A PROSPECTIVE OPEN-LABEL STUDY TO ASSESS EFFICACY AND SAFETY OF RT001 IN SUBJECTS WITH INFANTILE NEUROAXONAL DYSTROPHY

PROTOCOL NUMBER: RT001-008

Version 9
Protocol Date: 14 July 2021

Confidentiality Statement

1. SIGNATURE PAGE

Title of Protocol: A Prospective Open-label Study to Assess Efficacy and Safety of RT001 in Subjects with Infantile Neuroaxonal Dystrophy

Protocol No: RT001-008

1.1. Sponsor Approval

14 July 2021

Peter Milner, MD Date
Chief Medical Officer/Sponsor Representative

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1.2. Investigator Agreement

By signing this page, I attest that I have read and understand the contents of Clinical Protocol RT001-008. I agree to adhere to the design, conduct, and reporting requirements of the study as stated in the clinical protocol and to my obligations to the Sponsor as described in the protocol, The Declaration of Helsinki, relevant 21 CFR sections, Good Clinical Practices, and the executed contracts between myself, my Institution, and the Sponsor.

Signature **Investigator Signature** Date

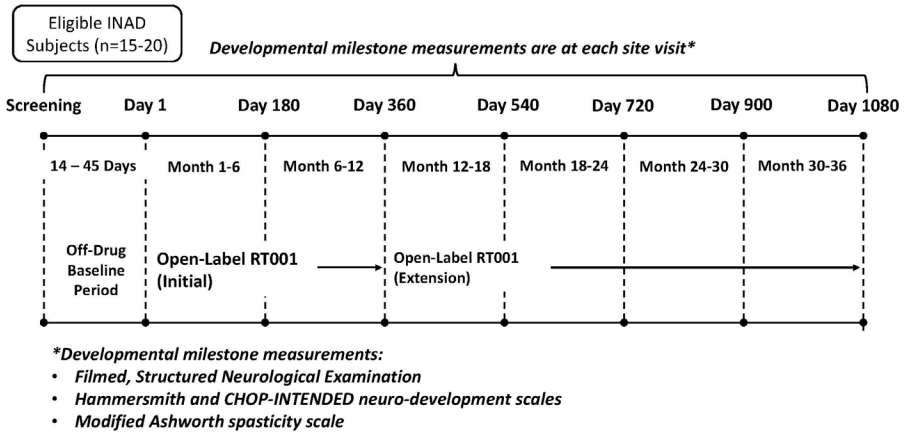
Name and Address of Institution: _____

2. STUDY SYNOPSIS

Sponsor:	Retrotope, Inc. 4300 El Camino Real, Suite 201 Los Altos, CA 94022
Title:	A Prospective Open-label Study to Assess Efficacy and Safety of RT001 in Subjects with Infantile Neuroaxonal Dystrophy
Protocol No:	RT001-008
Study Drugs:	RT001, 960 mg soft gelatin capsules (9-*cis*, 12-*cis*-11,11-D_2-linoleic acid ethyl ester [deuterated ethyl linoleate, or D_2-LA])
Study Population:	Patients with infantile neuroaxonal dystrophy (INAD) who meet all entry criteria.
Number of Subjects:	The planned enrollment for RT001 treated subjects is 15-20 subjects. A total of 19 subjects were enrolled. For the treatment extension, all enrolled subjects will be eligible.
Dosing Schedule:	Subjects will receive the content of four capsules daily (3,840 mg total dose) given as 2 capsules twice daily with meals for the first month of treatment. After this initial period, the dose will be reduced to three capsules daily (2,880 mg total dose) given as 1 capsule TID (three times a day) with meals. If a subject is unable to tolerate study drug during the initial period because of adverse events (AEs), the dose may be spread over three meals. If at any time a subject is unable to tolerate study drug after spreading it over three meals, the total dose may be reduced by 960-1,920 mg/day as needed (1-2 capsules/day). Subjects on enteral feeding in whom follow-up PK shows a plasma D2-LA ratio of less than 15% will need to take RT001 with blended food 2 hours away from the enteral feed given twice daily (2 capsules with first blended feed and 1 capsule with the second blended feed) or once daily (3 capsules with a single blended feed).
Route of Administration:	Oral or by feeding tube
Study Centers:	Up to 5 study centers can be used in this trial. The name and address and a statement of the qualifications of each investigator, and the name of each sub-investigator working under the supervision of the investigator; the name and address of the research facilities to be used; and the name and address of each reviewing Institutional Review Board will be provided in Form FDA 1572 and will be submitted to the US Food and Drug Administration (FDA) prior to activation of the sites.
Objectives:	**Primary objective:** To evaluate the effect of treatment with RT001 on INAD subjects **Secondary objectives:** • To evaluate the safety and tolerability of RT001 in INAD subjects • To explore the relationship between concentration of D_2-LA (RT001) and its primary metabolite deuterated arachidonic acid (D_2-ARA) to clinical

	response in INAD

Endpoints:	**<u>Primary endpoint:</u>** Change from baseline in the Modified Ashworth spasticity scale **<u>Secondary endpoints:</u>** 1. Change from baseline in an INAD progression composite (matched patient population only) 2. Progression Free Survival time (mortality or pneumonia; Same as Safety analysis) **<u>Exploratory endpoints:</u>** 1. Change from baseline in the modified Infantile Neuroaxonal Dystrophy Rating Scale (mINAD-RS24) 2. Change from baseline in the mPRS22 3. Change from baseline in original INAD-RS40 4. Change from baseline in the PRS33 5. Change from baseline in the GCIS 6. Change from baseline in the visual analogue scale (VAS) 7. Change from baseline in plasma lactate dehydrogenase (LDH; safety) 8. Survival (death only) 9. Progression Free Survival time (mortality, pneumonia, or 5-point decline on mINAD-RS24 or mPRS22; Same as Safety) 10. Change from baseline in the CHOP-INTEND neuro-development scale 11. Change from baseline in the Hammersmith neuro-development scale 12. Within active group comparisons: 13. Change from baseline in neuro-ophthalmology exam 14. Change from baseline in electroencephalography (EEG) measurements 15. Change from baseline in nerve conduction velocity (NCV) 16. Change from baseline in brainstem evoked response audiometry (BERA) test **<u>Safety endpoints:</u>** Safety assessments include physical and neurologic examinations, vital signs, and clinical laboratory tests [hematology, clinical chemistry including liver function test (LFT) and lactase dehydrogenase (LDH) test, lipid profile, and coagulation] to identify AEs. AEs are evaluated for incidence, severity, and relationship to study drug.

Study Methodology:	This is a single arm open-label study with a structured observation of INAD patients treated with RT001. Enrolled subjects will undergo observation and testing to determine the effect of RT001 treatment. Fifteen to 20 eligible subjects will be treated with RT001 for long-term evaluation of efficacy, safety, tolerability, and PK. Subject caregivers will administer the study drug orally or through feeding tube with meals. Subjects will be asked to consume a restricted polyunsaturated fatty acid (PUFA) diet, keeping PUFA consumption at approximately 5g/day (diet only) or lower. Dietary guidance will be provided to the parent/caregiver to assist them in making appropriate food choices in their child's consumption of non-deuterated PUFAs. Dietary modification to a restricted PUFA diet will begin after the screening visit and continue through the end of the drug treatment extension period including the extensions.
Study Duration:	Participation is anticipated to require at least 13 months and can be extended for a period of an additional 24 months. • **Screening**: can be done at least 14 and no more than 45 days prior to start of treatment • **Off-drug natural history observation period:** Subjects will undergo two structured neurological examinations with at least 14 and no more than 45 days in between the first and second exam. The first exam can be done during the screening visit. The second exam can be done at the Day 1 visit. The exams will be videotaped. • **Treatment Period:** Treatment with RT001 for a period of at least 12 months. • **Open-Label Study Extension:** All patients will be invited to an open-label study extension. Treatment with RT001 will be extended for a period of 24 months. In order to participate into the extension trial, a new informed consent will need to be signed for each 12 month extension. • **Follow-up**: 30 days after the last dose
Study Design:	Fifteen to 20 subjects will be enrolled in an open label RT001 treatment arm.  During the initial treatment period, the subject's parent/caregiver will be asked to obtain a monthly video of required ADL. In addition, a parental severity assessment and VAS will be applied by phone interview once per month.

Safety Assessments:	Safety assessments include physical and neurologic examinations, vital signs, and clinical laboratory tests [hematology, clinical chemistry including liver function test (LFT) and lactase dehydrogenase (LDH) test, lipid profile, and coagulation] to identify AEs. AEs will be evaluated for incidence, severity, and relationship to study drug.

PK/Biomarker Analysis:	Bioanalytical measurements will be performed to determine the concentration of D2-LA, the primary metabolite D2-ARA, nondeuterated linoleic acid (H2-LA) and nondeuterated arachidonic acid (H2-ARA) in plasma, and D2-LA, H2-LA, D2-ARA and H2-ARA in red blood cells (RBC). Blood samples will be collected for the plasma measurements as follows: Screening: in a fasting state (or 4 hours after the last meal) Month 3 (Day 90 ± 7 days): Pre-dose (prior to breakfast) Month 6 (Day 180 ± 14 days): Pre-dose (prior to breakfast) Month 12 (Day 300 + 90 days): Pre-dose (prior to breakfast) **Extension Period:** Month 18 (Day 540 ± 90 days AND at least 90 days after actual Month 12 visit): Pre-dose (prior to breakfast) Month 24 (Day 720 ± 90 days AND at least 90 days after actual Month 18 visit): Pre-dose (prior to breakfast) Month 30 (Day 900 ± 90 days AND at least 90 days after actual Month 24 visit): Pre-dose (prior to breakfast) Month 36 (Day 1080 ± 90 days AND at least 90 days after actual Month 30 visit): Pre-dose (prior to breakfast) **Washout period**: 1 Week (+7 days) following the last dose: pre-breakfast 1 Month (±7 days) following the last dose: pre-breakfast Blood samples for the assessments in RBC will be collected as follows: Screening: in a fasting state (or 4 hours after the last meal) Month 3 (Day 90 ± 7 days): Pre-dose (prior to breakfast) Month 6 (Day 180 ± 14 days): Pre-dose (prior to breakfast) Month 12 (Day 300 + 90 days): Pre-dose (prior to breakfast) **Extension Period:** Month 18 (Day 540 ± 90 days AND at least 90 days after actual Month 12 visit): Pre-dose (prior to breakfast) Month 24 (Day 720 ± 90 days AND at least 90 days after actual Month 12 visit): Pre-dose (prior to breakfast) Month 30 (Day 900 ± 90 days AND at least 90 days after actual Month 24 visit): Pre-dose (prior to breakfast) Month 36 (Day 1080 ± 90 days AND at least 90 days after actual Month 30 visit): Pre-dose (prior to breakfast)

	Washout period: 1 Week (+7 days) following the last dose: pre-breakfast 1 Month (±7 days) following the last dose: pre-breakfast

Efficacy Assessments:	The following efficacy assessments will be conducted at screening visit, at Day 1 visit, at Day 180 (± 14 days) visit and at Day 360 (± 30 days) visit: 1. Structured neurological assessment (Appendix 1) 2. Hammersmith and CHOP-INTEND neuro-development scales 3. Modified Ashworth spasticity scale Neuro-ophthalmology examination, EEG, NCV, and BERA will be performed at screening visit, at Day 180 (± 14 days) visit and at Day 360 (± 30 days) visit. The subject's parent/caregiver will be asked to complete an assessment of severity (Appendix 3) and VAS (Appendix 4) at each of the 2 baseline visits and monthly thereafter. In addition, a parental GCIS will be applied at Day 180 (± 14 days) visit and at Day 360 (± 30 days) visit (Appendix 2). The Investigator will review these parental baseline and monthly scales as part of his/her assessment. The subject's parent/caregiver will be asked to keep a diary of the subject's bowel movements, eating habits and obtain a monthly 2 to 5-minute video of each of the following ADL, the "Required ADL", starting after the screening visit: 1. 2 to 5 min video of feeding 2. 2 to 5 min video of holding up head, sitting, standing, assisted or unassisted walking 3. 2 to 5 min video of subject bathing 4. 2 to 5 min video of interaction with parent/caregiver During the extension period, the following efficacy assessments will be conducted at Day 540 (± 90 days) visit, Day 720 (± 90 days) visit, Day 900 (± 90 days), and Day 1080 (± 90 days) 1. Structured neurological assessment (Appendix 1) 2. Hammersmith and CHOP-INTEND neuro-development scales 3. Modified Ashworth spasticity scale Neuro-ophthalmology examination, EEG, NCV, and BERA will be performed at Day 720 (± 90 days) visit and Day 1080 visit (± 90 days). In addition, all parental assessments will be applied at Day 540 (± 90 days) visit, Day 720 (± 90 days) visit, Day 900 (± 90 days), and Day 1080 (± 90 days) (Appendix 2).
Inclusion Criteria:	To be eligible for treatment with RT001, subjects must meet the following requirements: 1. Male or female 18 months to 10 years of age 2. Medical history consistent with the symptoms of classic INAD (onset of symptoms between the ages of 6 months and 3 years) 3. Homozygous for PLA2G6 deficiency (variant alleles may be mixed heterozygotes) 4. Must have impairment in at least 2 of the assessed categories at baseline 5. Signed informed consent form (ICF) prior to entry into the study 6. Able to provide the necessary blood samples

Exclusion Criteria:	Subjects meeting one or more of the following will not be eligible for treatment with RT001 in this protocol: 1. Received treatment with other experimental therapies within the last 30 days prior to the first dose 2. Requiring mechanical ventilation that is not positive air pressure support primarily for mitigation of sleep apnea 3. Have a life expectancy of less than one year 4. Diagnosis of atypical NAD (ANAD) 5. Unwilling or unable to comply with the requirements of this protocol, including the presence of any condition (physical, mental, or social) that is likely to affect the subject's ability to return for visits as scheduled
Follow-up:	Subjects will be contacted 30 days ± 7 days following the last dose for safety monitoring. Subjects will be instructed to contact the clinic at any time during this period if experiencing an AE or a serious AE or if there are any questions. The wash-out PK samples at 1 week (+7 days) and 1 month (±7 days) may be obtained at a qualified laboratory local to the subject or at home by an approved phlebotomy service provider.
Standardized meals and diet coaching:	Subjects will follow a restricted PUFA diet from baseline through the end of the treatment extension period. During the initial treatment period, subject's caregiver will record all food intake into a food journal. During the initial treatment period, the caregiver will have monthly phone calls with the diet coach to support them in making appropriate low-PUFA food choices for their child and discuss eating habits and food preparation. During the treatment extension period, the diet coach will contact the caregiver every 3 months (only for subjects participating in the treatment extension).
Statistical methods:	Demographic data will be presented using descriptive statistics (e.g., mean, standard deviation, median, and range). Exposure to study drug and reasons for discontinuation will be tabulated. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). The incidence of treatment-emergent AEs will be presented by system organ class and preferred term. AEs will be presented by severity and relationship to study drug. Changes from Baseline in clinical laboratory parameters and vital signs will be summarized across time. Shift tables will be provided for selected laboratory parameters. Physical examination results will be presented in listings. For quantitative efficacy assessments, the observed values and the change from baseline at each visit will be presented in graphs and with descriptive statistics (mean, standard deviation, median, and range). For categorical efficacy assessments, the number and proportion of subjects in each category at each visit, and change from baseline at each visit, will be reported. The statistical analysis will be described in detail in a Statistical Analysis Plan to be completed and filed before data collected in the study is reviewed. The PK of D2-LA, D2-ARA, H2-LA, and H2-ARA will be characterized in terms of minimum and maximum exposure at steady state. Change from baseline will also be correlated with plasma and RBC levels of D2-LA and D2-ARA.
Safety Monitoring:	The safety and efficacy data will be reviewed by the medical monitor on an ongoing basis.

Table 1: Schedule of Events

Assessments	Study Period	Screening	Initial Treatment Period					Treatment Extension				Follow-up
	Clinic Visit No.	1	2	Phone Call	3	Phone Call	4	5	6	7	8	Phone Call
	Study Day	45 to 14 days prior to Day 1[a]	Day 1[a]	Day 30, 60. 90, 120, and 150 (± 7 days)	Day 180 (± 14 days) [a]	Day 210, 240, 270, 300 and 330 (± 7 days)	Day 360 (± 30 days)[a, j]	Day 540 (± 90 days)[a,j]	Day 720 (± 90 days)[a;j]	Day 900 (± 90 days)[a;j]	Day 1080 (± 90 days)[a;j]	30 (± 7) Days after the last dose
Informed Consent		X					X[i]		X[i]			
Inclusion/Exclusion Criteria		X	X									
Demographics/Medical History		X	X									
Physical Exam		X	X		X		X	X	X	X	X	
Vital Signs (including height and weight)		X	X		X		X	X	X	X	X	
Safety Laboratory Tests[b,c,d]		X	X[d]		X		Xe,k	X[e]	X[e]	X[e]	X[e]	
PK Sampling[c, e]		X		X[e]	X		X[e,k]	X[e]	X[e]	X[e]	X[e]	X[e]
Lipid Profile[c]		X	X		X		X[e,k]	X[e]	X[e]	X[e]	X[e]	
In-Clinic Drug Administration			X									
Filmed structured neurological examination		X	X		X		X	X	X	X	X	
Hammersmith Neuro-development Scale		X	X		X		X	X	X	X	X	
CHOP-Intend Neuro-development Scale		X	X		X		X	X	X	X	X	
Modified Ashworth spasticity scale		X	X		X		X	X	X	X	X	
Neuro-ophthalmology examination		X			X		X[l]	X[l]	X[l]	X[l]	X[l]	
Electroencephalography (EEG)		X			X		X		X		X	
Nerve Conduction Velocity (NCV)		X			X		X		X		X	
Brainstem Evoked Response Audiometry (BERA)		X			X[f]		X[f]		X[f]		X[f]	
Parent/Caregiver videos		X	X	X	X	X	X					
Assessment of Severity and VAS by Parent/Caregiver		X	X	X	X	X	X	X	X	X	X	
Parental Global Clinical Impression Scale					X		X	X	X	X	X	
Study Drug Dispensing			X		X		X[g]	X[g]	X[g]	X[g]	X[g]	
Perform Drug Accountability					X		X	X	X	X	X	
Food and Eating Habits Diary		X →	→	→	→	→	→ X					
Bowel Movements Diary		X		X[h]		X[h]						
Monitor AE/Concomitant Medication			X	X	X	X	X	X	X	X	X	X

[a] Each site visit can be done over several days as needed to accommodate for scheduling flexibility.
[b] Safety laboratory tests include chemistry, hematology, and coagulation.
[c] The safety laboratory tests, lipid profile, and PK sampling should be performed in a fasting state. During the initial treatment period, all fasting blood draws should be done on a different day than the neurological examinations. During the extension phase, the neuro exam can be done on the same day as the blood draw, with the neuro exam completed after the study participant has been allowed a meal and rest period.
[d] The safety laboratory tests and lipid profile need not be repeated at Day 1 visit unless the screening results are both out of range and deemed clinically significant by the Investigator.
[e] The sample may be obtained at a qualified laboratory local to the subject or at home by an approved phlebotomy service provider.
[f] BERA does need not be repeated at subsequent visits (Day 180, 360, 720, and Day 1080) in those participants in whom baseline BERA yielded uninterpretable data due to artifact or other technical difficulty.
[g] Study drug will be dispensed to all subjects participating in the study treatment extension period.
[h] The subject's parent/caregiver will keep a bowel movements diary from Day 150 to Day 180 and from Day 330 to Day 360.
[i] Only for subjects who are participating in the treatment extension.
[j] Visits may be coordinated remotely if the participant is unable to travel to the study site.
[k] Window for Month 12 labs is Day 300 + 90 days (Day 300 to Day 390) .
[l] If the visit must be done remotely and it is not possible to secure a neuro-ophthalmologist to perform a full neuro-ophthalmology exam, a regular ophthalmology exam may be performed instead.

