Poster number 23

Safety and Biodistribution Assessment in Non-human Primates (NHPs) of a miniMECP2 AAV9 Vector for Gene-replacement Therapy of Rett Syndrome

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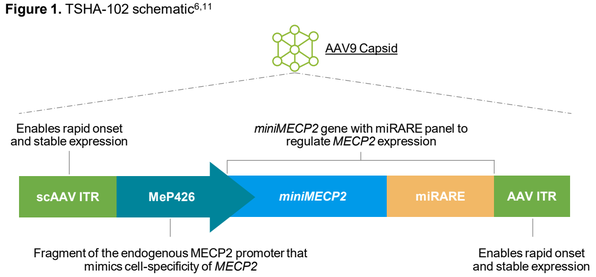
INTRODUCTION

- MeCP2 loss-of-function is a major cause of Rett syndrome, with *MECP2*-inactivating mutations found in >90% of females with typical Rett syndrome and 50–70% of individuals with variant presentation.[1-3] In males with Rett syndrome, *MECP2* mutations have been documented in 56% of cases.[4]
- Enabling MeCP2 expression through gene replacement represents a key strategy for treating the underlying cause of Rett syndrome due to MeCP2 loss-of-function mutations.[5]
- However, it is critical to regulate MeCP2 overexpression, which can be damaging,[6,7] as evidenced by *MECP2* duplication syndrome and mouse models of overexpression toxicity.[8]
- Furthermore, X-chromosome inactivation and mosaicism lead to heterogeneous MeCP2 expression within individuals.[9,10] Therefore, finding the right balance between under- and overexpression is key to disease management,[11] and an MeCP2-expression regulating approach is required on a cell-by-cell basis.
- TSHA-102 is an investigational AAV9 vector containing a truncated, but therapeutically active, version of the human *MECP2* gene (*miniMECP2*), a novel miRNA binding site panel (miRARE), self-complementary AAV inverted terminal repeat elements, and a fragment of the endogenous *MECP2* promoter that contains both regulatory and putative silencer elements (MeP426) (Figure 1).[6,12]

Figure 1. TSHA-102 schematic[6,11]



- In preclinical studies, regulated *miniMECP2* gene replacement was not associated with early death in wild type mice and extended survival in a *Mecp2* knockout mouse survival study.[6]

STUDY OBJECTIVES

- Primary endpoint: to determine the potential safety / toxicity of TSHA-102 when administered intrathecally to cynomolgus monkeys (*Macaca fascicularis*) up to 6 months post-administration.
- Secondary endpoint: to evaluate the biodistribution of TSHA-102 in the same model.

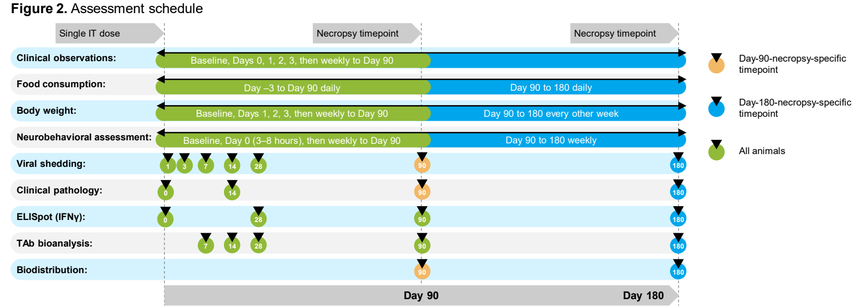
METHODS

- Preliminary safety and biodistribution data are presented from the TSHA-102 300 NHP study and the TSHA-102 200 rat study.
- Female cynomolgus monkeys (N=24; n=3/dose/timepoint; approximately 2 years of age and 2–3.5 kg in weight) were assigned to receive either vehicle or a dose of TSHA-102 as a single intrathecal lumbar puncture injection (Table 1). The highest corresponds to 4x the initial human clinical starting dose (planned at human equivalent dose [HED]). All animals receiving TSHA-102 had AAV9 NAb titers of ≤5. Assessments were carried out over 90 or 180 days (Figure 2).
  - Figure 2 shows the assessment schedule of the study. Blood, urine and feces samples were collected throughout the study for various bioanalytical assays and for determination of viral shedding. Tissue samples were collected at necropsy for biodistribution of vector and transgene expression analyses.

