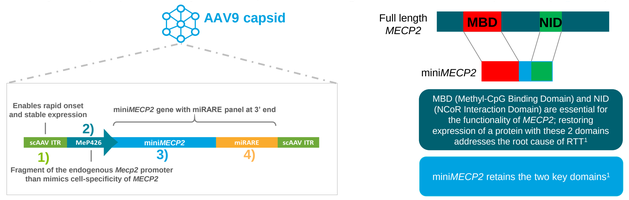
TSHA-102 Investigational Gene Therapy for Rett Syndrome: First-cohort Data from the REVEAL Adolescent/Adult and Pediatric Studies

E Rossignol,[1] EM Berry-Kravis,[2] C Buhrfiend, [2] J Downs, [3] T Clarkson,[4] B Seremula,[5] E Tesfaye,[4] R Haque-Ahmed,[4] E Squiers, [4] B Maru,[4] M Schultz,[4] N Devidze [4]

[1]Departments de Neurosciences and Pediatric, Université de Montréal and Centre Universitaire Hospitalier Sainte-Justine, Montreal, Quebec, Canada; [2]Departments of Pediatrics, Neurological Sciences, Anatomy and Cell Biology, Rush University Medical Center, Chicago, IL, USA; [3]Telethon Kids Institute, Centre for Child Health Research, University of Western Australia, Nedlands, Australia; [4]Taysha Gene Therapies, 3000 Pegasus Park Drive, Suite 1430. Dallas, Texas, USA; [5]Atorus Research Inc., Newtown Square, PA, USA.

Introduction

TSHA-102 is a gene therapy product designed to treat adolescent/adult and pediatric patients with Rett Syndrome (RTT). To safely restore Methyl CpG-binding Protein 2 (MeCP2) function, this construct includes a microRNA-responsive target sequence (miRARE) designed to modulate transgene expression in the central nervous system (CNS) on a cell-by-cell basis, thus minimizing the risk of toxic overexpression (Figure 1).



MBD (Methyl-CpG Binding Domain) and NID (NCoR Interaction Domain) are essential for the functionality of *MECP2*; restoring expression of a protein with these 2 domains addresses the root cause of RTT[1]

miniMECP2 retains the two key domains[1]

Figure 1: Key features of TSHA-102: 1) Inverted terminal repeats (ITR) that allow efficient packaging of the viral genome. 2) A *Mecp2* promoter fragment driving transgene expression in neurons/other brain cells. 3) mini*MECP2*, encoding a truncated version of the human MeCP2, with two essential MeCP2 domains (MBD (red) and NID (green)), and a nuclear localization signal (blue). 4) The miRARE, a post-transcriptional regulatory element designed to allow for silencing of the transgene in cells with MeCP2 function.[1-4]

The REVEAL Adolescent/Adult (AA; NCT05606614) and Pediatric (Ped; NCT06152237) studies are first-in-human trials of TSHA-102 gene therapy. For both studies, the complete first cohorts (n=2, for each) received a 5.7×10^{14} vg dose of TSHA-102 and are undergoing follow-up assessments (Figure 2). The patients have diverse clinical histories and varied *MECP2* mutations, including deletions and point mutations. Safety and efficacy data are available for variable periods, extending to Week 52 for the first dosed patient (AAP1).

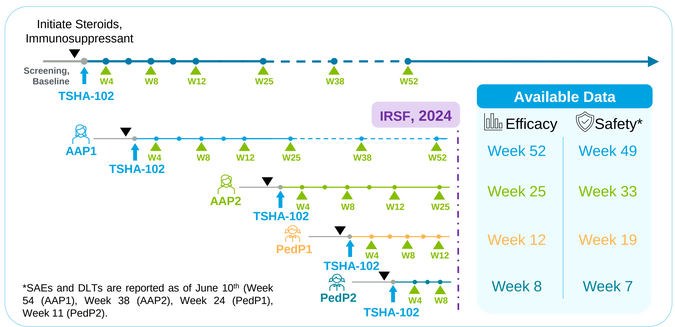


Figure 2: The REVEAL Clinical Trials (Cohort 1; green triangles indicate scheduled visits for efficacy endpoints evaluation).

