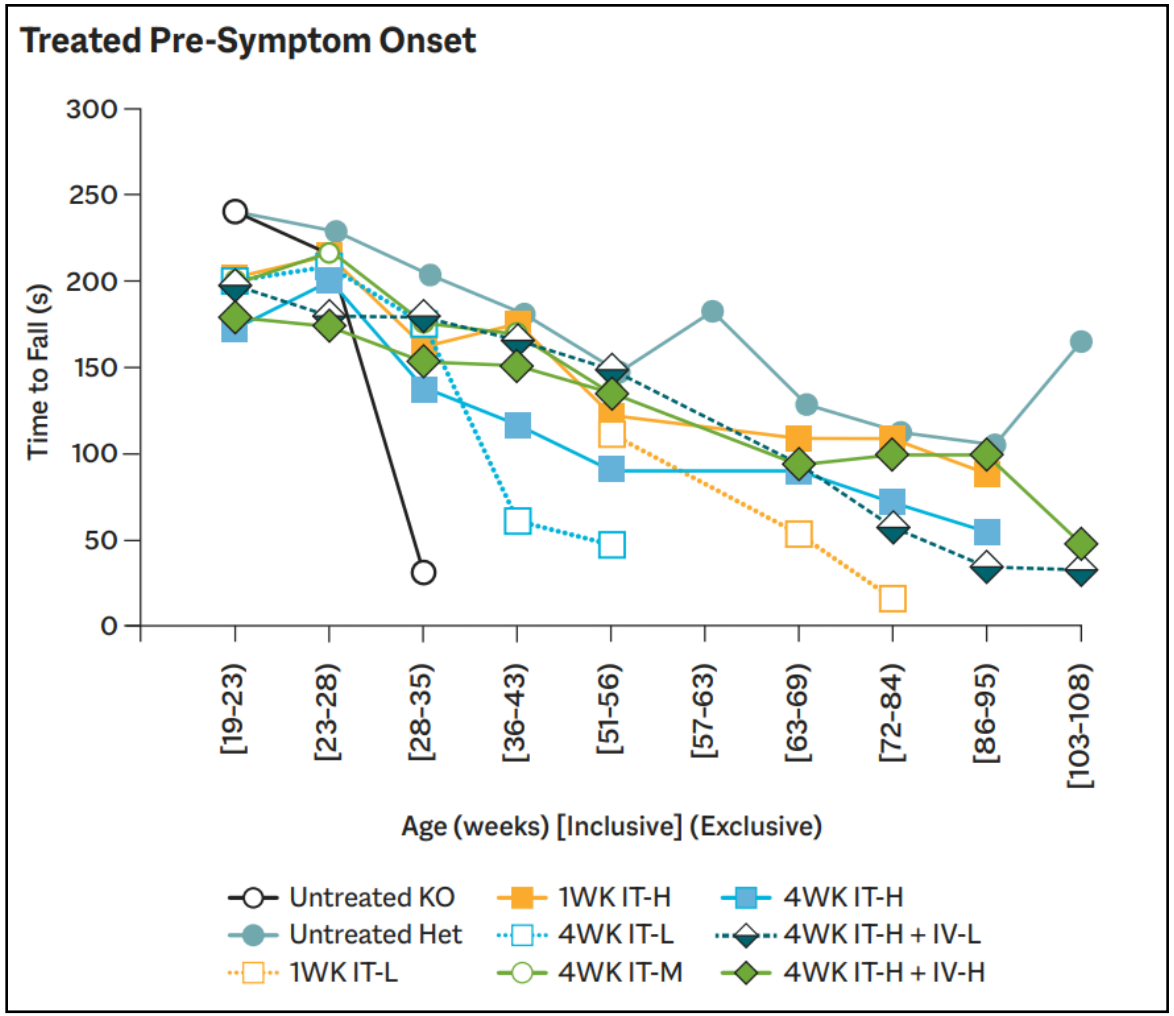
TSHA-118
CLN1 disease

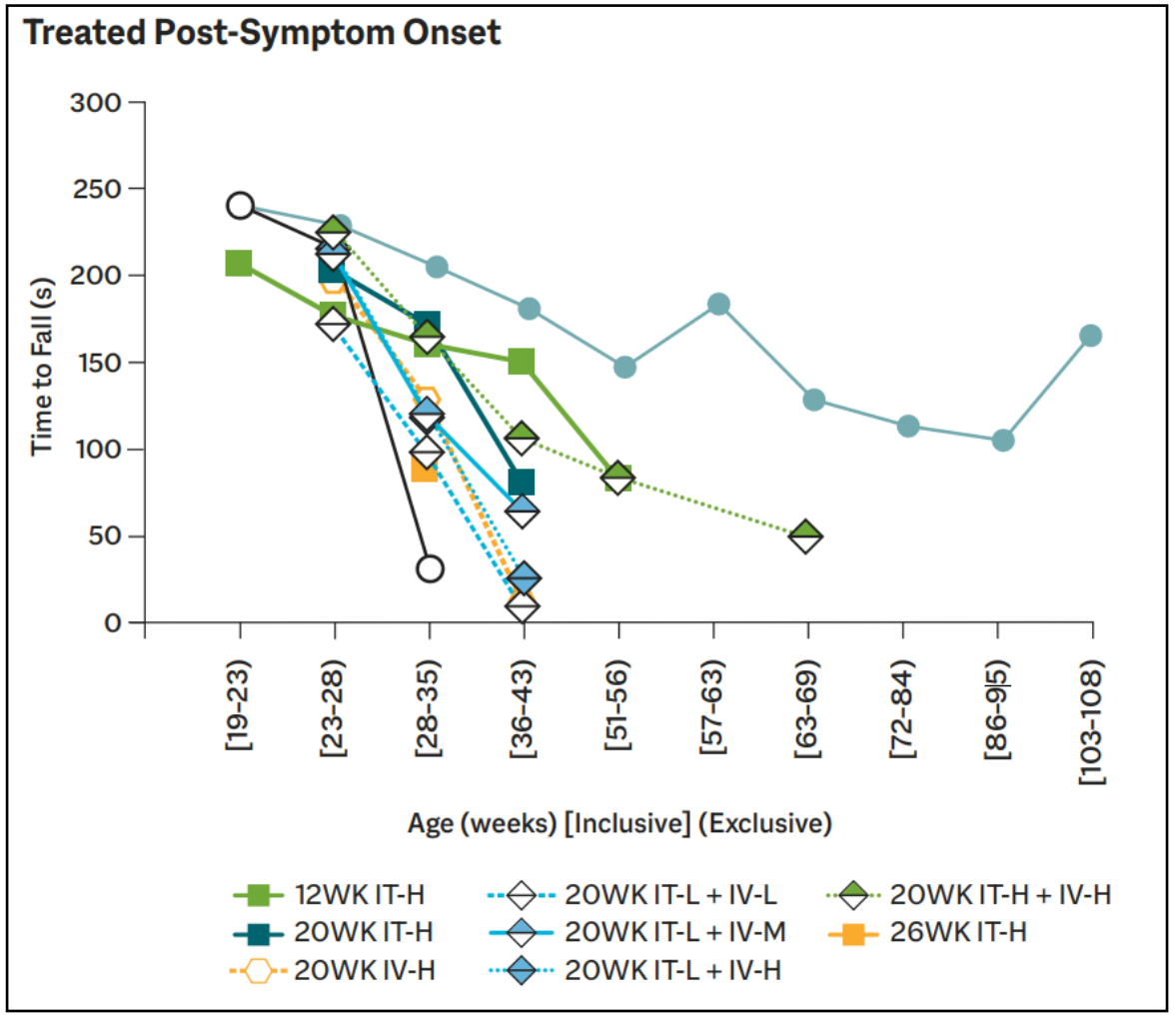




L - $7.0x10^{10}$ vg/mouse M - $2.2x10^{11}$ vg/mouse H - $7.0x10^{11}$ vg/mouse

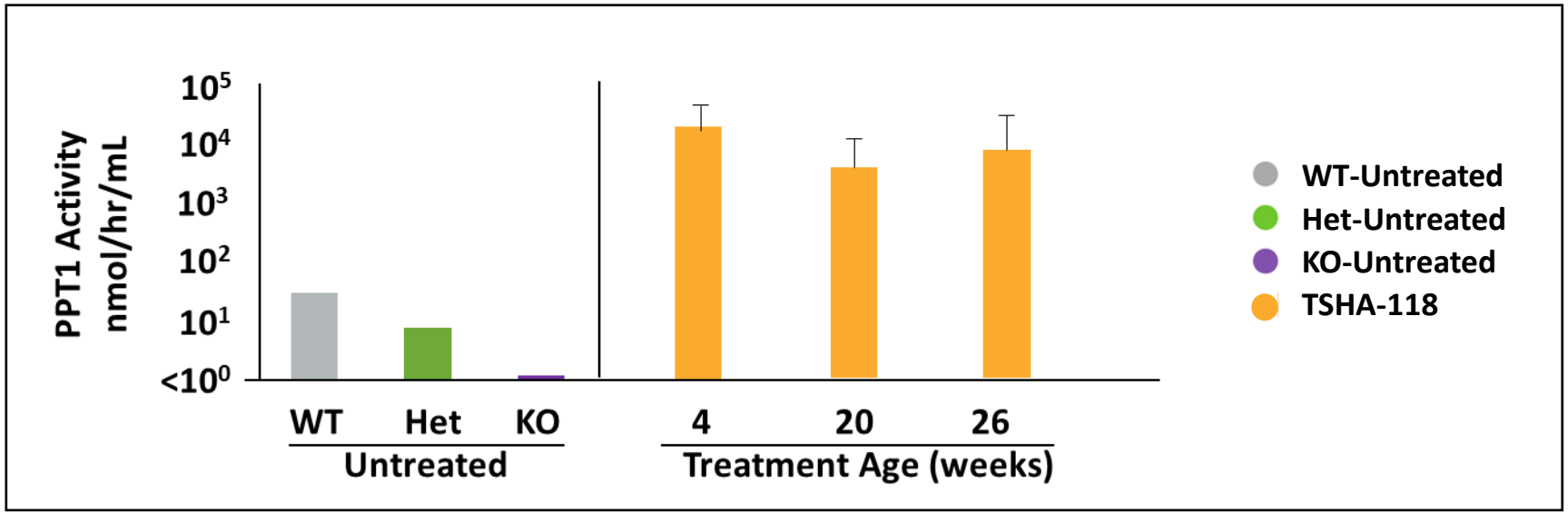
TSHA-118-treated CLN1 KO mice had sustained preservation of motor function





L - $7.0x10^{10}$ vg/mouse M - $2.2x10^{11}$ vg/mouse H - $7.0x10^{11}$ vg/mouse

TSHA-118-treated CLN1 mice had increased and sustained plasma PPT1 activity



- Supraphysiological levels of active PPT1 were observed in all TSHA-118 treated mice and persisted through the study endpoint
- Persistence of effect after animal sacrificed up to 8.5 months post-treatment

Phase 1/2 adaptive trial for TSHA-118 in CLN1

Goals and Targets of Trial

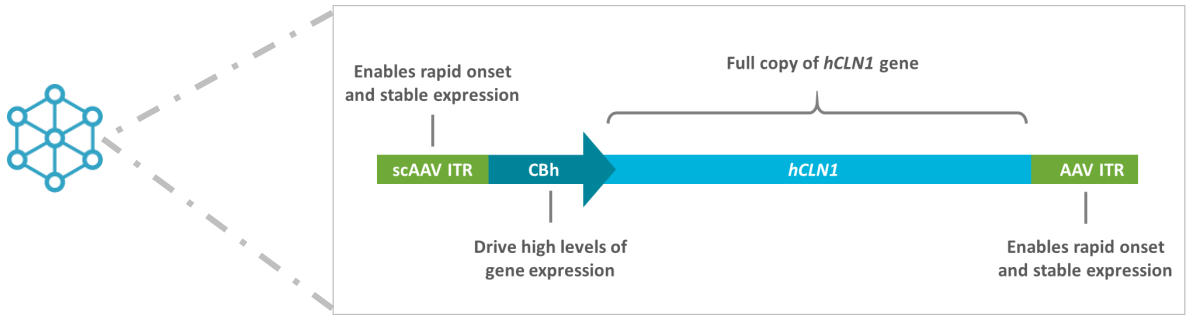
Goals

- **Primary** – Safety: clinical and laboratory assessments
- **Secondary** – Efficacy: pathologic, physiologic, functional and clinical markers

Target Recruitment

- Up to 18 subjects
- Each cohort will include at least one participant with infantile onset (classic or late, screened within one or two years from symptom onset, respectively) and one participant with juvenile onset (screened within four years from symptom onset)

Product Details and Dose Cohorts



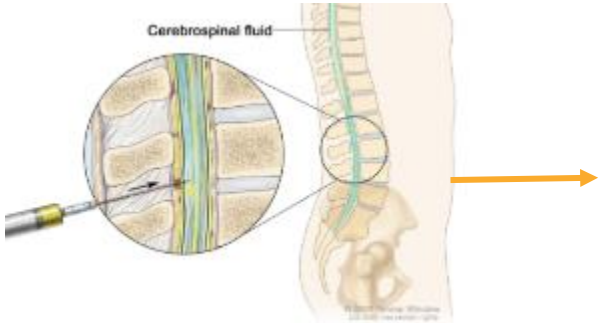
Dose Cohorts

- 5 x 10^{14} total vg (n=3)
- 1.0 x 10^{15} total vg (n=3)
- Dose expansion – TBD

Route and Method of Administration

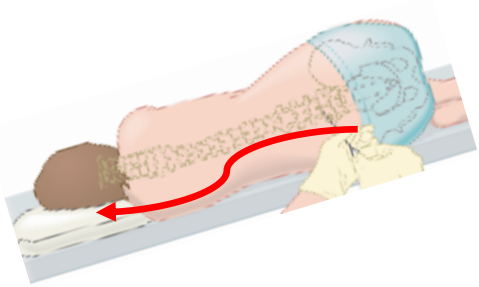
Administration

- Lumbar Intrathecal Infusion (IT)
- Amount and rate: 1 mL/min for total of 10-12 mL
- Immunosuppression regimen of prednisolone and sirolimus



Technique to Improve Transduction

- Trendelenburg position (15-30^{o})
- During infusion & 1 hour post infusion



TSHA-118 Phase 1/2 clinical assessments

Disease-Specific/Global Assessments
- Unified Batten Disease Rating Scale (UBDRS)
- CHOP INTEND
- Hamburg Scale: motor, visual, language, and seizure scores
- Seizures assessed by UBDRS and seizure diary
- Adaptive score assessed by Vineland-III
- Bayley-III / WPPSI-IV / WISC-V

Ophthalmological Assessments
- ERG, OCT, and preferential looking test

Imaging
- Brain MRI, 60-minute electroencephalogram (EEG)
- Brain MRI using Diffusion Tensor Imaging (DTI) technology

Biomarkers
- PPT1 enzyme activity in CSF & serum

Communication Assessments
- Observer Reported Communication Assessment (ORCA)

Quality of Life/Other Assessment
- PedsQL™ Generic Core Scales
- Pittsburgh Sleep Quality Index (PSQI)
- Parenting Stress Index, 4th Edition (PSI-4) Parental Global Impression (PGI) Form
- Clinician Global Impression Improvement (CGI-I)

Anticipated next steps for TSHA-118 by the end of 2021

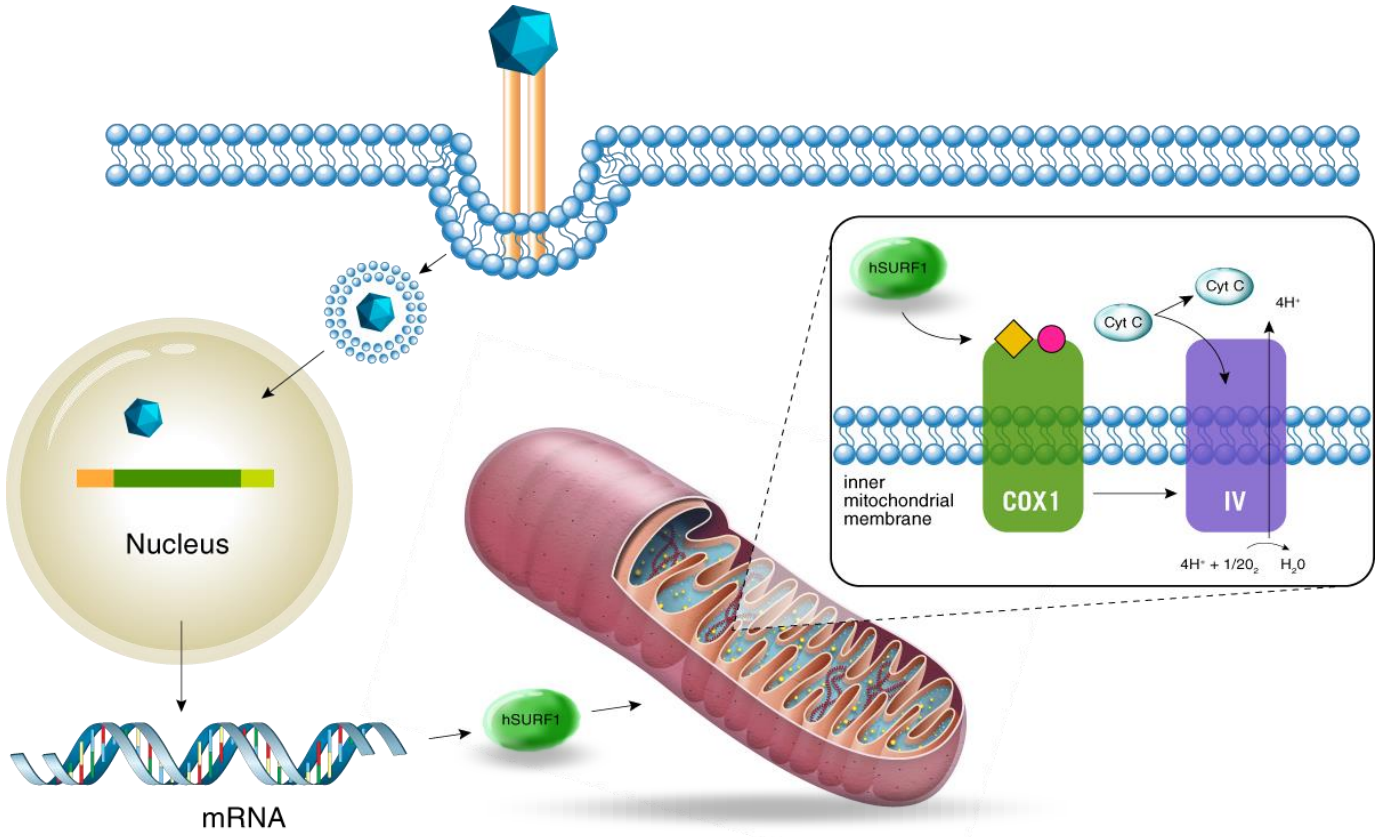
Initiate Phase 1/2 clinical study in 2H 2021

CTA scientific advice meetings underway to open European site

Patient finding activity in collaboration with UTSW, Rochester, Hamburg

SURF1 deficiency is the most common cause of Leigh syndrome

- A monogenic mitochondrial disorder
- Most common cause of cytochrome c oxidase deficient Leigh syndrome
- Leigh syndrome – severe neurological disorder that presents in the first year of life
  - Initially often presents with gastrointestinal symptoms
  - Progressive loss of mental and movement abilities, often regression is episodic in nature
  - Can result in death within two to three years
  - ~10-15% have SURF1 mutation
- No approved therapies
- Estimated prevalence of SURF1 deficiency is 300 to 400 patients (US+EU)



SURF1 deficiency natural history study – Initial symptoms

- Review of 44 cases with SURF1 deficiency
- Median age for first symptom onset was 9.5 months (range 0–60 months); majority presented in the first year (32/44, 73%)
- Most frequently noted initial symptoms included poor feeding/vomiting (frequently attributed to gastroesophageal reflux) and poor weight gain
- Neonatal period uneventful in majority of patients (41/44, 93%)
- Most (26/44, 59%) presented with combination of GI symptoms, poor weight gain and hypotonia
- Developmental regression (loss of cognitive or motor skills) was initial symptom in 3/44 (7%) patients

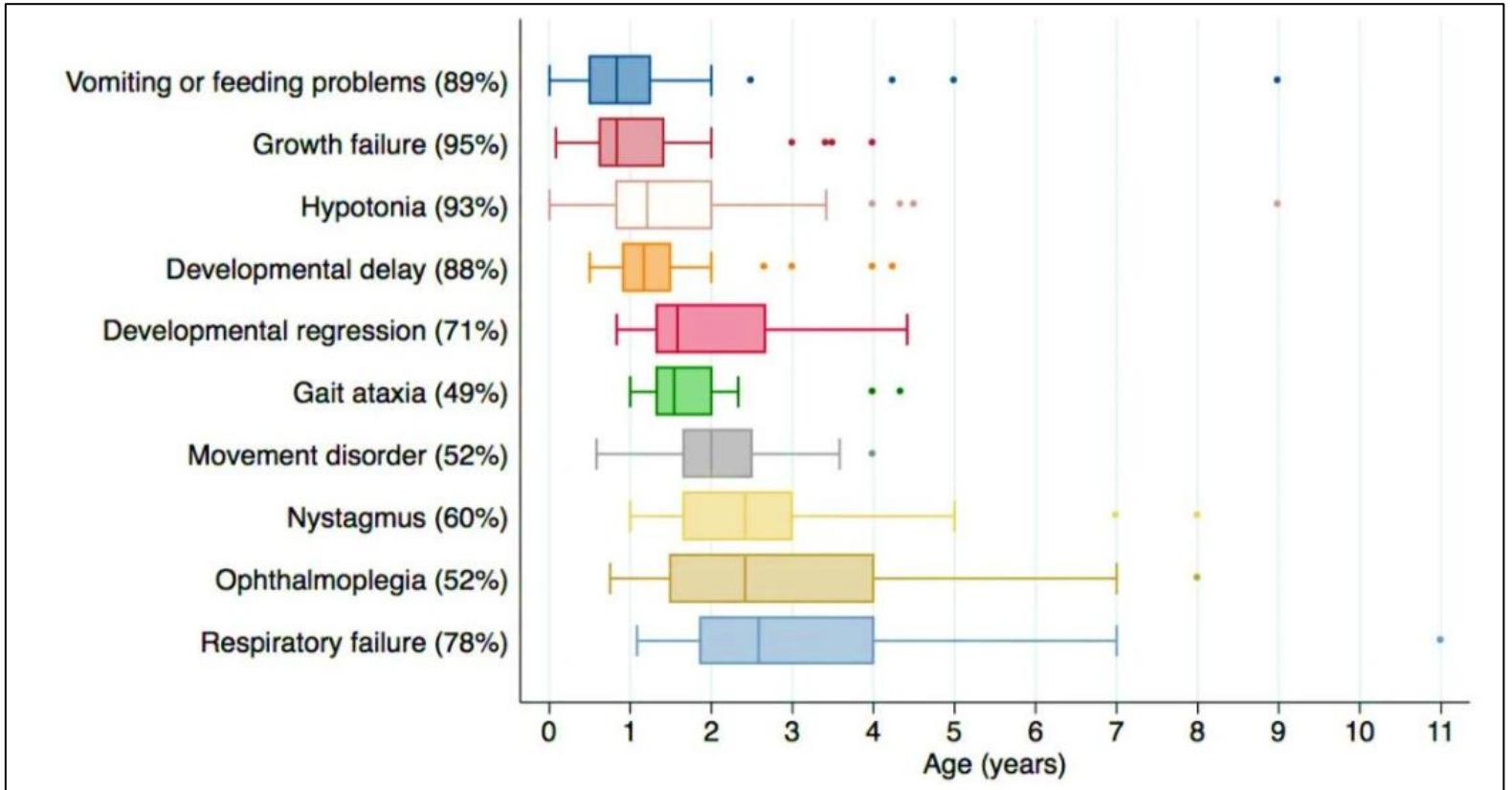
Initial symptoms in 44 patients with SURF1 deficiency

Initial symptoms	Number of patients (%)	Age range of initial presentation (months)
poor feeding/vomiting	20 (46)	0-24
poor weight gain	19 (43)	1.5-20
developmental delay	10 (23)	9-51
hypotonia	9 (21)	0-10
movement disorder	3 (7)	10-24
developmental regression	3 (7)	10-18
ataxia	2 (5)	14-60

Wedatilake et al. *Orphanet J Rare Dis* 2013

SURF1 deficiency natural history study – Major clinical features

- Symptoms occurring in ~80%+: vomiting / feeding problems, growth failure, hypotonia, developmental delay and respiratory failure.
- Median time to onset of most symptoms was 1-2 years

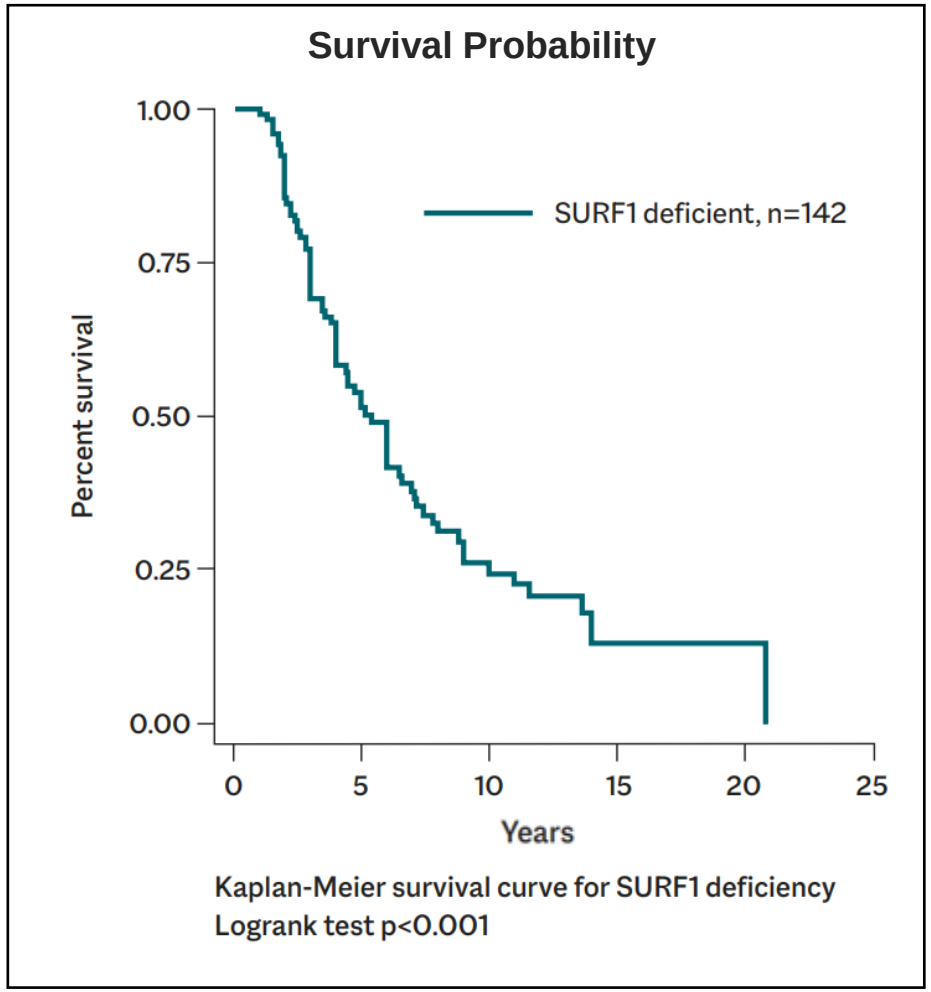


Clinical features in 44 patients with SURF1 deficiency. The x axis indicates the age of onset (years) and the y axis indicates the clinical features. Percentages denote the proportion of patients with a given clinical feature, and box-and-whisker plots show the age of onset. The median age of onset is indicated by the vertical line within the boxes. Boxes represent upper and lower quartiles, whiskers represent extreme values, and dots represent outliers which are ≥1.5 times the interquartile range from the median. Other less commonly observed features included hypertrichosis (41%), optic atrophy (23%), encephalopathy (20%), seizures (14%) and cardiomyopathy (2%).

Wedatilake et al. *Orphanet J Rare Dis* 2013

SURF1 deficiency natural history study – Survival

- Among 44 patients with detailed clinical data, 5 were alive at time of writing (ages 2–19 years); current vital status was unknown for 3
- Of the 36 deceased patients with known cause of death, the **cause was central respiratory failure in 29/36 (80%)**
- Seven patients survived beyond 10 years of age
  - Of these, 6 had neurological symptoms such as ataxia and motor developmental delay; of note, GI symptoms were not the prominent presenting feature in these cases. Furthermore, **these six patients also did not experience developmental regression**
- Literature searches identified 98 SURF1-deficient cases with available survival data, which were pooled together with the data from the 44 cases. The Kaplan-Meier analysis compares the survival experience of 142 SURF1-deficient cases to two other groups with LS due to nuclear gene mutations (56 with LRPPRC deficiency and 63 with nuclear-encoded complex I-deficient LS/“Leigh- like” disease)
- **Median survival length for SURF1 deficiency was 5.4 years (25th centile 3.0, 75th centile 10 years)**

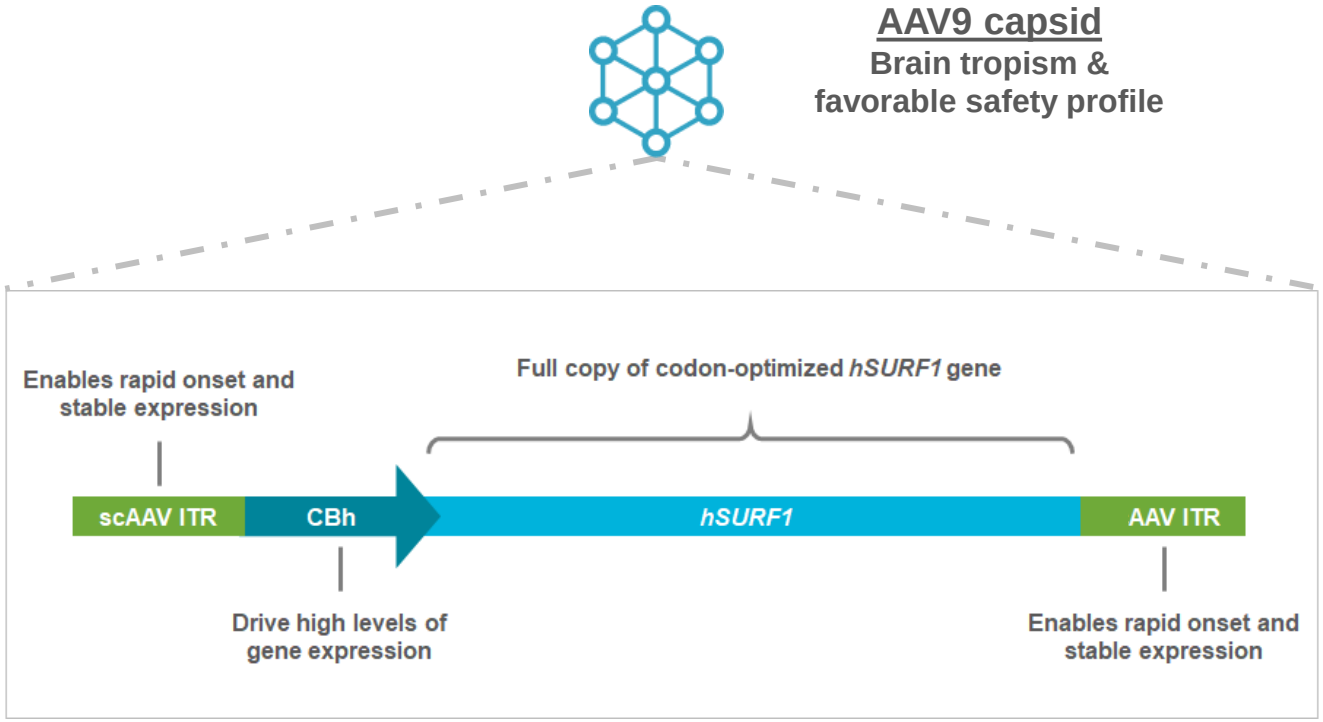


Kaplan-Meier survival curve for SURF1 deficiency
Logrank test p<0.001

Wedatilake et al. *Orphanet J Rare Dis* 2013

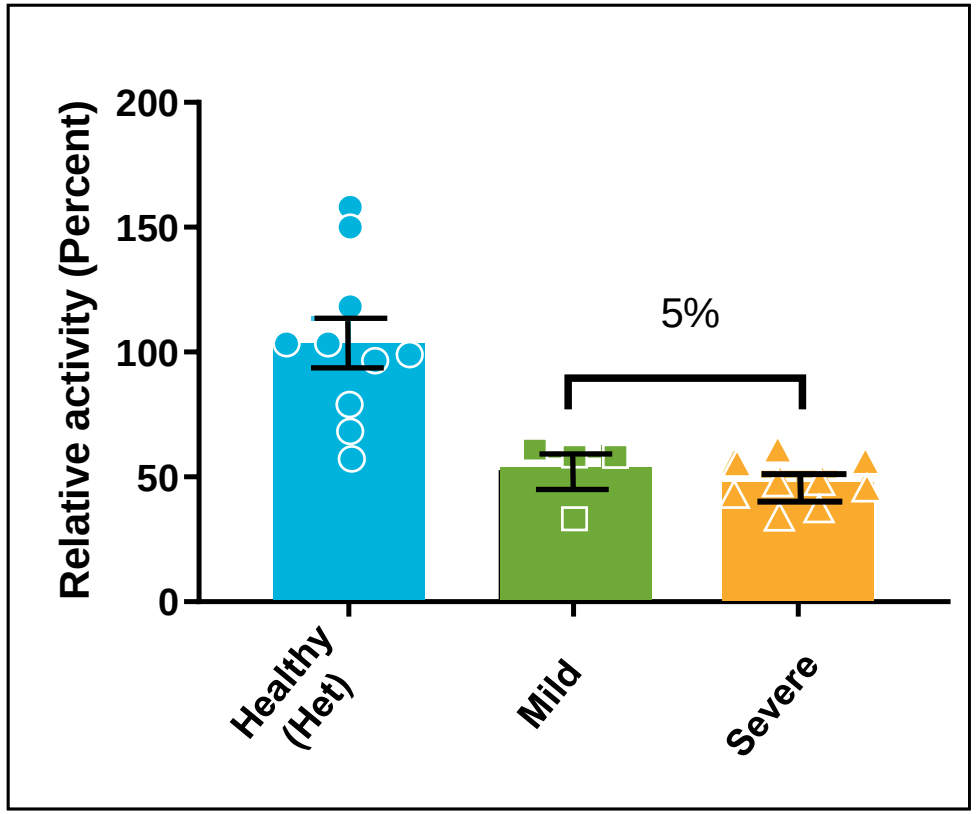
TSHA-104 to deliver functional copy of *SURF1* gene

- Recombinant AAV9 viral vector with engineered transgene encoding the human SURF1 protein
- Designed to deliver a functional copy of the *SURF1* gene
- Received orphan drug and rare pediatric disease designations

