Preliminary Safety Results from the Ph1/2 Study of NGN-401, a Novel Regulated Gene Therapy for Rett Syndrome

Suter B[1], Lieberman D[2], Benke T[3], Neul J[4] ,Jaggumantri S[5], Feng C[5], Albanis E[5], Cobb S[5], Jordan J[5]

[1]Department of Pediatrics & Neurology, Texas Children's Hospital, Baylor College of Medicine, Houston TX, [2]Department of Neurology, Boston Children's Hospital, Harvard Medical School, Boston, MA, [3]Department of Pediatrics-Neurology, University of Colorado School of Medicine, Children's Hospital Colorado, Anschutz Medical Campus, Aurora, CO, [4]Department of Pediatrics-Neurology, Monroe Carell Jr. Children's Hospital at Vanderbilt, Vanderbilt University Medical Center, Nashville, TN, [5]Neurogene Inc., New York, NY

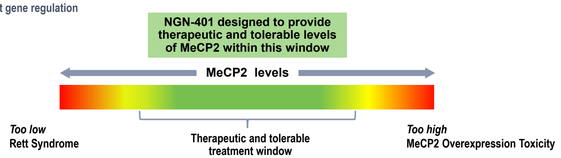
RETT SYNDROME

- Rett syndrome (RTT) is a severe X-linked neurodevelopmental disorder, occurring predominately in females.
- Most cases of RTT are caused by loss-of-function variants in the *MECP2* gene that lead to deficiency of methyl CpG binding protein 2 (MeCP2), a ubiquitously expressed nuclear protein critical for brain function[1,2].
- The cardinal clinical features of the disease phenotype include impairments in language/communication (i.e., verbal and non-verbal), ambulation, hand function, as well as autonomic dysfunction (e.g., severe daytime apnea episodes, hyperventilation).

RATIONALE FOR GENE THERAPY IN RETT SYNDROME

Gene therapy has potential to address the root cause of RTT by delivering functional copies of the *MECP2* gene to the brain, thereby potentially restoring MeCP2 protein.

Fig. 1. RTT requires tight gene regulation



- RTT disease severity is correlated with the amount of functional MeCP2 protein.
- *MECP2* duplication disorder is a distinct disease resulting from expression of two or more copies of the *MECP2* gene.
- Gene therapy for RTT requires tightly controlled MeCP2 protein expression to deliver therapeutic levels of MeCP2 (in the green range) without overshooting into levels that would be toxic[3].

NGN-401 GENE THERAPY INVESTIGATIONAL PRODUCT FOR RTT

NGN-401 is designed to be a best-in-class therapy for RTT.

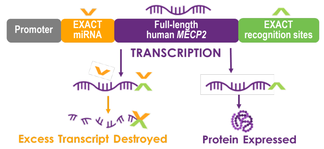
Fig 2. NGN-401 construct design

NGN-401:

- Includes EXACT™ gene regulation technology, designed to tightly control MeCP2 protein expression on a cell-by-cell basis. (Fig.3-4)
- Contains full-length human *MECP2* gene, which provides potential to maximize efficacy by creating a fully functional MeCP2 protein.
- Delivered by intracerebroventricular (ICV) administration, which has been shown to have the broadest targeting of brain regions underlying RTT pathophysiology compared to other routes of administration[4].

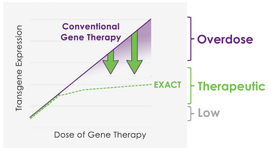
EXACT technology is designed to self-regulate transgene expression to maximize therapeutic potential while minimizing the risk of MeCP2 overexpression toxicity associated with conventional gene therapy.

Fig. 3. Self-regulation of transgene expression using EXACT construct



EXACT technology embeds a non-mammalian miRNA element and recognition sites to self-regulate gene expression in each cell, designed to maintain the desired level and prevent overexpression toxicity.

