UNITED STATES
SECURITIES AND EXCHANGE COMMISSION
WASHINGTON, D.C. 20549

FORM 8-K

CURRENT REPORT
Pursuant to Section 13 or 15(d)
of the Securities Exchange Act of 1934

Date of Report (Date of earliest event reported): June 28, 2021

Taysha Gene Therapies, Inc.
(Exact name of registrant as specified in its Charter)

Delaware	**001-39536**	**84-3199512**
(State or Other Jurisdiction of Incorporation)	**(Commission File Number)**	**(IRS Employer Identification No.)**

3000 Pegasus Park Drive, Suite 1430
Dallas, Texas **75247**
(Address of Principal Executive Offices) **(Zip Code)**

(214) 612-0000
(Registrant's Telephone Number, Including Area Code)

Not Applicable
(Former Name or Former Address, if Changed Since Last Report)

Check the appropriate box below if the Form 8-K filing is intended to simultaneously satisfy the filing obligation of the registrant under any of the following provisions (see General Instructions A.2. below):

☐ Written communications pursuant to Rule 425 under the Securities Act (17 CFR 230.425)

☐ Soliciting material pursuant to Rule 14a-12 under the Exchange Act (17 CFR 240.14a-12)

☐ Pre-commencement communications pursuant to Rule 14d-2(b) under the Exchange Act (17 CFR 240.14d-2(b))

☐ Pre-commencement communications pursuant to Rule 13e-4(c) under the Exchange Act (17 CFR 240.13e-4(c))

Securities registered pursuant to Section 12(b) of the Act:

Title of each class	Trading Symbol(s)	Name of each exchange on which registered
Common Stock, $0.00001 par value	**TSHA**	**The Nasdaq Stock Market LLC**

Indicate by check mark whether the registrant is an emerging growth company as defined in Rule 405 of the Securities Act of 1933 (§230.405 of this chapter) or Rule 12b-2 of the Securities Exchange Act of 1934 (§240.12b-2 of this chapter).

Emerging growth company ☒

If an emerging growth company, indicate by check mark if the registrant has elected not to use the extended transition period for complying with any new or revised financial accounting standards provided pursuant to Section 13(a) of the Exchange Act. ☐

Item 7.01 Regulation FD Disclosure.

On June 28, 2021, Taysha Gene Therapies, Inc. (the "Company") will host the first day of a two-day virtual research and development day (the "R&D Day") for analysts and investors to highlight the Company's research and development progress, focused on advancement of its early- and late-stage investigational programs. A copy of the presentation, dated June 28, 2021, that the Company intends to use on the first day of the Company's R&D Day is furnished as Exhibit 99.1 to this Current Report on Form 8-K.

The information in this Item 7.01 of this Current Report on 8-K (including Exhibit 99.1) is furnished and shall not be deemed "filed" for purposes of Section 18 of the Securities Exchange Act of 1934, as amended, or subject to the liabilities of that section or Sections 11 and 12(a)(2) of the Securities Act of 1933, as amended. The information shall not be deemed incorporated by reference into any other filing with the Securities and Exchange Commission made by the Company, whether made before or after today's date, regardless of any general incorporation language in such filing, except as shall be expressly set forth by specific references in such filing.

Item 9.01 Financial Statements and Exhibits.

(d) Exhibits

Exhibit No.	Description
99.1	Company presentation, dated June 28, 2021

SIGNATURES

Pursuant to the requirements of the Securities Exchange Act of 1934, as amended, the Registrant has duly caused this report to be signed on its behalf by the undersigned hereunto duly authorized.

Taysha Gene Therapies, Inc.

Dated: June 28, 2021

By: /s/ Kamran Alam
Kamran Alam
Chief Financial Officer

TAYSHA GENE THERAPIES

Bringing New Cures to Life

RESEARCH & DEVELOPMENT DAY

DAY 1 – June 28, 2021 | 9:00 AM – 12:00 PM CT

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. 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.

Introductions & Company Overview

RA Session II

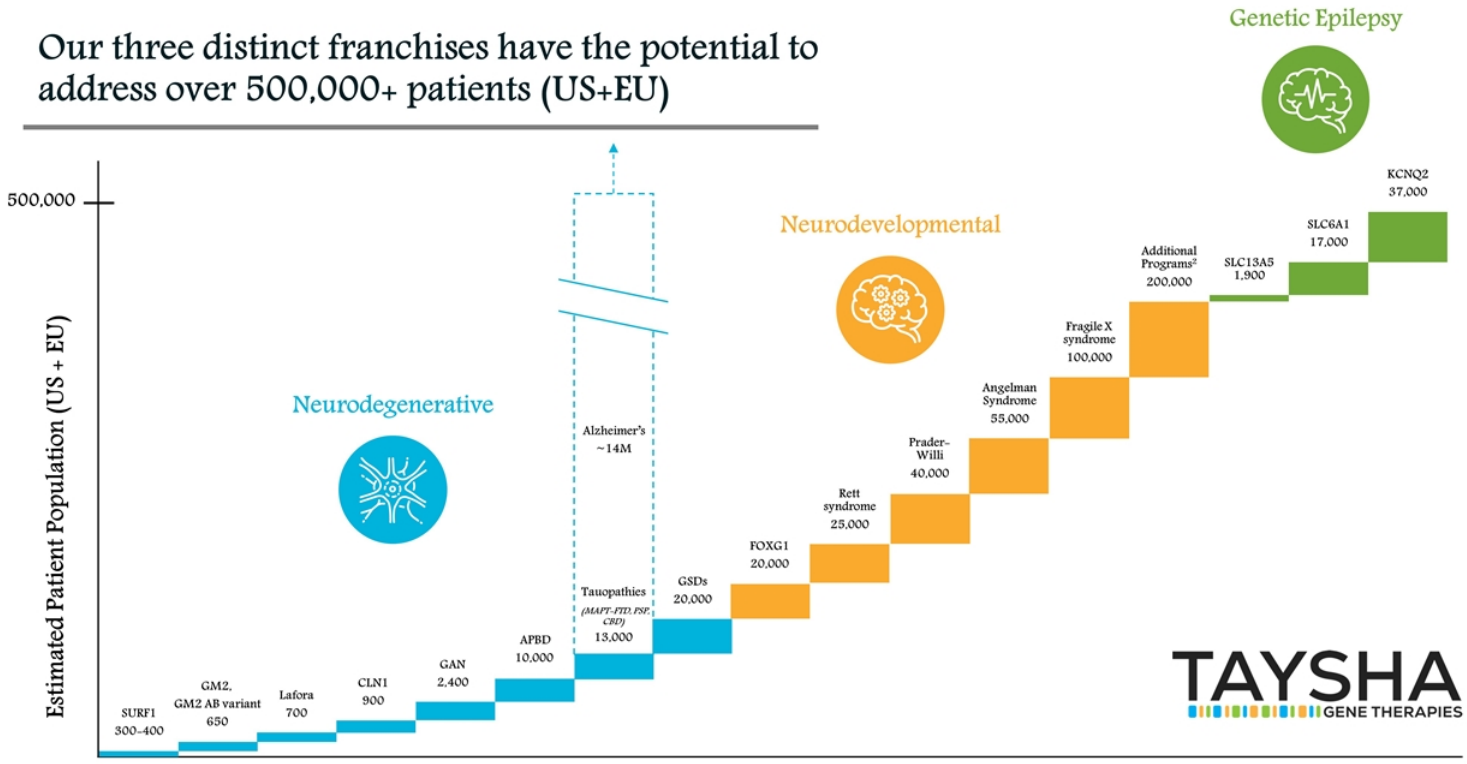
President, Founder & CEO

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-112	*miRNA*	APBD					
TSHA-111-LAFORIN	*miRNA*	Lafora Disease					
TSHA-111-MALIN	*miRNA*	Lafora Disease					
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-110	mini-gene	KCNQ2					
Undisclosed	mini-gene	Undisclosed					

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

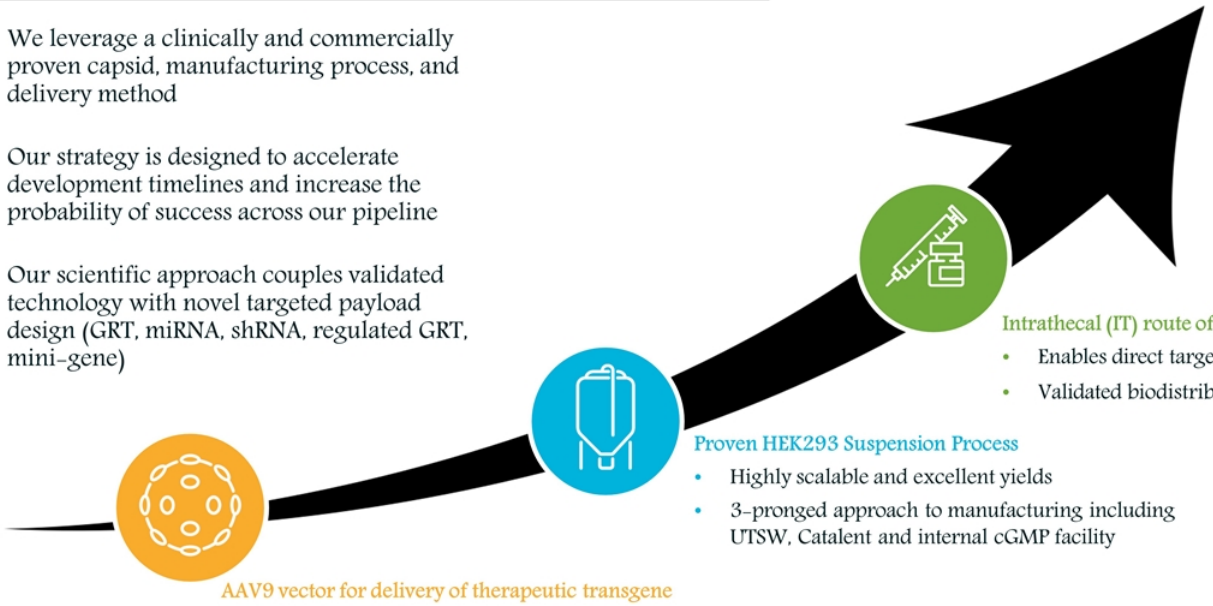
Our three distinct franchises have the potential to address over 500,000+ patients (US+EU)



[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)



AAV9 vector for delivery of therapeutic transgene

- Demonstrated safety and efficacy across multiple CNS indications

Proven HEK293 Suspension Process

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

Intrathecal (IT) route of administration

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

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

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

TSHA-120 for Giant Axonal Neuropathy

Suyash Prasad, MBBS, MSc, MRCP, MRCPCH, FFPM
Chief Medical Officer and Head of R&D

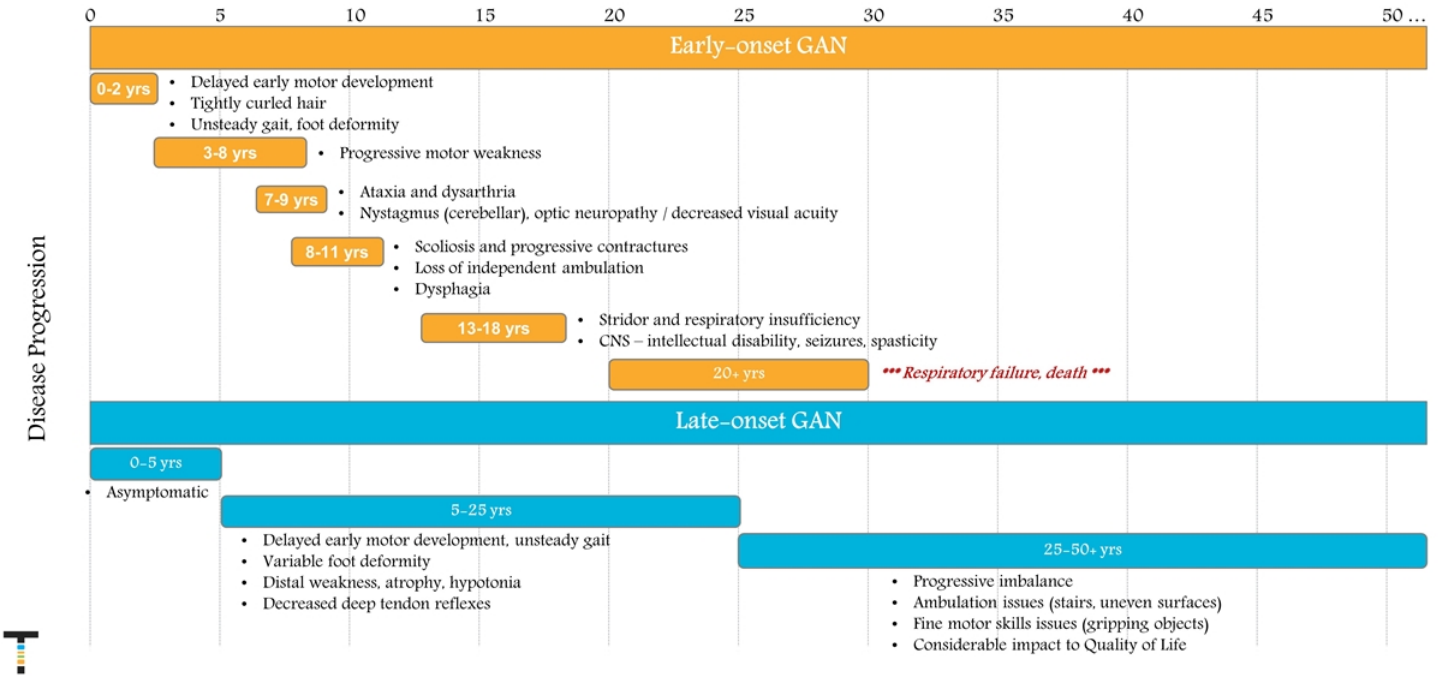
Steven Gray, PhD
Chief Scientific Advisor, UTSW Gene Therapy Program

Giant axonal neuropathy (GAN) is a rare inherited genetic disorder that affects both 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
- 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)

GAN natural history and disease progression

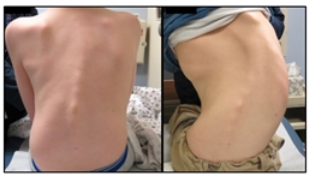


Clinical manifestations of GAN

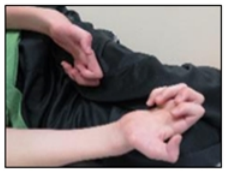
- Tightly curled hair are hallmark of early-onset GAN cohort– characterized by a dull appearance and course texture with tight curls
- Rapid progression of rotational and S-shaped scoliosis in the same male with GAN at age 12 and 15 years
- Severe finger flexor contractures develop as seen here in a 15-year-old male with GAN
- In neuronal cells GAN results in:
  - Accumulation and altered distribution of neurofilaments (NFs)
  - Enlarged (giant) axons (asterisks) surrounded by abnormally thin myelin sheaths, which impairs nerve conduction
  - White matter abnormalities (demyelination)

