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Antisense oligonucleotide therapy for rare pediatric genetic disease: SCN2A

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Summary of Changes from Previous Version:

Affected Section(s)	Summary of Revisions Made	Rationale
• 6.2.1 Dosing and Administration	• Revised requirements for reducing the dosing interval length to include changes in movement/motor function and gastrointestinal issues in addition to the already included seizure control	This change was implemented at the request of the FDA to ensure all assessments are taken into account when reducing the dosing interval.

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1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title:

Personalized antisense oligonucleotide therapy for rare pediatric genetic disease: SCN2A

Study Description:

This research project entails delivery of a personalized antisense oligonucleotide (ASO) drug designed for a single pediatric participant with SCN2A associated developmental epileptic encephalopathy. This rare genetic condition is associated with early onset severe epilepsies including frequent seizures, neurocognitive developmental delay, involuntary movements, and autistic behaviors. Currently there are no available treatments for SCN2A associated neurological disorders. A personalized targeted ASO drug nL-SCN2A-002 will be delivered to a single participant with a pathogenic de novo p.R853Q gain of function amino acid substitution mutation in the *SCN2A* gene to selectively decrease expression of the mutant allele and decrease production of the mutant protein.

Objectives:

Primary Objectives: To assess seizure frequency, motor function (assessed via the Vineland-3, Dyskinetic Cerebral Palsy Functional Impact Scale, and Bayley-4 with GSV as a performance measure), and gastrointestinal issues at 12 and 24 months post nL-SCN2A-002 administration in a participant with *SCN2A* gene mutation

Secondary Objectives: To assess improvement in neurodevelopmental scores at 24 months post nL-SCN2A-002 administration in a participant with SCN2A gene mutation

Exploratory Objective: To assess improvement in gait at 24 months post nL-SCN2A-002 administration in a participant with SCN2A gene mutation

Endpoints:

Primary Endpoint: Quantitative change in baseline at 12 and 24 months post nL-SCN2A-002 administration in

- frequency of seizures from a seizure diary (defined as average number of daily seizures over a 3-month time period at baseline compared to the average number of daily seizures over a 3-month time period at 12 and 24 months)

- movement and motor domain scores of Vineland-3, Bayley-4 and Dyskinetic Cerebral Palsy Functional Impact Scale (parent/teacher reported measures)
- gastrointestinal issues as measured via the Bristol Stool Form scale

Secondary Endpoints: Quantitative change in baseline at 24 months post nL-SCN2A-002 administration in neurodevelopmental and behavioral scores (per standardized parent/teacher report measures including the Aberrant Behavior Checklist, Vineland-3, ORCA and Bayley-4 with GSVs as a performance measure)

Exploratory Endpoint: Change in gait at 24 months post nL-SCN2A-002 administration as assessed from video recordings using the Scale for the Assessment and Rating of Ataxia (SARA) gait criterion

Study Population:	A single pediatric participant with a pathogenic de novo *SCN2A* mutation associated developmental encephalopathy
Description of Sites/Facilities Enrolling Participants:	UC San Diego Rady Children's Hospital
Description of Study Intervention:	This is an interventional study to evaluate the safety and efficacy of treatment with an individualized antisense oligonucleotide treatment in a single pediatric participant with a de novo pathogenic gain of function *SCN2A* mutation associated with severe developmental epileptic encephalopathy. A personalized ASO drug nL-SCN2A-002 will be delivered intrathecally via lumbar puncture over a 24-month period, to target the pre-RNA transcript containing the mutation and reduce mutant protein expression with the goal of reducing clinical impact of the abnormal gene mutation.
Study Duration:	This is an investigator-initiated study for an Investigational New Drug (IND) to study the safety and effect of nL-SCN2A-002 on a participant with SCN2A-associated severe developmental epileptic encephalopathy. The study is scheduled for 24 months. If the participant does not exhibit AEs requiring the termination of the treatment, and if the physician overseeing the study deems it would be beneficial to continue, the participant will enter a maintenance phase after 24 months. The participant will have access to treatment for life as long as the investigator deems the treatment beneficial for the subject.
Study Duration:	24 months

1.2 SCHEMA

SCHEDULE FOR EVALUATION OF TREATMENT

PATIENT ID	nL-00001
Intervention	An open-label single center, single participant study of an experimental antisense oligonucleotide treatment for SCN2A associated neurological disorder
Genetic Diagnosis	The participant being treated with nL-SCN2A-002 has a pathogenic de novo hg38:Chr2:165342465G>A, NM_001040142.2 c.2558G>A single base substitution leading to a p.R853Q gain-of-function amino acid substitution mutation in the *SCN2A* gene. The *SCN2A* gene encodes the voltage-gated sodium channel Na(v)1.2, which plays an important role in the initiation and conduction of action potentials. SCN2A is expressed in axon initiation segments and at nodes of Ranvier in myelinated nerve fibers (Wolff et al., 2017). Voltage-sensitive sodium channels are heteromeric complexes consisting of a large glycosylated alpha subunit (approximately 260 kD) and 2 smaller beta subunits (33-39 kD). In most types of excitatory neurons, the Na(v)1.2 sodium channel is responsible for generating action potentials. The human *SCN2A* gene spans approximately 120 kb and has 29 exons, including a noncoding alternative first exon and alternative exon 6.
Clinical Presentation	Both inherited and de novo mutations are associated with disease in humans, ranging in severity from benign familial neonatal-infantile seizures (BFNIS) to EIEE11. Mutations in *SCN2A* can be categorized as loss-of-function or gain-of-function. SCN2A gain-of-function mutations cause early-onset severe epilepsies, while loss-of-function mutations cause autism with occasional seizures (Sanders et al., 2018). In late onset developmental and epileptic encephalopathies (DEE) cases loss of sodium channel function is a consistent finding but genetic findings that show an overwhelming absence of nonsense mutations suggest other mechanisms associated with the mutant allele are contributing to the more severe than autism alone DEE phenotypes. De novo mutations in *SCN2A* are found in approximately 3% of early onset seizures (Trump et al., 2016). The p.R853Q allele is a recurrent mutation associated with seizure onset at 6-8 months and frequent co-occurrence of involuntary movements, including choreoathetosis, dystonia, and pyramidal signs (Berecki et al., 2018). The p.R853Q variant displays both GOF and LOF effects when expressed in cells, and it is thought that the GOF effect is responsible for the DEE clinical phenotype (Berecki et al., 2018) beyond autism predicted by haploinsufficiency alone.
Antisense approach	RNase H1 ASO targeted to decrease expression of the pathogenic mutant allele

Primary Objectives	Reduction in seizure frequency, stabilization/improvement in movement and motor function, and reduction in gastrointestinal issues
Secondary Objective(s)	Improvement in neurodevelopmental/behavioral measures
Exploratory objective	Improvement in gait
Evaluation Methods	1. Seizure Incidence – Seizure diary 2. Motor function – motor domains of the Vineland-3, Bayley-4 with GSV as a performance measure and the Dyskinetic Cerebral Palsy Functional Impact Scale 3. Developmental/behavioral scores – neurodevelopmental and behavioral scores (per standardized parent/teacher report measures such as the Aberrant Behavior Checklist, Vineland-3, ORCA and Bayley-4 with GSVs as a performance measure) 4. Gastrointestinal issues – as measured by the Bristol Stool Form scale 5. Gait – as measured by SARA gait criterion from video recordings
Safety Objective(s)	To evaluate the safety and tolerability of ASO based on incidence and severity of treatment-emergent adverse events (TEAEs), serious adverse events (SAEs), including changes in vital signs, EEG and clinical laboratory results.
Study Design	24 month study period with option for open label treatment for the lifetime of the participant if safe and efficacious
Drug Dosage and Administration	**Dosing Regimen** A dose of 20 mg will be utilized based on the potency similarity of nL-SCN2A-002 to other intrathecally delivered ASOs in the same chemical class evaluated at this pediatric age. From preclinical testing, the safety margin for the 20 mg dose is 25-fold to the 1 mg/month dose in rats which was well tolerated. Doses will be administered on Day 1, Day 28, Day 84, and every 3 months after (maintenance).The dosing window may be adjusted to 60-90 days (at the highest tolerated dose or lower) if the Principal Investigator determines the clinical effects are waning prior to the end of the 3-month interval. Dose escalation, as illustrated in the table below (at 60 mg and upwards), will not proceed until further regulatory approval is received. When regulatory approval is received, if there is potential for increased clinical benefit (e.g., reduction in seizure frequency on seizure diary post nL-SCN2A-002 without seizure freedom), and if adequate safety has been demonstrated in the participant, the physician may choose to continue the escalation by 10-20 mg increments every 3 months, up to a maximum of 100 mg. The 100 mg dosage level maintains a safety margin of ~15-fold to the well-tolerated 1 mg/month rat dose, which itself is lower than the maximum tested dose level

from preclinical testing. The PI may also choose to remain at the same dose-escalation step for multiple doses to better assess drug efficacy, for safety monitoring, or for any other reason determined to be necessary.

Dosing will continue until the physician determines clinical benefit has been attained and as long as no adverse events are found.

Dose Escalation Steps for nL-SCN2A-002

PROPOSED REGIMEN*	STARTING DOSE	STEP 1	STEP 2	STEP 3	STEP 4	STEP 5
Default	20 mg	40 mg	60 mg	60 mg	60 mg	60 mg
Option 1	20 mg	40 mg	60 mg	80 mg	80 mg	80 mg
Option 2	20 mg	40 mg	60 mg	80 mg	100 mg	100 mg
Option 3	20 mg	20 mg	30 mg	30 mg	40 mg	40 mg

* Refer to the paragraph above for criteria; “default regimen” should be followed if 60 mg provides adequate efficacy and safety; Options 1 and 2 should be followed for increased clinical benefit if safety allows. Option 3 allows for a slower dose escalation per FDA feedback.