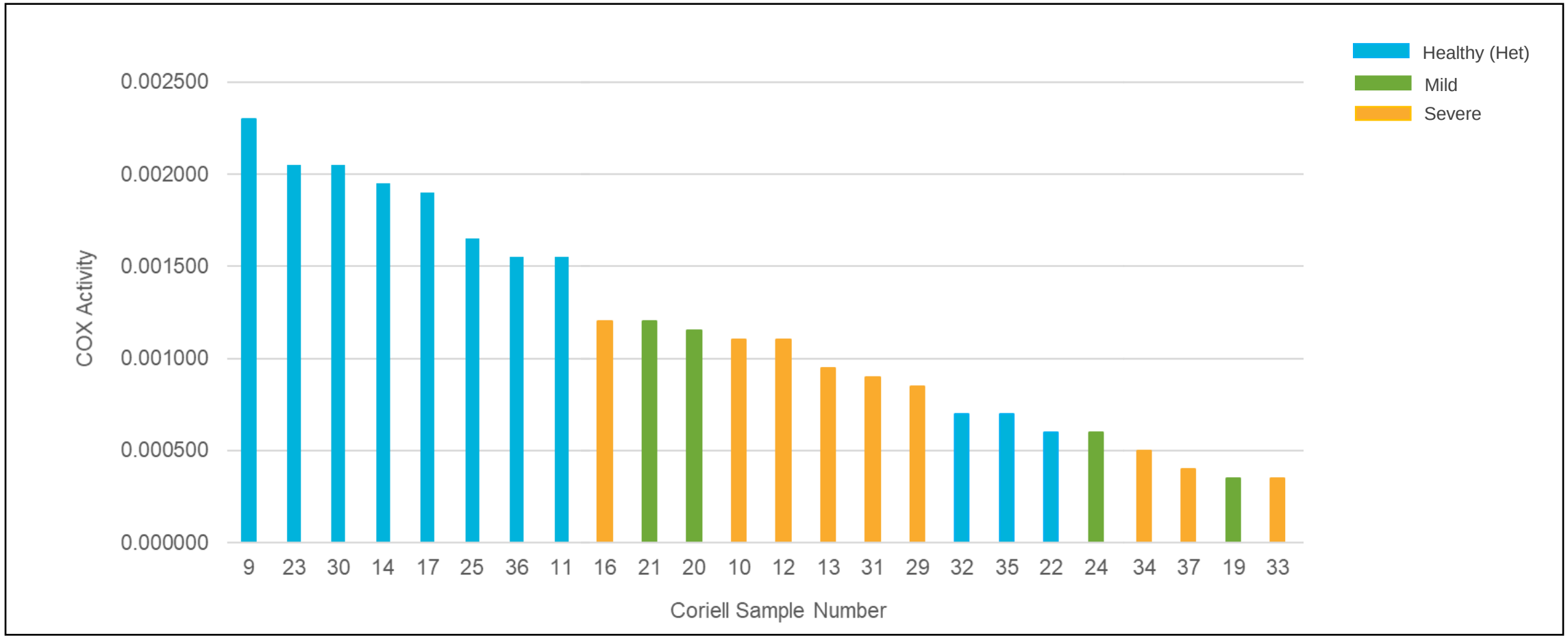


Slight increase in COX1 activity significantly improved clinical phenotype

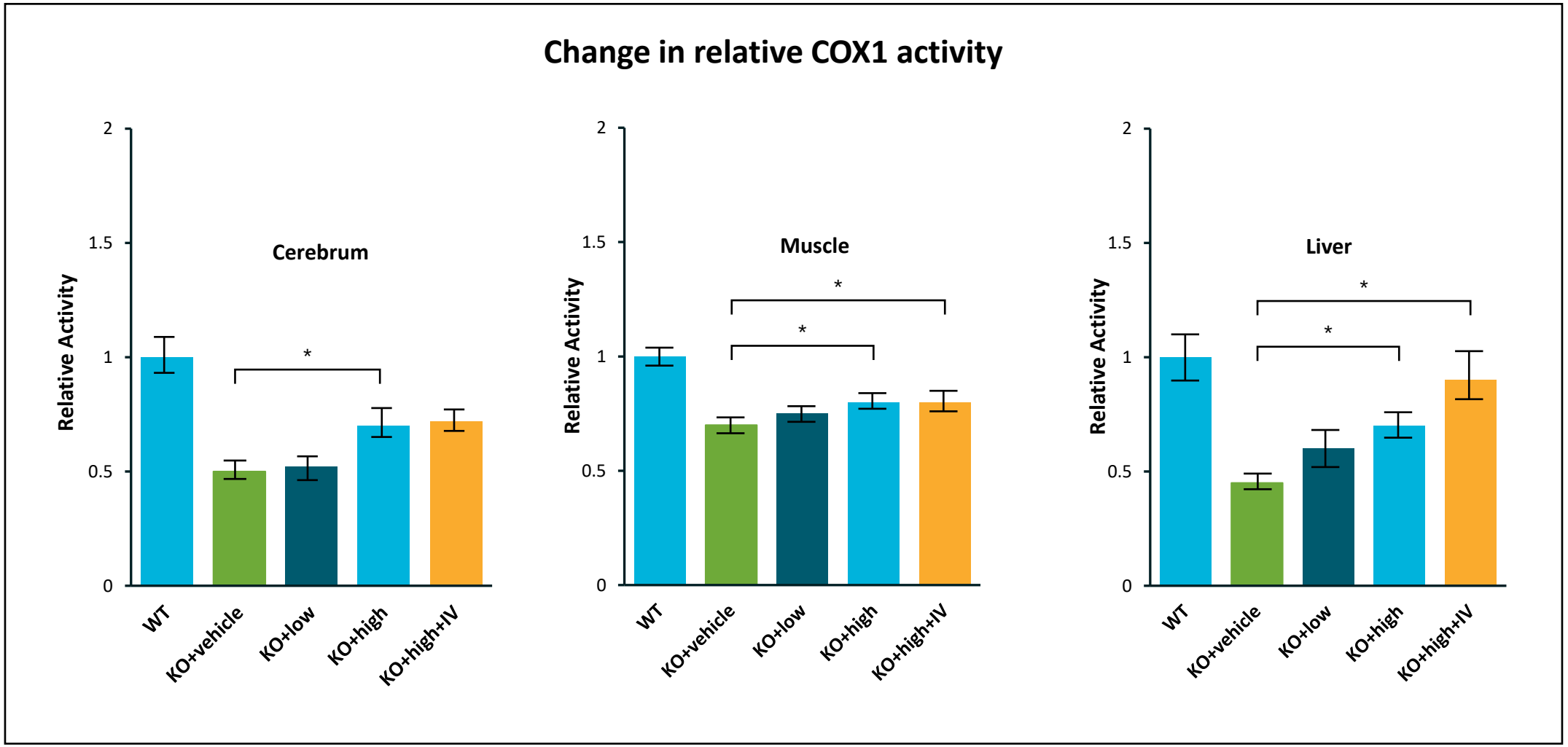
- Of 17 samples, 14 were assayed
  - 8 Leigh syndrome & 6 healthy donors (hets)
  - Severely affected individuals had roughly 50% of normal activity
  - Mildly affected individuals had roughly 55% of normal activity
- A relatively small difference in activity could have significant clinical consequences
- Other studies measuring COX activity have shown roughly 20% of normal activity for affected individuals
- Potential reason – “healthy” donors are heterozygote parents of affected children; this and their age may affect their COX activity. In other studies, healthy children were used for reference



Reduction in COX activity correlated with disease worsening – Patient fibroblast data

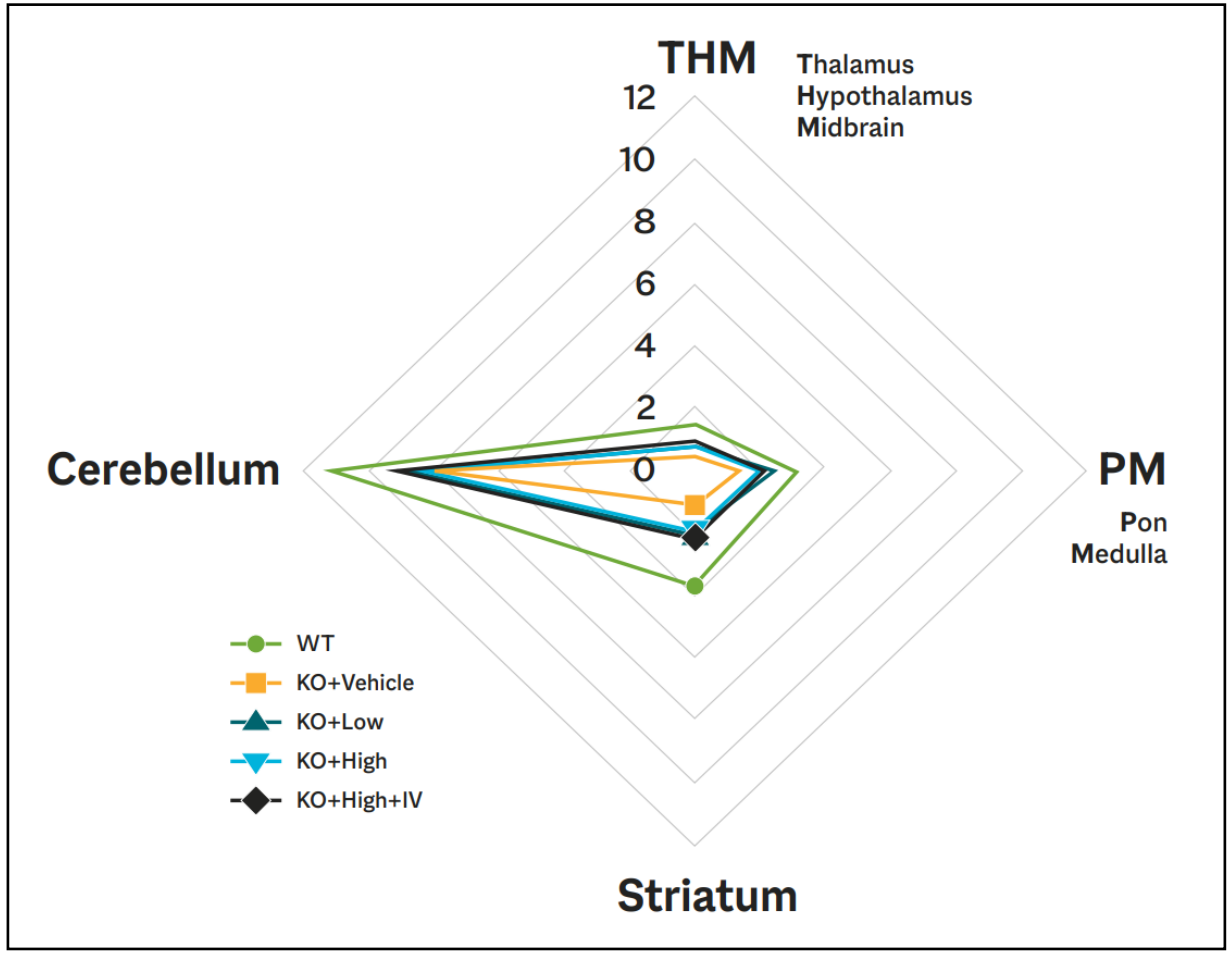


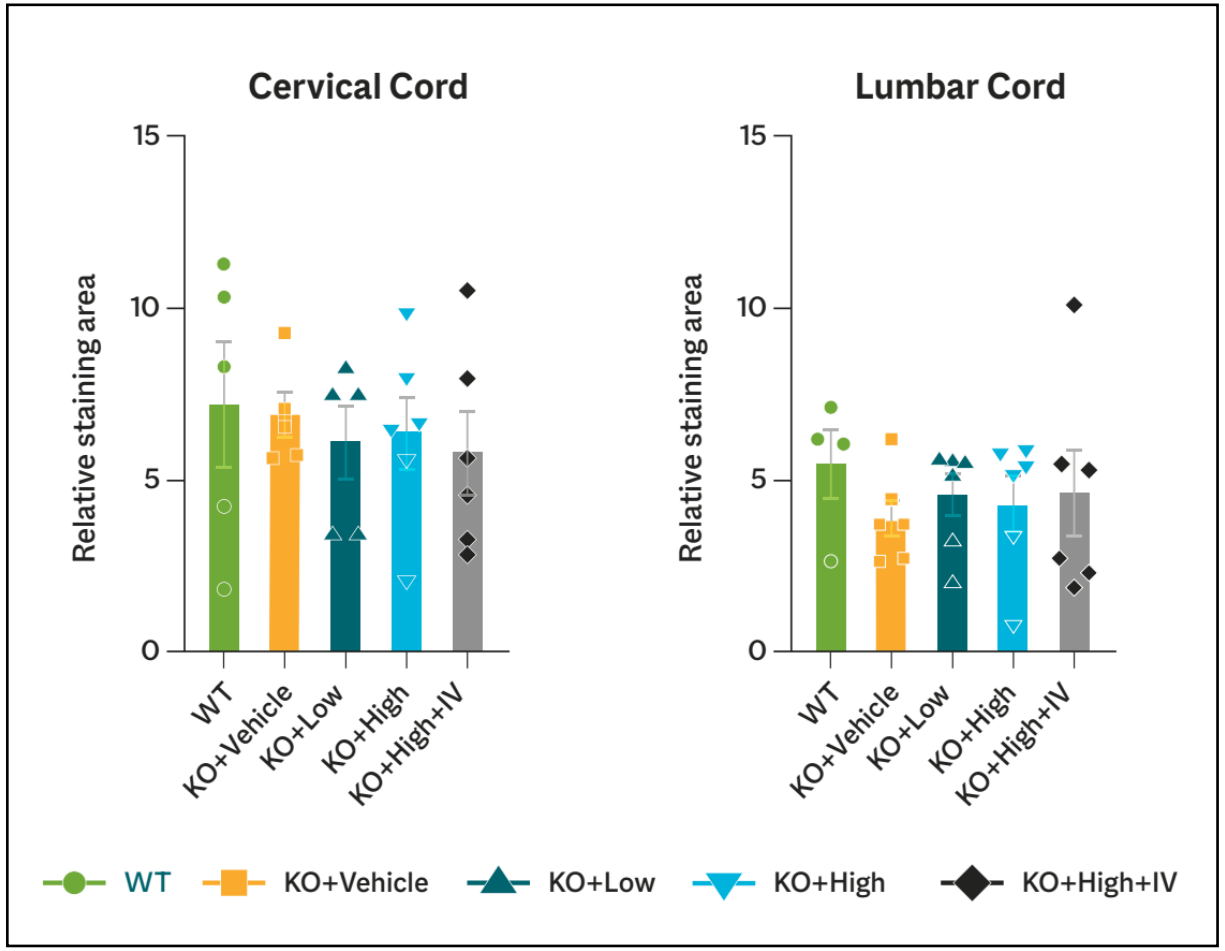
TSHA-104 increased COX1 activity in brain and muscle in dose-dependent manner in SURF1 KO mice



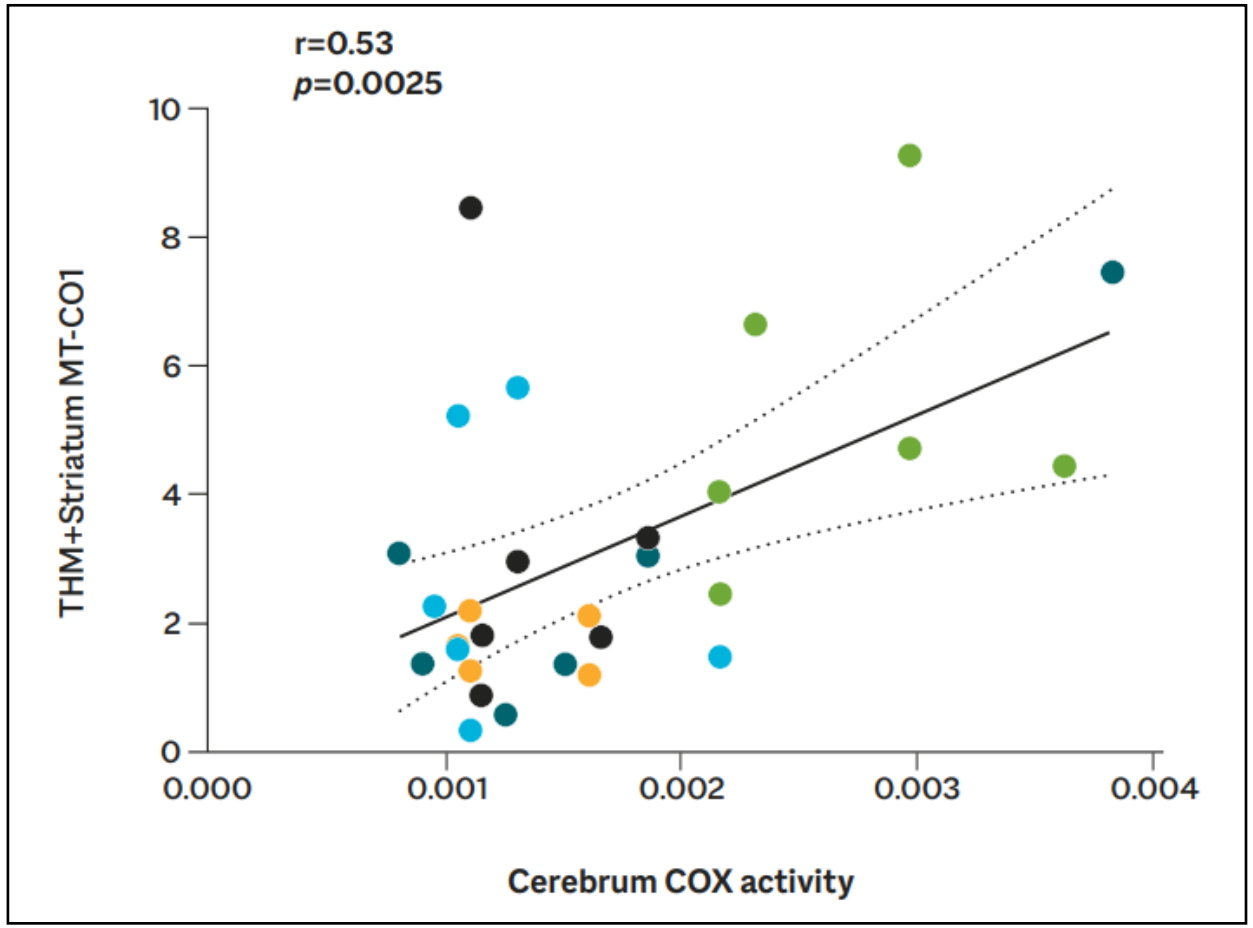
Ling Q et al. *ASGCT* 2020

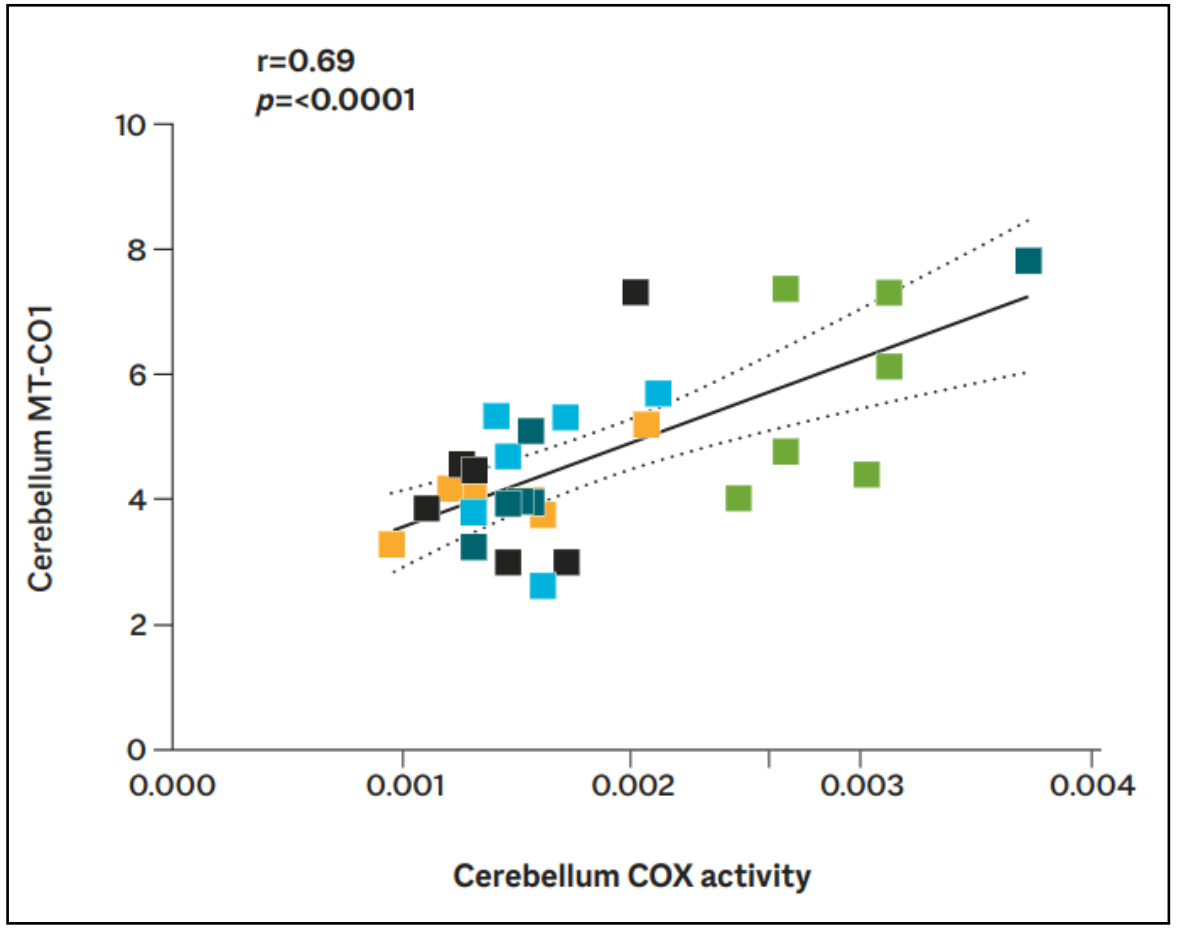
Improvement in MT-CO1 abundance 4 weeks post-injection



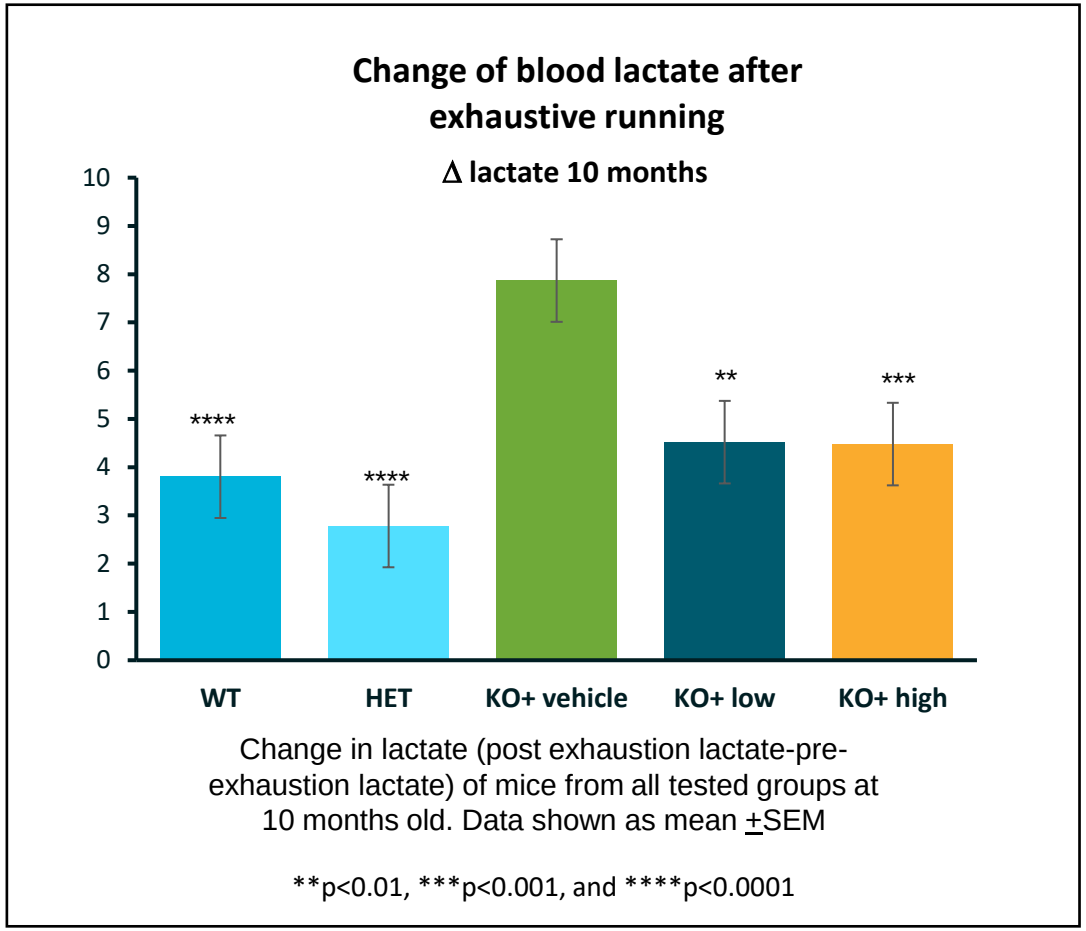


Biochemical COX activity correlated with histological COX content level





TSHA-104 restored elevation of blood lactate on exhaustive exercise in dose-dependent manner in SURF1 KO mice

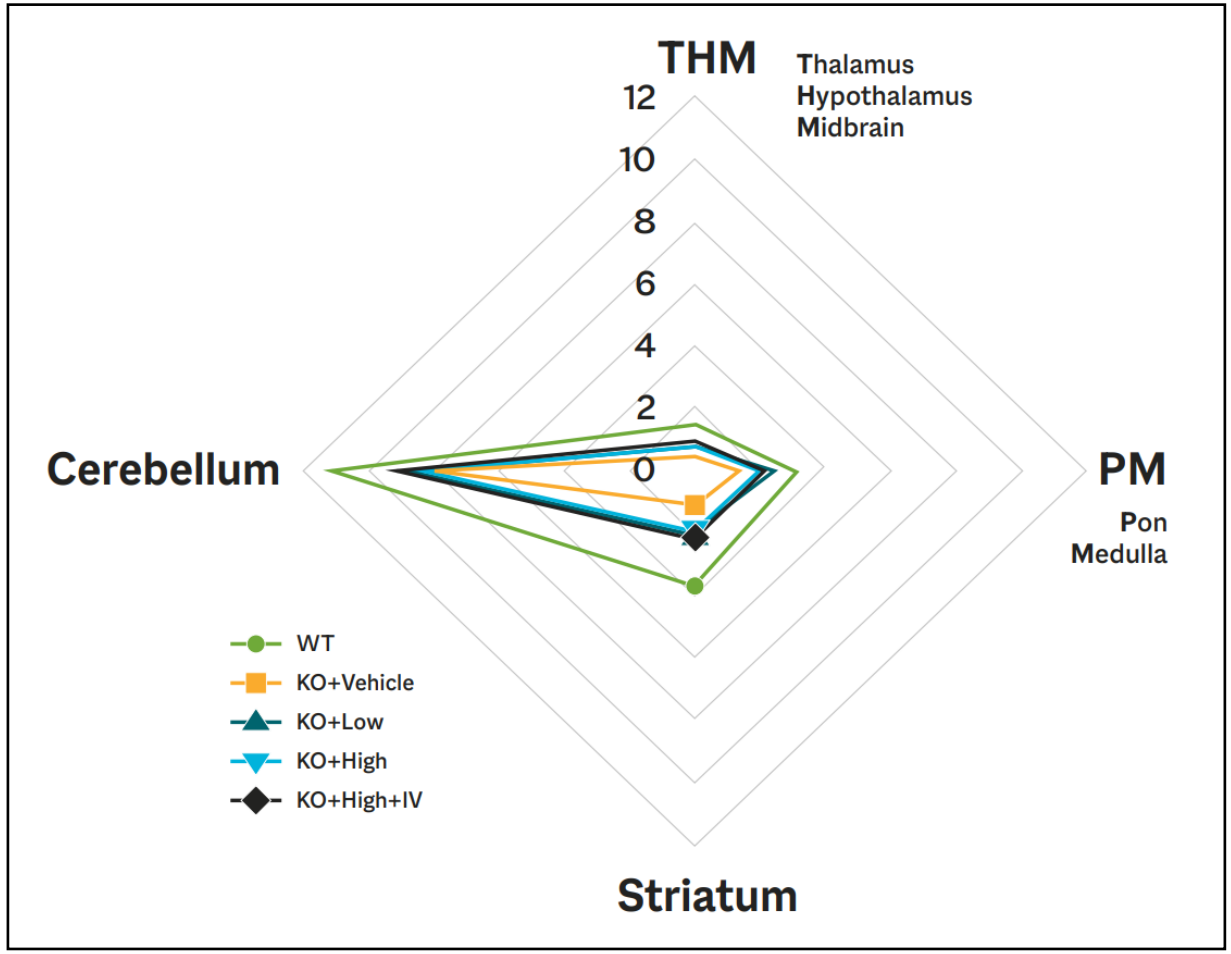


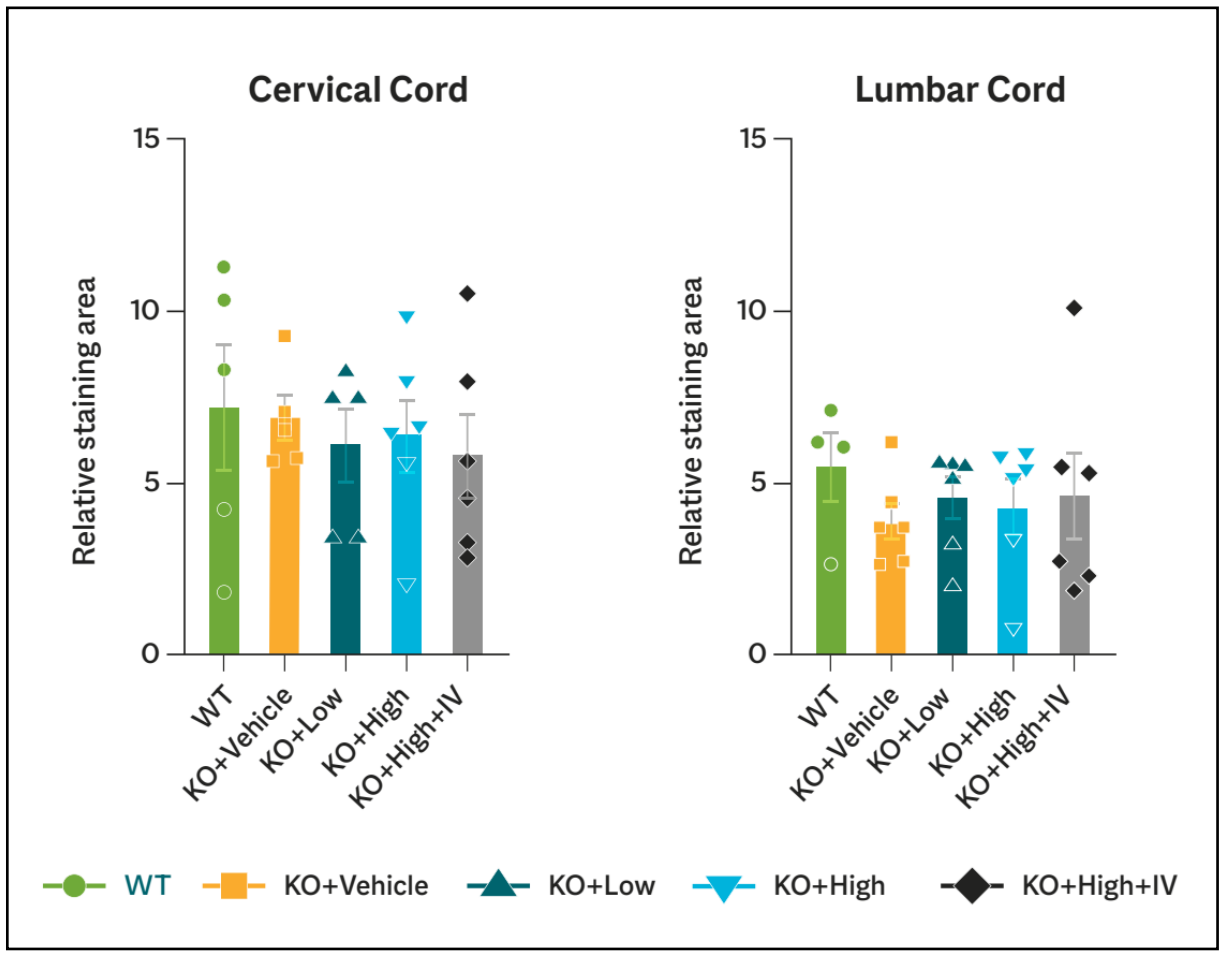
Change in lactate (post exhaustion lactate-pre-exhaustion lactate) of mice from all tested groups at 10 months old. Data shown as mean ±SEM

**p<0.01, ***p<0.001, and ****p<0.0001

Ling Q et al. *ASGCT* 2020

Improvement in MT-CO1 abundance 4 weeks post-injection





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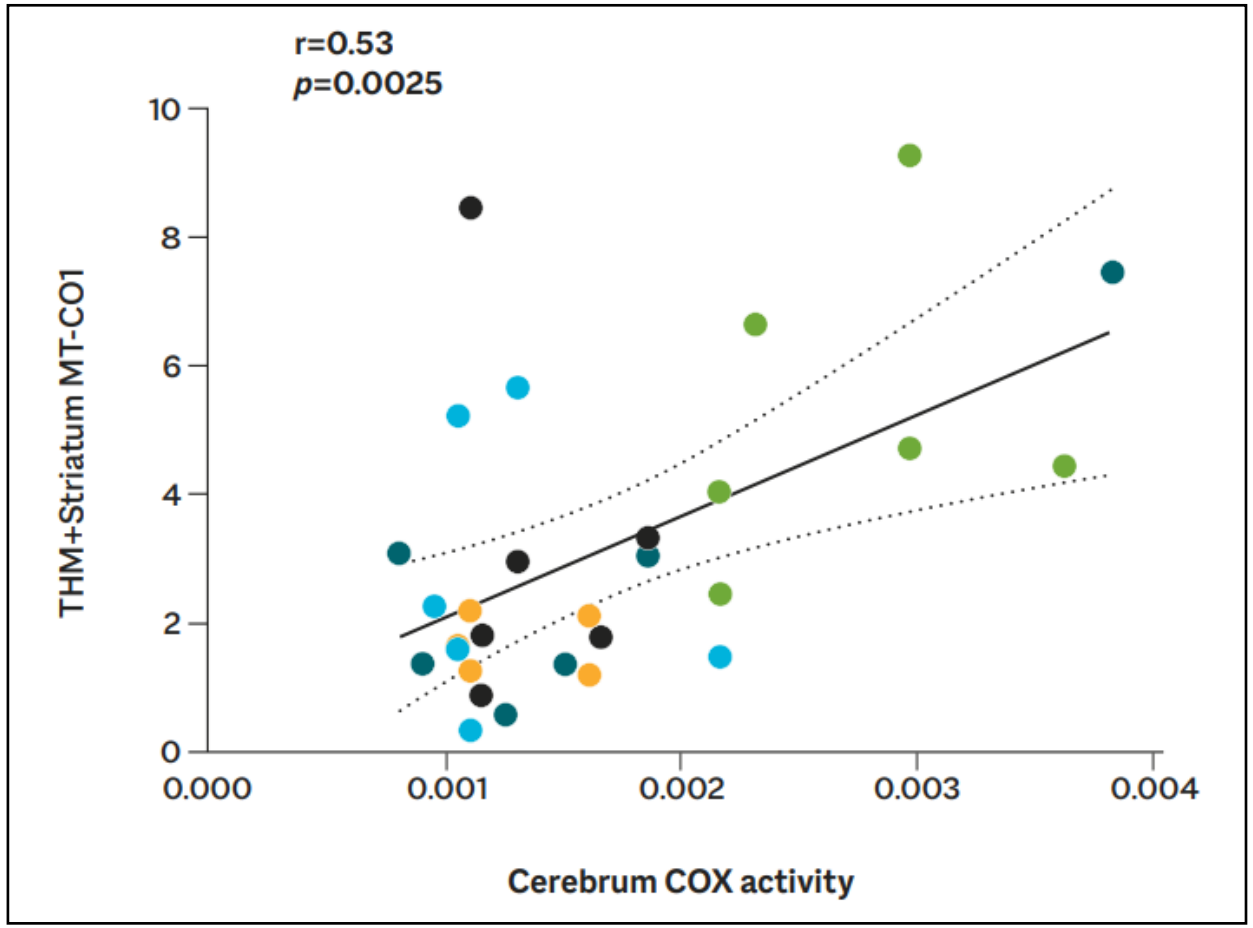
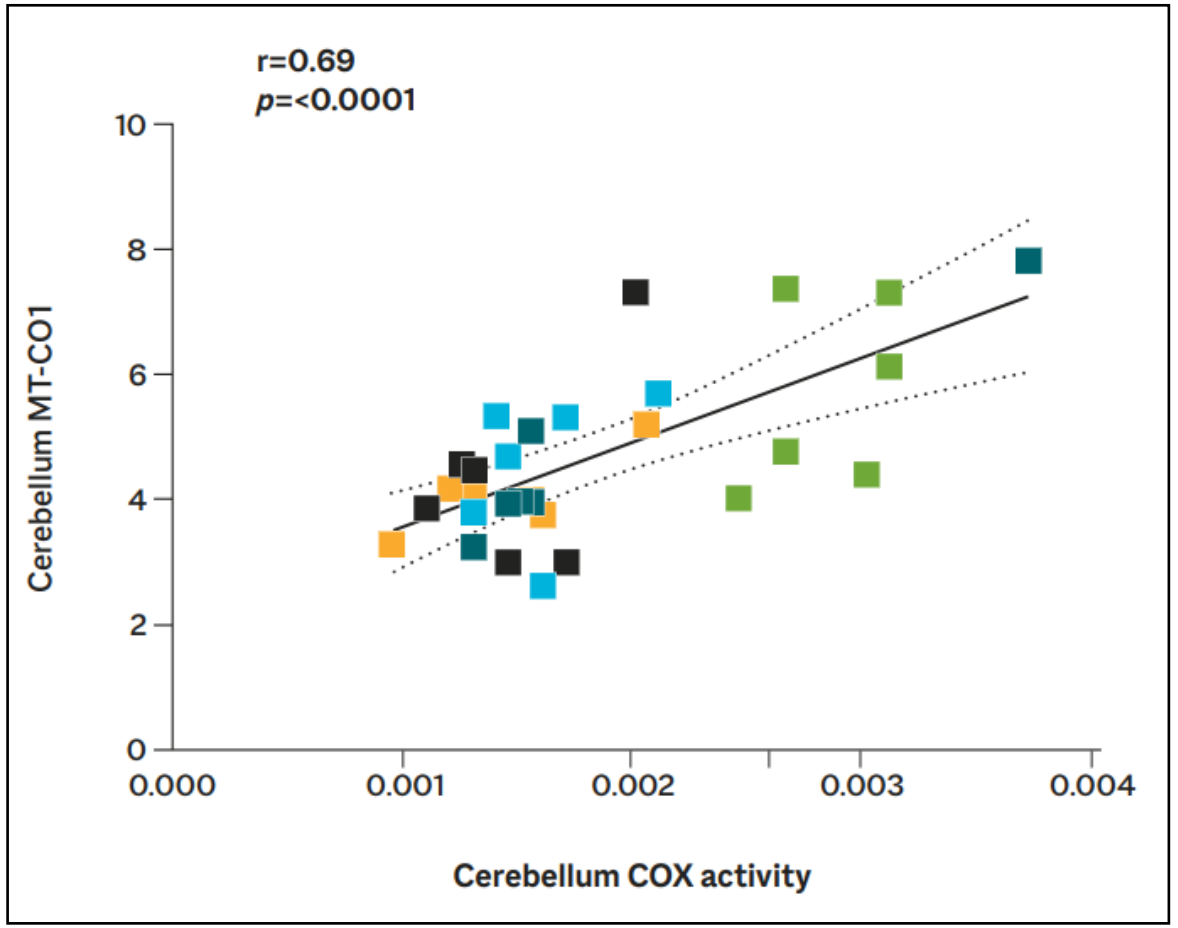




TSHA-104 Phase 1/2 study design plan

Goals and Targets of Trial

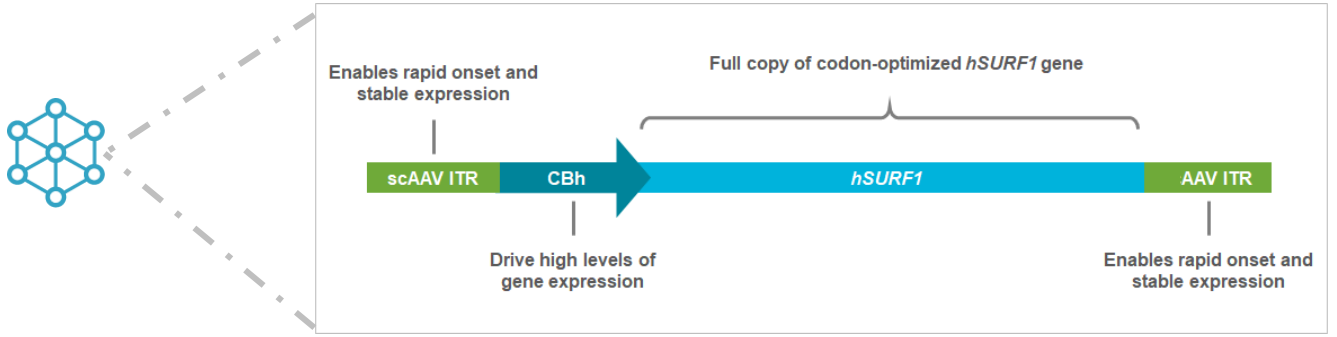
Goals

- **Primary** – Safety: clinical and laboratory assessments
- **Secondary** – Efficacy: pathologic, physiologic, functional and clinical markers

