Route Matters: Intracerebroventricular (ICV) Delivery of NGN-401 Drives Superior Transgene Expression to Key Areas of the Brain When Compared to Lumbar Intrathecal (IT-L) Delivery at a Clinically Relevant Dose – Implications for a One-Time Gene Therapy for Rett Syndrome

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NGN-401 Gene Therapy for Rett syndrome

- Rett syndrome is a severe X-linked neurodevelopmental disorder, occurring predominately in females.
- Most cases of Rett syndrome 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].
- Rett syndrome is a dosage-sensitive disorder in which too little MeCP2 causes the disorder and excess levels of MeCP2 can be toxic.
- NGN-401 is an AAV9 gene therapy for Rett syndrome that contains the full-length human *MECP2* transgene and the EXACT™ transgene regulation technology designed to deliver controlled MeCP2 protein expression on a cell-by-cell basis.
- NGN-401 is being evaluated in a Phase 1/2 clinical trial at a dose of 1E15 vg and will be evaluated in a registrational trial (Embolden™) at the same dose.

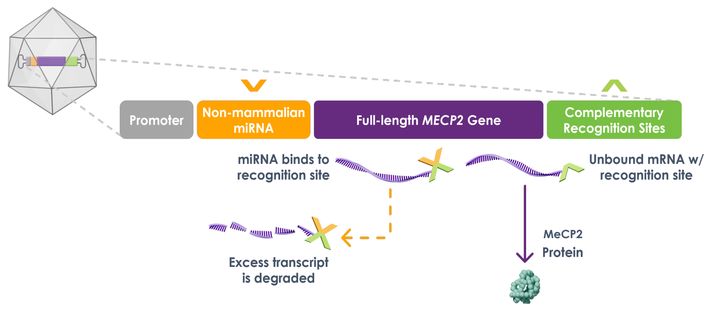

Figure 1. NGN-401 construct design. NGN-401 contains a ubiquitous promoter expressing the full-length human *MECP2* gene and a self-regulating miRNA circuit consisting of a non-mammalian miRNA and complementary miRNA recognition sites.

Route of Administration Selection for NGN-401

- Rett syndrome phenotypes include impairments in gross motor function, fine motor function, motor planning, and communication.
- Given that AAV gene therapies are currently limited to a one-time dose, selecting the route of administration that is optimal for clinical benefit is critical.
- NGN-401 utilizes one-time intracerebroventricular (ICV) delivery to achieve the broadest transduction to cortical structures and other key brain regions underlying disease pathophysiology.

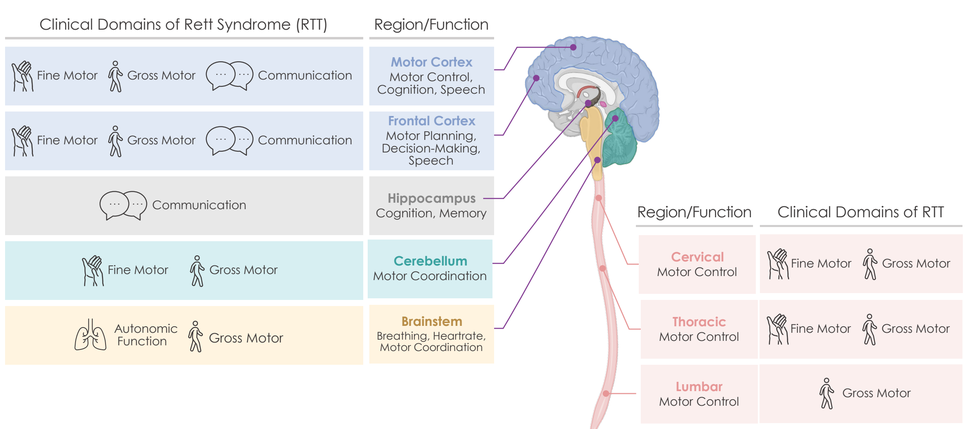

Figure 2. Central nervous system regions associated with clinical phenotypes of Rett syndrome

- Published literature has shown the effect of route of administration on AAV biodistribution, and multiple studies have demonstrated greater brain biodistribution following delivery to the cisterna magna (ICM) or ventricle (ICV) when compared to lumbar intrathecal delivery (IT-L)[3-5].
- ICV delivery was chosen for NGN-401 based on a previously conducted study in nonhuman primates (NHPs) comparing various routes of delivery of AAV9.
- This study demonstrated superior biodistribution to key brain regions relevant for Rett syndrome with ICV compared to IT-L using a different gene of interest[6].