Dose Modifications

The dose of nL-SCN2A-002 would be reduced in a stepwise manner as depicted in the table below if the participant exhibits adverse events deemed as requiring dose reduction by the physician overseeing the study, with the objective of finding a well-tolerated dose which would then be maintained quarterly, as long as there is a potential for clinical benefit and no new adverse events are found.

If the participant continues to show undesired effects, subsequent dosing will then go to the next reduction step.

Dose Reduction Steps for nL-SCN2A-002

DOSE*	STEP 1	STEP 2	STEP 3	STEP 4
60 mg	40 mg	20 mg	10 mg	Discontinue
40 mg	20 mg	10mg	Discontinue	N/A
30 mg	20 mg	10 mg	Discontinue	N/A
20 mg	10mg	Discontinue	N/A	N/A

*If the dose was escalated to 80 mg or 100 mg, each reduction step would lower the dose by 20 mg, with the last step going from 20 mg to 10 mg, until

	stabilization or discontinuation. Dose can also be lowered in 10 mg decrements. The Investigator may consider another dose escalation after 1-2 lower doses have been tolerated or choose to stop dose escalation if there is evidence of a favorable benefit-risk. The Investigator may then choose to attempt another dose escalation after 1-2 lower doses have been tolerated or choose to stop dose escalation if there is evidence of a favorable benefit-risk.
Safety Endpoint(s)	Safety and tolerability of ASO will be assessed by determining the incidence and severity of the below parameters: • Treatment-emergent AEs, serious AEs and changes in concomitant medications • Physical examination and complete neurological examination • Vital signs (HR, BP, weight, respiration) • CSF safety labs (cell count, protein, glucose) • Plasma laboratory tests (clinical chemistry, hematology, coagulation, liver and renal function) • Worsening of disease biomarkers and clinical efficacy assessments that exceeds that expected from worsening of the underlying disease The totality of the safety assessment described above and the clinical picture of the patient, including all assessments listed in the primary objectives, will be taken into consideration for dose-escalation decisions.
Sponsor	Rady's Childrens Hospital UC San Diego

1.3 SCHEDULE OF ACTIVITIES (SOA)

Study Visits (Days)	**Baseline**	**Initial dose D1**		**Follow-up visit 1 D8**	**Second dose D28**		**Follow-up visit 2 D35**	**Third dose D84**		**Follow-up visit 3 D91**	**Fourth dose D174**		**Follow-up visit 4 D181**	**Maintenance**	
Study Window	45 Days	(+/- 7 days) [a]		(+/- 3days) [b]	(+/- 7 days) [a]		(+/- 3days) [b]	(+/- 7 days) [a]		(+/- 3days) [b]	(+/- 7 days) [a]		(+/- 3days) [b]	Doses every 60-90 days [a] (+/- 7 days)	
Location	Onsite	Onsite		Telephone or onsite	Onsite		Telephone or onsite	Onsite		Telephone or onsite	Onsite		Telephone or onsite	Onsite	
		Pre-Dose[c]	**Post-Dose**		**Pre-Dose[c]**	**Post-Dose**		**Pre-Dose[c]**	**Post-Dose**		**Pre-Dose[c]**	**Post-Dose**		**Pre-Dose[c]**	**Post-Dose**
Informed/surrogate consent	X														
Review eligibility	X														
Medical history[d]	X														
Ancillary Procedures	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Review of Seizure Diary: historical vs present	X	X			X			X			X			X	
Physical examination	X	X			X			X			X			X	
Neurological exam	X	X			X			X			X			X	
Clinical lab testing[e]	X	X			X			X			X			X	
MRI (magnetic resonance imaging) [f]	X														X
Neuropsychology Formal clinical testing[g]	X														X
Neuropsychology testing Caregiver Scales[h]	X	X			X			X			X			X	
CDC HRQOL-14[i]	X	X			X			X			X			X	
Bristol Stool Form Scale	X	X			X			X			X			X	
Gait Analysis											X			X	
12-lead ECG	X		X			X			X			X			X
Vital signs	X	X	X		X	X		X	X		X	X		X	X
Weight	X	X			X			X			X			X	
Electroencephalography (EEG) (historical vs. present)	X		X			X			X			X			X
CSF Sampling [j]		X			X			X			X			X	
Study drug injection		X			X			X			X			X	
Adverse event monitoring		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant medication	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

a. Visit window may be adjusted to +7 days to accommodate scheduling

b. Visit window may be adjusted to +3 days to accommodate scheduling

c. Pre-dose assessments should occur on the same day as dosing; however due to scheduling, pre-dose assessments may be conducted +/- 7- days prior to the scheduled dosing.

d. General medical history and assessments relating to primary and secondary endpoints prior to enrollment will be collected as part of the baseline history.

e. Clinical laboratories assessments include complete blood count (with differential), serum chemistry, liver panel, and coagulation factors. Total blood drawn will be approximately 30 mL and will not exceed 40 mL (approximately 8 teaspoons).

f. Neuroimaging with brain MRI will be obtained at the completion of the 24 month study and during the study as needed if any signs of SAE such as hydrocephalus, with and w/out contrast with sedation. Clinical testing performed within six months of enrollment AND judged by the PI to be a sufficient baseline measure will be used as baseline in comparison to planned safety or outcome measures to be performed during this study.

g. Bayley-4 (with GSVs) as a performance measure conducted by a clinical psychologist

h. Aberrant behavior checklist, the Vineland-3, the ORCA, and the Dyskinetic Cerebral Palsy Functional Impact Scale – caregiver/teacher reported questionnaires. The ORCA should be collected within +14-21 days postdose.

i. CDC HRQOL-14 – caregiver reported questionnaire

j. CSF sampling will be conducted pre-dose of study drug administration on the same day as dosing. CSF analysis will be conducted as noted in Section 6.1.2.

2 INTRODUCTION

2.1 STUDY RATIONALE

Currently there is no standard therapy for SCN2A related epileptic encephalopathy. The participant being treated with nL-SCN2A-002 has a pathogenic de novo hg38:Chr2:165342465G>A, NM_001040142.2 c.2558G>A single base substitution leading to a p.R853Q gain-of-function amino acid substitution mutation in the *SCN2A* gene. The *SCN2A* gene encodes the voltage-gated sodium channel Na(v)1.2, which plays an important role in the initiation and conduction of action potentials. SCN2A is expressed in axon initiation segments and at nodes of Ranvier in myelinated nerve fibers (Wolff et al., 2017). Voltage-sensitive sodium channels are heteromeric complexes consisting of a large glycosylated alpha subunit (approximately 260 kD) and 2 smaller beta subunits (33-39 kD). In most types of excitatory neurons, the Na(v)1.2 sodium channel is responsible for generating action potentials. The human *SCN2A* gene spans approximately 120 kb and has 29 exons, including a noncoding alternative first exon and alternative exon 6. Both inherited and de novo mutations are associated with disease in humans, ranging in severity from benign familial neonatal-infantile seizures (BFNIS) to EIEE11. The study drug nL-SCN2A-002 is an individualized antisense oligonucleotide targeting the subject's SCN2A mutation to reduce mutant RNA expression. We hypothesize that lowering the levels of mutant protein will allow more normalized brain function.

2.2 BACKGROUND

Mutations in *SCN2A* can be categorized as loss-of-function or gain-of-function. SCN2A gain-of-function mutations cause early-onset severe epilepsies, while loss-of-function mutations cause autism with occasional seizures (Sanders et al., 2018). In late onset DEE cases loss of sodium channel function is a consistent finding but genetic findings that show an overwhelming absence of nonsense mutations suggest other mechanisms associated with the mutant allele are contributing to the more severe than autism alone DEE phenotypes.

De novo mutations in SCN2A are found in approximately 3% of early onset seizures (Trump et al., 2016). The p.R853Q allele is a recurrent mutation associated with seizure onset at 6-8 months frequent co-occurrence of involuntary movements, including choreoathetosis, dystonia, and pyramidal signs (Berecki et al., 2018). The p.R853Q variant displays both GOF and LOF effects when expressed in cells, and it is thought that the GOF effect is responsible for the DEE clinical phenotype (Berecki et al., 2018) beyond autism predicted by haploinsufficiency alone. The plan we would like to discuss is to administer an ASO to target a SNP that is phased to the preRNA transcript containing the mutation.

These deleterious effects of the mutant allele may be mitigated using antisense oligonucleotides, or ASOs that specifically lower the expression of the mutant transcript.

Currently, there is no existing treatment for SCN2A associated neurologic disorders. n-Lorem works with expert research physicians in personalized medicine centers to treat participants under investigator-initiated INDs and n-Lorem's activities are governed by and consistent with the individualized ASO drug

products draft guidance documents for extremely rare patients. Though not all patients with nano-rare diseases are candidates for ASO treatment, many can be treated with ASOs.

Consequently, the behavior of a new ASO that is a member of a well characterized ASO chemical class, such as potency, route of delivery, duration of effect, and adverse events can be predicted (Crooke, 2021, 2022) . Importantly, n-Lorem relies on published analyses of integrated safety data bases for the ASO chemical classes used by n-Lorem as a critical guide (Crooke et al., 2016; Crooke, Baker, et al., 2018; Crooke et al., 2017; Crooke et al., 2019). To assure that potential on target adverse events is understood and any sequence specific adverse events are identified, the functions of the causative gene are fully characterized, and cellular and toxicity studies are performed in accordance with the draft guidance issued in 2021.