Target Recruitment

- Patients will roll in from ongoing prospective natural history study
- Up to 12 subjects, ages 1-18 years old
- Pathogenic confirmation of mutation in SURF1 gene
- All cohorts will be open for accrual concurrently

Product Details and Dose Cohorts



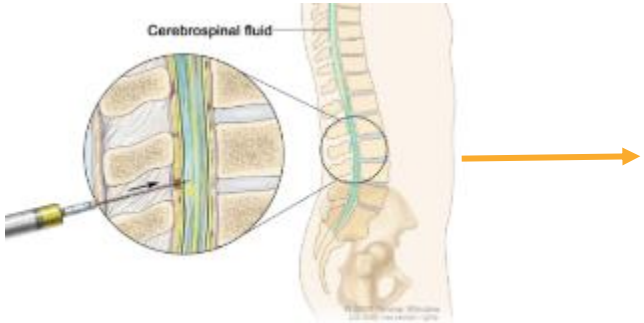
Dose Cohorts

- Participants below the age of 4 years will receive a calculated dose based on brain volume
- 5 x 10^{14} total vg (n=4)

Route and Method of Administration

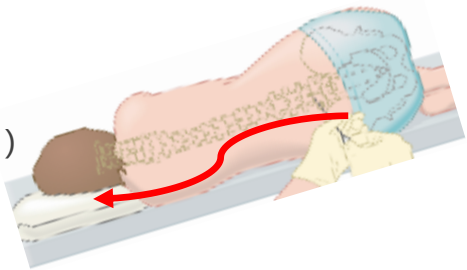
Administration

- Lumbar Intrathecal Infusion (IT)
- Amount and rate: 1 mL/min for total of 10-12 mL
- Immunosuppression regimen of prednisolone and sirolimus



Technique to Improve Transduction

- Trendelenburg position (15-30º)
- During infusion & 1 hour post infusion



TSHA-104 study clinical assessments

Disease-Specific/Global Assessments
- Newcastle Paediatric Mitochondrial Disease Scale (NPMDS)
- Gross Motor Function Measure (GMFM)
- Swallowing / Dysphagia Assessment
- Seizure Diary
- 100-Meter Walk Test
- Pediatric Balance Scale (PBS)
- Head Control Scale
- Scale for the Assessment and Rating of Ataxia (SARA)
- Vineland-3
- Bayley-III / WPPSI-IV / WISC-IV

Communication Assessments
- Expressive and Receptive One-Word Picture Vocabulary Test (ROWPVT-4, EOWPVT-4)
- Observer-Reported Communication Ability (ORCA)

Biomarkers
- COX activity
- COX expression
- Lactate
- Pyruvate

Quality of Life/Other Assessment
- Quality of Life Inventory Disability (QI-D)
- Infant Toddler Quality of Life Questionnaire (ITQOL)
- PedsQL Scales and Family Impact Module
- Study Participant Feedback Questionnaire
- Clinical Global Impression Scale (CGI)

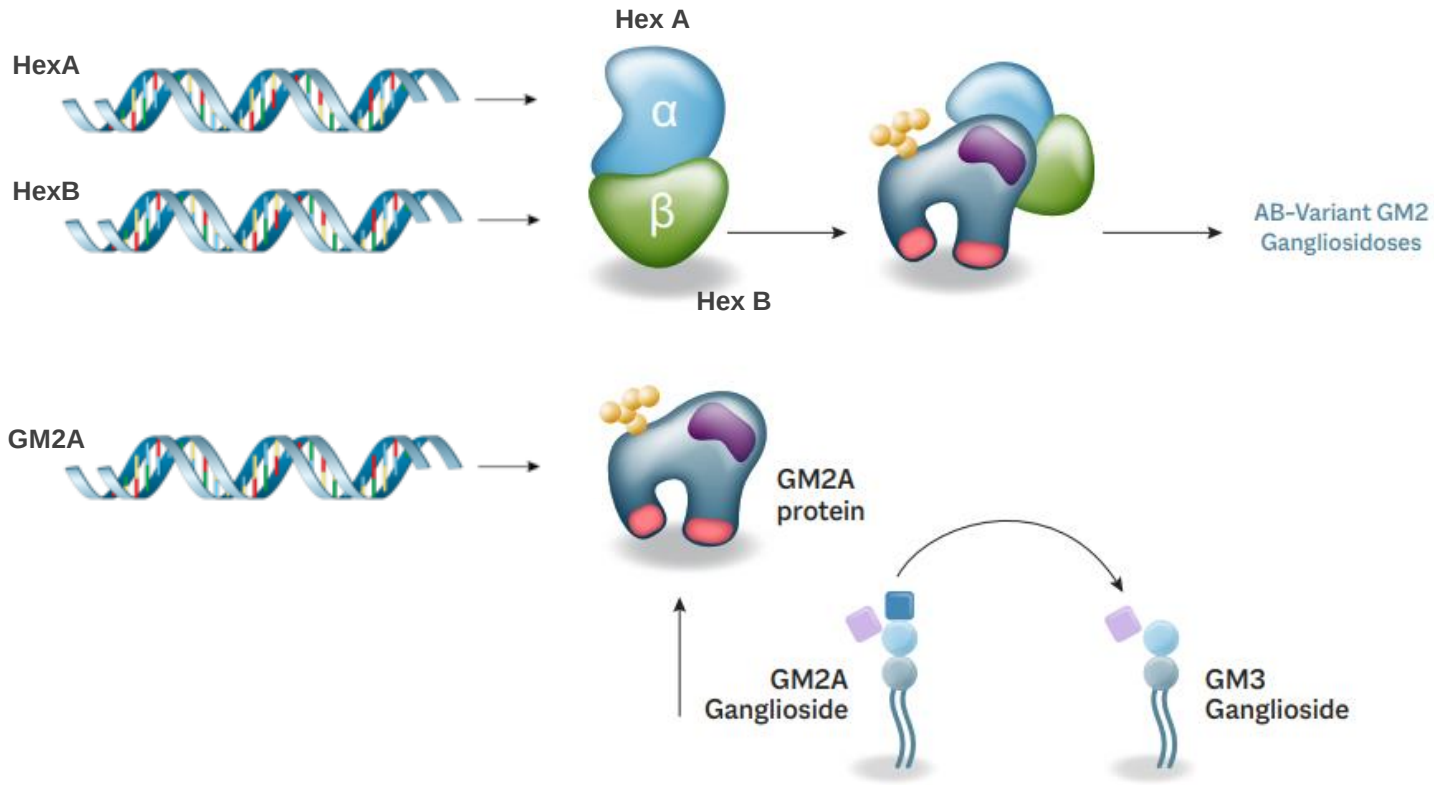
Neurological Assessment
- EEG
- MRI/MRS

Anticipated next steps for TSHA-104

- UTSW to complete IND-enabling toxicology study
- Continue enrollment in natural history study

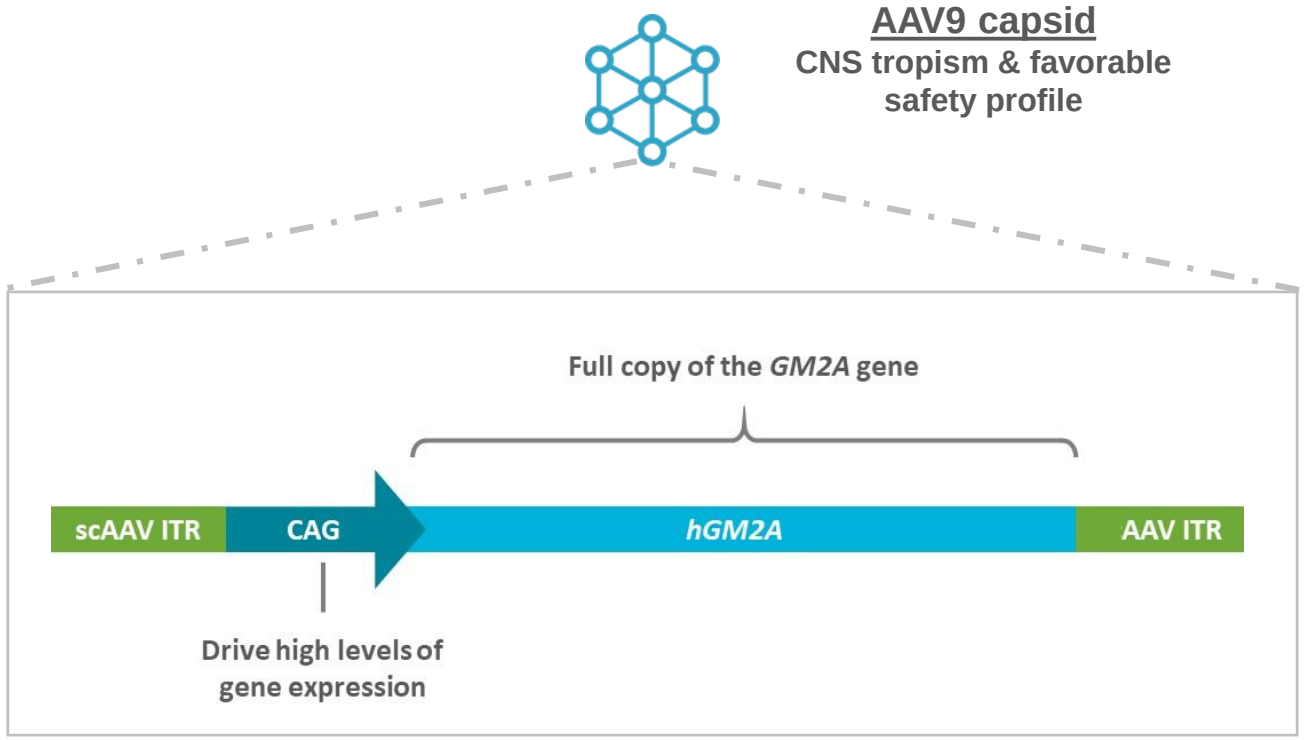
GM2 gangliosidosis, AB variant

- Characterized by a mutation in the GM2A gene, leading to a deficiency of the GM2-activator protein (GM2AP), a required co-factor for the breakdown of GM2-gangliosides by the protein Hex A
- Loss-of-function mutations result in a deficiency of GM2AP causing intra-lysosomal accumulation of GM2 and other glycolipids in neuronal cells ultimately resulting in cell death.
- Signs, symptoms and progression mirror that of infantile GM2, and include seizures, vision and hearing loss, intellectual disability and paralysis and early death
- No approved therapies

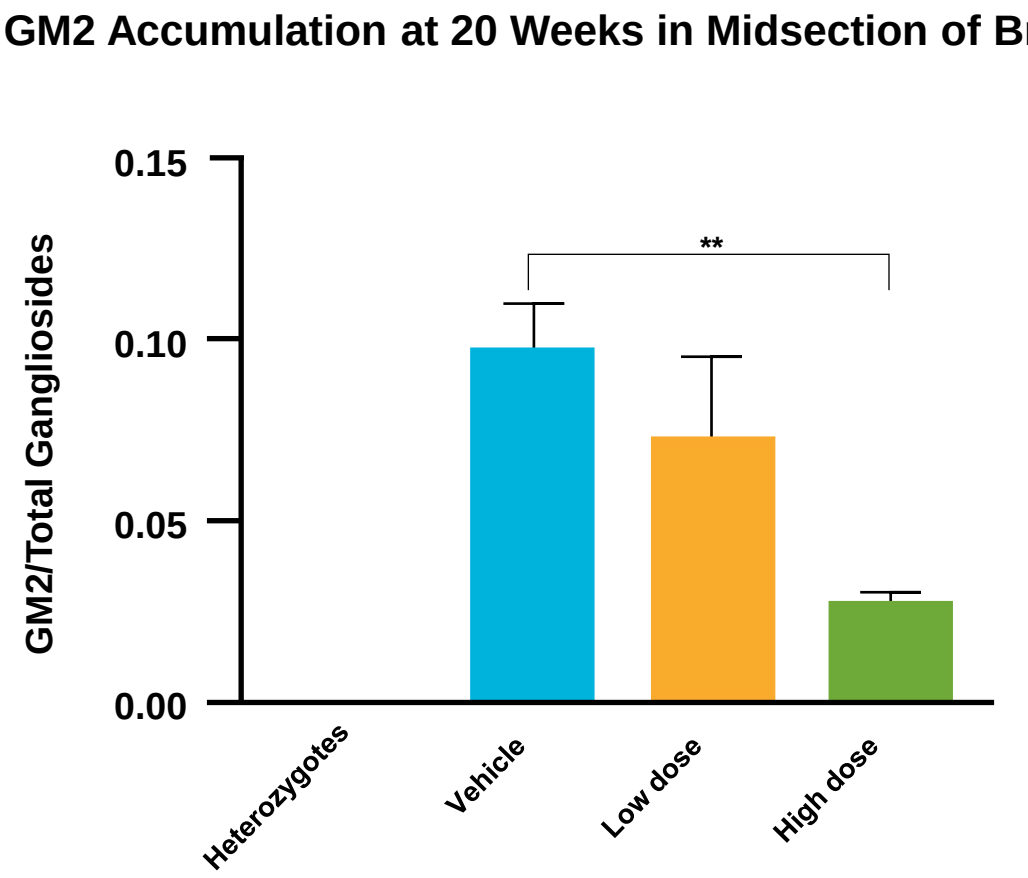


TSHA-119 in preclinical development

- Self-complementary AAV9 viral vector for rapid activation and stable expression
- Designed to deliver a functional copy of the GM2A gene
- CAG promoter drives high levels of expression
- Proof-of-concept demonstrated in GM2A KO mouse model
- Currently in IND/CTA-enabling studies

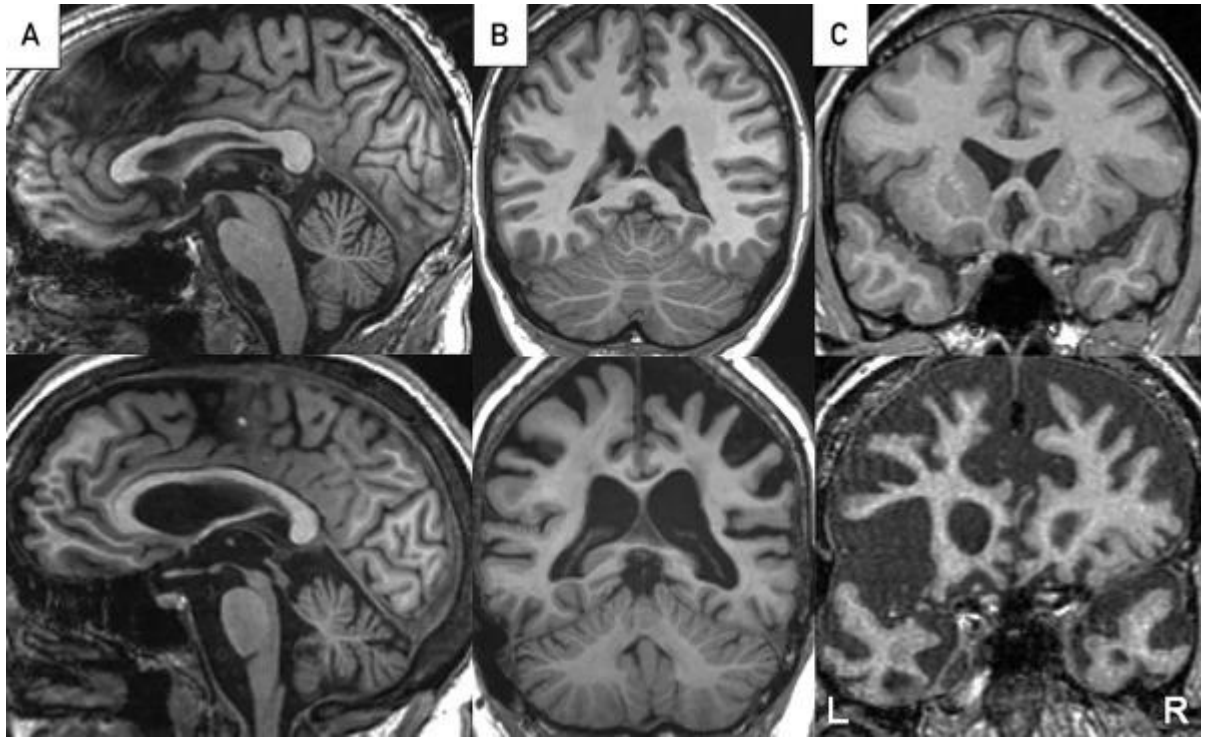


TSHA-119 caused a dose-dependent reduction of GM2 accumulation in mice



Tauopathies – Microtubule associated Protein Tau (MAPT)

- Tauopathies are characterized by the accumulation of toxic tau protein in the brain that results in widespread neuronal dysfunction and loss
- Tau accumulation is thought to underpin several neurodegenerative diseases, including Alzheimer's, frontotemporal dementia (FTD), progressive supranuclear palsy, corticobasal degeneration, chronic traumatic encephalopathy and parkinsonism linked to chromosome 17
- Tau isoforms are expressed in the central and peripheral nervous systems
- Estimated prevalence of 13,000 patients with MAPT-FTD, PSP, CBD in the US and EU
- Estimated 6.2 million Americans and 7.8 million Europeans are living with Alzheimer's disease

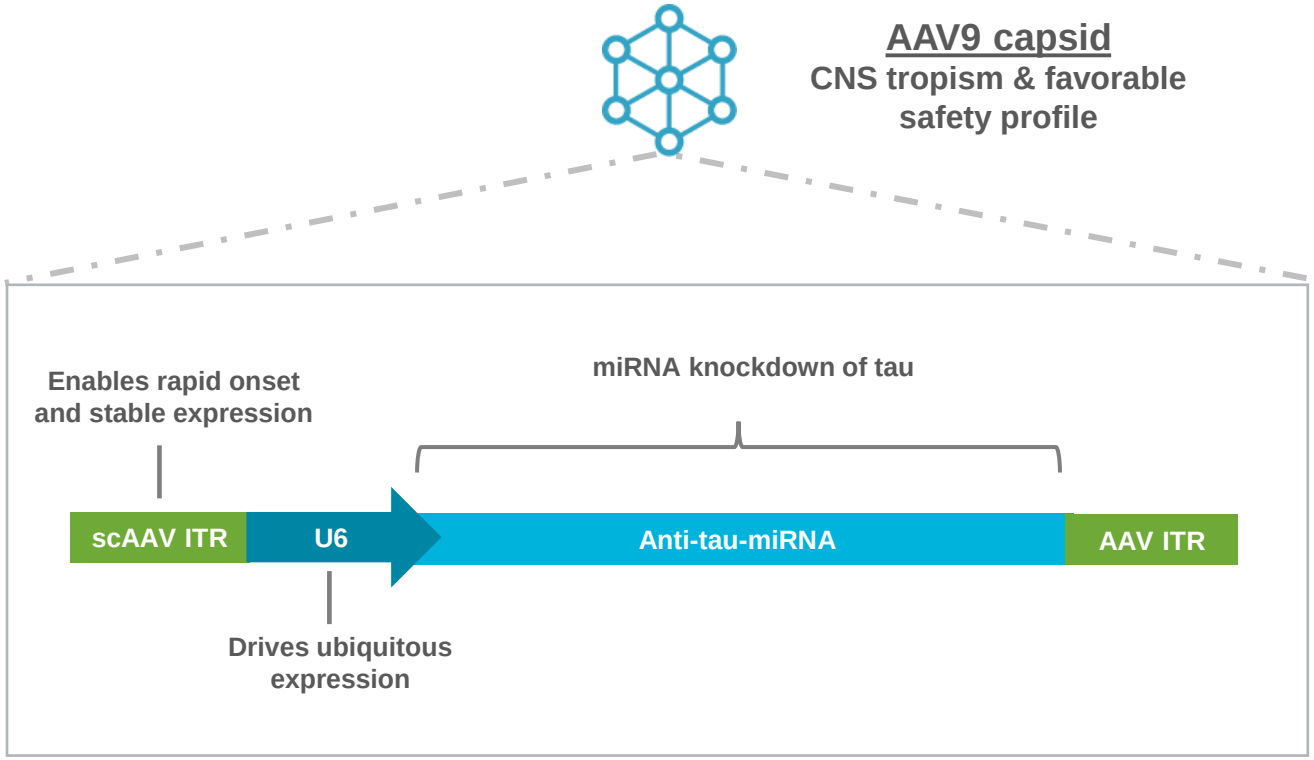


Mayo Clinic Proceedings 2017 921291-1303DOI: (10.1016/j.mayocp.2017.04.016)

FTD – frontotemporal dementia; PSP – progressive supranuclear palsy; CBD – corticobasal degeneration

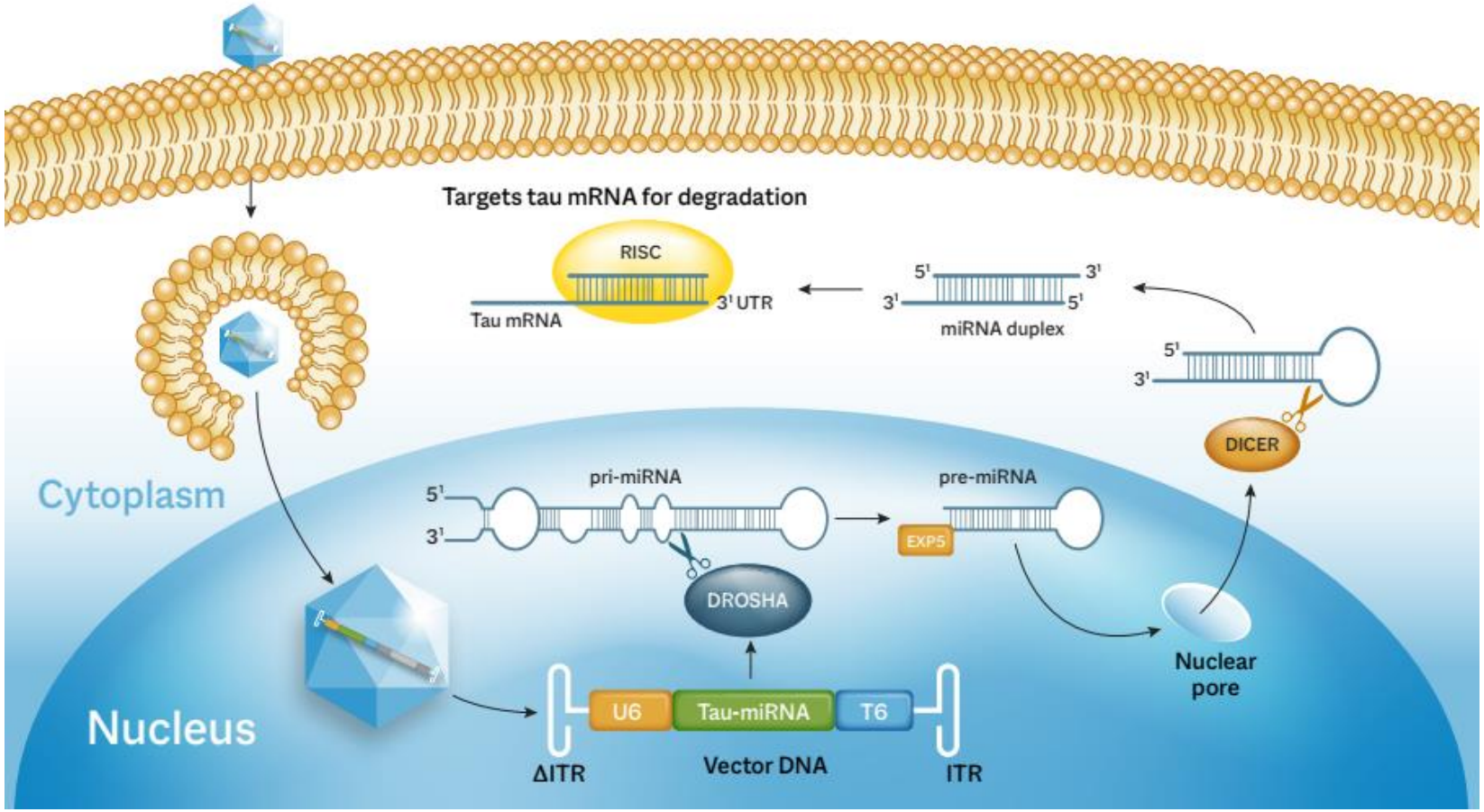
TSHA-113 in preclinical development

- Self-complementary AAV9 viral vector for rapid activation and stable expression
- Utilizes AAV-mediated gene silencing to deliver life-long reduction of tau protein levels in neurons following administration of a single dose
- U6 promoter drives ubiquitous expression
- Currently in preclinical development

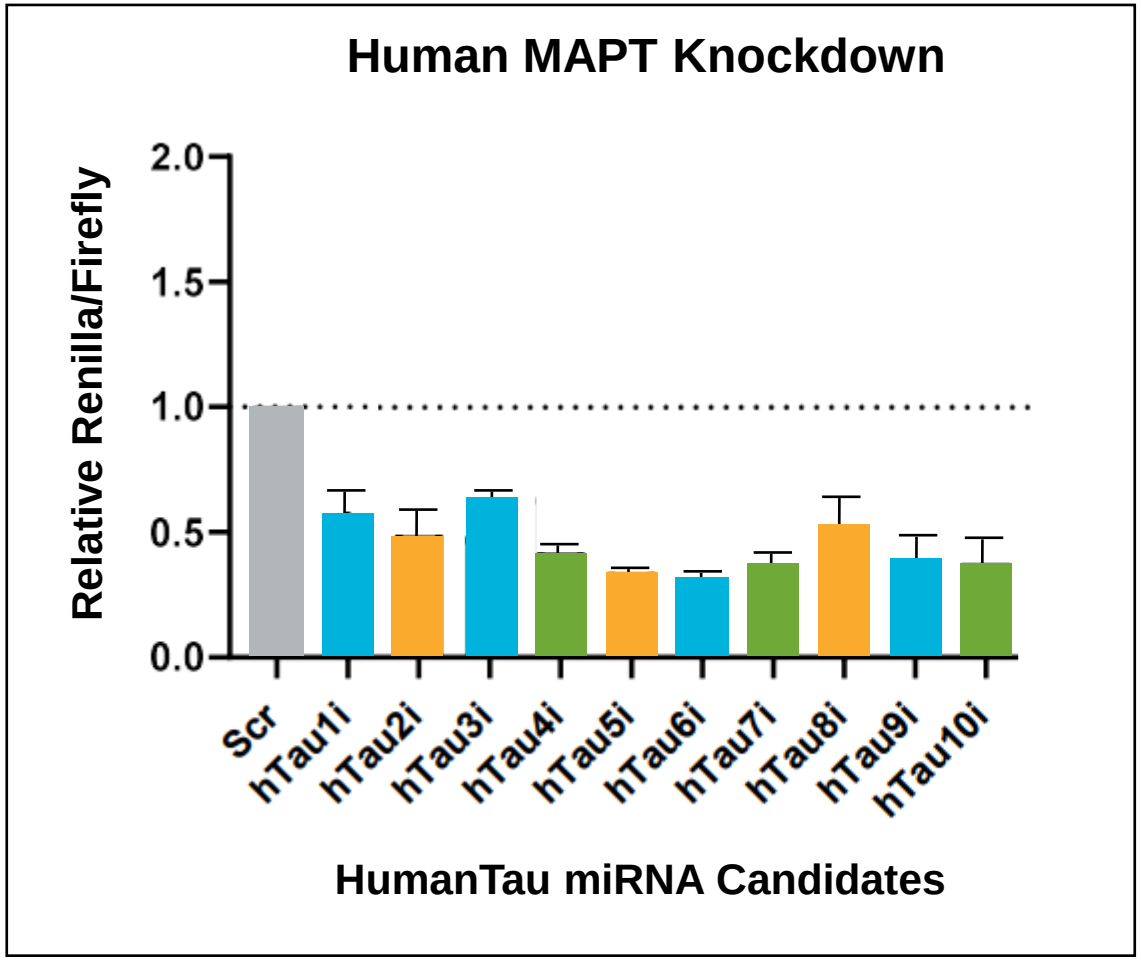


Our approach to treat MAPT

- We are employing tau-specific miRNA shuttles that have been designed to target mRNA for all six isoforms of tau found in the human brain and/or mouse brain



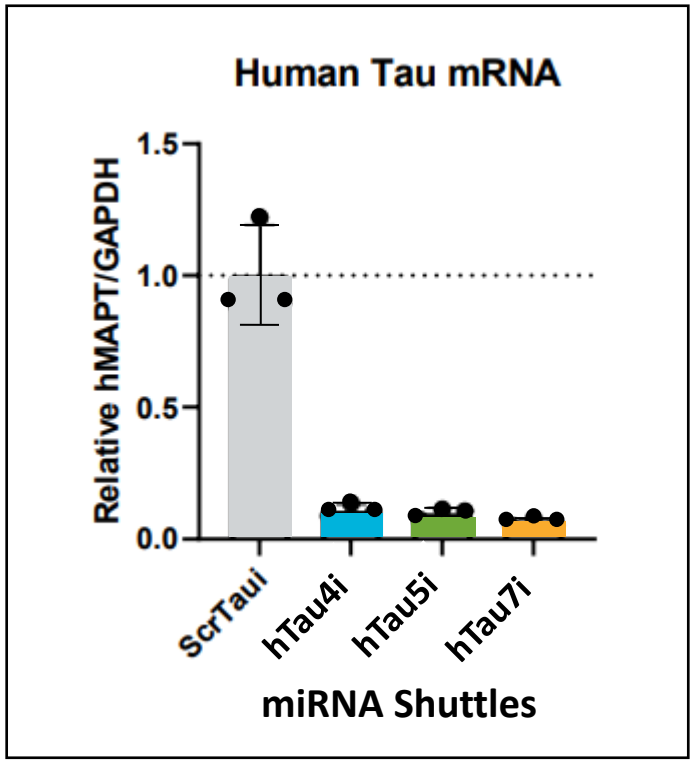
Primary screen of human tau miRNA candidates

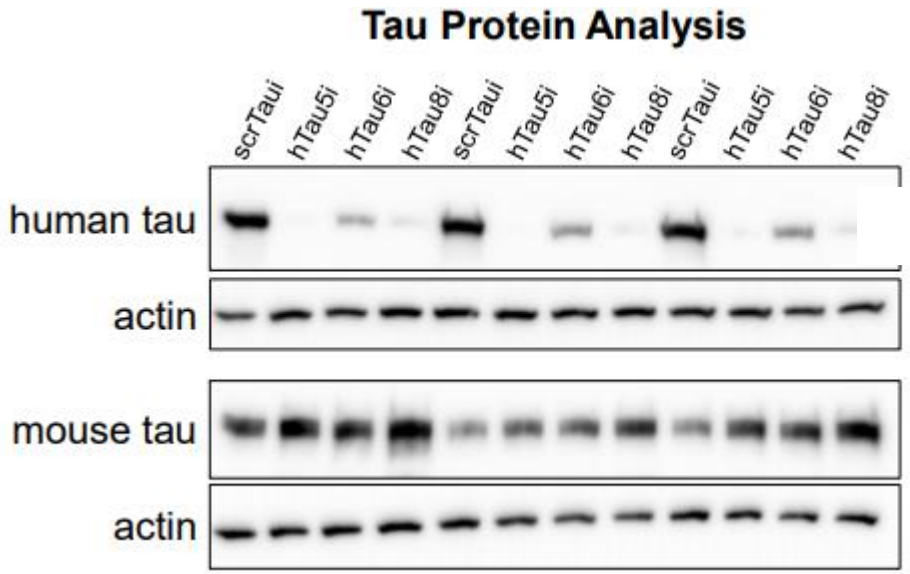


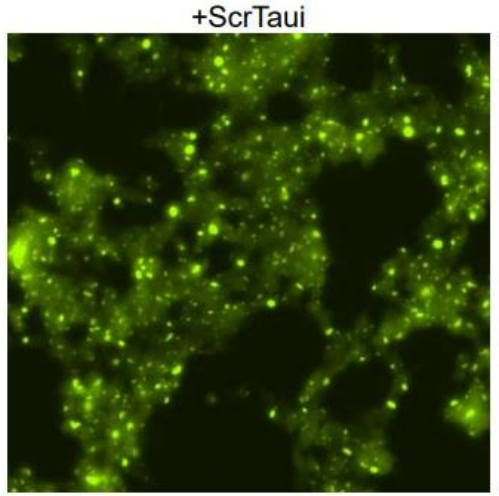
Secondary screening of top candidates:
hTau4i, hTau5i, and hTau7i

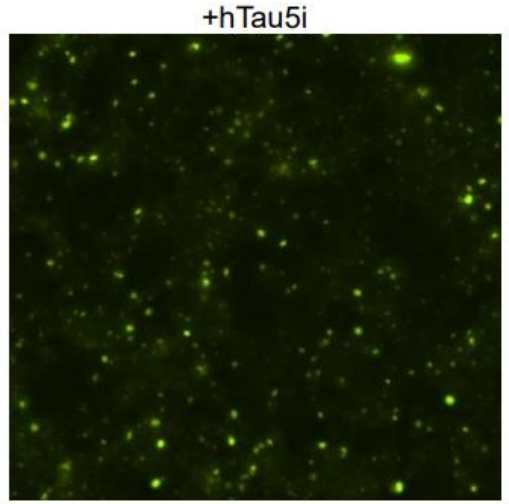
Scr = scrambled tau miRNA; hTau = human-specific tau miRNA

TSHA-113 reduced tau expression

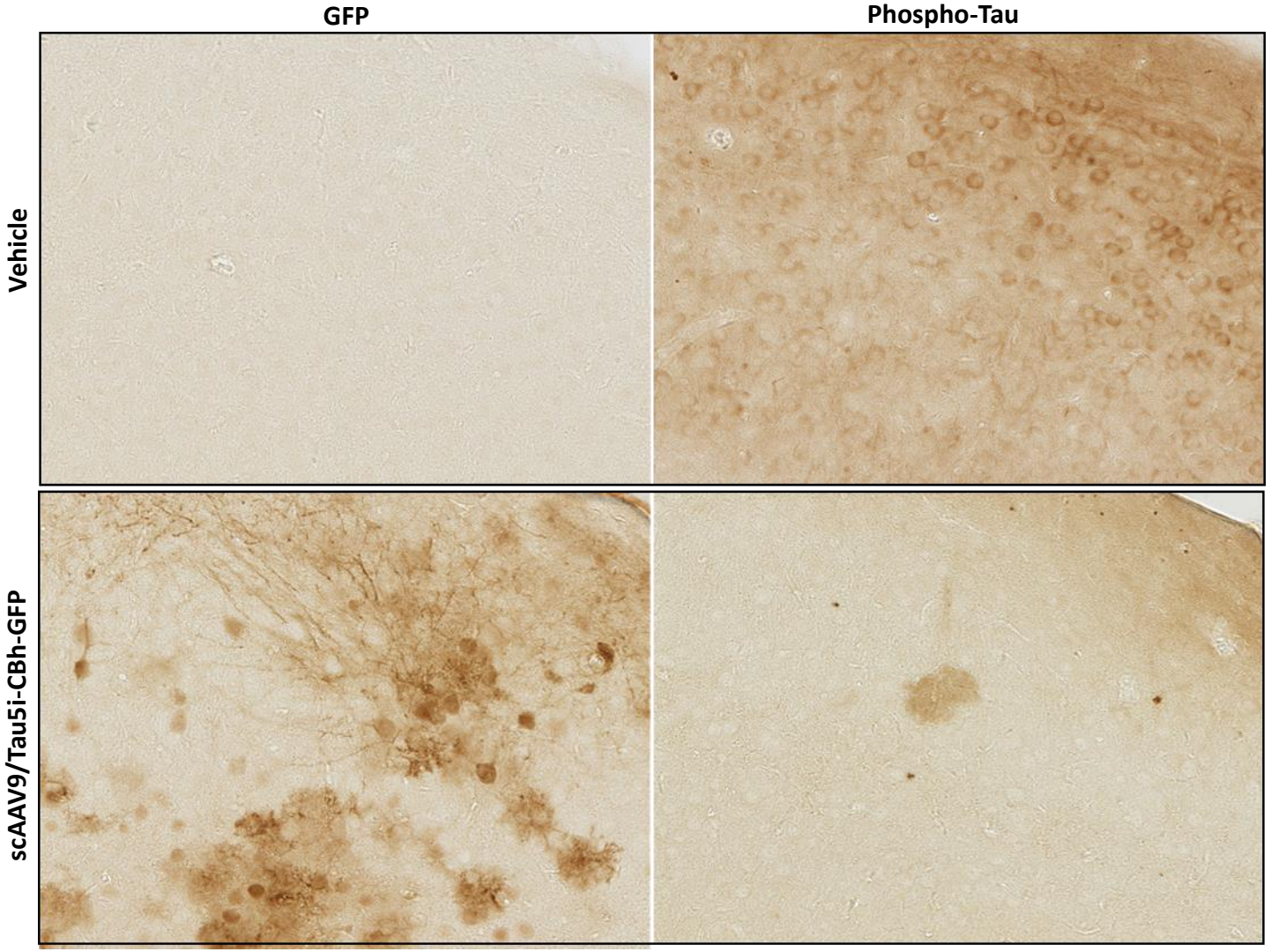


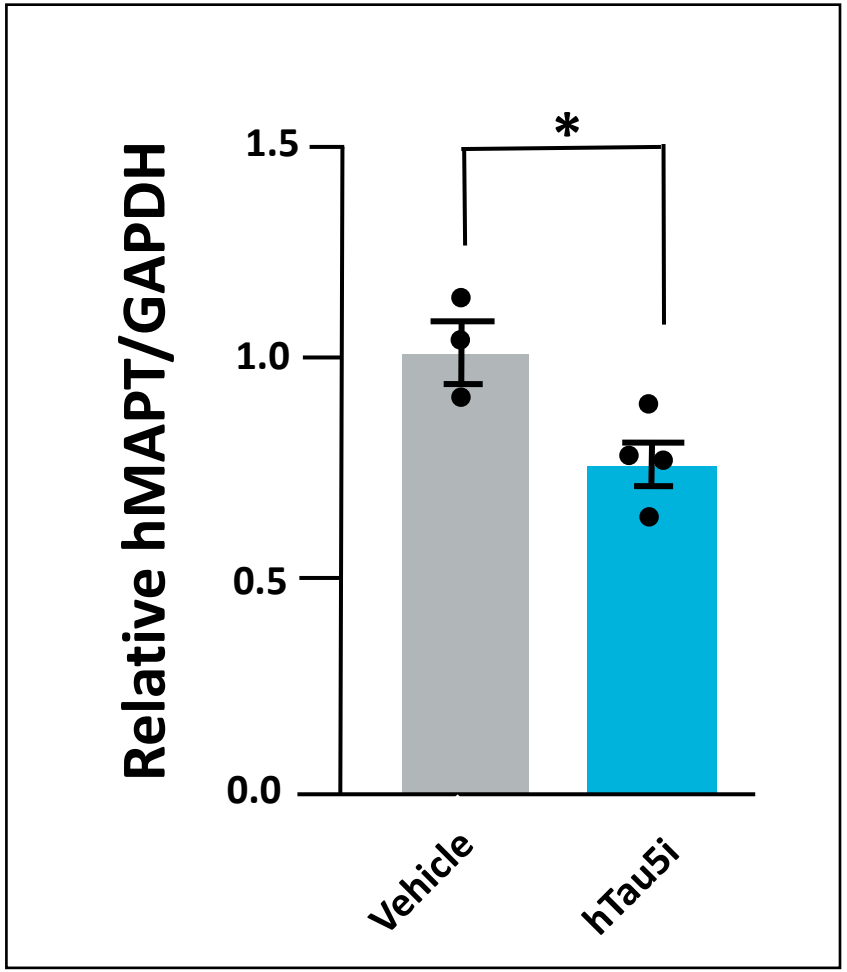






Tau mice dosed with TSHA-113 demonstrated function and GFP expression in neurons and glia