Table 2: Schedule for Dietary Counseling, and Monitoring

Procedure	Interval	Schedule of Days
Dietary Counseling[a]	Diet coach to call monthly or as needed during the initial treatment period and every 3 months during the treatment extension period	To be scheduled with the subjects

[a] The subject's parent/caregiver will have monthly phone calls with the diet coach to support them in making appropriate low-PUFA food choices for their child and discuss eating habits and food preparation.

Table 3: Schedule of Events for Pharmacokinetic Analysis

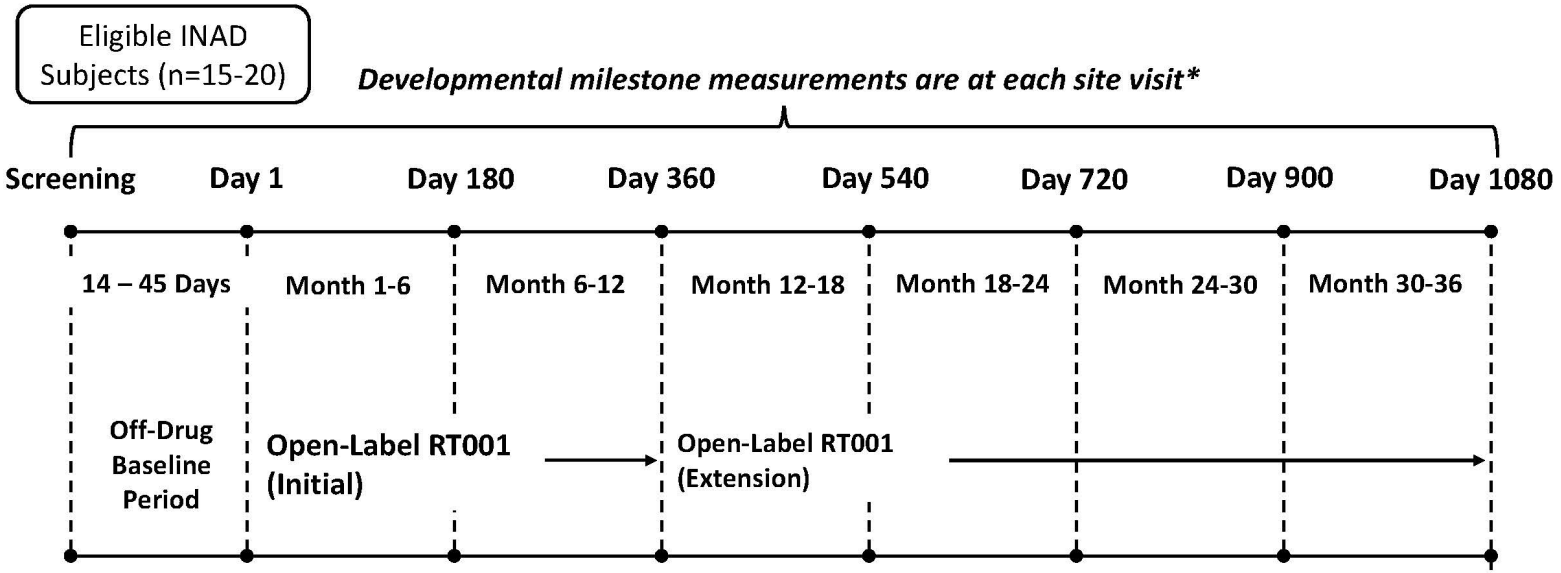
	Schedule of Days[a]
Screening	**Plasma PK (in a fasting state or 4 hours after the last meal)** **RBC PK (in a fasting state or 4 hours after the last meal)**
Month 3 (Day 90 ± 7 days) [b]	**Plasma PK**: Pre-dose (prior to breakfast) **RBC PK**: Pre-dose (prior to breakfast)
Month 6 (Day 180 ± 14 days)	**Plasma PK**: Pre-dose (prior to breakfast) **RBC PK**: Pre-dose (prior to breakfast)
Month 12 (Day 300 + 90 days) [b, c]	**Plasma PK**: Pre-dose (prior to breakfast) **RBC PK**: Pre-dose (prior to breakfast)
Month 18 (Day 540 ± 90 days) [b, c]	**Plasma PK**: Pre-dose (prior to breakfast) **RBC PK**: Pre-dose (prior to breakfast)
Month 24 (Day 720 ± 90 days) [b, c]	**Plasma PK**: Pre-dose (prior to breakfast) **RBC PK**: Pre-dose (prior to breakfast)
Month 30 (Day 900 ± 90 days) [b, c]	**Plasma PK**: Pre-dose (prior to breakfast) **RBC PK**: Pre-dose (prior to breakfast)
Month 36 (Day 1080 ± 90 days) [b, c]	**Plasma PK**: Pre-dose (prior to breakfast) **RBC PK**: Pre-dose (prior to breakfast)
1 week + 7 days following the last dose [b, c]	**Plasma PK**: Pre-breakfast
1 month ± 7 days following the last dose [b, c]	**Plasma PK**: Pre-breakfast

[a] All blood draws should be done on a different day than the neurological examinations during the initial treatment phase. During the extension phase, neuro assessments may be performed the same day following a meal and rest period. Actual collection dates and times must be recorded.

[b] Samples may be obtained at a qualified laboratory local to the subject or at home by an approved phlebotomy service provider.

[c] Samples must be separated by a minimum of 90 days.

3. STUDY SCHEMATIC



**Developmental milestone measurements:*

- *Filmed, Structured Neurological Examination*
- *Hammersmith and CHOP-INTENDED neuro-development scales*
- *Modified Ashworth spasticity scale*

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5. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Table 4: Abbreviations and Specialist Terms

Abbreviation or Specialist Term	Explanation
AD	Alzheimer's disease
ADL	Activities of Daily Living
ALS	Amyotrophic lateral sclerosis
ARA	Arachidonic acid
AE(s)	Adverse event(s)
ALT	Alanine transaminase
ANAD	Atypical neuroaxonal dystrophy
AST	Aspartate transaminase
ATP	Adenosine triphosphate
BERA	Brainstem evoked response audiometry
BMI	Body mass index
BUN	Blood urea nitrogen
CHOP-INTEND	The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders
CNS	Central nervous system
CO_2	Carbon dioxide
COVID-19	Coronavirus disease of 2019
CPEX	Cardiopulmonary exercise testing
CRF	Case report form
CSR	Clinical study report
D_2-ARA	Deuterated arachidonic acid
D_2-LA	Deuterated ethyl linoleate
DHA	Docosahexaenoic acid
EEG	Electroencephalography
EP	Evoked potentials
EPA	Eicosapentaenoic acid
ER	Endoplasmic reticulum
ERP	Event-related potential
FDA	Food and Drug Administration

FFT	Fast Fourier transform
GCIS	Global Clinical Impression Scale
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GM1	GM1 gangliosidosis
GRAS	Generally recognized as safe
H_2-ARA	Nondeuterated arachidonic acid
H_2-LA	Nondeuterated linoleic acid
HDL	High density lipoprotein
HINE	Hammersmith Infant Neurological Examination
HIPAA	Health Insurance Portability and Accountability Act
ICF	Informed consent form
ICH	International Conference for Harmonization
INAD	Infantile neuroaxonal dystrophy
IND	Investigational New Drug
INR	International normalized ratio
IRB	Institutional Review Board
IV	Intravenous
LA	Linoleic acid
LDH	Lactase dehydrogenase
LDL	Low density lipoprotein
LFT	Liver function test
LOTS	Late-onset Tay Sachs disease
MAS	Modified Ashworth scale
MedDRA	Medical Dictionary for Regulatory Activities
MUPA	Motor unit action potential
NBIA	Neurodegeneration with brain iron accumulation
NCV	Nerve conduction velocity
NS	Neuroserpinosis
O_2	Oxygen
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic
PLAN	PLA2G6-associated neurodegeneration

PSD	Power spectral densities
PSP	Progressive supranuclear palsy
PT	Prothrombin time
PTT	Partial thromboplastin time
PUFAs	Polyunsaturated fatty acids
RBC	Red blood cell
ROS	Reactive oxygen species
SAE	Serious adverse event
SOC	Standard of care
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
TID	Three times a day
TMF	Trial Master File
ULN	Upper limit of normal
US	United States
WBC	White blood cell
WHODD	World Health Organization Drug Dictionary
VAS	Visual analogue scale

6. INTRODUCTION

6.1. Infantile Neuroaxonal Dystrophy

Infantile neuroaxonal dystrophy (INAD) is a neurological disorder, inherited in an autosomal recessive pattern. It begins within the first few years of life, and leads to a progressive impairment of movement, cognition, and vision. Death usually occurs before puberty. Various scientific and regulatory sources specify INAD as an extremely rare disorder. The Genetic and Rare Diseases Information Center, a program of the National Center for Advancing Translational Sciences and the National Institutes of Health, includes INAD in its list of rare diseases (GARD 2018). Prevalence data from this source cites GeneReviews.org for its prevalence data. Although this source states that the disease prevalence is "not established," it is estimated at 1:1,000,000 (Gregory et al., 2018). The National Organization for Rare Disorders, a patient advocacy organization dedicated to individuals with rare diseases and the organizations that serve them, also lists INAD as an extremely rare disease. It cites a prevalence of 1:200,000 children (NORD 2018). Orphanet, an organization with an inventory of rare diseases, also lists INAD as a rare disease, with "more than 150 cases reported." As an upper limit, its prevalence is reported as 1.5:1,000,000 (Orphanet 2018). The current US population is about 330,000,000, and the number of persons under the age of 18 is about 75,000,000. Calculation of the absolute prevalence range using the relative prevalence values with the appropriate population size from the various references is displayed in the table below.

Table 5. Prevalence of INAD in US population

Reference	NORD	GARD	Orphanet
Relative prevalence (max)	1:200,000	1:1,000,000	1.5:1,000,000
Population size	75,000,000	330,000,000	330,000,000
Absolute prevalence (max)	375	330	495

Despite these theoretical gene prevalence calculations, INAD patient support groups with registries estimate only 15-30 current US living patients with classical INAD.

INAD affects axons, the part of a nerve cell that carries messages from the brain to other parts of the body, causing progressive loss of vision, muscular control, and mental skills. The pathological hallmark is the presence of large spheroids containing accumulated material (*e.g.*, tubulovesicular membrane structures likely stemming from lysosomes, mitochondria and ER) in distal axons and nerve terminals both in the central and peripheral nervous systems. Although the cerebellum is the region most affected in INAD, many cases of INAD are also characterized by iron deposition in the globus pallidus and substantia nigra, indicating overlap with spectrum of diseases classified as neurodegeneration with brain iron accumulation (NBIA). Both INAD and NBIA often present with Lewy bodies and neurofibrillary tangles.

Many disease-causing mutations have been identified in PLA2G6, a gene encoding Ca^{2+}-independent phospholipase A2 beta (iPLA2β or iPLA2-VIA); > 90% of mutations causing INAD are in this gene (only 20% for NBIA). iPLA2β hydrolyzes phospholipids, releasing fatty acids (typically, arachidonic acid) and lysophospholipids. The enzyme is cytosolic, although it has also been described in mitochondria, nuclei, Golgi and ER. Its main function appears to be the maintenance, repair and remodeling of phospholipids; in particular, it can hydrolyze peroxidized phospholipids, facilitating the reacylation of lysophospholipids by acyl-transferases and ensuring the regeneration of normal phospholipids.

Mutations in PLA2G6 prevent phospholipid repair, causing PLA2G6-associated neurodegeneration (PLAN) and conditions such as INAD. The PLAN phenotype likely originates from excessive oxidative stress and lipid peroxidation in mitochondria and perhaps also in lysosomes; neurodegeneration may also be related to iron toxicity (ferroptosis), a further lipid peroxidation trigger. Partial rescue of cellular damage and behavioral phenotype has been seen in fibroblasts, human embryonic stem cells, and fly models of PLAN treated with deuterated polyunsaturated fatty acids, including RT001, which are resistant to and reduce lipid peroxidation.

INAD symptoms are the result of an abnormal build-up of toxic substances in nerves that communicate with muscles, skin, and the conjunctive tissue around the eyes. Symptoms usually begin within the first 2 years of life, with the loss of psychomotor skills, such as head control and the ability to sit, crawl, or walk, accompanied by deterioration in vision and speech. Some children may have seizures. Distinctive facial deformities may be present at birth, including a prominent forehead, crossed eyes, an unusually small nose or jaw, and large, low-set ears. INAD is an autosomal recessive disorder, which means that both parents must be carriers of the defective gene that causes INAD to pass it on to their child. Electrophysiology (nerve conduction velocities) may be helpful for diagnosis, although diagnosis is usually confirmed by tissue biopsy of skin, rectum, nerve or conjunctive tissue to confirm the presence of characteristic swellings (spheroid bodies) in the nerve axons.

INAD is a progressive disease. Once symptoms begin, they will worsen over time. Generally, an INAD-affected baby's development starts to slow down between the ages of 6 months to 3 years. The first symptoms may be slowing of motor and mental development, followed by loss or regression of previously acquired skills. Rapid, wobbly eye movements and squints (strabismus and nystagmus) may be the first symptoms, followed by floppiness in the body and legs (more than in the arms). For the first few years, a baby with INAD will be alert and responsive, despite being increasingly physically impaired. Eventually, because of deterioration in vision, speech, and mental skills, the child will lose touch with its surroundings. Death usually occurs between the ages of 5 to 10 years.

Currently no specific treatment for INAD exists, and there is no treatment that can stop the progress of the disease. Physicians can prescribe medications for pain relief and sedation. Physiotherapists and other physical therapists can teach parents and caregivers how to position

and seat their child, and to exercise arms and legs to maintain comfort. These measures may provide comfort and support for patients with INAD; however, no current therapies address the causal pathway of the disease, and none are disease modifying. Currently, all patients with INAD can expect a varying, but inexorable pattern of disease progression, neurological impairment, and early death.

6.2. Oxidative Stress and Neurodegenerative Disease

A great deal of evidence exists which demonstrates the involvement of oxidative damage to lipids in the pathology of neurological disorders (e.g. Reed 2011). Moreover, the vulnerability of the central nervous system to reactive oxygen species (ROS) mediated injury is well established. Neurons consume large amounts of oxygen, the brain has many areas containing high iron content, and neuronal mitochondria generate large amounts of hydrogen peroxide. Furthermore, neuronal membranes are rich in polyunsaturated fatty acids, which are particularly susceptible to oxidative stress. The biological roles of products produced by lipid peroxidation have received much attention, not only for their pathological mechanisms associated with neurological disorders, but also for their practical clinical applications as biomarkers. ROS are likely to play a pathophysiological role in many neurological disorders, including Alzheimer's disease, Down syndrome, Parkinson's disease, and stroke.

The brain is extremely sensitive to oxidative damage for several reasons. The brain consumes an inordinate amount of oxygen (around 20%), particularly when considering the fact that the brain accounts for only 2% of body weight (Halliwell, 2006). A major reason for the high oxygen uptake is the vast amounts of adenosine triphosphate (ATP) needed to maintain neuronal intracellular ion homeostasis in the face of ion channels need for action potentials and neurosecretion. Additionally, several brain areas, including the substantia nigra, caudate nucleus, putamen, and globus pallidus, are rich in iron content (Zecca et al., 2004). It is generally accepted that iron accumulates in the brain of older individuals, and iron ions that are released following brain damage can catalyze free radical reactions. The brain is a source of ROS. Complex I-dependent hydrogen peroxide generation in brain mitochondria is greater than that in skeletal muscle mitochondria (Malinska et al., 2009). Lastly, neuronal membranes are rich in polyunsaturated fatty acids (PUFAs), particularly arachidonic acid (ARA), docosahexaenoic acid (DHA), and eicosapentaenoic acid (EPA) (Chen et al., 2008). These PUFAs are particularly vulnerable to oxidative stress due to their degree of unsaturation (multiple double bonds). For these reasons, neural cells are more susceptible to oxidative damage when compared to other body tissues.