Table 1. TSHA-102 dosing in the NHP study

Dose	TSHA-102 HED Dose (vg/NHP)	TSHA-102 HED (total vg)	90-day necropsy animals (N)	180-day necropsy animals (N)
Vehicle	N/A	N/A	3	3
Low	2.88×10^{13}	2.5×10^{14}	3	3
Medium	5.77×10^{13}	5×10^{14}	3	3
High	2.31×10^{14}	2×10^{15}	3	3

Figure 2. Assessment schedule



RESULTS

Immunogenicity:

- All animals developed anti-AAV9 antibodies and low levels of anti-miniMeCP2 antibodies were observed at 3- and 6-month timepoints.
- One animal had IFNγ reactivity by ELISpot to AAV9 capsid peptides
  - No reactivity was observed in other animals for AAV9 capsid or *miniMECP2*-derived peptides.

Food consumption versus controls and body weight:

- Significant differences between all dose groups and vehicle-treated group occurred on individual days; however, food consumption across the study was not significantly different for all groups.
- There were no significant differences for low, medium and high dose groups' body weight trend over study days compared with vehicle control.

Organ weights:

- No TSHA-102-related differences were observed.

Survival and Clinical Pathology

- Survival: no unscheduled deaths or removals.
- Hematology and coagulation: no significant difference between TSHA-102-treated animals and vehicle-treated animals at any timepoint.
- Serum chemistry:
  - Total protein – significantly lower (<0.9-fold) in the medium- and high-dose groups at Baseline (P<0.05; Dunnett's adjusted P-value compared to vehicle control)
  - ALP – significantly increased (>1.8-fold) in low- and medium-dose groups versus vehicle group at Day 180 necropsy (P<0.05; Dunnett's adjusted P-value compared to vehicle control)
- No significant difference between TSHA-102-treated animals and vehicle-treated animals at other timepoints and in other parameters.

Histopathology

Day 90

- Liver findings: mild to moderate mononuclear cell infiltrates in and around the portal areas.
- Spinal cord / DRG findings: some minimal nerve fiber degeneration (Figure 3).
- Peripheral nerve findings: minimal nerve fiber degeneration occurred in the sciatic, tibial and radial nerves.

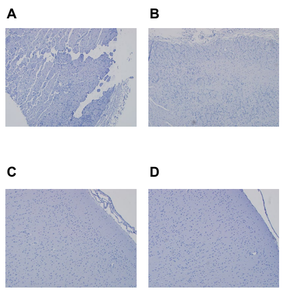
Day 180

- TSHA-102 findings were reduced in incidence or severity by Day 180.

Across both timepoints:

- **Liver findings: non-adverse, no functional deficiency or notable liver enzyme changes.**
- **Nervous system findings: non-adverse, no in-life functional neurological deficits. Observed nerve fiber degeneration and DRG findings were consistent with other AAV9-gene-therapy treated NHPs.[13]**
- **No TSHA-102-related macroscopic findings were observed.**

Figure 3. Representative Day-90 histopathology images of the DRG in vehicle-(A) and medium-dose-treated (B) animals, and the brain in vehicle- (C) and medium-dose-treated (D) animals.



Hematoxylin without eosin-stained tissues (magnification 100x). Medium dose: 5.77×10^{13} vg/animal (5×10^{14} total vg HED).

Biodistribution and gene expression

- Broad Central Nervous System (CNS) biodistribution of vector demonstrated by vector genome detection across tissues (Table 2).
- Low mRNA expression of *miniMECP2* expression in the CNS suggests downregulation by miRARE regulatory element (Table 3).