Anticipated next steps for TSHA-113

Animal proof-of-concept achieved in KO mouse model

Evaluate dose response and age response and finalize dose from pharmacology

Interventional trial protocol development underway

Additional candidates targeting neurodegenerative diseases

TSHA-115 *miRNA*
GSDs
Preclinical

- miRNA targeting *GYS1* to inhibit glycogen synthase in the brain to decrease abnormal glycogen formation
- This approach may enable the treatment of several glycogen storage disorders
- Identical construct as TSHA-111-LAFORIN and TSHA-111-MALIN for Lafora disease and TSHA-112 for APBD
- Estimated prevalence of 20,000 patients in the US and EU

Neurodevelopmental Disorder Franchise

Rett syndrome is one of the most common genetic causes of intellectual disabilities in women

TSHA-102
Rett Syndrome

- Rett Syndrome is caused by mutations in the X-linked *MECP2* gene
- *MECP2* regulates the expression of many genes involved in normal brain function
- A brief period of normal development is followed by a devastating loss of speech and purposeful hand use along with the emergence breathing abnormalities
- Disease reversibility described in animal models as demonstrated by Sir Adrian Bird[1]
- Estimated prevalence of Rett syndrome is 25,000 patients (US+EU)

STAGE I

6-18 months (typical)
≤6 months (early)

Developmental Arrest Symptom Onset

Infants are generally described as having normal development until approximately 6 to 18 months of age

STAGE II

1-4 years

Rapid Deterioration Symptom progression-regression

Hallmark Rett symptoms appear: Hand wringing or squeeze, clapping, rubbing, washing, or hand to mouth movements

STAGE III

4-10 years

Pseudo stationary Symptoms stabilize/improve

After a period of rapid deterioration neurological symptoms stabilize, with some even showing slight improvements

STAGE IV

>10 years

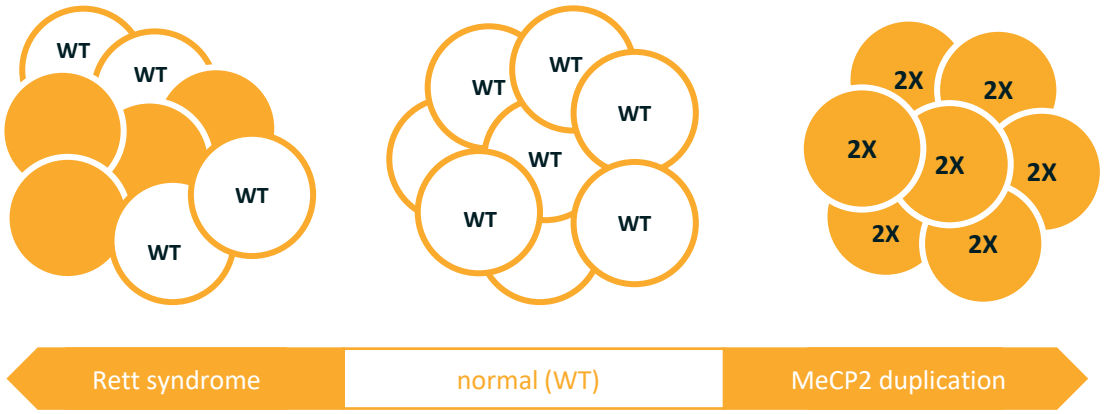
Late Motor Deterioration Muscle wasting with age

85-90% of affected people may **experience growth failure** and **muscle wasting** that worsens with age

Guy J et al. *Science* 2007

Rett syndrome (RTT) is an X-linked neurodevelopmental disorder

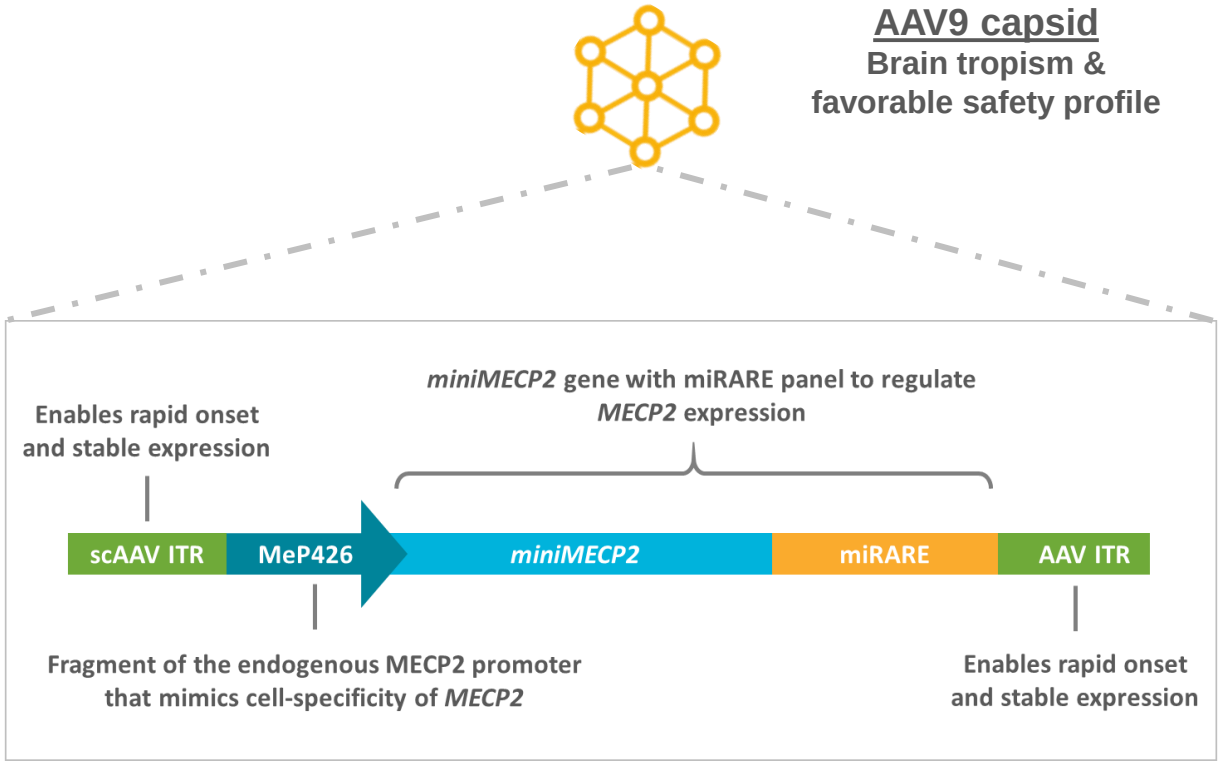
- Characterized by mutations in methyl CpG-binding protein 2 (MECP2), a protein that is essential for neuronal and synaptic function in the brain.
- Female heterozygous RTT patients are mosaic carriers of normal and mutated *MECP2*
- RTT falls along a spectrum of *MECP2* activity and toxicity from gene therapies is linked to unregulated expression of *MECP2*
- *MECP2* expression must be regulated to correct the deficiency, while avoiding toxicity associated with overexpression



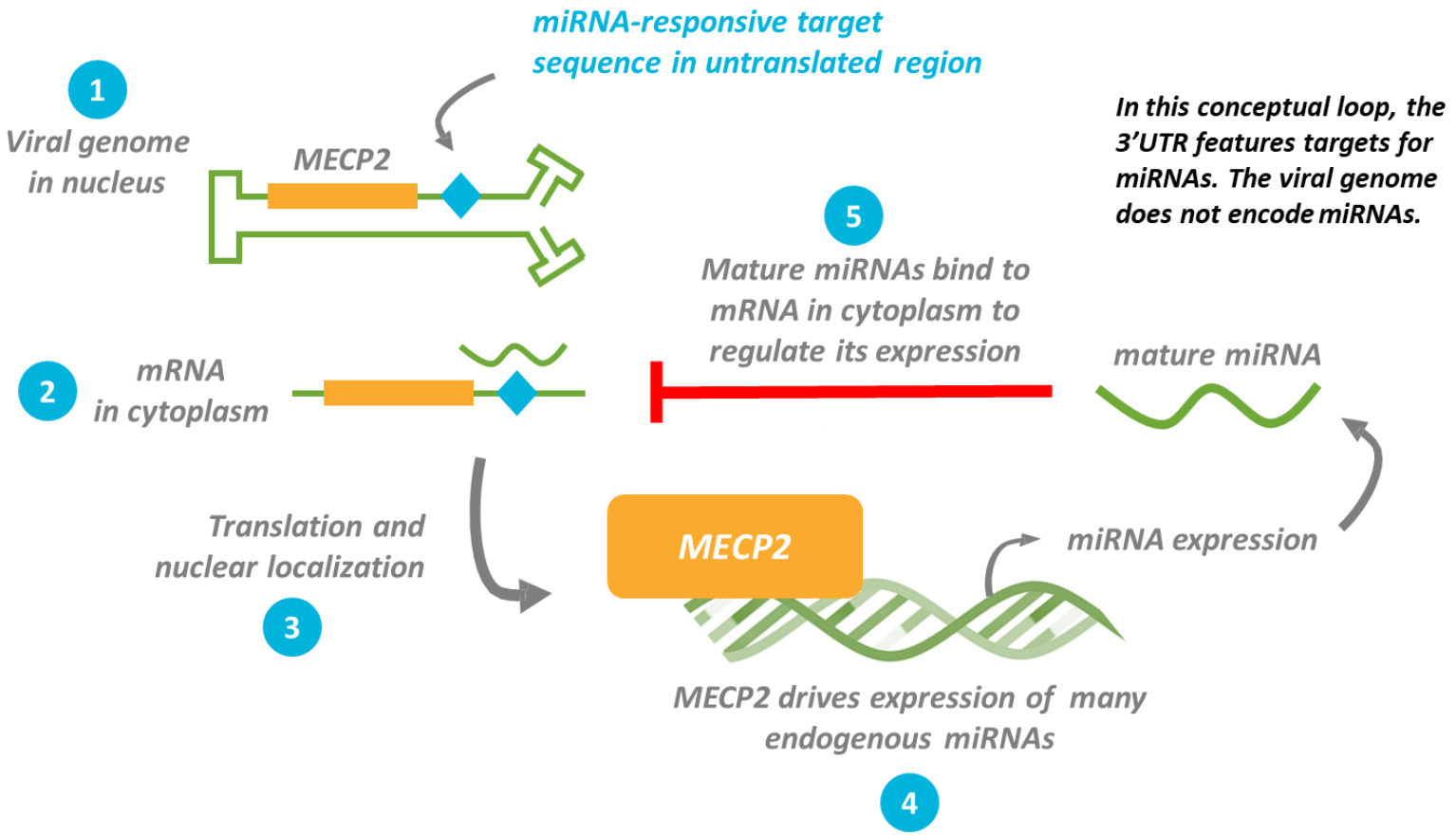
Development of a gene therapy for Rett syndrome requires regulated expression of MECP2

TSHA-102
Rett Syndrome

- AAV9/*MECP2* caused dose-dependent side effects after intraCSF administration in WT and KO mice
- We have developed a novel miRNA-responsive target sequence (miRARE) that regulates the expression of the *MECP2* transgene
- Our approach provides a superior therapeutic profile to that of unregulated *MECP2* gene replacement



miRARE is a targeting panel for endogenous miRNAs which regulate MECP2 expression



Preclinical data for TSHA-102 in Rett syndrome recently published in *Brain*

ACCEPTED MANUSCRIPT

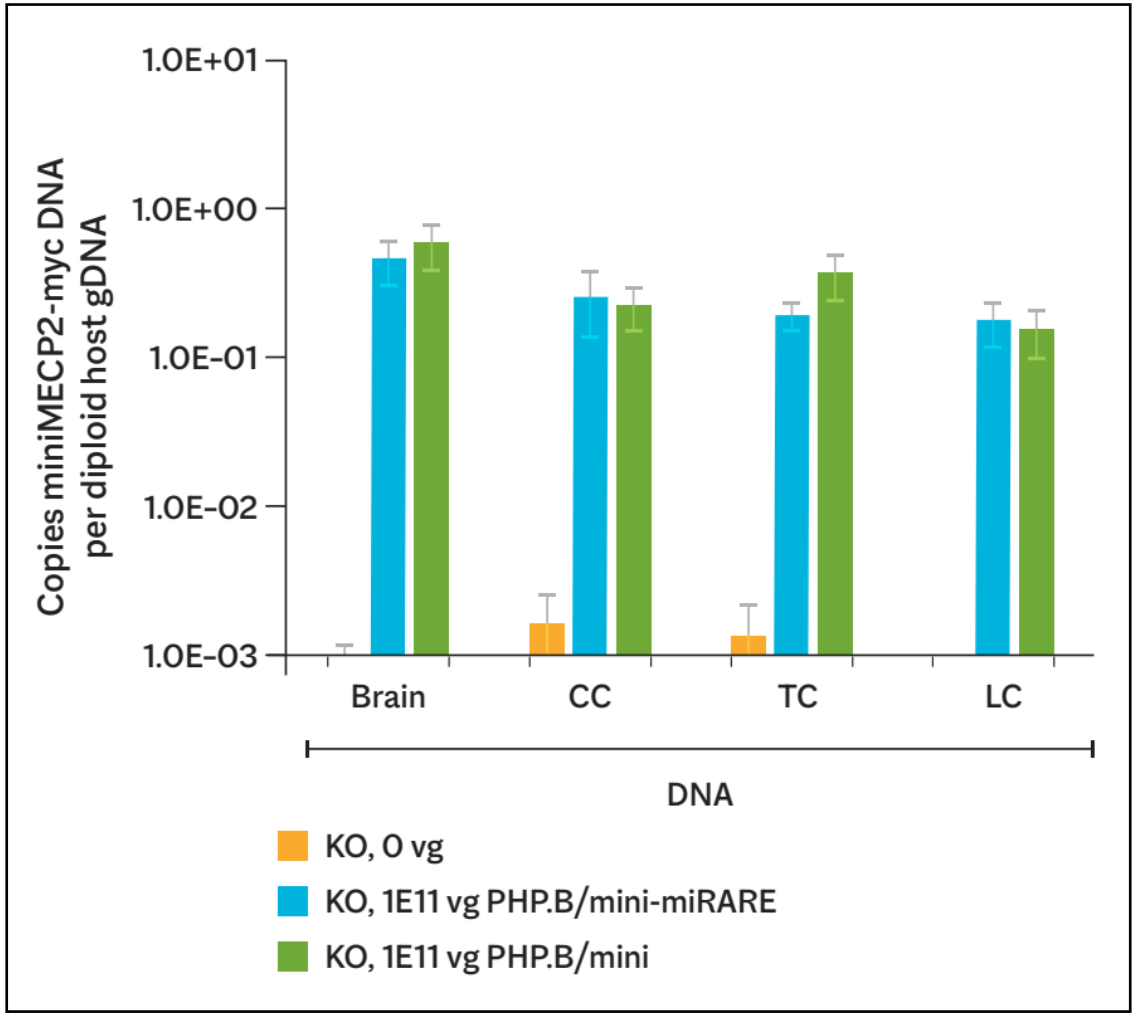
Engineered microRNA-based regulatory element permits safe high-dose mini*MECP2* gene therapy in Rett mice

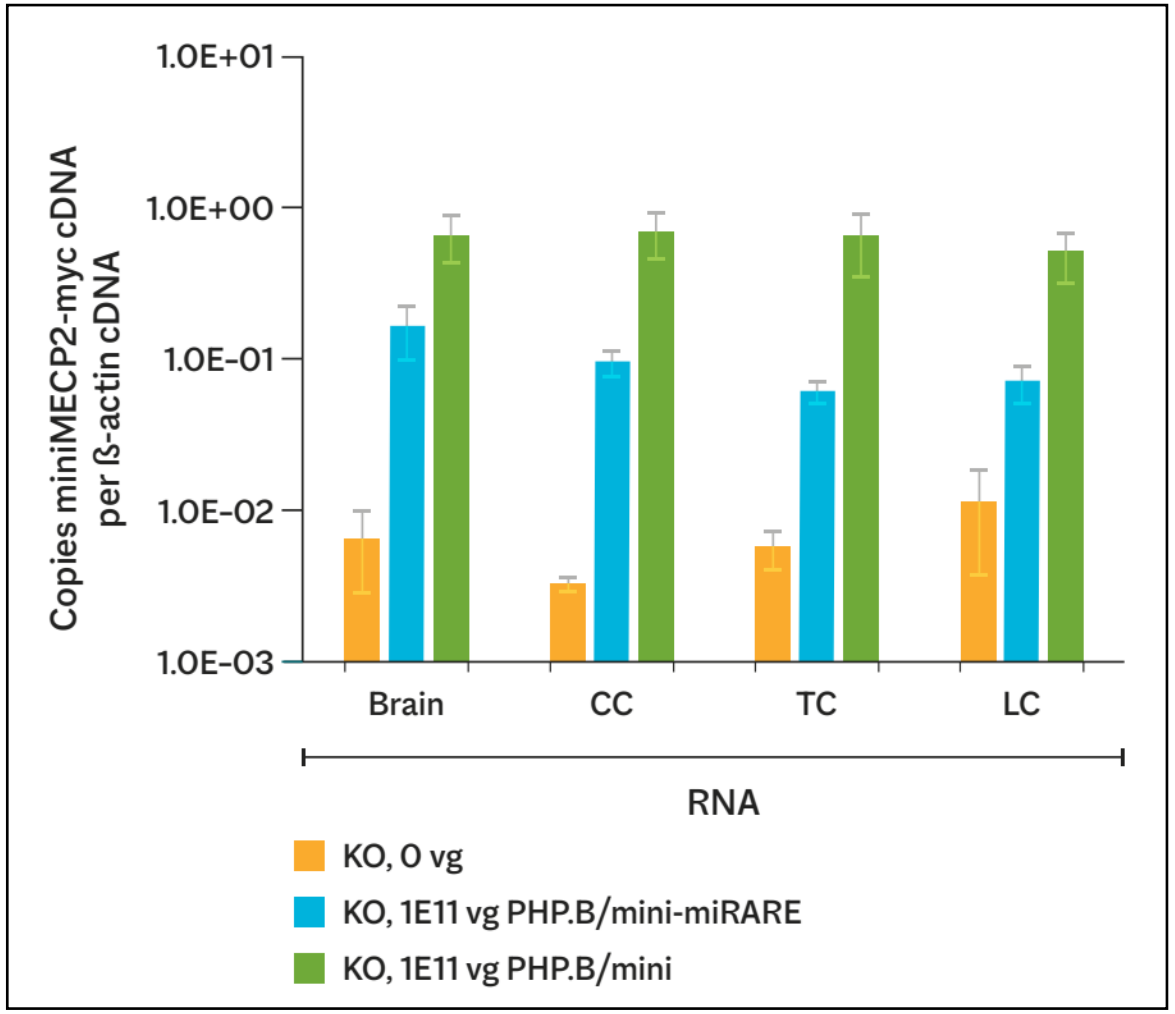
Sarah E Sinnett, Emily Boyle, Christopher Lyons, Steven J Gray ✉

Abstract

MECP2 gene transfer has been shown to extend the survival of *Mecp2*$^{-/y}$ knockout (KO) mice modeling Rett syndrome (RTT), an X-linked neurodevelopmental disorder. However, controlling deleterious overexpression of MeCP2 remains the critical unmet obstacle towards a safe and effective gene therapy approach for RTT. A recently developed truncated mini*MECP2* gene has also been shown to be therapeutic after AAV9-mediated gene transfer in KO neonates. We show that AAV9/mini*MECP2* has a similar dose-dependent

miRARE reduced overall expression of mini*MeCP2* transgene expression compared to unregulated mini*MeCP2* in WT mice

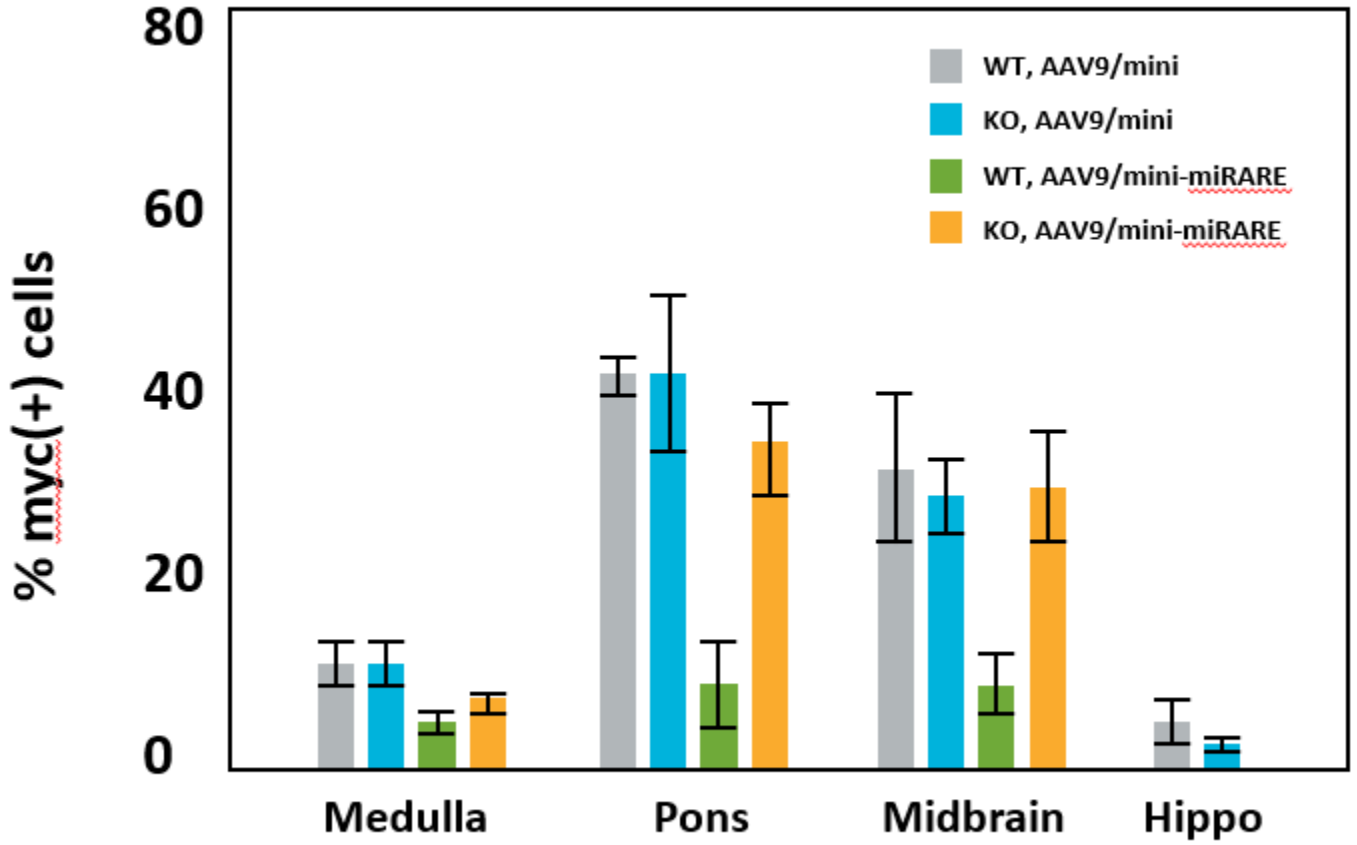




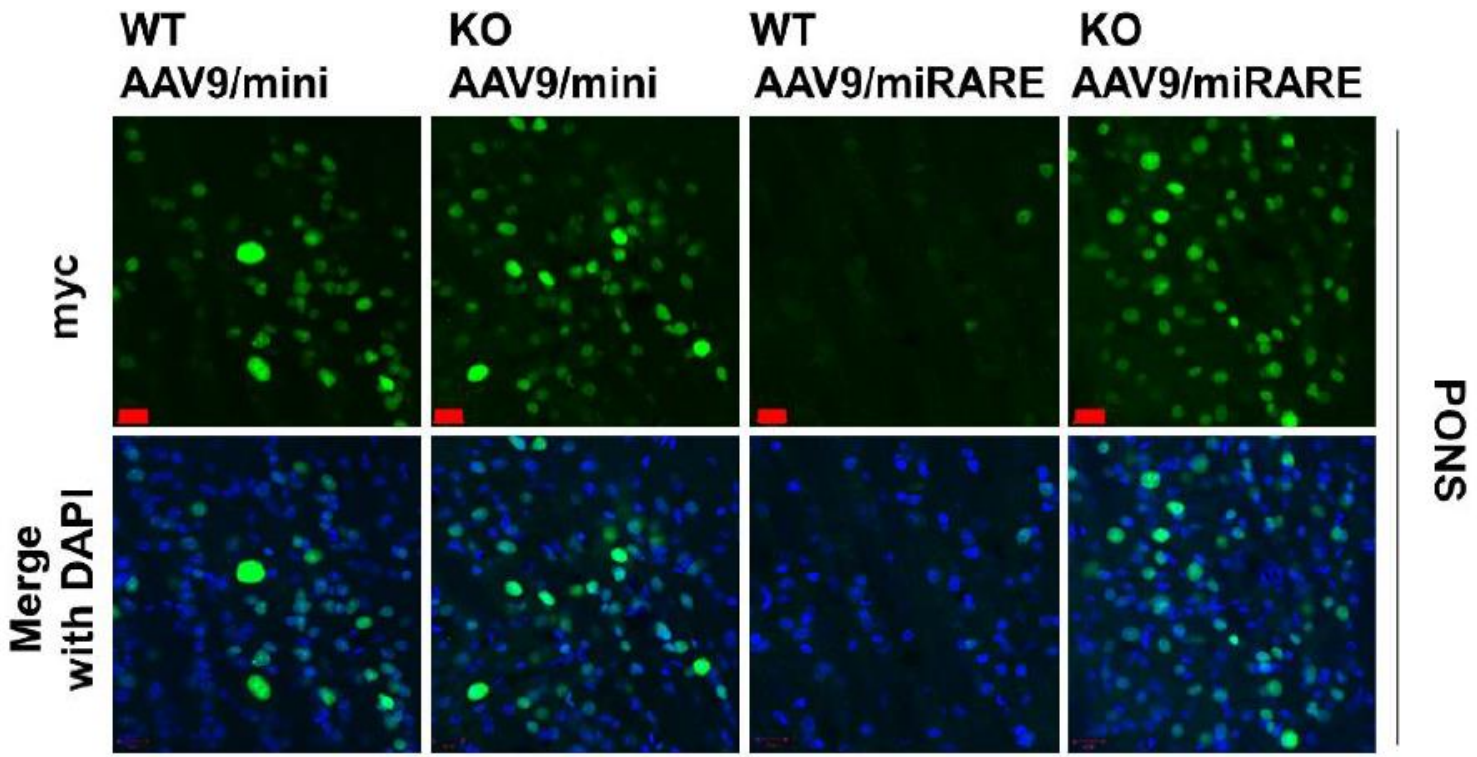
CC: Cervical Cord; TC: Thoracic Spinal Cord; LC: Lumbar Cord

miRARE regulated genotype-dependent *MECP2* expression across different brain regions in wild type and Rett KO mouse models

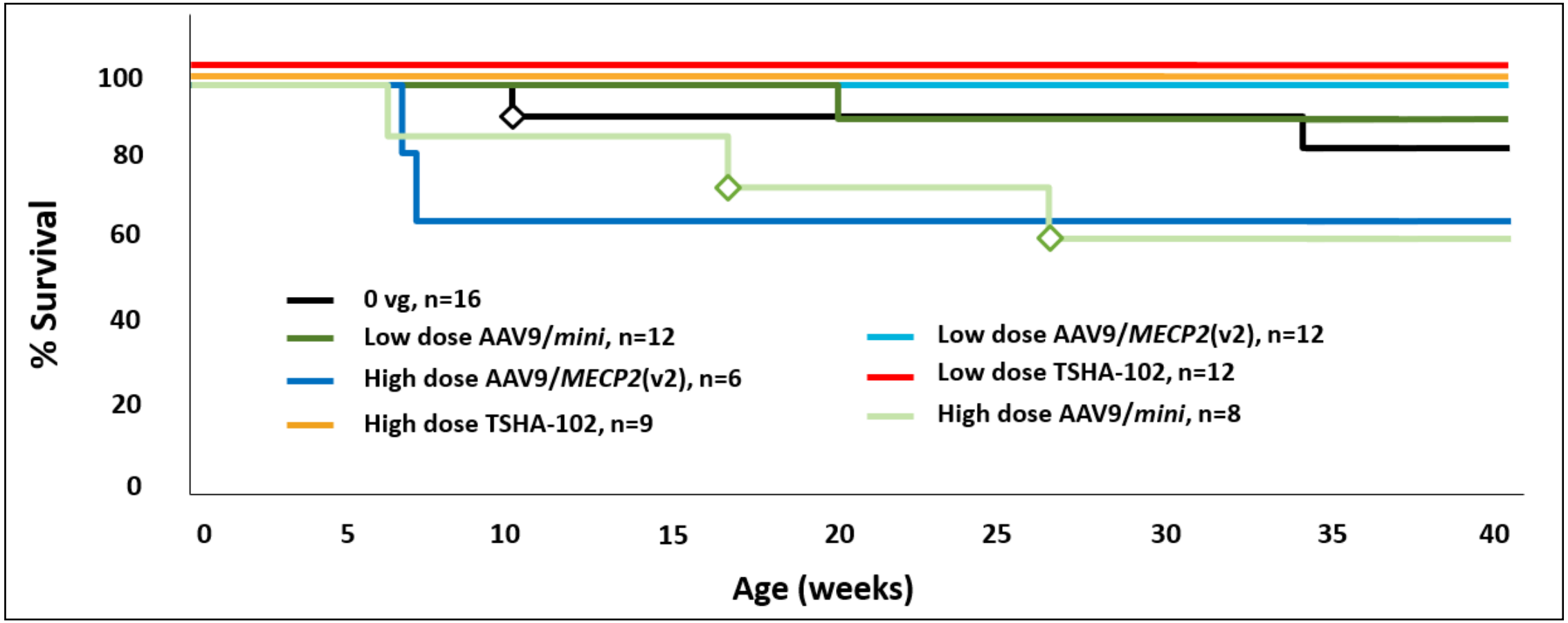
TSHA-102
Rett Syndrome



miRARE regulated expression in pons and midbrain based on a cell-by-cell basis



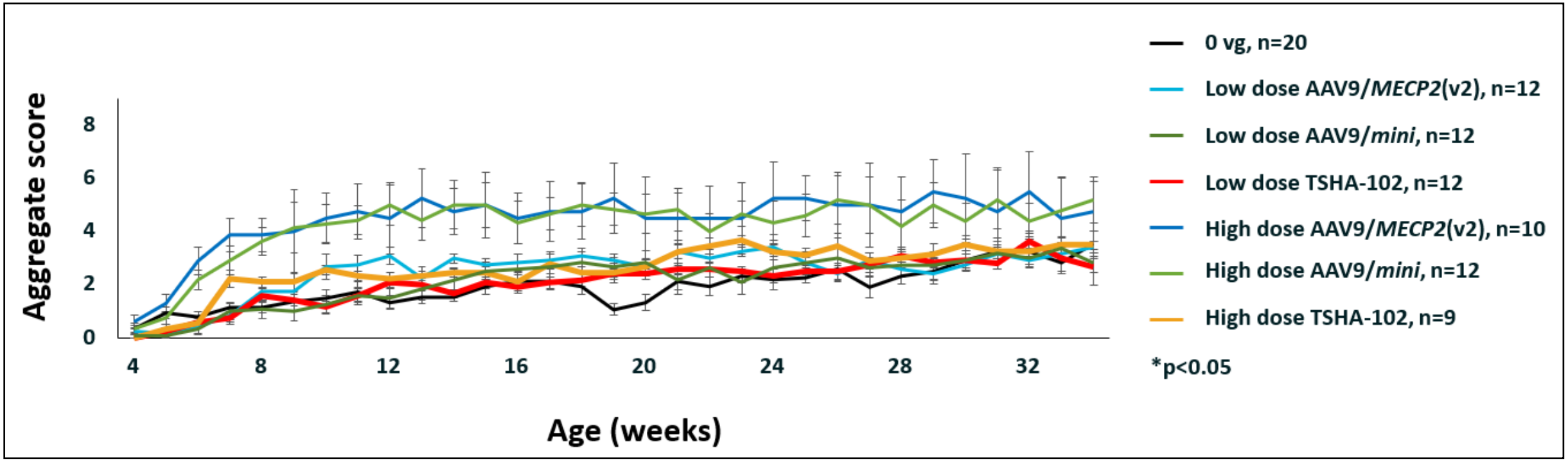
Safety – Intrathecal TSHA-102 was not associated with early death in WT mice



Mice were dosed P28-35

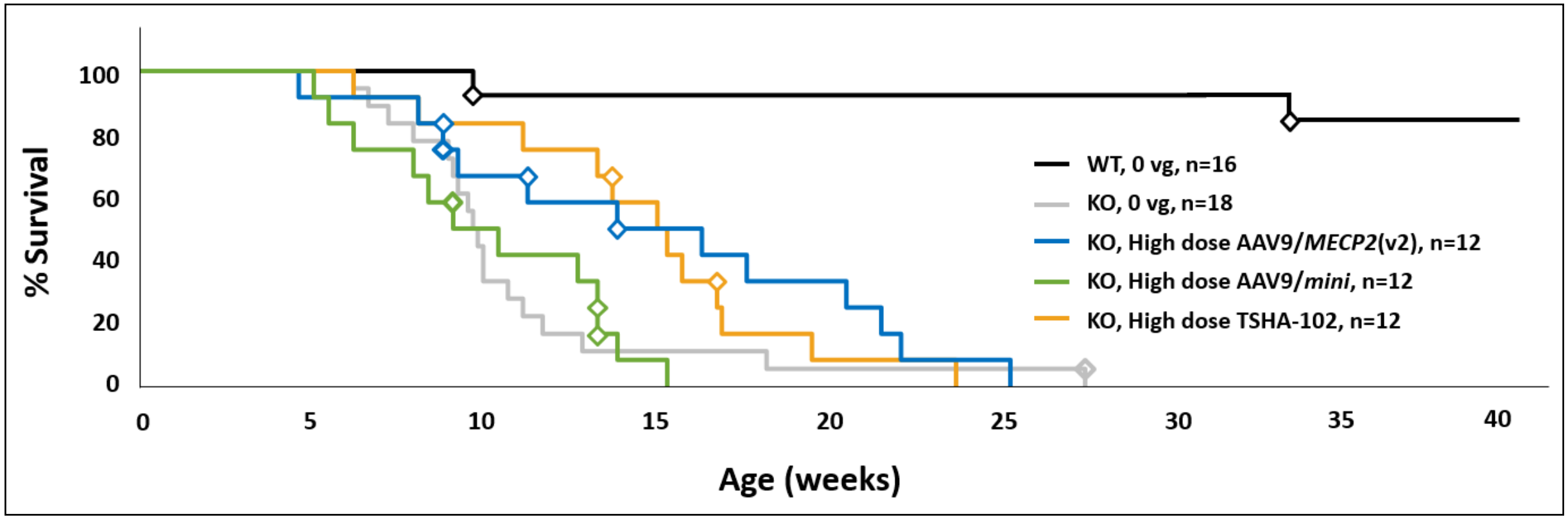
Diamond = vet-requested euthanasia for prolapse or bullying-related injury

Safety – TSHA-102 did not cause adverse behavioral side effects in WT mice



Mice were dosed P28-35

Efficacy – TSHA-102 outperformed unregulated AAV9/mini in *MECP2* KO mouse survival study



Mice were dosed P28-35

Diamond = vet-requested euthanasia, primarily for lesions. Lesions have been observed with varying frequencies among saline-treated KO mice, virus-treated WT and KO mice, as well as untreated RTT weanlings.