To date, more than 10,000 participants have been treated with PS 2'MOE ASOs in controlled clinical trials and commercially. After intrathecal administration, PS 2'MOE ASOs distribute broadly throughout the spinal cord and CNS and have been shown to be pharmacologically active equally broadly. The most extensive experience is with nusinersen (Spinraza®). Commercially, more than 10,000 participants have been treated with nusinersen, some for as long as 9 years. In addition, other 2'MOE ASOs have also been studied in multiple clinical controlled trials at a range of doses (10-120 mg) and a broad range of ages from newborns to > than 40 years of age (MAPT-NCT03186989, SOD1-NCT02623699, Htt-NCT02519036, SNCA-NCT04165486, LRRK2- NCT03976349, C9orf72- NCT03626012, ATXN2-NCT04494256, GFAP- NCT04849741, FUS- NCT04768972). There is substantial clinical trial experience with many other members of the PS 2'MOE chemical class administered intrathecally (Chiriboga et al., 2016; Crooke, Baker, et al., 2021; Crooke, Liang, Baker, et al., 2021; Crooke, Liang, Crooke, et al., 2021; Korobeynikov et al., 2022; Miller et al., 2020; Miller et al., 2022; Tabrizi et al., 2019). HALOS is an additional relevant clinical trial that is evaluating the safety and tolerability of an intrathecally administered PS 2'MOE ASO (NCT05127226) in Angelman syndrome.

The experimental ASO developed for this participant has been based on 1] in vitro toxicology screens, 2] proof-of-concept data in participant-derived cellular model, 3] single and repeat-dose toxicology studies in rodents, and 4] GMP drug substance and drug product manufacturing.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 KNOWN POTENTIAL RISKS

Certain ASO drugs have been associated with inflammatory effects, including increases in plasma chemokines or cytokines. In general, these effects are considered related to the proinflammatory effects of ASOs at high drug concentrations. In humans, influenza-like constitutional symptoms such as fever, chills, increase in body temperature, and arthralgias have occasionally been observed following parenteral administration of ASOs at high doses, mostly during the initial dosing period.

Post-dose transient absence of the patellar and foot reflexes is considered a well described, non-adverse class finding for ASOs administered via intrathecal injection (Korte et al., 2020) and the absence of any direct effects on respiratory, or cardiovascular parameters is consistent with previous experience of the ASOs of this chemical class (Kim et al., 2014). Rare adverse events that have been associated with ASO intrathecal administration include sporadic low incidence of myelitis, radiculopathy and hydrocephalus (Miller et al., 2020; Tabrizi et al., 2019). Thrombocytopenia and renal toxicity seen with some peripherally administered ASOs is highly unlikely due to the low systemic exposures expected with intrathecal administration and has not been observed in intrathecal programs to date. More commonly, transient local injection site discomfort, headaches and nausea may be seen after intrathecal lumbar puncture.

The study participant will be closely monitored for safety events as has been the case for all intrathecally administered ASOs for signs and symptoms of neurologic and neuropsychiatric changes. Study investigators have the primary responsibility for study conduct and for monitoring participant safety during the trial. The schedule of safety assessments includes evaluation of AEs, physical examination, neurological examination, vital signs, ECGs, and safety laboratory testing. The participant will undergo standardized safety testing prior to each dose of nL-SCN2A-002 to ensure suitability to undergo the lumbar puncture procedure. Potential systemic adverse events will be monitored using standard clinical measures of organ function. Additionally, should clinical observations suggest potential adverse events of concern, MRI evaluation may be performed. Neurological examinations will be conducted following each intrathecal injection during the study to monitor for potential changes in patellar and/or foot reflexes. Identified risks associated with the lumbar puncture will be minimized with use of small gauge atraumatic needles, aseptic procedures, assessment of signs and symptoms associated with increased risk of CNS infection and herniation, assessment for health conditions that increase risks associated with lumbar puncture, assessment of platelets and coagulation parameters before each study drug administration and close participant monitoring during and after study drug administration.

Overall, the risks, both identified and potential, associated with this investigational ASO are balanced by the anticipated benefits to the participant.

2.3.2 KNOWN POTENTIAL BENEFITS

This will be a first-in-human administration of personalized nL-SCN2A-002 antisense oligonucleotide drug in a pediatric participant with a pathogenic de novo p.R853Q gain-of-function amino acid substitution mutation in the *SCN2A* gene. Therefore, there is no prior clinical research data available. The dose and dosing regimen to be administered is determined based on preclinical pharmacology, toxicology and pharmacodynamic (PD) observations. The potential benefits of the treatment include reduction of seizures and anticonvulsant medications as well as improvement in developmental milestones, reduction in abnormal movements and autistic behaviors.

2.3.3 ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

The study participant is a child with severe neurodevelopmental disorder presenting with intractable epilepsy and severe neurodevelopmental delay (nonverbal, non-ambulatory) due to a rare pathogenic de novo p.R853Q gain of function S*CN2A* mutation for which there are currently no effective or targeted therapies. The study drug will selectively target the participant's mutant allele and decrease expression of the mutant allele and decrease production of the mutant protein. Individualized ASO drug will be delivered by intrathecal injection with the goal of reducing clinical impact of the abnormal gene. The study participant will have personalized investigational ASO drug based on his individualized genetic mutations developed through a non-profit foundation (n-Lorem Foundation) if found to be efficacious and safe after a 24-month study period. Potential direct benefits to the participant are listed above (Section 2.3.2) and improvements in any domains would result in substantial increase in quality-of-life to the participants and their family.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS
Primary	
To assess improvement in baseline seizure frequency, motor function (assessed via the Vineland-3, Dyskinetic Cerebral Palsy Functional Impact Scale, and Bayley-4 with GSV as a performance measure), and gastrointestinal issues at 12 and 24 months post nL-SCN2A-002 administration	Quantitative change from baseline at 12 and 24 months post nL-SCN2A-002 administration in • frequency of seizures from a seizure diary (defined as average number of daily seizures over a 3-month time period at baseline compared to the average number of daily seizures over a 3-month time period at 12 and 24 months) • movement and motor domain scores of Vineland-3, Bayley-4 and Dyskinetic Cerebral Palsy Functional Impact Scale (parent/teacher reported measures) • gastrointestinal issues as measured via the Bristol Stool Form scale
Secondary	
To assess improvement in neurodevelopmental scores at 24 months post nL-SCN2A-002 administration	Quantitative change from baseline at 24 months post nL-SCN2A-002 administration in neurodevelopmental and behavioral scores (per standardized parent/teacher report measures including the Aberrant Behavior Checklist, Vineland-3, ORCA and Bayley-4 with GSVs as a performance measure)
Tertiary/Exploratory	
To evaluate EEG biomarkers and spike quantification on a machine EEG learning platform and to assess improvement in gait at 24 months post nL-SCN2A-002	Quantitative change from baseline at 24 months post nL-SCN2A-002 administration in: • EEG spike counts and disease specific biomarkers on a machine learning platform

OBJECTIVES	ENDPOINTS
	• Change in gait as assessed from video recordings using the SARA gait criterion

4 STUDY DESIGN

4.1 OVERALL DESIGN

This is an interventional study to evaluate the effect of personalized antisense oligonucleotide treatment in a single participant with a rare pathogenic gene mutation (n=1 trial). This protocol aims to provide investigational personalized antisense oligonucleotide treatment to a single participant with a specific gene-associated neurological disorder. The objectives of the study are to determine whether long-term administration of personalized intrathecal nL-SCN2A-002 treatment can stabilize or improve neurological function using clinical endpoints including number of average daily seizures recorded via seizure diary and level of cognitive and neurodevelopmental function as measured by standardized neuropsychological assessments, and to evaluate the safety of nL-SCN2A-002 when administered via intrathecal injection.

We hypothesize that lowering the levels of mutant protein in our participant will lead to improvement in neurodevelopmental brain function.

4.2 SCIENTIFIC RATIONALE FOR STUDY DESIGN

There are no current standard treatments for patients with these rare gene mutations, for whom commercial drug development is not feasible. A personalized ASO drug which reduces expression of abnormal gene in an individual participant may allow for more normalized neurodevelopmental brain function and decrease clinical impact of the abnormal gene.

Use of ASO to knock down pre-mRNA transcripts is well established and has demonstrated reduced steady state levels by upwards of 90%. An example of a preclinical model of a SCN2A R-to-Q substitution (p.R1882Q) that shows predominantly gain-of-function effects on the channel demonstrated that ASO intracerebroventricular administration significantly extended lifespan and markedly reduced spontaneous seizure occurrence. Across a range of cognitive and motor behavioral tests, ASO treated p.R1882Q mice were largely indistinguishable from wildtype mice (Li et al., 2021).

4.3 JUSTIFICATION FOR DOSE

4.3.1 NONCLINICAL DATA

The final SCN2A pathogenic allele targeting ASO, nL-SCN2A-002, is homologous to the annotated genomic sequence of human SCN2A as the normal allele in this patient exhibits variation from genomic annotation. In contrast, the pathogenic allele exhibits the genomic annotated sequence at the ASO binding site. Thus, nL-SCN2A-002 is designed to specifically bind the pathogenic allele.

nL-SCN2A-002 was identified as a prospective treatment based on primary *in vitro* screening of approximately 520 ASOs designed to bind to the human *SCN2A* pre-mRNA. nL-SCN2A-002 was identified as the most potent ASO tested in the screening assay in patient cells with an IC_{50} of 0.5458 µM for the pathogenic allele compared to an IC_{50} of 9.582 µM for the wild type SCN2A allele, representing >17-fold allele-selectivity.

nL-SCN2A-002 has been studied in two non-GLP 8-week tolerability studies in mice and rats to avoid nonspecific toxicities. The rodent screening studies included a single-dose ICV study in mice and a single-dose IT study in rats. The studies in each of these cases were designed to look at a single dose level, 15- to 25-fold above the typical high dose in humans administered by repeated intrathecal injection, to assess tolerability for this sequence compared to previous experience. Although these studies are single-dose, non-GLP studies, they have been used to effectively screen and eliminate poorly tolerated ASO sequences. Results from nL-SCN2A-002 can be compared to other representative MOE-mixed backbone ASOs that have progressed through toxicology and human safety studies. The findings in these two studies were limited to transient effects on gross motor function, which resolved within 24 hours.