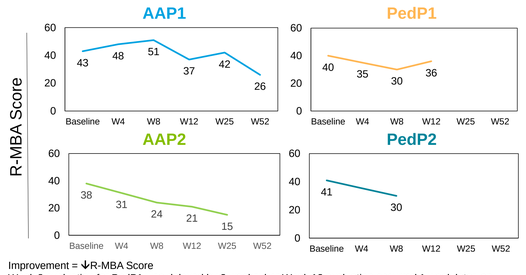
Methods

Following immunosuppression initiation at Day –7, TSHA-102 (5.7×10^{14} vector genomes) is infused intrathecally via lumbar puncture (LP) on Day 0. Patients were placed in Trendelenburg position post-dosing. Regular safety monitoring includes clinical and laboratory evaluations. Prespecified efficacy measures used in REVEAL are shown below.

Outcome	Evaluated by	Scoring	REVEAL Adolescent / Adult	REVEAL Pediatric
Revised Motor Behavior Assessment (R-MBA)	Physician	• 24-item clinician-evaluated scale. • Possible scores range from 1 to 58. • Higher values indicate more severe motor deficits.	✓	✓
Clinician's Global Impression-Severity (CGI-S)	Physician	• 7-point scale indicating global clinical severity at a single time point. • Higher scores indicate greater severity.	✓	✓
Clinician's Global Impression-Improvement (CGI-I)	Physician	• 7-point scale indicating global change in clinical severity. • Values <4 (Unchanged) represent clinical improvement relative to Baseline.	✓	✓
Caregiver/Parental Global Impression-Improvement (PGI-I)	Caregiver	• 7-point scale indicating global change in clinical severity. • Values <4 (Unchanged) represent improvement relative to Baseline.	✓	✓
RTT Behavior Questionnaire (RSBQ)	Caregiver	• 8-domain scale. • Possible overall scores from 0 to 90. • Higher values indicate more severe functional deficits.	✓	✓
Mullen Scale Adapted for use in RTT (MSEL-A)	Psychologist	• 4-domain scale permitting independent evaluation of receptive and expressive language, visual reception, and fine-motor function. • Higher scores indicate improved cognitive skills.		✓
RTT Hand Function Scale (RSHFS)	Physical Therapist	• Separate evaluations of dominant and non-dominant hand. • Higher scores indicate improved hand function.	✓	

Results

Efficacy Summary



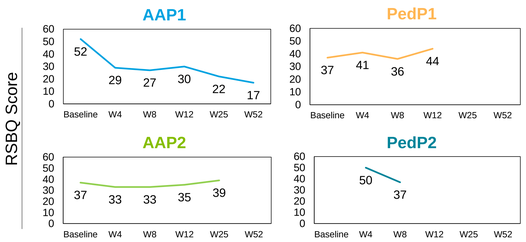
Improvement = ↓R-MBA Score
Week 8 evaluation for PedP1 was delayed by 3 weeks; her Week 12 evaluation occurred 1 week later, on schedule.

Figure 3: R-MBA
All 4 patients showed improvement in R-MBA score at the latest time point analyzed, relative to Baseline.
- While AAP1 did not show a change at early timepoints, she experienced a further 16-point improvement 9 weeks after immunosuppressant taper (completed at Week 43).
- Three of the patients (AAP1, AAP2, and PedP2) showed improvement that appears to be clinically meaningful.

	AAP1	AAP2	PedP1	PedP2
CGI-S				
Baseline	6 (Severely ill)	4 (Moderately ill)	5 (Markedly ill)	4 (Moderately ill)
Week 4	5	4	5	4
Week 8	5	4	5	4
Week 12	5	4	5	--
Week 25	5	4	--	--
Week 52	5	--	--	--
CGI-I				
Baseline	NA	NA	NA	NA
Week 4	2	3	3	3
Week 8	2	3	3	2
Week 12	2	3	3	--
Week 25	2	3	--	--
Week 52	3	--	--	--
PGI-I				
Baseline	NA	NA	NA	NA
Week 4	3	3	3	3
Week 8	3	3	3	2
Week 12	2	3	3	--
Week 25	2	3	--	--
Week 52	1	--	--	--

Bolded values indicate improvement relative to Baseline. NA=Not applicable.