Fig. 4. Self-regulation of transgene expression using EXACT in highly transduced cells

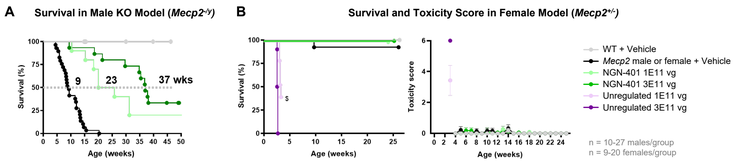


EXACT designed to provide therapeutic and tolerable levels of transgene expression on a cell-by-cell basis, even as dose increases AAV levels in highly transduced cells.

NON-CLINICAL SAFETY DATA

Efficacious doses of NGN-401 established in male murine knock-out (KO) model of RTT were well-tolerated in female heterozygous murine model of RTT, which has mosaic MeCP2 expression.

Fig. 5. NGN-401 led to dose-dependent increase in survival in male murine KO model (*Mecp2*$^{-/y}$); unregulated gene therapy led to rapid overexpression toxicity in female murine model (*Mecp2*$^{+/-}$), while NGN-401 was well-tolerated through 26 weeks

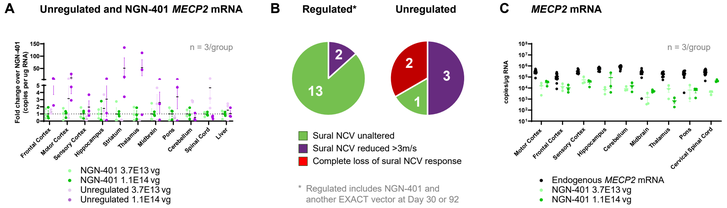


$ Animals culled due to toxicity in high-dose unregulated vector group.

- In *Mecp2*$^{+/-}$ heterozygous female mice with mosaic MeCP2 expression, NGN-401 exhibited no signs of toxicity at clinically relevant doses. In contrast, unregulated gene therapy was not tolerated with mice showing severe toxicity, requiring euthanasia by 3 weeks of age (Fig. 5B). The timing of toxicity onset was consistent with timing of peak transgene expression.

NGN-401 was well-tolerated while unregulated gene therapy drove early toxicity in wild-type non-human primates (NHPs).

Fig. 6. NHP data confirm EXACT regulation technology and support encouraging tolerability profile for human testing

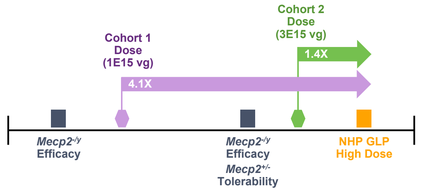


- In juvenile NHP ICV biodistribution study, an unregulated vector produced higher and more variable *MECP2* mRNA expression compared to NGN-401 30 days post-dosing, demonstrating EXACT regulation in NHPs. (Fig. 6A)
- EXACT regulated vectors were well-tolerated whereas unregulated vector demonstrated a loss of sural nerve conduction (NCV) in NHPs by Day 30. (Fig. 6B)
- Expression of transgene mRNA 30 days post-dosing of NGN-401 in NHPs was below the level of endogenous *MECP2* mRNA, avoiding overexpression concerns. (Fig. 6C)

Both NGN-401 clinical trial doses are expected to be safe and efficacious based on non-clinical data.

Fig. 7.