In addition, a GLP compliant 13-week repeat dose study was conducted in Sprague Dawley rats. The in-life portion of the study is completed, and the histopathology and audited draft report are in preparation. Upon preliminary evaluation of the available data, changes were limited to transient clinical observations with or without correlating neurobehavioral findings, including decreased activity, incoordination, abnormal gait, and limited use of the hindlimbs. Based on this preliminary evaluation, the NOAEL for this study is anticipated to be 1 mg/dose.

4.4 END OF STUDY DEFINITION

This is an investigator-initiated study for an Investigational New Drug Application (IND) to study the safety and effect of ASO nL-SCN2A-002 in a participant with a de novo pathogenic monoallelic p.R853Q gain-of-function amino acid substitution SCN2A gene mutation. The study is scheduled for 24 months. If the participant does not exhibit AEs requiring the termination of the treatment, and if the physicians overseeing the study deem it would be beneficial to continue, the participant would enter a long term follow up after 24 months.

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

- Informed consent provided by the participant's parent(s)/guardian(s)
- Ability to travel to the study site, adhere to study-related follow-up examinations and/or procedures, and provide access to participant’s medical records.
- Genetically confirmed neurological disorder

5.2 EXCLUSION CRITERIA

- Use of an investigational medication within less than 5 half-lives of the drug at enrollment
- Thrombocytopenia (< 100K)

- Bleeding diathesis

5.3 LIFESTYLE CONSIDERATIONS

Not applicable

5.4 SCREEN FAILURES

Not applicable

6 STUDY INTERVENTION

6.1 STUDY INTERVENTION(S) ADMINISTRATION

6.1.1 STUDY INTERVENTION DESCRIPTION

nL-SCN2A-002 was designed to promote selective degradation of the mutant SCN2A RNA through recruitment of RNase H1 to the RNA-oligonucleotide heteroduplex (Crooke, Witztum, et al., 2018; Wu et al., 2007). This ASO was designed as a chimeric 2'-O-methoxyethyl/DNA modified oligonucleotides with five 2'-O methoxyethyl modifications on the 5'- and 3'- ends of the oligonucleotide and the central 10 nucleotides being deoxynucleotides (DNA). The ASO is a mixture of phosphorothioate, in which one of the non-bridging oxygen atoms is replaced with sulfur, and phosphodiester backbone.

Since personalized nL-SCN2A-002 is a research drug, allergic reactions are possible.

Overall, the risks, both identified and potential, associated with allele targeting ASO are balanced by the anticipated benefits to the study participant with SCN2A associated neurological disorder.

6.1.2 DOSING AND ADMINISTRATION

DOSING, ROUTE AND REGIMEN:

Dosing Regimen:
nL-SCN2A-002 will be administered intrathecally via lumbar puncture. A dose of 20 mg will be utilized based on the potency similarity of nL-SCN2A-002 to other intrathecally delivered ASOs in the same chemical class evaluated at this pediatric age. From preclinical testing, the safety margin for the 20 mg dose is 25-fold to the 1 mg/month dose in rats which was well tolerated. Doses will be administered on Day 1, Day 28, Day 84, and every 3 months after (maintenance). The dosing window may be adjusted to 60-90 days (at the highest tolerated dose or lower) if the Principal Investigator determines the clinical effects are waning prior to the end of the 3-month interval.

Dose escalation, as illustrated in the below table (at 60 mg and upwards), will not proceed until further regulatory approval.

When regulatory approval is received, if there is potential for increased clinical benefit (e.g. reduction in seizure frequency on seizure diary post nL-SCN2A-002 without seizure freedom), and if adequate safety

has been demonstrated in the participant, the physician may choose to continue the escalation by 10-20 mg increments every 3 months, up to a maximum of 100 mg. The 100 mg dosage level maintains a safety margin of ~15 fold to the well-tolerated 1 mg/month rat dose, which itself is lower than the maximum tested dose level from preclinical testing. The escalation will be stopped if there is an adverse event due to the ASO, there are significant lab abnormalities that are probably or definitely due to the ASO, there is worsening of a physical or neurological exam parameter due to ASO, or there is observable improvement (i.e., no more seizures and anticonvulsants have been weaned) based on the seizure diary.

Dose Escalation Steps for nL-SCN2-001

PROPOSED REGIMEN*	STARTING DOSE	STEP 1	STEP 2	STEP 3	STEP 4	STEP 5
Default	20 mg	40 mg	60 mg	60 mg	60 mg	60 mg
Option 1	20 mg	40 mg	60 mg	80 mg	80 mg	80 mg
Option 2	20 mg	40 mg	60 mg	80 mg	100 mg	100 mg
Option 3	20 mg	20 mg	30 mg	30 mg	40 mg	40 mg

* Refer to the paragraph above for criteria; “default regimen” should be followed if 60 mg provides adequate efficacy and safety; Options 1 and 2 should be followed for increased clinical benefit if safety allows. Option 3 allows for a slower dose escalation per FDA feedback.

The PI may also choose to remain at the same dose-escalation step for multiple doses to better assess drug efficacy, for safety monitoring, or for any other reason determined to be necessary.

Prior to study drug dosing on Day 1, procedures will be performed as outlined in the Schedule of Assessments.

During the initial dosing visits (initial dose and subsequent first dose of each dose escalation) the surveillance of the participant will be done in an inpatient setting at Rady Children’s Hospital. Vital signs including heart rate, respiratory rate, blood pressure, and pulse oximetry will be monitored every 30 minutes for the first hour following injection, every hour for 2-6 hours and then every 4 hours for the remaining 24 hours observation period. Daily phone calls will be performed for the 3 days after the injection. After a given dose level has been achieved, this inpatient observation may be shortened to a 6-hour outpatient observation at the study physician's discretion. In this case, the participant may be asked to return or respond to a phone call the day following a dose for a 24-hour post-dose assessment, which may include adverse events and concomitant meds.

After each dose, the participant will be observed to closely monitor for acute, serious adverse events precipitated by the administration procedure or by the ASO drug product itself (e.g., hemodynamic instability, severe hypersensitivity reactions/anaphylaxis, CNS adverse effects).

After a given dose level has been achieved, and administered without observable adverse events, less frequent vital sign monitoring will be permitted at the discretion of the investigator. In the event that abnormalities are detected, then appropriate medical intervention will occur, including the possibility of hospitalization and/or subsequent testing. Additional testing may be performed per standard of care guidelines. In the unlikely event that a severe allergic reaction should occur, the medical and nursing staff will follow institutional anaphylaxis guidelines.

Sedation and Analgesia

Considerations for sedation and analgesia for this participant reflect balancing comfort versus risk. If necessary, according to the judgment of the treating physicians, Monitored Anesthesia Care (MAC) will be provided to titrate sedation and analgesia as needed for comfort and spine positioning. This will be most readily accomplished by titrated incremental dosing of midazolam, fentanyl, and propofol. Standard American Society of Anesthesiologists (ASA) monitors will be used. Lidocaine 1% will be infiltrated for local anesthesia. The aim will be to maintain spontaneous ventilation, but assisted ventilation by mask may be used if necessary.

Intrathecal Access

A 22, 25 or 27 gauge one 1/2-inch atraumatic needle will be used. Needle direction will be guided with surface landmarks, although ultrasound guidance may be used if necessary. A certified clinician from Rady Children's Hospital with hospital privileges for intrathecal drug delivery will be performing the lumbar punctures.

CSF Sampling

A total volume of 20 mL of CSF will be obtained at each dose administration. Part of the CSF sampled will be sent to the hospital lab for safety testing (protein, glucose, and cell count). The remaining CSF will be banked for possible future analyses *(e.g*. pharmacokinetic analysis, neurofilament light chain, SCN2A protein levels).

SCN2A ASO Injection

nL-SCN2A-002 will be administered as a 20-ml bolus injection over 1-3 minutes. A lumbar puncture will be performed in accordance with the study site's standard of care (including verification of the platelets count and INR before performing the lumbar puncture).

After each dose is administered, the participant will remain inpatient for observation for 24 (up to 48) hours post-dose, or 6 hours outpatient observation.

Procedures for Treatment (1-24 months) and Follow Up

Following completion of the Day 1 dosing, all study treatments will be administered as described in the below sections and procedures will be performed as shown in Schedule of Assessments.

