

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

Suyash Prasad¹, Govinder Flora¹, Mary Newman¹, Steven J. Gray², Sarah Sinnett², Chanchal Sadhu¹

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

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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 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.

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.

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 (2.31x10¹⁴ 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.¹⁶**
- No TSHA-102-related macroscopic findings.**

Figure 1. TSHA-102 schematic^{6,14}

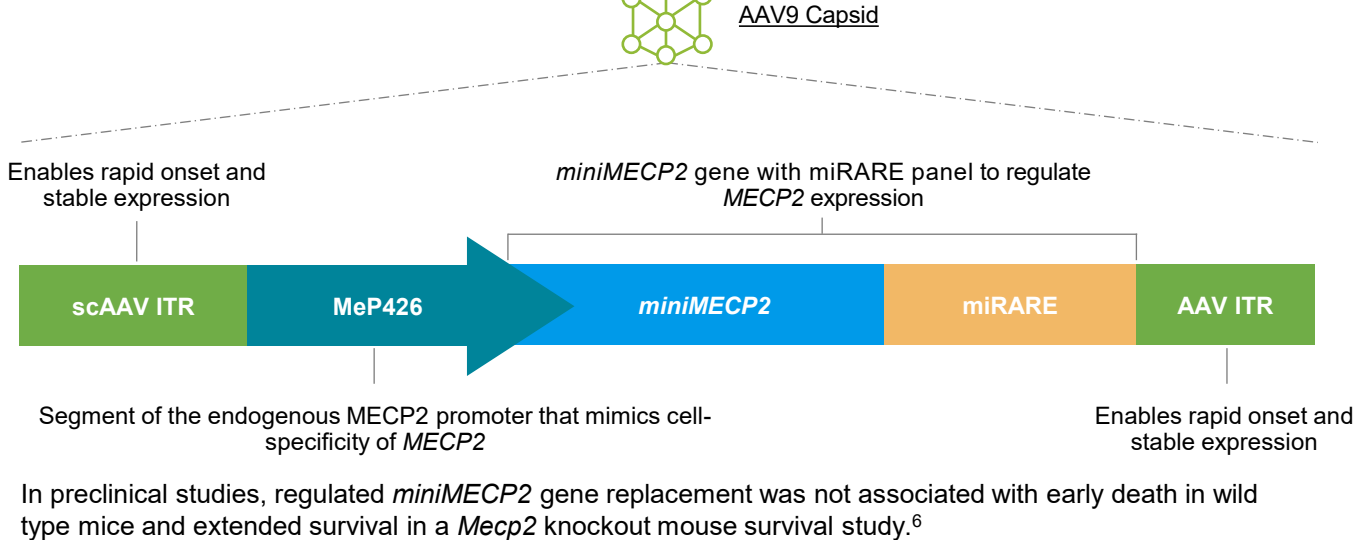
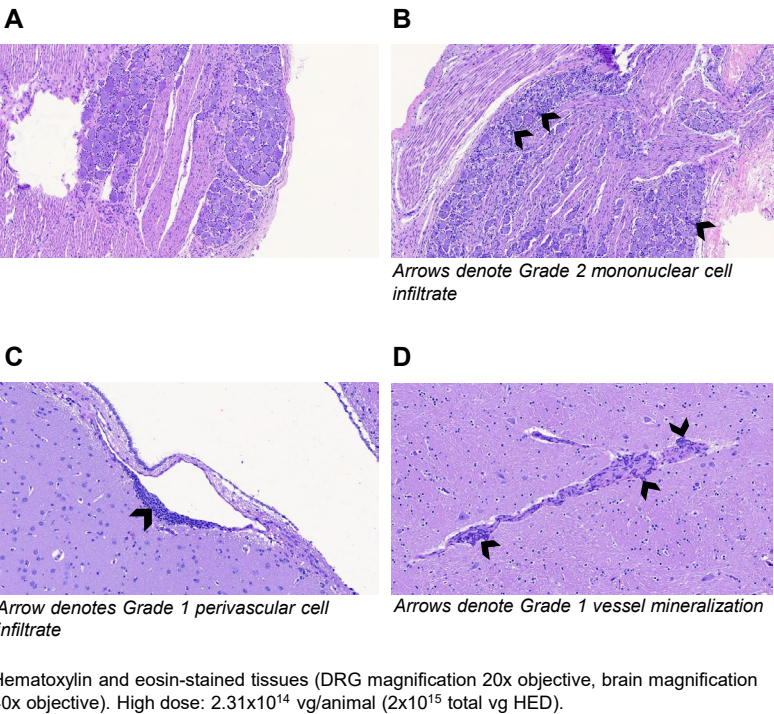