TSHA-102 Phase 1/2 study design plan

Goals and Targets of Trial

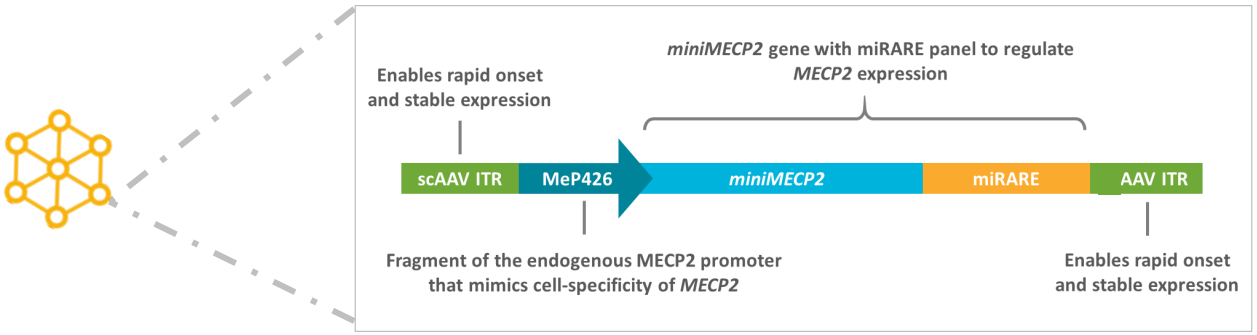
Goals

- **Primary** – Safety: clinical and laboratory assessments
- **Secondary** – Efficacy: pathologic, physiologic, functional and clinical markers

Target Recruitment

- 8 subjects
- Adults with pathogenic confirmation of mutation in *MECP2*

Product Details and Dose Cohorts



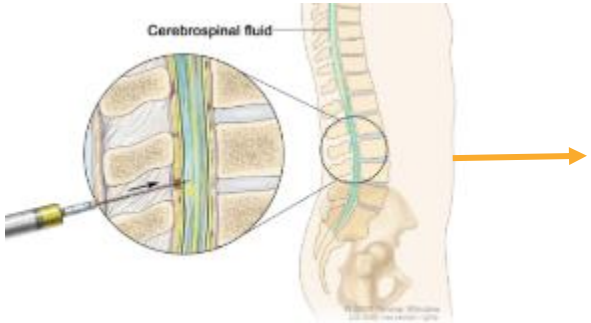
Dose Cohorts

- Each cohort randomized 3:1 (one patient is a delayed treatment control)
- 5.0 x 10^{14} total vg (n=4)
- 1.0 x 10^{15} total vg (n=4)

Route and Method of Administration

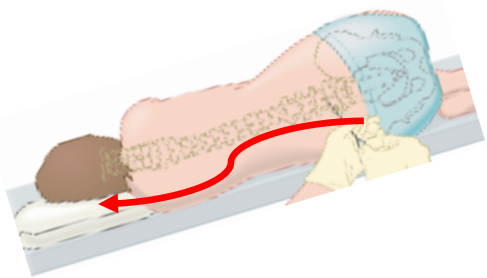
Administration

- Lumbar Intrathecal Infusion (IT)
- Amount and rate: 1 mL/min for total of 10-12 mL
- Immunosuppression regimen of prednisolone and sirolimus



Technique to Improve Transduction

- Trendelenburg position (15-30^{o})
- During infusion & 1 hour post infusion



TSHA-102 Phase 1/2 clinical assessments

Rett-Specific/Global Assessments
- Motor Behavior Assessment Scale (MBA)
- Rett Syndrome Hand Apraxia Scale (RHAS)
- Rett Syndrome Behavior Questionnaire (RSBQ)
- Functional Mobility Scale in Rett Syndrome (FMS)
- Clinical Global Impression

Behavior/Mood Assessments
- Anxiety, Depression, and Mood Scale (ADAMS)
- Aberrant Behavior Checklist (ABC)

Seizure Assessments
- EEG and neurophysiology
- Seizure diary

Respiratory Assessments
- Respiratory Disturbance Index (RDI)
- Sleep apnea, sleep study

Communication Assessments
- Observer Reported Communication Assessment (ORCA)

Quality of Life/Other Assessment
- SF-36 – Quality of life assessment from principal caregiver
- RTT-CBI – Caregiver burden inventory

Wearables
- Hexoskin: cardiac, respiratory, sleep & activity

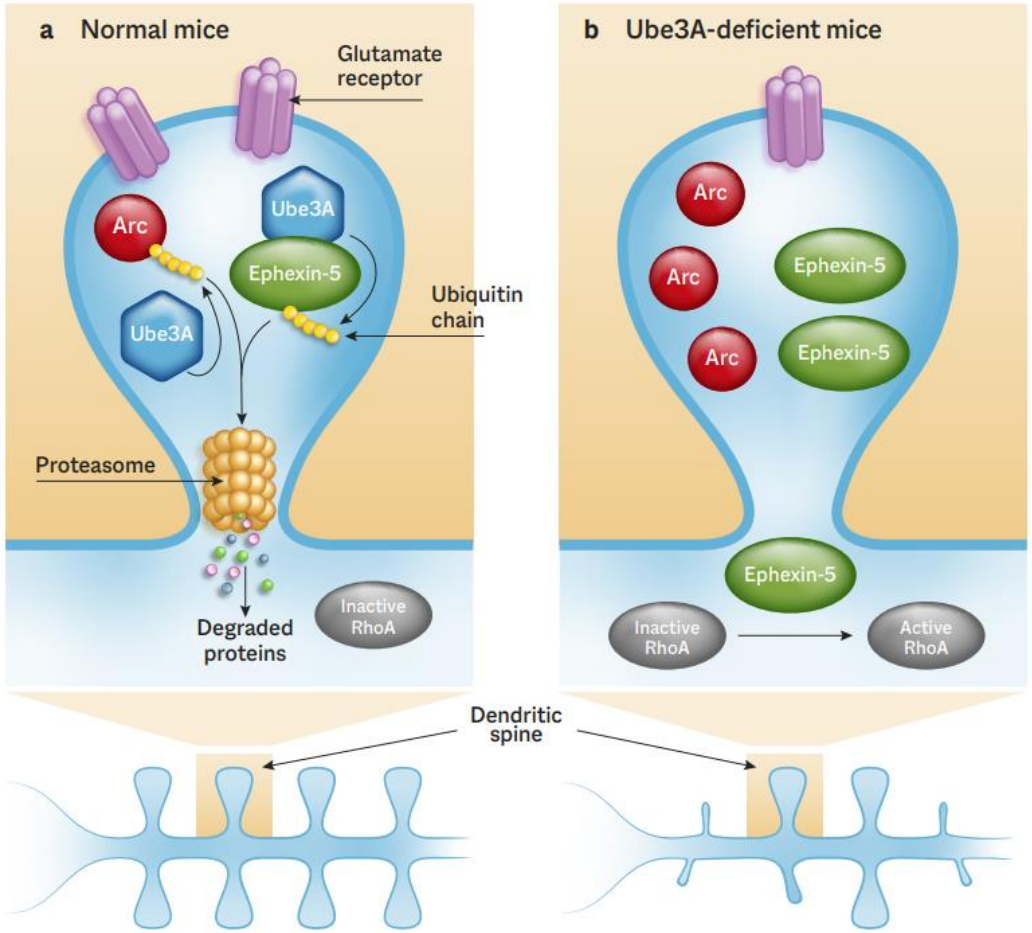
TSHA-102
Rett Syndrome

Anticipated next steps for TSHA-102 by the end of 2021

- Submit IND/CTA in 2H 2021
- Initiate Phase 1/2 study by YE 2021
- Complete GMP manufacturing using commercial process
- Pre-IND/CTA and Scientific Advice meetings underway

Angelman syndrome is a rare, neurogenic disorder due to genomic imprinting

- Caused by a deletion or loss of function of the maternally inherited allele of the UBE3A gene resulting in loss of the UBE3Q protein expression in neurons and abnormal communications between neurons
- Maternal-specific inheritance pattern due to genomic imprinting of UBE3A in neurons
- Maternal UBE3Q allele is expressed; paternal allele is silenced by a long non-coding RNA, UBE3A antisense transcript, or UBE3A-ATS
- No signs of Angelman syndrome at birth. Disorder usually diagnosed at 6-12 mo
- Signs and symptoms include developmental delay, severe impairments in behavior, motor function, communication and sleep, as well as intellectual disability, debilitating seizures and ataxia
  - Frequent smiling and laughter (happy puppet syndrome)
  - Feeding issues, no speech or minimal speech
  - Abnormal sleep-cycles and diminished need for sleep
  - Seizures may begin at 2 to 3 years
- Normal lifespan but unable to live independently
- No currently approved therapies; current treatment focused on managing medical and developmental issues
- Estimated prevalence of Angelman syndrome is 55,000 patients (US+EU)

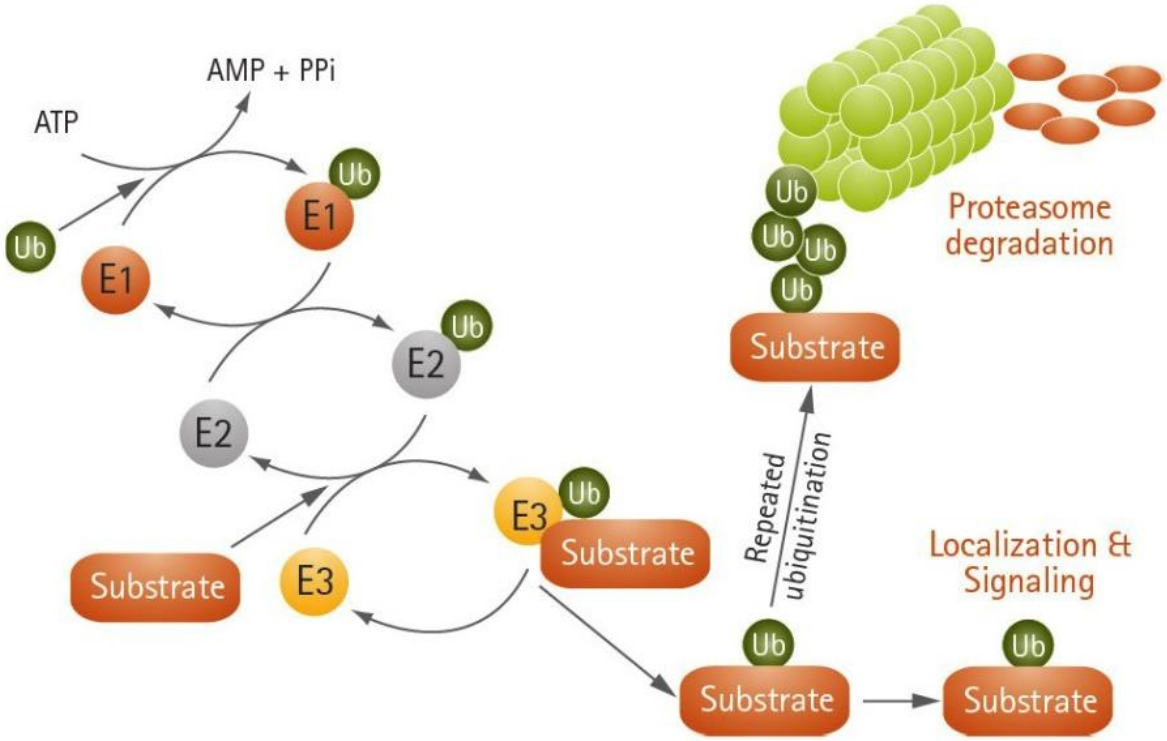


UPD – Uniparental disomy

Scheiffele, P. et al. *Nature* 2010

Molecular pathology of Angelman syndrome

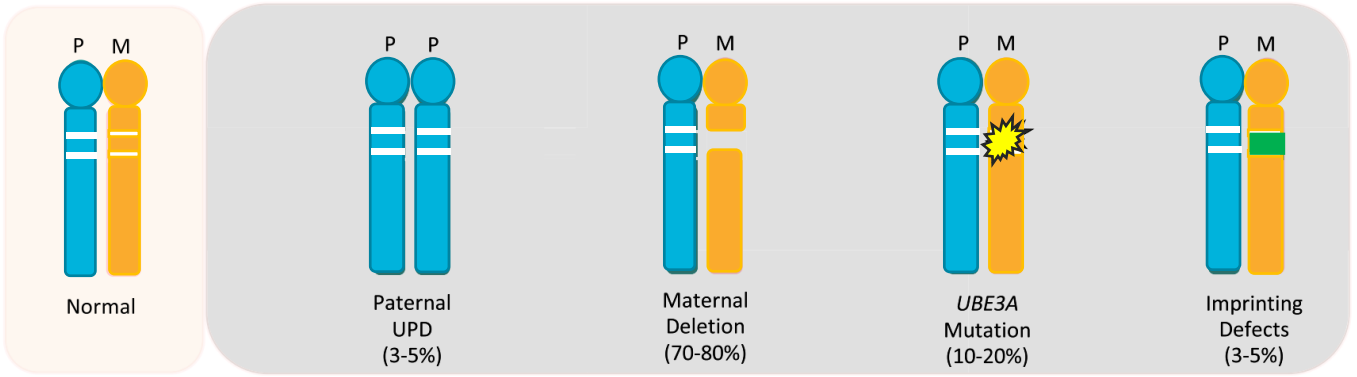
- UBE3A, an E3 ligase in the ubiquitin-proteasomal system, plays important roles in brain development and normal function
- UBE3A conjugates polyubiquitin chains to specific lysine residues in its substrates, regulating the expression and function of these proteins
- Deletion or loss-of-function mutations of the maternally inherited allele of UBE3A result in Angelman syndrome while maternal duplication or triplication of the chromosome 15q11–13 region is associated with autism spectrum disorder
- Impairments in ubiquitin-mediated protein degradation can lead to deficits in neuronal development and the maintenance of synaptic connections



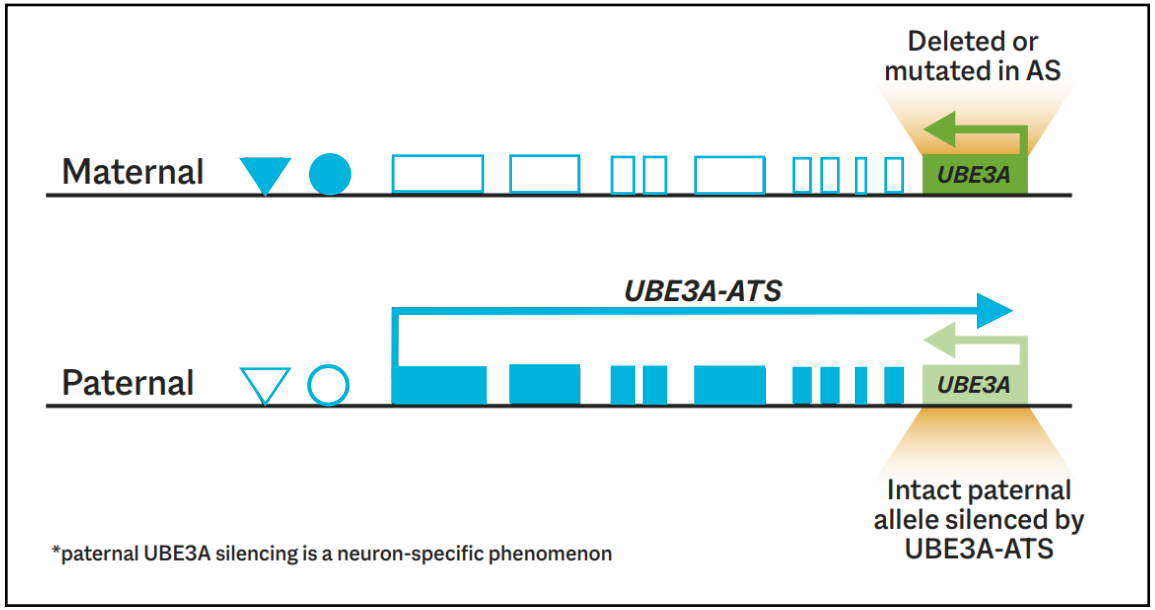
The ubiquitination process

Two novel approaches to treat Angelman syndrome

- Targeting entire Angelman syndrome population using two approaches
  - Knockdown of UBE3A-ATS to unsilence paternal allele
  - Gene replacement strategy on *UBE3A* to mimic maternal UBE3Q allele expression
- Currently developing both approaches

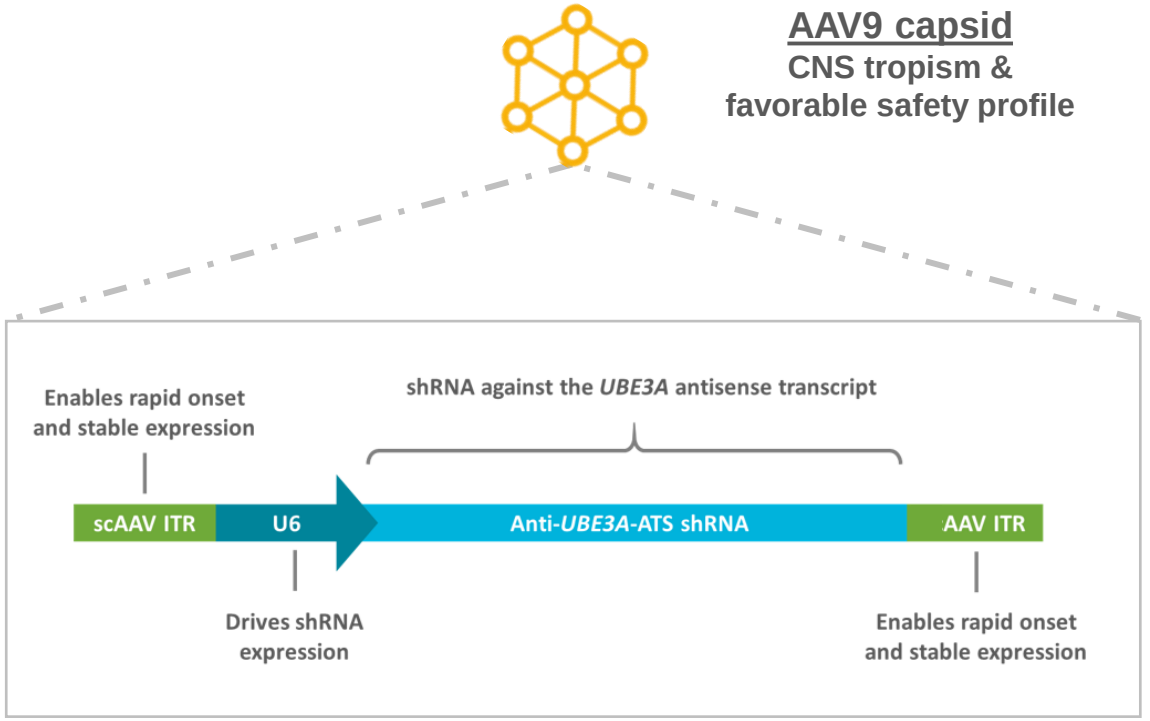


In neurons, the **maternally inherited UBE3A allele is the only active allele**, since the **paternally inherited UBE3A allele is silenced through cell type–specific imprinting**

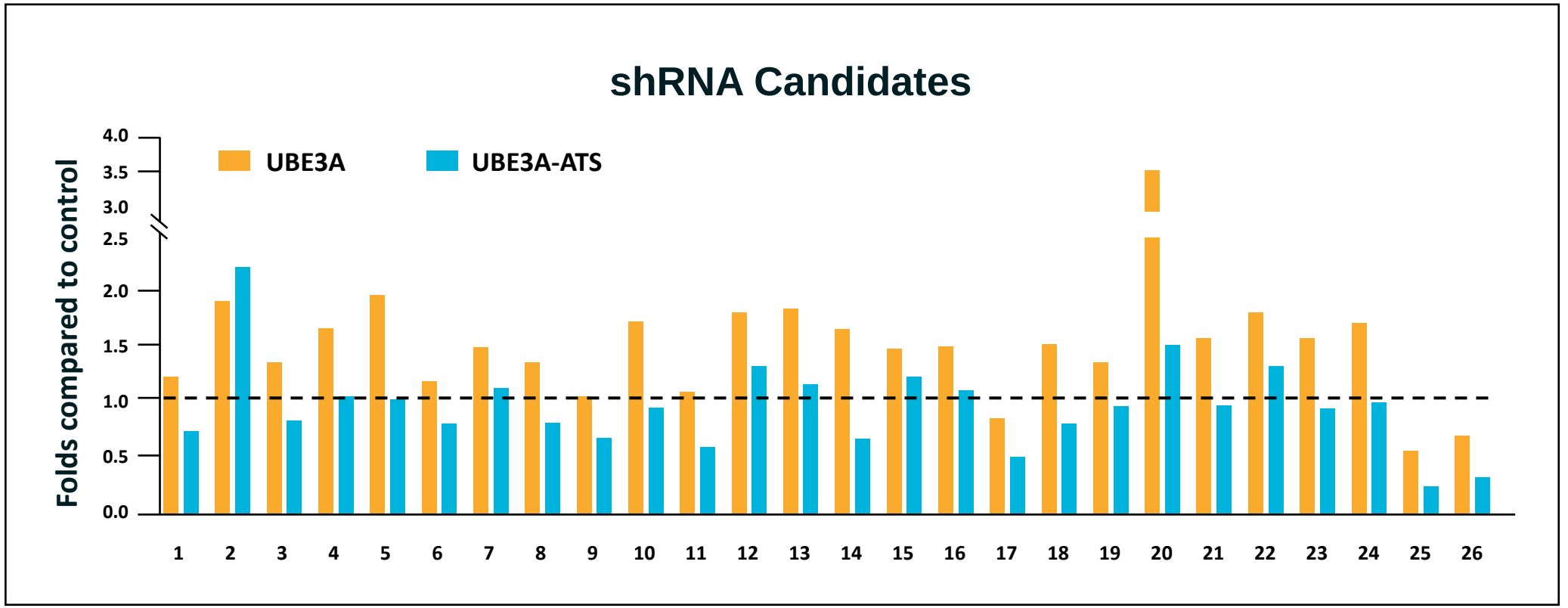


Knockdown of *UBE3A-ATS* established, placing TSHA-106 in a competitive position to advance rapidly

- AAV9 viral vector designed for shRNA-mediated knockdown of *UBE3A-ATS*, the antisense transcript governing the expression of UBE3A through the paternal allele
- First-in-human study in Angelman syndrome demonstrated that knockdown of *UBE3A-ATS* to unsilence the paternal allele led to meaningful clinical improvement
  - Five patients treated with an antisense oligonucleotide (ASO) therapy had significant improvement in the Clinical Global Impression (CGI) scale of change in Angelman syndrome after 128 days of treatment
  - CGI-I-AS measures several domains of function, including global, fine motor, gross motor, communication, behavior, and sleep
  - Treatment with the ASO was generally well tolerated but led to SAEs, including transient lower extremity weakness in all 5 patients treated
- Gene therapy to unsilence the paternal allele offers a unique profile of advantages
  - One-time dosing
  - Widespread transduction through the CNS using an AAV9 vector
  - Established safety as seen with other intrathecal clinical trials



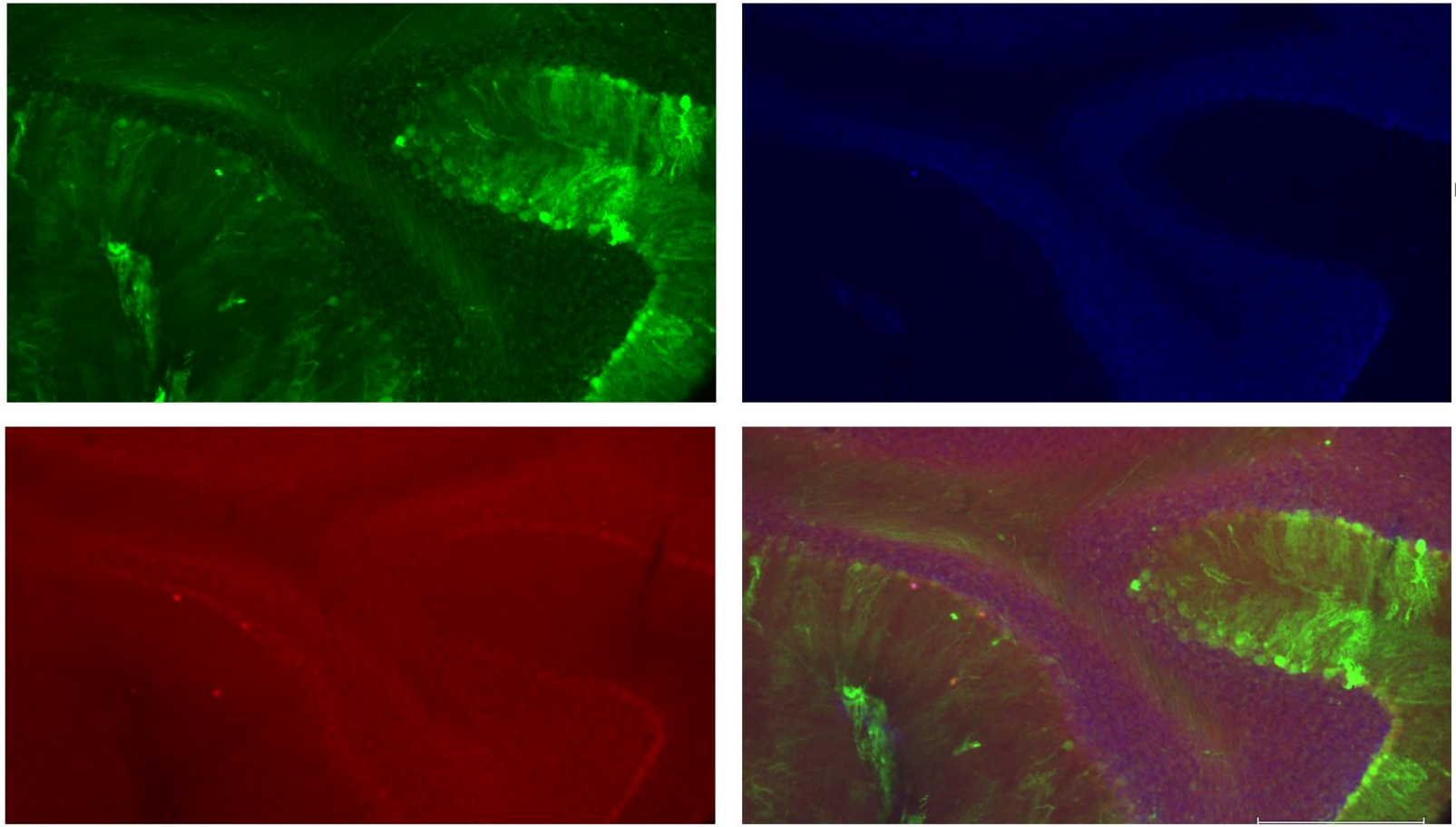
TSHA-106 targets UBE3A-ATS transcript through shRNA knockdown



Testing in neuroblast cell line demonstrated consistent knockdown of UBE3A-ATS and a subsequent increase in UBE3A expression across 26 distinct shRNA candidates

UBE3A expression following administration of shRNA candidate

Cerebellum



Anticipated next steps for TSHA-106

Interim expression and safety data from confirmatory NHP studies

Evaluate dose response and age response and finalize dose from pharmacology

Interventional trial protocol development underway

Additional candidates targeting neurodevelopmental disorders

TSHA-114 GRT
Fragile X syndrome
Preclinical

- FMR1 is the most common single gene cause of autism and cognitive impairment
- Fragile X Syndrome is characterized by anxiety, aggression, hyperactivity, attention deficits, and sleep/communication disruption
- Estimated prevalence of 100,000 patients in the US and EU

TSHA-116 shRNA
Prader-Willi syndrome
Preclinical

- Loss of function of genes along 15q11-q13 chromosome region due to an imprinting defect
- Patients have developmental delay, insatiable eating habits accompanied by obesity and overt diabetes
- Estimated prevalence of 40,000 patients in the US and EU

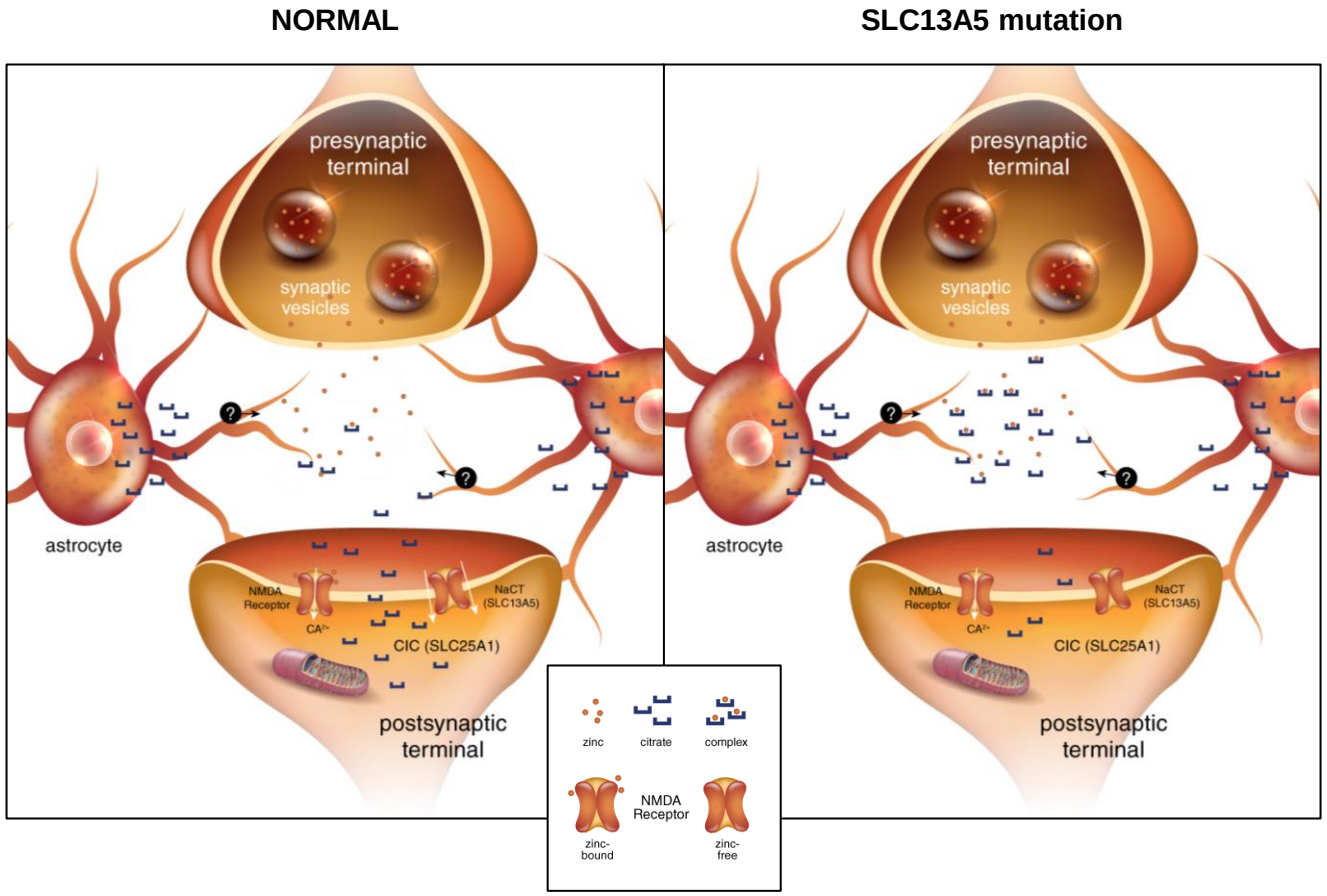
TSHA-117 regulated GRT
FOXG1 syndrome
Preclinical

- Newly discovered gene with prevalence expected to steadily rise as more children as tested with autism spectrum disorder
- Development and intellectual disabilities, growth restriction with microcephaly, epilepsy, and hyperkinetic-dyskinetic movement disorder
- Estimated prevalence of 20,000 patients in the US and EU

Genetic Epilepsy Franchise

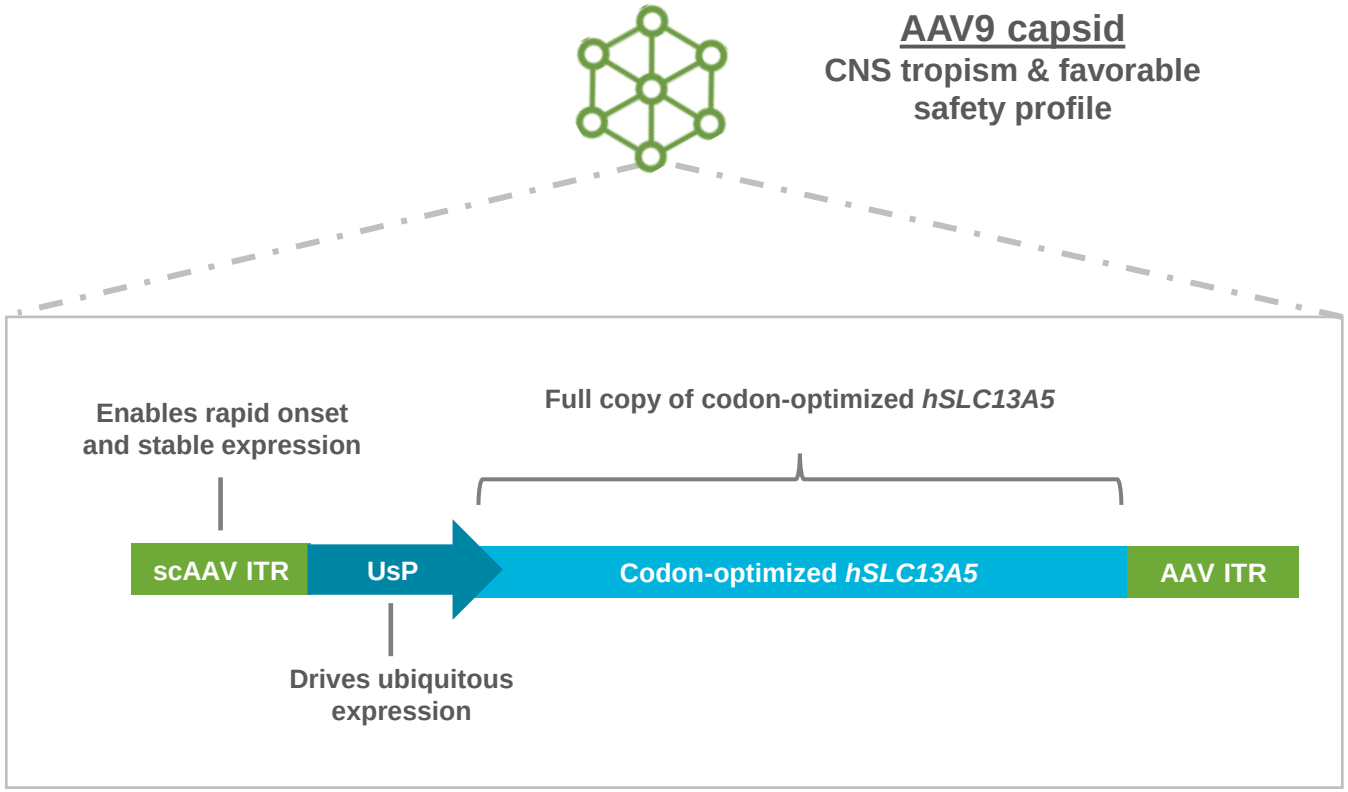
SLC13A5 deficiency is a rare autosomal recessive disorder

- Bi-allelic loss of function in the SLC13A5 gene, resulting in a loss or reduction in citrate transport and aberrant cellular metabolism
- Patients have impaired motor function, speech production and seizures
- Signs and symptoms include seizures within a few days of birth, persisting through life, encephalopathy, delayed speech/language development, developmental regression and abnormalities in tooth enamel
- First-line treatment is anti-seizure medications
- Estimated prevalence of SLC13A5 deficiency is 1,900 patients (US+EU)

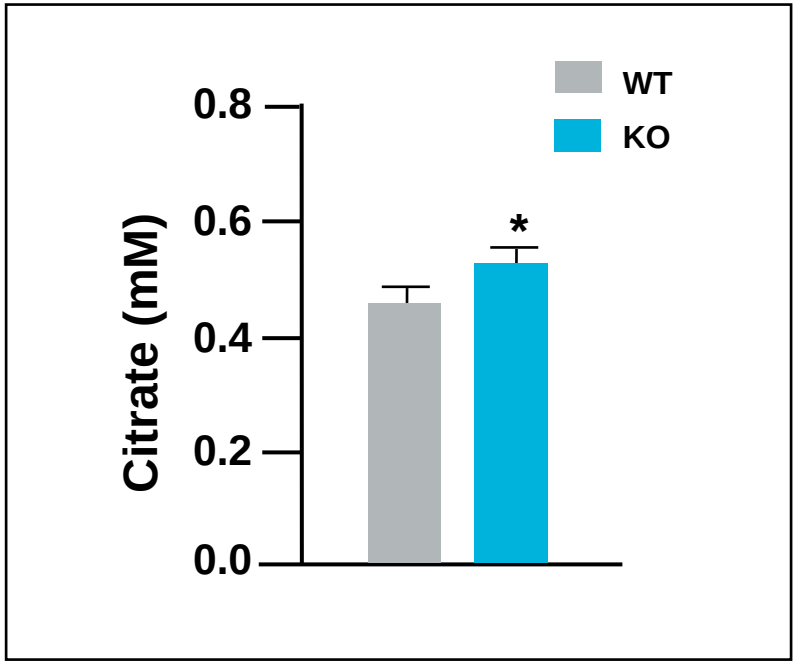


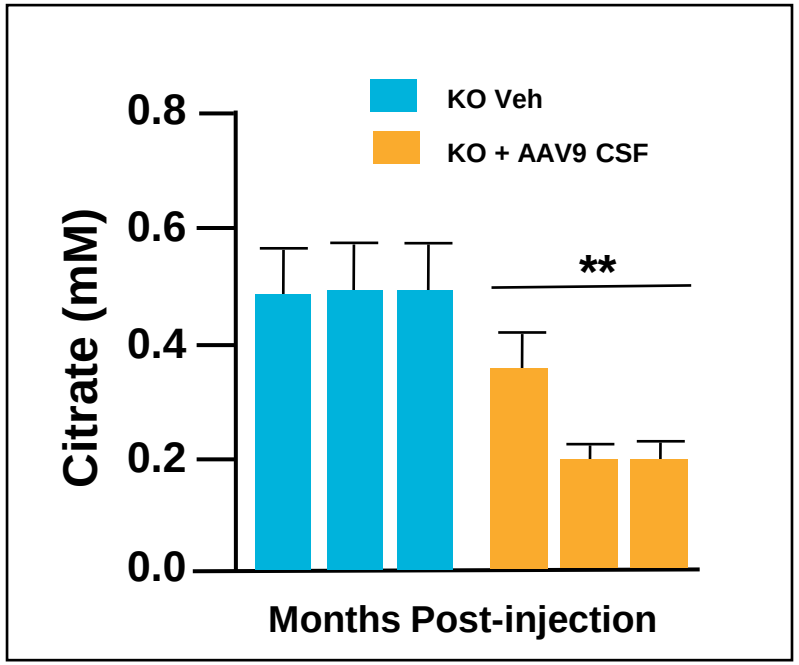
TSHA-105 currently in IND/CTA-enabling studies

- Self-complementary AAV9 expressing human SLC13A5 protein under the control of a ubiquitous promoter
- Delivered intrathecally
- Received orphan drug and rare pediatric disease designations
- Currently in IND/CTA-enabling studies