Tightly Curled Hair

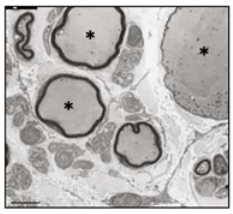
Progressive Scoliosis



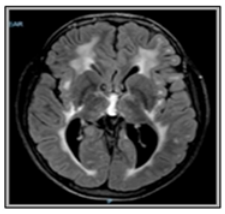
Contractures



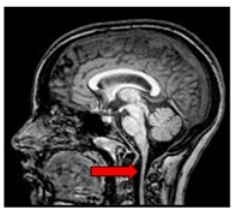
Giant Axons



White Matter Abnormality



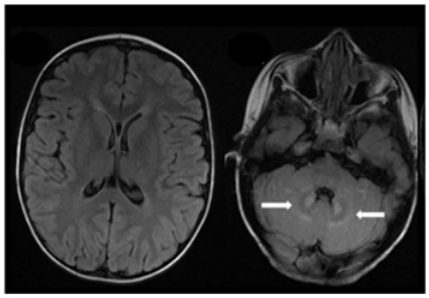
Spinal Cord Atrophy



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

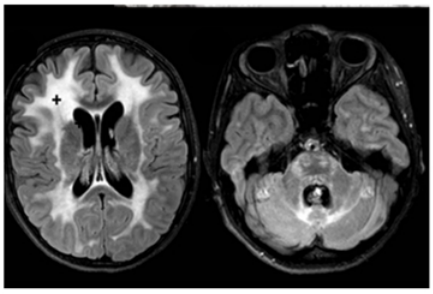
MRIs demonstrate progression of CNS symptoms with age

- Distinctive increased T2 signal abnormalities within cerebellar white matter surrounding the dentate nucleus of the cerebellum observed
  - One of the earliest brain imaging findings in individuals with GAN
  - Findings precede the more widespread periventricular and deep white matter signal abnormalities associated with advanced disease
- Cortical and spinal cord atrophy appear to correspond to more advanced disease severity and older age



Axial FLAIR Brain MRI in a 3-year-old female with GAN

No significant signal abnormalities within cerebral white matter Early hyperintense signal abnormalities within cerebellar white matter in the region surrounding cerebellar nuclei (white arrows)



Axial FLAIR brain MRI in the same female at 12-years-of-age

Confluent hyperintense signal abnormalities within the white matter (plus signs) of the cerebrum, cerebellum and brainstem

Bharucha-Goebel D et al. *Brain* 2021

Impaired pulmonary function in GAN patients

- Forced vital capacity (FVC%) correlated well with several functional outcomes
  - MFM32
  - Neuropathy impairment score
  - FARS
  - Ambulatory status
    - With independently ambulant individuals having better performance than the non-ambulant group
- Nocturnal hypoventilation and sleep apnea progress over time
  - Sleep apnea worsens as ambulatory function deteriorates

GAN patients report significant autonomic nervous system impairments

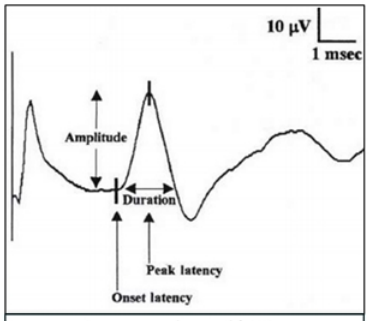
- GAN patients in this study reported significant autonomic dysfunction
- Patient or parent report of autonomic dysfunction were based upon the COMPASS 31 self-assessment questionnaire, specifically affecting the domains of autonomic function: orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor
  - Gastrointestinal, vasomotor, and pupillomotor (eye) were the most frequently reported dysfunctions
  - The gastrointestinal domain had the highest mean score (corresponding to worse reported function)

COMPASS 31	Orthostatic Intolerance	Vasomotor	Secretomotor	Gastrointestinal	Bladder	Pupillomotor
Percent Individuals Reporting Symptoms	**21.43%**	**57.14%**	**35.71%**	**78.57%**	**28.57%**	**57.14%**
Mean Scores by Domain [Range]	**3.43** [0 - 20.00]	**1.43** [0 - 3.33]	**1.68** [0 - 8.57]	**4.97** [0 - 14.29]	**0.56** [0 - 2.22]	**1.07** [0 - 3.33]
Total Score	**Average Total Weighted Score (TWS) = 13.14 [Range: 2.11 - 40.19]**					

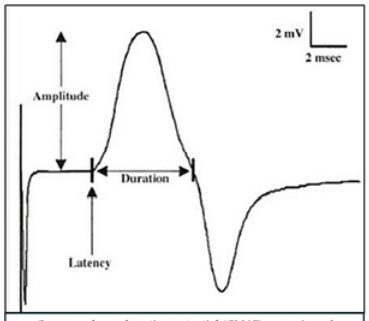
Bharucha-Goebel D et al. *Brain* 2021

Neurophysiology in GAN

- Nerve conduction function showed progressive sensorimotor polyneuropathy with age
- Significantly diminished Compound Motor action potential (CMAP) amplitudes
  - Overall, upper extremity CMAP amplitudes correlated significantly to the MFM32% score and the total NIS score, and appear to be the best electrophysiologic measures to follow over time
  - The median CMAP amplitude correlated significantly with other upper extremity measures of strength including grip and pinch strength
  - In the lower extremity, peroneal CMAP amplitudes correlated to lower extremity strength measures (percent predicted strength/myometry) in knee flexion, knee extension, and hip abduction
- Significantly diminished Sensory Nerve Action Potential (SNAP) amplitudes
- Sensory nerve responses were affected earlier than motor responses and were frequently absent as follows:
  - Median sensory response absent in 50% (n=32)
  - Ulnar sensory response absent in 57% (n=21)
  - Sural sensory response absent in 78 % (n=27)



Sensory nerve action potential (SNAP):
sum of all the individual sensory fibers that depolarize

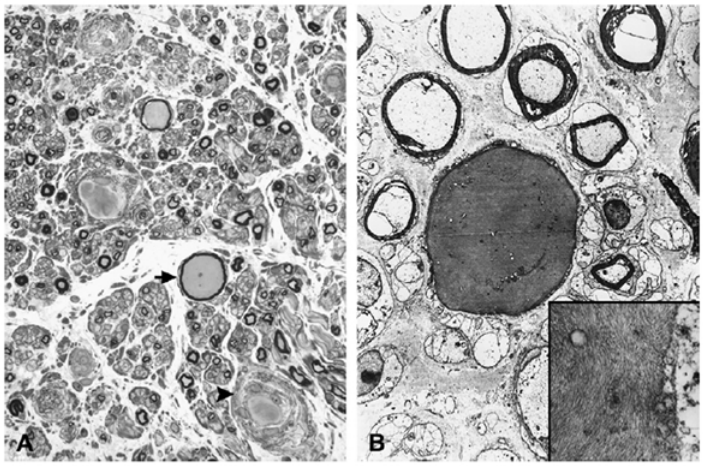


Compound muscle action potential (CMAP) comprises of latency, amplitude, duration, and area of the CMAP

Bharucha-Goebel D et al. *Brain* 2021

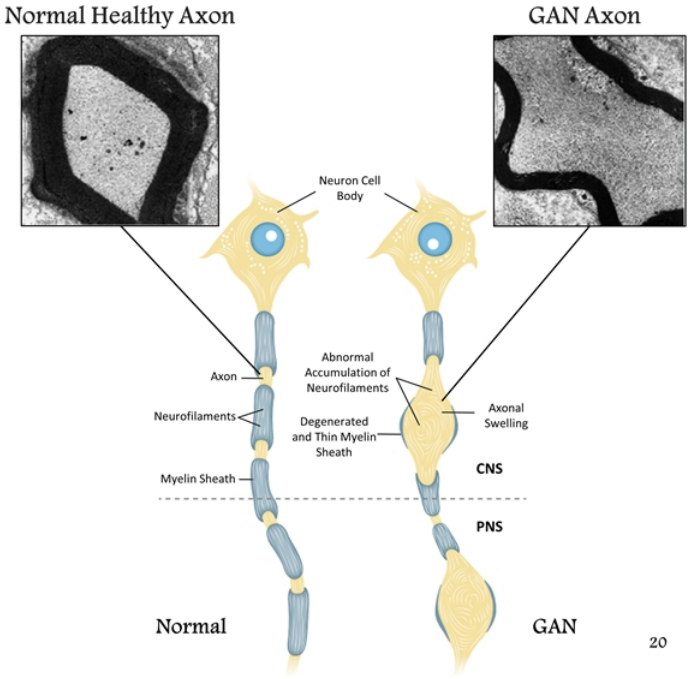
Giant Axonal Neuropathy (GAN)

- Sensory and Motor Peripheral Neuropathy, "ALS in kids"
- Cognition is mostly unaffected in the early stages of disease
- 3-4 yrs old: clumsiness, loss of coordination
- ~10 yrs old: unable to walk
- Late teens: highly reduced coordination and use of arms/hands
- ~20 yrs old: Fatal



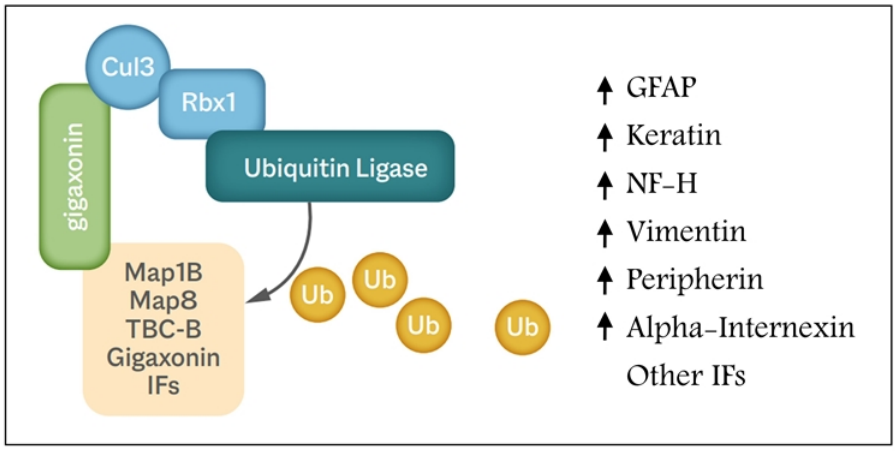
Rationale for targeting the *GAN* gene

- Gigoxonin is an E3 ligase enzyme that attach ubiquitin to substrate proteins (Ubiquitination), marking them for degradation by either proteosome or autophagy
- Mutations affect production of the protein gigaxonin
  - Leads to dysregulation and the progressive accumulation of intermediate filaments (IFs) affecting endothelial cells, skin fibroblasts, muscle fibers, Schwann cells, astrocytes and neurons, which in turn impairs host cell functions
  - Neurons are particularly sensitive to IF accumulation, causing axonal dysfunction and eventually neuronal death
- 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



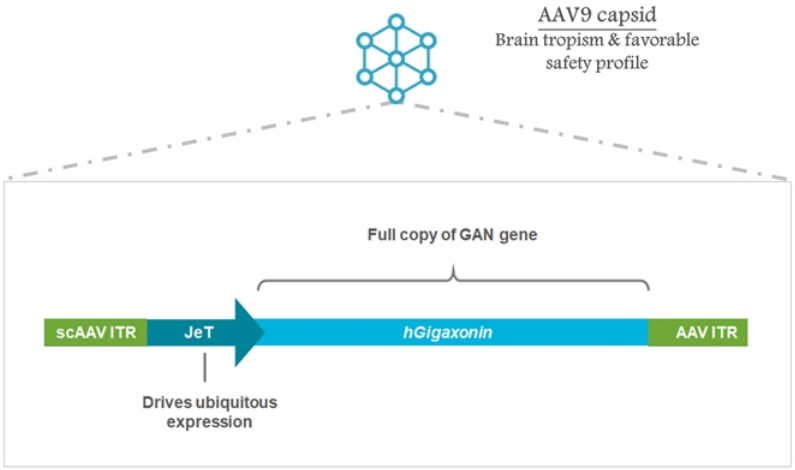
Molecular underpinnings of GAN

- Evidence that gigaxonin targets itself, providing some amount of theoretical autoregulation of gigaxonin protein levels
- Full list of gigaxonin targets unknown, with lack of clarity around whether gigaxonin targets intermediate filaments for degradation directly
- Loss of gigaxonin function leads to the accumulation and/or disregulation of a broad class of proteins called intermediate filaments
- Intermediate filaments important for cell and axon structure and transport of certain macromolecules within the cell



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

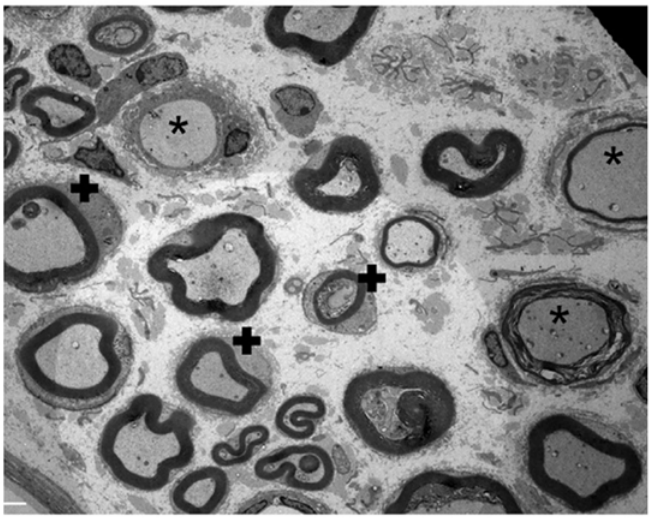
Comprehensive preclinical results demonstrated:

- 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

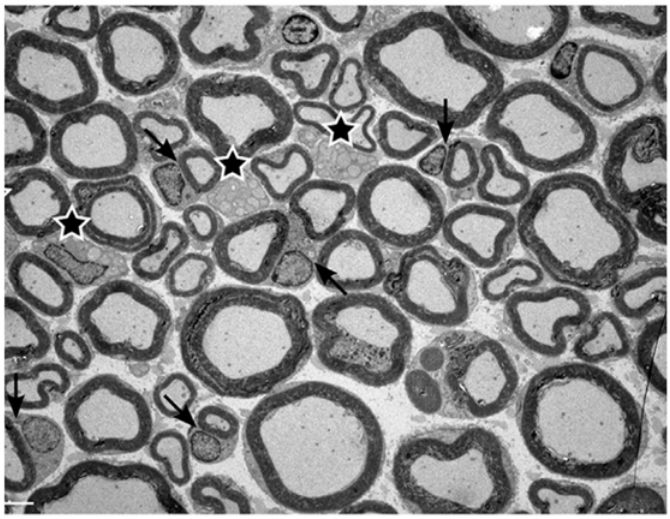
TSHA-120 improved pathology of the sciatic nerve in the GAN KO mice

TSHA-120
GAN

KO



KO + AAV9/GAN



✱ Dense, disorganized accumulations of NFs in fibers

✚ Accumulation of IFs in Schwann cell cytoplasm associated with myelinated fibers