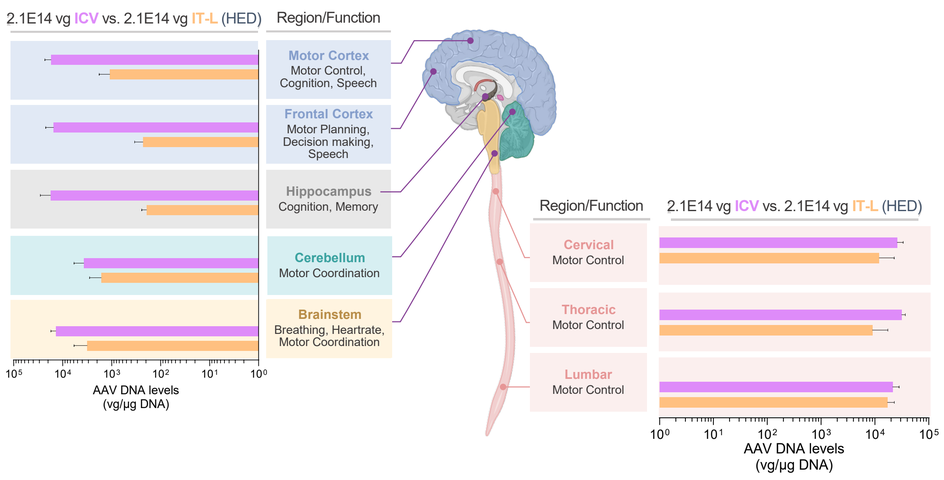

Figure 3. Data from initial biodistribution study comparing ICV delivery in NHPs. Vector genome DNA levels measured by qPCR one month following delivery of a self-complementary AAV9 vector at a dose of 1.1E13 total vg (Human equivalent dose = 2.1E14 vg). n = 3-6/group.

Study Design

- We sought to reproduce the findings from our previous study using NGN-401 at a dose representative of the clinical dose.
- ICV and IT-L administration were compared head-to-head at a dose that approximates the human dose (1E15 vg) being evaluated in the NGN-401 Phase 1/2 clinical trial and for the planned Embolden clinical trial, when allometrically scaled based on brain weight.
- Female cynomolgus macaques (age 26-37 months, 1.9-2.9 kg) without pre-existing AAV9 neutralizing antibodies (<1:10) were enrolled in a GLP study.

Table 1. Nonhuman primate study design

Group	Number of animals	Treatment	Dose (total vg)	Human Equivalent Dose (HED, vg)[a]	Dose margin to Embolden clinical trial
IT-L Vehicle	3	Vehicle	0	N/A	N/A
ICV Dose A	6	NGN-401	6.6×10^{13}	1.2×10^{15}	1.2x
IT-L Dose A	6	NGN-401	6.6×10^{13}	1.2×10^{15}	1.2x
IT-L Dose B – High Dose	6	NGN-401	2.1×10^{14}	3.9×10^{15}	3.9x

[a]Based on a human brain weight of 1200g[7] and a NHP brain weight of 64g (study data)

- Animals received daily oral prednisolone (1 mg/kg) beginning two weeks prior to dosing.
- Dose volume of 1.9 mL and infusion rate of 0.1 mL/min were consistent in all groups.
- IT-L cohorts were maintained in a modified Trendelenburg position post-dosing to model common clinical practice to maximize biodistribution to the brain.
- Tissues were collected 3 months after dosing for analysis of vector genome DNA levels (qPCR) or transgene mRNA levels (qRT-PCR).
- PCR assays targeted a unique sequence within the 3' UTR of NGN-401.
- For CNS tissues (except brainstem and thoracic spinal cord), two tissues aliquots per region per animal were evaluated (Fig. 4).

References

(1) www.orpha.net (2) Neul JL, et al. Ann Neurol 2010;68:944-50. (3) Ohno K et al. Mol Ther Methods Clin Dev. 2018; 13:47-54. (4) Hinderer C. et al. Mol Ther Methods Clin Dev 2014; 1: 14051. (5) Belle et al. Molecular Therapy Vol 27 No 4S1, April 2019. (6) Daily et al. Molecular Therapy Vol 29 No 4S1, April 2021. (7) Dekaban AS Ann Neurol. 1978 4(4) 345-56. (8) Rosenberg JB et al. Hum Gene Ther 2023; 34(21-22): 1095-1106. (9) Meseck EK et al. Toxicol Pathol 2022; 50(4): 415-431.

