Expression Kinetics and Clinical Onset of Benefit Following Single-Stranded AAV Delivery of NGN-401 in Rett Syndrome

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INTRODUCTION

- Rett syndrome (RTT) is a rare neurodevelopmental disorder caused by loss-of-function *MECP2* variants resulting in deficiency of functional MeCP2 protein.[1]
- Unlike neurodegenerative disorders characterized by progressive neuronal loss, RTT is driven by impaired neuronal function resulting from MeCP2 deficiency.[2]
- Disease-causing variants occur throughout *MECP2*; delivery of the full-length human *MECP2* transgene has the potential to provide the most complete restoration of MeCP2 biology to maximize therapeutic potential.[3,4]
- NGN-401 was purposefully designed to deliver the full-length human *MECP2* transgene incorporating EXACT™ transgene regulation technology in an AAV9 cassette. A single-stranded (ss) construct was required; the alternative self-complementary (sc) construct comparatively has 50% packaging capacity and cannot accommodate full-length *MECP2* together with its regulatory elements.[4]
- Self-complementary vectors bypass second-strand synthesis and are frequently cited as conferring faster onset of transgene expression compared with ss vectors.[5,6] Although sc vectors may enable faster onset of transgene expression, the key therapeutic objective in RTT is sustained, regulated expression of full-length *MECP2* to support durable clinical benefit.
- MeCP2 is a transcriptional regulator acting on >1,000 target genes.[7] Clinical benefit in RTT therefore requires a sequence of events after protein restoration: transcriptional normalization, synaptic and circuit remodeling, and re-acquisition of developmental milestones, processes that occur over weeks to months, not days.
- We therefore characterized the onset and durability of ssAAV-driven MeCP2 expression in a preclinical model, asking whether there is a rapid onset of expression that remains durable and in place well before and throughout the period over which clinical benefit emerged in the NGN-401 Phase 1/2 study.

METHODS AND STUDY DESIGNS

Phase 1/2 clinical trial design

- The Phase 1/2 open-label trial (NCT05898620) in females with classic RTT, with a baseline Clinical Global Impression–Severity (CGI-S) score of 4–6, is evaluating the safety, tolerability, and efficacy of NGN-401.[8]
- 10 participants received a one-time 1E15 vg dose of NGN-401 (age 4–10 years cohort, N=8; age ≥11 years cohort, N=2).
- The data cutoff was June 16, 2026, yielding a minimum of 12 months and up to 30 months of follow-up across the 10 participants.
- Study endpoints included:
  - Safety and tolerability
  - Efficacy assessments included Clinical Global Impression-Improvement (CGI-I) and functional ability in core RTT domains
  - Developmental milestone gains from baseline were scored by independent central rating using prespecified criteria.

Preclinical expression kinetics study design

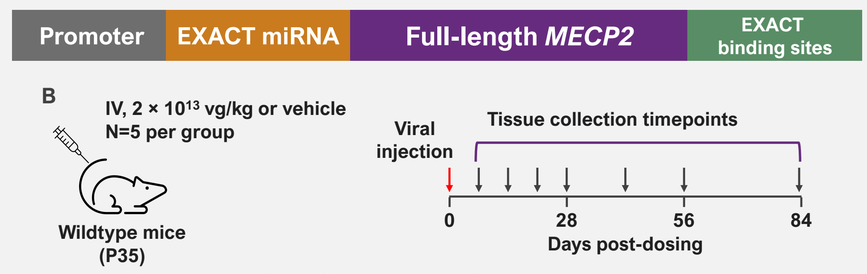


Figure 1. In vivo kinetics of expression study design
Schematic of **(A)** construct design and **(B)** in vivo study design.

- FLAG-tagged full-length human *MECP2* with EXACT regulation mimicking NGN-401 was vectorized in ssPHP.eB. The BBB-penetrant capsid was administered IV to achieve broad brain biodistribution, facilitating more accurate quantification of transgene expression in the adult brain.
- P35 wild type mice were dosed with vector or vehicle and brain (neocortex) sampled at various timepoints post-dosing to assess expression kinetics.
- FLAG intensity was assessed by immunoblot in cortical tissues or in single neurons using single nuclei flow cytometry.
- Biodistribution was assessed at each timepoint using absolute qPCR.