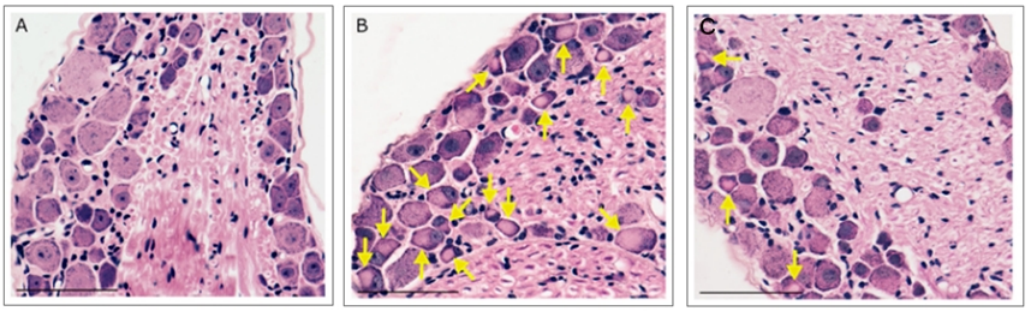
★ Intact unmyelinated fibers and associated Schwann cells

→ Normal Schwann cell cytoplasm associated with myelinated fibers

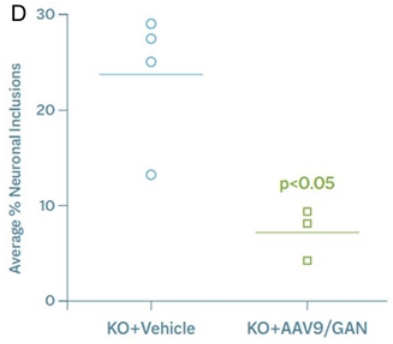
Bailey, R. et al, *MTMCD* 2018

Scale bar: 5 mm

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



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



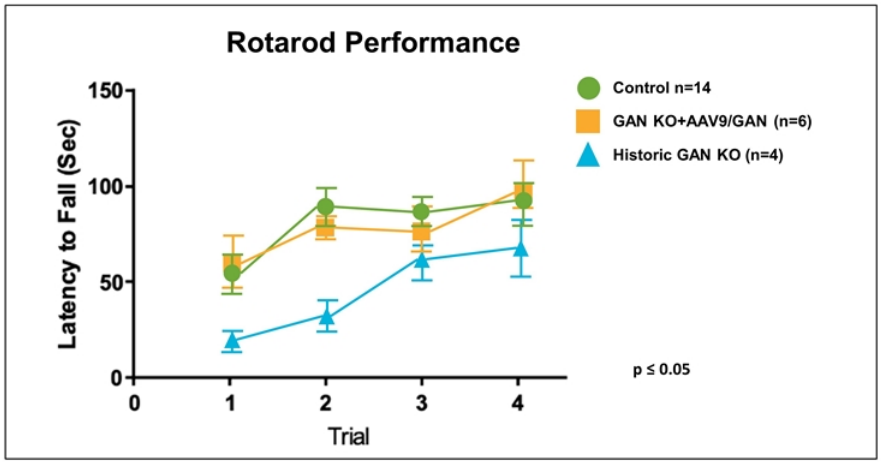
Representative images of light microscopic evaluation of lumbar DRG in 24-month-old normal control (A), vehicle IT-injected GAN /Y KO mice (B), and AAV9/JeT-GAN IT-injected GAN/Y KO mice (C). H&E staining shows unremarkable DRG neurons in control mice (A) versus abundant, brightly eosinophilic inclusion-bearing 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

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

TSHA-120
GAN

- 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



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

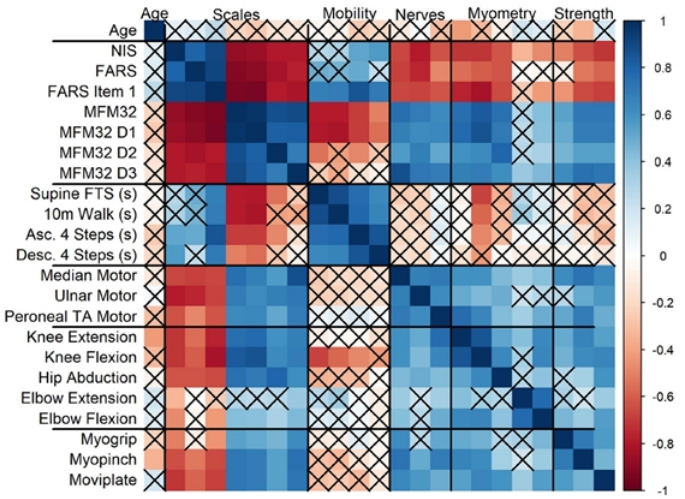
Examples of tasks

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), and distal grip and pinch strength)
- MFM32 correlates 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 correlate 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

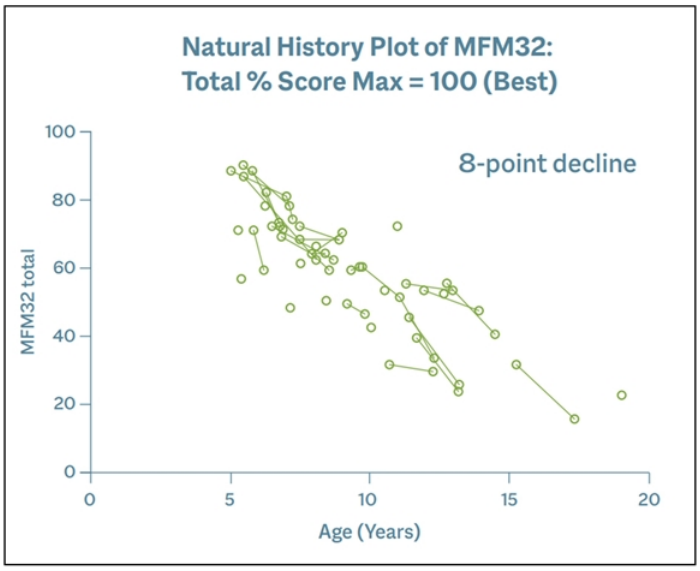


Bharucha-Goebel D et al. *Brain* 2021

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

- 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



GAN natural history study data – Cohort characteristics

TSHA-120
GAN

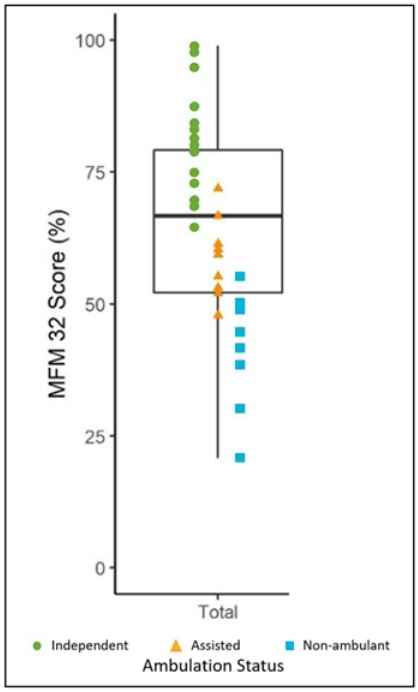
- Of ninety total alleles analyzed in this cohort, forty-six different pathogenic variants (mutations) in the *GAN* gene were observed, and included:
  - Missense mutations (53.3%)
  - Splice site mutations (16.7%)
  - Frameshifting deletions (15.6%)
  - In-frame deletions (4%)
  - Nonsense mutations (7.8%)
  - Whole gene deletions (2%)

	Early Onset (n=35)	Late Onset (n=10)	Overall (n=45)
Age (years)			
Mean (SD)	8.7 (3.3)	12.7 (4.8)	9.6 (4.0)
Median [IQR]	7.9 [7.3, 10.8]	11.9 [8.8, 16.1]	8.8 [6.8, 11.4]
Range	3.2 – 19.0	7.3 – 21.3	3.2 – 21.3
Age < 6 years MFM administered			
Yes	8 (23%)	0 (0%)	8 (18%)
Sex			
Male	18 (51%)	2 (20%)	20 (44%)
Female	17 (49%)	8 (80%)	25 (55%)
Ambulation Status			
Independent	16 (46%)	9 (90%)	25 (56%)
Assisted	9 (26%)	1 (10%)	10 (22%)
Non-Ambulant	10 (29%)	0 (0%)	10 (22%)

Bharucha-Goebel D et al. *Brain* 2021

Total MFM32 score correlated with ambulatory status

- Only includes individuals over age 6 where MFM32 was performed (n=37)
- Eighteen individuals were independently ambulant, 10 required assistance to walk, and 9 were non-ambulant
- Independently ambulant individuals having better performance and higher MFM32 scores than the non-ambulant group
- MFM32 scores track well with ambulatory status and, therefore, may be a relevant marker of function



Bharucha-Goebel D et al. *Brain* 2021

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

TSHA-120 GAN

Goals and Targets of Trial

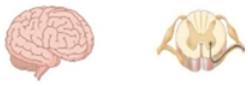
Goals

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

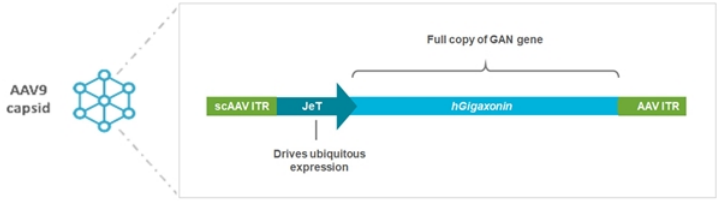
Target Recruitment

- 14 subjects injected
- > 5 years old

Target Areas to Transduce



Product Details and Dose Cohorts



Dose Cohorts*

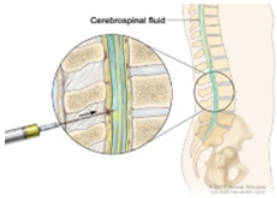
- 1x 5 x 10^{13} total vg (n=2)
- 3.3x 2 x 10^{14} total vg (n=4)
- 5x 8 x 10^{14} total vg (n=5)
- 10x 5 x 10^{14} total vg (n=3)

*Doses calculated by qPCR

Route and Method of Administration

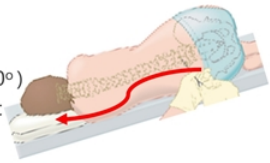
Administration

- 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

TSHA-120 interventional study endpoints

Disease-Specific/Global Assessments
- Motor Function Measure 32 (MFM32) total score (and domains)
- Motor symptoms (10m walk, 4 stair climb, 4 stair descent)
- Muscle strength (myometry)
- Sensory symptoms (NIS, FARS, clinical examination, reflexes)

Neurophysiology Assessments
- Nerve conduction
- Electrical impedance myography

Imaging
- MRI of the brain and spine

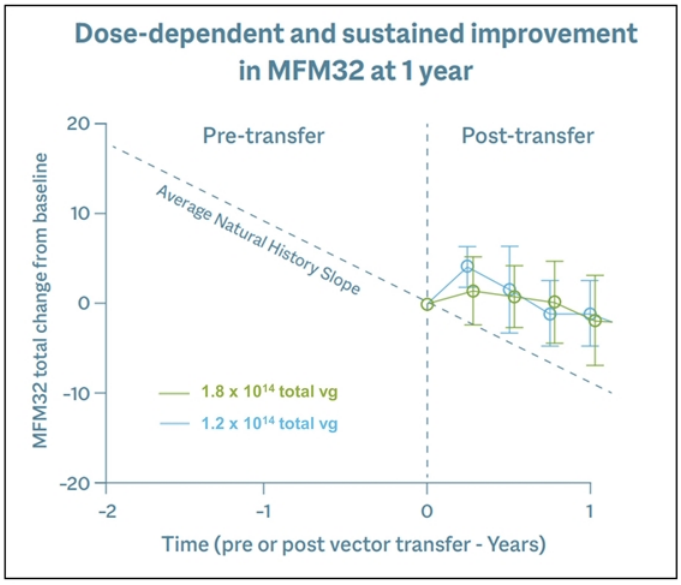
Biomarkers
- DNA/RNA/Protein
- Neurofilament

Neuropathological
- Peripheral nerve biopsies
- DNA/RNA/Protein
- Markers of inflammation

Examination of visual/ophthalmologic parameters
- Optical coherence tomography (OCT) assessment of retinal nerve fiber layer (RNFL) thickness

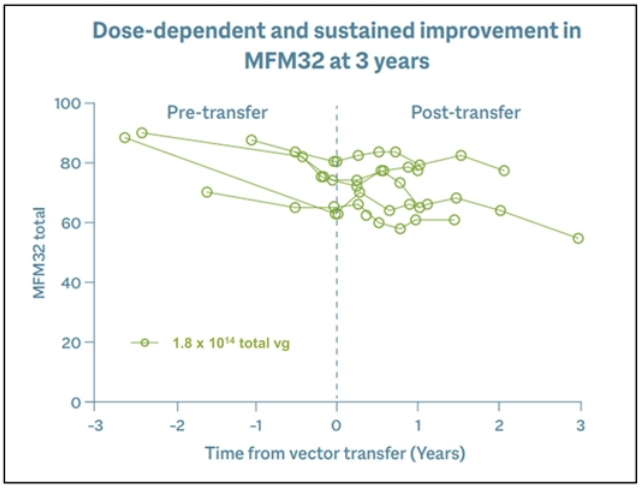
TSHA-120 achieved sustained improvement in primary efficacy endpoint and was well tolerated at multiple doses

- 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

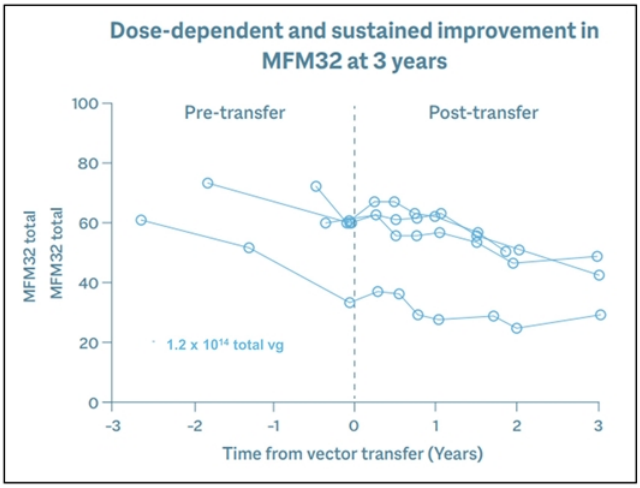


Bönnemann, C. et al; 2020

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



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

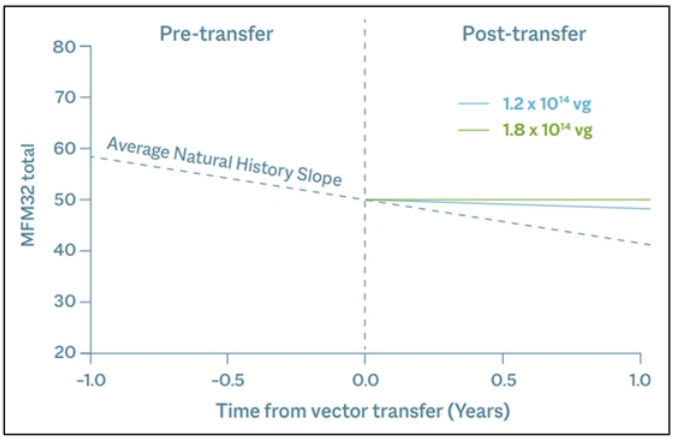


- 6 patients treated for 3+ years supporting long-term durability
- Plan to engage with agencies in US, EU and Japan to discuss regulatory pathway as soon as possible