The mechanism of ROS production differs in each neurological disorder; excess amounts of oxidized lipids causing neurological degeneration may be the result of excessive generation of these compounds, reduced clearance, or some combination of the two processes. These differences provide multiple targets for therapeutic intervention. However, any intervention that

results in less lipid peroxidation is likely to exhibit some therapeutic benefit in neurological degeneration.

6.3. Rationale Supporting RT001 Development for INAD

RT001 is a site-specific (C11) di-deutero synthetic homologue of linoleic acid (LA) ethyl ester. Arachidonic acid (ARA) is a polyunsaturated fatty acid present in abundance in the phospholipids in the membranes of the central nervous system (CNS). Most of the ARA in the CNS is synthesized in the liver from dietary LA. It has been established that substitution of hydrogen with deuterium at specific bis-allylic sites, as in RT001, decreases the production of lipid peroxidation products. In PLAN disorders such as INAD, the normal mechanism for maintenance, repair, and remodeling of oxidized membrane phospholipids is defective resulting in decreased clearance of these compounds in the mitochondria and lysosomes. Because RT001 prevents lipid peroxidation, it offers a novel yet specific approach to preventing the harmful oxidative attack on lipids in mitochondrial and other membranes. This has the potential to reduce the neurodegenerative consequences of lipid peroxidation compound accumulation seen in conditions such as INAD.

Kinghorn (Kinghorn et al., 2015) demonstrated in the fruit fly (Drosophila melanogaster) model that the fruit fly PLA2G6 homologue, iPLA2-VIA, plays a major role in maintaining normal mitochondrial function. When iPLA2-VIA is absent in a KO model, the fly has reduced survival, locomotor deficits and organismal hypersensitivity to oxidative stress. Loss of iPLA2-VIA function leads to a number of mitochondrial abnormalities, including reduced mitochondrial membrane potential, mitochondrial respiratory chain dysfunction and reduced ATP synthesis. There are significantly elevated levels of phospholipid peroxidation in the fly brain. They also showed significant decreases in basal mitochondrial membrane potential and excessive ROS generation in 2 cultured fibroblast cell lines derived from patients with demonstrated mutations in PLA2G6. The cell lines displayed mitochondrial membrane abnormalities and raised levels of cytoplasmic and mitochondrial ROS with lipid peroxidation levels greatly elevated compared to normal controls, especially in the mitochondria.

When the aged iPLA2-VIA KO fruit flies were fed a diet enriched with RT001 they had significantly improved locomotor activity with a trend to increased climbing ability. Likewise, when they pretreated human PLA2G6 mutant fibroblasts for 24 h with RT001, there was decreased lipid peroxidation in the cells to below control levels and a rescue of the reduced mitochondrial membrane potential. Moreover, specific assessment of lipid peroxidation levels in digitonin-permeabilized cells, which destroys all cell membranes except mitochondrial membranes, demonstrated that lipid peroxidation was substantially increased in the mutated PLA2G6 fibroblast cell lines, but reversed when incubated with RT001.

RT001 is currently in use in two children (age 4 years treated for 17 months, and age 5 years treated for 10 months) under Expanded Access Investigational New Drug (IND) #134385 and

#136908 for the treatment of INAD. Both affected infants have documented genetic mutations in both alleles of PLA2G6. At present, no adverse drug effects have been identified or reported. In the first patient treated with RT001 for INAD, plasma levels of D-LA > 40% of total plasma LA were achieved within one month of study drug initiation, well in excess of therapeutic levels deemed necessary for clinical effect. Promising delays in milestone loss and reversal of symptoms have been observed in videotaped recordings of standard pediatric development assessments in the subject who has been treated longest. Retrotope now seeks to conduct a clinical trial to evaluate further the safety and efficacy of RT001 in INAD.

6.4. Non-Clinical Safety Summary

Refer to the Investigator Brochure for a summary of the nonclinical studies conducted with RT001. There was no adverse finding in any parameter in Good Laboratory Practice (GLP) dietary toxicity rat studies of RT001 (di-deutero LA) up to 26-weeks at the highest doses tested, averaging 452 mg/kg (401 and 502 mg/kg in males and females; respectively).

In animal studies, RT001 (di-deutero LA ethyl ester) was found to be equivalent to, and biologically interchangeable with normal dietary LA as a sole source (100% replacement) of LA, indicating that the substitution of hydrogen with deuterium does not interfere with the normal biology of the fat. If this were not the case, essential lipid deficiency diseases (Burr & Burr, 1929 and 1930) would be observed in the treated populations. In addition, LA is recognized by the Food and Drug Administration (FDA) as generally recognized as safe (GRAS), and has no upper limit under the GRAS designation. Additionally, IntraLipid® is approved as an IV drug at doses up to 93 g/day LA (2.5 g/kg/day) based on a 60-kg person (IntraLipid Prescribing Information, 2006) representing a 16.3-fold safety margin over the dose of 5.7 g/day.

6.5. Clinical Experience with RT001

6.5.1. Controlled Clinical Studies

6.5.1.1. Friedreich's Ataxia

In a recent double blind, placebo controlled, Phase 1b/2a clinical trial protocol in Friedreich's ataxia (FA) patients under IND 115977, to which right of reference has been granted for this study, RT001 showed a statistical improvement in peak exercise capacity, as measured by cardiopulmonary exercise testing (CPEX), and an improvement trend in peak volume of oxygen consumption (VO_2MAX) during CPEX, the Friedreich's Ataxia Rating Scale (FARS) Neurological test, and an electronically timed stride speed during a timed 25-foot walk (eT25FW). In addition, RT001 appeared safe and well tolerated, similar to other high dose polyunsaturated fatty acid supplementation drugs, and displayed an excellent pharmacokinetic (PK) profile, indistinguishable from non-deuterated LA. Gastrointestinal disorder adverse events (diarrhea) were the only treatment-emergent adverse event (AE) in this study. Refer to the Investigator Brochure for a summary of this study, safety and PK profile.

6.5.2. Expanded Access Protocols

6.5.2.1. Infantile Neuroaxonal Dystrophy

RT001 is currently in use in two children (age 4 years treated for 17 months, and age 5 years treated for 10 months) under Expanded Use INDs #134385 and #136908 for the treatment of INAD. Both affected infants have documented genetic mutations in both alleles of PLA2G6. At present, no adverse drug effects have been identified or reported.

The first subject is a 4-year-old child with a severe form of INAD who has been on RT001 for 17 months. At enrollment, the subject had lost virtually all of her previously acquired development milestones and was mostly unresponsive, unable to sit, hold her head up, or interact meaningfully with her parents at the trial start. She had lost her ability to take liquids from a child's 'sippy' cup and was being given liquids by syringe. Expected progression would have required a feeding tube within the next few months per the treating physician. Immediately after being on RT001 at 3.6 g/day with food, her constipation resolved, and she has been regular since. Prior treatment with normal dietary omega 3/6 supplements at high doses had not resolved the constipation in the past. Plasma collected at her month 1, month 3, month 6, and month 9 protocol visits showed a high level of drug absorption and a steady state with > 30% of total plasma LA as RT001. Her neurology exams at baseline, month 1, month 3, month 6, and month 9 were videotaped.

Since the start of the trial, the caregiver and physician observations have been:

- RT001 is safe and well-tolerated at high dose in a 3-year old patient with INAD
- Subject's disease appears not to have progressed
- Parents noted and documented signs of steady improvements/reversals
  - Regained ability to grasp spoon and hold
  - Regained ability to use sippy cup (no longer feeding liquids by syringe)
  - Better muscle strength (holding head up, pushing off parent when held)
  - Growing and putting on weight (age appropriate)
  - Child is newly responsive to verbal requests/more interactive
  - Constipation resolved; subject completely regular
  - Excessive drooling resolved; subject is observed swallowing her saliva

The second subject is a 5-year-old child with INAD. In two baseline neurological examinations one month apart, he had severe defects in both fine and coarse motor skills; exhibited a combination of overall hypotonia, and had spasticity in certain muscle groups; initiated only occasional semi-purposeful movements; was non-verbal and was not able to follow commands. He was drooling profusely; unable to stand or walk; exhibited poor fine motor skills, and had marked strabismus and nystagmus. The child was unable to raise his head from prone position or

sit unaided. Since he has started RT001 approximately ten months ago, PK data indicates strong drug uptake. At the 1-month post drug neurology exam, the videotaped exams were compared to the two baseline visits. The treating pediatric neurologist made the following observation immediately after the one-month post drug exam, "I will be uploading all of his videos and data tomorrow, but wanted to share that he has seen meaningful improvements, both by history and by exam. Today, he showed increased alertness and participation in the exam, as well as somewhat improved fine motor control. He initiated several steps (with ankle-foot orthesis on and significant support from mom), which he has not done for months. His lab work remains stable and he has had no known side effects to date. His PK samples were sent off as well. Thank you all. It is a true joy to see him making gains." The parents noted, and it was confirmed by video during the exam, that he now could follow simple commands, and executed coordinated, purposeful movement of the hands and arms in response to a "wheels on the bus" jingle, a previously lost skill. At his 3-month exam, the treating physician noted that the improvements observed at the one-month visit were sustained with continued improvements.

6.5.2.2. Other Expanded Access Experience

RT001 has been used in 7 additional expanded access protocols in 6 different conditions including APOE4+ Alzheimer Disease (AD), Late-onset Tay Sachs Disease (LOTS), GM1 gangliosidosis (GM1), progressive supranuclear palsy (PSP), amyotrophic lateral sclerosis (ALS), and neuroserpinosis (NS). The duration of exposure in this group varies between 30 and 240 days. Because of the diversity of the conditions under treatment and the variability in treatment duration, observations of efficacy are not made at this time. Safety experience is summarized in the next section.

6.5.2.3. Expanded Access Safety Summary

Overall tolerability has been good, with few side effects or adverse events associated with the drug. A summary of exposure in expanded access protocols is listed in the Table below, with the duration of exposure, the number of AEs observed, and whether or not AEs were associated with the need to discontinue the drug. Below the table, brief narratives of the AEs are included.

Condition	N	Mean duration (d)	AE (#)	Permanent Discontinuation? (yes/no)
INAD	2	360	2	1Y, 1N
AD	1	240	0	N
LOTS	1	150	0	N
GM1	1	60	1	N
PSP	2	60	0	N
ALS	1	60	0	N
NS	1	30	1	Y

- For INAD subject #1, cramping and abdominal pain developed after 360 days of exposure. RT001 was held for one week and re-started at a reduced dosage of 1 capsule TID instead of 2 tablets BID. The subject has improved.
- For INAD subject #2, repeated rupture of the balloon tipped G-tube prompted the subject's parents to withdraw from the study after 210 days of enrollment. The relationship of the G-tube malfunction was not clearly linked to RT001.
- For the GM1 subject, liver function abnormalities at baseline were noted to rise after 6 weeks of treatment with RT001. ALT rose from 130 IU/L to 435 IU/L. Total Bilirubin rose from 4.8 mg/dL to 22 mg/dL. RT001 was held for 16 days. LFTS returned to baseline levels, and RT001 has been restarted for 6 additional weeks without incident. The LFT elevations were thought secondary to underlying GM1 pathology and a concurrent infection which resolved.
- For the NS subject, a complicated history of poorly controlled seizure disorder was present at baseline. The subject had continued poorly-controlled seizures within 6 weeks of starting RT001, and the parents decided to withdraw the subject from the study drug. The poorly controlled seizure disorder was not linked to RT001 as the condition had been pre-existing.

6.6. Pediatric Subjects Considerations

The decision to include pediatric subjects with INAD in a clinical trial requires a careful analysis of the risks and potential benefits to the subjects. Inclusion of pediatric subjects also involves an analysis of whether or not the subjects would be representative of the population of subjects under study, and whether or not the pediatric subjects would be able to comply with the protocol requirements.

RT001 is a deuterated formulation of LA, the predominant fatty acid in several approved drug products for IV nutritional supplementation, including the widely used IntraLipid^{TM}. RT001 is being developed for INAD using the 505(b)2 pathway. Pediatric subjects in the clinical trials that led to the approval of the nutritional supplementation drugs containing LA constituted more than half of all enrolled patients. Most adverse reactions were related to the route of administration rather than to the drug itself; the warning for use in pediatric patients limiting use to less than 3g/kg/day was related to a side effect that was clearly linked to IV administration. These studies included extensive safety assessments on all subjects and can be incorporated by right of reference into our trial via FDA's determination in our pre-IND meeting minutes that RT001 may use the 505(b)(2) approval pathway. RT001 is planned for oral administration only, and the maximum daily dosage will be 3.84g/d. The average weight of a 3-year old patient is approximately 10 kg. Therefore, the maximum dosage administered orally in our clinical trial will be 0.384 g/kg/day, far less than the maximum recommended IV dosage for pediatric patients using IntraLipid. The use of RT001 in pediatric patients is likely to be safe, as evidenced by the many years of safe use of the nutritional supplements in this age group. In the clinical trials of RT001 in adults, diarrhea has been the only significant adverse reaction, and this appears to be easily manageable with dose adjustment.

6.7. Potential Risks and Benefits to Human Subjects

INAD is a rare, inherited recessive disorder characterized by progressive degenerative changes, in particular to mitochondria, which lead to morbidity and mortality at a young age. There is currently no approved therapy for this condition. RT001 offers a novel yet specific approach to mitigation of the harmful oxidative attack on lipids of mitochondrial membranes, which has potential benefit if the hypothesis is proven in this and subsequent efficacy studies. RT001 is based on a common, essential dietary sourced fatty acid, LA. The slight modification to the chemical structure of LA, the site-specific replacement of 2 hydrogen atoms with 2 deuterium atoms, does not impact any of the key properties and fate of the lipid, yet remains functionally active against unwanted, pathological oxidative attack. The deuterium presents low risk (see below) in that it is stably incorporated in covalent bonds, and the amount dosed is relatively low. Thus, the risk-benefit profile is thought to be favorable and suggests a future potential benefit of RT001 treatment in this intractable condition.

RT001 has been demonstrated as safe in extensive IND-enabling studies resulting in FDA approval of IND 115977 in Friedreich's ataxia. The first in human clinical trial of RT001 based on that IND went on to demonstrate excellent human safety and tolerability, with signals of efficacy in multiple measures of disease progression in a genetic disease characterized, like INAD, with mitochondrial iron accumulation, mitochondrial deficits, and neuromuscular deficiency. Finally, multiple preclinical models of genetic diseases in which accelerated lipid peroxidation is observed, including human embryonic cell studies of PKAN patients and cell and drosophila models of PLA2G6 deficiency, showed robust reductions in cell death and/or phenotype mitigation. Combined with the profound safety of RT001, a deuterium modified LA drug, we believe further use of RT001 is likely to be low risk with a large potential upside for INAD patients.

6.8. Description and Justification for the Dosing Regimens to Be Studied

Based on the observed gastrointestinal disorder AEs, we believe that a maximal tolerated dose (MTD) of 9.0 g/d (150 mg/kg for a 60-kg patient or approximately 391 mg/kg/m^2) in adults may have been reached. We have dosed two infants, one for up to 17 months, at 3.6 g/d (360 mg/kg for a 10-kg patient or approximately 240 mg/kg/m^2) and safely achieved up to 40 % of plasma and red blood cell (RBC) LA as drug at steady state. This appears therapeutic, is above the threshold levels needed for RT001's mechanism of action, and is already much higher in mg/kg than the MTD in adults.

For this study, we selected a starting dose of 3.84 g/d (4 capsules) divided between 2 or 3 meals. This dose is similar to the dose that has been verified as adequate, safe and effective for infants in two expanded access protocols. On a mg/BMI (allometric) basis this dose is about half of the adult MTD, on a mg/kg basis this dose is about 2.5 times above the adult MTD.

A planned dose adjustment has been added to the protocol to try to avoid possible gastrointestinal disturbance AEs. No reports of diarrhea have been observed or reported in any infants using RT001 at the 3.84 g/day dose.

7. STUDY OBJECTIVES AND ENDPOINTS

This study is designed to evaluate the effect of RT001 in subjects with INAD:

7.1. Study Objectives

7.1.1. Primary Objective:

To evaluate the effect of treatment with RT001 on INAD subjects

7.1.2. Secondary Objectives:

6. To evaluate the safety and tolerability of RT001 in INAD subjects
7. To explore the relationship between concentration of D_2-LA (RT001) and its primary metabolite deuterated arachidonic acid (D_2-ARA) to clinical response in INAD

7.2. Study Endpoints

7.2.1. Primary endpoint:

Change in score from baseline at 12 months derived from a structured pediatric neurological development exam (Appendix 1) tailored for INAD, involving elements of activities of daily living (ADL) and vital function

7.2.2. Secondary endpoints:

1. Change in score from baseline at 12 months in the bulbar function category derived from a structured pediatric neurological development exam tailored for INAD involving elements of ADL and vital functions
2. Change from baseline at 12 months in the Modified Ashworth spasticity scale
3. Change from baseline at 12 months in the composite score of the Hammersmith and CHOP-INTEND neuro-development scales
4. Assessment (serial) at 6 months and 12 months of parental global clinical impression scale (GCIS) (Appendix 2)
5. Longitudinal assessment of monthly parental severity assessment (Appendix 3) and visual analogue scale (VAS) (Appendix 4)
6. Longitudinal assessment at 6 months and 12 months of change in score derived from a structured pediatric neurological development exam tailored for INAD, involving elements of ADL and vital functions

7.2.3. Exploratory endpoints:

1. Change from baseline in neuro-ophthalmology exam

8. Change from baseline in electroencephalography (EEG) measurements
9. Change from baseline in nerve conduction velocity (NCV)
10. Change from baseline in brainstem evoked response audiometry (BERA) test
11. Assessment of monthly video recordings of Required ADL
12. Change from baseline in weekly frequency of bowel movements
13. PK of trough levels of RT001 and metabolite (D2-ARA) and correlation with pharmacodynamic (PD) assessments

7.2.4. Safety endpoints:

1. Mortality and morbidity in INAD subjects
2. Safety and tolerability of RT001 in INAD subjects

8. INVESTIGATIONAL PLAN

8.1. Study Design

This is a single arm open-label study with a structured observation of INAD patients treated with RT001. Enrolled subjects will undergo observation and testing to determine the effect of RT001 treatment. Fifteen to 20 eligible subjects will be treated with RT001 for long-term evaluation of efficacy, safety, tolerability, and PK. Subject caregivers will administer the study drug orally with meals or through feeding tube.