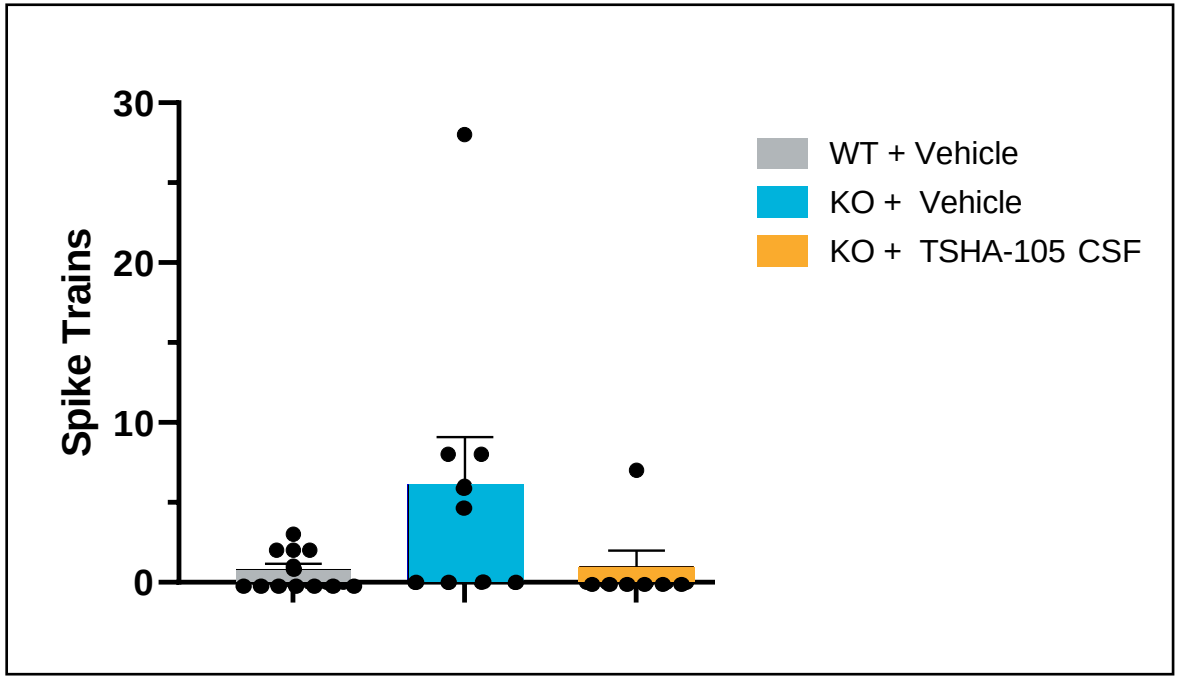
TSHA-105 decreased plasma citrate levels in 3-month old SLC13A5 KO mice

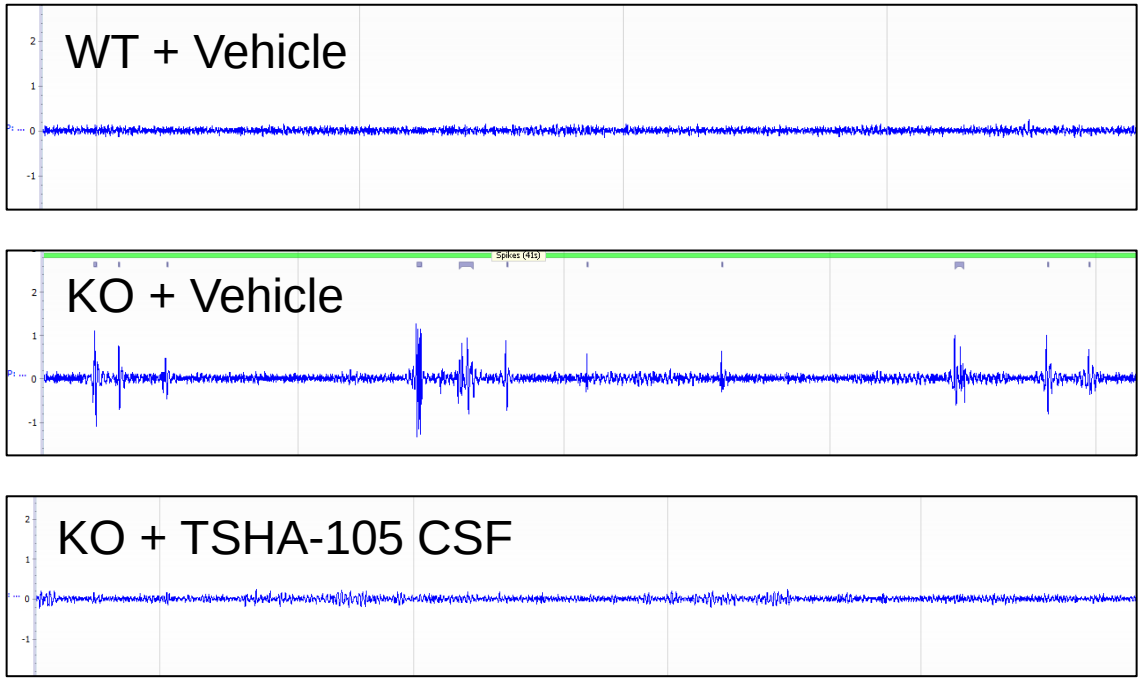




Bailey, R. 2020

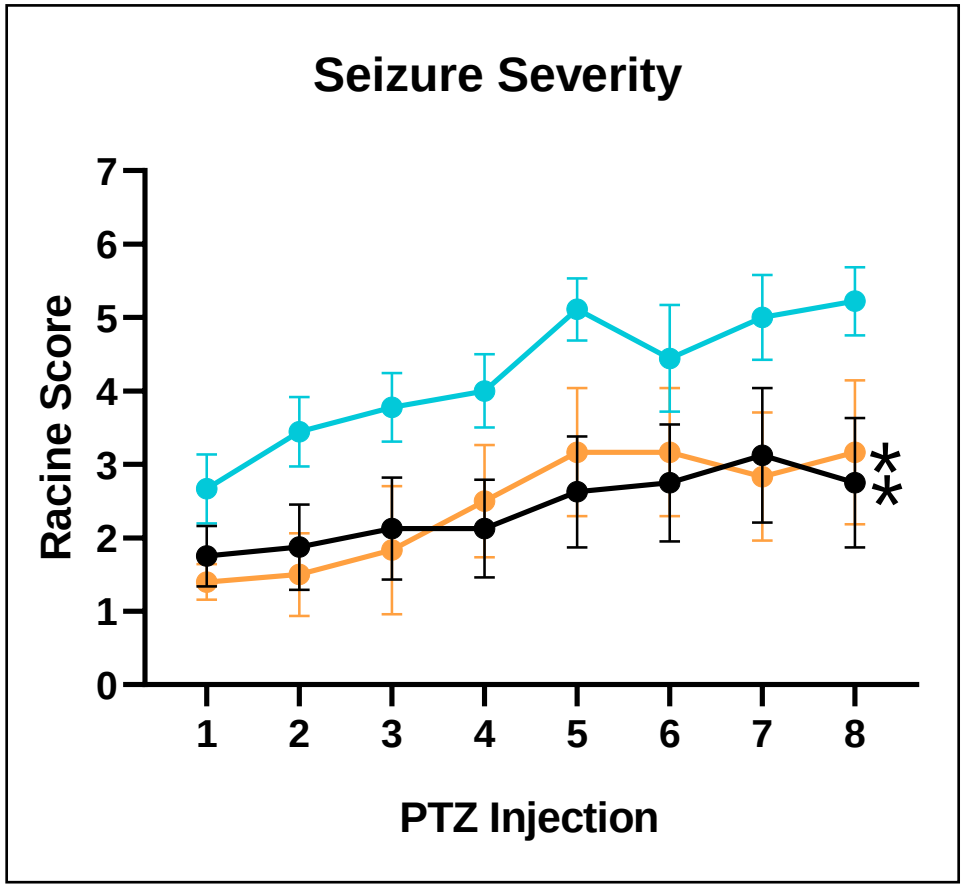
TSHA-105 improved EEG activity in 3-month old SLC13A5 KO mice

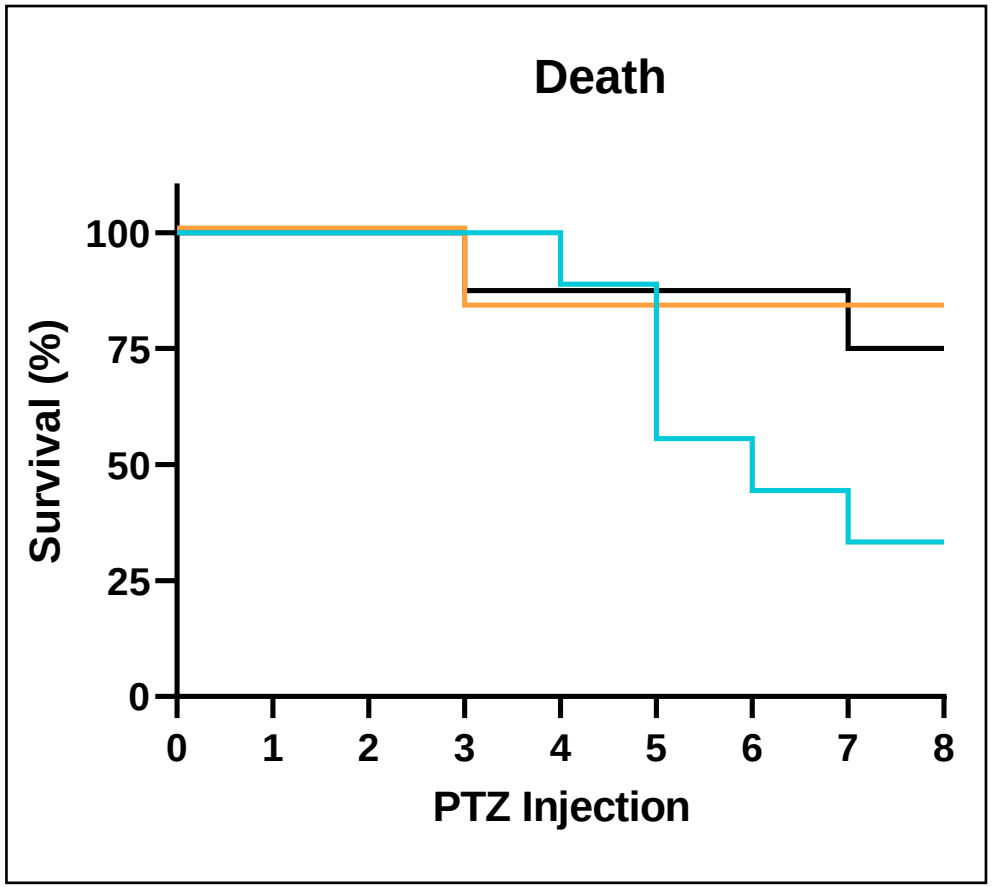




Bailey, R. 2020

TSHA-105 reduced seizures and associated deaths in SLC13A5 KO mice





WT + Vehicle (n=8)
KO + Vehicle (n=9)
KO + TSHA-105 CSF (n=6)

PTZ - pentylenetetrazol

Bailey, R. 2020

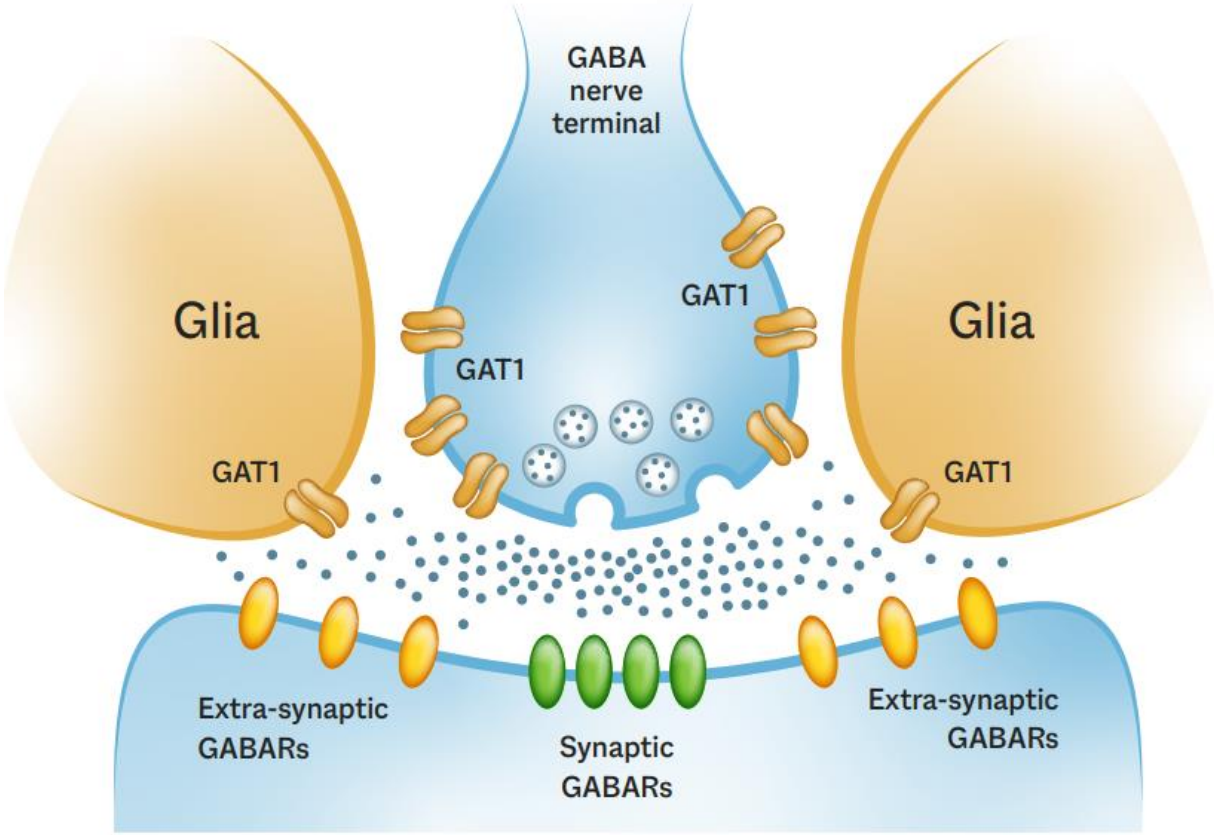
Moving TSHA-105 towards clinical study – Clinical considerations

- Ongoing IND/CTA-enabling studies for TSHA-105
- Patients will roll in from ongoing prospective natural history study
- Current clinical trial considerations:

Considerations for the TSHA-105 Phase 1/2 first-in-human study		
Study design	• Open-label, randomized, dose-escalation study of the safety, tolerability and preliminary efficacy of TSHA-105 for the treatment of epileptic encephalopathy caused by SLC13A5 deficiency • CSF dosing in children	
Clinical assessments	**Disease-Specific/Global Assessments** • CHOP-INTEND • Peabody Developmental Motor Scale • Bayley Scales of Infant Development • Developmental quotient score • Clinical global impression of improvement (CGI-I) • Quality of Life scales **Communication Assessments** • Observer-reported communication ability (ORCA) **Imaging** • MRI of Brain	**Biomarkers** • Citrate levels in plasma, urine, and CSF **Seizure Monitoring** • Seizure frequency • Incidence of status epilepticus • EEG • Ratio of slow wave activity (SWA) during first hour of non rapid eye movement (NREM) sleep (measured by interictal EEG)

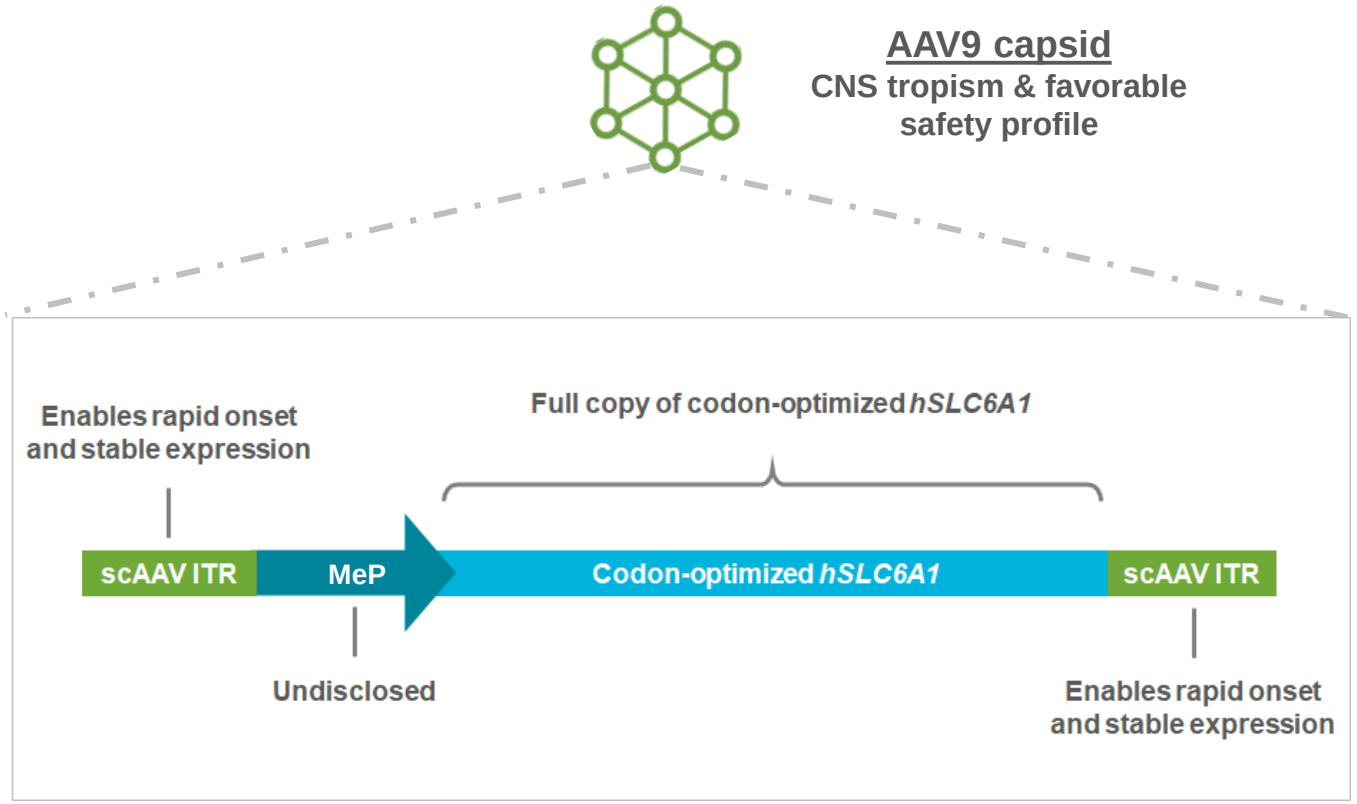
SLC6A1 haploinsufficiency disorder results in persistent seizures and developmental delays

- Autosomal dominant genetic disorder characterized by the loss of function of one copy of the *SLC6A1* gene
- SLC6A1 encodes the GABA transporter protein type 1 (GAT1), which is responsible for the reuptake of GABA into presynaptic neurons and glia
- Clinical manifestations include epilepsy, developmental delays, including mild or moderate intellectual disability, ataxia and autism
- No approved therapies
- Estimated prevalence of SLC6A1 haploinsufficiency disorder is 17,000 patients (US+EU)

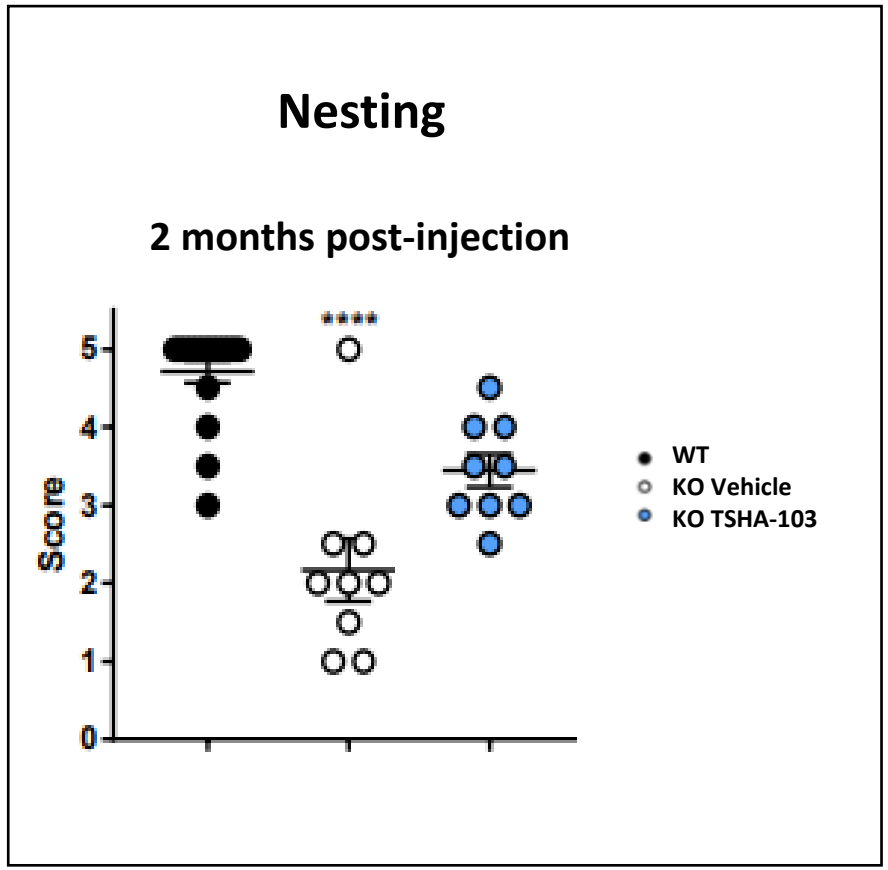


TSHA-103 in IND/CTA-enabling studies

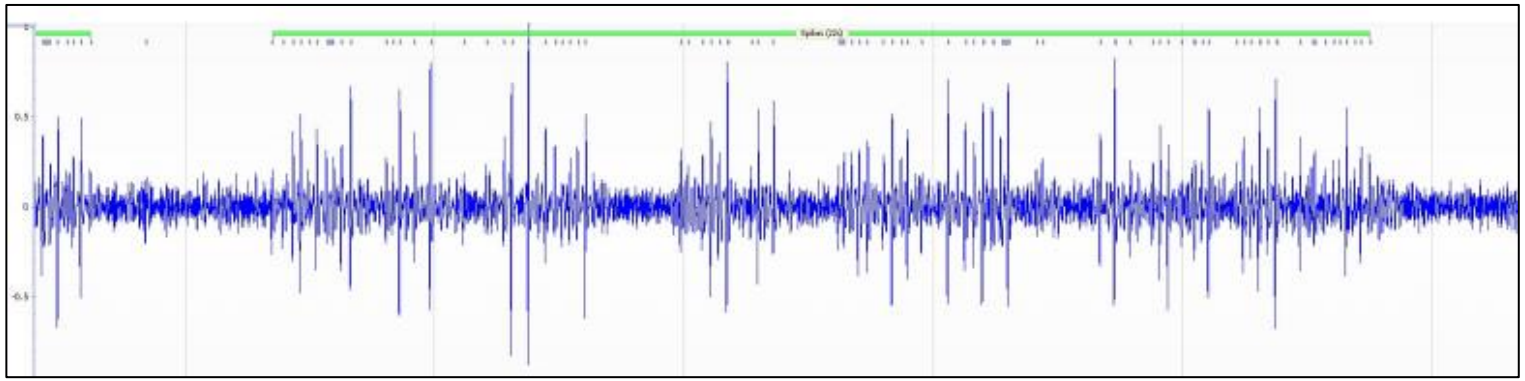
- Self-complementary AAV9 viral vector designed to deliver a functional copy of hSLC6A1
- Proof-of-concept demonstrated in knockout SLC6A1 mouse model
- Delivered intrathecally
- Received orphan drug and rare pediatric disease designations
- Currently in IND/CTA-enabling studies



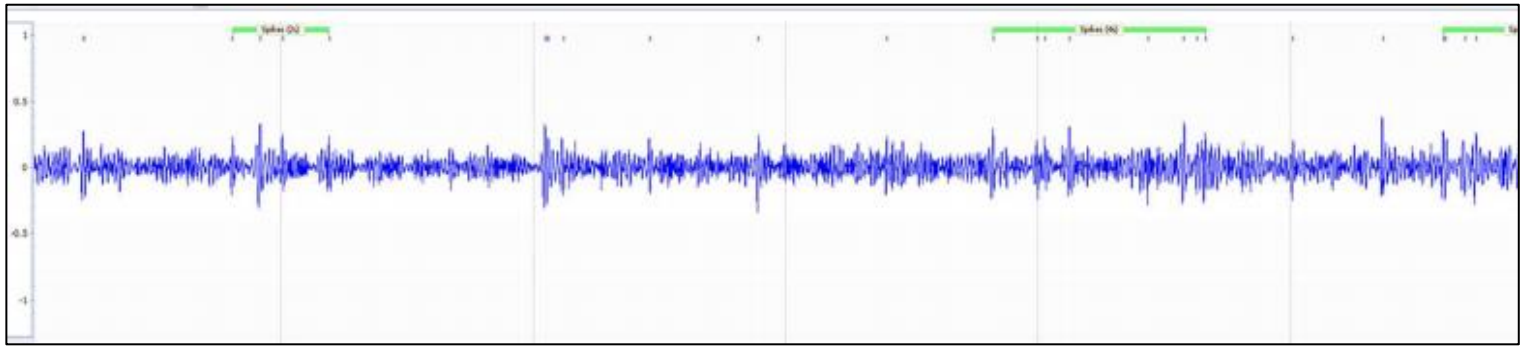
TSHA-103 improved nesting and EEG activity in SLC6A1 KO mouse model



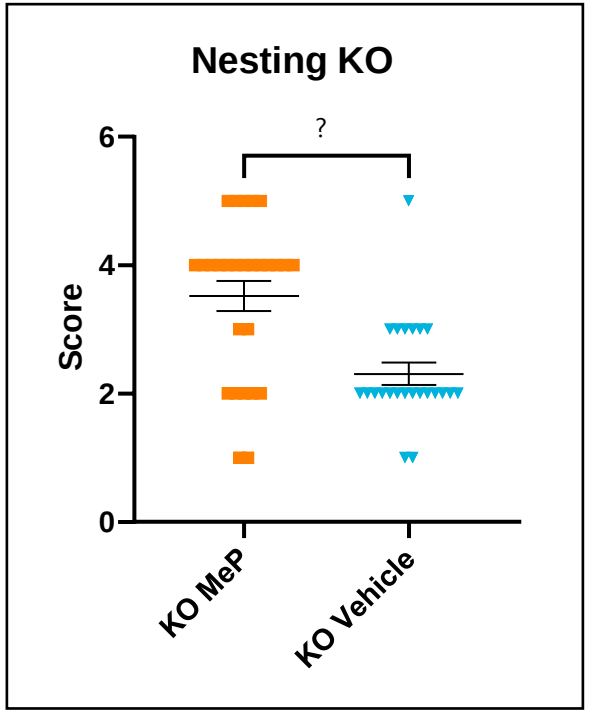
KO + vehicle

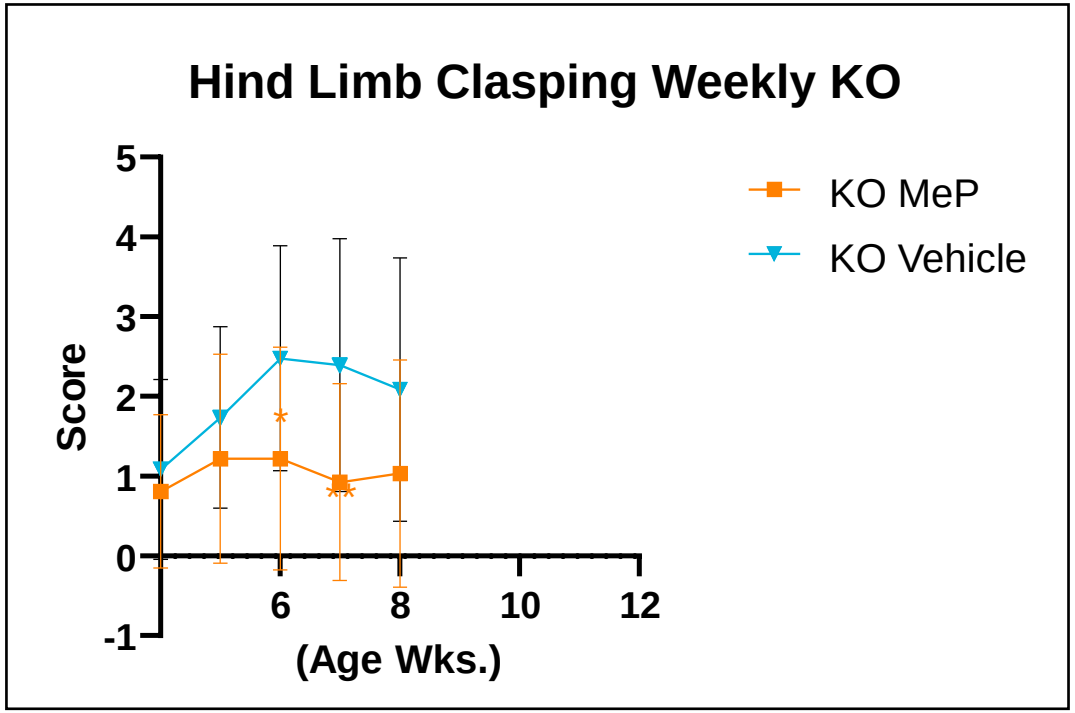


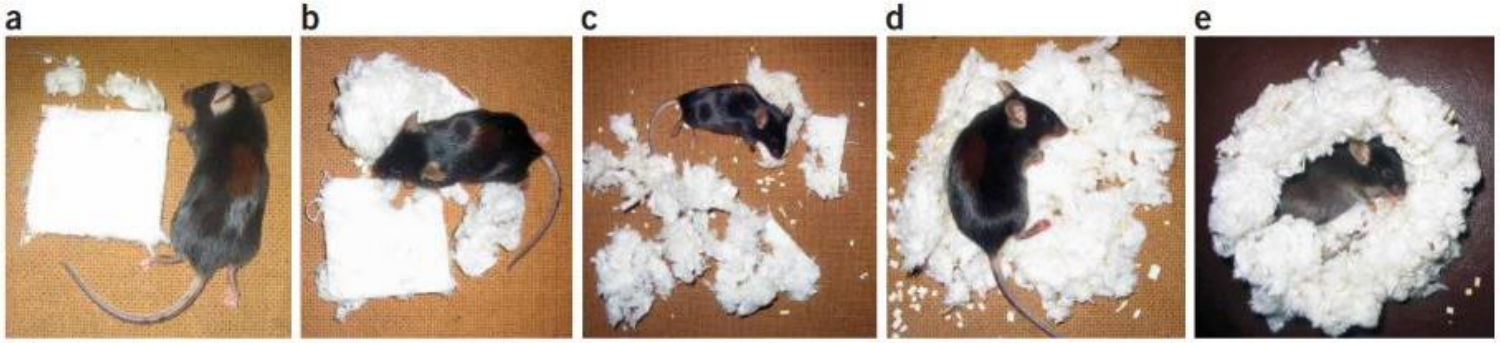
KO + TSHA-103



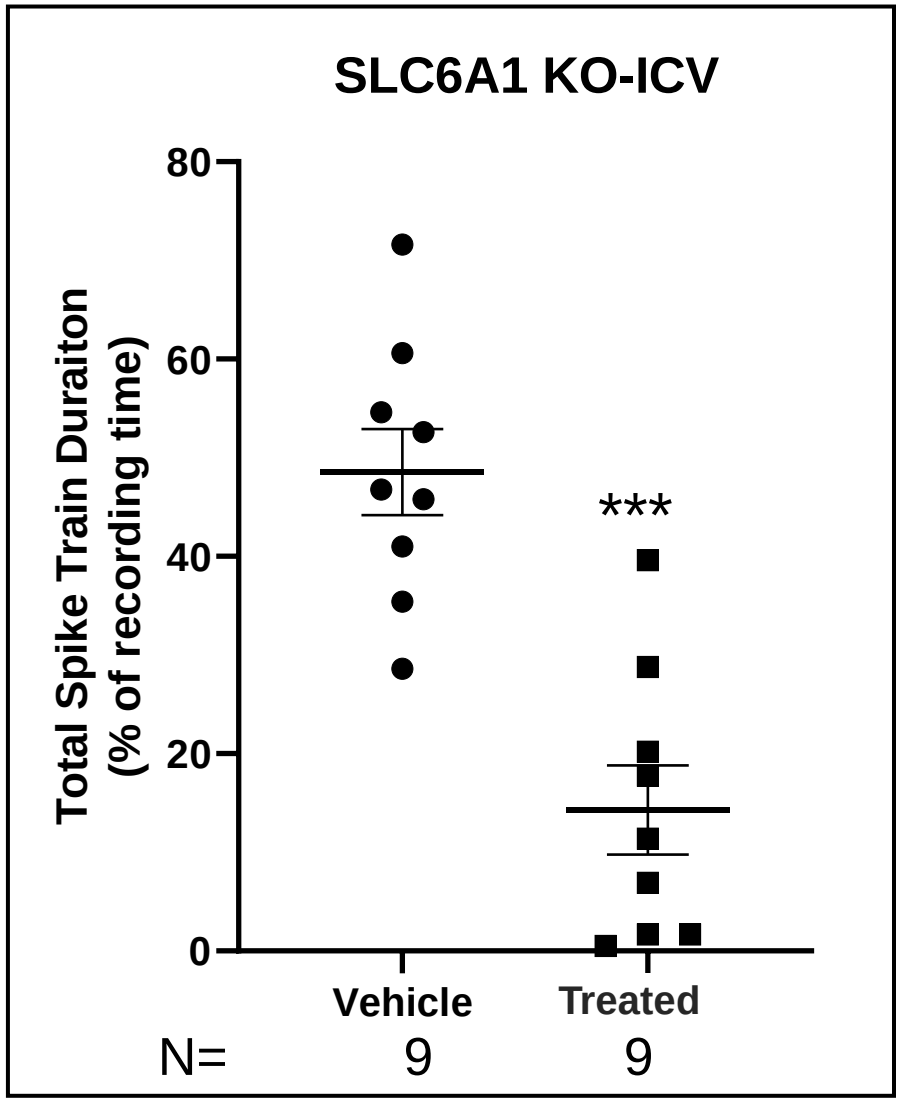
TSHA-103 improved nesting and hind limb clasping in SLC6A1 KO mouse model

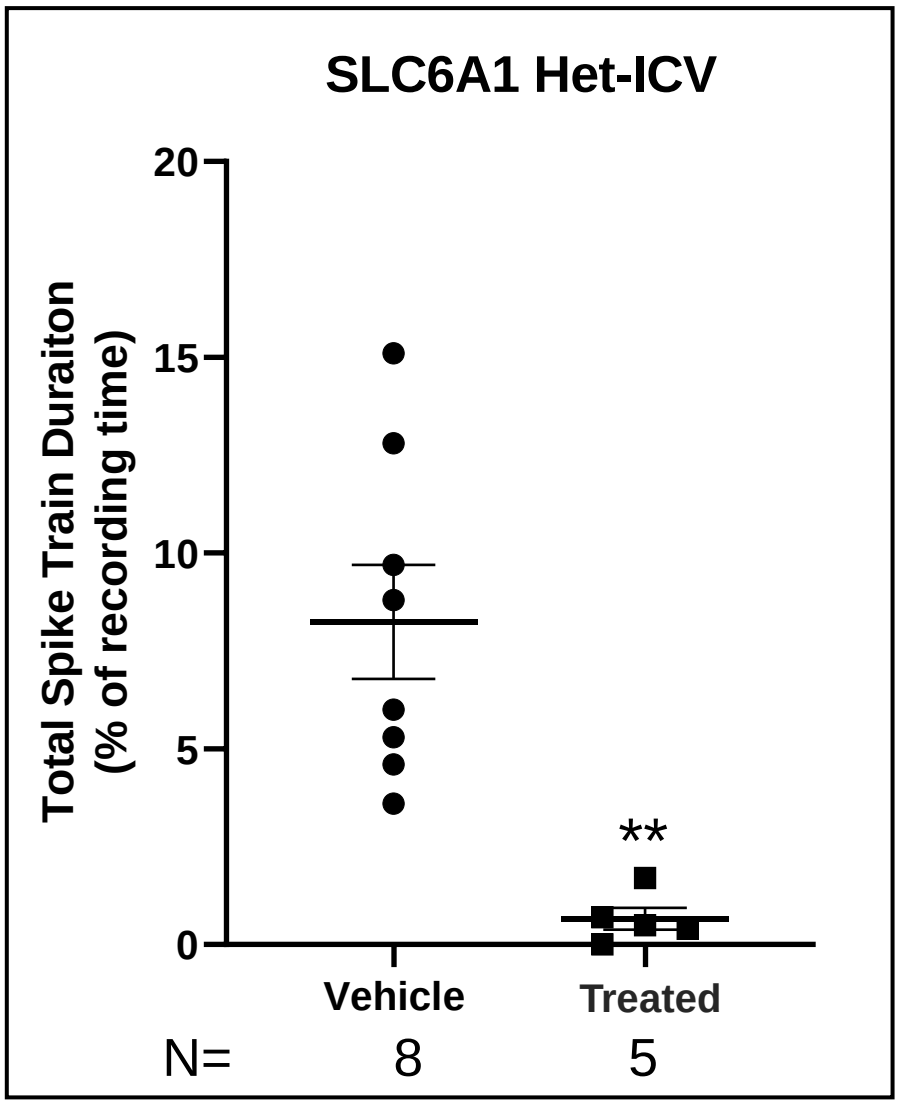




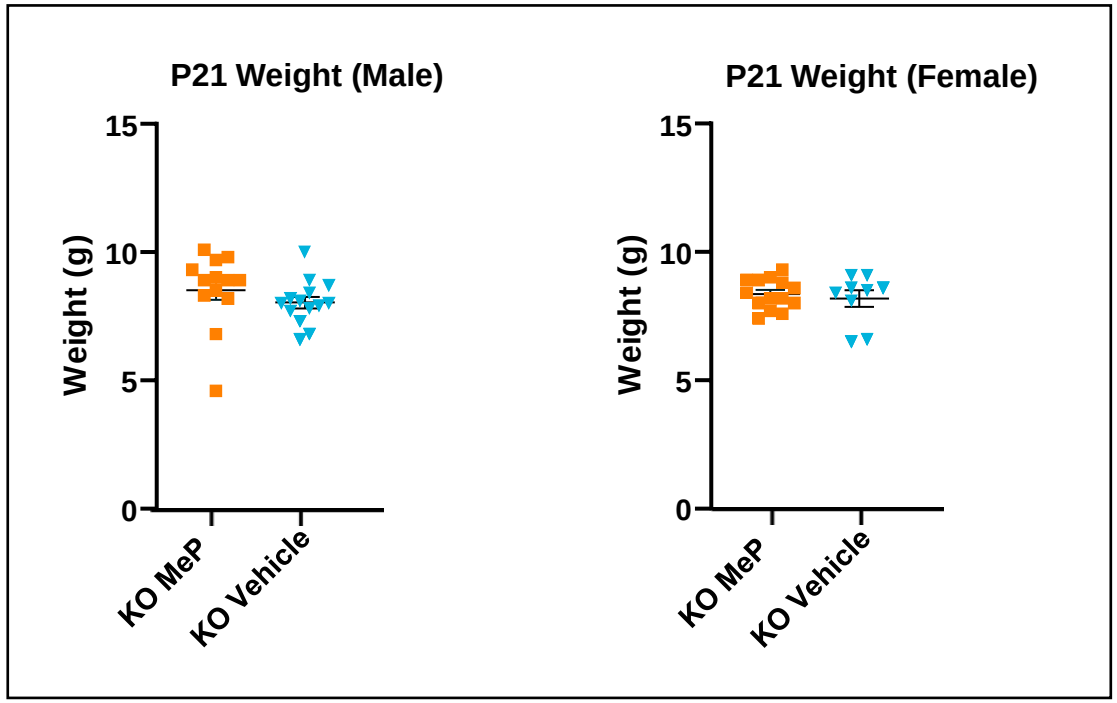


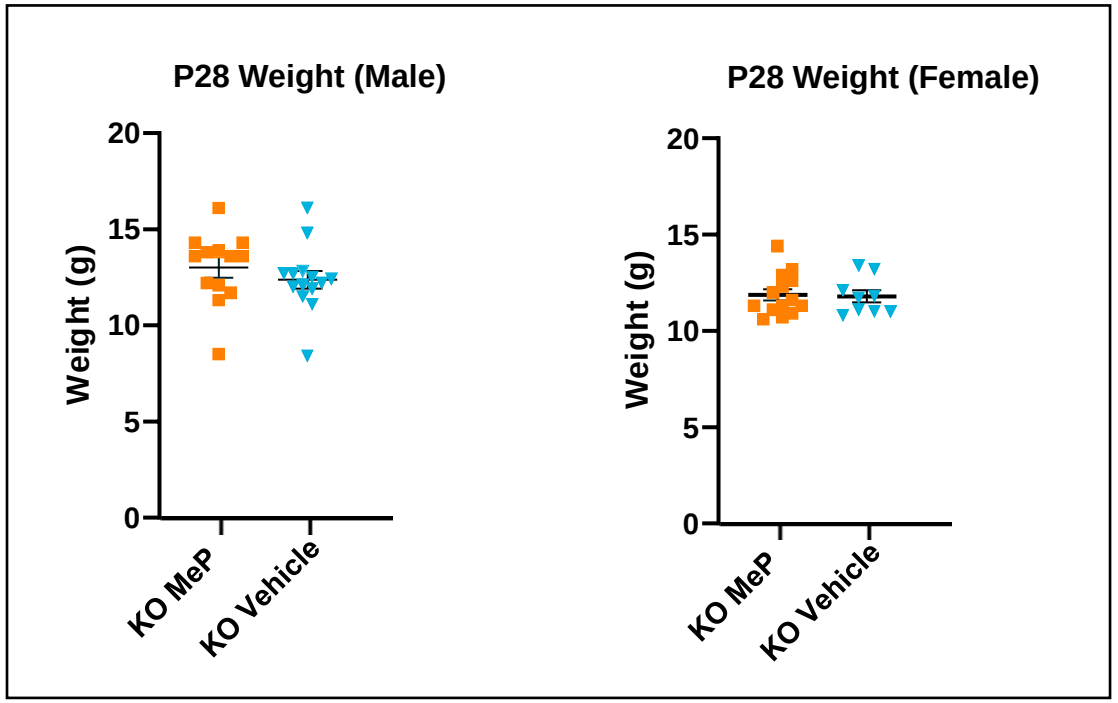
Neonatal ICV administration of TSHA-103 rescued abnormal EEG in SLC6A1 KO and heterozygous mouse models