Bönnemann, C. et al; 2020

Additional analysis using Bayesian methodology confirmed arrest of disease progression

- 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



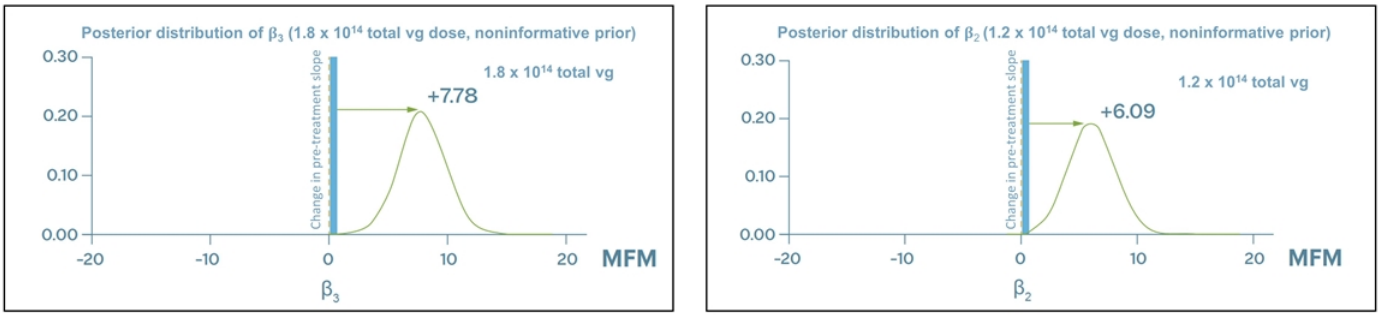
	Bayesian Analysis		Frequentist Analysis		
	Mean	Std Dev	Estimate	Std Error	p-Value
Post infusion, $1.8x10^{14}$ total vg	7.78	1.94	7.78	1.89	<0.001
Post infusion, $1.2x10^{14}$ total vg	6.09	2.11	6.07	2.05	0.004
Natural history decline	-8.19	0.74	-8.18	0.72	<0.001

Bönnemann, C. et al; 2020

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

Bayesian Efficacy Analysis

Compared to individual historical data



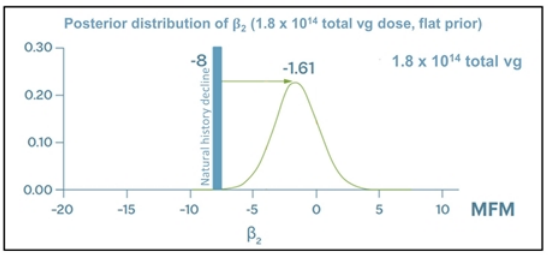
X-axis = change in slope compared to pre-gene transfer
Blue line = pre-treatment change in slope = 0

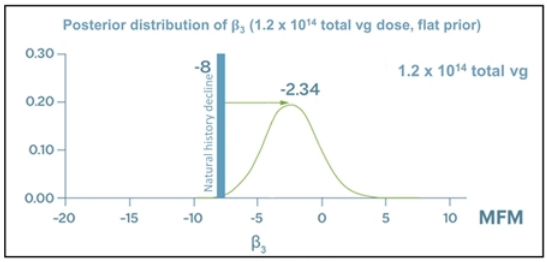
- Treated population average annual post-treatment decline for both the $1.8x10^{14}$ total vg cohort and $1.2x10^{14}$ total vg cohort
- $1.8x10^{14}$ vg halted patient pre-treatment rate of decline, avg annual slope improvement of 7.78 points
- $1.2x10^{14}$ vg resulted in clinically meaningful slowing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

- 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





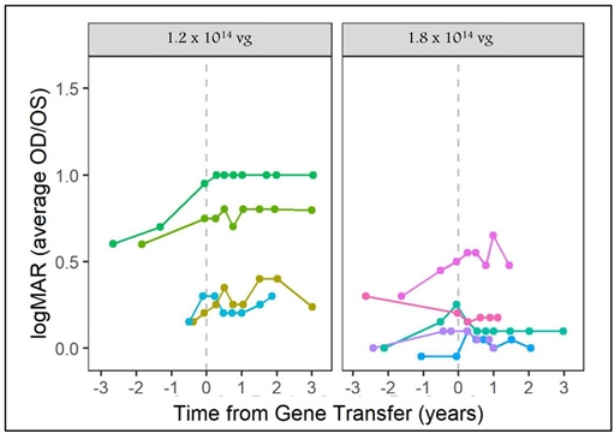
X-axis = annual decline in MFM32 total % score
Blue line = natural history decline (-8 points per year)

	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

Exploratory endpoints – Ophthalmology biomarkers

- 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

Summary of safety findings

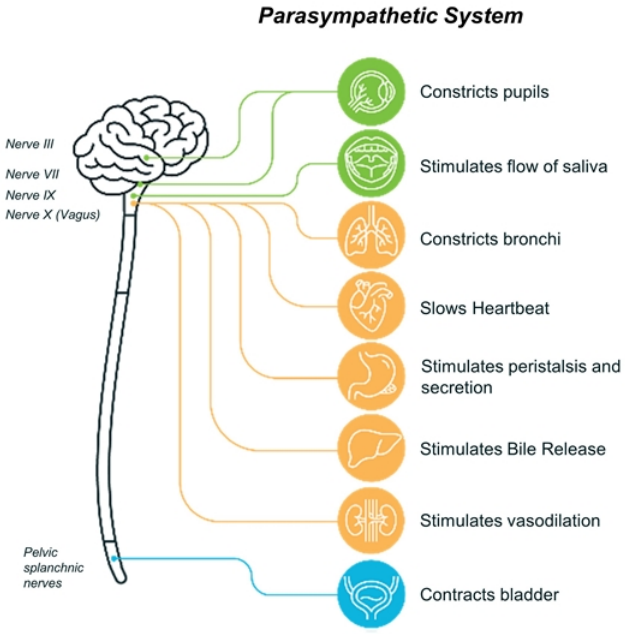
- Clinically well tolerated
- Some evidence of asymptomatic cerebrospinal fluid pleocytosis in earlier dosed patients
- No dose-limiting toxicity
- No transaminitis
- No sign by neuroimaging or clinically of new enhancement or inflammation
- No clinical signs of acute or subacute inflammation (i.e., encephalopathy, persistent headaches, seizures, or vision changes outside of related to underlying disease)
- No sudden sensory changes or evidence by spine MRI of nerve root/ DRG inflammation
- No evidence of thrombocytopenia

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 the 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

Opportunity to achieve human POC for vagus nerve redosing

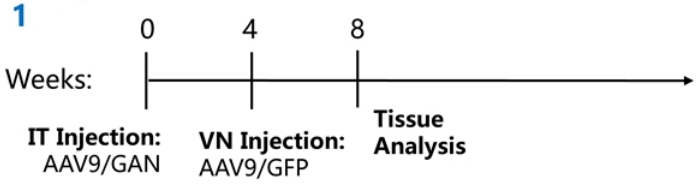
- The vagus nerve represents the main component of the autonomic nervous system
- Direct delivery to the vagus nerve may provide broad coverage of the autonomic nervous system and enable redosing by subverting the humoral immune response
- Proof-of-concept established in rodent and canine models; oral 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

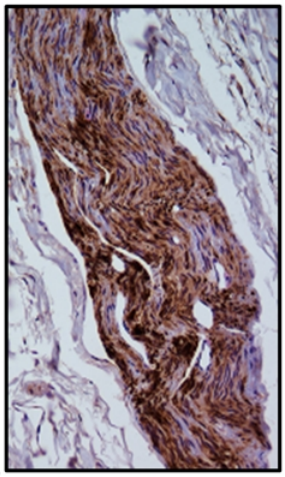
TSHA-120 GAN

Study 1

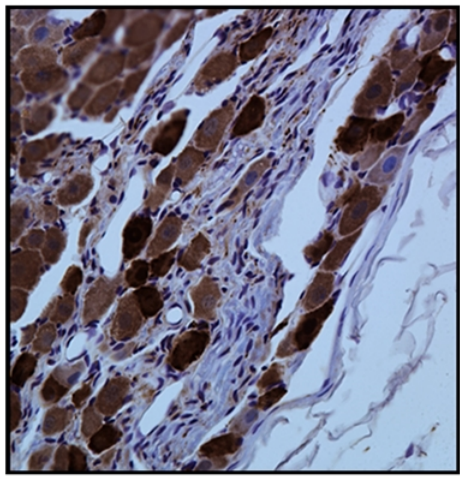


Study 2

Vagus Nerve

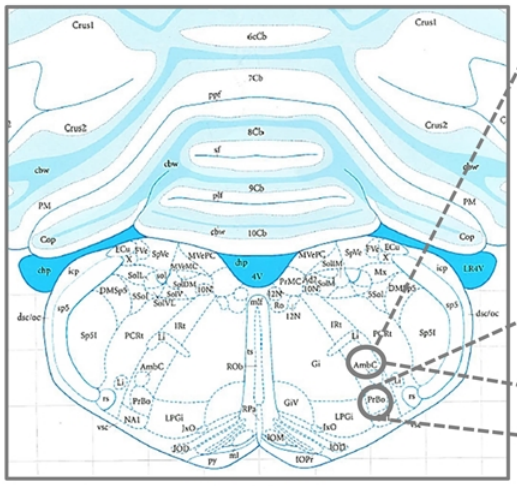


Nodose Ganglia

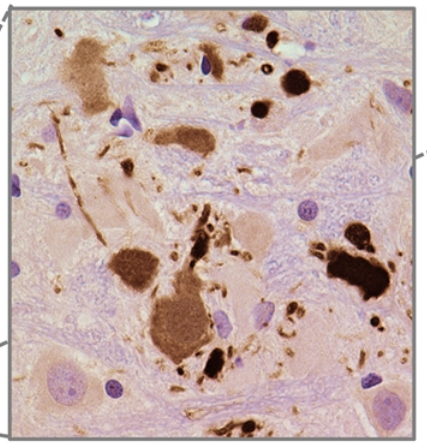


GFP – green fluorescent protein
Courtesy of Dr. Diane Armao

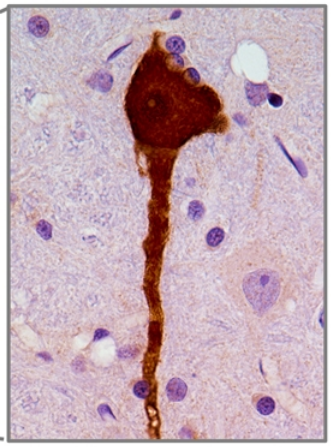
Successful transduction of relevant brain neurons following redosing via vagus nerve injection



Medulla



Nucleus Ambiguous

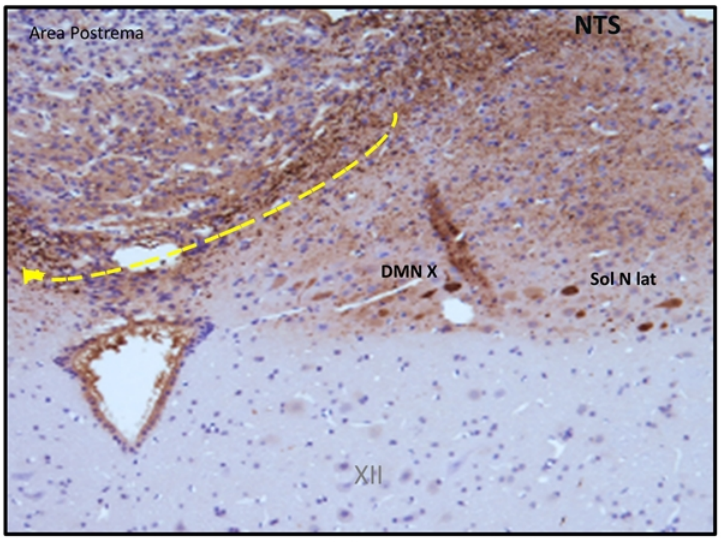


Pre-Botzinger Complex

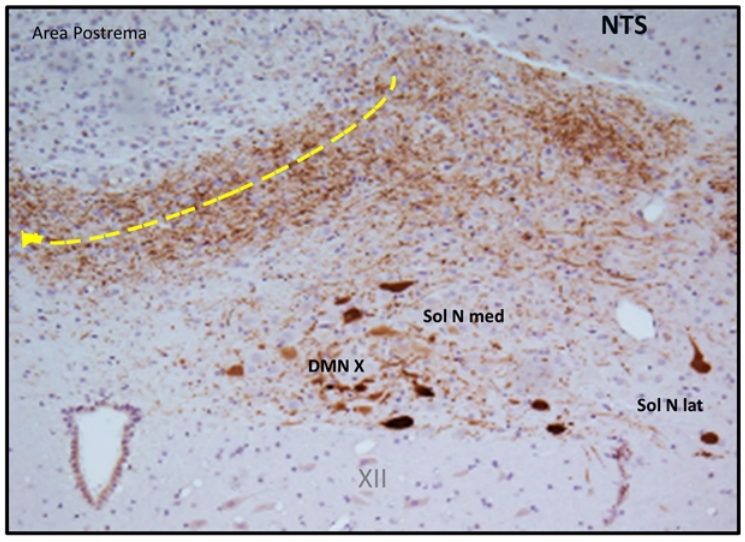
Courtesy of Dr. Diane Armao

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

TSHA-120
GAN



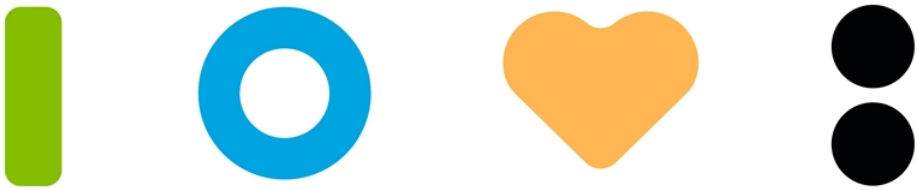
Naive



AAV9 Pre-immunized

Courtesy of Dr. Diane Armao

Q & A

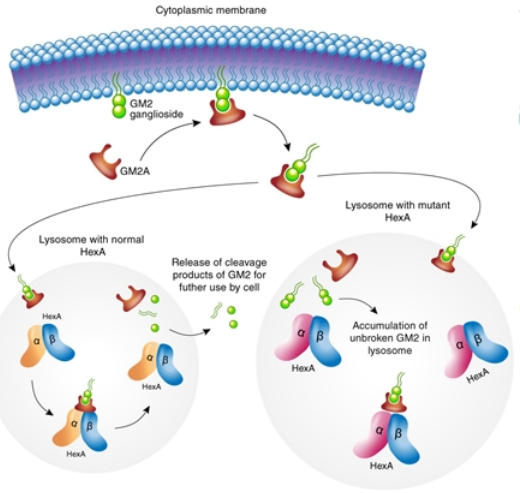


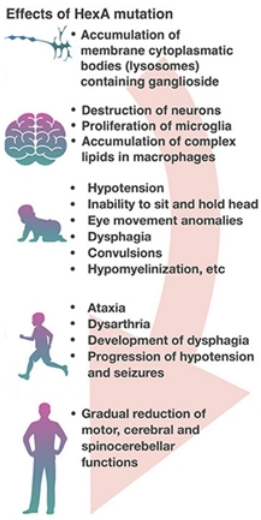
TSHA-101 for GM2 Gangliosidosis

Suyash Prasad, MBBS, MSc, MRCP, MRCPCH, FFPM
Chief Medical Officer and Head of R&D

