Hemophagocytic Lymphohistiocytosis (HLH)/Hyperinflammatory Syndrome following High Dose AAV9 Therapy

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Rett Syndrome and Rationale for Gene Therapy

- Rett syndrome (RTT) is a severe X-linked neurodevelopmental disorder, occurring predominately in females caused by mutations in the *MECP2* gene[1,2]
- The cardinal clinical features of the disease phenotype include impairments in hand function/fine motor, ambulation/gross motor, language/communication and autonomic dysfunction (e.g., constipation, sleep, and dysphagia)
- In the natural history of RTT[3], simple developmental skills (e.g., raking grasp, pincer grasp, babbling) are generally acquired but the majority are lost during the regression phase (~1-3 years). More complex skills (e.g., using utensils to eat, climbing up/down stairs without help, and pointing for wants) are generally not acquired
- Gene therapy has the potential to address the root cause of RTT by delivering functional copies of the MECP2 gene to the brain and nervous system, thereby potentially restoring MeCP2
- NGN-401 is an AAV9 gene therapy for Rett syndrome that utilizes one-time intracerebroventricular (ICV) delivery to achieve broadest transduction to cortical structures and other key brain regions underlying disease pathophysiology
- 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

NGN-401: Positioned to be Best-in-Class Gene Therapy for Rett Syndrome

Figure 1

Requirements for One-Time Gene Therapy for Rett Syndrome	NGN-401 Design
Deliver wild-type MeCP2 protein	**Full-length human *MECP2* gene** translates a fully functioning MeCP2 protein
Protect against MeCP2 overexpression toxicity	**EXACT™ transgene regulation** technology designed to deliver consistent and tightly controlled MeCP2 protein expression on a cell-by-cell basis
Reach areas of the brain and nervous system that underly Rett syndrome pathophysiology	**ICV delivery** has shown in preclinical models to have the broadest targeting of brain and nervous system regions underlying Rett syndrome pathophysiology

See poster #P0372 for full presentation of data

Phase 1/2 Methods and Study Design

- The Phase 1/2 open-label trial enrolled participants with classic RTT (NCT05898620) to receive a one-time ICV administration of NGN-401 at a dose of 1E15 vg (total vector genomes). Eligibility criteria included a clinical diagnosis and genetic confirmation of a MECP2 pathogenic variant and a baseline Clinical Global Impression-Severity (CGI-S) score of 4-6
- All participants received prophylactic immunosuppression with prednisolone
- 10 participants received NGN-401 (1E15 vg)
  - 8 females in age 4–10 cohort
  - 2 females in ages > 11 cohort
- Initial efficacy data reported from 4 pediatric participants receiving NGN-401 1E15 total vg with a data cutoff date of October 17, 2024; updated clinical data expected to be reported 2H 2025
- 3 participants received higher dose of NGN-401 (3E15 vg) in a since discontinued cohort

Improvements in Clinician and Caregiver Assessments with 23 Skills Acquired Across 4 Participants* Administered 1E15 vg NGN-401

Table 1

	CGI-I	CGI-S Total Score		RSBQ		Gain of Skills, Developmental Milestones and Symptom Improvement in RTT Clinical Domains				
	Improved?	How many points?	Improved?	How many points?	Improved?	How many points? (% Change)	Hand Function	Gross Motor	Communi-cation	Autonomic	Attentive-ness
Pt:1 15 mos. post-NGN-401	✓	2 pts.			✓	10 pts. (-28%)	✓	✓	✓	✓	✓
Pt:2 12 mos. post-NGN-401	✓	2 pts.	✓	1 pt.	✓	32 pts. (-52%)	✓	✓	✓	✓	✓
Pt:3 9 mos. post-NGN-401	✓	2 pts.			✓	5 pts. (-29%)	✓	✓		✓	✓
Pt:4 3 mos. post-NGN-401	✓	2 pts.			✓	8 pts. (-28%)	✓			✓	✓

Consistent Improvement Across Key Rett Syndrome Scales, Bolstered by Functional Improvements in Core Clinical Domains

CGI-I: Clinical Global Impression-Improvement; CGI-S: Clinical Global Impression-Severity; RSBQ: Rett Syndrome Behavioral Questionnaire

Multi-Domain Improvements Deepened Over Time, and Not Expected Based on Rett Syndrome Natural History*

Figure 2

Baseline – 7 Yrs Old
Mild Disease

Select Pt:1 Developmental Skills Post-NGN-401		Months Post-NGN-401				
		3	6	9	12	15
Uses a pincer grasp	Fine Motor (Raking, no ability to hold objects)		✓	✓	✓	✓
Holds bottle or cup unpropped			✓	✓	✓	✓
Uses spoon/fork to self-feed						✓
Transfers objects between hands						✓
Heel-to-toe walking	Gross Motor (Walking, ataxic gait, no ability to climb stairs)			✓	✓	✓
Climbs up stairs without help			✓	✓	✓	✓
Climbs down stairs without help					✓	✓
Follows a command without gesture	Communication (Severe impairment, unable to follow commands)		✓	✓	✓	✓
Waves hello†					✓	✓
Taps for wants					✓	✓