Maintenance dosing will continue on a two to three-month (every 60-90 days ± 5 business days) interval at the investigator's discretion according to the same protocol. The interval for dosing will be based on the length of initial seizure control after dosing (presence of a clear deterioration in control before the next dose), changes in movement/motor function (presence of clear regression in independent mobility, i.e. walking independently), and gastrointestinal issues (requirement for use of suppositories to support bowel movements). If based on these parameters, a pattern of "wearing off" of the ASO is seen at 90 day dosing intervals, the interval may be decreased by up to 30 days (to 60 day intervals). If this pattern is not seen towards the end of dosing again, the interval would remain at 60 days. The dosing interval will not be decreased to less than 60 days (- 7 day window). Each dose will be followed by 24-hour post-dose monitoring (or option to observe outpatient doses, per investigator's discretion) and a follow-up visit in person, by call or videoconferencing 7 days (±3 days) post-dose. Each maintenance dose date will be anchored off the previous maintenance dose date. Therefore, if a maintenance dose takes place out of window (early or postponed), the following maintenance dose will occur every 60-90 days ± 5 days after out of window maintenance dose. After each dose, the investigator will review safety data to determine whether or not to continue with the scheduled dosing.

Follow-up visits to assess clinical status and adverse events may be conducted by the investigator or a member of the study team by phone and documented in the participant's medical record. The investigator or a study co-investigator will speak to the participant and caregivers to assess adverse events, current health status, and medications. If a medical event or clinical change of concern is noted, the investigator will determine the need and level of urgency for an in-person evaluation and contact the participant's local primary care provider if necessary.

Unscheduled Procedures and Visits

An unscheduled procedure or visit may be performed at any time during the study at the participant's request or as deemed necessary by the investigator.

6.2 TOXICITY MANAGEMENT AND DOSE MODIFICATIONS

The dose of nL-SCN2A-002 would be reduced in a stepwise manner as depicted in the table below if the participant exhibits adverse events deemed as requiring dose reduction by the physician overseeing the study, with the objective of finding a well-tolerated dose which would then be maintained quarterly, as long as there is a potential for clinical benefit and no new adverse events are found. If the participant continues to show undesired effects, subsequent dosing will go to the next reduction step.

Table 1 Dose Reduction Steps for nL-SCN2A-002

DOSE	STEP 1	STEP 2	STEP 3	STEP 4
60 mg	40 mg	20 mg	10 mg	Discontinue
40 mg	20 mg	10 mg	Discontinue	N/A
30 mg	20 mg	10 mg	Discontinue	N/A

20 mg	10mg	Discontinue	N/A	N/A

*If the dose was escalated to 80 mg or 100 mg, each reduction step would lower the dose by 20 mg, with the last step going from 20 mg to 10 mg, until stabilization or discontinuation. Dose can also be lowered in 10 mg decrements.

The investigator may then choose to attempt another dose escalation after 1-2 lower doses have been tolerated or choose to stop dose escalation if there is evidence of a favorable benefit-risk.

Rare adverse events that have been associated with ASO intrathecal administration include sporadic low incidence of myelitis, radiculopathy, and hydrocephalus (Miller et al., 2020; Tabrizi et al., 2019). Thrombocytopenia and renal toxicity found in some peripherally administered ASOs is highly unlikely in this case due to the low exposures expected with intrathecal administration and has not been observed in intrathecal programs to date.

The participant will be monitored for safety events as has been the case for all intrathecally administered PS 2'-MOE ASOs. The study investigator has the primary responsibility for study conduct and for monitoring participant safety during the trial. The schedule of safety assessments includes evaluation of AEs, physical examination, neurological examination, vital signs, ECGs, and safety laboratory testing. The participant will undergo standardized safety testing prior to each dose of nL-SCN2A-002 to ensure suitability to undergo the lumbar puncture procedure. Potential systemic adverse events will be monitored using standard clinical measures of organ function. Additionally, should clinical observations suggest potential adverse events of concern, MRI evaluation may be performed.

6.3 PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

n-Lorem will supply nL-SCN2A-002 to the study investigator and/or pharmacist at Rady Children's Hospital. Drug supplies will be maintained in a secure, limited access storage area under refrigerated conditions. The Investigator/pharmacist/study staff is responsible for ensuring adequate accountability of all study drug provided to the participant. The Investigator/pharmacist/study staff will dispense nL-SCN2A-002 to the participant according to the Schedule of Assessments.

Accountability records will be utilized to record:

- Date received and quantity of study drug
- Date study drug dispensed
- Date quantity of used and unused study drug, along with the initials of the person recording the information

nL-SCN2A-002 will be labelled at a minimum, according to the local regulatory requirements. Please see the pharmacy manual for detailed information about the packaging, labeling, formulation and appearance.

Until dispensed for administration to the participant, all study drug will be stored in a securely locked area, accessible only to authorized site personnel. To ensure stability and proper identification, the drug substance should not be stored in a container other than the container in which it was supplied.

nL-SCN2A-002 will be stored under controlled refrigerated temperature at 2-8°C, protected from light until needed for administration.

nL-SCN2A-002 will be supplied as a sterile solution with 2-mL deliverable volume in stoppered and sealed glass vials under sterile condition. Before injection, nL-SCN2A-002 will be diluted with sterile artificial CSF (aCSF) to the indicated dosage level to an injection volume of 20 mL per dose.

Unused study drug may be returned to n-Lorem or may be destroyed at Rady Children's Hospital with appropriate standard operating procedure (SOP) in place.

6.4 MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

Not applicable

6.5 STUDY INTERVENTION COMPLIANCE

The study participant will be followed at regular intervals per the schedule of assessments. Seizure diary and neuropsychological rating scales from caregivers will be routinely collected at each scheduled interval. An unscheduled visit may be performed at the participant's request or as deemed necessary by the study investigators for any compliance concerns during the 24-month study period.

6.6 CONCOMITANT THERAPY – CONCOMITANT MEDICATIONS AND ANCILLARY PROCEDURES

Any medication and non-medication interventions, supportive interventions, or concomitant procedures that the participant is receiving at the time of enrollment or receives during the study must be recorded along with:

- reason for use.
- dates of administration including start and end dates.
- dosage information including dose and frequency.

Prior use of medications taken by the participant within 180 days prior to screening will be recorded, as well as medications taken by the participant during the study.

The Investigator or qualified designee will record medication and non-medication interventions or procedures, if any, taken or received by the participant during the study through the last visit. Medications will be captured as concomitant medications and procedures as ancillary procedures on the electronic case report forms (eCRF).

7 STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

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7.1 DISCONTINUATION OF STUDY INTERVENTION

The investigator may suspend or terminate the study when:

- A serious life-threatening adverse event considered by the investigator as related to the investigational medicine occurs.
- There is no stabilization of neurological status during the treatment period.
    - The treating physician may apply to extend the protocol by amendment if there is sufficient evidence of safety, if not efficacy.
- The participant's neurological function appears to be adversely affected by the injections.
- The treating team determines the benefit/risk ratio is not favorable, including the consideration of risks related to repeated lumbar punctures.

7.2 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

The study participant is free to withdraw from participation in the study at any time upon request.
An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant study intervention non-compliance
- If any clinical adverse event (AE), laboratory abnormality, or other medical condition or situation occurs such that continued participation in the study would not be in the best interest of the participant
- Disease progression which requires discontinuation of the study intervention
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF).

7.3 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he fails to return for 2 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit within 14 days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary,

a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.

- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

8 STUDY ASSESSMENTS AND PROCEDURES

8.1 EFFICACY ASSESSMENTS

The specific timing of procedures/evaluations to be done at each study visit are captured in **Section 1.3, Schedule of Activities** (SoA). The following efficacy assessments will be performed per SOA.

Seizure Diary

The number and severity of seizures are considered part of the disease progression. The participant will be tracked for number of seizures. Parents/Caregivers will complete a seizure diary daily to document seizure activity.

Electroencephalography

Overnight EEG will be performed for seizure and spike quantification at baseline evaluation (D-14) and at each dosing visit with an overnight stay. This EEG iteration is designed to closely follow the course of epilepsy after the first injection. A Board-certified epileptologist, Dr Olivia Kim-McManus, Department of Neurology Rady Children's Hospital, or a trained neurologist from the same department will be reviewing the results of the EEG.

Vineland Adaptive Behavior Scales – Version 3 (Vineland – 3)

The Vineland-3 is a valid and reliable measure of a person's adaptive level of functioning from birth to 90 years of age (Sparrow et al., 1984). It is commonly used in clinical care and research to measure the development and functioning of individuals with and without disabilities and has been discussed with FDA as an outcome for clinical trials. It is an informant-based measure that yields a composite score and domain standard scores in domains (and subdomains) of: Communication (receptive, expressive, and written adaptive language functions), Daily Living Skills (personal, domestic, and community skills), Socialization (interpersonal relationships, play and leisure time, and coping abilities), and Motor Skills (gross and fine motor skills). This structure corresponds to the 3 broad Domains of adaptive functioning recognized by the American Association of Intellectual and Developmental Disabilities (AAIDD, 2010). The Survey Interview Edition is administered to parents or caregivers using a semi-structured interview format. In addition to standard scores and age-equivalents, growth score values (GSVs, person ability scores) are available for all subdomain scores allowing reliable assessment of change even in those who floor the measure or appear to make no progress when using standard scores due to functional level far below chronological age (Farmer et al., 2020). The caregiver rating assessments will be performed by the

study participant's parent and teacher and reviewed at each study interval as specified by SOA by a clinical neuropsychologist at UCSD Rady Children's Hospital or a qualified designee.

Aberrant Behavior Checklist – Community Edition (ABC-C)

The Aberrant Behavior Checklist- Community Edition (ABC-C) is a 58-item parent/caregiver rating scale used to assess the severity of a range of problem behaviors commonly observed in individuals with intellectual disability across five dimensions or subscales: irritability, hyperactivity, lethargy/withdrawal, stereotypy, and inappropriate speech (Aman et al., 1985). Items are evaluated on a 4-point Likert scale ranging from 0 (not at all a problem) to 3 (the problem is severe in degree). The caregiver completing the assessment will remain the same at all applicable visits throughout the trial. The measure will be completed as specified by the SOA and reviewed at each study interval as specified by SOA by a clinical neuropsychologist at UCSD Rady Children's Hospital or a qualified designee.