GM2 gangliosidosis is a severe neurodegenerative disease

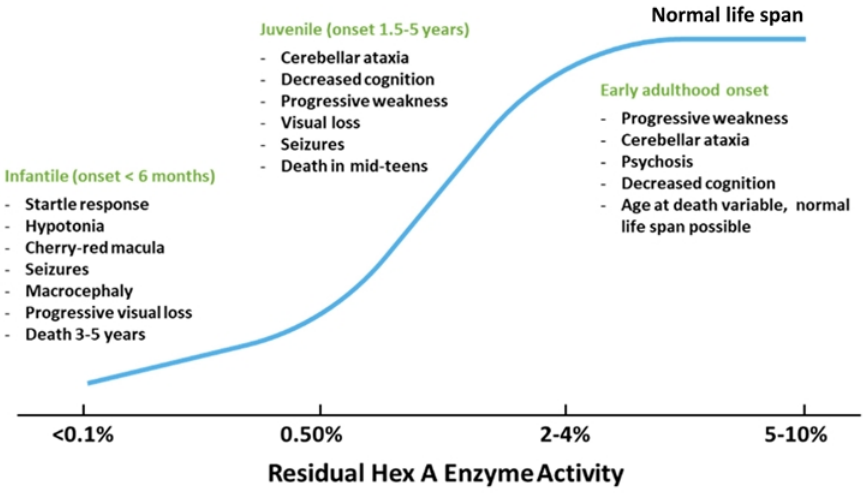
- GM2 gangliosidosis results from a deficiency in the β-hexosaminidase A (Hex A) enzyme
- Hex A is comprised of 2 subunits encoded by the alpha-subunit, HEXA, coded for by the HEXA gene, and the beta-subunit, HEXB, coded for the HEXB gene
- Mutations of the HEXA gene cause Tay-Sachs disease (TSD) while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





Residual Hex A activity determines the severity of GM2

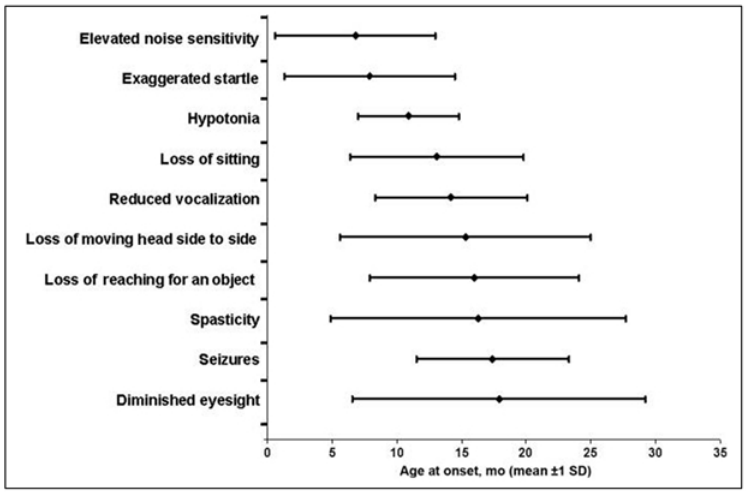
- Small increases in Hex A activity may lead to significant improvements in clinical outcomes and quality of life
- Infantile onset is the most severe form of GM2
- Infantile forms may die within the first 4 years of life, and juvenile onset patients rarely survive beyond mid-teens



What does natural history tell us about disease progression?

Bley A.E. et al; A retrospective study through NTSAD

- Patients experience significant diagnostic delays:
  - Mean age at onset of earliest symptom was 5 +/- 3.3 months
  - Average age of diagnosis was 13.3 +/- 5.3 months
  - Diagnosis usually occurs on the presence of a hallmark cherry red macula
- Most common initial symptoms:
  - Developmental arrest (83%); startle response (65%); Hypotonia (60%)
  - Loss of head control by ~ 9.7 mo.
  - Loss of ability to vocalize by ~14 mo.
  - Loss of ability to reach for an object by ~16 mo.
  - Loss of ability to sit up by ~13.1 mo.
  - Dysphagia / gastric tube placement: no specific data reported, but could deduce from 'ability to vocalize' data
- Symptom progression
  - Majority of infants can gain some early motor milestones such as head control but lose achieved motor milestones
  - Most patients develop seizures (98%) and require multiple anti-convulsants
- Early mortality despite use of supportive care such as gastric tube placement
  - Median survival: 47 months



Bley AE et al. *Pediatrics* 2011

What does natural history tell us about motor development delay?

Utz J. et al; Prospective Nat Hx. study

- Similar age of diagnosis reported compared to Bley et al.:
  - Median age of diagnosis was 15 mo.
- Most patients experienced motor developmental delays within the first 6 months of life, and all patients had documented motor developmental delays by 12 months of age
- Most common initial symptoms:
  - Hypotonia within 6 months of life (in 67% of patients)
  - Dysphagia / feeding tube placement between 7–13 months of age
  - Seizure onset between 7–18 months of age
  - Cherry red spots between 7–13 months of age or later
  - Cognitive and motor declines between 18–28 months or later with severe neurological impairment present long before diagnosis is made
  - All patients developed excessive salivary and respiratory secretions as well as recurrent respiratory infections
- Symptom progression
  - Motor skills gained within the first 6–12 months of life were lost by 2 years of age
  - Median survival: 43.3 months

Utz J et al. *Mol Genet Metab* 2017

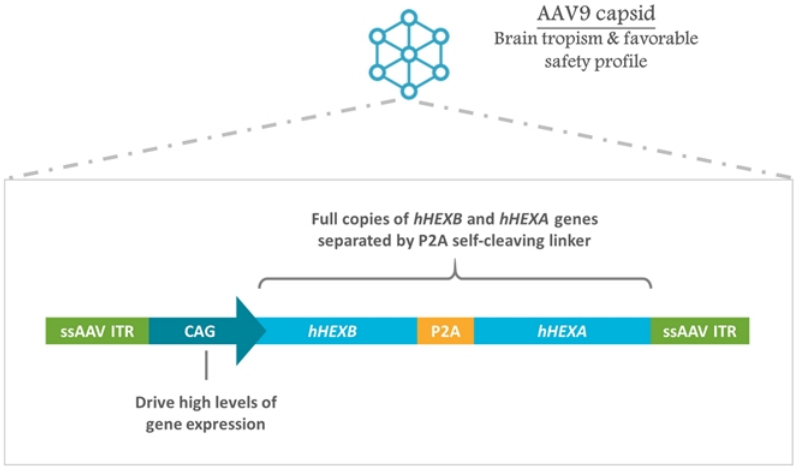
What does natural history tell us about motor development delay?

Motor Developmental Delay Timeline								
Motor Skills	Diagnosis (N=# of patients assessed)	% Never Gained	% Experienced	Age (months) divided into 6 month intervals at which motor developmental milestones occurred				
				0–6	7–12	13–18	19–24	Age unknown
Gained independent head control	n=14	0%	100%	79%	7%	–	–	14%
Lost independent head control	n=14	–	93%	–	57%	21%	7%	7%
Gained ability to sit independently	n=13	62%	39%	31%	8%	–	–	–
Lost ability to sit independently	n=13	–	39%	–	23%	15%	–	–
Gained ability to crawl	n=13	100%	0%	–	–	–	–	–
Lost ability to crawl	n=14	–	7%	–	–	7%	–	–

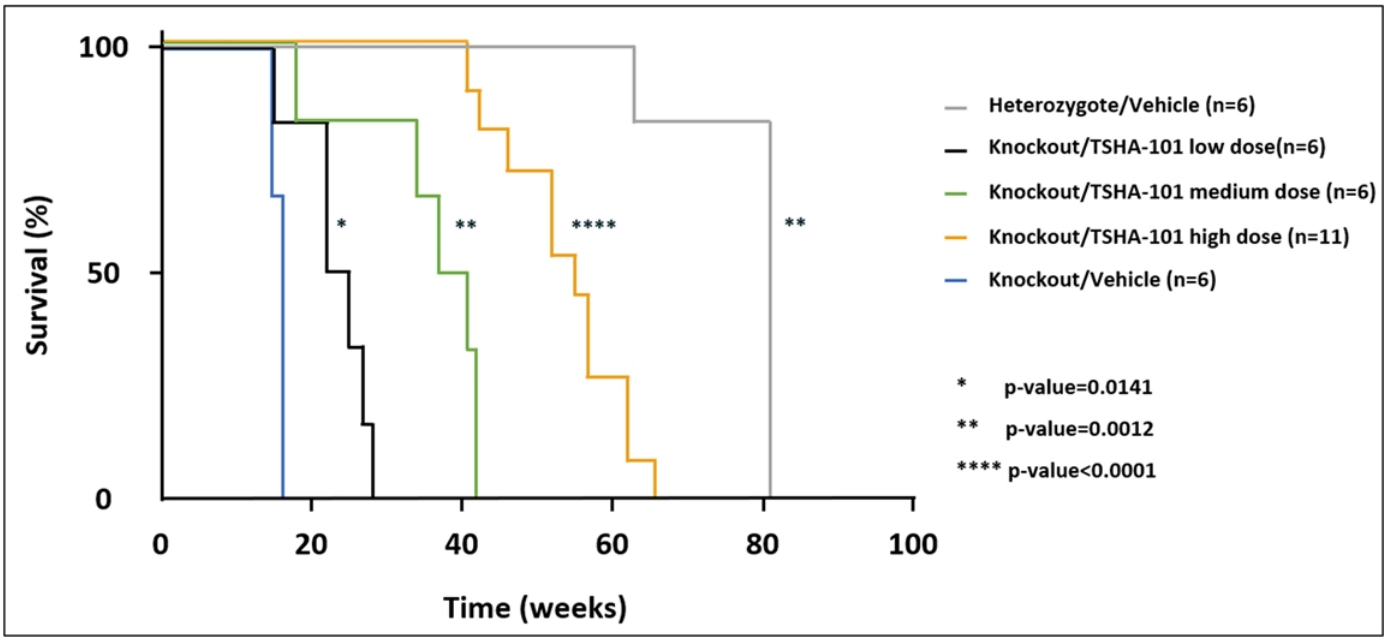
Utz J et al. *Mol Genet Metab* 2017

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

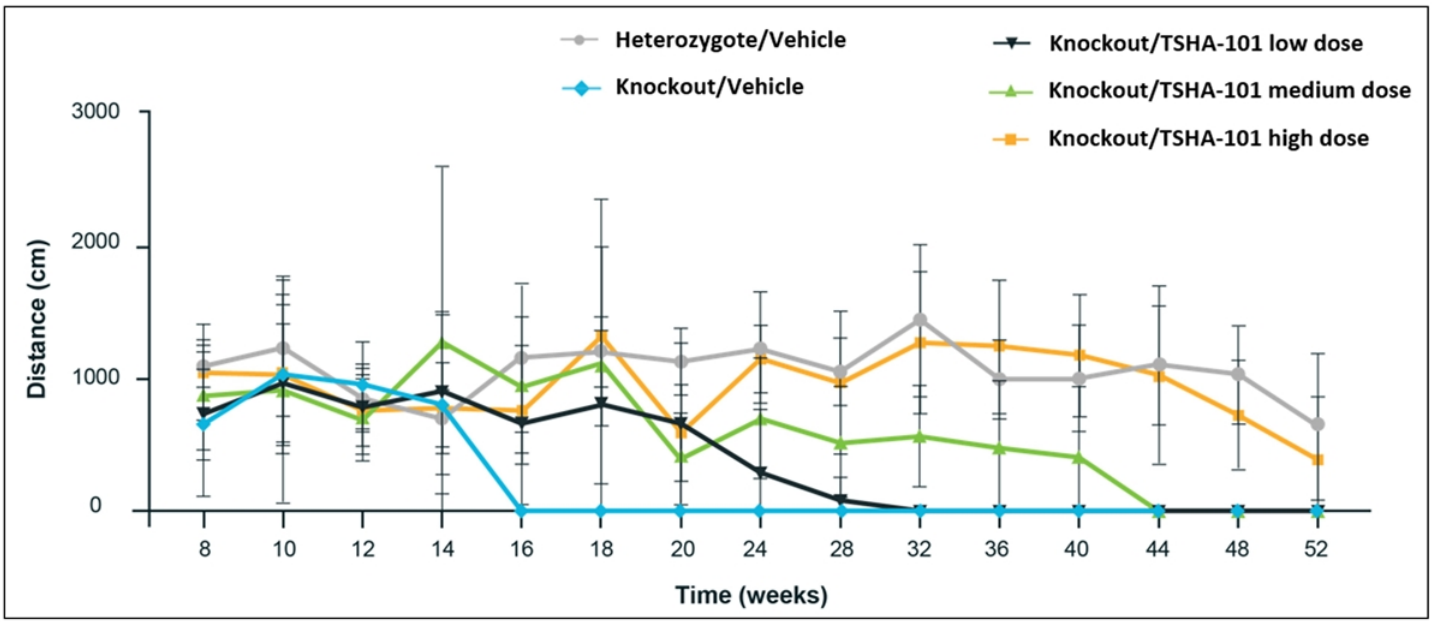
- *HEXA* and *HEXB* genes are required to produce the subunits of the beta-hexosaminidase A enzyme
- The novel bicistronic vector design enables 1:1 expression of the alpha-subunit, *HEXA*, and the beta-subunit, *HEXB*, under the control of a single promoter with a P2A-self-cleaving linker
- SD mice 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



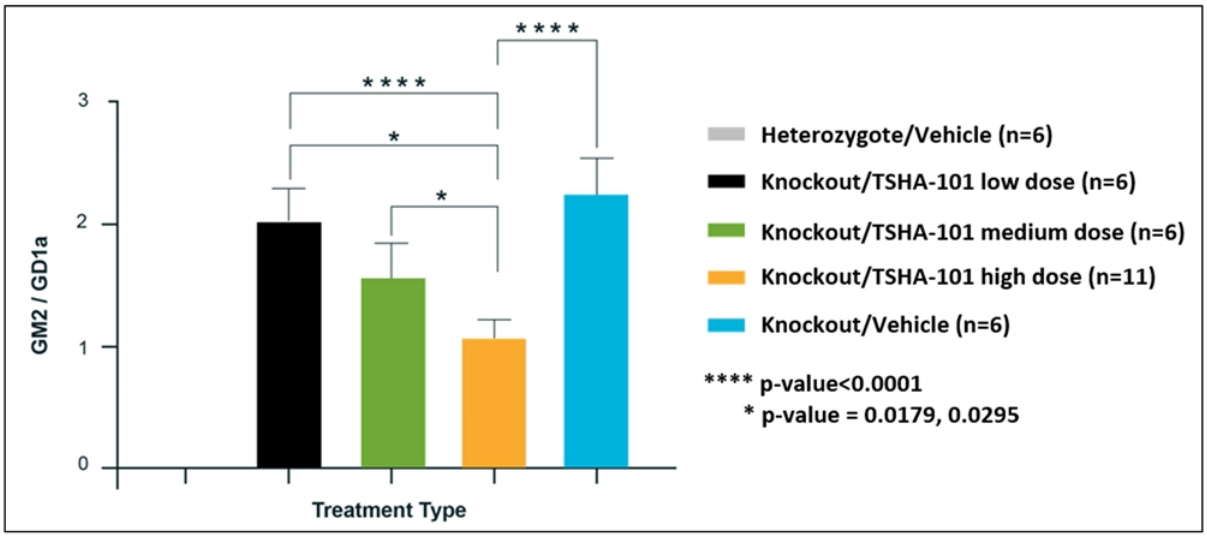
Preclinical Pharmacology: Significant, dose-dependent improvement in survival observed in mice treated with TSHA-101