Figure 3. Representative Day-90 histopathology images of the DRG in vehicle-treated animals (A) and high-dose-treated animals (B), and the brain in vehicle-treated animals (C) and high-dose-treated animals (D).



METHODS

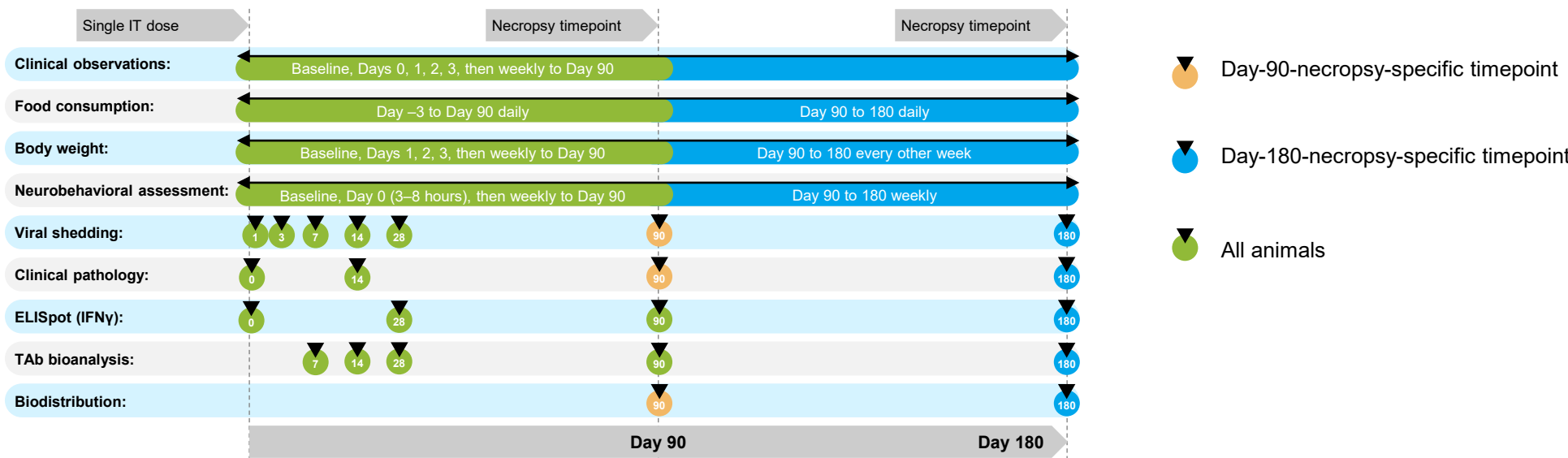
- Preliminary safety and biodistribution data are presented from the TSHA-102 300 NHP study
- Female cynomolgus monkeys (n=24; n=3/dose/timepoint; approximately 2 years of age and 2–3.5 kg in weight) were assigned to receive either vehicle or a dose of TSHA-102 as a single intrathecal lumbar puncture 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



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

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 IFNy reactivity by ELISpot to AAV9 capsid peptides.
 - No reactivity was observed in other animals for AAV9 capsid or *miniMECP2*-derived peptides.

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	
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

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.

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