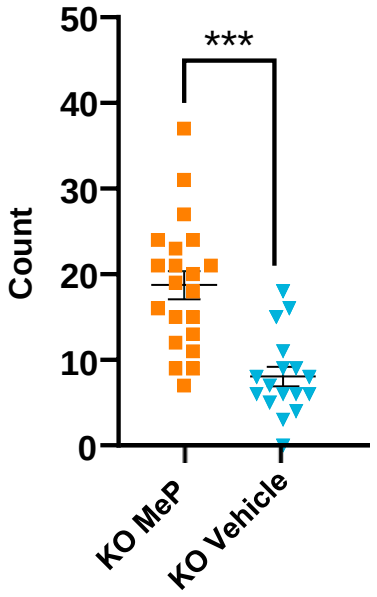
Neonatal ICV administration of TSHA-103 was safe in SLC6A1 knockout mice



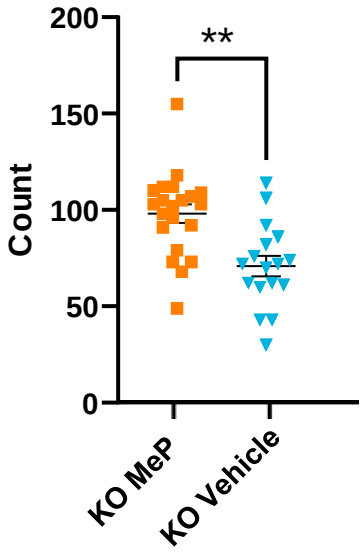


Neonatal ICV administration of TSHA-103 reduced spike train activity

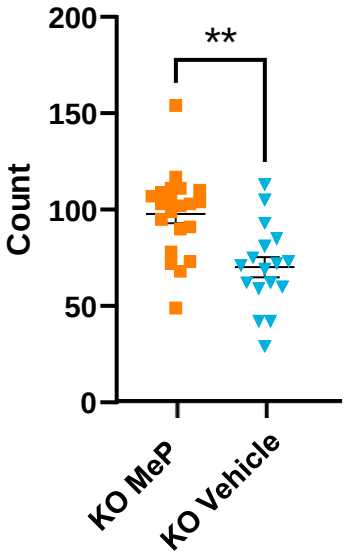
Center Frequency



Periphery Frequency

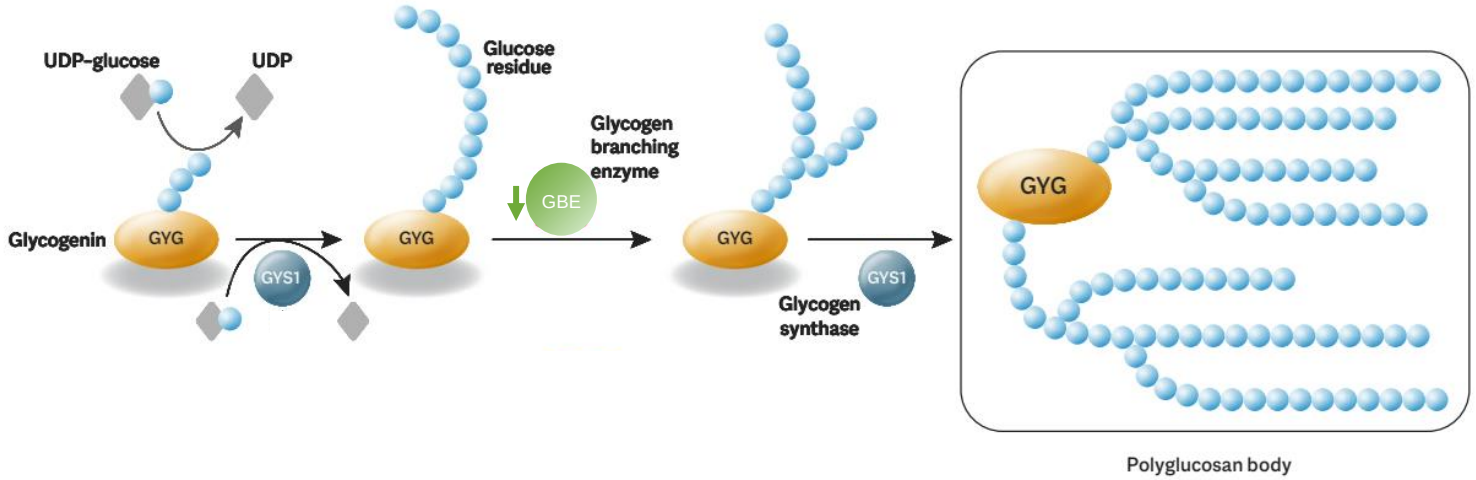


Non-Periphery Frequency



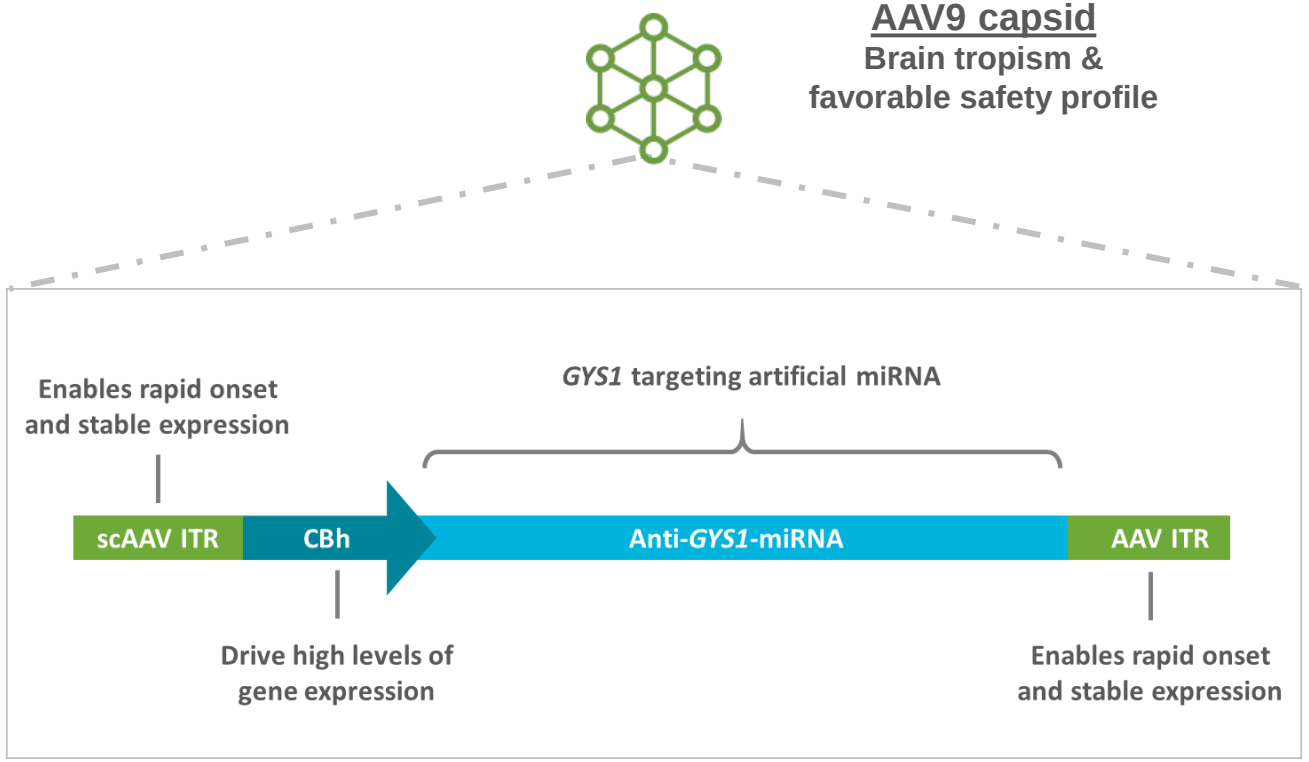
Adult polyglucosan body disease (APBD)

- Caused by a mutation in the *GBE1* gene, responsible for the creation of branches during glycogen synthesis
- Reduction in glycogen synthesis yields elongated glycogen changes that form poorly soluble aggregates in the liver, muscle and CNS
- Prime of life disease, with onset between 40-50 years
- Signs and symptoms include sensory loss in the legs, progressive muscle weakness, gait disturbances, mild cognitive impairment and urinary difficulties
- Often misdiagnosed as multiple sclerosis
- No approved therapies
- Estimated prevalence of APBD is 10,000 patients (US+EU)

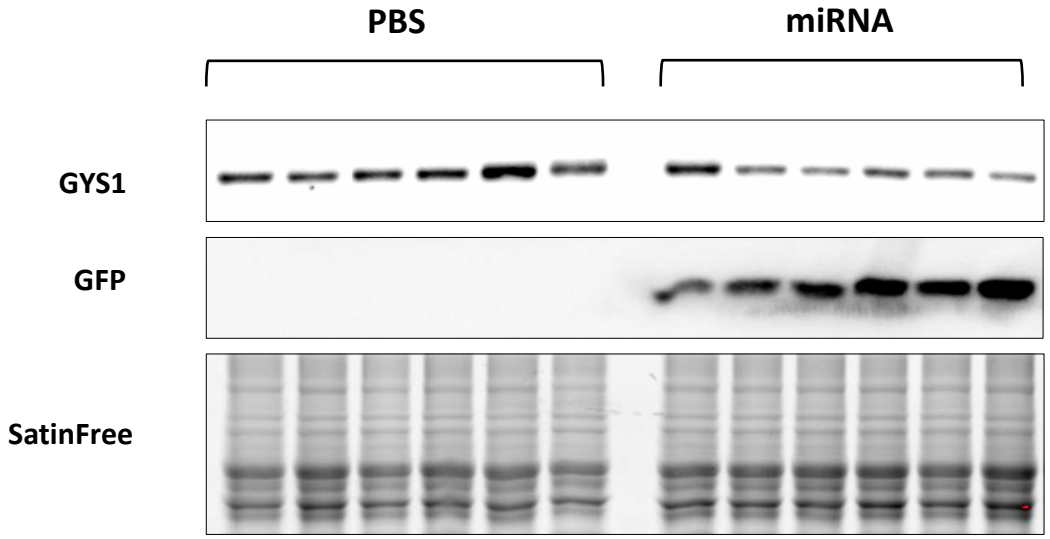


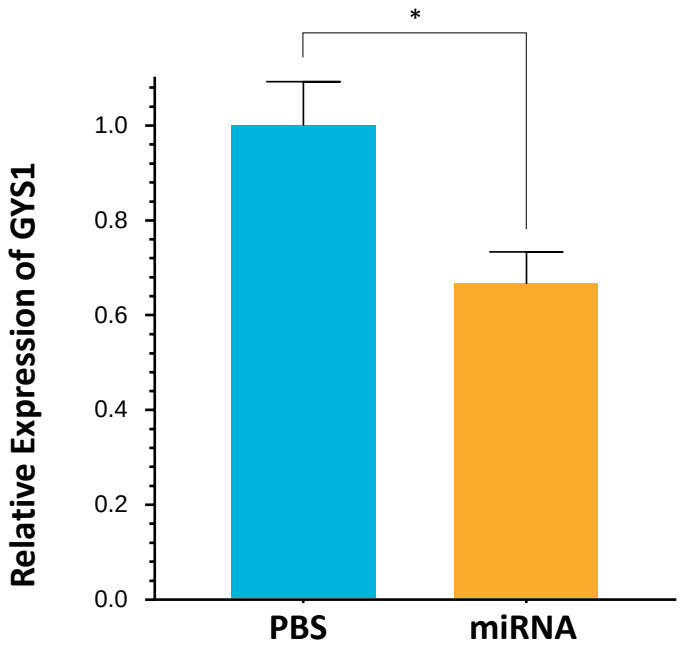
TSHA-112 expected to advance in IND/CTA-enabling studies in 2021

- Recombinant AAV9 viral vector designed for miRNA-mediated knockdown of the *GYS1* gene to treat APBD
- Self-complementary AAV capsid (scAAV) for rapid activation and stable expression
- CBh promoter drives high levels of expression
- Currently in IND/CTA-enabling study



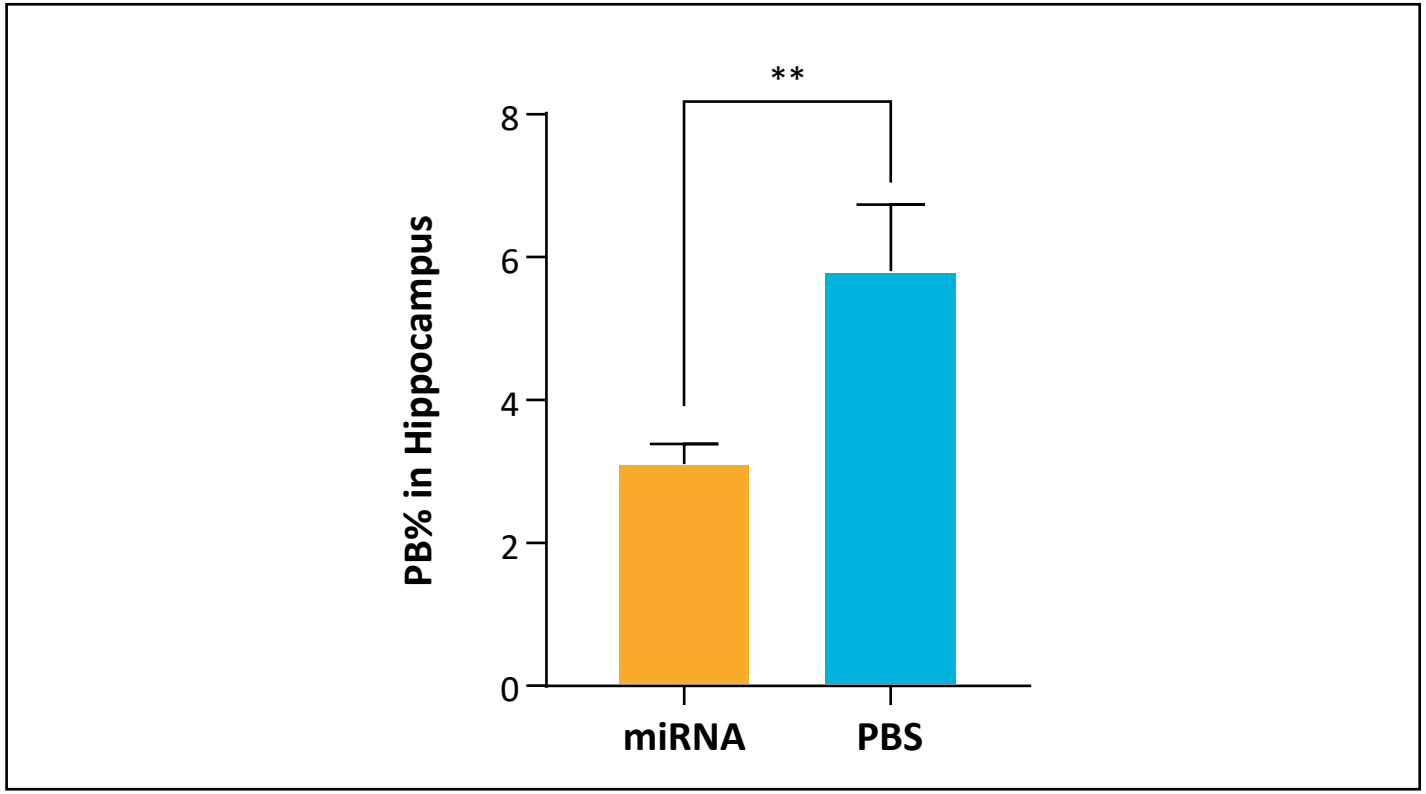
TSHA-112 reduced GYS1 expression in the APBD KO model





Gumusgoz, E. 2020

TSHA-112 decreased polyglucosan body formation in mice hippocampus



TSHA-112 decreased polyglucosan body formation in the hippocampus

Anticipated next steps for TSHA-112

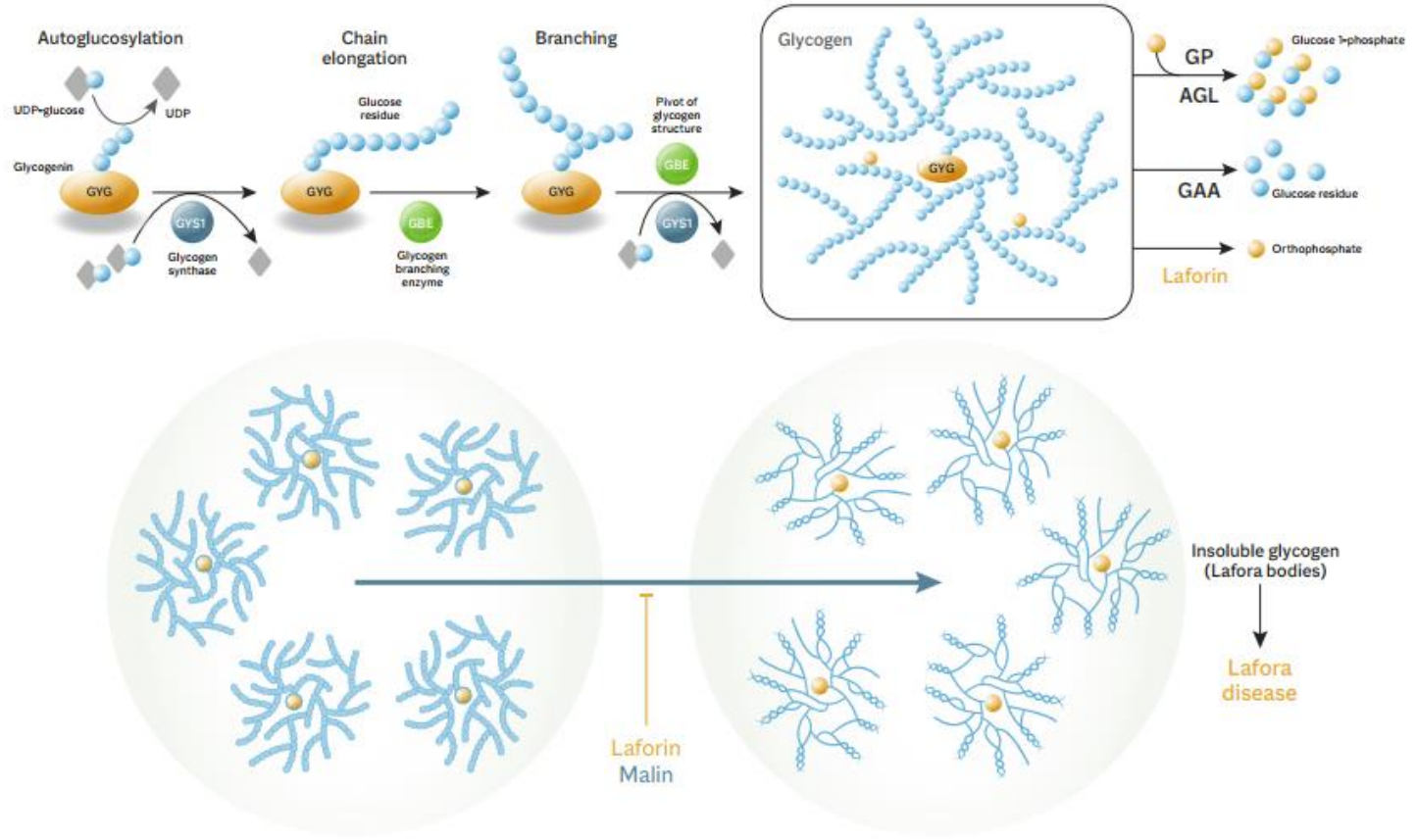
Animal proof-of-concept achieved in KO mouse model

Evaluate dose response and age response and finalize dose from pharmacology

Interventional trial protocol development underway

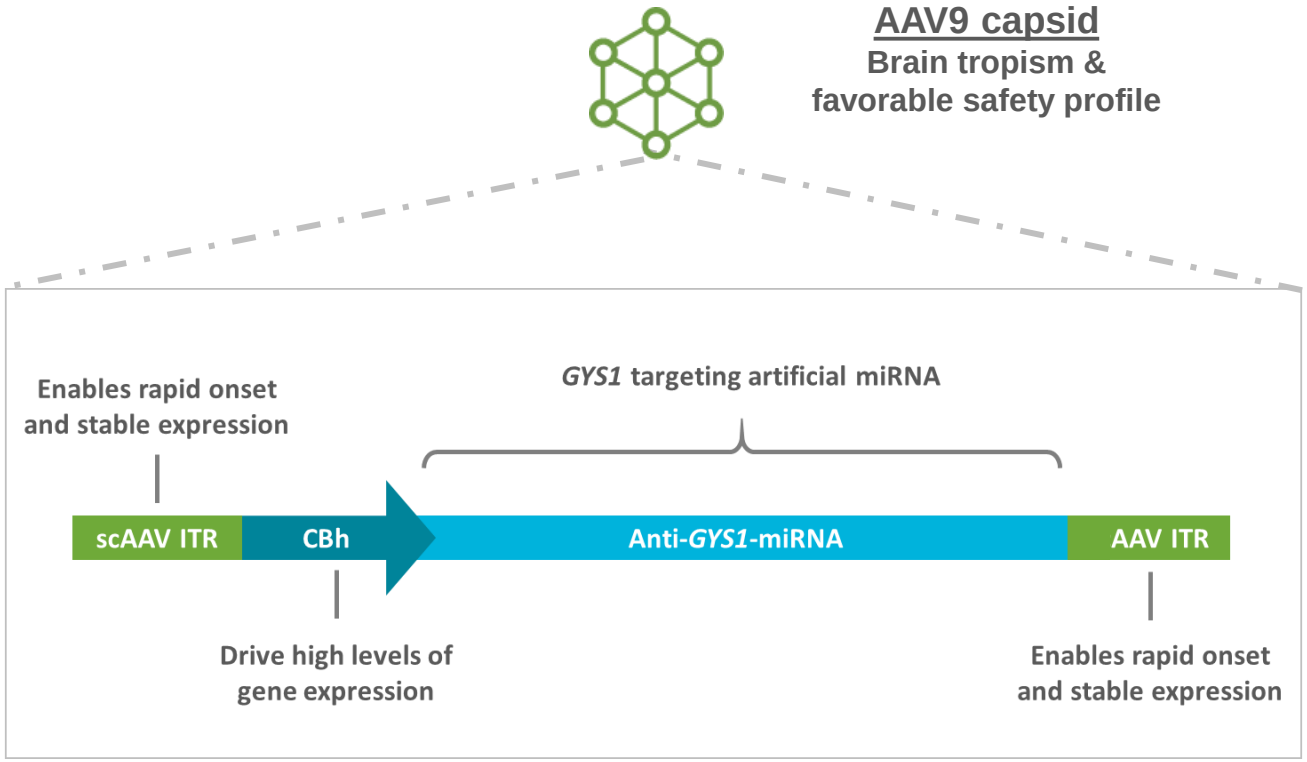
Lafora disease is a progressive and fatal neurodegenerative disorder

- Inherited, severe form of progressive myoclonus epilepsy
- Caused by loss of function mutations in the *EPM2A* (laforin) or *EPM2B* (malin) genes responsible for glycogen metabolism
- Absence of laforin or malin results in aggregates of polyglucosans or abnormally shaped glycogen molecules known as Lafora bodies
- Signs and symptoms include recurrent epileptic seizures in late childhood or adolescence, difficulty walking, muscle spasms and dementia
- Fatal within 10 years of onset
- No approved therapies
- Estimated prevalence of Lafora disease is 700 patients (US+EU)

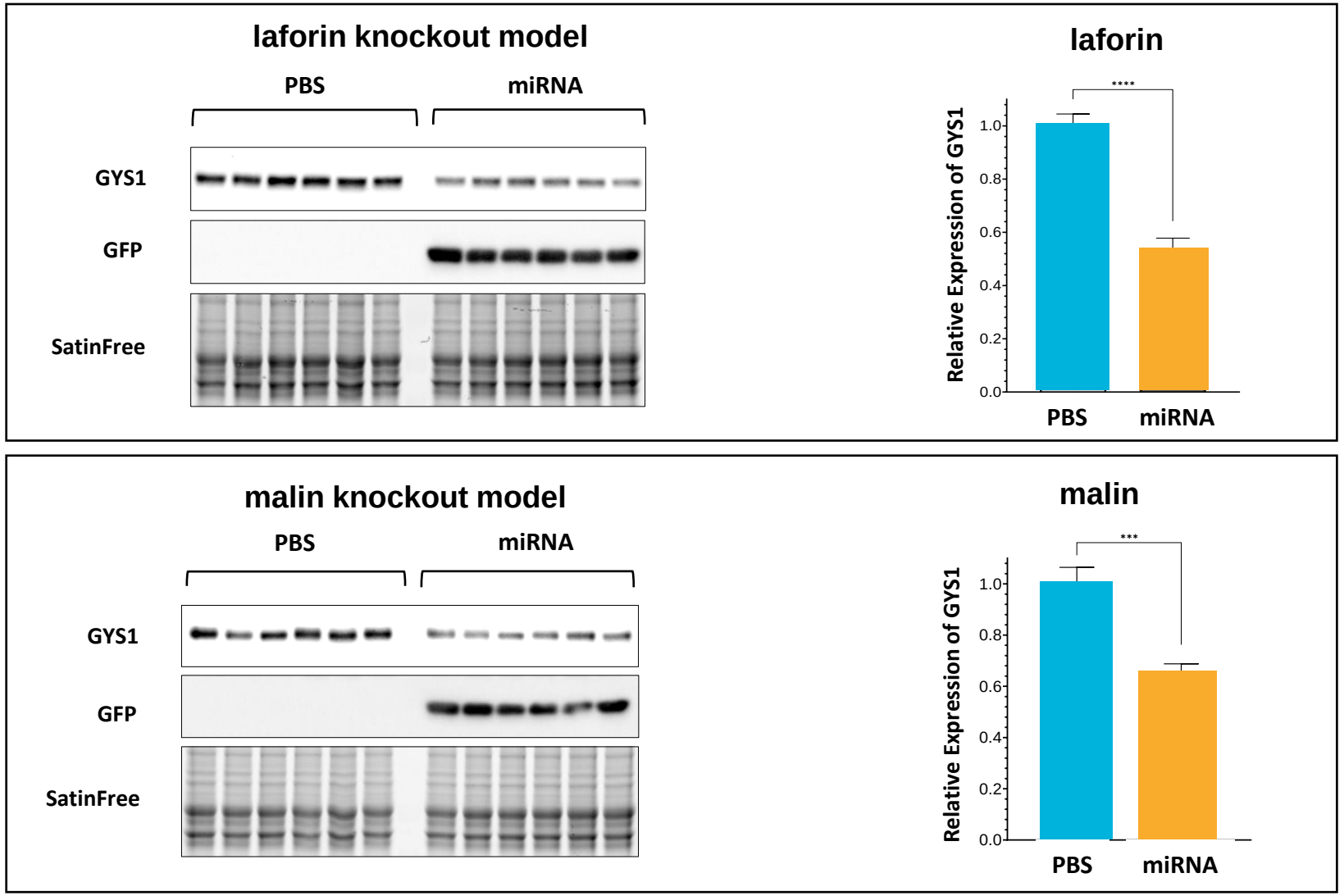


TSHA-111-LAFORIN and TSHA-111-MALIN, miRNA approaches

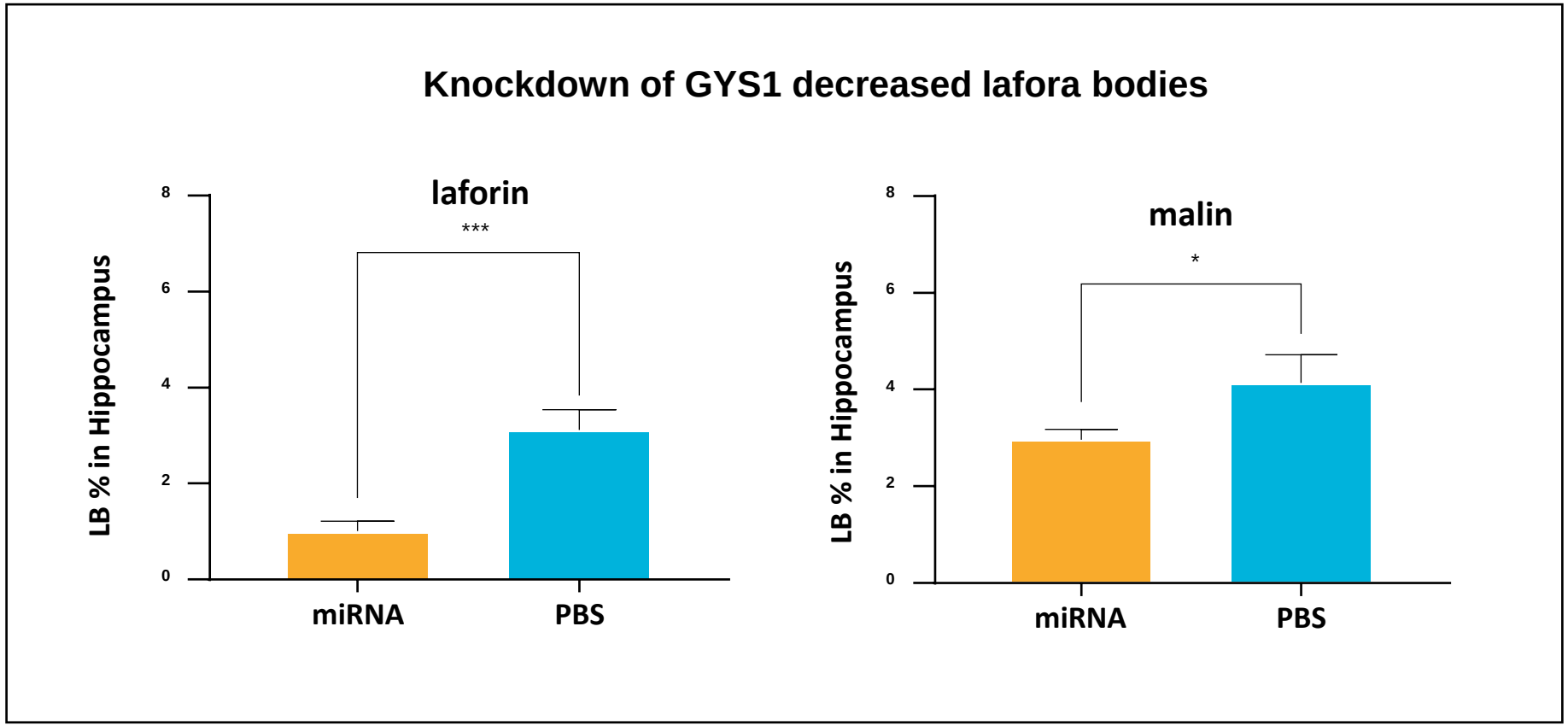
- Recombinant AAV9 viral vector designed for miRNA-mediated knockdown of the *GYS1* gene
- GYS1 knockdown designed to reduce Lafora bodies and improve clinical condition
- Self-complementary AAV capsid (scAAV) for rapid activation and stable expression
- CBh promoter drives high levels of expression
- Currently in IND/CTA-enabling studies



TSHA-111-LAFORIN and TSHA-111-MALIN reduced GYS1 expression in the laforin and malin KO models



TSHA-111-LAFORIN and TSHA-111-MALIN decreased Lafora body formation in mice brain



Anticipated next steps for TSHA-111-LAFORIN and TSHA-111-MALIN

Animal proof-of-concept achieved in KO mouse model

Evaluate dose response and age response and finalize dose from pharmacology

Interventional trial protocol development underway

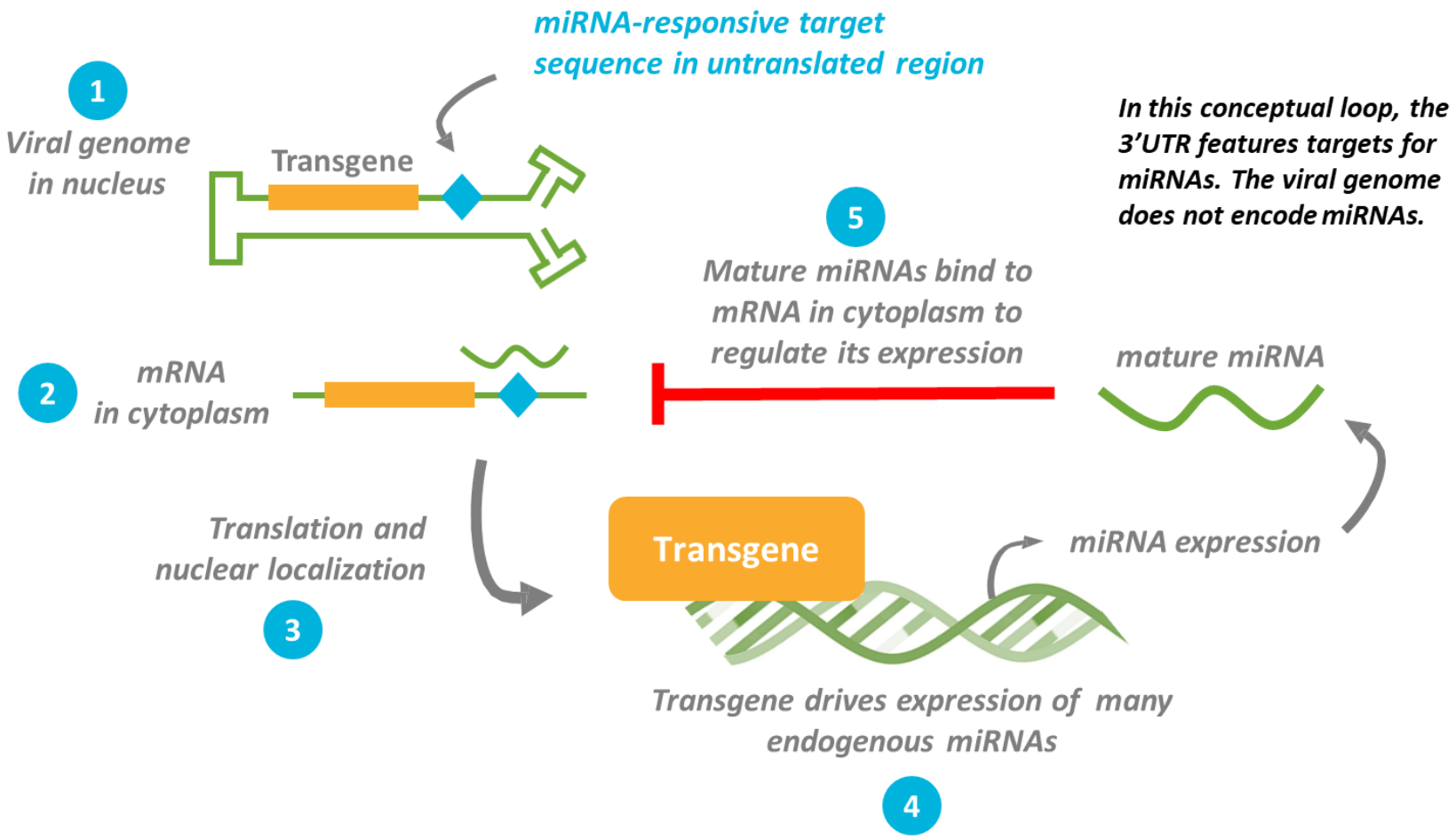
Deep pipeline of gene therapies targeting genetic epilepsies