Preclinical Pharmacology: Dose-dependent improvements observed in rotarod assessments in mice treated with TSHA-101



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

Goals and Targets of Trial

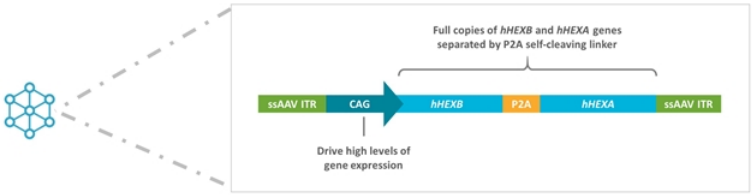
Goals

- **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



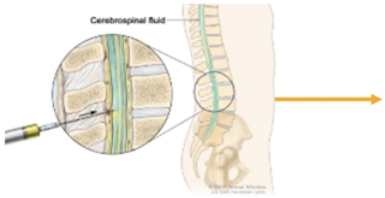
Dose Cohorts

- 5×10^{14} total vg (n=4)

Route and Method of Administration

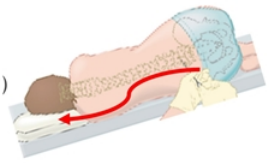
Administration

- 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 (15-30°)
- 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

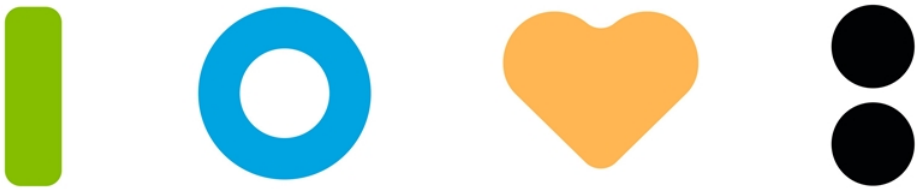
Preliminary Phase 1/2 biomarker data (Queen's University study) in 2H 2021

US study utilized material from commercial process

Submit IND in 2H 2021

Initiate U.S. Phase 1/2 study in 2H 2021

Q & A



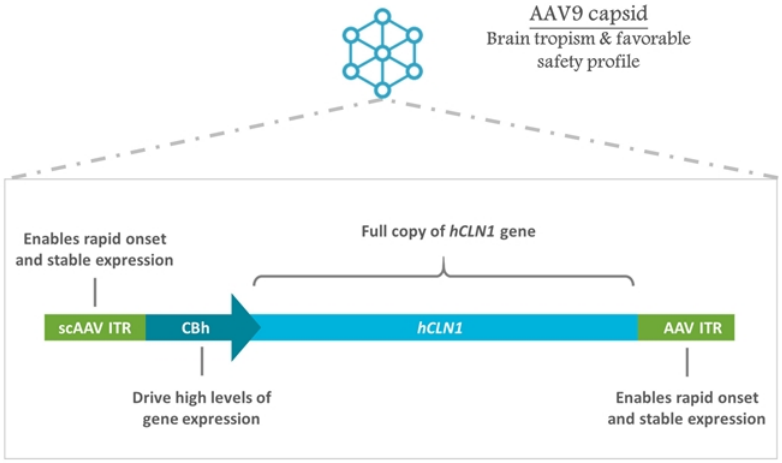
TSHA-118 for CLN1 Disease

Suyash Prasad, MBBS, MSc, MRCP, MRCPCH, FFPM
Chief Medical Officer and Head of R&D

Steven Gray, PhD
Chief Scientific Advisor, UTSW Gene Therapy Program

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
- The estimated prevalence of CLN1 disease is 900 patients (US+EU)

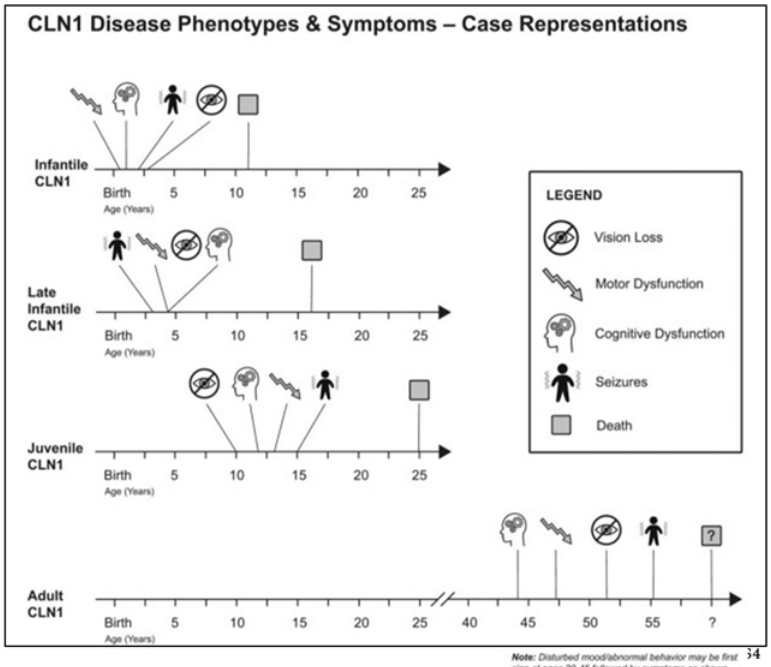


CLN1 disease onset and progression

ONSET	COMMON SYMPTOMS	DISEASE PROGRESSION
• Typically between 2–24 months of age when mental and motor development declines • Late infantile onset between 2–4 years • Juvenile onset between 4–10 years	• Developmental regression; rapid loss of motor function and cognitive abilities • Decreased muscle tone (hypotonia)	• Ataxia, muscle twitches (myoclonus), spasticity, recurrent seizures (epilepsy), and vision loss/blindness • Overall loss of brain tissue (brain atrophy) and microcephaly • Severe feeding difficulties that often require a feeding tube

CLN1 disease phenotypes and symptom progressions

- Ages of symptom onset derived from clinical experience and recently published guidelines
- Specific occurrence, order, and age at symptom onset are variable
  - In general, individuals with the infantile phenotype have the most aggressive course, with age at death in the first or second decade (published reports range from three to 12 years)
- Late infantile phenotype develop severe impairment phase by age 6 to 12 years and may survive into the second or third decade
- Juvenile phenotype reach severe state in the third decade and typically live into the third or fourth decade
- Median age of death was 9.5, 16.6, and 27 years for infantile, late infantile and juvenile forms, respectively



Augustine EF et al. *Pediatr Neurol* 2021; Simonati A et al. *NCL* 2018

Clinical spectrum of CLN1 disease phenotypes varies

- CLN1 disease phenotypes vary by age at onset, order of symptom onset, rate of disease progression, and life expectancy
- There are at least 71 different disease-causing pathogenic variants in *CLN1* reported to date, with strong genotype-phenotype correlations for certain mutations
- Ascertainment of the specific CLN1 disease phenotype is key in informing the anticipated clinical course, prognosis, and care needs

Phenotype	Typical Ages at Symptom Onset	Rate of Progression	Clinical Features
Infantile	6–18 months	Rapid	Cognitive and motor decline, hypotonia, ataxia, myoclonus, seizures, hand stereotypies, vision loss, acquired microcephaly
Late infantile	>18 months–4 years	Rapid	Developmental delay, early cognitive decline, later vision loss, ataxia, myoclonus, seizures
Juvenile	>4 years–early adolescence	Slow	Cognitive decline, seizures, motor decline, ataxia, spasticity, later vision loss
Adult	Late adolescence and older	Protracted	Cognitive decline, depression, ataxia, parkinsonism, vision loss

No clinical management guidelines or consensus statements specific to CLN1 disease

- 15 CLN1 disease experts and 39 caregivers responded to the surveys, and 14 experts met to develop consensus-based recommendations
- Found a limited evidence base for treatment and no clinical management guidelines specific to CLN1 disease
  - Disease-modifying therapies are not presently available for CLN1 disease, although clinical trials are being planned
  - Current management strategies focus on symptom relief and palliative care
  - Due to disease rarity, many clinicians lack experience treating individuals with *any* NCL disorder
- Early diagnosis is critical for providing optimal symptom management, minimizing complications, and connecting families to appropriate psychosocial support and genetic counseling.
  - Because CLN1 disease is rare and its presentation is nonspecific, it is not uncommon for diagnosis to take two years or more
- CLN1 disease often requires individualized, multidisciplinary care

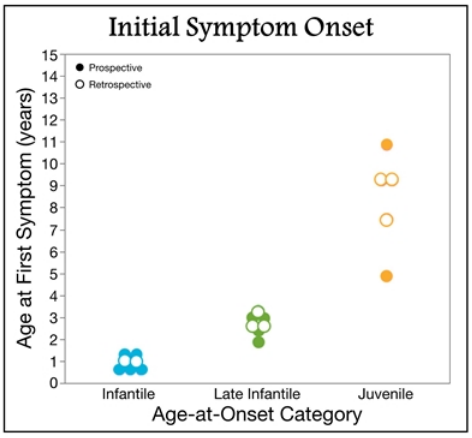
CLN1 disease natural history data

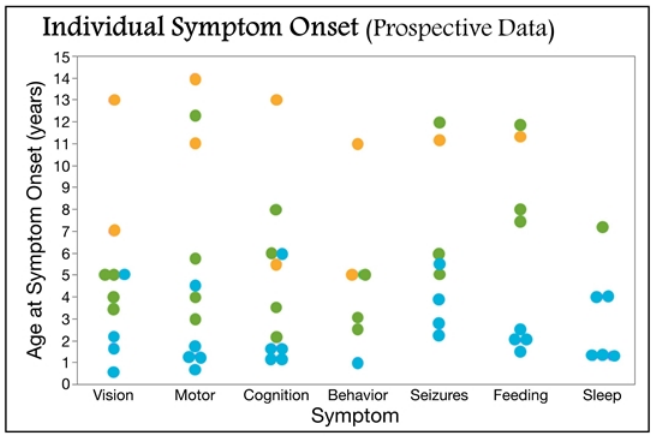
- Ongoing observational study that aims to assess natural history of NCL diseases (including CLN1) as part of the international DEM-CHILD Database (Angela Schulz, Universitätsklinikum Hamburg-Eppendorf)
- University of Rochester NHS used a combined retrospective and prospective approach to characterize age-at-onset of major symptoms and the 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)



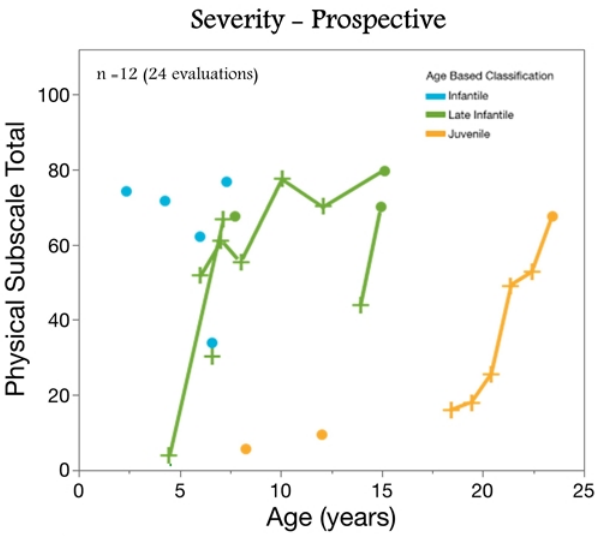


- Infantile: 0 – 1.5 yrs.
- Late Infantile: >1.5 – <5 yrs.
- Juvenile: ≥5 yrs.

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



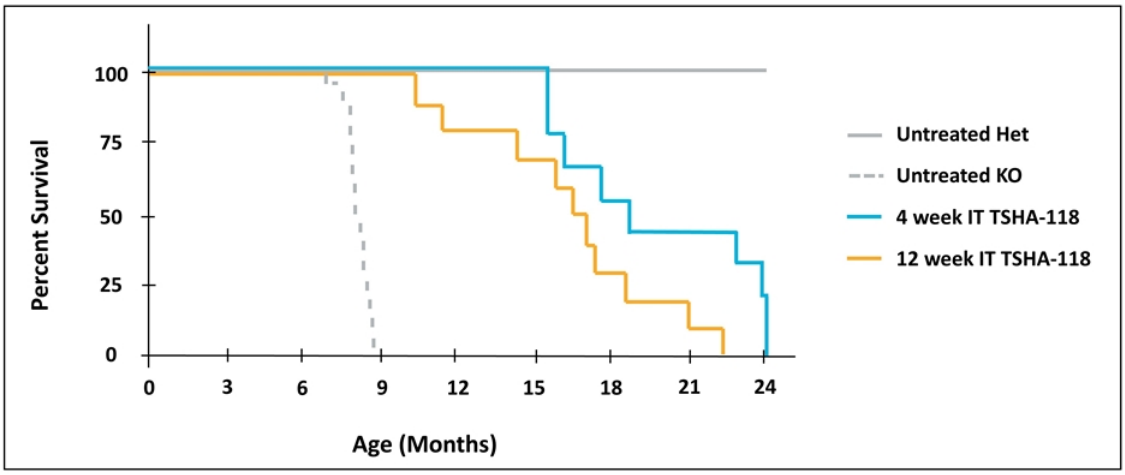
UBDRS physical subscale total score against age in years. Data from individuals with multiple data points connected by lines. Dots represent most recent evaluation. Colors represent age-based classification.

