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

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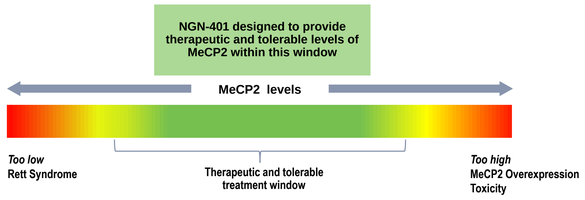
## RETT SYNDROME

- 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 transgene 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.**

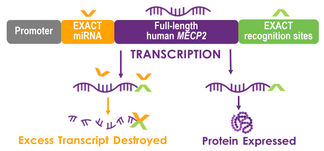
Fig 2. NGN-401 construct design


**NGN-401:**

- Includes EXACT™ transgene 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].

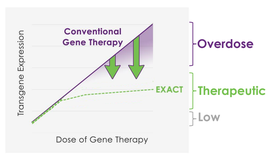
**EXACT technology is designed to self-regulate transgene expression to maximize therapeutic potential while minimizing the risk of MeCP2 overexpression toxicity associated with conventional gene therapy.**

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




EXACT technology embeds a non-mammalian miRNA element and recognition sites to self-regulate transgene 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

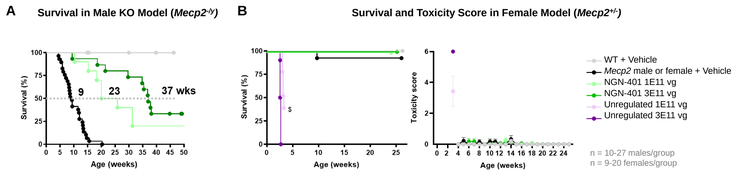



EXACT designed to provide therapeutic and tolerable levels of transgene expression on a cell-by-cell basis, even as dose increases AAV levels in highly transduced cells.

## NON-CLINICAL 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.

**Intracerebroventricular (ICV) dosing resulted in significantly better distribution than intrathecal-lumbar (IT-L) to key areas of the nervous system underlying RTT pathophysiology.**

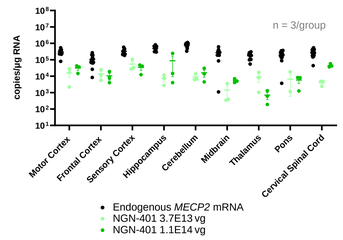
Fig. 6. NHP data support ICV as route of administration chosen for NGN-401 clinical trial[4]




- ICV is a common neurosurgical procedure, performed approximately 30,000 times annually in the U.S. alone.

**EXACT provided consistent levels of *MECP2* mRNA in wild-type NHPs.**

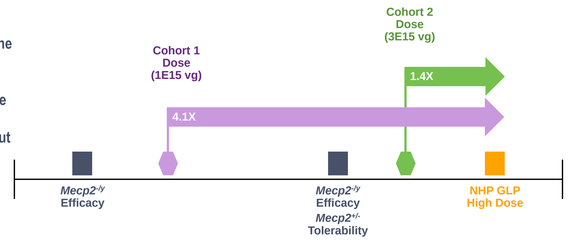
Fig. 7. Expression of transgene mRNA 30 days post-dosing of NGN-401 in NHPs was below the level of endogenous *MECP2* mRNA, avoiding overexpression concerns




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

Fig. 8.

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. 9. 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

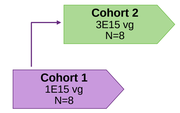



Table 1: Baseline characteristics of the first three participants dosed

| | 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 | ~11 months | ~8 months | ~5 months |

**High Dose Cohort 2: First participant recently dosed and thus far, NGN-401 has been well-tolerated**

## NGN-401 SAFETY PROFILE IS FAVORABLE TO DATE

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 for first three low-dose participants: May 31, 2024

## CONCLUSIONS

- **NGN-401 gene therapy candidate is designed to be a best-in-class treatment for RTT.**
- **ICV administration in NHPs resulted in significantly better distribution than IT-L to key areas of the nervous system underlying RTT pathophysiology, supporting route of administration in NGN-401 trial.**
- **NGN-401 safety profile is favorable to date in the first three participants who have been dosed in the low-dose cohort, at ~11, ~8 and ~5 months post-dosing.**
  - **There have been no signs or symptoms indicative of MeCP2 overexpression toxicity reported, including the participant with a mild variant (~11 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 serious AEs.**
- **Enrollment in the low-dose and high-dose cohorts is ongoing; first high-dose participant recently dosed. High-dose NGN-401 has been well-tolerated thus far with an early favorable safety profile.**
- **Interim efficacy data are 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) American Society of Gene & Cell Therapy 24th Annual Meeting. May 2021. (5) Ta D, et al. *Orphanet J Rare Dis* 2022;7:131.



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