TSHA-110 GRT
KCNQ2
Preclinical

- Diminished KCNQ2 function results in seizures in the first week of life, accompanied by developmental delay involving one or more domains of motor, social, language, or cognition
- Some children may have autistic features
- Estimated prevalence of 37,000 patients in the US and EU

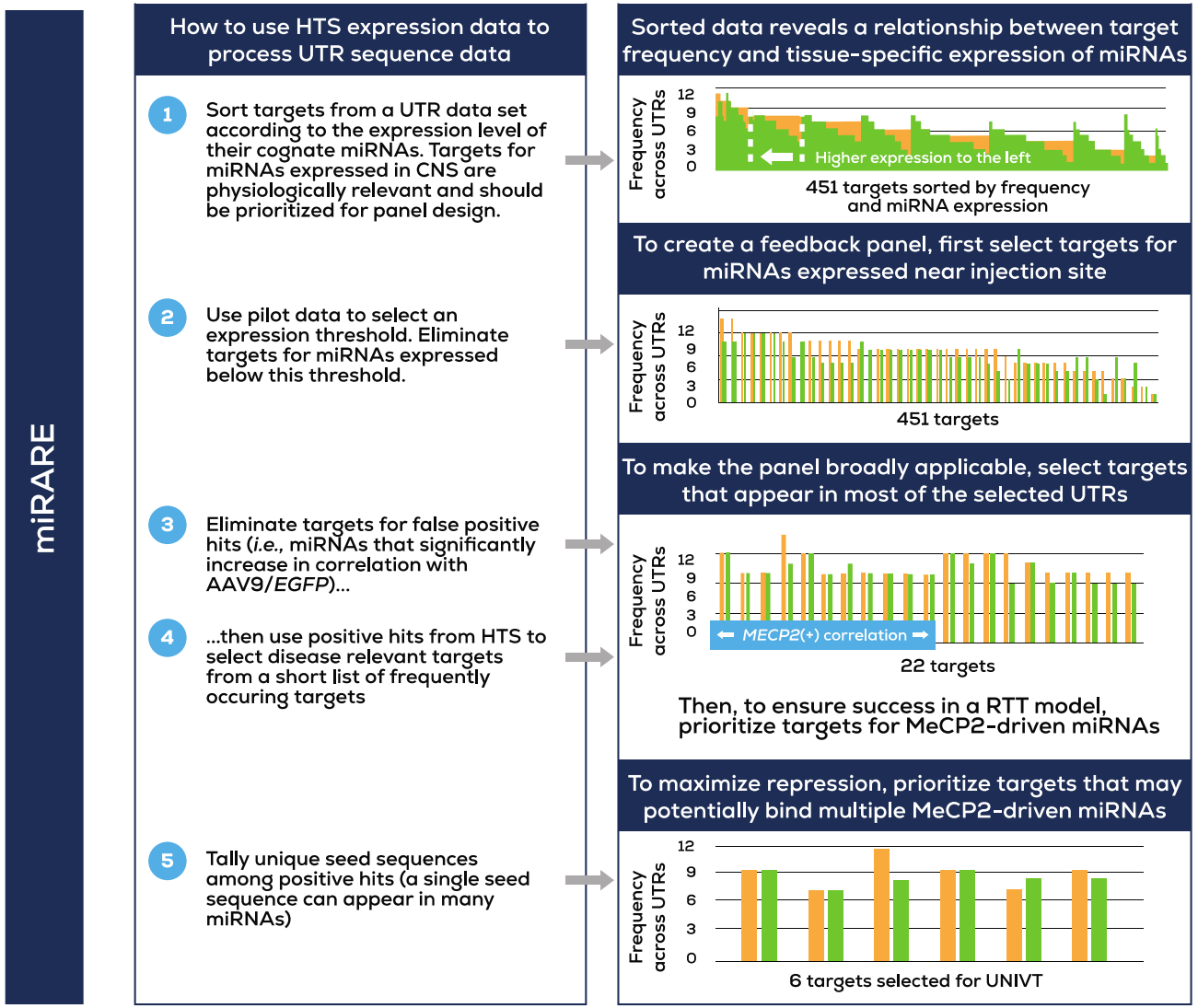
Platform Technologies

miRARE is a targeting panel for endogenous miRNAs which can regulate various transgenes

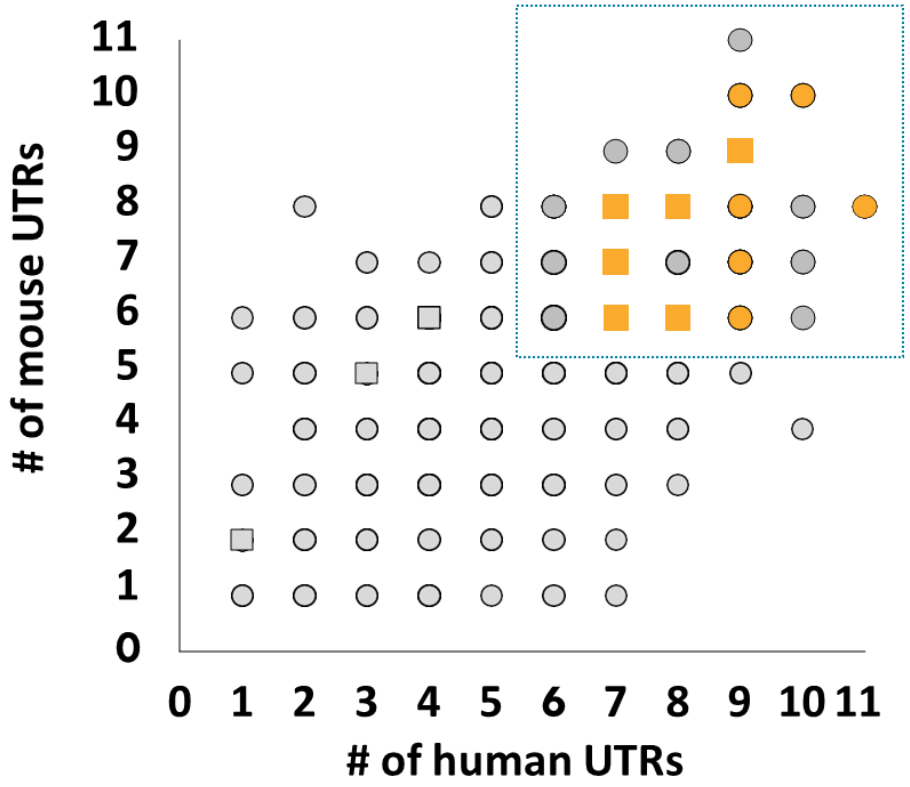


Approaches to create a miRNA target panel for regulating *MECP2* expression

- High-throughput screening of mouse CNS miRNAs upregulated after *MECP2* gene therapy overdose
- Identify endogenous miRNA targets that are conserved across species and appear frequently among the UTRs of dose-sensitive genes regulating intellectual ability
- Use positive results from high-throughput screening to filter and rank bioinformatics data
- Merge screening data and genomic sequence information
- Create a small synthetic (and potentially broadly applicable) regulatory panel



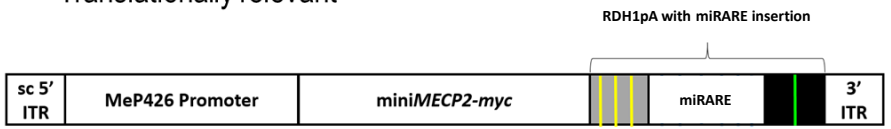
451 targets annotated across both species for selected 3’UTRs



- Many targets appear frequently among the 3’UTRs of dose-sensitive genes mediating disorders characterized by intellectual disability
- Bounded area: targets appear across ≥ 6 selected 3’UTRs
- Orange data points: corresponding miRNAs expressed in CNS tissue
- Squares: corresponding miRNAs are potentially MeCP2-responsive

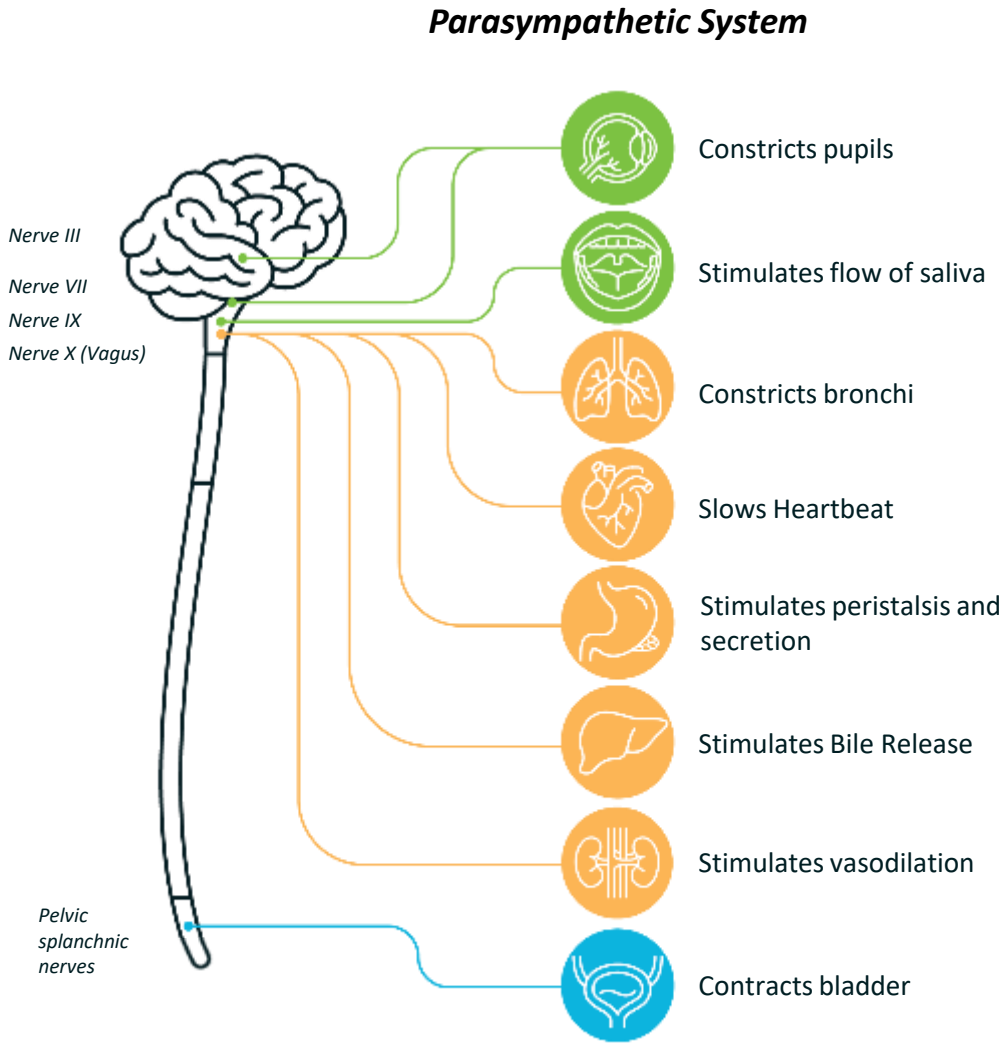
New target panel miRARE

- Compact (6 targets)
- Useful for RTT
- Possibly multipurpose
- Translationally relevant



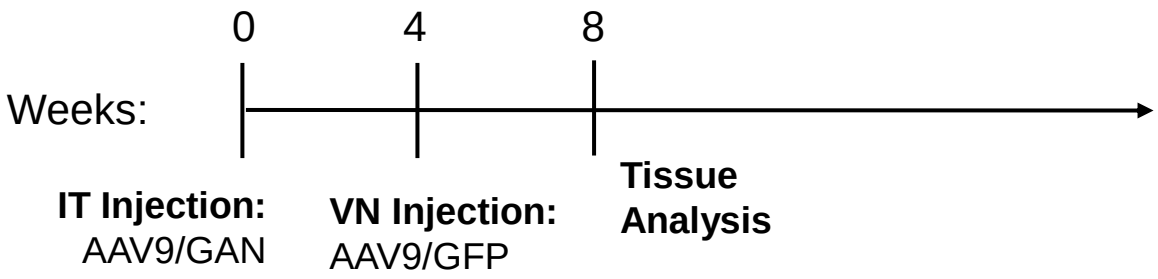
Opportunity to achieve human POC for vagus nerve redosing

- The vagus nerve represents the main component of the autonomic nervous system
- Direct delivery to the vagus nerve may provide broad coverage of the autonomic nervous system and enable redosing by subverting the humoral immune response
- Proof-of-concept established in rodent and canine models; oral presentation of data at ASGCT 2020
- Plan to execute confirmatory preclinical studies in canines
- Platform may be utilized to facilitate redosing of previously treated patients in the GAN AAV9 clinical trial as well as other indications

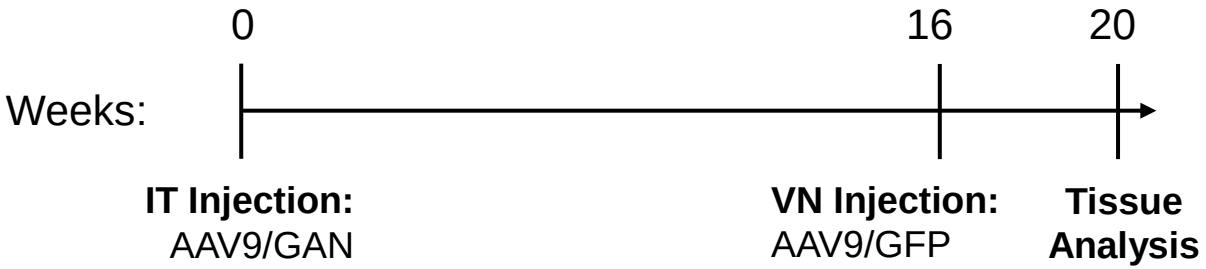


Robust expression of GFP in the vagus nerve and associated nodose ganglia in rats support redosing via vagus nerve injection

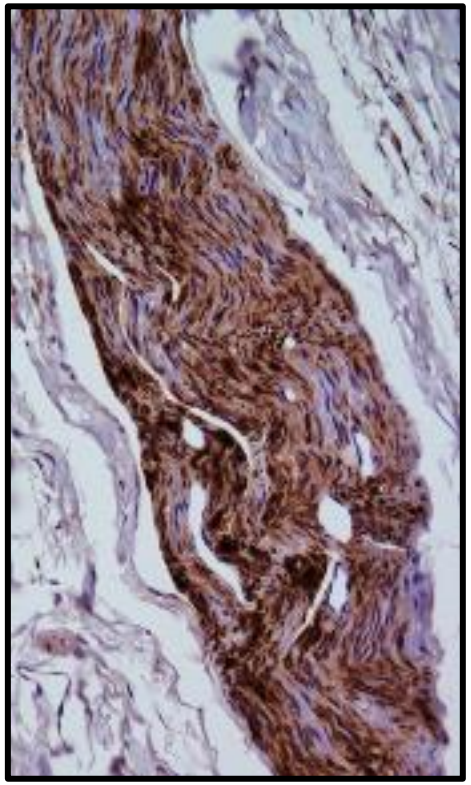
Study 1



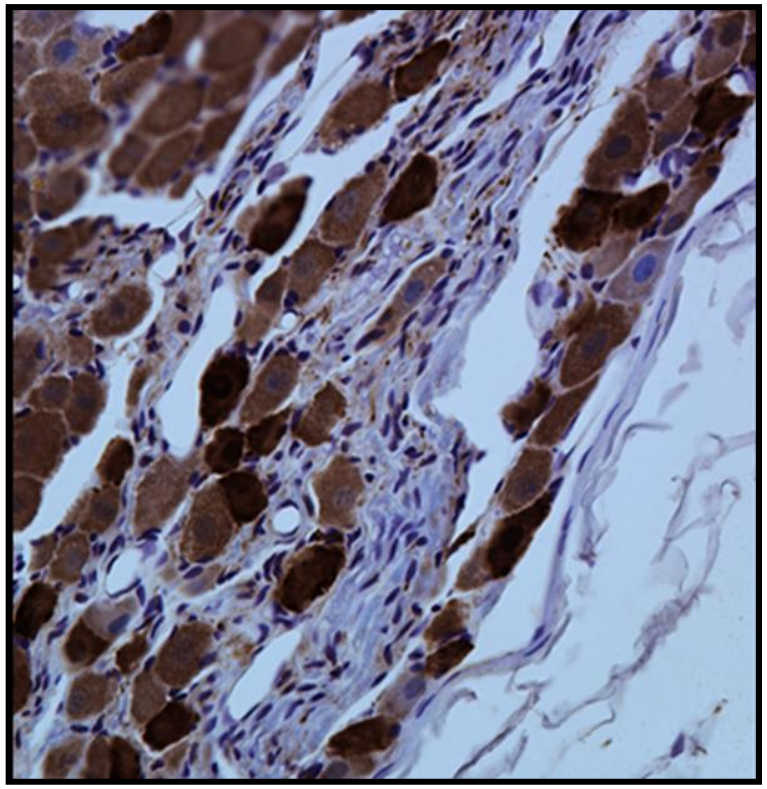
Study 2



Vagus Nerve

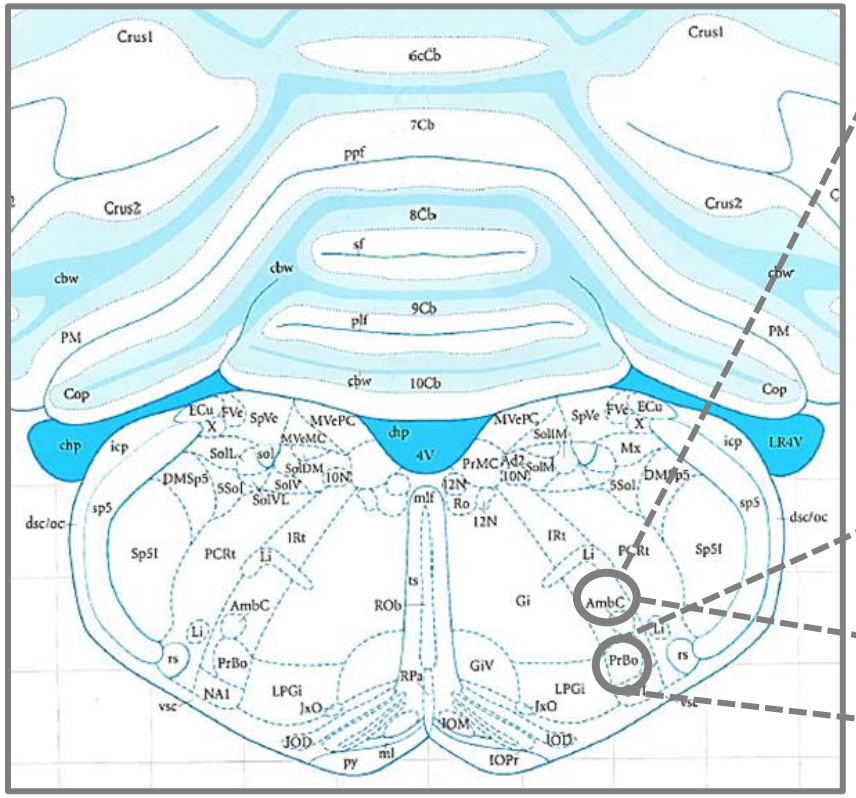


Nodose Ganglia

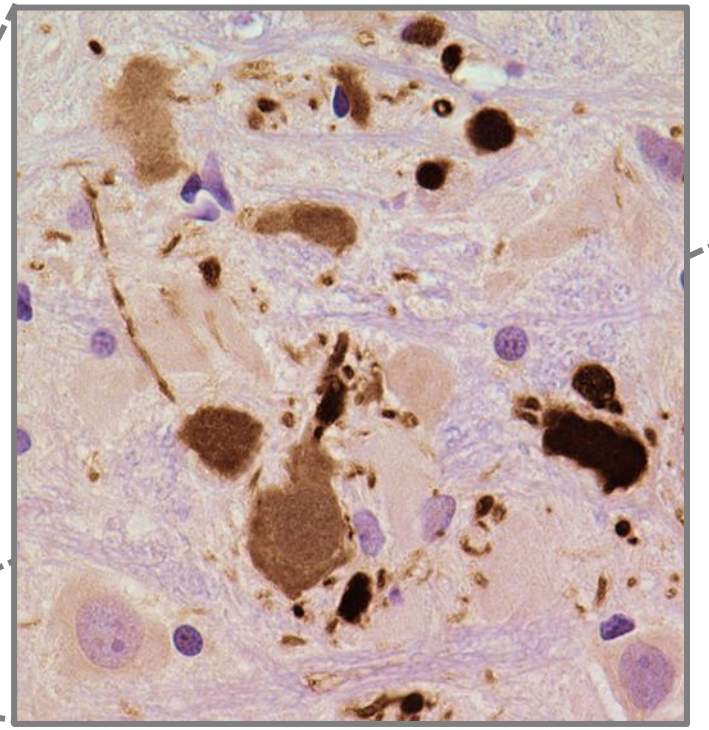


GFP – green fluorescent protein
Courtesy of Dr. Diane Armao

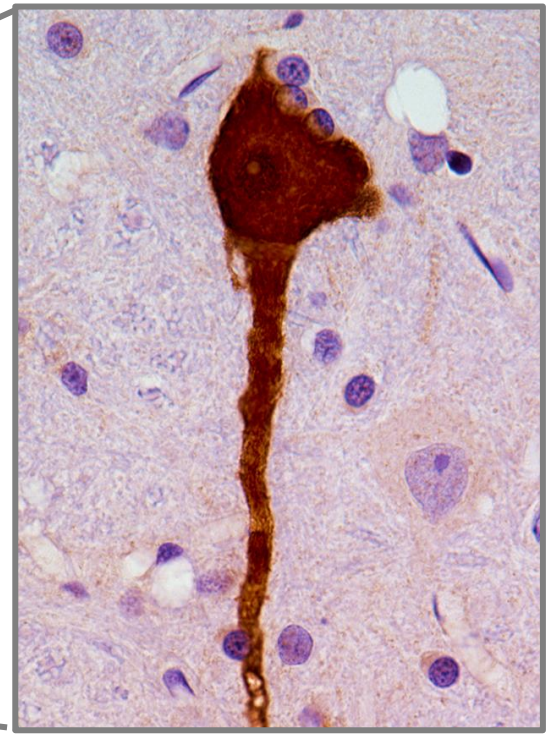
Successful transduction of relevant brain neurons following redosing via vagus nerve injection



Medulla



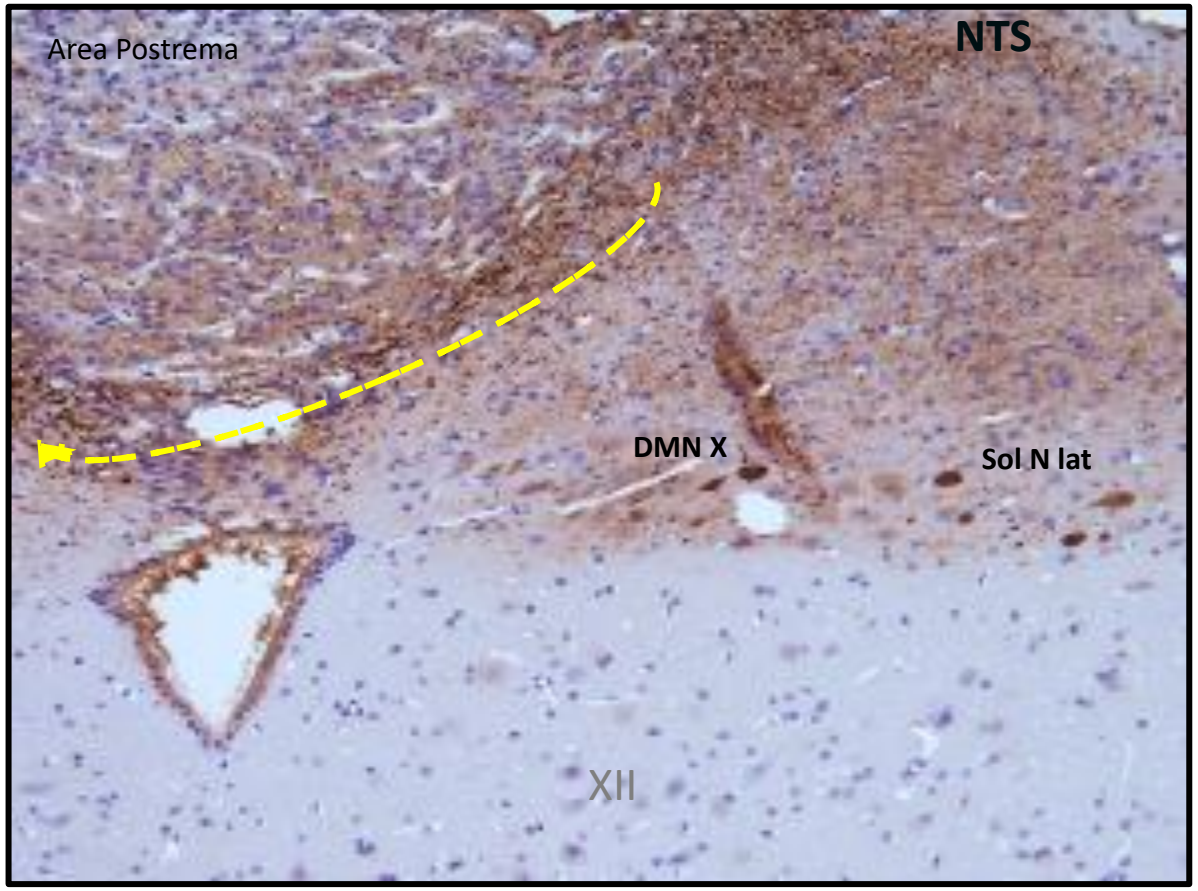
Nucleus Ambiguous



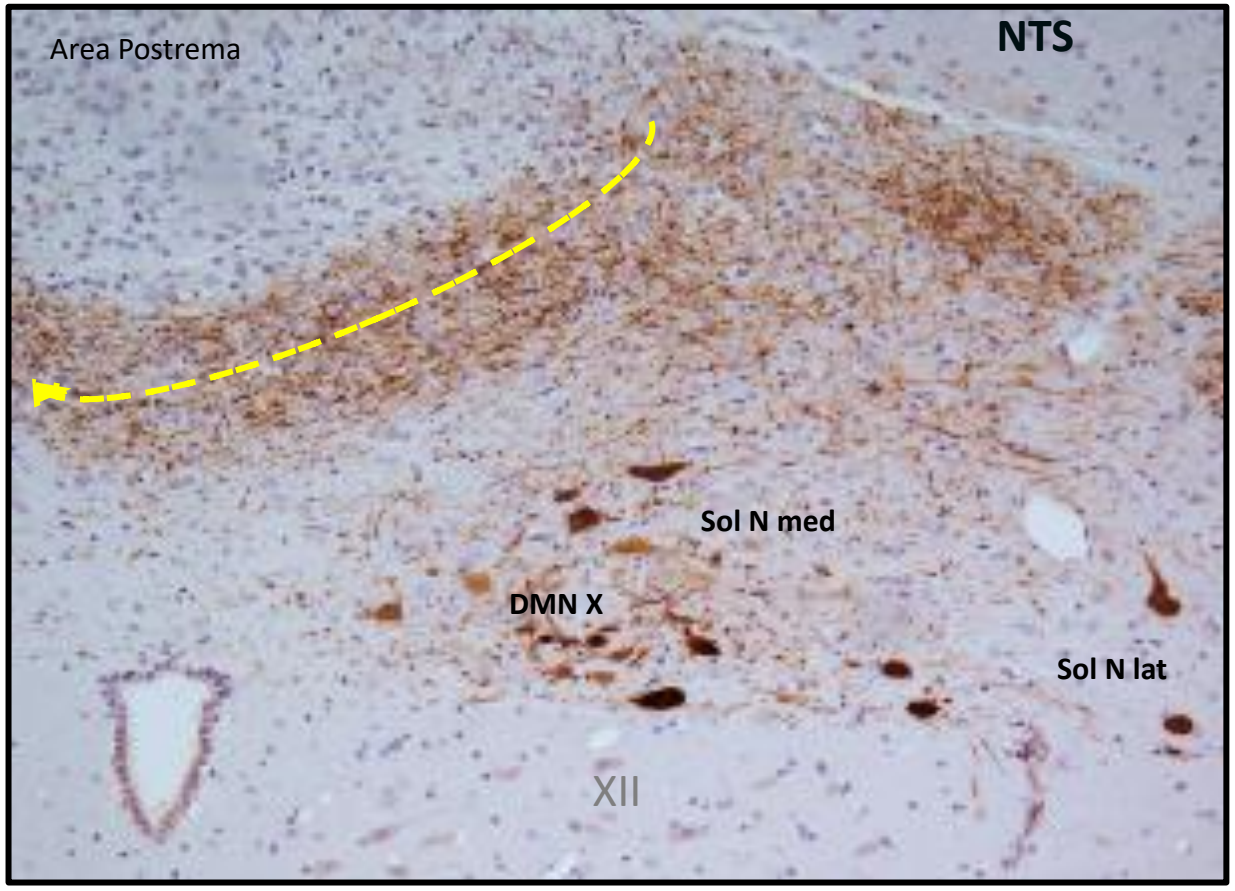
Pre-Botzinger Complex

Courtesy of Dr. Diane Armao

Vagus nerve injection permits AAV9 redosing; confirmed in brain slices of AAV9-immunized rats



Naive

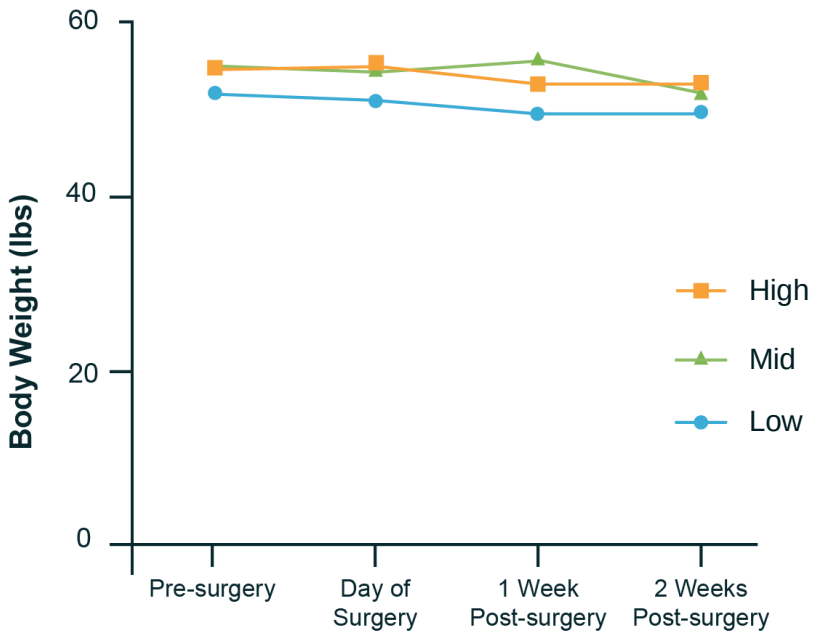


AAV9 Pre-immunized

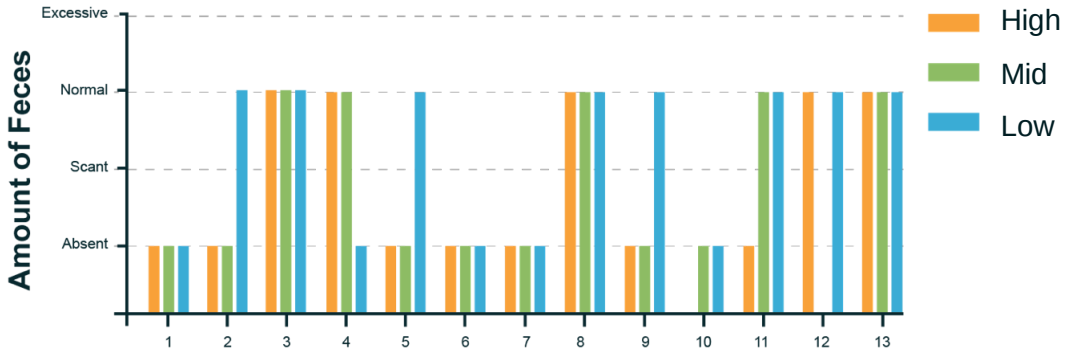
Courtesy of Dr. Diane Armao

Vagus nerve injection of increasing doses of AAV delivery were well-tolerated in hounds observed over 13 days

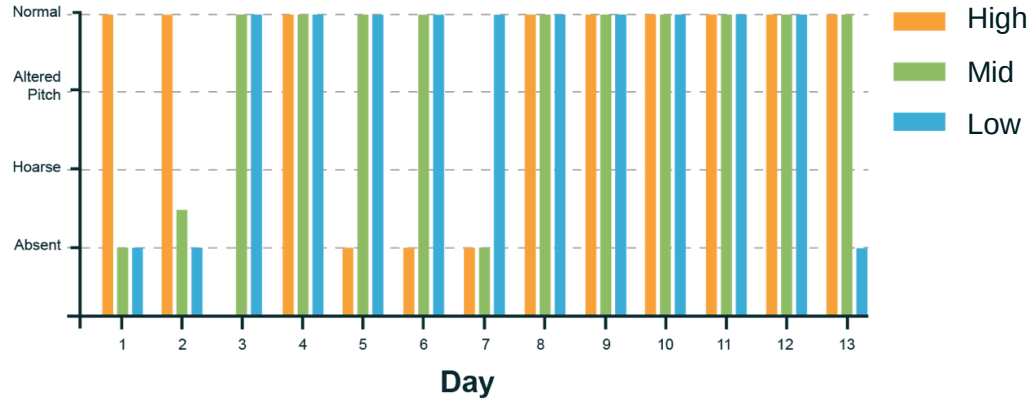
Testing VN Delivery Rates



Post-surgery Afternoon Fecal Output



Post-surgery Vocalization

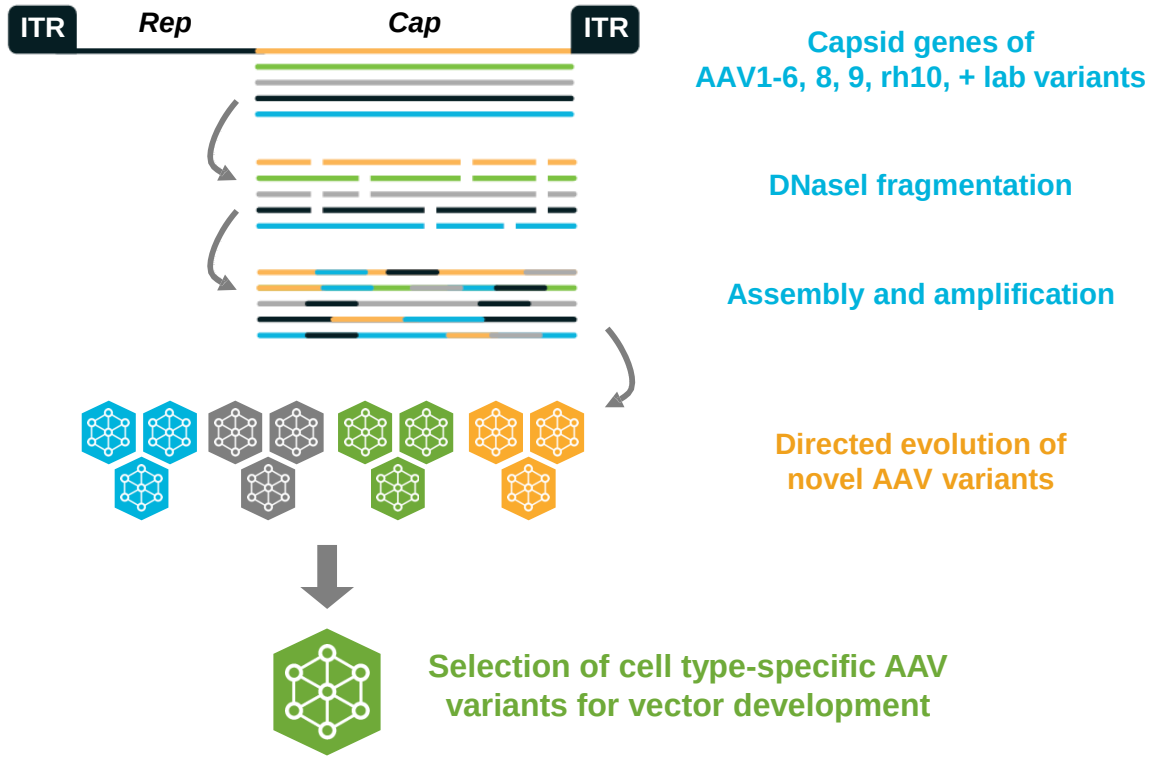


Post-mortem vagal nerves and brain were microscopically normal

[1]Taysha has exclusive rights to the vagus nerve redosing platform in select indications.

Utilizing machine learning, DNA shuffling, and directed evolution for capsid discovery

- High-content sequencing of recovered capsid pools
- Using sequencing data from *in vivo* selection to feed machine learning algorithms, for *in silico* design of novel capsids
- Development of new libraries, based on capsid-spanning modifications rather than just peptide insertions
- Directed evolution to generate CNS-directed capsids, cross-compatible between mice and NHPs



Focused on achieving anticipated near-term milestones in 2021 and building long-term value

GAN clinical program update, including 3.5 x 10^{14} total vg cohort
GM2 gangliosidosis preliminary biomarker data in 2H 2021
CLN1 program to dose first patient in 2021 under open IND
Preliminary CLN7 data for first-generation construct by YE 2021

5 open IND/CTAs expected by the end of 2021, including Rett syndrome

Major demolition and detailed design of internal cGMP facility completed

5 additional programs currently in IND-enabling studies
TSHA-106 Angelman Investor Day October 2021
Numerous value generating catalysts over the next 18 months

TAYSHA GENE THERAPIES

Thank you