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

TSHA-118 preclinical studies to date

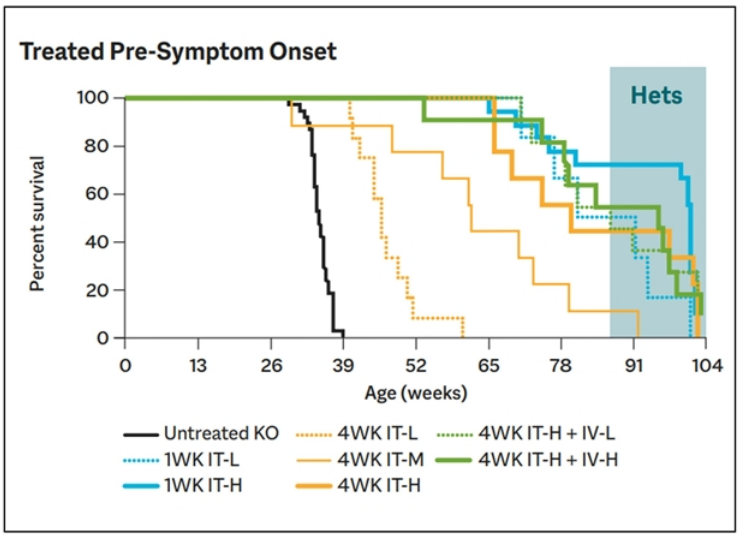
#	Study Scope (ID)	Model System	Age at dosing	Route of Administration & Dose (vg/animal)	Major Findings
1	Proof of Concept; (UNC-2014-001)	PPT1$^{-/-}$ mice	1, 4, 12, 20, 26 weeks	IT: 7E+10, 2.2E+11, 7E+11	• Elevated levels of active PPT1 in serum • Significant survival benefit and functional improvements • Rescue of behavioral deficits
2	Safety and Efficacy (UNC-2015-001)	PPT1$^{-/-}$ and PPT1$^{+/-}$ mice	P0 – P2	IV: 2.8E+11	• Significant survival benefit: median life-span 21 months in treated mice vs. 8.3 months in untreated mice
3	Efficacy of Combination IT and IV Dosing; (UNC-2016-001)	PPT1$^{-/-}$ mice	20 weeks	IT: 7E+10, 7E+11 IV: 7E+11 IT: 7E+10, 7E+11 each in combination with IV: 7E+10, 2.2E+11, or 7+E11	• Dose-dependent survival benefit and improvements in function • Single routes and lower doses provided some benefit • Maximum benefit with high IT plus high IV dose at this stage of disease (i.e. ~ 20 week old mice)
4	Efficacy of Combination IT and IV Dosing; (UNC-2017-001)	PPT1$^{-/-}$ mice	4 weeks	IT: 7E+11; IT: 7E+11 in combination with IV: 7E+10 or 7+E11	• Testing up to 12 months demonstrated survival or behavioral benefits for the combination treatment similar to IT dose alone, which had a median lifespan of 18.7 months
5	Biodistribution and PPT1 Activity Comparison; (UNC-2017-002)	C57B1/6 mice & Fischer rat	Mouse: 9 wks Rat: 11 wks	IT: M: 9.1E+11 R: 3.64E+12	• Wild-type mice and rats had similar biodistribution and enzyme activity after IT injection of TSHA-118
6	Toxicology Study in Rat; (MPI-2389-010)	Wister Hans rat	6 weeks	IT: 2E+11, 2E+12 IV: 5.6E+12, 2E+13 IT: 2E+12 in combination with IV: 2E+13	• Administration of TSHA-118 was not associated with any mortality, clinical observations, bodyweight, or food consumption changes

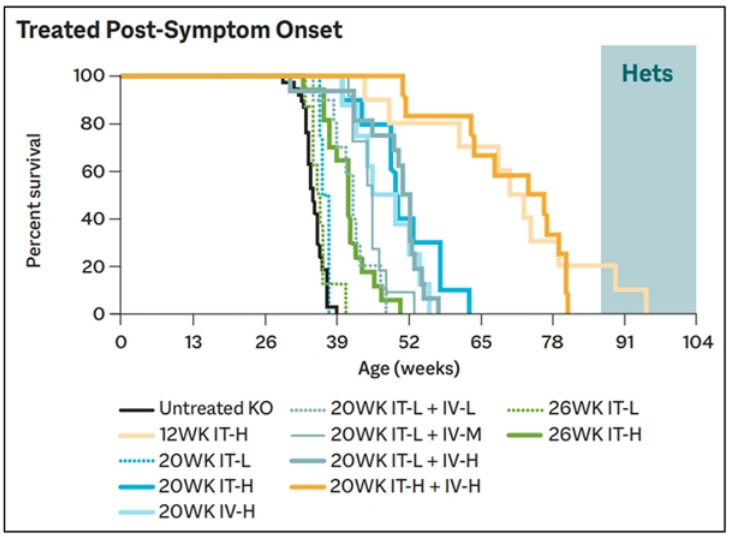
TSHA-118-treated CLN1 KO mice had improved survival rates



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

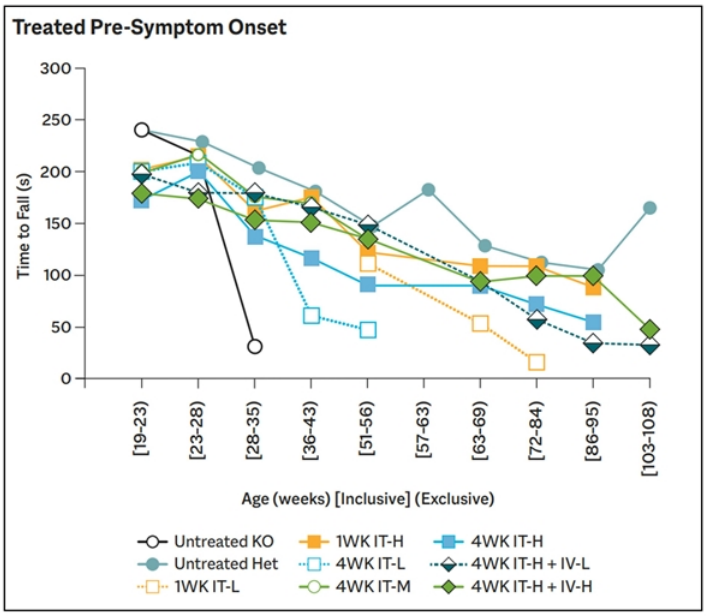
Higher doses of TSHA-118 and earlier intervention mediated stronger rescue of CLN1 KO mice

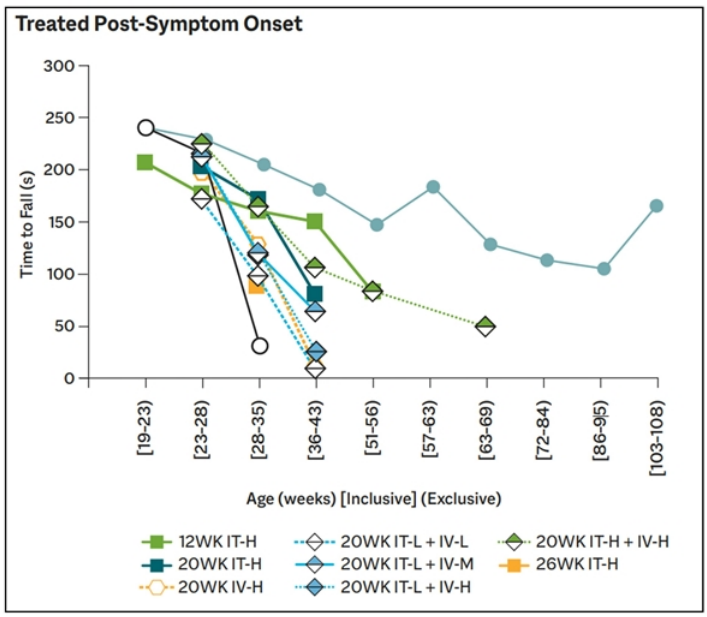




L - $7.0x10^{10}$ vg/mouse M - $2.2x10^{11}$ vg/mouse H - $7.0x10^{11}$ vg/mouse

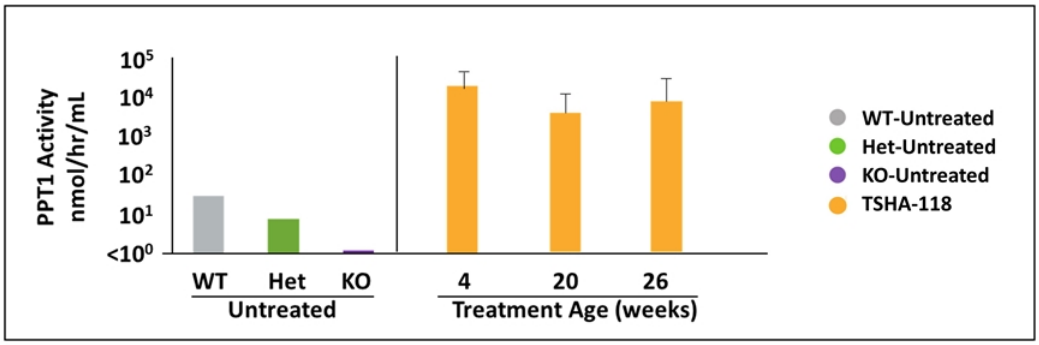
TSHA-118-treated CLN1 KO mice had sustained preservation of motor function





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

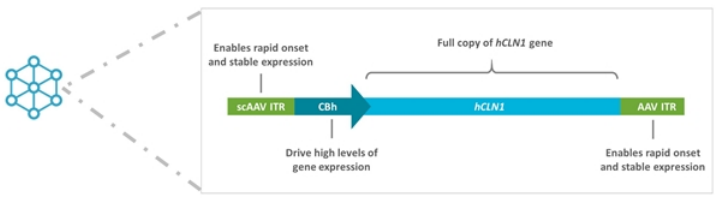
Goals

- **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



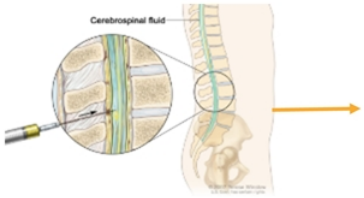
Dose Cohorts

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

Route and Method of Administration

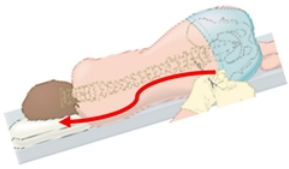
Administration

- 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°)
- 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)

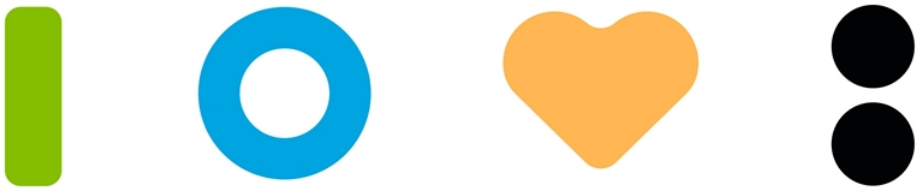
Anticipated next steps for TSHA-118 by the end of 2021

Initiate Phase 1/2 clinical study in 2H 2021 under open IND

CTA scientific advice meetings underway to open European site

Patient finding activity in collaboration with UTSW, Rochester, Hamburg

Q & A



TSHA-102 for Rett Syndrome

Suyash Prasad, MBBS, MSc, MRCP, MRCPCH, FFPM
Chief Medical Officer and Head of R&D

Steven Gray, PhD
Chief Scientific Advisor, UTSW Gene Therapy Program

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

- 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]
- The estimated prevalence of Rett syndrome is 25,000 patients in the US and 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

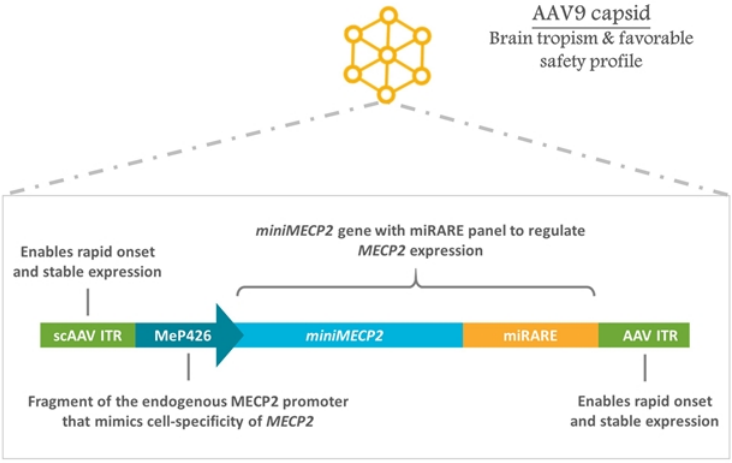
Guy J et al. *Science* 2007

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

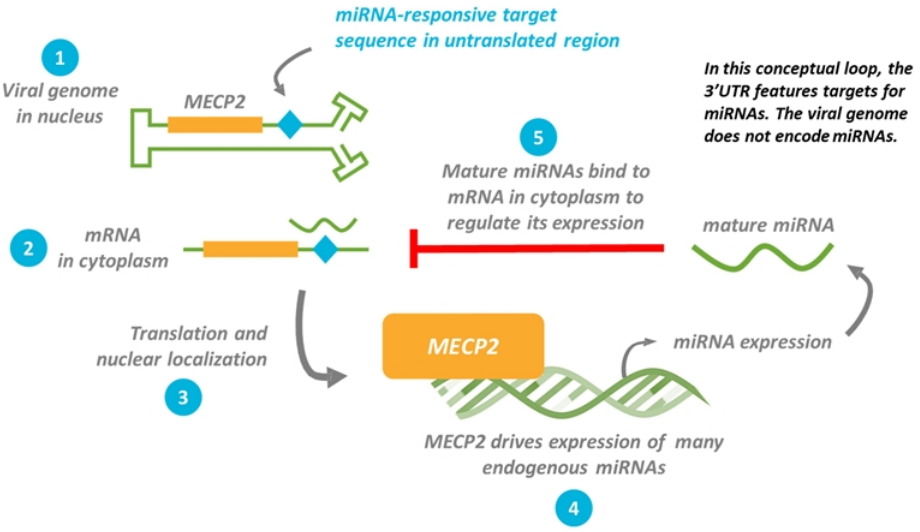
Development of a gene therapy for Rett syndrome requires regulated expression of MECP2

