Assessment of Safety of *miniMECP2* AAV9 vector (TSHA-102) for Gene-replacement Therapy of Rett Syndrome in Rats

Poster: P206

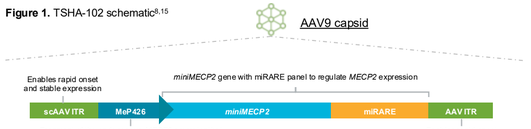
Suyash Prasad[1], Govinder Flora[1], Nagarajan Ramesh[1], Mary Newman[1], Steven J. Gray[2], Sarah E. Sinnett[2], Chanchal Sadhu[1]

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

INTRODUCTION

- Rett syndrome is a rare disorder that affects approximately one in every 10,000 to 15,000 live female births, and rarely occurs in males.[1,2] This neurodevelopmental disease is defined by a constellation of clinical features that, in females, typically occur after the first 6 months of life, including loss of motor skills, loss of acquired spoken language, gait abnormalities and stereotypic hand movements.[1]
- MeCP2 loss of function is a major contributor to Rett syndrome, with *MECP2* inactivating mutations found in >90% of individuals with typical Rett and 50–70% of individuals with variant/atypical presentation.[3,4] MeCP2 expression is associated with neuronal differentiation, maturation and maintenance, and regulates a range of features including dendritic growth, spine maturation and neuronal connectivity in the CNS.[5,6]
- Enabling MeCP2 expression through gene replacement represents a key target for treating the underlying cause of *MECP2*-derived Rett syndrome.[7] However, regulating MeCP2 expression is critical to avoid overexpression, which can be damaging,[8] as evidenced by *MECP2* duplication syndrome and mouse models of 150% overexpression.[9]
- Furthermore, X-chromosome inactivation and mosaicism lead to heterogeneous MeCP2 expression within individuals;[10,11] therefore, a MeCP2-expression regulating approach is required on a cell-by-cell basis.
- TSHA-102 is an investigational AAV9 vector containing a truncated version of the human *MECP2* gene (*miniMECP2*), a novel transcription regulator (miRARE), self-complementary AAV ITR elements, and a fragment of the endogenous mouse *MECP2* promoter containing both regulatory and putative silencer elements (MeP426) (Figure 1).[12,13] The miRARE transcription regulator is an inhibitory element designed to respond to high MeCP2 expression.[8] TSHA-102 is delivered to the CNS via intrathecal lumbar administration.[14]

Figure 1. TSHA-102 schematic[8,15]



- In preclinical trials, TSHA-102 extended survival in *Mecp2* knockout mice, demonstrating the efficacy of miniMeCP2. Additionally, the regulatory elements prevented MeCP2-overexpression-related mortality in wild-type mice.[8]
- In this study, the safety of TSHA-102 was further evaluated in wild-type Sprague Dawley rats, including analyses of the effects of the treatment on sensory and motor nerve conduction to evaluate gene therapy-related DRG toxicity.

METHODS

- The objectives of this study are to determine the potential toxicity and biodistribution of TSHA-102 for the treatment of Rett syndrome when given as a single intrathecal injection.
- The following key parameters and endpoints were evaluated: mortality; clinical observations; body weight; food consumption; body temperature; clinical pathology including clinical chemistry, hematology, urinalysis; organ weights; macroscopic and microscopic examinations; biodistribution of vector DNA and transgene mRNA expression, total AAV9 and anti-transgene product antibodies.
- Analyses are ongoing and here we report mortality, weights, behaviors, clinical pathology, histopathology and key nerve conduction assessments.
- Sprague Dawley rats between 3.4 and 6.1 weeks of age received a single intrathecal lumbar injection according to the study design (Table 1). The high dose corresponded to 4 times the initial human planned dose for clinical trials.

