Poster: Tu-147
Abstract: 642

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

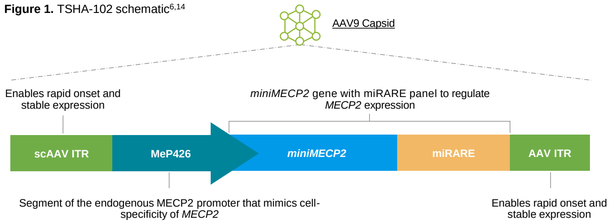
Suyash Prasad[1], Govinder Flora[1], Mary Newman[1], Steven J. Gray[2], Sarah Sinnett[2], Chanchal Sadhu[1]

1. Department of Research & Development, Taysha Gene Therapies R&D, Dallas, Texas, USA; **2.** Department of Pediatrics, The University of Texas Southwestern Medical Center, Dallas, Texas, USA

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 Rett syndrome due to MeCP2 loss-of-function mutations.[5]
- However, it is critical to regulate MeCP2 overexpression, which can be toxic[6,7] as evidenced by *MECP2* duplication syndrome and mouse models of Mecp2 overexpression.[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 AAV2 inverted terminal repeat elements, and a fragment of the endogenous mouse *MECP2* promoter that contains both regulatory and putative silencer elements (MeP426) (Figure 1).[12,13] The miRARE transcription regulator is an inhibitory element.

Figure 1. TSHA-102 schematic[6,14]



- 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 : Potential safety / toxicity of TSHA-102 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.

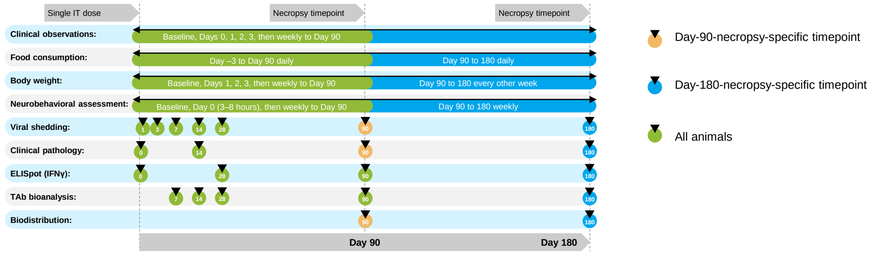
METHODS

- Preliminary safety and biodistribution data are presented from the TSHA-102 300 NHP study
- 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 corresponded to 4 times 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 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	$2.88x10^{13}$	$2.5x10^{14}$	3	3
Medium	$5.77x10^{13}$	$5x10^{14}$	3	3
High	$2.31x10^{14}$	$2x10^{15}$	3	3

Figure 2. Assessment schedule



RESULTS

Food consumption and body weight:
- Overall, test article exposure groups had no significant changes in food consumption compared to vehicle control.
- During the third week post-dosing, the low-dose group had significantly increased food consumption compared with the group receiving vehicle.

Organ weights:
- No TSHA-102-related differences were observed compared to the vehicle control group.

Survival and Clinical Pathology
- Survival: no unscheduled deaths or removals.
- Hematology and coagulation: no significant difference between TSHA-102-treated and vehicle-treated animals at any timepoint.
- Serum chemistry:
  - 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 and vehicle-treated animals at other timepoints and in other parameters.

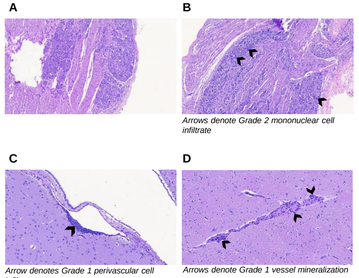
Histopathology

Day 90
- Test article-related changes were mostly minimal to mild in severity, with moderate liver and spinal cord changes associated with the medium dose.
- Liver findings: mild to moderate mononuclear cell infiltrates in and around the portal areas.
- Spinal cord / DRG findings: minimal nerve fiber degeneration (Figure 3).
- Peripheral nerve findings: minimal nerve fiber degeneration in the sciatic, tibial, and radial nerves.

Day 180
- TSHA-102 findings were reduced in incidence and severity by Day 180.
- Test article-related changes were minimal to mild in severity, with a single instance of moderate changes in the spinal cord of one animal dosed at the highest dose ($2.31x10^{14}$ vg / animal).