- 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



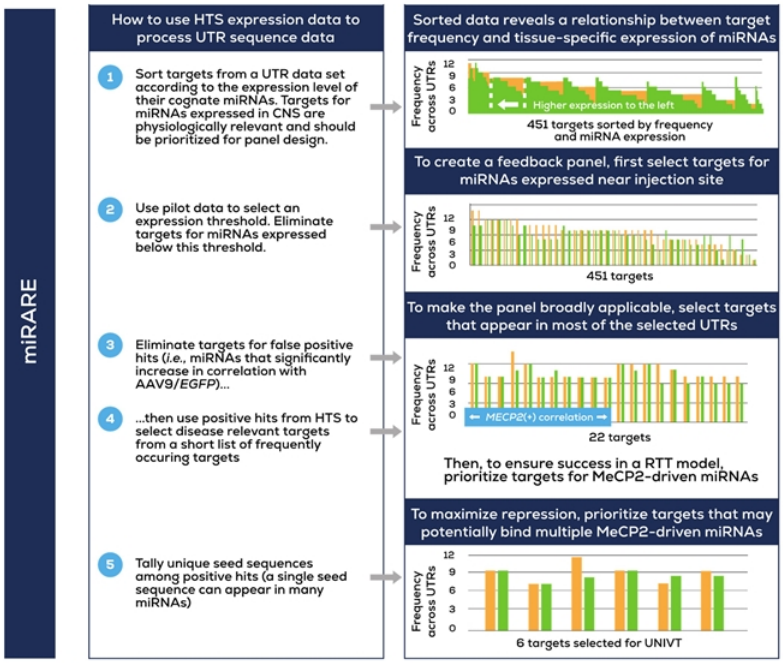
*myc-tagged version of TSHA-102

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



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



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

ACCEPTED MANUSCRIPT

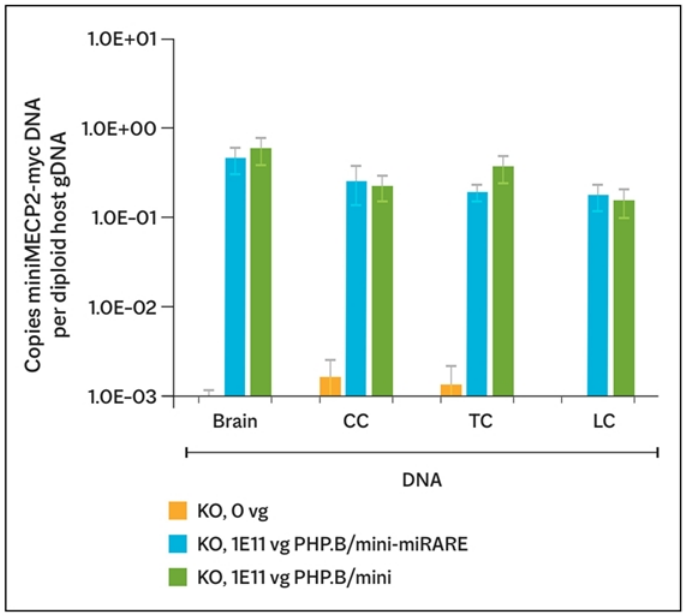
Engineered microRNA-based regulatory element permits safe high-dose mini*MECP2* gene therapy in Rett mice

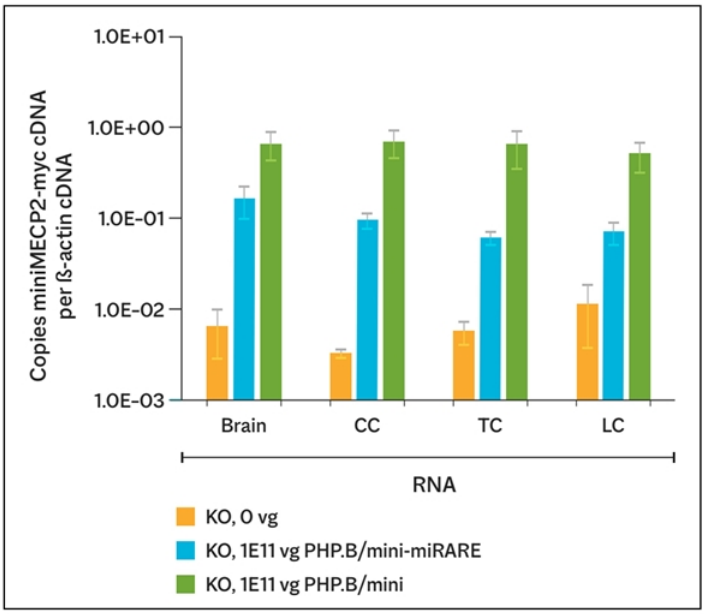
Sarah E Sinnett, Emily Boyle, Christopher Lyons, Steven J Gray

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

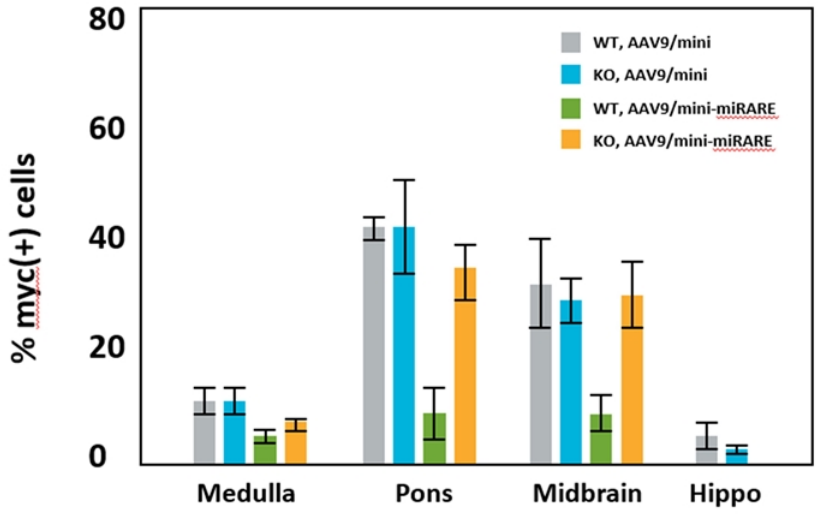
miRARE reduced overall expression of miniMeCP2 transgene expression compared to unregulated miniMeCP2 in WT mice



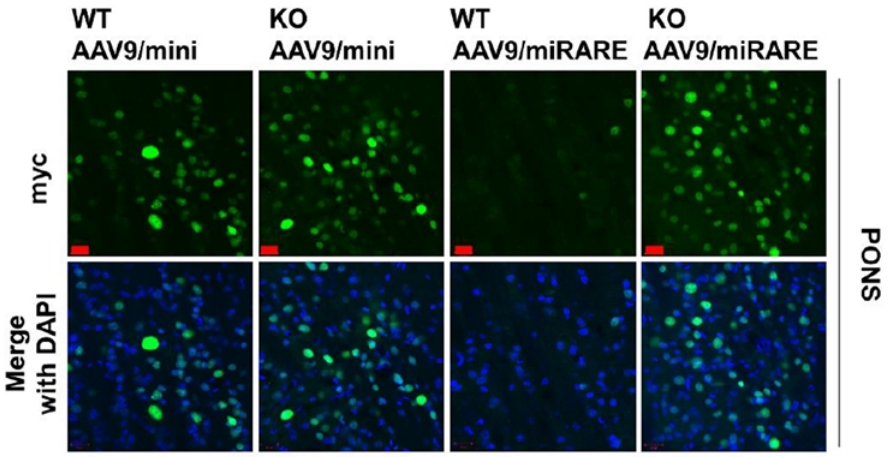


CC: Cervical Cord; TC: Thoracic Spinal Cord; LC: Lumbar Cord

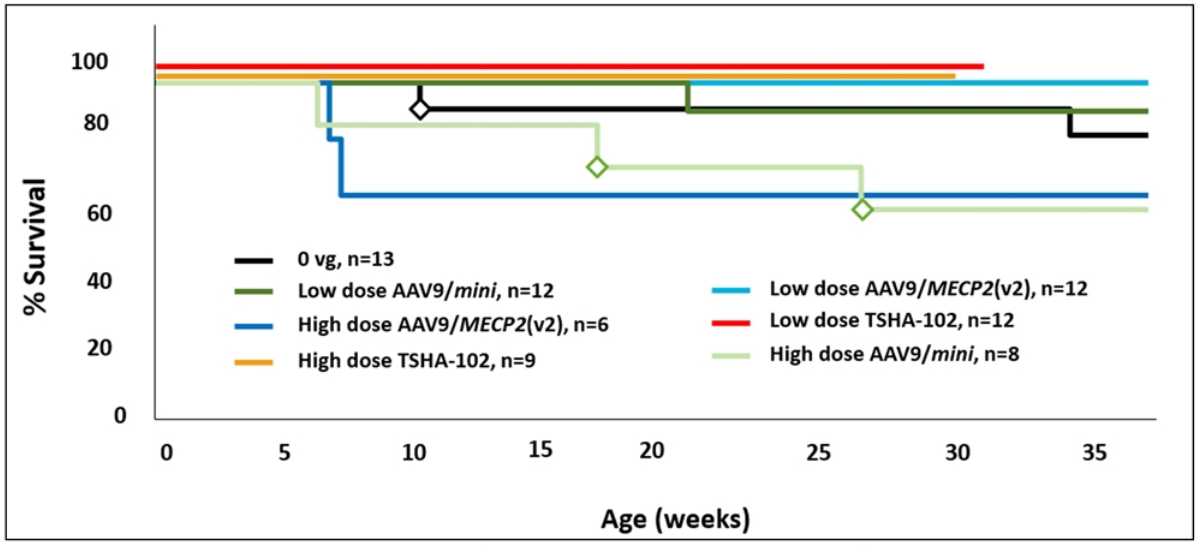
miRARE regulated genotype-dependent *MECP2* expression across different brain regions in wild type and Rett KO mouse models



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



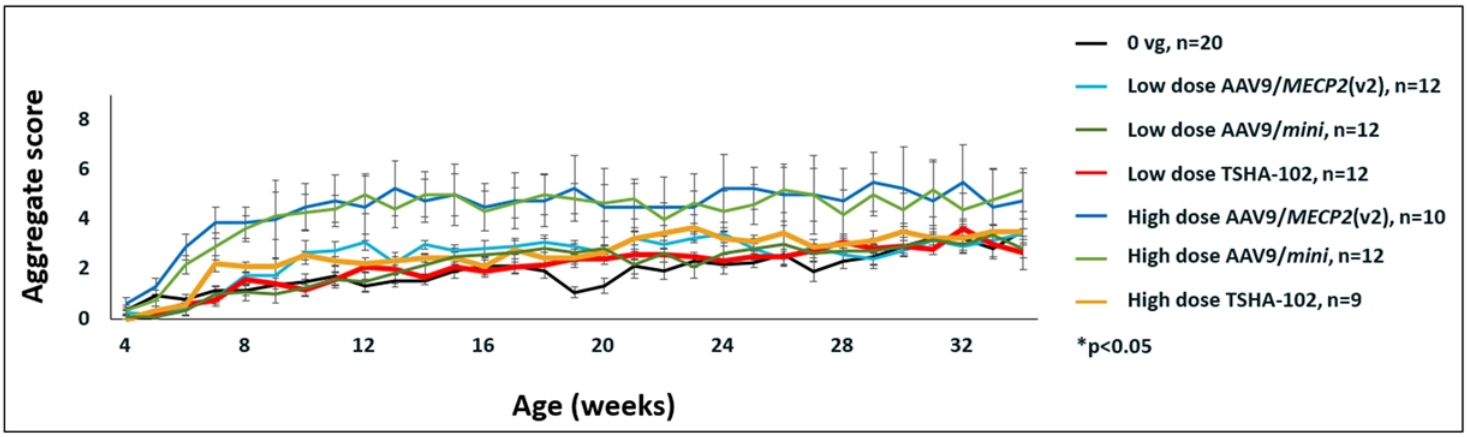
Safety: Intrathecal TSHA-102 was not associated with early death in WT mice



Mice were dosed P28-35

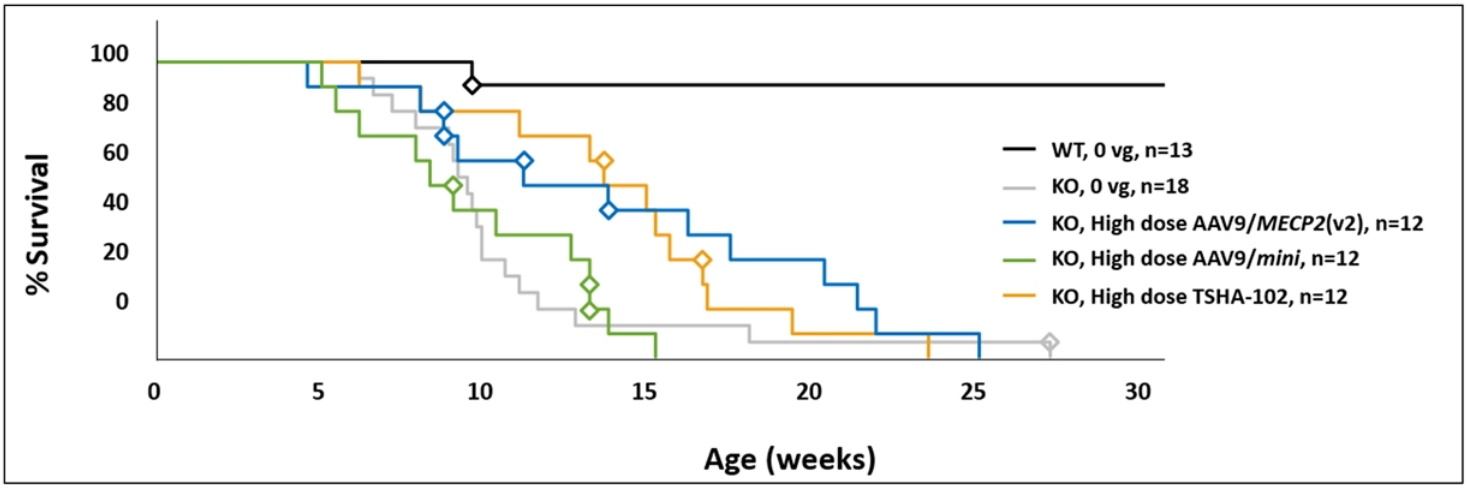
Diamond = vet-requested euthanasia for prolapse or bullying-related injury

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.

*myc-tagged version of TSHA-102

TSHA-102 Phase 1/2 study design plan

Goals and Targets of Trial

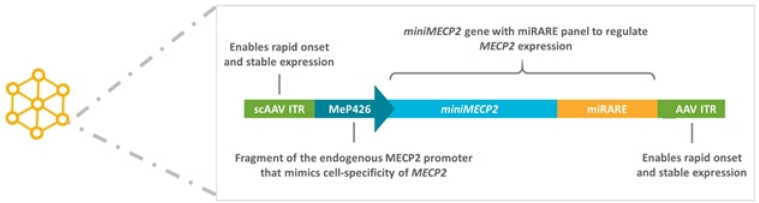
Goals

- **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



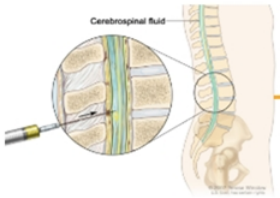
Dose Cohorts

- Each cohort randomized 3:1 (one patient is a delayed treatment control)
- 5×10^{14} total vg (n=4)
- 1.0×10^{15} total vg (n=4)

Route and Method of Administration

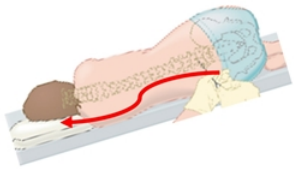
Administration

- 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°)
- 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

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

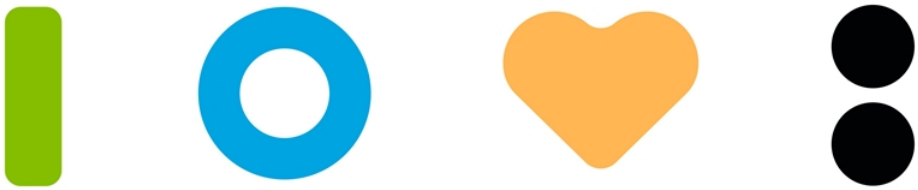
Submit IND/CTA in 2H 2021

Initiate Phase 1/2 study by YE 2021

Complete GMP manufacturing using commercial process

Pre-IND/CTA and Scientific Advice meetings underway

Q & A



Closing Remarks

RA Session II

President, Founder & CEO

TAYSHA
GENE THERAPIES

Thank you