A Human-ready Regulated AAV9/mini*MECP2*- miRARE Gene Therapy (TSHA-102) Improves Survival, Weight, and Behavior After Intracerebroventricular (ICV) Dosing in the Neonatal Knockout Rett (RTT) Mouse Model

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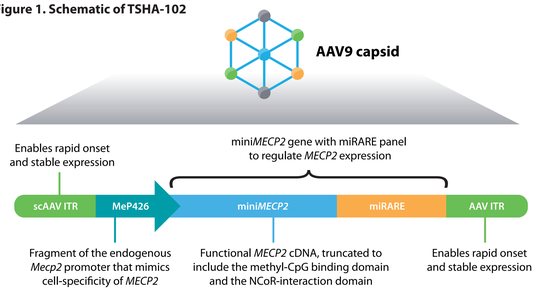
INTRODUCTION

- Methyl-CpG binding protein 2 (MeCP2) is an X-linked transcriptional regulator and chromatin binding factor that is essential for normal neurodevelopment[1-3] and adult neuronal function[4]
- In girls and women with typical Rett syndrome (*MECP2*$^{-/+}$ heterozygotes), *MECP2* expression is silenced in a random and cell-autonomous manner due to X chromosome inactivation[5]
- Mouse studies of Rett syndrome typically employ *Mecp2*$^{-/Y}$ males, which display developmental, physiological and behavioral features recapitulating the human disease. These males express Rett-like phenotypes as early as Week 3[6] and die in early adulthood[5,7]
- Rett syndrome represents a challenging case for human gene therapy because the therapeutic window for *MECP2* transgene expression is narrow; as with *MECP2* deficiency, *MECP2* gene duplication leads to serious neurodevelopmental disease[8,9]
- One approach to safe therapeutic reversion of Rett syndrome is to engineer post-transcriptional feedback repression into a therapeutic *MECP2* transgene, mitigating risk of MeCP2 overexpression
- TSHA-102 (**Figure 1**) is a human-ready adeno-associated virus- (AAV-) based gene therapy construct for use in Rett syndrome. This construct contains:[9]
  - A truncated but functional MECP2 cDNA (mini*MECP2*)[10] under control of a *Mecp2* promoter fragment (MeP426)
  - A microRNA- (miRNA-) responsive autoregulatory element (miRARE), designed to regulate expression of mini*MECP2* in the CNS

Figure 1. Schematic of TSHA-102



- The miRARE, inserted in the 3′ UTR of mini*MECP2*, carries targets for 6 miRNAs; these miRNAs were selected based on:[9]
  - Expression in CNS tissues near the expected site of delivery, and/or
  - Expression correlating with that of MeCP2, suggesting that they could participate in feedback repression of mini*MECP2*
- Studies with earlier AAV vectors that preceded TSHA-102 demonstrated that:[9,11,12]
  - Inclusion of miRARE in a mini*MECP2-myc* construct injected intrathecally via lumbar puncture in 4 to 5-week-old *Mecp2*$^{-/Y}$ males extended the animals' lifespan significantly, relative to a construct lacking miRARE
  - Mini*MECP2* constructs encoding a Myc tag (but otherwise identical to TSHA-102) generated a functional MeCP2 protein localizing to nuclei in the CNS
  - When the miRARE was included in the construct, transgene expression was regulated throughout the brain, with genotype-dependent expression in the pons and midbrain
- Although the most complete therapeutic correction of Rett syndrome is expected to follow from early treatment, TSHA-102 has not been previously studied in mice injected early in postnatal life

OBJECTIVE

To explore the safety and pharmacologic effects of the human-ready construct TSHA-102, delivered by intracerebroventricular (ICV) injection to *Mecp2*$^{-/Y}$ (KO) and control (WT) male mice on postnatal day 2 (P2)

METHODS

GENERATION OF MOUSE PUPS

- Heterozygous females (B6.129P2(C)-*Mecp2*$^{tm1.1bird}$/J) were bred with C57BL/6J males, and offspring were genotyped. Pups were allocated to TSHA-102 or vehicle treatment. Mice were maintained for up to 36 weeks. Prior to this time, they could be euthanized for humane reasons if they lost 20% of bodyweight or per veterinarians' judgment
- Injections, health monitoring and phenotypic testing were performed blind to genotype and treatment allocation

ICV INJECTION

- On P2, up to 48 hours after birth, anesthetized pups were injected bilaterally ICV with 1 µL per ventricle TSHA-102 (8.8 x 10^{10} viral genomes/mouse) or vehicle
- This route of administration was chosen due to the technical challenges of intrathecal dosing in early postnatal mice

ASSESSMENTS

- Weight and Bird Score[13] were assessed twice weekly for 2 weeks following injection; weekly thereafter. Pups losing 10% from peak bodyweight were assessed every other day
- Bird Score (range 0 – 12; higher score indicating more severe abnormality) consists of 6 sub-scores (range 0 – 2), all evaluated by visual inspection. These include mobility, gait, hindlimb clasping, tremor, breathing, and general condition

STATISTICS

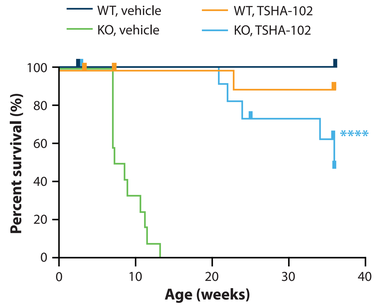
- GraphPadPrism 9 was used to calculate significance. Employing GraphPad's recommended tests, the Gehan-Breslow-Wilcoxon test was used to assess survival differences. For weight and aggregate Bird Score, significance was evaluated at all time points where comparison was possible, using two-way ANOVA followed by Sidak's multiple comparison test. Student's *t* test was used to assess significance of age-of-onset differences in gait and hindlimb clasping