Across both timepoints:
- **Liver findings: non-adverse, no functional deficiency or notable liver enzyme changes.**
- **Nervous system findings: non-adverse, no functional neurological deficits. Nerve fiber degeneration and DRG findings are consistent with other AAV9-gene-therapy treated NHPs.[15]**
- **No TSHA-102-related macroscopic findings.**

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



Hematoxylin and eosin-stained tissues (DRG magnification 20x objective, brain magnification 40x objective). High dose: $2.31x10^{14}$ vg/animal ($2x10^{15}$ 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: $5.77x10^{13}$ vg/animal ($5x10^{14}$ 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: $5.77x10^{13}$ vg/animal ($5x10^{14}$ 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

*Assumes 10 pg of total RNA / cell

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.

Immunogenicity:
- All animals developed anti-AAV9 antibodies and low levels of anti-miniMeCP2 antibodies 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.

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 on body weight, 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-mediated 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 in 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.
Funding sources: A grant from Rett Syndrome Research Trust (RSRT) to SJG, a donation from Abeona Therapeutics to SJG, and general laboratory funds of SJG (UTSW Dept of Pediatrics) funded preclinical work for miRARE. A grant from Taysha Gene Therapies to SS now provides additional support.
Medical writing support was provided by dna Communications. Writing support was funded by Taysha Gene Therapies Inc.

References:
1. Neul, J.L., et al., *Rett syndrome: revised diagnostic criteria and nomenclature*. Ann Neurol, 2010. 68(6): p. 944-50; **2.** Neul, J.L., et al., *Specific mutations in methyl-CpG-binding protein 2 confer different severity in Rett syndrome*. Neurology, 2008. 70(16): p. 1313-21; **3.** Percy, A.K., et al., *Rett syndrome: North American database*. J Child Neurol, 2007. 22(12): p. 1338-41; **4.** Reichow, B., et al. *Brief Report: Systemic Review of Rett Syndrome in Males*. J Autism Dev Disord, 2015. 45: p. 3377-3383; **5.** Gadalla, K.K., M.E. Bailey, and S.R. Cobb, *MeCP2 and Rett syndrome: reversibility and potential avenues for therapy*. Biochem J, 2011. 439(1): p. 1-14; **6.** Sinnett, S.E.B., E. Lyons, C. Gray, S.J., *A New Approach for Designing a Feedback-Enabled AAV Genome Improves Therapeutic Outcomes of MiniMeCP2 Gene Transfer in Mice Modelling Rett Syndrome (RTT)*. Molecular Therapy (2020 ASGCT Annual Meeting Abstracts), 2020. 28(4, Supplement 1): p. 1-592; **7.** Shao, Y., et al., *Identification and characterization of conserved noncoding cis-regulatory elements that impact Mecp2 expression and neurological functions*. Genes Dev, 2021. 35(7-8): p. 489-494; **8.** Ramocki, M.B., Y.J. Tavyev, and S.U. Peters, *The MECP2 duplication syndrome*. Am J Med Genet A, 2010. 152A(5): p. 1079-88; **9.** Shah, R.R. and A.P. Bird, *MeCP2 mutations: progress towards understanding and treating Rett syndrome*. Genome Med, 2017. 9(1): p. 17; **10.** Zhang, Q., et al., *Genomic mosaicism in the pathogenesis and inheritance of a Rett syndrome cohort*. Genet Med, 2019. 21(6): p. 1330-1338; **11.** Chao, H.T. and H.Y. Zoghbi, *MeCP2: only 100% will do*. Nat Neurosci, 2012. 15(2): p. 176-7; **12.** Liu, J. and U. Francke, *Identification of cis-regulatory elements for MECP2 expression*. Hum Molec Genet, 2006. 15(11): p. 1769-1782; **13.** Adachi, M., E.W. Keefer and F.S. Jones, *A segment of the Mecp2 promotor is sufficient to drive expression in neurons*. Hum Molec Genet, 2005. 14(23): p 3709-3722. **14.** Gadalla, K.K.E., et al., *Development of a Novel AAV Gene Therapy Cassette with Improved Safety Features and Efficacy in a Mouse Model of Rett Syndrome*. Mol Ther Methods Clin Dev, 2017. 5: p. 180-190; **15.** Hordeaux, J., et al., *Adeno-Associated Virus-Induced Dorsal Root Ganglion Pathology*. Hum Gene Ther, 2020. 31(15-16): p. 808-818.