Dose group	Dose level (vg/animal)	Human equivalent dose (vg)	Number of animals – male / female
Control (vehicle)	0	N/A	20 / 20
Low-dose TSHA-102	4.81×10^{11}	2.5×10^{14}	20 / 20
Mid-dose TSHA-102	9.62×10^{11}	5.0×10^{14}	20 / 20
High-dose TSHA-102	3.85×10^{12}	2×10^{15}	20 / 20

- In addition, functional observational battery evaluations were made throughout the study including activity, excitability, autonomic function, neuromuscular function, sensorimotor response and physiological parameters.
- The effect of TSHA-102 on nerve conduction was determined using neuro-electrophysiological evaluations at baseline and Weeks 4, 13 and 25 for five animals per treatment group. Nerve conduction was assessed for the sural / digital nerves (sensory), the left sciatic / tibial nerve (motor), and caudal nerve (mixed sensory and motor – data not shown).
  - In addition to conduction velocity, sensory assessments included SNAP and motor assessments included CMAP, taken at the hock and sciatic notch.
  - F-waves were used to assess conduction over long neuronal pathways including the proximal spinal segments and nerve roots.
  - Statistical significance ($P<0.05$) was identified across groups using ANOVA, with baseline adjusted values at each timepoint.

RESULTS

Mortality, weights and behaviors

- No TSHA-102 mortality and no adverse TSHA-102-related clinical observations occurred. Two deaths occurred, both unrelated to study drug.
  - Body weights and body weight gains, food consumption and body temperature were unaffected by TSHA-102 administration.
  - No TSHA-102-related organ weight changes were noted for all available timepoints (through Day 92).
  - No TSHA-102-related effects on the functional battery throughout the study.

Clinical pathology

- Hematology, clinical chemistry and urinalysis parameters were not associated with any TSHA-102-related changes.

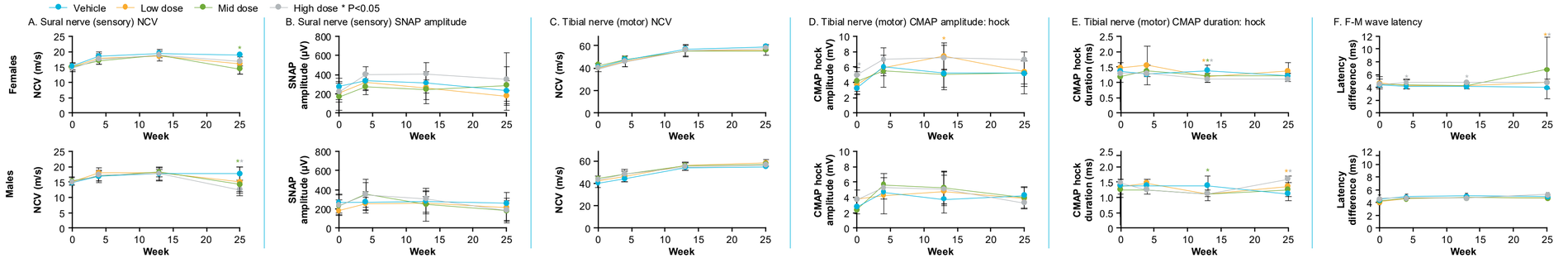
Histopathology

- No macroscopic findings were noted.
- Microscopic evaluations (through Day 92) revealed a low incidence of minimal to mild, rarely moderate, axonal degeneration in the sciatic, tibial and sural nerves as well as nerve fibers associated with the DRG in all groups, including the controls.
- No apparent TSHA-102 dose association in the incidence or severity of the microscopic changes in the nerves.
- Microscopic changes in nerves were consistent with those described in association with rAAV-based gene therapies.[16]

Key nerve conduction assessments

- The sural nerve was used to assess conduction in sensory neurons:
  - No statistically significant or physiologically relevant changes in NCV versus time-matched controls were observed at Weeks 4 and 13.
  - At Week 25, there were statistically significant reductions in NCV in mid-dose male and females, and high-dose males compared to control animals (Figure 2A). Despite minor slowing in the sensory conduction velocity in mid-dosed males and females compared to control animal, NCV remained within physiologically functional limits overall.
  - There were no statistically significant or physiologically relevant differences in SNAP amplitude (Figure 2B) or duration (data not shown) in the TSHA-102 groups versus time-matched controls at all timepoints.
- The tibial nerve was used to assess conduction in motor neurons:
  - No statistically significant or physiologically relevant changes in NCV versus time-matched controls were observed for all timepoints and treatment groups (Figure 2C).
  - No statistically significant reductions or physiologically relevant changes in CMAP amplitude were observed post baseline at both the hock (Figure 2D) and sciatic notch (data not shown) locations versus the control group. Some statistically significant increases in CMAP amplitude compared to control were noted; however, these are not considered physiologically relevant.
  - Statistically significant reduction in CMAP duration at the hock was noted at Week 13 for low-, mid- and high-dose female groups, and mid-dose males versus time-matched controls (Figure 2E). At Week 25, a statistically significant increase in CMAP duration was observed in low- and high-dose male groups compared to the control group (Figure 2E).
- Minor but statistically significant F-M wave latency was observed in high-dose females at all timepoints and mid-dose females at Week 25 (Figure 2F). No significant differences were observed in males (Figure 2F).
- There were no statistically significant differences in caudal NCV between control and treatment groups at any timepoints (data not shown). However, there was a >3 m/s reduction in the mean (not statistically significant) of mid-dose groups compared to control at Week 25 (data not shown).