RESULTS

45 pups were treated with TSHA-102 or vehicle (27 KO male and 18 WT male). For survival analysis (**Figure 2**):

- Among vehicle-treated KO pups (n=14), all animals died, with a median survival of 8.1 weeks
- Median survival of WT animals treated with vehicle (n=6) or TSHA-102 (n=12) was 36 weeks, i.e., the duration of follow-up in this study. No significant difference in mortality was seen between these groups
- TSHA-102-treated KO mice (n=13) survived a median 36.0 weeks, representing a significant (p<0.0001) >4-fold extension of their lifespan versus vehicle-treated KO mice

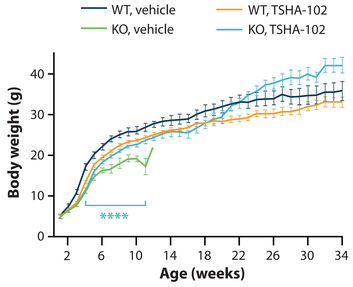
Figure 2. Extended survival of *Mecp2*$^{-/Y}$ neonatal mice following ICV injection with TSHA-102. ****, p<0.0001 (KO TSHA-102 vs vehicle)



Weight increase (**Figure 3**) was analyzed across the 4 groups of pups to postnatal Week 11 (for comparisons involving vehicle-treated KO mice) or Week 34 (for other comparisons)

- Vehicle-treated KO mice showed reduced growth compared to other groups (p<0.0001)
- TSHA-102-treated WT mice showed a trend to lower bodyweight versus vehicle-treated WT mice; their growth followed a generally normal trajectory to Week 34
- TSHA-102 treatment of KO mice led to bodyweight increase (p<0.05 versus vehicle-treated KO mice). This improvement in growth trajectory was significant starting at Week 6

Figure 3. Normalization of bodyweight in *Mecp2*$^{-/Y}$ neonatal mice following ICV injection with TSHA-102. ****, p<0.0001 (KO TSHA-102 vs vehicle)



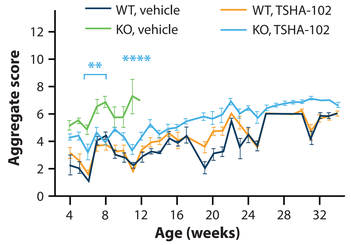
Rett symptom-like phenotypes were assessed with the Bird scoring system (**Figure 4**)

- In WT mice, this analysis showed no consistent difference between TSHA-102 and vehicle treatment
- Conversely, in KO mice, TSHA-102-treated animals had a significantly milder phenotype than vehicle-treated animals at several time points. Bird Scores in these animals increased gradually over 36 weeks

These differences in phenotypic severity arose in part due to gait abnormalities[7] and hind-limb clasping behaviors[9] in vehicle-treated mice. These phenotypes were significantly (p<0.001) delayed with TSHA-102 treatment:

- Onset of severely abnormal gait (e.g., tremor when feet are lifted; backward walking; hopping) was significantly delayed from mean 8 weeks to 20 weeks
- Onset of severe clasping behavior (legs pulled in tightly toward the body or toward one another) was delayed from mean 7 weeks to 21 weeks

Figure 4. Early sustained improvement in Rett-like phenotypes (aggregate Bird Score) in *Mecp2*$^{-/Y}$ neonatal mice following ICV injection with TSHA-102. **, p<0.01 (KO TSHA-102 versus KO vehicle); ****, p<0.0001 (KO TSHA-102 versus KO vehicle)



DISCUSSION

Administered by ICV injection in male P2 mice, TSHA-102 met expectations[9,11,12] for safety and pharmacologic efficacy. Thus, in the current study:

- In WT animals, TSHA-102 treatment showed no toxicity, relative to vehicle treatment.
  - Survival of treated WT mice was not reduced over 36 weeks
  - Bird Score analysis (for Rett syndrome-like behaviors and pathologies) showed no treatment effect
  - However, WT animals' growth trajectory was reduced with TSHA-102 treatment, contrasting with the restoration of normal and faster-than-normal growth seen in KO animals
- While vehicle-treated KO pups survived for ≤13.3 weeks in all cases, half (47%) of TSHA-102-treated KO mice survived for the duration of this 36-week study
  - The >4-fold extension of KO mouse lifespan observed here is substantially greater than has been observed with other gene therapy vectors and dosing protocols[9,11,12,14]
- In KO mice, aggregate Bird Score was significantly improved by TSHA-102 treatment at several time points, with an overall significant treatment effect
- This improvement was reflected in a significant delay in the onset of severe gait and limb abnormalities

These findings, combined with earlier studies with a *myc*-tagged version of this construct,[9] suggest that the miRARE element supports therapeutic expression of miniMeCP2 protein in MeCP2-deficient CNS tissues while suppressing toxic overexpression of the transgene. The use of a miRARE-like element may be broadly useful for engineering gene therapies where gene dosage must be tightly controlled

CONCLUSIONS

- **Early postnatal ICV administration of TSHA-102 profoundly increased longevity in *Mecp2*$^{-/Y}$ KO mice. The miRARE appears to regulate transgene expression in this mouse model, which may translate to clinical benefits for treated Rett syndrome patients. The safety of this therapeutic in WT males, along with the pharmacologic benefits seen in KO males, provide indirect support for use of this human-ready construct in *MECP2*$^{-/+}$ Rett patients, where the virus will transduce mosaic tissues in which cells express either WT or mutant MeCP2**

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DISCLOSURES

SS and **SJG** have received royalties and other revenue for miRARE-related IP licensed to Taysha Gene Therapies

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