Observer-Reported Communication Ability (ORCA)

The ORCA measure is a parent-reported questionnaire developed at Duke University for the evaluation of expressive, receptive, and pragmatic communication skills of children with Angelman syndrome and can be applied to other individuals with severe ID and communication impairment due to other neurodevelopmental disorders. The measure provides a more granular assessment of communication impairment than provided by standard language scales used with the typical population, and thus allows detection of change in populations with severe impairment that would not be possible with standard language scales. The measure is designed to observe the overall communication ability of the participant from the perspective of their primary caregiver and consists of 84 questions with 70 behavior items within 22 concepts. The assessment considers the heterogeneity of communication modalities and assesses the individual's unique methods of communicating. The total ORCA score reflects the primary caregiver's assessment of the participant's ability to perform behaviors frequently and consistently, indicating the participant's total communication ability. The caregiver completing the assessment will remain the same at all applicable visits throughout the trial. The measure will be completed as specified by the SOA and reviewed at each study interval as specified by SOA by a clinical neuropsychologist at UCSD Rady Children's Hospital or a qualified designee.

Bayley-4 with growth scale values (GSV)

The Bayley Scales of Infant Development-4 (BSID-4) is a standardized developmental assessment measure used by clinicians to evaluate key domains in early childhood development for individuals between 16 days of birth and 42 months of age (Bayley & Aylward, 2020). This measure has been used in intellectual disability out of the age range for use in typically developing populations when mental age falls in the range of the BSID-4 (Sadhwani et al., 2023). The domains in the BSID-4 include cognition, language (subdomains receptive and expressive), and motor function (subdomains gross and fine). The BSID-4 will be administered to the participant by a trained psychologist. In addition to standard scores and age-equivalents, growth score values (GSVs, person ability scores) are available for all subdomain scores allowing reliable assessment of change even in those who floor the measure or appear to make no progress when using standard scores due to functional level far below chronological age.Bayley-4

with growth scale values (GSV) will be performed at each study interval as specified by SOA by a clinical neuropsychologist at UCSD Rady Children's Hospital or a qualified designee.

Dyskinetic Cerebral Palsy Functional Impact Scale (D-FIS)

The D-FIS is a parent-reported measure used to assess the impact of abnormal muscle tone, such as spasticity (increased tone or stiffness), floppiness (decreased tone), or dyskinesia (fluctuating tone) on the patient's ability to perform daily activities.

Bristol Stool Form Scale

The Bristol stool scale is a diagnostic medical tool designed to classify the form of human faeces into seven categories. It has been used to track and diagnose irritable bowel syndrome and is sensitive to change in intestinal transit time following several medications.

Exploratory Biomarkers

De-identified EEG data obtained at study intervals as specified by SOA will be evaluated on a machine learning EEG platform for spike quantification and identification of any SCN2A disease specific EEG biomarkers by contracting firm, Beacon Biosignals.

Quality of Life Inventory-Disability Questionnaire

Quality of Life will be evaluated using the parental version of the Quality of Life Inventory-Disability questionnaire per SOA schedule.

Gait Analysis:

Parent/caregiver will be asked to record the child walking with or without assistance. Gait index from each video timepoint will be rated using a blinded rater based on the **Scale for Assessment and Rating of Ataxia (SARA)** gait criterion.

8.2 SAFETY AND OTHER ASSESSMENTS

The participant will be monitored at baseline and throughout the course of treatment according to the Schedule of Assessments (See Section 1.3). The following assessments will be performed to monitor safety:

PHYSICAL EXAMINATION

Physical examinations will be conducted at visits as outlined in the Schedule of Assessments (see Section 1.3) A complete physical examination will include recording, height, weight and full body system check: examination of general appearance, skin, neck (including thyroid), eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities, neurologic and vascular.

ECG

A single 12-lead ECG will be performed at the timepoints outlined in the Schedule of Assessments.

VITAL SIGNS AND GROWTH MEASUREMENTS

- Body temperature (route can be oral, tympanic, rectal, axillary, skin, or temporal artery), heart rate, respiratory rate, oxygen saturation via pulse oximetry, and blood pressure
- Blood pressure and heart rate measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Blood pressure and heart rate measurements should be preceded by at least 5 minutes of rest for the participant in a quiet setting without distractions (e.g. television, cell phone).
- Vital signs including systolic and diastolic blood pressure and radial pulse rate and temperature will be collected as outlined in the Schedule of Assessments (See Section 1.3).
- Height and weight

CLINICAL LABORATORY TESTING

Laboratory tests will be performed according to the Schedule of Assessments (see Section 1.3). Laboratory test results (blood and urine) will be reviewed by the investigator prior to any study treatment. The medical personnel performing the dose administration will review the platelet count before performing the lumbar puncture. The clinical significance of out-of-range laboratory findings is to be determined and documented by the investigator/sub-investigator who is a qualified physician.

Specific laboratory tests to be performed are listed in Table 2. Total blood drawn will be approximately 30 mL and will not exceed 40 mL (approximately 8 teaspoons).

Table 2 Clinical Laboratory Tests

Serum Chemistry	**Hematology**	**Urinalysis**	**Cerebrospinal fluid analysis**
Albumin Blood Urea Nitrogen (BUN) Calcium Bicarbonate Chloride Creatinine Glucose Magnesium Phosphate Potassium Sodium Total Bilirubin Total Protein Alanine Aminotransferase (ALT)	Hematocrit (Hct) Hemoglobin (Hgb) Red Blood Cell Count (RBC) White Blood Cell Count (WBC) / WBC differential Absolute Neutrophil Count (ANC) / Platelets Mean Corpuscular Volume (MCV) Mean Corpuscular Hemoglobin (MCH) Mean Corpuscular Hemoglobin Concentration (MCHC) PT PTT INR	Color Clarity/turbidity pH Specific gravity Glucose Ketones Nitrites Leukocyte esterase Bilirubin Urobilinogen Blood Protein RBCs WBCs	Cell count Total protein Glucose

Serum Chemistry	Hematology	Urinalysis	Cerebrospinal fluid analysis
Alkaline Phosphatase (ALP) Aspartate Aminotransferase (AST) Creatinine Clearance			

IMAGING

MRI brain with sedation will be performed at the completion of the 24-month study per Schedule of Assessments and will be obtained during the study per investigator discretion if any concern for SAE such as hydrocephalus.

8.3 ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1 DEFINITION OF ADVERSE EVENTS (AE)

The FDA definition of an Adverse event is any untoward medical occurrence associated with the use of an intervention in humans, whether or not considered intervention-related (21 CFR 312.32 (a)). An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of medicinal (investigational) product, whether or not the AE is considered related to the medicinal (investigational) product.

An AE can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition)
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline
- Any deterioration in a laboratory value or other clinical test (e.g., ECG, X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from Study Drug

8.3.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

A Serious Adverse Event (SAE) is any AE that in the view of the Investigator or Sponsor, meets any of the following criteria:

- Results in death

- Is life threatening: that is, poses an immediate risk of death at the time of the event

An AE or suspected adverse reaction is considered "life-threatening" if, in the view of either the Investigator or Sponsor, its occurrence places the patient at immediate risk of death. It does not include an AE or suspected adverse reaction that, had it occurred in a more severe form, might have caused death

- Requires inpatient hospitalization or prolongation of existing hospitalization
- Hospitalization is defined as an admission of greater than 24 hours to a medical facility and does not always qualify as an AE
- Results in a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Results in a congenital anomaly or birth defect in the offspring of the patient (whether the patient is male or female)
- Important medical events that may not result in death, are not life-threatening, or do not require hospitalization may also be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an AE (e.g., rated as mild, moderate, or severe). Severity and seriousness need to be independently assessed for each AE recorded on the eCRF.

All adverse events that do not meet any of the criteria for serious, should be regarded as non-serious adverse events.

8.3.3 CLASSIFICATION OF AN ADVERSE EVENT

For adverse events (AEs) not included in the protocol defined grading system, the following guidelines will be used to describe severity.

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".]

8.3.3.1 RELATIONSHIP TO STUDY INTERVENTION

All adverse events (Aes) must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

The relationship of an AE to the Investigational Drug is a clinical decision by the investigator or sub-investigator (SI) based on all available information at the time of the completion of the CRF and is graded as follows:

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (DE challenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "definitely related", as appropriate.
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

8.3.3.2 EXPECTEDNESS

Study investigators will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.4 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All Aes including local and systemic reactions not meeting the criteria for SAEs will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of

onset, clinician's assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All Aes occurring while on study must be documented appropriately regardless of relationship. All Aes will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE.

Changes in the severity of an AE will be documented to allow an assessment of the duration of the event at each level of severity to be performed. Aes characterized as intermittent require documentation of onset and duration of each episode.

Study investigators will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious Aes) or 30 days (for SAEs) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of AE/SAEs since the last visit. Events will be followed for outcome information until resolution or stabilization. For this study, the study treatment follow-up period is defined as 30 days following the last administration of study treatment. The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up.

8.3.5 ADVERSE EVENT REPORTING

Rare adverse events that have been associated with ASO intrathecal administration include sporadic low incidence of myelitis, radiculopathy, and hydrocephalus (Miller et al., 2020; Tabrizi et al., 2019). Thrombocytopenia and renal toxicity found in some peripherally administered ASOs is highly unlikely in this case due to the low exposures expected with intrathecal administration and has not been observed in intrathecal programs to date.