Subjects will be asked to consume a restricted PUFA diet, keeping PUFA consumption at approximately 5g/day (diet only) or lower. Dietary guidance will be provided to the parent/caregiver to assist them in making appropriate food choices in their child's consumption of non-deuterated PUFAs. Dietary modification to a restricted PUFA diet will begin after the screening visit and continue through the end of the drug treatment or if participating in the treatment extension through the end of the extension period.

Subjects will receive the content of four capsules daily (3,840 mg total dose) given as 2 capsules twice daily with meals for the first month of treatment. After this initial period, the dose will be reduced to three capsules daily (2,880 mg total dose) given as 1 capsule TID (three times a day) with meals. If a subject is unable to tolerate study drug during the initial period because of AEs, the dose may be spread over three meals. If at any time a subject is unable to tolerate study drug after spreading it over three meals, the total dose may be reduced by 960-1,920 mg/day as needed (1-2 capsules/day).

Participants on total entering feeding in whom the Day 90, 180, 360, 540, 720, or 900 PK plasma D2-LA ratio is less than 15%, will need to take RT001 with a small volume of blended food 2 hours away from the enteral feed. In these cases, the daily administration of RT001 can be split into 2 dosing times (i.e. 2 capsules in the am and 1 capsule in the pm) or given as one dose (i.e. 3 capsules).

Participation is anticipated to require at least 13 months and can be extended for a period of an additional 24 months.

- **Screening**: can be done at least 14 and no more than 45 days prior to start of treatment.
- **Off-drug natural history observation period:** Subjects will undergo two structured neurological examinations with at least 14 and no more than 45 days in between the first and second exam. The first exam can be done during the screening visit. The second exam can be done at the Day 1 visit. The exams will be videotaped.
- **Treatment Period:** Treatment with RT001 for a period of at least 12 months. Subjects will remain in the treatment arm until the last subject enrolled completes at least 12 months of treatment.

- **Open-Label Study Extension:** All patients will be invited to an open-label study extension. Treatment with RT001 will be extended for a period of an additional 24 months. In order to participate into the extension trial, a new informed consent form will need to be signed. Patients in the extension may remain on RT001 until the last participant completes the 36-month (Day 1080) follow-up.
- **Follow-up**: 30 days after the last dose.

Safety assessments include physical and neurologic examinations, vital signs, and clinical laboratory tests [hematology, clinical chemistry including liver function test (LFT) and lactase dehydrogenase (LDH) test, lipid profile, and coagulation] to identify AEs. AEs will be evaluated for incidence, severity, and relationship to study drug.

The first dose of the study drug will be taken on Day 1. Bioanalytical measurements will be performed to determine the concentration of D2-LA, D2-ARA, nondeuterated linoleic acid (H2-LA) and nondeuterated arachidonic acid (H2-ARA) in plasma, and D2-LA, H2-LA, D2-ARA and H2-ARA in RBC. Blood samples will be collected at screening in a fasting state (or 4 hours after the last meal), at month 3 (Day 90 ± 7 days), at month 6 (Day 180 ± 14 days), and at month 12 (Day 300 + 90 days). PK samples will also be obtained at least 90 days apart on all subjects who are participating in the extension period at month 18 (Day 540 ± 90 days), month 24 (Day 720 ± 90 days), month 30 (Day 900 ± 90 days), and month 36 (Day 1080 ± 90 days). All samples are to be obtained prior to dosing and prior to breakfast. Additional PK samples will be obtained 1 week (+ 7 days) and 1 month (± 7 days) following the last dose. These samples are to be obtained prior to breakfast. All fasting blood draws should be done on a different day than the neurological examinations during the initial treatment phase.

A structured neurological examination will be conducted at screening visit, at Day 1 visit, at Day 180 (± 14 days) visit, and at Day 360 (± 30 days) visit; and a score will be derived. The examination will be videotaped. The Hammersmith and CHOP-INTEND neuro-development assessment and the Modified Ashworth spasticity assessment will be conducted at screening visit, at Day 1 visit, at Day 180 (± 14 days) visit, and at Day 360 (± 30 days) visit. These measurements will be repeated on all subjects who are participating in the extension period at least 90 days apart at month 18 (Day 540 ± 90 days), month 24 (Day 720 ± 90 days), month 30 (Day 900 ± 90 days), and month 36 (Day 1080 ± 90 days). Each site visit can be done over several days as needed to accommodate for scheduling flexibility.

EEG, NCV, and BERA will be performed at screening visit, at Day 180 (± 14 days) visit and Day 360 (± 30 days) visit. These measurements will be repeated on all subjects who are participating in the extension period at month 24 (Day 720 ± 90 days) and month 36 (Day 1080 ± 90 days). These procedures are to be done after neurological examinations or on a different day. BERA does not need to be repeated at Day 180, Day 360, Day 720, and Day 1080 in those patients in whom baseline BERA yields uninterpretable or incomplete results.

Neuro-ophthalmology examination will be performed at screening visit, at Day 180 (± 14 days) visit and Day 360 (± 30 days) visit. Neuro-ophthalmology exam will be repeated a minimum of 90 days apart on all subjects who are participating in the extension period at month 18 (Day 540 ± 90 days), month 24 (Day 720 ± 90 days), month 30 (Day 900 ±90 days), and month 36 (Day 1080 ± 90 days).

The subject's parent/caregiver will be asked to complete an assessment of severity (Appendix 3) and VAS (Appendix 4) at each of the 2 baseline visits (Screening and Day 1) and monthly thereafter. A parental GCIS will be applied at Day 180 (± 14 days) visit and at Day 360 (± 30 days) visit (Appendix 2). All parental assessments will be repeated a minimum of 90 days apart on all subjects who are participating in the extension period at month 18 (Day 540 ± 90 days), month 24 (Day 720 ± 90 days), month 30 (Day 900 ± 90 days), and month 36 (Day 1080 ± 90 days). The Investigator will review these parental scales as part of his/her assessment.

During the initial treatment period, the subject's parent/caregiver will be asked to keep a diary of the subject's bowel movements, eating habits and obtain a monthly 2 to 5-minute video of each of the following Required ADL, starting after the screening visit. ADL videos will not be required during the extension period.

1. 2 to 5-min video of feeding
2. 2 to 5-min video of holding up head, sitting, standing, assisted or unassisted walking
3. 2 to 5-min video of subject bathing
4. 2 to 5-min video of interaction with parent/caregiver

8.2. Randomization

This study is an open label study with one treatment arm. Therefore, there will be no randomization required in this study.

8.3. Blinding

This is an open-label study. All subjects will receive RT001.

8.4. Safety Monitoring

8.4.1. Safety Monitoring

The study site investigators assess each subject and record findings obtained during each visit. Subjects will be queried to report any observations, concerns or symptomatology, and a clinical assessment will also be made. This includes any evidence of disease exacerbation and/or progression. Safety reports made during the study (see Section 16) will be promptly reviewed by the Sponsor's Medical Monitor, and processed. This includes reporting to the FDA according to 21 CFR 312.32, "IND Safety Reporting," and taking any action necessary to protect subject safety.

8.4.2. Sponsor Safety Monitoring

The safety data will be reviewed by the study monitor on an ongoing basis. These data will not be made available to the Principal Investigators unless changes need to be made to the protocol for safety reasons.

8.5. Dietary Restrictions

Subjects will be asked to consume a restricted PUFA diet, keeping PUFA consumption at approximately 5g/day (dietary PUFAs only) or below. Controlling the amount of competition from dietary PUFAs will allow for better absorption of the study medication.

Dietary guidance will be provided to the parent/caregiver to assist them in making appropriate food choices in their child's consumption of non-deuterated PUFAs. Dietary modification to a restricted PUFA diet will begin after the screening visit and continue through the end of the treatment extension period. During the initial treatment period, subject's parent/caregiver will be asked to enter foods that the subject consumes in a diary. The diet coach will review these food journals and make any recommendations as necessary to stay around the designated PUFA consumption recommendation. It will not be considered a protocol deviation if diary entries are missed by the subject's parent/caregiver. No diet journals will need to be recorded during the treatment extension period.

During the initial treatment period, subject's parent/caregiver will have monthly phone calls with the diet coach to support them in making appropriate low-PUFA food choices for their child and discuss eating habits and food preparation. During the treatment extension period, parent/caregiver will have phone calls with the diet coach every 3 months. As part of the restricted PUFA diet, subjects will be prohibited from taking any fish oils or other oil-based supplements. Subjects will remain on the restricted PUFA diet for the duration of the study.

9. SELECTION AND WITHDRAWAL OF SUBJECTS

9.1. Inclusion Criteria

To be enrolled into the study subjects must meet the following requirements:

1. Male or female 18 months to 10 years of age
2. Medical history consistent with the symptoms of classic INAD (onset of symptoms between the ages of 6 months and 3 years)
3. Homozygous for PLA2G6 deficiency (variant alleles may be mixed heterozygotes)
4. Must have impairment in at least 2 of the assessed categories at baseline
5. Signed informed consent form (ICF) prior to entry into the study
6. Able to provide the necessary blood samples

9.2. Exclusion Criteria

Subjects meeting one or more of the following may not enter the study:

1. Received treatment with other experimental therapies within the last 30 days prior to the first dose

14. Requiring mechanical ventilation that is not positive air pressure support primarily for mitigation of sleep apnea
15. Have a life expectancy of less than one year
16. Diagnosis of atypical NAD (ANAD)
17. Unwilling or unable to comply with the requirements of this protocol, including the presence of any condition (physical, mental, or social) that is likely to affect the subject's ability to return for visits as scheduled

9.3. Withdrawal

9.3.1. Withdrawal Criteria

Subject's parent may choose to withdraw a subject from the study at any time for any reason. In addition, subjects may be withdrawn from the study by the Investigator for any of the following reasons:

- The subject or subject's caregiver is unwilling or unable to adhere to the protocol.
- Any serious adverse event (SAE), clinically significant AE, severe laboratory abnormality, intercurrent illness, or other medical condition that indicates to the Investigator that continued participation is not in the best interest of the subject.

- Other medical reason, at the discretion of the Investigator and/or the Medical Monitor.

9.3.2. Withdrawal Procedures

- If it is necessary for a subject to discontinue the study drug/study earlier than planned, subjects should complete all Early Termination procedures (see Section 13.15) and return the study drug. Date of last dose should be recorded.
- The Investigator must notify the Sponsor and the Medical Monitor within 24 hours when a subject has been withdrawn from the study. Any subject withdrawn because of a related AE (whether serious or non-serious), including clinically significant abnormal laboratory test values, will be evaluated by the Investigator or a designee and be treated and/or followed until the symptoms resolve or values return to normal or acceptable levels, as judged by the Investigator.
- If a subject does not return for a scheduled visit, every effort should be made to contact the subject's caregiver. If a subject withdraws from the study, the Investigator or designee should inquire about the reason for withdrawal and will follow-up with the subject by phone as scheduled to collect AE information.
- If the subject withdraws from the study and the subject's legal representative also withdraws consent for disclosure of future information, no further evaluations will be performed, and no additional data will be collected. The Sponsor may retain and continue to use any data collected before such withdrawal of consent; these data will be included in the safety database.

9.3.3. Documentation of Withdrawal

The reason(s) for withdrawal from the study drug/study must be recorded in the subject's medical record and case report form (CRF).

9.3.4. Replacement of Subjects

Subjects who discontinue the study will not be replaced.

9.3.5. Termination of Study by Sponsor

Although the Sponsor has every intention of completing the study, the Sponsor reserves the right to discontinue the study at any time for clinical or administrative reasons, or if required by the FDA or other regulatory authorities. Both the Sponsor and the Medical Monitor will review the safety of RT001 throughout the study. The study may be halted at any time for safety concerns.

10. INVESTIGATIONAL PRODUCT

10.1. Identity of Investigational Product

RT001 is encapsulated 9-*cis*, 12-*cis*-11,11-D_2-linoleic acid ethyl ester, which is a site-specific (C11) di-deutero synthetic homologue of LA ethyl ester. Each capsule contains 960 mg of RT001 in an opaque brown oblong soft-gel capsules.

10.2. Packaging and Labeling of Clinical Supplies

RT001 is provided in glass bottles each containing 42 softgel capsules.

10.3. Storage of Clinical Supplies

Study drug should be stored either in a refrigerator at 2 to 8℃ or in controlled ambient at 15 to 30°C as indicated on the label.

10.4. Study Drug Dispensation

The study drug will be dispensed at the study sites. If a patient is unable to travel to the study site due to COVID-19 precautions (see Section 14), the study drug may be mailed to the patient from the study site or Sponsor's distribution center.

10.5. Drug Accountability

The subject's parent/caregiver will be asked to return the used and unused study bottles to the study site. It is the responsibility of the Investigator or his/her designee to maintain drug accountability at the clinical trials site and ensure that a current record of investigational product disposition is maintained. It is the responsibility of the Investigator or his/her designee to ensure that the investigational product is used only in accordance with the approved protocol. All records or logs must comply with applicable regulations and guidelines. The Sponsor will provide forms to facilitate accountability if the staff at the investigational site does not have an established system that meets these requirements.

If a patient is unable to return used, empty bottles to the study site due to the COVID-19 pandemic, used, empty bottles may be destroyed by the patient's parent provided that written attestation detailing number of empty bottles, date, and means of destruction of empty bottles signed and dated by the parent is collected by the study site. Unused medication must still be returned to the study site for destruction in person or by mail/courier service (see Section 14).

11. STUDY DRUGS AND OTHER MEDICATIONS, SUPPLEMENTS

11.1. Study Drug Administration

The subject's parent/caregiver will receive instructions on giving the study drug with meals. Subjects will receive the content of four capsules daily (3,840 mg total dose) given as 2 capsules twice daily with meals for the first month of treatment. After this initial period, the dose will be reduced to three capsules daily (2,880 mg total dose) given as 1 capsule TID with meals. Capsules can be softened in warm liquid (milk, water) and can be drunk or added to food. The caregiver should make sure that all food is consumed. On PK collection days all subjects will need to take the morning dose of study drug after the PK samples are obtained.

If a subject is unable to tolerate study drug during the initial treatment period because of AEs, the dose may be spread over three meals. If at any time a subject is unable to tolerate study drug after spreading it over three meals, the total dose may be reduced by 960-1,920 mg/day as needed (1-2 capsules/day).

Participants on total enteral feeding for whom the Day 90, 180, 360, 540, 720, 900, or 1080 PK shows a plasma D2-LA ratio of less than 15%, will need to take RT001 with a small volume of blended food 2 hours away from the enteral feed. In these cases, the daily administration of RT001 can be split into 2 dosing times (i.e. 2 capsules in the am and 1 capsule in the pm) or given as a single dose (i.e. 3 capsules). Because the PK samples are analyzed intermittently, the PK data may not be available for all subjects who are on enteral feeding supplementation. Dosing adjustment in subjects on enteral feeding will be made only for those subjects whose PK data is available.

11.2. Prior and Concomitant Medications

Stable, ongoing therapies will be permitted during this study. Any drug therapy initiated during the study should be discussed with the Medical Monitor prior to administration, if possible. Data on concomitant medications will be collected at each visit.

Fish oils or other oil-based supplements should not be taken for the duration of the study. In addition, if subjects have participated in prior therapeutic trials, they will need to have been off therapy for at least 30 days prior to receiving the first dose of study drug. Herbal remedies, dietary supplements and off-label use of approved compounds for the treatment of INAD need to be approved by the Medical Monitor.

11.3. Subject Compliance

Compliance will be assessed through counting the number of bottles/capsules returned to the investigational site by the subject's parent/caregiver. If subjects are unable to visit the

investigational site in-person due to the COVID-19 pandemic, the number of bottles/capsules will be obtained by the study site via parent/caregiver interview (see Section 14).

12. EFFICACY ENDPOINTS, SAFETY, PHARMACOKINETIC, AND OTHER ASSESSMENTS

12.1. Efficacy Endpoints

Efficacy endpoints include changes from baseline in the following:

- Structured neurological examination. The examination will be recorded.
- Composite score of the Hammersmith and CHOP-INTEND neuro-development scales
- Modified Ashworth spasticity scale
- Neuro-ophthalmology exam
- Electroencephalography (EEG) measurements
- Nerve conduction velocity (NCV)
- Brainstem evoked response audiometry (BERA)

There will also be a longitudinal assessment (serial) monthly over 12 months of parental severity assessment and VAS; and monthly video recordings of ADL, including feeding, bathing, ambulation, communication, social skills and fine motor skills.

12.1.1. Structured Neurological Examinations by Investigator

A structured neurological examination (Appendix 1) will be conducted at screening visit, at Day 1 visit, at Day 180 (± 14 days) visit and at Day 360 (± 30 days) visit; and a score will be derived (Appendix 1). This examination will be repeated on all subjects who are participating in the extension period at month 18 (Day 540 ± 90 days), month 24 (Day 720 ± 90 days), month 30 (Day 900 ± 90 days), and month 36 (Day 1080 ± 90 days), provided there is a minimum of 90 days between assessments. The examination will include five main categories of pediatric developmental evaluation: 1) gross motor skills, 2) fine motor skills, 3) bulbar function, 4) ocular function, 5) temporo-frontal function, and an evaluation of the functional part of the autonomic nervous system. The examination will be videotaped. When possible, the neurological examinations should be performed by the same examiner, under identical circumstances for each examination when possible, and under well-lit conditions. If a study participant is unable to return to the study site due to the coronavirus disease of 2019 (COVID-19) pandemic associated travel concerns and restrictions, the neurological exam can be performed remotely by a local physician who has been trained on the structured neuro exam. In such cases, the Principal Investigator will score the exam by joining the examination live via videoconference or by watching the recording of the exam (see Section 14).