Figure 2: Mean female (top row) and male (bottom row) nerve conduction outcomes (± standard deviation)



CONCLUSIONS

- TSHA-102 administration was not associated with mortality or changes in weights, body temperature, behaviors or clinical pathology parameters.
- Minimal to mild microscopic axonal degeneration was observed related to treatment. This type of observation is consistent with AAV9 administration.[16]
- The microscopic histopathology observations did not convert into observable changes in behavior.
- Overall, with the exception of individual outliers, nerve conduction metrics remained within functional physiological ranges for all groups and timepoints. Week 25 reductions in nerve conduction suggest possible slow progression of functional loss with late onset symptoms.
  - Reduced sensory NCV indicates a reduction in nerve myelination or loss of fast-conducting fibers. However, the sensory conduction velocity remained within physiologically functional limits overall and the SNAP data suggest limited overall axonal loss.
  - The reduction in motor CMAP duration observed at Week 13 suggests a small gain of function with unclear significance. The small increase in CMAP duration at Week 25 is indicative of possible minor diffuse demyelination; however, the lack of change in CMAP amplitude at all timepoints and doses indicates the absence of significant axonopathies.
  - The limited increases in F-M wave latency indicate minimally impacted conduction in the proximal regions of the motor nerve.
- Overall, TSHA-102 was well tolerated in wild-type Sprague Dawley rats with no observed adverse effects.
- Preclinical safety and efficacy data has led to the progression of TSHA-102 to Phase 1/2 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; AAV9, adeno-associated virus serotype 9; CMAP, compound muscle action potential; CNS, central nervous system; DRG, dorsal root ganglion; ITR, inverted terminal repeat; MeCP2, methyl CpG binding protein 2; MeP, methyl CpG binding protein 2 promoter; miRARE, microRNA-responsive auto-regulatory element; N/A, not applicable; NCV, nerve conduction velocity; rAAV, recombinant adeno-associated virus; scAAV, self-complementary adeno-associated virus; SNAP, sensory nerve action potential; vg, vector genome

Disclosures

SP is the Chief Medical Officer and Head of Research and Development for Taysha Gene Therapies. GF is a former employee of Taysha Gene Therapies. NR are employees of Taysha Gene Therapies. NR, MN and CS are employees 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.

This study was funded by Taysha Gene Therapies.

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.** Lauvick, C.L., et al., *Rett syndrome in Australia: a review of the epidemiology.* J Pediatr, 2006. **148**(3): p. 347-52. **3.** 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. **4.** Percy, A.K., et al., *Rett syndrome: North American database.* J Child Neurol, 2007. **22**(12): p. 1338-41. **5.** Zhou, Z., et al., *Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation.* Neuron, 2006. **52**(2): p. 255-69. **6.** Gulmez Karaca, K., D.V.C. Brito, and A.M.M. Oliveira, *MeCP2: A Critical Regulator of Chromatin in Neurodevelopment and Adult Brain Function.* Int J Mol Sci, 2019. **20**(16). **7.** 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. **8.** Sinnett, S.E., et al., *Engineered microRNA-based regulatory element permits safe high-dose miniMECP2 gene therapy in Rett mice.* Brain, 2021. **144**(10): p. 3005-3019. **9.** 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. **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.** Shah, R.R. and A.P. Bird, *MeCP2 mutations: progress towards understanding and treating Rett syndrome.* Genome Med, 2017. **9**(1): p. 17. **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.** Gray, S.J., et al., *Global CNS gene delivery and evasion of anti-AAV-neutralizing antibodies by intrathecal AAV administration in non-human primates.* Gene Ther, 2013. **20**(4): p. 450-9. **15.** 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. **16.** Bolt, M.W., et al., *Development challenges associated with rAAV-based gene therapies.* J Toxicol Sci, 2021. **46**(2): p. 57-68.