RESULTS

NGN-401 achieved a rapid response post-treatment that deepened over time

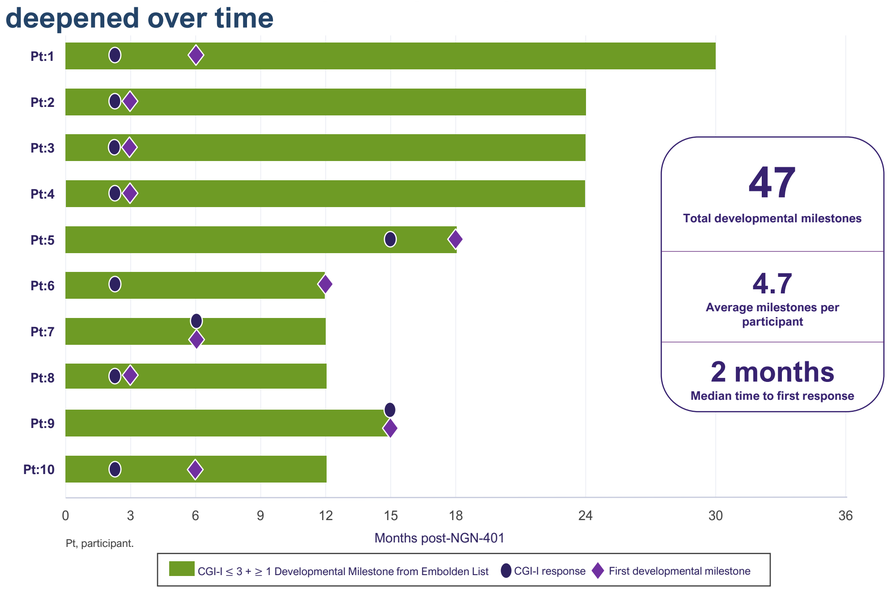
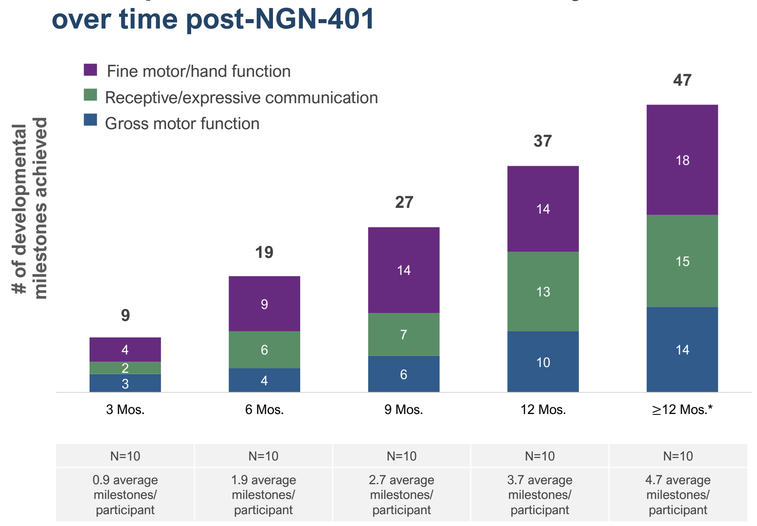


Figure 2. Responder analysis, time to CGI-I improvement, and gain of first developmental milestone

- 100% of participants gained ≥1 developmental milestone and improved on CGI-I.
- Median time to first response was at the first assessment (2 months), as measured by CGI-I.
- Responses deepened over time.

Developmental milestones increased by domain over time post-NGN-401



Four previously disclosed developmental milestones included are derived from other validated scales and are not included in the Embolden list: Pt.1 – Heel to toe walking – 9 mos.; Pt.2 – Bent down, touched floor and recovered – 9 mos.; Stepped off curb with help – 12 mos.; Pt.8 – Bent down, touched floor and recovered – 3 mos.
*n=6 participants with data >12 mos. post-NGN-401 and n=4 participants with 12-month data.
Mos, months.

Figure 3. Developmental milestones over time across core RTT domains

- 47 total milestones gained; 4.7 avg milestones gained per participant.
- NGN-401 drove durable accumulation of multidomain milestones across core domains that matter most to caregivers.

Restarting developmental progression is the goal for one-time gene therapy

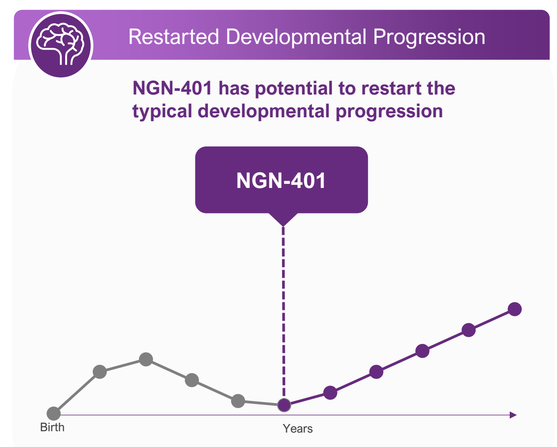


Figure 4. Illustrative to show anticipated gains over years for a developmental restart following gene therapy in RTT