Table 2. TSHA-102 vector genome biodistribution

NHP Tissue Analyzed Dose: 5.77×10^{13} vg/animal (5×10^{14} total vg HED)	vg / diploid genome Necropsy Day 180 (n = 3)	
	Mean	SD
Brain - Frontal Lobe (Right)	1.49	0.45
Brain - Parietal Lobe (Right)	2.56	1.09
DRG Cervical (Right)	2.09	2.76
DRG Lumbar (Right)	0.50	0.43
DRG Thoracic (Right)	1.82	2.58
Sciatic Nerve (Right)	0.05	0.01
Spinal Cord Cervical	2.77	0.38
Spinal Cord Lumbar	2.39	1.00
Spinal Cord Thoracic	1.95	1.58
Tibial Nerve (Right)	0.04	0.01

Table 3. *miniMECP2* gene expression

NHP Tissue Analyzed Dose: 5.77×10^{13} vg/animal (5×10^{14} total vg HED)	miniMeCP2 mRNA copies/ug total RNA Necropsy Day 180 (n = 3)		Calculated miniMeCP2 mRNA copies/cell* Necropsy Day 180 (n = 3)
	Mean	SD	Mean
Brain - Frontal Lobe (Right)	8	14	0.000042
Brain - Parietal Lobe (Right)	0	0	0
DRG Cervical (Right)	0	0	0.000042
DRG Lumbar (Right)	527	892	0.0039
DRG Thoracic (Right)	8	14	0.000083
Sciatic Nerve (Right)	0	0	0
Spinal Cord Cervical	8	14	0.000042
Spinal Cord Lumbar	583	506	0.003
Spinal Cord Thoracic	230	377	0.0012
Tibial Nerve (Right)	0	0	0

**miniMeCP2 mRNA copies/cell values were calculated by assuming that each cell contains 10 pg of total RNA*

Vector shedding

- TSHA-102 remained at low but detectable levels in the blood to end of study.
- TSHA-102 was undetectable in urine after Day 91 and feces after Day 28.

CONCLUSIONS

- TSHA-102, a gene therapy candidate in development utilizing the miRARE regulatory element, has the potential to allow for regulated expression of the therapeutic *MECP2* gene.
- Following single IT administration of TSHA-102 across three doses:
  - TSHA-102 was not associated with any adverse treatment-related findings at doses 4-fold over the clinical starting dose
  - Throughout the study, there were no TSHA-102-related abnormal clinical observations, body weight or food consumption, or neurobehavioral signs. A significant decrease in ALP at Day 180 necropsy was the only change in clinical pathology associated with TSHA-102
  - Broad distribution of TSHA-102 in CNS with low miniMeCP2 expression (i.e. – mRNA levels) was observed – suggesting miRARE downregulation of transgene expression in these MeCP2-expressing NHPs
- Preclinical safety and efficacy data has led to the progression of a Phase 1/2 trial clinical trial.
  - Open CTA in March 2022
  - Preliminary Phase 1/2 clinical safety and efficacy data from adult females by end of 2022
  - Completed GMP manufacturing using commercial process
  - Pediatric studies to be initiated in 2023

Abbreviations
AAV, adeno-associated virus; ALP, alkaline phosphatase; CNS, central nervous system; DRG, dorsal root ganglion; HED, human equivalent dose; IFNγ, interferon gamma; IT, intrathecal; LLoD, lower limit of detection; MeCP2, methyl-CpG-binding protein 2; miRNA, microRNA; mRNA, messenger RNA; N/A, not applicable; NAb, neutralizing antibody; NHP, non-human primate; PBMC, peripheral blood mononuclear cell; qPCR, real-time quantitative polymerase chain reaction; RT-qPCR, reverse transcription qPCR; SD, standard deviation; vg, vector genome.

Disclosures
SP is the Chief Medical Officer and Head of Research and Development for Taysha Gene Therapies. MN and CS are employees of Taysha Gene Therapies. GF is a former employee of Taysha Gene Therapies. SJG declares a conflict of interest with Asklepios Biopharma, from which he has received patent royalties for IP that are not used in this study. SS and SJG declare a conflict of interest with Abeona Therapeutics, from which they have received royalties for miRARE or related IP. Additional IP related to the TSHA-102 viral genome design, for which SJG and SS are co-inventors, has been licensed to Taysha Gene Therapies.
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