The participant will be monitored for safety events as has been the case for all intrathecally administered PS 2'-MOE ASOs. The Sponsor's study team and study investigators have the primary responsibility for study conduct and for monitoring participant safety during the trial. The schedule of safety assessments includes evaluation of Aes, physical examination, neurological examination, vital signs, ECGs, and safety laboratory testing. The study participant will undergo standardized safety testing prior to each dose of nL-SCN2A-002 to ensure suitability to undergo the lumbar puncture procedure. Potential systemic adverse events will be monitored using standard clinical measures of organ function. Additionally, should clinical observations suggest potential adverse events of concern, MRI evaluation may be performed.

At screening, any clinically significant abnormality should be recorded as a preexisting condition. A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens

during the study period. Any abnormal laboratory test result (e.g., hematology, clinical chemistry or urinalysis) or other safety assessment (e.g., ECGs, radiology scans, vital signs measurements, physical examination), including those that worsen from baseline, that is considered to be clinically significant in the medical and scientific judgement of the investigator and not related to underlying disease, is to be reported as an AE.

Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should not be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator

At each contact with the participant, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event section of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should be recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs during the Adverse Event Reporting Period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

The investigator will use the following definitions to rate the severity of each adverse event

- **Mild**: No disruption of normal daily activities
- **Moderate**: Affect normal daily activities
- **Severe**: Inability to perform daily activities

8.3.6 SERIOUS ADVERSE EVENT REPORTING

The sponsor-investigator will report to the FDA all unexpected, serious suspected adverse reactions according to the required IND Safety Reporting timelines, formats and requirements. The sponsor should also report any serious adverse events to n-Lorem and will work with n-Lorem on any reporting to the FDA.

Unexpected fatal or life threatening suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 7 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Other unexpected serious suspected adverse reactions where there is evidence to suggest a causal relationship between the study drug/placebo and the adverse event, will be reported as a serious suspected adverse reaction. This will be reported to the FDA on FDA Form 3500A, no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

Any clinically important increase in the rate of serious suspected adverse reactions over those listed in the protocol or product insert will be reported as a serious suspected adverse reaction. This will be reported to the FDA-on-FDA Form 3500A no later than 15 calendar days after the sponsor-investigator's initial receipt of the information about the event.

The study sponsor/investigator will be responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor's initial receipt of the information. In addition, the sponsor must notify FDA of potential serious risks, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting.

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable.

8.3.7 REPORTING EVENTS TO PARTICIPANTS

It is the responsibility of the study investigators to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above. Medical monitoring will include a regular assessment of the number and type of serious adverse events. The study participant will be notified immediately within 24 hours of any serious adverse events.

8.3.8 EVENTS OF SPECIAL INTEREST

For the purposes of this protocol, the following are considered an Aes of Special Interest (AESI):

- Hydrocephalus:

- Brain CT scan or MRI will be performed to rule out hydrocephalus if any two or more of these symptoms appear in the patient without any obvious other underlying reason.
  - Nausea and vomiting
  - Worsening of gait abnormalities
  - Changes in behavior
  - Increasing frequency and severity of seizures
  - Poor appetite

If hydrocephalus is confirmed, the treatment will be stopped and the patient will be addressed to a neurosurgeon to be treated according to the standard of care.

8.3.9 DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.3.10 UNANTICIPATED PROBLEM REPORTING

The investigator will report unanticipated problems (Ups) to the reviewing Institutional Review Board (IRB). The UP report will include the following information:

- Protocol identifying information: protocol title and number, PI's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;
- An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

An investigator-sponsor will submit to the reviewing Institutional Review Board (IRB) a report of any unanticipated adverse events occurring during an investigation as soon as possible, but in no event later

than 10 working days after the investigator first learns of the effect (21 CFR 312.32(a)). A sponsor who conducts an evaluation of an unanticipated adverse event shall report the results of such evaluation to the Food and Drug Administration (FDA) within 10 working days after the investigator-sponsor first receives notice of the event. Thereafter the investigator-sponsor shall submit such additional reports concerning the event as FDA requests (21 CFR 312.32(b)).

8.3.11 REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

The study participant with be notified of any Ups within 10 working days after the investigator-sponsor first receives notice of the effect.

9 STATISTICAL CONSIDERATIONS

9.1 STATISTICAL HYPOTHESES

Efficacy Endpoint:

- Quantitative change in baseline at 12 and 24months post nL-SCN2A-002 administration in
    - frequency of seizures from a seizure diary (defined as average number of daily seizures over a 3-month at baseline compared to the average number of daily seizures over a 3-month time period at 12 and 24 month)
    - movement and motor domain scores of Vineland-3, Bayley-4 and Dyskinetic Cerebral Palsy Functional Impact Scale (parent/teacher reported measures)
    - gastrointestinal issues as measured via the Bristol Stool Form scale

- Quantitative change in baseline at 24 months post nL-SCN2A-002 administration in neurodevelopmental and behavioral scores (per standardized parent/teacher report measures including the Aberrant Behavior Checklist, Vineland-3, ORCA and Bayley-4 with GSVs as a performance measure)

9.2 SAMPLE SIZE DETERMINATION

Not applicable.

9.3 POPULATIONS FOR ANALYSES

This is an interventional experimental treatment study for a single participant (n=1) at a single center with rare SCN2A associated genetic disease.

9.4 STATISTICAL ANALYSES

A participant profile will be created using the data collected on the individual participant in the study. This will include, as appropriate, data listings, narratives, and graphical displays such as bar charts, spaghetti plots, and trend graphs over time.

The frequency of rate data will be summarized as the number of events divided by the observation time. The percentage change in a rate prior to starting treatment vs on treatment will be calculated, if applicable, as (1-rate ratio)*100, where the rate ratio is equal to the on-treatment rate divided by the pre-treatment rate.

For continuous score data, change from the scores obtained during baseline evaluation (up to 30 days prior to first treatment) over time will be described. The data will be plotted over time and a slope will be estimated if applicable. The percentage change in a mean or actual score prior to starting treatment vs on treatment will be calculated as (the difference in score between on-treatment minus pre-treatment divided by pre-treatment score)*100.

For categorical data, shifts over time will be described.

In order to assess the overall treatment effect on multiple outcomes over time, each individual assessment will be ranked using scores from the common timepoints among the outcomes. An average ranking across all outcomes at each timepoint will then be calculated.

To investigate potential relationships between outcomes, correlations between changes over time will be examined.

Analysis and reporting for adverse events will be based on treatment emergent adverse events (TEAEs). Intensity and causality of TEAEs will be evaluated by the investigator. A listing of all TEAEs will be provided including additional information such as action taken with study treatment, other action taken, and outcome.

Listings of laboratory parameters (hematology, chemistry, urinalysis) will be provided by study time point including changes from baseline. Any abnormal values will be flagged with ‘L’ for values below the lower limit of the clinical reference range and ‘H’ for values above the upper limit of the clinical reference range and included in the listings. Listings of all vital signs, ECG and physical examination parameters by study time point will also be provided.

A safety narrative describing any death, serious adverse event, or other adverse event that is judged to be of special interest will be provided. Specifically, the safety narrative will include the clinical course of the event, with an indication of the timing of event corresponding to study drug administration; the nature, intensity/severity, and outcome of the event; relevant laboratory findings; any treatment administered for the event; action taken with respect to the study drug; and investigator's assessment on causality. In addition, it should also include the participant’s age, sex, race, height and weight (if relevant), disease being treated and duration of disease, prior and concomitant medications, medical history, and active/ongoing medical conditions**.**

10 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1 REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1 ETHICAL CONDUCT OF THE STUDY

The study will be conducted in accordance with the protocol, ICH guidelines, applicable regulations and guidelines governing clinical study conduct and the ethical principles that have their origin in the Declaration of Helsinki.

10.1.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to the study participant, investigator, funding agency, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform the study participant, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. The study participant will be contacted, as applicable, and be informed of changes to the study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to the participant
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

10.2 WHEN AND HOW TO WITHDRAW PARTICIPANTS

The study participant will be informed that they are free to discontinue study drug or withdraw from the study at any time and for any reason. The Investigator may discontinue study drug or withdraw a participant from the study if, in the Investigator's opinion, it is not in the best interest of the participant to continue the study.

The investigator may terminate this study prematurely for reasonable cause provided that written notice is submitted in advance of the intended termination. If the investigator terminates the study for safety reasons, the investigator will immediately notify n-Lorem and subsequently provide written instructions for study termination.

The Investigator will work with the participant and n-Lorem to determine follow-up study visits and assessments after treatment termination. Ideally, if the participant discontinues nL-SCN2A-002 early they will be encouraged to return within 3 months to collect efficacy endpoints and safety data. After

that, study participation will end, and the participant can still return for regular clinical follow-up visits with the providers.

10.2.1 CONFIDENTIALITY AND PRIVACY

Information about study participants will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the participant of the following:

- What protected health information (PHI) will be collected from participants in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI

In the event a participant revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization. For participants who have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the participant is alive) at the end of their scheduled study period.

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.
The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

10.2.2 FUTURE USE OF STORED SPECIMENS AND DATA

Electronic CRFs specifically designed for this study must be completed for the participant after providing informed consent. All participant data must have supportive original source documentation in the medical records or equivalent. De-identified, archived data will be transmitted to and stored at Electronic Data Capture (EDC) system prepared by n-Lorem Foundation for use by other researchers including those outside of the study. Permission to transmit data to the EDC system prepared by n-Lorem Foundation will be included in the informed consent.