Post Treatment with NGN-401

†Skill learned is "Wave hello;" however, RNHS tracked "Waves Bye Bye"

Baseline – 4 Yrs Old

Select Pt:2 Developmental Skills Post-NGN-401		Months Post-NGN-401			
		3	6	9	12
Reaches for an object	Fine Motor (Severe impairment, unable to use hands)	✓	✓	✓	✓
Uses raking grasp to retrieve an object				✓	✓
Self-feeds				✓	✓
Stands independently from seated position	Gross Motor (Impaired, ataxic, help to stand)	✓	✓	✓	✓
Bends down, touches floor, and recovers				✓	✓
Steps off curb with help					✓
Follows a command without a gesture	Communication (Severe impairment, unable to follow commands, non-verbal)	✓	✓	✓	✓
Uses words with meaning		✓	✓	✓	✓

Post Treatment with NGN-401

Baseline – 6 Yrs Old
Severe dysphagia

Select Pt:3 Developmental Skills		Months Post-NGN-401		
		3	6	9
Uses a pincer grasp	Fine Motor (Raking grasp)		✓	✓
Able to self-feed				✓
Sits independently	Gross Motor (Cannot sit, stand or walk)	✓	✓	✓

Post Treatment with NGN-401

Baseline – 7 Yrs Old

Select Pt:4 Developmental Skills		Months Post-NGN-401
		3
Uses a pincer grasp	Fine Motor (Raking grasp, unable to hold objects)	✓
Can use utensils to self-feed (without assistance)		✓

Post Treatment with NGN-401

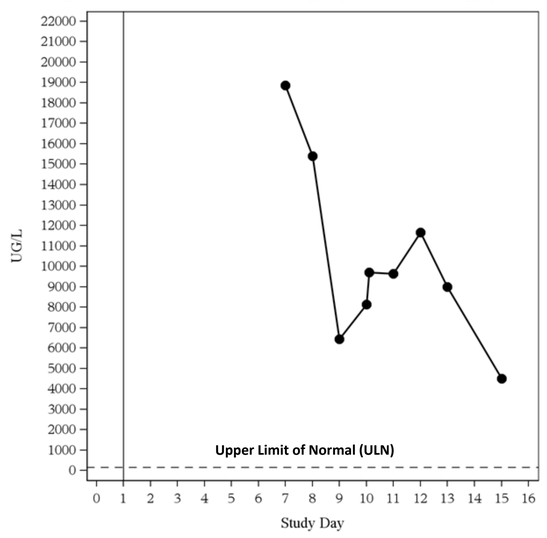
HLH Has Been Rarely Reported Following High-dose AAV Gene Therapy

- Hemophagocytic lymphohistiocytosis (HLH) is a rare, life-threatening hyperinflammatory syndrome characterized by immune dysregulation, cytokine storm, and multi-organ damage[4-6]
- Most cases of HLH are triggered by infections, malignancy, autoimmune disease, or CAR-T (3.5% incidence); but rarely been reported in gene therapy literature and there is not currently standard monitoring in AAV trials
- Over 90% of patients with HLH present with the initial "three F's" seen in the HLH-2004 study: Fever, elevated Ferritin, and Falling blood counts (cytopenia)
- Only one published case report[4] and mention of similar cases[5] reported HLH-like symptoms with high-dose systemic AAV treatment (1E14 vg/kg or higher)
- Cases treated successfully with early administration of either high dose steroids or anakinra (IL-1 receptor antagonist)
- All cases of HLH have been reported at or above AAV doses of 1E14 vg/kg in published literature or FAERS database

HLH Suspected in Patient Receiving 3E15 vg NGN-401 – Maximal Care Provided

- Three participants were dosed with the 3E15 vg dose, which translates to ~ 1.5E14 vg/kg
- All participants at the 3E15 vg dose level received an augmented immunosuppression regimen of corticosteroids, sirolimus, and rituximab
- No hyperinflammatory reaction was experienced by the first two participants
- The third participant experienced an acute hyperinflammatory response in the first week after dosing with symptoms including fever, markedly elevated ferritin, and cytopenias, consistent with HLH/hyperinflammatory syndrome
- TMA considered unlikely (negative blood smear)

Figure 3 - Ferritin level 18,850 ug/L (normal <150 ug/L)



- Participant experienced rapid clinical decline with respiratory failure, acute kidney injury, elevated liver enzymes, coagulopathy, hypotension; intubated, admitted to ICU and placed on CVVH and inotropic support
- Treatment with dexamethasone, anakinra, and a single dose of eculizumab administered despite negative blood smear
- Multi-system failure was too far advanced and despite maximal supportive care, the participant's clinical status declined, and she passed away approximately two weeks post-dosing
- The 3E15 vg dose was discontinued from further development