Detailed videotaping instructions will be provided in a separate manual. All neuro examinations should be done at approximately the same time for each visit. Exams should occur in the morning and prior to any other procedures. Patients may get a breakfast prior to the exam.

12.1.2. Hammersmith Infant Neurological Examination

The Hammersmith Infant Neurological Examination (HINE) is designed to be a simple and scorable method for evaluating infants from 2 months to 2 years of age. The HINE includes 3 sections containing 26 items that assess different aspects of neurologic function. Section 1 assesses cranial nerve function, posture, movements, tone, reflexes, and reactions. Section 2 assesses developmental milestones (head control, sitting, voluntary grasp, ability to kick, rolling, crawling, standing, and walking). Section 3 is a behavioral assessment (state of consciousness, emotional state, social orientation) (Haataja et al., 1999). Each section includes 7-10 items scored on a 5-point scale with 0 as the absence of activity, and a maximum score of 4 points. A HINE will be conducted at screening visit, Day 1 visit, Day 180 (± 14 days) visit, and Day 360 (± 30 days) visit. The HINE will be repeated on all subjects who are participating in the extension period at month 18 (Day 540 ± 90 days), month 24 (Day 720 ± 90 days), month 30 (Day 900 ± 90 days), and month 36 (Day 1080 ± 90 days), provided there is a minimum of 90 days between assessments.

12.1.3. CHOP-Intend Neurological Examination

The Children's Hospital of Philadelphia Infant Test of Neuromuscular Disorders (CHOP-INTEND) may be used to evaluate the motor skills of infants. It was developed by evaluating infants and has been shown to be valid for the assessment of children ranging in age from 3.8 months to over 4 years (Glanzman et al., 2010). CHOP-Intend includes 16 items used to assess motor skills. Each item is graded on a scale of 0-4 and total score ranges from 0-64. CHOP-Intend neurological examination will be conducted at screening visit, at Day 1 visit, at Day 180 (± 14 days) visit, and at Day 360 (± 30 days) visit. The CHOP-Intend neurological examination will be repeated on all subjects who are participating in the extension period at month 18 (Day 540 ± 90 days), month 24 (Day 720 ± 90 days), month 30 (Day 900 ± 90 days), and month 36 (Day 1080 ± 90 days), provided there is a minimum of 90 days between assessments.

12.1.4. Modified Ashworth Spasticity Assessment

The Modified Ashworth scale (MAS) measures resistance during passive soft-tissue stretching and is used as a simple measure of spasticity. It is performed in the supine position, while moving the limb at the same speed that the limb would move if dropped naturally. It is performed a maximum of three times for each joint, and prior to any stretching of the limb (Bohannon and Smith, 1987) and is scored as follows:

- 0: No increase in muscle tone

- 1: Slight increase in muscle tone, manifested by a catch and release or by minimal resistance at the end of the range of motion when the affected part(s) is moved in flexion or extension
- 1+: Slight increase in muscle tone, manifested by a catch, followed by minimal resistance throughout the remainder (less than half) of the ROM
- 2: More marked increase in muscle tone through most of the ROM, but affected part(s) easily moved
- 3: Considerable increase in muscle tone, passive movement difficult
- 4: Affected part(s) rigid in flexion or extension

A MAS assessment will be conducted at screening visit, at Day 1 visit, at Day 180 (± 14 days) visit, and at Day 360 (± 30 days) visit. A MAS assessment will be repeated on all subjects who are participating in the extension period at month 18 (Day 540 ± 90 days),month 24 (Day 720 ± 90 days), month 30 (Day 900 ± 90 days), and month 36 (Day 1080 ± 90 days), provided there is a minimum of 90 days between assessments.

12.1.5. Parental Assessments

The subject's parent/caregiver will be asked to complete an assessment of severity (Appendix 3) and VAS (Appendix 4) at each of the 2 baseline visits (Screening and Day 1) and monthly thereafter. A parental GCIS will be applied at Day 180 (± 14 days) visit and at Day 360 (± 30 days) visit (Appendix 2). All parental assessments will be repeated on all subjects who are participating in the extension period at month 18 (Day 540 ± 90 days), 24 (Day 720 ± 90 days), month 30 (Day 900 ± 90 days), and month 36 (Day 1080 ± 90 days), provided there is a minimum of 90 days between assessments. The Investigator will review these parental scales as part of his/her assessment.

12.1.6. Parental Diaries

During the initial treatment period, the subject's parent/caregiver will be asked to keep a diary of the subject's bowel movements from screening to Day 1, between Day 150 and Day 180, and between Day 330 and Day 360. Bowel movement diaries will be collected and reviewed at each visit. During the initial treatment period, daily food intake and eating habits will be entered into a food journal. These diary entries will start after screening and will be reviewed by the diet coach. It will not be considered a protocol deviation if diary entries are missed by the subject's parent/caregiver. Detailed diary instructions will be provided in a separate document. Diet journals will not be collected during the extension phase.

12.1.7. Monthly Parent Videos

During the initial treatment period, the subject's caregiver will be asked to obtain a monthly 2 to 5-minute video of each of the following Required ADL, starting after the screening visit. The

videos should be obtained under identical circumstances and under well-lit conditions. Detailed instructions for obtaining and uploading the videos will be provided in a separate document.

1. 2 to 5-min video of feeding
2. 2 to 5-min video holding up head, sitting, standing, assisted or unassisted walking
3. 2 to 5-min video of subject bathing
4. 2 to 5-min video of interaction with parent/caregiver

Monthly parent videos will not be collected during the extension phase.

12.1.8. Neuro-ophthalmology Examination

A complete neuro-ophthalmology examination will be obtained at screening visit, at Day 180 (± 14 days) visit, and at Day 360 (± 30 days) visit. This examination will be repeated on all subjects who are participating in the extension period at month 18 (Day 540 + 90 days), month 24 (Day 720 ± 90 days), month 30 (Day 900 + 90 days), and month 36 (Day 1080 ± 90 days), provided that there is a minimum of at least 90 days between ophthalmologic examinations. The examination will include assessment of nystagmus, strabismus, pupil response and optic disc measurement using dilated fundoscopy. The strabismus assessment will need to be scored as follows:

1. Constant exotropia
2. Exotropia > 50 % of the exam before dissociation
3. Exotropia < 50 % of the exam before dissociation
4. No exotropia unless dissociated, recovers in > 5 seconds
5. No exotropia unless dissociated, recovers in 1-5 seconds
6. No exotropia unless dissociated, recovers in < 1 second (phoria)

A copy of each neuro-ophthalmology examination report should be retained as a source document.

In cases where the visit is done remotely and it is not possible to perform a neuro-ophthalmology exam, a limited evaluation of optic disc measurement can be performed by an ophthalmologist (see Section 14).

12.1.9. Electroencephalography

EEG is an electrophysiological monitoring method to record electrical activity of the brain. It is typically noninvasive, with the electrodes placed along the scalp. EEG measures voltage fluctuations resulting from ionic current within the neurons of the brain. Derivatives of the EEG technique include evoked potentials (EP), which involves averaging the EEG activity time-locked to the presentation of a stimulus of some sort (visual, somatosensory, or auditory). Event-related potentials (ERPs) refer to averaged EEG responses that are time-locked to more complex processing of stimuli; this technique is used in cognitive science, cognitive psychology, and psychophysiological research. Recently, quantitative EEG has been used to assess fronto-

temporal neurodegeneration (Ratti et al., 2017) and dementia associated with Parkinson Disease (Klassen et al., 2011). EEG data may be converted from time to frequency domain using Fast Fourier Transform (FFT). Absolute power spectral densities (PSDs) can then be calculated for 1 second epochs (1–40Hz bins and standard bandwidths). These data can then be used to compare group PSDs and to perform LORETA analyses.

An EEG will be obtained at screening visit, at Day 180 (± 14 days) visit, and at Day 360 (± 30 days) visit. An EEG will be repeated on all subjects who are participating in the extension period at month 24 (Day 720 ± 90 days) and month 36 (Day 1080 ± 90 days), with a minimum of at least 90 days between EEGs.

A copy of each EEG report should be retained as a source document.

12.1.10. Nerve conduction velocity

NCV is an important aspect of nerve conduction studies. It is the speed at which an electrochemical impulse propagates down a neural pathway. Conduction velocities are affected by a wide array of factors, including age, sex, and various medical conditions. Studies allow for better diagnoses of various neuropathies, especially demyelinating conditions as these conditions result in reduced or non-existent conduction velocities. Distal motor latency, as well as sensory and motor nerve conduction velocities can be measured and compared with baseline measurements. These parameters have been measured for the motor ulnar, median and peroneal nerves and the sensory ulnar nerve in a natural history study of Charcot-Marie-Tooth disease (Manganelli et al., 2016). NCV will be conducted at screening visit, at Day 180 (± 14 days) visit, and at Day 360 (± 30 days) visit. NCV will be repeated on all subjects who are participating in the extension period at month 24 (Day 720 ± 90 days) and month 36 (Day 1080 ± 90 days), provided that there is a minimum of at least 90 days between NCVs. A copy of each NCV report should be retained as a source document.

12.1.11. Brainstem evoked response audiometry

BERA is an auditory evoked potential extracted from ongoing electrical activity in the brain and recorded via electrodes placed on the scalp. It is used for newborn hearing screening, auditory threshold estimation, intraoperative monitoring, determining hearing loss type and degree, and auditory nerve and brainstem lesion detection, and in development of cochlear implants. Amplitude (the number of neurons firing), latency (the speed of transmission), interpeak latency (the time between peaks), and interaural latency (the difference in wave V latency between ears). The BERA represents initiated activity beginning at the base of the cochlea and moving toward the apex over a 4ms period. BERA has been used as an endpoint in clinical trials of interventions performed on icteric babies (Sharma et al., 2006). BERA will be conducted at screening visit, at Day 180 (± 14 days) visit, and at Day 360 (± 30 days) visit. BERA will be repeated on all subjects who are participating in the extension period at month 24 (Day 720 ± 90 days) and month 36 (Day 1080 ± 90 days), with a minimum of 90 days between BERA. BERA does not need to be repeated

at Day 180, Day 360,Day 720, and Day 1080 visits in those participants in whom screening BERA yields uninterpretable or insufficient results. A copy of each BERA report should be retained as a source document.

12.2. Safety

Safety assessments include AEs, physical and neurologic examinations, vital signs, and clinical laboratory tests (hematology, clinical chemistry, lipid profile, and coagulation). Safety signals must be promptly communicated to the Medical Monitor.

12.2.1. Adverse Events

Subjects will be assessed for AEs at each study visit after the ICF is obtained through the 30-day safety follow-up period. AEs will be evaluated for incidence, severity, and relationship to study drug. The safety evaluation will include an assessment of all AEs, SAEs, and dose discontinuations due to AE. Monitoring and grading of AEs will be done in accordance with Medical Dictionary for Regulatory Activity (MedDRA) during the study. See Section 16 for additional information.

12.2.2. Physical Examination

Complete physical examinations will be conducted at screening visit, at Day 1 visit, at Day 180 (± 14 days) visit, and at Day 360 (± 30 days) visit. Physical examinations will be repeated on all subjects who are participating in the extension period at month 18 (Day 540 ± 90 days), month 24 (Day 720 ± 90 days), month 30 (Day 900 ± 90 days), and month 36 (Day 1080 ± 90 days), provided there is a minimum of 90 days between examinations. Physical examinations include skin, HEENT, respiratory, cardiovascular, gastrointestinal, endocrine/metabolic, genitourinary, neurological, blood/lymphatic, musculoskeletal, hepatic, allergies and psychological examination.

12.2.3. Vital Signs

Vital signs (systolic and diastolic blood pressure, heart rate, respiration rate, temperature), height and weight will be measured at each study visit. Blood pressure and heart rate measurements should be performed in the supine position after the subject has rested in that position for at least 3 minutes. Blood pressure will be obtained with an automated or manual blood pressure apparatus. Blood pressure and pulse should be performed on the same extremity throughout the study and documented as such.

12.2.4. Clinical Laboratory Tests

The following clinical laboratory tests will be performed at each visit at a local approved laboratory. The safety laboratory tests and lipid profile should be performed in a fasting state. All fasting blood draws should be done on a different day than the neurological examinations.

- Hematology: hematocrit, hemoglobin, mean corpuscular hemoglobin, mean corpuscular volume, platelet count, RBC count, and white blood cell (WBC) count with differential (absolute).
- Coagulation: prothrombin time (PT), international normalized ratio (INR), and partial thromboplastin time (PTT).
- Clinical chemistry: Alanine transaminase (ALT), aspartate transaminase (AST), LDH, alkaline phosphatase, total bilirubin, amylase, lipase, calcium, magnesium, glucose, sodium, potassium, carbon dioxide (CO_2), chloride, blood urea nitrogen (BUN), and creatinine. ALT and AST values that are > 2 x upper limit of normal (ULN) should be repeated within 14 days from the first elevated value.
- Lipid profile: total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides

12.3. Pharmacokinetics

12.3.1. Sample Collection

Bioanalytical measurements will be performed to determine the concentration of D2-LA, D2-ARA, H2-LA and H2-ARA in plasma, and D2-LA, H2-LA, D2-ARA and H2-ARA in RBC. Blood samples will be collected at Screening, on Day 90 ± 7 days (pre-breakfast and pre-dose), on Day 180 ± 14 days (pre-breakfast and pre-dose), and on Day 360 (window Day 300 +90 days) (pre-breakfast and pre-dose). Blood samples will also be collected from all subjects who are participating in the extension period at month 18 (Day 540 ± 90 days), month 24 (Day 720 ± 90 days), month 30 (Day 900 ± 90 days), and month 36 (Day 1080 ± 90 days), with a minimum of 90 days between sample collection.

All fasting blood draws should be done on a different day than the neurological examinations during the initial treatment phase. In the extension phase, it is permissible to perform the neuro exam the same day as the fasting blood draw, following a meal and rest period Additional PK samples will be obtained 1 week (+ 7 days) and 1 month (+/- 7 days) following the last dose. These samples are to be obtained prior to breakfast. Actual collection dates and time points should be recorded and will be used for PK analysis. Best efforts to collect the samples at the planned time should be made.

Protocol-specific instructions will be provided to each site in PK Instructions for the collection, handling, storage, and shipping of PK samples. The date and actual time each sample is collected will be recorded. Date and time of last dose prior to sample collection will also be recorded. The samples obtained on Day 90, and post-dose may be obtained at a qualified laboratory local to the subject or at home by an approved phlebotomy service provider.

12.3.2. Washout Pharmacokinetic sampling

The RT00l-008 Trial includes bioanalytical measurements to determine the concentration of study drug and its metabolites in plasma and red blood cells (RBCs). These PK samples are collected approximately every 6 months while on treatment and during washout at 7 and 30 days following RT00I cessation.

Recent PK analysis 30 days after RT00l was discontinued reveals that significant levels of the primary metabolite of RT00I (D2-Arachidonic acid) remain. Therefore, additional washout PK sampling and analysis is optimal.

To this end, study sites may contact parents study participants who have discontinued RT001 to obtain consent for follow-up PK samples, safety labs, and physical exams. These would be performed upon consent and repeated every 6 months until the (D2-Arachidonic acid) level is less than 25% of the peak level measured while the subject was receiving RT00I. The parents of study participants will need to read and sign an IRB-approved informed consent form (ICF) addendum prior to conducting any study procedures.

13. STUDY PROCEDURES AND SCHEDULE

It is preferred that all study visits, assessments, and procedures involving study subjects will be performed at a Sponsor-approved investigator site, and assessments and procedures will be performed by study personnel with the supervision of an Investigator. However, when ability to travel to the study site is impaired due to the COVID-19 pandemic, study procedures and visits may be performed by Sponsor-approved local providers as outlined in COVID-19 Contingency Plan (see Section 14). All measurements and results obtained as part of the protocol will be recorded in the CRF. The Schedule of Events is summarized in Table 1. Each site visit can be done over several days as needed to accommodate for scheduling flexibility. During the initial treatment phase, all fasting blood draws should be done on a different day than the neurological examinations. During the extension period, it is permissible to perform fasting blood draw in the morning followed by the neurological examinations later the same day, following a meal and rest period.

13.1. Visit 1: Screening

Screening procedures can be performed at least 14 days and no more than 45 days prior to the first dose on Day 1. A signed and dated ICF will be obtained from the subject's parents/legal representative as required by the IRB before any screening procedures are conducted. A signed copy of the ICF will be given to the subject's parent/legal representative. Screening procedures include the following:

Fasting blood draws (to be done on a different day than the neurological exams)

- Obtain blood samples for hematology, chemistry, coagulation, and lipid profile in a fasting state
- Obtain blood sample for PK

- Neurological Examinations

- Perform a filmed, structured neurological examination (by Investigator)
- Perform a Hammersmith and CHOP-Intend neurological assessment
- Perform a modified Ashworth spasticity assessment

- Other Procedures (to be done **after** neurological examinations or on a different day)

- Evaluate eligibility per inclusion/exclusion criteria
- Record medical history and demographics
- Perform a physical examination

- Record vital sign measurements, height, and weight
- Obtain the parental baseline assessment of severity
- Perform neuro-ophthalmology examination
- Obtain EEG, NCV and BERA

13.2. After the screening visit (to be done by the subject's parent/caregiver)

- Phone call with the diet coach to review the diet requirements. Subject will start to consume a restricted PUFA diet, keeping PUFA consumption at approximately 5g/day (diet only) or lower.
- The subject's parent/caregiver will obtain a 2 to 5-minute video for each of the Required ADL
- The parent/caregiver will start to keep a diary of the subject's bowel movements, eating habits and food intake. Bowel movement diaries will need to be collected at site visits.