ICV Administration Shows Greater RNA Expression in Key Brain Regions Underlying Rett Syndrome Pathophysiology, Compared to IT-L

- NGN-401 delivered via ICV substantially outperformed the equivalent and the high IT-L dose in sampled cortical brain regions by a margin of ~10-100x more transgene RNA expression.
- Compared to IT-L, ICV delivery of the equivalent dose resulted in enhanced levels of RNA expression across multiple brain structures and the upper spinal cord, which are integral to Rett syndrome phenotypes, including gross motor function, fine motor function, motor planning, and communication.
  - Even when compared against the IT-L cohort receiving ~4x the clinically-relevant dose, ICV administration led to higher RNA expression in key areas of the brain
- RNA expression levels in lumbar spinal cord were more similar between routes of administration, as expected.

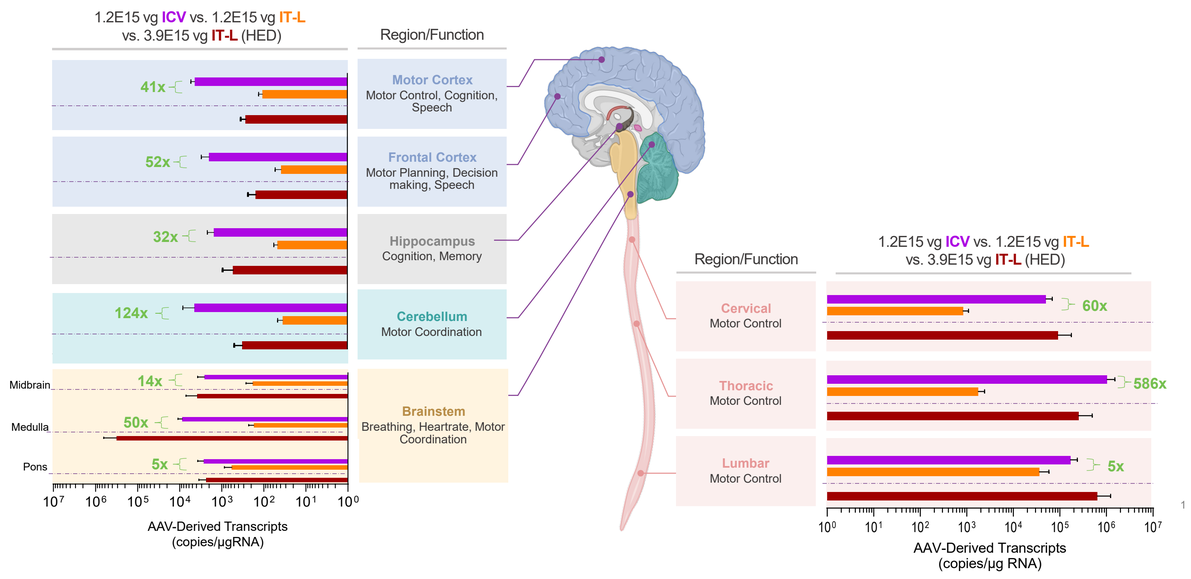

Figure 4. RNA expression measured by qRT-PCR in central nervous system tissues. n=6/group. In tissues where 2 aliquots were evaluated, average value is included. Mixed effect ANOVA (FDR = 0.05) on log-transformed data from 1.2E15 vg HED groups indicated statistically significant differences between routes of administration in all tissues shown except midbrain.

- Vector genome DNA biodistribution data (not shown) are consistent with RNA expression data
- As transduction of a greater number of cells in cortical and other brain structures expressing the therapeutic transgene is expected to be crucial for the magnitude of treatment benefit of a Rett syndrome gene therapy, these data provide mechanistic rationale for the multi-domain improvements and skill acquisition observed in the previously reported interim clinical efficacy data for NGN-401 (see poster P0322).

Biodistribution to the Periphery, including the Liver, is Comparable between Routes of Administration

- Vector genome biodistribution in peripheral organs demonstrated comparable peripheral exposure between ICV and IT-L at equivalent doses, consistent with other NHP studies comparing intra-CSF delivery routes[8,9].