No Evidence of HLH/Hyperinflammatory Syndrome at the 1E15 vg Dose Level

A total of 10 participants have been dosed at the 1E15 vg dose level and no evidence of HLH/hyperinflammatory syndrome has been observed

1E15 vg translate into ~E13 vg/kg

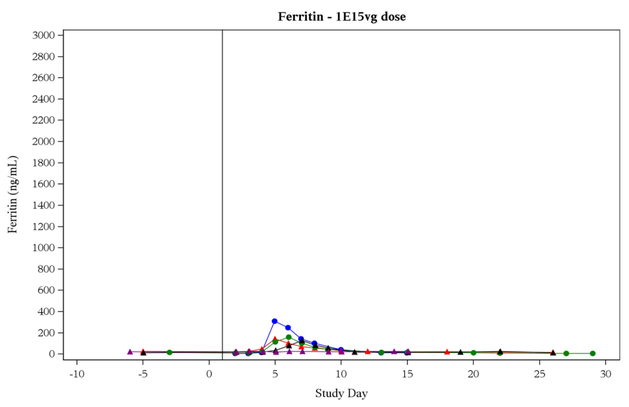
5 of the participants were dosed following the implementation of HLH risk mitigation and monitoring protocol, which includes:

- Dose level at/above 1E14 vg/kg not allowed
- In the first week post dosing: employ daily monitoring of HLH, focused on "the three Fs" – Ferritin, Fever, and Falling blood counts (cytopenia)
- Exclude subjects with any illness within 30 days of dosing and COVID within 6 weeks of screening
- Prior to dosing, require sites to have anakinra available and encourage availability of a local HLH expert
- Included HLH treatment algorithm: 1st line – high-dose corticosteroids, 2nd line – anakinra

Results following additional monitoring:

- No clinical symptoms of HLH/hyperinflammatory syndrome observed
- Transient, non-clinically significant ferritin elevations were observed in 4 of 5 participants, peaking at Study Day 5-6 with recovery to Baseline by Day 10-12 with no intervention

Figure 4 - Ferritin Levels of Most Recently Dosed Participants – 1E15vg



Galletta et al. publication reported case of HLH following high-dose systemic AAV treatment (1.1E14 vg/kg); the patient presented with the "three Fs" - fever, falling blood counts (cytopenia) and ferritin of 2,959 ng/ml 36 hours post-dose. The patient was treated with high-dose steroids and recovered.[7]

Embolden Registrational Clinical Trial Start-up Activities Underway

Figure 5

Ages ≥ 3 years

N = 20

All participants will receive corticosteroids alone for prophylactic immunosuppression

Open-label, baseline-controlled trial evaluating single dose of NGN-401 (1E15 vg)

Key Eligibility Criteria:

- Females with Classic Rett syndrome in post regression stage of illness
- Clinical diagnosis and genetic confirmation of pathogenic *MECP2* variant
- Clinical Global Impression-Severity (CGI-S) score of 4–6

Primary Endpoint: Responder-based composite endpoint at 12 months with responders defined as participants who:

- Attain a CGI-I score of ≤3 ("minimally improved"); and
- Gain any one developmental milestone/skill from a list of 28, as captured by standardized video recordings and independently verified by blinded central raters

Conclusions

- HLH is rarely described but has occurred in the setting of high-dose AAV therapy of 1E14 vg/kg or higher
- Key early signs and symptoms include the "**3 F's**" - **Fever, elevated Ferritin, and Falling blood counts (cytopenias)**
- Delayed recognition of HLH can lead to rapid clinical decline and may be life-threatening
- We incorporated an HLH monitoring and treatment algorithm into the Phase 1/2 trial for NGN-401 to enable early detection and intervention if warranted and discontinued the 3E15 vg dose cohort (~1.5E14 vg/kg)
- No clinical signs or symptoms of HLH have been observed in patients receiving the 1E15 vg dose of NGN-401 (N=10)
- We encourage others evaluating AAV treatments to monitor for HLH
- Encouraging efficacy data has been observed with NGN-401 at the 1E15 vg dose, which translates to ~E13 vg/kg range, below the dose level where HLH has been reported in the literature
- We believe NGN-401 has been generally well tolerated at the 1E15 vg dose in the Phase 1/2 trial
- Start-up activities are underway for Embolden, a registrational open-label, baseline-controlled trial evaluating a single 1E15 vg dose of NGN-401
- Embolden also incorporates early HLH monitoring and treatment protocol
- Additional clinical data from the Phase 1/2 trial is planned to be reported in second half 2025

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*Data cut-off date of October 17, 2024

References: (1) www.orpha.net. (2) Neul JL, et al. *Ann Neurol* 2010;68:944-50. (3) Neul J, et al. *Journal of Neurodevelopmental Disorders* (2014) 6:20. (4) Soy et al. Rheumatol Int. 2021;41(1):7-18. 2Mérad et al., 2019; (5) Jordan et al. Blood. 2011;118(15):4041-52; (6) Henter J et al 2007 Feb; 48(2):124-31. (7) Galletta et al. J Clin Pharm Ther. 2022:47(9):1478-1481