13.3. Visit 2: Study Day 1

The following procedures will be performed:

Fasting blood draws (to be done pre-dose and on a different day than the neurological exams)

- Obtain blood samples for hematology, chemistry, coagulation, and lipid profile in a fasting state. Only those labs for which screening results are both out of range and deemed clinically significant by the Investigator need to be repeated at Day 1.

Neurological Examinations (to be done pre-dose)

- Perform a filmed, structured neurological examination (by Investigator)
- Perform a Hammersmith and CHOP-Intend neurological assessment
- Perform a modified Ashworth spasticity assessment
    - Other Procedures (to be done pre-dose; and **after** neurological examinations or on a different day)
- Reassess inclusion/exclusion criteria
- Update medical history and demographics
- Record vital sign measurements, height and weight
- Perform a physical examination
- Obtain the parental baseline assessment of severity

Dosing (to be done after all baseline testing is complete)

- Subjects will be administered the first dose of study drug at the clinic with a meal after all baseline testing is complete.

Post-Dose

- Record concomitant medications
- Record AEs
  - At home:
- Parent/caregiver will be contacted by the diet coach to review the food diary and discuss eating habits and food preparation. Subject continues to consume a restricted PUFA diet, keeping PUFA consumption at approximately 5g/day (diet only) or lower.
- The subject's parent/caregiver will obtain a 2 to 5-minute video for each of the Required ADL
- The parent/caregiver continues to keep a diary of the subject's eating habits and food intake.

13.4. Phone Call: Day 30 (± 7 days), Day 60 (± 7 days), Day 90 (± 7 days), Day 120 (± 7 days), and Day 150 (± 7 days)

- Provide blood samples for PK prior to the morning meal and morning dose of study medication only on Day 90 (± 7 days). Samples may be obtained at a qualified laboratory local to the subject or at home by an approved phlebotomy service provider.
- The subject's parent/caregiver will be contacted by phone by a study site staff member to assess AEs and obtain the parental assessment of severity and VAS.
- The subject's parent/caregiver will be contacted by the diet coach to review the food diary and discuss eating habits and food preparation. Subject continues to consume a restricted PUFA diet, keeping PUFA consumption at approximately 5g/day (diet only) or lower.
- The subject's parent/caregiver will obtain a 2 to 5-minute video for each of the Required ADL.
- The subject's parent/caregiver continues to keep a diary of the subject's eating habits and food intake.
- Between the Day 150 phone call and the Day 180 visit, the subject's parent/caregiver will keep a diary of the subject's bowel movements.

13.5. Visit 3: Day 180 (± 14 days)

The following procedures will be performed:

Fasting blood draws (to be done pre-dose and on a different day than the neurological exams)

- Obtain blood samples for hematology, chemistry, coagulation, and lipid profile in a fasting state
- Obtain blood samples for PK prior to the morning meal

Neurological Examinations

- Perform a filmed, structured neurological examination (by Investigator)
- Perform a Hammersmith and CHOP-Intend neurological assessment
- Perform a modified Ashworth spasticity assessment
    - Other Procedures (to be done **after** neurological examinations or on a different day)
- Record vital sign measurements, height and weight
- Perform a physical examination
- Obtain the parental GCIS, assessment of severity and VAS
- Perform neuro-ophthalmology examination
- Obtain EEG, NCV and BERA
- Record concomitant medications
- Record AEs
- Perform drug accountability
    - At home:
- The subject's parent/caregiver will be contacted by the diet coach to review the food diary and discuss eating habits and food preparation. Subject continues to consume a restricted PUFA diet, keeping PUFA consumption at approximately 5g/day (diet only) or lower.
- The subject's parent/caregiver will obtain a 2 to 5-minute video for each of the Required ADL.
- The subject's parent/caregiver continues to keep a diary of the subject's eating habits and food intake.

13.6. Phone Call: Day 210 (± 7 days), Day 240 (± 7 days), Day 270 (± 7 days), Day 300 (± 7 days), and Day 330 (± 7 days)

- Parents will be contacted by phone by a study site staff member to assess AEs and obtain the parental assessment of severity and VAS.

- Parents will be contacted by the diet coach to review the food diary and discuss eating habits and food preparation. Subject continues to consume a restricted PUFA diet, keeping PUFA consumption at approximately 5g/day (diet only) or lower.
- The subject's parents will obtain a 2 to 5-minute video for each of the Required ADL.
- The parent/caregiver continues to keep a diary of the subject's eating habits and food intake.
- Between Day 330 phone call and the Day 360 visit, the subject's parent/caregiver will keep a diary of the subject's bowel movements.

13.7. Visit 4: Day 360 (± 30 Days)

The following procedures will be performed:

Fasting blood draws (to be done pre-dose and on a different day than the neurological exams)

- Obtain blood samples for hematology, chemistry, coagulation, and lipid profile in a fasting state
- Obtain blood samples for PK prior to the first daily dose of RT001 and the morning meal
- Due to the COVID-19 pandemic the window for fasting blood draws for Visit 4 (Month 12) is widened to Day 300 + 90 Days

Neurological Examinations

- Perform a filmed, structured neurological examination (by Investigator or local trained physician)
- Perform a Hammersmith and CHOP-Intend neurological assessment
- Perform a modified Ashworth spasticity assessment
    - Other Procedures (to be done **after** neurological examinations or on a different day)
- Record vital sign measurements, height and weight
- Perform a physical examination
- Obtain the parental GCIS, assessment of severity and VAS
- Perform neuro-ophthalmology examination
- Obtain EEG, NCV and BERA
- Record concomitant medications
- Record AEs
- Perform drug accountability

- Dosing: The last dose of study drug will be given in the clinic (for subjects who are not participating in the treatment extension)
- At home: The subject's parents will obtain a 2 to 5-minute video for each of the Required ADL.

13.8. Visit 5: Day 540 (± 90 days) (For subjects in extension period)

The following procedures will be performed:

Fasting blood draws (to be done pre-dose)

- Obtain blood samples for hematology, chemistry, coagulation, and lipid profile in a fasting state
- Obtain blood samples for PK prior to first daily dose of RT001 and the morning meal

Neurological Examinations

- Perform a filmed, structured neurological examination (by Investigator or trained local physician)
- Perform a Hammersmith and CHOP-Intend neurological assessment
- Perform a modified Ashworth spasticity assessment
    - Other Procedures (to be done **after** neurological examinations or on a different day)
- Record vital sign measurements, height and weight
- Perform a physical examination
- Perform a neuro-ophthalmology exam (or ophthalmology exam)
- Obtain the parental GCIS, assessment of severity and VAS
- Record concomitant medications
- Record AEs
- Perform drug accountability

13.9. Visit 6: Day 720 (± 90 Days) (For subjects in extension period)

The following procedures will be performed:

- Fasting blood draws (to be done pre-dose) Obtain blood samples for hematology, chemistry, coagulation, and lipid profile in a fasting state
- Obtain blood samples for PK prior to the first daily dose of RT001 and the morning meal

Dosing: The last dose of study drug will be given in the clinic (for subjects who are not continuing in the second treatment extension)

Neurological Examinations

- Perform a filmed, structured neurological examination (by investigator)
- Perform a Hammersmith and CHOP-Intend neurological assessment
- Perform a modified Ashworth spasticity assessment
    - Other Procedures (to be done **after** neurological examinations or on a different day)
- Record vital sign measurements, height and weight
- Perform a physical examination
- Obtain the parental GCIS, assessment of severity and VAS
- Perform neuro-ophthalmology examination (or ophthalmology exam)
- Obtain EEG, NCV and BERA
- Record concomitant medications
- Record AEs
- Perform drug accountability

13.10. Visit 7: Day 900 (± 90 days) (For subjects in extension period)

The following procedures will be performed:

- Fasting blood draws (to be done pre-dose) Obtain blood samples for hematology, chemistry, coagulation, and lipid profile in a fasting state
- Obtain blood samples for PK prior to the morning meal and first daily dose of RT001

Neurological Examinations

- Perform a filmed, structured neurological examination (by Investigator or trained local physician)
- Perform a Hammersmith and CHOP-Intend neurological assessment
- Perform a modified Ashworth spasticity assessment
    - Other Procedures (to be done **after** neurological examinations or on a different day)
- Record vital sign measurements, height and weight
- Perform a physical examination
- Perform neuro-ophthalmology examination (or ophthalmology exam)
- Obtain the parental GCIS, assessment of severity and VAS
- Record concomitant medications

- Record AEs
- Perform drug accountability

13.11. Visit 8: Day 1080 (± 90 Days) (For subjects in extension period)

The following procedures will be performed:

- Fasting blood draws (to be done pre-dose). Obtain blood samples for hematology, chemistry, coagulation, and lipid profile in a fasting state
- Obtain blood samples for PK prior to the morning meal and before first daily dose of RT001.

Dosing: The last dose of study drug will be given in the clinic (for subjects who are not continuing in the treatment extension)

Neurological Examinations

- Perform a filmed, structured neurological examination (by Investigator or trained local physician)
- Perform a Hammersmith and CHOP-Intend neurological assessment
- Perform a modified Ashworth spasticity assessment
    - Other Procedures (to be done **after** neurological examinations or on a different day)
- Record vital sign measurements, height and weight
- Perform a physical examination
- Obtain the parental GCIS, assessment of severity and VAS
- Perform neuro-ophthalmology examination (or ophthalmology exam)
- Obtain EEG, NCV and BERA
- Record concomitant medications
- Record AEs
- Perform drug accountability

13.12. Quarterly Diet Call (For subjects in extension period)

Parents will be contacted by the diet coach to discuss their child's eating habits, food preparation and assess compliance to the restricted PUFA recommendations. Subject continues to consume a restricted PUFA diet, keeping PUFA consumption at approximately 5g/day (diet only) or lower. Calls will be performed every 90 days (± 14 days) after completion of the Month 12 neuro-assessment visit.

13.13. Safety Follow-up

Subjects will be contacted by phone by a study site staff member 30 days (+7 days) after the last dose to collect AE information and confirm any change in concomitant medication since the last visit.

PK samples will be obtained 1 week (+ 7 days) and 1 month (± 7 days) following the last dose. These samples are to be obtained prior to breakfast. Actual collection dates and time points should be recorded and will be used for PK analysis. The sample may be obtained at a qualified laboratory local to the subject or at home by an approved phlebotomy service provider.

13.14. Unscheduled Visits

Procedures to be performed at any unscheduled visit are at the discretion of the Investigator, but should at least include safety, laboratory, and health examinations necessary for collection of AE information. Any procedure can be repeated at an unscheduled visit as needed.

13.15. Early Termination Visit

The following procedures should be performed if a subject terminates early from the study if possible:

- Fasting blood draws (to be done on a different day than the neurological exams)
- Obtain blood samples for hematology, chemistry, coagulation, and lipid profile in a fasting state
- Obtain blood samples for PK prior to the morning meal

Neurological Examinations

- Perform a filmed, structured change from baseline neurological examination (by Investigator)
- Perform a Hammersmith and CHOP-Intend neurological assessment
- Perform a modified Ashworth spasticity assessment
    - Other Procedures (to be done **after** neurological examinations or on a different day)
- Record vital sign measurements, height and weight
- Perform a physical examination
- Obtain the parental GCIS, assessment of severity and VAS
- Perform neuro-ophthalmology examination
- Obtain EEG, NCV and BERA
- Record concomitant medications

- Record AEs
- Perform drug accountability
    - At home: The subject's parents will obtain a 2 to 5-minute video for each of the Required ADL.

PK: PK samples will be obtained 1 week (+ 7 days) and 1 month (± 7 days) following the last dose. These samples are to be obtained prior to breakfast. Actual collection dates and time points should be recorded and will be used for PK analysis. The sample may be obtained at a qualified laboratory local to the subject or at home by an approved phlebotomy service provider.

14. COVID-19 CONTINGENCY PLAN

The COVID-19 crisis has disrupted the ability of study participants to travel to the study sites for necessary assessment of safety and efficacy. The usual method of the re-supply of RT001 to these patients has also been interrupted. The overriding principle behind all the measures listed below is the safety of the patients, family members, caregivers, and clinical trial sites involved in the RT001-008 clinical trial.

In instances where subject and subject families are unable/unwilling to travel to clinical site, due to government restrictions, clinical site closures or risk of contracting COVID-19 virus during travel, visits can be conducted as remote visits as follows:

(1) If the PI is willing to travel to the patient's home, the protocol-specific structured neurological assessments will be conducted at the patient's home. OR
(2) If the PI is unable to travel to the patient's home, a local physician will be trained by the PI, or a representative from the study sponsor, on the protocol-specific structured neurological assessments, which will then be administered by the local physician.
    a. Whenever possible, the PI will proctor live via videoconference. If live proctoring is not possible, the PI will apply final score via review of the video-recorded assessment.
    b. Comprehensive training to ensure competence in the examinations will be provided which may include a combination of 1:1 training and review of a select set of previously recorded exams. AND
(3) Local lab/phlebotomy service will draw safety labs, lipid panel, and PK samples as outlined in the protocol. AND
(4) Physical exams, vital signs, weight and height measurements will be performed by the traveling PI or local physician. AND
(5) Safety assessment and parental assessment will be conducted telephonically by the site or by traveling PI. AND
(6) If possible, attempt will be made to have local ophthalmologist perform limited eye exam to measure optic atrophy/pallor (needed for the structured neuro assessment).

The Month 12 visit window is extended from 360 days ± 14 days to 360 days ± 30 days. During the extension phase (Month 18, 24, 30 and 36) visit windows are extended to ± 90 days, provided that visits are separated by a minimum of 90 days. The rationale for the expanded window is to allow maximum flexibility during the COVID-19 pandemic when clinic hours may be reduced and the ability to travel impacted.

Every attempt will be made to obtain electroencephalography (EEG), nerve conduction velocity (NCV), eye exam, and brainstem evoked response audiometry (BERA) per protocol, but failure to do so will not be considered a protocol deviation. If an EEG, NCV, eye exam, or BERA cannot be done at any visit, it will be attempted at the next visit.

It is acknowledged that the timing of the actual visits will need to coincide with the ability of local physicians to safely conduct the visits in their clinics or at the subjects' homes depending on

local restrictions, health authority guidance, the judgement of the subjects' legal guardians, and other unforeseen factors. Deviations from the current protocol will be documented.

Parental interview to assess for safety (adverse events), Parental Global Clinical Impression Scale, and Parental Assessment of Severity, Visual Analogue Scale (VAS), study drug accountability, and review of concomitant medications will be conducted by the study site telephonically or via email on the same day or the within 5 business days of the actual performance of remote neuro-exam for any of the visits impacted by COVID-19 travel interruption (Months 12, 18, 24, 30, and 36).

Retrotope has authorized resupply of RT001 from the clinical site or Retrotope drug depot shipped directly to the patient/family as allowed by regulations in place in the participant's country of residence and subject to the following provisions: (1) the patient's parent(s) must express verbal consent to continue RT001; (2) PI must feel that the patient's known clinical status is suitable for continuation of RT001; and (3) the PI must place orders for the drug to be dispensed, specifying the dosing, quantity to be dispensed.

Maintenance of drug accountability will remain the responsibility of the study sites. In instances where drug is shipped from the Sponsor's distribution center instead of the study site's pharmacy, the Sponsor will provide documentation of shipment to the study site. The number of used and unused bottles and capsule count will be ascertained via communication between the study site and parents as part of each remote visit interview. Unused study medication must still be returned to the study site, but used, empty bottles may be destroyed remotely if authorized by the study site per requirements in Section 10.5 (Drug Accountability).

15. STATISTICS

Full details of planned analyses, including exploratory analyses, will be provided in a separate statistical analysis plan.

15.1. Analysis Sets

All Patients Population

The All Patients Population is defined as patients with any record in the database. This population includes both screening and enrolled subjects. This population will be utilized for descriptive patient counts that include screening subjects.

Intent-To-Treat Analysis Set

The Intent-To-Treat (ITT) Analysis Set is defined as all subjects enrolled in the open-label and natural history studies.

Modified Intent-To-Treat Analysis Set

The Modified Intent-To-Treat (mITT) Analysis Set is defined as all ITT subjects who had both baseline and at least one post-baseline efficacy measurement. The mITT Analysis Set will be the data set used for primary, secondary, and exploratory efficacy analyses unless otherwise specified.

Natural History Set

The Natural History Set is defined as all subjects in the natural history study.

Safety Analysis Set

The Safety Analysis Set is defined as all subjects who received any amount of study drug (RT001) in the treated study or are in the Natural History Set.

Matched Patient Set

The natural history dataset was matched 1:1 to the treated dataset using propensity score matching (N=19 for both groups). The sponsor was blinded to all data, while Pentara used only baseline data for patient matching.

15.2. Efficacy Analyses

15.2.1. General Considerations for Efficacy Analyses

Efficacy analyses will be performed in the mITT population.

15.2.2. Primary Analyses

The primary analysis of the effect of RT001 on the modified Ashworth scale will compare treatment groups across both studies, using a joint rank analysis, Combined Assessment of Function and Survival (CAFS) model. CAFS analyses allows survival (death or pneumonia) outcomes to be combined with functional decline (Ashworth). A CAFS analysis ranks each

subject by their outcome with the worst outcome assigned to the subject who dies first in the study and the best outcomes assigned to the surviving subject with the least functional decline. All patients who die will be classified worse than patients who survive, and patients with pneumonia will be classified worse than all patients who survive without pneumonia. Joint rank scores will be calculated according to Berry et. al.[5]

An MMRM with individual modified Ashworth score without survival integration will be performed as a sensitivity analysis (see secondary and exploratory below). An additional sensitivity model will be performed by running the primary analysis but including a random effect for matched pair number and excluding the 19 patients in the NH study who were not matched to active treatment patients. The final sensitivity analysis will omit scores with missing data.

15.2.3. Analysis Method for Effect of RT001 on secondary and exploratory efficacy outcomes

All secondary and exploratory functional endpoints will use the same primary model as described above for the primary outcome (CAFS analysis). Because the composite was derived using individuals not within the matched patient set, the composite endpoint will be run using the matched patient set. All other secondary and exploratory outcomes will use the mITT set.