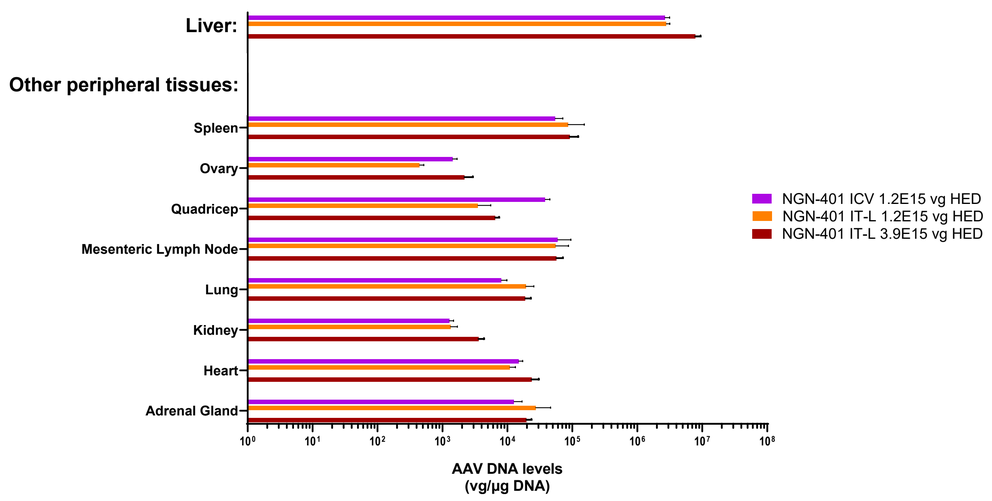

Figure 5. Vector genome DNA levels measured by qPCR in peripheral organs. n=6/group.

Participants in the NGN-401 Phase 1/2 Trial Gained 23 Skills, Representing Multi-Domain Improvement Requiring Transduction of Multiple Regions of the Central Nervous System

- Previously shared data from the first four participants in the Phase 1/2 trial of NGN-401 showed consistent, concordant improvements across key Rett syndrome scales, achieving meaningful gains of function and developmental milestones in the core clinical domains of Rett syndrome – hand function/fine motor, communication/language, and ambulation/gross motor. These data were as of the data cut-off date of October 17, 2024.
- For example, a participant who at baseline could not follow simple commands demonstrated improvements in executive function and motor planning, which involves multiple central nervous system regions.

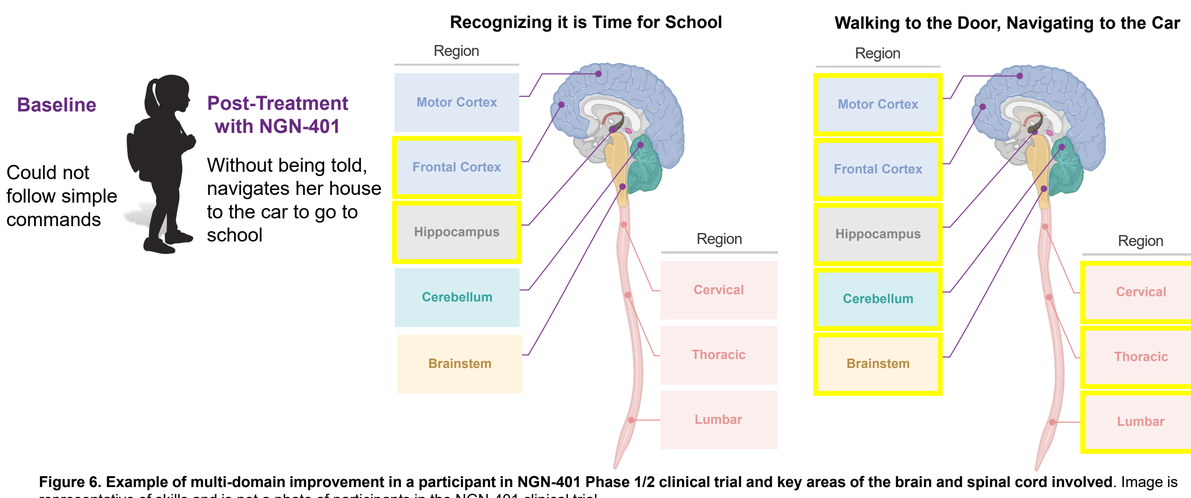

Figure 6. Example of multi-domain improvement in a participant in NGN-401 Phase 1/2 clinical trial and key areas of the brain and spinal cord involved. Image is representative of skills and is not a photo of participants in the NGN-401 clinical trial.

Conclusions

- Results of this second NHP biodistribution study provide further definitive evidence consistent with our and other prior NHP studies that ICV delivery is a superior route to maximize AAV9 biodistribution in the brain when compared to IT-L at translationally relevant doses selected for clinical trials.
- Comparable systemic exposure of NGN-401 between ICV and IT-L delivery suggests there is no added safety benefit for IT-L as it relates to liver exposure, a well-known safety risk of AAV treatment.
- Together, these data reinforce the importance of using ICV delivery in Rett syndrome gene therapy clinical studies to maximize efficacy with this one-time treatment.

For more information on the NGN-401 clinical program, please visit Poster P0322.

Acknowledgements

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