Table 1: Global efficacy measures up to Week 52 in Cohort 1 patients.
- CGI-S indicated variable severity at Baseline, ranging from 4 (Moderately ill) to 6 (Severely ill).
- Despite this variability in severity:
  - All patients showed early (Week 4) improvement in CGI-I, which has continued to their latest available visit.
  - Clinical improvement has been evaluated as 3 (Minimally improved) or 2 (Much improved).
- Caregiver-evaluated global change (PGI-I) was generally consistent with the clinician evaluation.
  - All patients showed early (Week 4) improvement, which has continued to their latest available visit.
  - Two patients (AAP1 and PedP2) experienced further improvement at Weeks 12 and 52 and at Week 8, respectively.
  - Improvement has been evaluated as 3 (A little better), 2 (Much better), or 1 (Considerably better).



Improvement = ↓RSBQ Score
PedP2 was not evaluated at Baseline.

Figure 4: RSBQ
Caregiver-evaluated RSBQ has been inconsistent across patients.
- AAP1 and PedP2 have experienced improvement in RSBQ total scores early on, relative to the first available timepoint.
- AAP1 experienced a further improvement 9 weeks after immunosuppressant taper.
- Two patients (AAP2 and PedP1) remain largely unchanged up to the latest timepoint evaluated.

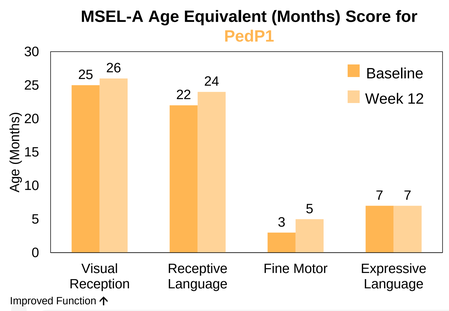


Figure 5: Multidomain improvement at Week 12 on the MSEL-A scale in PedP1. Examples of functional improvements reflected in these scores include:
- VR: gained ability to identify an object from memory.
- RL: gained ability to follow two unrelated commands and identify the function of objects and action words.
- FM: gained ability to use refined thumb grasp.

Based on the MSEL-A Baseline assessment and caregiver history, the patient was not able to demonstrate the above gained functional improvements before treatment.

Clinical Observations

Improvement seen across multiple clinical domains in all patients based on clinical observations.

	AAP1	AAP2	PedP1	PedP2
Gross and Fine Motor Skills	• Improved hand function, with hands more open • Gained ability to sit unassisted and move legs for the first time in over a decade	• Improved hand stereotypies	• Improved hand function and grasping with ability to hold object up to three-minutes vs 12 seconds at Baseline • Improved truncal stability and balance; able to sit unassisted for longer duration	• Improved gait and balance when walking; new abilities of walking up a stair and standing up from a chair • Improved hand function, with ability to reach and grab/swat
Communication/ Socialization	• Improved social interest, vocalization, and use of eye-driven communication device	• Improved social interest and increased response to words and eye contact	• Improved communication and use of eye-driven communication device, with new words communicated using device	• Improved social interest and eye contact‡
Autonomic Function/ Oral Motor	• Improved breathing patterns and circulation • Normalized sleep quality/duration	• Improved breathing patterns and circulation	• Improved breath holding, swallowing, and oral intake	• Improved breathing patterns
Seizures	• Stable seizure events with lower levels of anti-seizure medication, relative to Baseline	• Seizure-free for 8.5 months following treatment with TSHA-102 compared to 2-4 seizures per week pre-treatment†	• Stable seizure events, relative to Baseline	• Increase in seizure-free days, relative to historical seizure frequency§

†Findings based on Week 36 data. ‡Has experienced irritability thought to be secondary to immunosuppression. §Phenobarbital was added to regimen at Week 4 due to event of increased seizures.

Safety Summary

In these clinical trials, TSHA-102 has been well tolerated, with a total of 34** treatment-emergent adverse events (TEAEs). Of these, 33 were mild or moderate.
- One serious adverse event (SAE), seizure (severe, grade 3), has been reported, unrelated to TSHA-102. This patient has a medical history of seizures requiring hospitalization for anti-epileptic loading, as occurred during this event; with multiple confounders (e.g., anesthesia, urinary tract infection (UTI), sleep deprivation). Overall, she has experienced an increase in seizure-free days since dosing.
- One SAE (constipation, moderate, grade 2) unrelated to TSHA-102 was reported after TEAE listing cut off. Patient was admitted for irritability and had a medical history of chronic constipation and concurrent non-serious event of parainfluenza virus, which may have been contributory.
- The most common AE overall was vomiting (5 TEAEs), a known risk of sirolimus.