A sensitivity model will analyze CFB in the outcomes comparing treatment groups using a mixed model with repeated measures (MMRM). The MMRM will compare the estimated change from baseline between treatments for each endpoint.

For the MMRM, A risk score will be calculated for the efficacy variable to adjust for baseline covariates as well as those that interact with time and will be included in the model. To calculate the risk score, the residuals are regressed on the covariates listed below. Individual risk score coefficients for each covariate are multiplied by individual covariate values and summed. A separate covariate composite is calculated for baseline covariates and baseline covariates interacting with time (slope risk score).

The MMRM will implement a separate means model in which visits will be windowed as outlined above into the prespecified visits. Only windowed visits will be included in the analysis. The baseline and slope risk scores (described below) will be subtracted off the CFB at each visit using observed time for the slope risk score.

- Baseline risk score
  - Sex
  - Age
  - Time since symptom onset
  - Baseline modified Ashworth score (% total score)
  - Baseline mPRS score (% total score)
  - Baseline mINAD-RS24 (% total score)

- Slope risk score
  - Sex*time

- Age*time
- Time since symptom onset*time

The MMRM with baseline and slope risk score adjusted primary outcome CFB values as the response variable will include the following covariates and fixed effects:

- Treatment
- Time (discrete)
- Treatment by time interaction
- Subject (random effect covariance = VC)

The covariance structure for the repeated measures in this model will be unstructured (UN). If UN does not converge for the model, the MMRM model will be simplified to allow convergence as described in the following paragraph. Variance components will be used as the covariance structure for the random site effect in the model.

Any efficacy outcomes that do not converge using the specified primary model will be rerun using a first-order heterogeneous autoregressive (ARH[1]) covariance structure, and then a compound symmetry (CS) structure if ARH[1] doesn't converge. If CS does not converge variance components (VC) will be used.

Least-squares means will be estimated at each visit. The LS mean at the endpoint (360 days) is interpreted as the expected CFB in the primary outcome at final visit with the estimate drawn from the model within each group. Least squares means and standard errors will be estimated from the mixed model at all timepoints and will be shown for all analyses. In addition, treatment differences, p-values, 95% confidence intervals for the difference, effect size, and 95% confidence interval for the effect size will be displayed. Effect size will be calculated by taking the difference of LSMEANS and dividing by the standard deviation (i.e. the standard error of the estimated difference multiplied by the squared degrees of freedom).

The number of subjects with an observed efficacy outcome, mean, standard deviation, median, 25th percentile (Q1), 75th percentile (Q3), minimum and maximum will all be reported and accompany the estimates from the MMRM outlined in this section.

15.2.4. Statistical Hypotheses

The null hypothesis is that the treatment estimates at 1 and 2 year for the two treatment arms are equal. The alternative hypothesis is that the treatment estimates for the two treatment arms are not equal at 1 and 2 years.

Normal Distribution of Residuals: Use of Log Transformed or Rank Values

For analyses of quantitative response variables that assume normal distribution of the residuals, normality will be tested using the Anderson-Darling test. If the residuals are not normally distributed ($p < 0.05$), then the original values will be log-transformed, and normality will be re-tested. If the residuals after log transformation are not normally distributed, the original values

will be replaced by their rank values, where the ranking is over all observed values for the variable, and the analysis will be performed using the rank values. If the change over time of a response variable is exponential rather than linear, the time values will be log transformed. If analyses are performed using log-transformed or rank values, the analysis using the original values will be performed as an exploratory analysis.

15.3. Other Analyses

Other analyses will be described in detail in the Statistical Analysis Plan.

15.4. Determination of Sample Size

The primary analysis of the endpoint of change from baseline to 12 months in ADL scores will use a one-sample t-test with alpha = 0.025. The null hypothesis is that the ADL scores decline by 10% or more. The alternative hypothesis is that the ADL scores do not decline by 10% or more. For the purpose of estimating power, assume that the mean change from baseline is an increase of 10% with a standard deviation of 20%. The number of subjects is 15 to 20. For 15 subjects the power is 0.95.

Subjects who discontinue from the study will not be replaced.

15.5. Interim Analysis

An interim analysis is not planned.

15.6. Deviation from Original Analysis Plan

Deviations from the original statistical or PK analysis plans will be provided in the final clinical study report (CSR).

16. ADVERSE EVENTS

All subjects in the study will have INAD and the Investigators will take the subjects' baseline status into account when reporting AEs. Investigators will also consider the underlying disease when designating relationship of AE to study drug, and if AEs are due to the INAD disease, disease progression or symptomatology exacerbation. Common characteristics of INAD include:

- Loss of coordination
- Loss of mobility
- Impaired vision
- Nystagmus, strabismus
- Loss of developmental milestones
- Inability to communicate or use words
- Inability to swallow and aspiration
- Constipation
    - In another clinical trial with RT001, the only significant AE clearly related to study drug was steatorrhea/diarrhea.

All adverse physical findings/laboratory results identified prior to initiation of study drug will be reported on the medical history and/or physical examination CRFs. After initiation of study drug, any clinically significant changes in physical findings/laboratory results or any new adverse physical findings/laboratory results will be reported as AEs.

16.1. Adverse Event Definitions

16.1.1. Adverse Event

An AE means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, and does not imply any judgment about causality.

Because INAD is a progressive disease resulting in continued decline, changes that might ordinarily be expected to occur as a result of the natural progression of disease and, in line with the reported natural history, are not intended to be reported as adverse events. The progression of disease will be collected and captured during the efficacy assessments and parental assessments throughout the trial. For example, an increase in the loss of coordination or loss of a developmental milestone are not to be reported as adverse events. If a loss of coordination were to

result in a fall requiring a healthcare intervention, this would be an example of an adverse event that needs to be reported.

16.1.2. Suspected Adverse Reaction

A suspected adverse reaction means any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of IND safety reporting, ‘‘reasonable possibility’’ means there is evidence to suggest a causal relationship between the drug and the AE. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug.

16.1.3. Life-Threatening Adverse Event or Life-Threatening Suspected Adverse Reaction

An AE or suspected adverse reaction is considered ‘‘life-threatening’’ if, in the view of either the Investigator or Sponsor, its occurrence places the subject 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.

16.1.4. Serious Adverse Event or Serious Suspected Adverse Reaction

An AE or suspected adverse reaction is considered ‘‘serious’’ if, in the view of either the Investigator or Sponsor, it results in any of the following outcomes:

- Death
- A life-threatening AE - see definition above
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject 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.

16.1.5. Unexpected Adverse Event or Unexpected Suspected Adverse Reaction

An AE or suspected adverse reaction is considered ‘‘unexpected’’ if it is not listed in the Investigator Brochure or is not listed at the specificity or severity that has been observed.

For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the Investigator Brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the Investigator Brochure listed only cerebral vascular accidents.

‘‘Unexpected,’’ as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the Investigator Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

16.2. Adverse Event Classification

16.2.1. Relationship to Investigational Drug

The Investigator’s assessment of causality must be provided for all AEs (serious and non-serious). An Investigator’s causality assessment is the determination of whether or not there exists a reasonable possibility that the study drug caused or contributed to an AE, as described below:

- **None:** No relationship between the experience and the administration of study drug; related to other etiologies such as concomitant medications or subject’s clinical state.
- **Unlikely:** The current state of knowledge indicates that a relationship is unlikely.
- **Possible:** A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction might have been produced by the subject’s clinical state or other modes of therapy administered to the subject, but this is not known for sure.
- **Probable:** A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug. The reaction cannot be reasonably explained by the known characteristics of the subject’s clinical state or other modes of therapy administered to the subject.
- **Definite:** A reaction that follows a plausible temporal sequence from administration of the study drug and follows a known response pattern to the suspected study drug and can be confirmed with a positive re-challenge test or supporting laboratory data.
-

16.2.2. Severity

All AEs will be graded for severity using the following terms:

Grade 1	Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate	Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL[a]

Grade 3	Severe	Medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL[b]
Grade 4	Life threatening consequences	Urgent intervention indicated
Grade 5	Death	Death related to an AE

[a] Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
[b] Self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

16.3. Monitoring of Adverse Event Data

The Investigator and the Sponsor or its representative will be responsible for the following:

- Reviewing AE data on an ongoing basis throughout the study. Should any AE report suggest that an unexpected Grade 3 or higher, and probably or definite study drug-related occur, the Investigator and Sponsor will take prompt and appropriate action to protect subject safety. These actions may range from no action (proceed with the study) to halting the study for safety reasons. The evaluation and action decided upon will be documented.
- Assessing the safety data and providing recommendations if the Sponsor should stop or modify the study.

See Section 8.4 for additional details.

16.4. Documentation of Adverse Events by Investigator

Subjects will be evaluated and questioned generally to identify AEs during the course of the study. Any events occurring prior to administration of the first dose will be recorded on the Medical History CRF. Events occurring after administration of the first dose of study drug will be recorded on the AE CRF. AEs that occur up to and including the 30 days after the last dose of study drug must be reported.

Record all AEs spontaneously reported by the subject's caregivers and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures on the AE Form for that visit. Any clinically relevant deterioration in laboratory assessments or other clinical findings is considered an AE and must be recorded on the AE Form. In addition, an abnormal test finding must be classified as an AE if one or more of the following criteria are met:

- The test finding is accompanied by clinical symptoms.
- The test finding necessitates additional diagnostic evaluation(s) or medical/surgical intervention; including significant additional concomitant drug treatment or other therapy.

(Note: simply repeating a test finding, in the absence of any of the other listed criteria, does not constitute an AE.).

- The test finding leads to a change in study drug dosing or discontinuation of subject participation in the clinical research study.
- The test finding is considered an AE by the Investigator.

Wherever possible, a specific disease or syndrome rather than individual associated signs and symptoms should be identified. However, if an observed or reported sign or symptom is not considered a component of a specific disease or syndrome by the Investigator, it should be recorded as a separate AE. Laboratory data are to be collected as stipulated in this protocol. Clinical syndromes associated with laboratory abnormalities are to be recorded as appropriate (e.g., diabetes mellitus rather than hyperglycemia).

For SAEs, an SAE Form must also be completed with as much information as possible and submitted in the time frame described below in Section 16.5. When new significant information is obtained as well as when the outcome of an event become known, the Investigator should record the information on a new SAE Form, indicating that it is a follow-up report. If the subject was hospitalized, a copy of the discharge summary and any other relevant hospital records (e.g., admission report, laboratory test results) must be included as part of the subject medical file.

All AEs considered to be related (definitely, probably, or possibly related) to study drug and all SAEs will be followed until resolved or until a stable status has been achieved.

16.5. Notification about Serious Adverse Events and Serious and Unexpected Suspected Adverse Reactions

16.5.1. Investigator Reporting to Sponsor

All SAEs that occur during the course of the study must be reported by the Investigator to the Sponsor and to the Medical Monitor within 24 hours by telephone or by text message. Additionally, the SAE Form should be faxed/e-mailed within 1 working day from the point in time when the Investigator becomes aware of the SAE. In addition, all SAEs that occur up to and including 30 days after administration of the last dose of study drug must be reported to the Sponsor within 1 working day from when the Investigator becomes aware of the SAE.

Investigators must report to the Sponsor any SAE, whether or not considered drug related, including those listed in the protocol or Investigator Brochure. The report must include an assessment of causality.

For all SAEs, the Investigator is obligated to obtain and provide information to the Sponsor in accordance with the timeframes for reporting specified above. In addition, an Investigator may be requested by the Sponsor to obtain specific additional follow-up information in an expedited fashion. This information may be more detailed than that captured on the AE CRF. In general,

this will include a description of the AE in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes of the event, such as concomitant medications and illnesses must be provided. In the case of a subject death, a summary of available autopsy findings must be submitted as soon as possible to the Sponsor or its designated representative.

The Sponsor's Medical Monitor will review the information submitted, and seek further detail if deemed medically relevant and ideally concur on the severity and relatedness. However, the Medical Monitor may revise the severity and relatedness **upward**, but may not reduce severity and/or relatedness. Such revisions will be documented in the Sponsor file and provided to the Investigator.

Serious Adverse Event Reporting Contact Information:

Mark Midei, MD
Medical Monitor
Retrotope, Inc.
4300 El Camino Real, Suite 201
Los Altos, CA 94022
Tel: 410-472-6933; 410-371-5357
mark@retrotope.com

Geri Wohl, CNC
Clinical Consultant
Retrotope, Inc.
4300 El Camino Real, Suite 201
Los Altos, CA 94022
Tel: 650-619-5401
geri.wohl@retrotope.com

16.5.2. Reporting to Regulatory Agencies and Institutional Review Boards/Ethics Committees

If there is a serious and unexpected suspected adverse reaction, the Sponsor will notify the appropriate regulatory agency and all appropriate parties on an expedited basis. In addition, Sponsor must submit expedited reports of an increased rate of occurrence of serious suspected adverse reactions over that listed in the protocol or Investigator Brochure (§ 312.32(c)(1))(iv)).

It is the responsibility of the Investigator to promptly notify the Institutional Review Board (IRB)/Ethics Committee of all serious and unexpected suspected adverse reactions involving risk to human subjects. Provide a copy of this communication to the Sponsor.

16.6. Emergency Identification of Study Medication

This study is open label and all study medication will be RT001.

16.7. Emergency Sponsor Contact

In a medical emergency (such as an event that requires immediate attention regarding the treatment of a subject, operation of the clinical study, and/or the use of investigational drug), study site staff will apply appropriate medical intervention according to current standards of care, and contact the Medical Monitor and the Sponsor Contact.

Medical Monitor and Sponsor Contact Information:	
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17. ETHICS

17.1. Institutional Review Board/Ethics Committee

The IRB/Ethics Committee must comply with FDA requirements governing IRBs (21 CFR Part 56). The Investigator will provide the Sponsor (or designee) with documentation of IRB/Ethics Committee approval of the following documents before the study begins at the study site(s): protocol, ICF, and any other relevant materials intended for or directed to subjects (e.g., subject diaries, advertisements). The Investigator will supply documentation to the Sponsor of IRB/Ethics Committee requirements regarding continuing review and approval of revisions to any of these documents.

17.2. Ethical Conduct of the Study

This study will be conducted in accordance with the current IRB/Ethics Committee approved clinical protocol, International Conference for Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines, and relevant policies and requirements of the national regulations and laws, including the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

17.3. Subject Information and Informed Consent

Written ICF is required from each subject's legal representative prior to any testing under this protocol, including screening tests and evaluations. The ICF, as specified by the clinical site's IRB/IEC, must follow the Protection of Human Subjects regulations listed in 21 CFR Part 50.

The ICF will be used to explain the risks and benefits of study participation in simple terms before the subject will be entered into the study. The ICF will contain a statement that the consent is freely given, that the subject is aware of the risks and benefits of entering the study, and that the subject is free to withdraw from the study at any time. Written ICF must be given by the subject's legal representative after the receipt of detailed information on the study and sufficient time to read it and have any questions answered. It is the responsibility of the Investigator to obtain consent and to provide the subject with a copy of the signed and dated ICF. Confirmation of a subject's ICF must also be documented in the subject's medical record prior to any testing under this protocol, including screening tests and evaluations.

All ICFs used in this study must be approved by the appropriate IRB and by the Sponsor or its designee. The ICF must not be altered without the prior agreement of the relevant IRB and the Sponsor.

If a study participant is unable to return to the study site for in-person informed consent discussion due to the COVID-19 pandemic, remote informed consent is permitted. In such instances the ICF should be sent to the subjects in advance of informed consent discussion so that they have time to read and consider. The informed consent discussion needs to be done via

telephone conversation, with interpreter if needed, and the call must be documented in the participant's record. It is will not be necessary to obtain original wet signature in this case and copy of signed ICF sent via fax, email, scan or photo is permitted. Once received, the site will add site signatures, retain the original, and send a fully signed copy to the subject.

18. STUDY ADMINISTRATION

18.1. Administrative Structure

A list of individuals who will have key positions in this study will be saved in the Trial Master File (TMF). This list will include names, titles, and roles of selected individuals from the Sponsor and/or the contract research organization (CRO) that will contribute to this study.

18.2. Quality Control and Quality Assurance

18.2.1. Overview

According to the GCP Guidelines, the Sponsor is responsible for implementing and maintaining quality assurance and control systems with written standard operating procedures (SOPs).

QC will be applied to each stage of data handling. The following steps will be taken to ensure the accuracy, consistency, completeness, and reliability of the data:

- Investigator meeting(s)
- Site initiation visit
- Routine site monitoring
- Ongoing site communication and training
- Data management quality control checks
- Continuous data acquisition and cleaning
- Internal review of data; and
- QC checks of the final CSR

In addition, the Sponsor's (or designee) Clinical QA Department may conduct periodic audits of the study processes, including, but not limited to study site, site visits, vendors, clinical database, and final CSR. When audits are conducted, access must be authorized to Sponsor's representatives and regulatory authorities for all study-related documents, including medical history and concomitant medication documentation.

18.2.2. Monitoring

Site Monitors will work in accordance with Sponsor SOPs. Monitors will establish and maintain regular contact between the Investigator or designee and the Sponsor.

Monitors will evaluate the competence of each study site, informing the Sponsor about any problems relating to facilities, technical equipment or medical staff. During the study, monitors will check that written ICF has been obtained from all subjects correctly and that data are

recorded correctly and completely on the CRFs. Monitors are also required to compare entries in CRFs with corresponding source data and to inform the Investigator or designee of any errors or omissions. Monitors will also review adherence to the protocol and to regulatory requirements at the study site and discuss any deviations noted with the Investigator or designee. They will arrange for the study site to receive adequate supply of study drug and ensure appropriate storage conditions are maintained.

Monitoring visits will be conducted according to the US CFR Title 21 Parts 50, 56, and 312 and ICH Guideline for GCP. The monitor will make written reports to the Sponsor following each contact with the Investigator or designee, regardless of whether it is by phone or in person.