An n-Lorem hosted REDCap is a secure web application for building and managing online databases. N-Lorem hosted REDCap will be used collect participant data and is compliant with 21 CFR Part 11, HIPAA it is specifically geared to support online and offline data capture for research studies and operations. The data collected on the eCRFs will be captured in EDC that meets the technical requirements described in 21CRF Part 11 (USA). The EDC will be fully validated to ensure that it meets the scientific, regulatory and logistical requirement of the study before it is used to capture data from this study.

During the conduct of the study, an individual participant can choose to withdraw consent to have biological specimens stored for future research. However, withdrawal of consent with regard to biosample storage may not be possible after the study is completed.

When the study is completed, access to study data and/or samples will be provided through the n-Lorem hosted REDCap EDC system.

10.2.3 SAFETY OVERSIGHT

Safety oversight will be under the direction of the n-Lorem Data Safety Monitoring Board (DSMB) including a small group of experts including independent investigators at outside institutions who are independent of the protocol to review data from a particular study. The primary responsibility of the DSMB is to monitor participant safety. The DSMB considers study-specific data as well as relevant background information about the disease, intervention, and target population under study. The DSMB will meet at least semiannually to assess safety and efficacy data on the participant. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to sponsor-investigator.

10.2.4 DATA HANDLING AND RECORD KEEPING

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives,

microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

Electronic CRFs specifically designed for this study must be completed for the participant after providing informed consent. All participant data must have supportive original source documentation in the medical records or equivalent.

The sponsor-investigator will maintain records and essential documents related to the conduct of the study for the longest retention period that applies, based on all applicable regulations and requirements. These will include participant case history and regulatory documents.

The sponsor-investigator will retain all data and records will be maintained for the longest retention period that applies, based on all applicable regulations and requirements.

10.2.4.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Data will be gathered in an EDC system prepared by n-Lorem Foundation. N-Lorem hosted REDCap is a secure web application for building and managing online databases. N-Lorem hosted REDCap will be used collect patient data and is compliant with 21 CFR Part 11, HIPAA it is specifically geared to support online and offline data capture for research studies and operations. The data collected on the eCRFs will be captured in EDC that meets the technical requirements described in 21CRF Part 11 (USA). The EDC will be fully validated to ensure that it meets the scientific, regulatory and logistical requirements of the study before it is used to capture data from this study. Before using the EDC, all users will receive training on the system and study specific training. After they are trained, users will be provided with individual system access rights.

10.2.4.2 STUDY RECORDS RETENTION

Paper records will be retained as long as the participant is treated which could be for decades. All records will be maintained during treatment and for 20 years following treatment. Records in the

electronic medical record will be retained indefinitely. The sponsor-investigator will maintain records and essential documents related to the conduct of the study for the longest retention period that applies, based on all applicable regulations and requirements. These will include subject case histories and regulatory documents.

10.2.5 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the study protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations. Protocol deviations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator/study staff are responsible for knowing and adhering to the reviewing IRB requirements.

Any changes to the study that arise after approval of the protocol must be documented as protocol amendments: substantial amendments and/or non-substantial amendments. Depending on the nature of the amendment, either IRB/IEC, Competent Authority approval or notification may be required. The changes will become effective only after the approval of the sponsor, the investigator, the regulatory authority, and the IRB/IEC (if applicable).

Written verification of IRB/IEC approval will be obtained before any amendment is implemented. Modifications to the protocol that are administrative in nature do not require IRB/IEC approval but will be submitted to the IRB/IEC for their information.

10.3 ABBREVIATIONS

AAIDD	American Association of Intellectual and Developmental Disabilities
AE	Adverse Event
ANCOVA	Analysis of Covariance
ASO	Antisense Oligonucleotide
BP	Blood pressure
BSID	Bayley Scales of Infant Development
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
CNS	Central Nervous System
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
CSF	Cerebrospinal Fluid
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
EDC	Electronic Data Capture
ECG	Electrocardiogram
EEG	Electroencephalogram
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GSV	Growth Score Values
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
HR	Heart rate
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MAC	Monitored Anesthesia Care
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet

NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
ORCA	Observer-Reported Communication Ability measure
PD	Pharmacodynamic
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
TAEA	Treatment emergent adverse events
UP	Unanticipated Problem
US	United States

10.4 PROTOCOL AMENDMENT HISTORY

Version	Date	Description of Change	Brief Rationale
2.0	08 June 2023	The duration of treatment has been updated throughout the protocol from 12 to 24 months.	This change was based on comments from the FDA to allow for better assessment of behavioral/cognitive changes that may take longer to be measured.
2.0	08 June 2023	Primary endpoint has been changed from "Quantitative change in baseline at 12 months post nL-SCN2A-002 administration in frequency of seizures (defined as average number of daily seizures from a seizure diary), automated epileptiform spike detection/quantification on electroencephalogram (EEG), or both" to "Quantitative change in baseline at 12 and 24 months post nL-SCN2A-002 administration in frequency of seizures (defined as average number of daily seizures from a seizure diary)"	Based on comments from the FDA, the duration of the study was extended to 24 months and the automated epileptiform spike detection was removed from primary endpoint and kept as tertiary/exploratory endpoint.
2.0	08 June 2023	A few editorial changes were made throughout the protocol. One specific change to Section 8.3.10 was made to update "unanticipated adverse device effect" to unanticipated adverse events"	These changes were implemented to improve the readability of the protocol and to correct a previous oversight.
3.0	07 July 2023	The primary objectives and endpoints were expanded to include assessment of motor function and gastrointestinal issues, and to specify how change in seizures will be evaluated	This change was implemented to address questions from the FDA on the presentation and severity of the disease and seizure-related assessments over time
3.0	07 July 2023	A description of the Dyskinetic Cerebral Palsy Functional Impact Scale (D-FIS) has been added.	The D-FIS is a parent-reported questionnaire that was added to assess changes over time in the movement and motor function of the participant.
3.0	07 July 2023	The D-FIS was added to footnote 'h' of the schedule of activities.	To ascertain it is clear when the D-FIS needs to be implemented.
3.0	07 July 2023	Added details on how seizure frequency will be calculated	This change was made to address the FDA request to use a 3-month time period to calculate average frequency of seizures
4.0	25 August 2023	Drug dosage verbiage was updated to clarify that dose escalation will not proceed until further regulatory approval. Additionally, clarification that PI has oversight of dose escalation allowance was added. These updates	These changes were implemented to align with 20 mg dosage approval from the FDA

		were made to Section 1.2 "Schema – Drug Dosage and Administration" and Section 6.1.2 "Dosing and Administration".	
4.0	25 August 2023	Specified that safety data will be reviewed by the n-Lorem data safety monitoring board	Identified the DSMB to be used for this protocol
5.0	03 November 2023	Specified approximate total volume and volume limit of drawn blood for clinical labs. Corrected table reference for Clinical Laboratory Tests	Clarified blood volume needed for clinical labs.
5.0	03 November 2023	Corrected CSF section referenced in SoA footnote (previously written as "10.9.1"). Clarified potential CSF labs (previously written as "frozen for studies as described below"). Updated specified CSF safety labs to match that listed in Table 2 (cell count, protein, and glucose).	CSF lab information specified and clarified.
5.0	03 November 2023	Removed mention of video capture of patient's seizures.	Corrected text - video recordings will not be used to track seizures.
5.0	03 November 2023	Section 6.6 (Concomitant Therapy) renamed and updated with details for capturing "Concomitant Medications" and "Ancillary Procedures"	Clarified terms used in the Schedule of Activities
5.0	03 November 2023	Removed the word “assent” from Inclusion Criteria.	Corrected text as parent(s)/ guardian(s) will be providing consent
5.0	03 November 2023	Removed verbiage that study drug will be developed “at no cost and provided for life”.	Text not needed per 21 CFR Part 312.23(6i-iii)
6.0	15 May 2024	Updated dosage escalation from “20 mg increments” to “10-20 mg increments” and added “Option 3” for dose escalation table from 20 mg to 30 mg to 40 mg.	Updated dose escalation dosages and steps per FDA feedback.
6.0	15 May 2024	Added reduction steps from 30 mg to discontinuation for dose reduction table and included verbiage in dose reduction table footnote that dose may be reduced in 10 mg increments.	Added to complement updated dose escalation dosages and steps.
7.0	08 Jul 2024	Included Scale for Assessment and Rating of Ataxia (SARA), a clinical scale that is based on a semi-quantitative assessment of ataxia scored by a blinded reviewer/investigator using video recordings.	A SARA scale provides a semi quantitative assessment of gait as an additional outcome measure.
8.0	02 Aug 2024	Included gait assessment video recording and scoring from the Scale for Assessment and Rating of Ataxia	This assessment was incorporated to account for improvements in the patient’s motor function and the new ability to walk independently,

		(SARA) gait criterion and added as an exploratory objective.	enabling gait evaluation that was previously not possible.
8.0	02 Aug 2024	Editorial: added pre-existing exploratory endpoint of EEG biomarker and spike quantification evaluation (Section 3 – Objectives and Endpoints) to synopsis summary	This change was implemented to improve the readability of the protocol and to correct a previous oversight.
9.0	07 May 2025	Changed maintenance dosing interval from every 3 months to every 2-3 months (60-90 days).	This change was implemented to allow for reduced dosing interval if clinical data shows that the effects of the treatment are waning by the end of the 90-day dosing period.
9.0	07 May 2025	Confirmed dose escalation to 60 mg and above will not occur until further regulatory approval is received.	Updated following FDA approval of dose escalation to 40 mg.
9.0	07 May 2025	Updated window for ORCA assessment.	The ORCA assessment timing was updated to occur after dosing to better align with study drug exposure and more accurately capture potential efficacy signals.

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