Table 2: TEAE causality and events related to TSHA-102

		Relatedness Assessments††			
Event (MedDRA PT)	**Total Events (Patients)**	**TSHA-102**	**Pre-existing Disease**	**Immuno-suppression**	**Other Cause**
Any TEAE	34 (4)	10	6	13	9
Pyrexia	2 (2)	2	0	0	0
CSF protein increased	2 (2)	2	0	0	0
Lethargy	2 (2)	2	1	0	1
Vomiting	5 (2)	1	0	3	0
Irritability	4 (2)	1	1	2	0
Clonus	1	1	0	0	0
Seizures	1	1	1	0	0

**Listing 5.1 TSHA-102-CL-101 and TSHA-102-CL-102, 05/10/2024. ††An event may have more than 1 causality reported. Causality assessments updated per communication from investigator on 06/11/2024. The following TEAEs unrelated to TSHA-102, were also reported: Agitation, Aphthous Ulcer, Blister, Chronic Kidney Disease, Cystatin C increased, Dermatitis acneiform, Epilepsy, Escherichia UTI, Gastroenteritis, Infection, Myopathy, Papular rash, Seizure, Skin ulcer, Stress fracture, Ureteric dilatation.

Laboratory Measures

Liver function. Patients have experienced no clinically significant liver or cardiac abnormalities. Laboratory evaluations have shown some excursions, particularly in patients with high liver enzyme values (GGT/ALT/AST and AP) at Baseline. Two patients had abnormal liver enzymes at Baseline, consistent with concomitant medication. One patient showed mild (<1.5 × ULN) transient and self-limited ALT and AST elevations, that were not assessed as TEAEs.

Neurofilament light chain (NfL) levels. All four patients have experienced transient NfL increase, consistent with the NfL increase after LP alone, as reported in nonhuman primates in the absence of gene therapy and in a small human study.[5-7]
- NfL increases through Week 4, declining after Week 12.
- MRI Brain and Spine: No findings of inflammation or damage.
- SNAPs: No clinically significant changes from Baseline.
- No observed functional changes or clinical correlation with TEAEs.

Conclusions

Encouraging safety profile and clinical improvements observed across all Cohort 1 patients of both REVEAL studies strongly support the treatment potential of TSHA-102.
- TSHA-102 was generally well-tolerated.
  - No SAEs related to TSHA-102 or dose-limiting toxicities observed.
  - No clinically significant cardiac or liver abnormalities.
  - All such data have been reviewed by the Independent Data Monitoring Committee, who approved and recommended continuation of both studies without modification.
- In this first-in-human clinical trial, improvements were observed across clinical domains and efficacy measures, regardless of type of *MECP2* mutation, Baseline severity, and clinical presentation.
- Early improvements demonstrated across key efficacy measures sustained at longer-term assessments; continued improvements seen after immunosuppressant taper.
- Clinical improvements observed across motor skills, communication/socialization, autonomic function (i.e. improved sleep, breathing, and GI function), and seizures.

Acknowledgements

The authors wish to thank all patients and caregivers, as well as the study staff who make this work possible.

Abbreviations: AP, Alkaline Phosphatase; ALT, Alanine Aminotransferase; AST, Aspartate Aminotransferase; FM, fine motor; GGT, Gamma-Glutamyl Transferase; GI, gastrointestinal; RL, receptive language; VR, visual reception; ULN, upper normal limits.

References: 1. Tillotson R, et al. *Nature*. 2017;550(7676):398-401. 2. Sinnett SE, et al. A New Approach for Designing a Feedback-Enabled AAV Genome Improves Therapeutic Outcomes of MiniMeCP2 Gene Transfer in Mice Modeling RTT. *23rd Annual meeting for the American Society of Gene & Cell Therapy*; April 28,2020. 3. McCarty DM. *Mol Ther*. 2008;16(10):1648-1656. 4. Haque E et al. The microRNA-responsive autoregulatory element from TSHA-102 for Rett Syndrome modulates therapeutic transgene expression in response to cellular MeCP2 in mouse and human cell lines. *30th Annual Congress of the European Society for Gene and Cell Therapy*, 24–27 Oct 2023, Brussels, Belgium. Poster #P435. 5. Xu J, et al. *iScience*. 2024;27(4):109436. 6. Boehnke SE, et al. *Alzheimers Dement (Amst)*. 2020;12(1):e12069. 7. Olsson M, et al. *Fluids Barriers CNS*. 2019;16(1):37.