Cohort 1 dose is bracketed by efficacious doses in male murine knockout model, with a 4.1X safety margin to the NHP GLP toxicology study; Cohort 2 dose exceeds top efficacious dose studied in male murine knockout model and is still within the safety margins of the NHP GLP toxicology study



PHASE 1/2 NGN-401 PEDIATRIC TRIAL DESIGN AND BASELINE DEMOGRAPHICS

Objectives

- Safety, tolerability, and preliminary efficacy of NGN-401
- Evaluate two dose levels

Key Eligibility Criteria

- Female, age ≥4 to ≤10 years with Classic Rett syndrome
- Clinical diagnosis and genetic confirmation of pathogenic *MECP2* mutations
- Clinical Global Impression-Severity (CGI-S) score of 4-6

Key Efficacy Assessments

- Clinician Global Impression of Severity with RTT-specific anchors (CGI-S)
- Clinician Global Impression of Improvement (CGI-I)
- Rett Syndrome Behavior Questionnaire (RSBQ)

Fig. 8. RTT-200 Phase 1/2 pediatric study overview

The Phase 1/2 clinical trial of NGN-401 is concurrently enrolling low-dose and high-dose cohorts

The trial is utilizing a prophylactic immunosuppression regimen:

- Cohort 1: Corticosteroids
- Cohort 2: Targeted regimen of rituximab, sirolimus and shorter course of corticosteroids

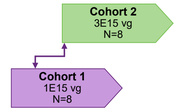


Table 1: Baseline characteristics of the first three participants dosed

	Low Dose: 1E15 vg (n = 3)		
	Participant 1	Participant 2	Participant 3
Age at Dosing	7 years old	4 years old	6 years old
Race	Asian	White	White
MECP2 mutation	Mild	Severe	Severe
Time post- NGN-401 administration	~9 months	~6 months	~3 months

NGN-401 HAS BEEN GENERALLY WELL-TOLERATED

Table 2:

- All treatment-emergent adverse events (TEAEs) related to NGN-401 have been mild/Grade 1 and transient or resolving, and most AEs are known potential risks of AAV
- There have been no treatment-emergent or ICV procedure-related serious AEs (SAEs)

	Number of Events [Number of Participants]
TEAEs related to NGN-401 (all mild/Grade 1)	13 [3]
Elevated ALT	5 [3]
Elevated AST	3 [2]
Elevated GGT	1 [1]
Decreased C3	1 [1]
Decreased C4	2 [2]
Vomiting	1 [1]

An extensive panel of studies are performed two to three times weekly for the first month post dosing, then on a weekly basis through five months, followed by decreasing frequency thereafter.

Table 3:

- No signs or symptoms indicative of MeCP2 overexpression toxicity have been reported in any participant

Clinical Sign or Symptom that May Indicate MeCP2 Protein Overexpression (derived from symptoms observed in *MECP2* duplication syndrome[5])	
Immunopathology (e.g., lymphadenopathy, recurrent respiratory infection)	None reported
New onset or worsening of persistent seizures	None reported
Worsening of constipation	None reported
New onset cardiovascular events	None reported

Data cut-off date: April 19, 2024

CONCLUSIONS

- **NGN-401 gene therapy candidate is designed to be a best-in-class treatment for RTT.**
- **NGN-401 has been generally well-tolerated in all three participants who have been dosed in the low-dose cohort, at ~9, ~6 and ~3 months post-dosing.**
- **There have been no signs or symptoms indicative of MeCP2 overexpression toxicity reported in any of the three participants, including the participant with a mild variant (~9 months post-dosing) predicted to result in residual MeCP2 expression.**
- **Mild, asymptomatic changes in laboratory assessments that are known risks of AAV administration were observed. There have been no treatment-emergent or ICV procedure-related SAEs.**
- **Enrollment in the low-dose and high-dose cohorts is ongoing and interim clinical data, including efficacy data, is expected fourth quarter 2024.**

References: (1) www.orpha.net. (2) Neul JL, et al. *Ann Neurol* 2010;68:944-50. (3) Anderson A, et al. *Orphanet J Rare Dis* 2014;9:87. (4) Daily JL, et al. *Molecular Therapy*, Vol. 29, No. 4, pp. 171-172. (5) Ta D, et al. *Orphanet J Rare Dis* 2022;7:131.

American Society of Gene and Cell Therapy 2024 | May 7-11, 2024 | Baltimore, MD I Abstract # 1408