- NGN-401 was associated with durable, multidomain developmental milestone gains that accrued and deepened over time.
- Milestones were acquired in a developmentally ordered sequence.[9]

In a preclinical mouse study, single-stranded AAV-mediated full-length human MeCP2 expression was detectable 7 days post-dosing and was sustained for the 3-month study duration

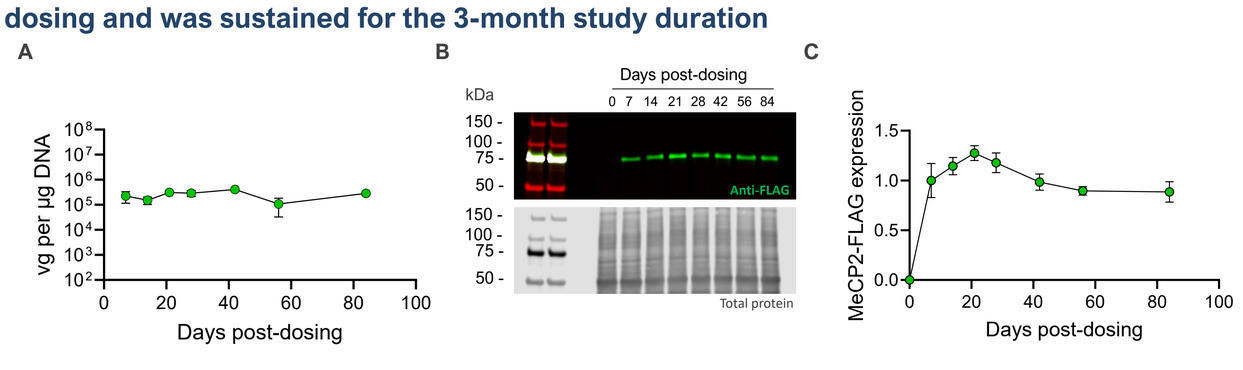


Figure 5. Analysis of expression kinetics from ssAAV in the mouse cortex
(A) Vector genome (vg) quantified in neocortex by qPCR showing stable genome signal over time. Untreated samples showed no signal. **(B)** Representative immunoblot showing with time course of anti-FLAG expression in treated neocortex. **(C)** Plot showing quantification of immunoblot data. All datapoints normalized to the mean FLAG intensity at day 7. **(D)** Single nuclei flow cytometry analysis of cortical neurons (NeuN positive) and comparing MeCP2-FLAG expression between transduced (FLAG positive) and non-transduced (FLAG negative) neurons across all timepoints collected. Datapoints are normalized to the mean FLAG intensity of transduced cells at the 7-day timepoint and show the geometric mean value. Data points in A, C and D represent mean ± SEM, N=3-5.

Transgene expression onset and durability precedes restart of developmental progression

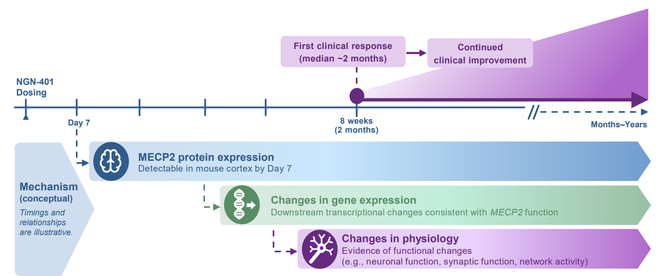


Figure 6. Model showing timing of MeCP2 transgene expression relative to clinical improvements.
MeCP2 and durable transgene expression drive changes in downstream gene expression, which lead to changes in physiology, and ultimately drive clinical improvements over time.

KEY CONCLUSIONS

1. **Single-stranded AAV-mediated delivery of EXACT-regulated full-length MeCP2 protein achieved durable expression in the mouse cortex by Day 7, the earliest time point assessed and remained stable through the 3-month preclinical study.**
2. **Clinical improvement post NGN-401 emerged by a median of 2 months post-treatment and continued to deepen, with durable, multidomain developmental milestone gains that accrued and deepened over time for up to 30 months, the longest follow-up available. These gains are consistent with a restart of developmental progression in RTT, a process measured in years.**
3. **The interval between protein restoration and measurable milestone gain reflects processes downstream of MeCP2 expression and the time required to acquire a developmental milestone, not the time required to express the transgene.**
4. **Expression precedes clinical response and is expected to be maintained throughout the period of clinical improvement. Sustained, regulated expression of fully functional, full-length *MECP2* is essential to support continued clinical benefit, an objective enabled by the single-stranded AAV platform.**

References

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