18.2.3. Data Management/Coding

Study data will be handled according to the relevant SOPs of the data management and biostatistics departments of the Sponsor or CRO.

AEs will be coded using MedDRA and medications will be coded using World Health Organization Drug Dictionary (WHODD).

18.2.4. Quality Assurance Audit

Study sites, the study database, and study documentation may be subject to a Quality Assurance audit by the Sponsor or designee on behalf of the Sponsor. In addition, inspections may be conducted by regulatory bodies at their discretion.

18.3. Data Handling and Recordkeeping

18.3.1. Electronic Data

When using electronic trial data handling and/or remote electronic trial data systems, the Sponsor will:

- Ensure and document that the electronic data processing system(s) conforms to the Sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance (i.e., validation).
- Maintain SOPs for using these systems.
- Ensure that the systems are designed to permit data changes in such a way that the data changes are documented and that there is no deletion of entered data (i.e., maintain an audit trail, data trail, edit trail).
- Maintain a security system that prevents unauthorized access to the data.
- Maintain a list of the individuals who are authorized to make data changes.
- Maintain adequate backup of the data.

- Safeguard the blinding, if any (e.g., maintain the blinding during data entry and processing).
- Documentation regarding electronic systems used in this protocol is available upon request.

18.3.2. Case Report Form Completion

CRFs will be completed for each study subject. It is the Investigator's responsibility to ensure the accuracy, completeness, legibility, and timeliness of the data reported in the subject's CRF. Source documentation supporting the CRF data should indicate the subject's participation in the study and should document the dates and details of study procedures, AEs, and subject status.

Electronic Data Capture (EDC) will be used for the study. Data will be recorded on source documentation at each study location. Data collected on each subject will be documented on the appropriate CRF in the EDC system. Completed CRFs in the EDC system are to be signed off by the Investigator or his/her designee.

18.3.3. Data Handling

If data are transformed during processing, records will be maintained so that it will be possible to compare the original data and observations with the processed data.

An unambiguous subject identification code will be used that allows identification of all the data reported for each subject.

18.3.4. Retention of Study Records

The Investigator must maintain essential study documents (protocol and protocol amendments, completed CRFs, signed ICFs, relevant correspondence, and all other supporting documentation) until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years after the formal discontinuation of clinical development of the investigational product. These documents will be retained for a longer period if required by the applicable regulatory requirements or the hospital, institution, or private practice in which the study is being conducted. Subject identification codes (subject names and corresponding study numbers) will be retained for this same period of time. These documents may be transferred to another responsible party, acceptable to the Sponsor, who agrees to abide by the retention policies. Written notification of transfer must be submitted to the Sponsor. The Investigator or designee must contact the Sponsor prior to disposing of any study records.

18.4. Financing and Insurance

Financing and insurance are addressed in a separate document.

18.5. Confidentiality

To maintain subject privacy, all CRFs, study drug accountability records, study reports and communications will identify the subject by the assigned subject number. The Investigator will grant monitor(s) and auditor(s) from the Sponsor or its designee and regulatory authority(ies) access to the subject's original medical records for verification of data gathered on the CRFs and to audit the data collection process. The subject's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

Subject will be notified that registration information, results, and other information about this study will be submitted to ClinicalTrials.gov, a publicly available trial registry database; however, protected health information of individual subjects will not be used.

All information regarding the investigational product supplied by the Sponsor to the Investigator is privileged and confidential information. The Investigator agrees to use this information to accomplish the study and will not use it for other purposes without consent from the Sponsor. It is understood that there is an obligation to provide the Sponsor with complete data obtained during the study. The information obtained from the clinical study will be used toward the development of the investigational product and may be disclosed to regulatory authority(ies), other Investigators, corporate partners, or consultants as required.

18.6. Publication Policy

The data generated by this study are considered confidential information and the property of Sponsor and shall not be published or disclosed without the prior written consent of Sponsor.

18.7. Direct Access to Source Data

The Investigators/institutions/clinical sites will permit trial-related monitoring, audits, IRB/Ethics Committee review, and regulatory inspections as requested by FDA, the Sponsor, or the Sponsor designee, including direct access to source data/documents (e.g., original medical records, laboratory reports, hospital documents, progress reports, signed ICF) in addition to CRFs.

The Investigator or designee will prepare and maintain adequate and accurate source documents to support all observations and other pertinent data recorded on the CRFs for each subject randomized into the study.

The Investigator will allow the Sponsor (or designee), and authorized regulatory authorities to have direct access to all documents pertaining to the study.

18.8. Protocol Amendments

Changes to the conduct of the study should be prepared as a protocol amendment and implemented only upon approval of the Sponsor, or a representative of the Sponsor. Protocol amendments should also receive written IRB/Ethics Committee approval prior to implementation,

except when necessary to eliminate immediate hazards to the subjects or when the changes involve only logistical or administrative aspects of the trial (e.g., change of monitor, telephone numbers). In this case, the Sponsor will amend and implement the protocol change and subsequently notify the regulatory authorities and/or the IRB/Ethics Committee, as appropriate.

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20. APPENDICES

20.1. Appendix 1: Neurology Assessment by Treating Pediatrician

Gross Motor Skills:

1) Hold head upright against gravity while sitting
   - Child cannot hold head erect for at least 3 seconds without support (Score = 0)
   - Child holds head erect for at least 3 seconds without support (Score = 1)
   - Child holds head erect and steady for at least 15 seconds without support (Score = 2)
2) Roll over
   - Child does not roll front to back or back to front (Score = 0)
   - Child rolls front to back or back to front, but not both (Score = 1)
   - Child rolls front to back and back to front (Score = 2)
3) Sit with support
   - Child cannot sit with support (Score = 0)
   - Child tenses muscles in an effort to maintain sitting position (Score = 1)
   - Child sits with slight support for at least 30 seconds (Score = 2)
4) Sit without support
   - Child cannot sit without support (Score =0)
   - Child sits without support for at least 5 seconds (Score = 1)
   - Child sits without proper support for at least 30 seconds (Score = 2)
5) Stand aided
   - Child cannot stand aided (Score = 0)
   - Child can stand aided (Score = 1)
   - Child raises self to a standing position, using a chair or other convenient object for support (Score = 2)
6) Stand unaided
   - Child cannot stand unaided (Score = 0)
   - Child can stand alone for at least 3 seconds after you release his or her hands (Score = 1)
   - Child comes to a standing position without using any support (Score = 2)
7) Does head lag with dynamic change of position?
   - Child cannot hold head when raised from supine to sitting by pulling on the arms (Score = 0)
   - Child has head lag when raised from supine to sitting by pulling on the arms (Score = 1)
   - Child has no head lag when raised from supine to sitting by pulling on the arms (Score = 2)
8) Peripheral limb function: Hand
   - Child has no hand grip and/or contractures (Score = 0)
   - Child shows finger grip (pincer) (Score = 1)

- Child holds object in hand (Score = 2)

9) Peripheral limb function: Feet
   - Child has contractures of both feet (Score = 0)
   - Child has pes equinus or pes cavus without contracture (Score = 1)
   - Child has no foot deformity (Score = 2)
10) Crawling
   - Child cannot crawl (Score = 0)
   - Child moves from lying prone to being up on hands and knees (Score = 1)
   - Child makes forward progress of at least 5 feet by crawling on hands and knees (Score = 2)
11) Walk aided
   - Child cannot walk with support (Score = 0)
   - Child walks with support by a person and initiates multiple steps (Score = 1)
   - Child walks independently while using or holding onto support (Score = 2)
12) Walk unaided
   - Child cannot walk without support (Score = 0)
   - Child takes at least 3 steps without support, even if gait is stiff-legged and wobbly (Score = 1)
   - Child takes at least 5 steps independently, displaying coordination and balance (Score = 2)

Fine Motor Skills:

1) Reaches for objects
   - Child does not reach for an object (Score = 0)
   - Child extends one or both arms forward to reach object, but does not touch object (Score = 1)
   - Child extends one or both arms forward to reach object, and touches object with any part of either hand (Score = 2)
2) Grasps small objects
   - Child cannot pick up block (Score = 0)
   - Child picks up block using one or both hands (Score = 1)
   - Child uses pad of his or her thumb and any fingertip to grasp block (Score = 2)
3) Picks up food or spoon
   - Child cannot pick up food pellet or spoon (Score = 0)
   - Child grasps food pellet or spoon, but does not bring it to his/her mouth (Score = 1)
   - Child grasps food pellet or spoon and brings it to his/her mouth (Score = 2)
4) Rings bell
   - Child does not reach for bell (Score = 0)
   - Child extends one or both arms forward to reach bell, and touches bell with any part of either hand (Score = 1)
   - Child picks up bell and attempt to ring bell (Score = 2)
5) Transfer objects
   - Child does not grasp ring when handed (Score = 0)
   - Child uses at least one hand to grasp ring for at least 2 seconds (Score = 1)
   - Child grasps ring and transfer from hand to hand (Score = 2)
6) Place one block on another
   - Child does not attempt to place one block on another (Score = 0)
   - Child attempts to place one block on another, but is unsuccessful (Score = 1)
   - Child is successful to place one block on another (Score = 2)

Bulbar Function:

1) Swallows saliva
   - Child drools most of the time, requiring bib or several shirt changes per day (Score = 0)
   - Child drools occasionally (does not require a bib or a shirt change) (Score = 1)
   - Child does not drool (Score = 2)
2) Swallows pureed food
   - Child cannot eat pureed food (Score = 0)
   - Child can occasionally eat pureed food (Score = 1)
   - Child can eat pureed food with no problem (Score = 2)
3) Swallows solid food (including soft foods)
   - Child cannot eat solid food (Score = 0)
   - Child can occasionally eat solid food (Score = 1)
   - Child can eat solid food with no problem (Score = 2)
4) Bite strength
   - Absent (Score = 0)
   - Weak (Score = 1)
   - Strong (Score = 2)
5) Nourishes liquids by syringe or tube feeding
   - Syringe feeding or tube feeding only (Score = 0)
   - Syringe feeding or tube feeding most of the time or occasional (Score = 1)
   - No syringe or tube feeding (Score = 2)
6) Tube feeding
   - Permanent (Score = 0)
   - Occasional (Score = 1)
   - Never (Score = 2)
7) Upper Airway
   - Tracheotomy or CPAP support (Score = 0)
   - Child has sleep apnea (Score = 1)
   - Child has normal sleep respiration (score = 2)

Ocular:

1. Nystagmus
   - Child has nystagmus most of the time (Score = 0)
   - Child has occasional nystagmus (Score = 1)
   - Child has no nystagmus (Score = 2)
2. Strabismus[a]
   - Severe (Score = 0)
   - Moderate (Score = 1)
   - Mild/No Strabismus (Score = 2)
3. Tracks human face
   - Child does not track human face (score = 0)
   - Child fixes gaze on a person for at least 2 seconds (score = 1)
   - Child turns head to follow a person through the room (score = 2)
4. Tracks object
   - Child does not track an object (score = 0)
   - Child's eyes follow an object that is moved horizontally or vertically (Score = 1)
   - Child's eyes follow an object that is moved in a circular motion (Score = 2)
5. Optic atrophy/temporal pallor
   - Child has severe optic atrophy/temporal pallor (Score = 0)
   - Child has moderate optic atrophy/temporal pallor (Score = 1)
   - Child has mild or no optic atrophy/temporal pallor (Score = 2)

[a] Severe Strabismus: Constant exotropia
Moderate Strabismus: Exotropia > 50 % of the exam before dissociation, or Exotropia < 50 % of the exam before dissociation
Mild Strabismus: No exotropia unless dissociated, recovers in > 5 seconds, no exotropia unless dissociated, recovers in 1-5 seconds, or no exotropia unless dissociated, recovers in < 1 second (phoria)

Temporo-frontal:

1) Interacts with parents or examiner
   - Child does not interact with parent or examiner (Score = 0)
   - Child clearly responds to the person's voice (Score = 1)
   - Child actively participates in at least one play routine (Score = 2)
2) Responds to verbal commands
   - Child does not respond to verbal comments (Score = 0)
   - Child stops reaching for objects in response to "no", and does not respond in an appropriate manner to other requests (Score = 1)
   - Child responds in an appropriate manner to at least one spoken request more complex than "no" (does not need to complete task) (Score = 2)
3) Repeats simple sounds
   - Child does not repeat simple sounds (Score = 0)
   - Child repeats a single vocalization only (Score = 1)
   - Child repeats two different, distinct vocalizations (Score = 2)
4) Smiles
   - Child does not smile nor vocalize mood (Score = 0)
   - Child expresses at least one mood (Score = 1)
   - Child's mood or focus can change in response to speaker's attention (Score = 2)
5) What is the child's affect?
   - Sad, distressed or crying a lot (Score = 0)
   - Neutral affect (Score =1)
   - Happy, ebullient, or cooperative (Score = 2)
6) Speaks individual words
   - Child does not speak individual words (Score = 0)
   - Child imitates at least one word, even if imitation consists of vowels only (Score = 1)
   - Child uses at least one word to make wants known (Score = 2)
7) Puts words together
   - Child does not use words (Score = 0)
   - Child uses at least one word to make wants known (Score = 1)
   - Child produces at least one utterance that includes two or more words (Score = 2)
8) Point to objects in a book
   - Child does not attempt to point to an object in a book (Score = 0)
   - Child points to object in a book, but does not identify object that was named (Score = 1)
   - Child points to object in a book that was named (Score = 2)

Autonomic Nervous System:

1) Constipation
   - Child has fewer than 2 bowel movement per week and is dependent on a laxative (Score = 0)
   - Child has 2 or more bowel movements per week and is dependent on a laxative (Score = 1)
   - Child has 2 or more bowel movements per week without a laxative (Score = 2)
2) Urinary
   - Indwelling catheter or dependent upon catherization (Score = 0)
   - Catherization no more than once per day (Score = 1)
   - No catherization required (Score = 2)

20.2. Appendix 2: Parental Clinical Impression Scale

Category	Milestone	Markedly Worse (-2)	Slightly Worse (-1)	Unchanged since last visit (0)	Slightly Better (+1)	Markedly Better (+2)	Score
Gross Motor	Hold head upright against gravity while sitting						
	Roll over						
	Sit with support						
	Sit without support						
	Stand aided						
	Stand unaided						
	Crawl						
	Walk aided						
	Walk unaided						
Category Total							
Fine Motor	Reaches for objects						
	Grasps small objects						
	Picks up food or spoon						
	Rings bell						
	Transfers objects between hands						
	Place one block on another						
Category total							

Category	Milestone	Markedly Worse (-2)	Slightly Worse (-1)	Unchanged since last visit (0)	Slightly Better (+1)	Markedly Better (+2)	Score
Bulbar	Can swallow saliva (control of drooling)						
	Swallows pureed food						
	Swallows solid food						
	Strong bite						
	Nourish liquids without syringe or feeding tube						
	Can nourish without feeding tube						
Category Total							
Ocular	Are eye movements normal?						
	"Lazy" eye in one or both eyes						
	Tracks human face						
	Tracks object						
Category total							
Temporo-frontal	Interacts with parents/family						
	Responds to verbal commands						
	Repeats simple sounds						
	Smiles and is happy						
	Speaks individual words						
	Puts words together						
	Point to objects in a book						
Category total							
Grand Totals							

Parental Global Clinical Impression Scale

1) How are the symptoms of your child's disease compared to when your child was not taking study medication?	
Markedly worse	1
Slightly worse	2
About the same	3
Slightly better	4
Markedly better	5
2) How satisfied are you with the effect of your child's study medication?	
Not satisfied at all	1
A little bit satisfied	2
Somewhat satisfied	3
Very satisfied	4
Completely satisfied	5

20.3. Appendix 3: Assessment of Severity by Parent or Caregiver

Category	Milestone	Never (+1)	Rarely (+2)	Sometimes (+3)	Frequently (+4)	Score
Gross Motor	Hold head upright against gravity while sitting					
	Roll over					
	Sit with support					
	Sit without support					
	Stand aided					
	Stand unaided					
	Crawl					
	Walk aided					
	Walk unaided					
Category Total						
Fine Motor	Reaches for objects or bell					
	Picks up food or spoon					
	Grasps small objects					
	Rings bell					
	Transfers objects between hands					
	Place one block on another					
	Point to objects in book					
Category total						

Category	Milestone	Never (+1)	Rarely (+2)	Sometimes (+3)	Frequently (+4)	Score
Bulbar	Can swallow saliva (control of drooling)					
	Swallows pureed food					
	Swallows solid food					
	Strong bite					
	Can nourish without syringe or tube feeding					
	Can nourish without feeding tube					
Category Total						
Ocular	Are eye movements normal?					
	No "Lazy" eye in one or both eyes?					
	Tracks human face					
	Tracks object					
Category total						
Temporo-frontal	Interacts with parents/family					
	Responds to verbal commands					
	Repeats simple sounds					
	Smiles and is happy					
	Speaks individual words					
	Puts words together					
	Point to objects in a book					
Category total						
Grand Totals						

Describe any changes in your child over the last month for the following categories.
Sleep:
Feeding:
Social skills (how he/she responds & interacts with you and others:
Pain and distress:
Mobility:
Movement of limbs:
Vision and hearing:
Breathing:

Please describe any additional improvements or regressions observed in your child over

the last month.

20.4. Appendix 4: Parental Visual Analogue Scale for Child's mood

Rate your child's mood in the last month. Please circle the appropriate happy/sad face.

0 = Unhappy mood is very typical. Child cries, frowns or whines most of the time.
1 = Unhappy mood is somewhat typical. Child cries, frowns or whines some of the time.
2 = Mood is neutral. Child cries, frowns or whines rarely, but more often than smiles
3 = Mood is neutral. Child smiles and laughs rarely, but more often than cries.
4 = Happy mood is somewhat typical. Child smiles and laughs some of the time.
5 = Happy mood is very typical. Child smiles and laughs most of the time. Child shows enthusiasm or excitement in response to parent's attention.