CLINICAL TRIAL PROTOCOL

An Open-Label Study of the Safety, Tolerability, and Pharmacokinetics of Oral NNZ-2591 in Pitt Hopkins Syndrome

Protocol Number:	NEU-2591-PTHS-001
Drug Development Phase:	2
Protocol Version No.:	3.0
Release Date:	October 30, 2023
NCT Number:	NCT05025332
Sponsor Address:	Neuren Pharmaceuticals Ltd. Suite 501, 697 Burke Road Camberwell, VIC 3124 Australia
Medical Monitor:	[redacted]
Program Director:	[redacted]

Clinical trial No. Neu-2591-PTHS will be conducted in accordance with this protocol, ICH GCP, as published in the Federal Register on 09 May, 1997, and all applicable regulations.

CONFIDENTIALITY STATEMENT

All information concerning the Investigational Medicinal Product, supplied by Neuren Pharmaceuticals Ltd. in connection with this study and not previously published, is considered confidential and proprietary information. This information includes the Investigator's Brochure, Clinical Protocol, and Case Report Forms. This confidential information shall remain the sole property of Neuren Pharmaceuticals Ltd. and shall not be disclosed to others without prior written consent from Neuren Pharmaceuticals Ltd. and shall not be used except in the performance of this study.

The information developed during the conduct of the clinical study is also considered confidential and will be used by Neuren Pharmaceuticals Ltd. in connection with the development of the Investigational Medicinal Product. This information may be disclosed as deemed necessary by Neuren Pharmaceuticals Ltd.

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1. LIST OF ABBREVIATIONS

2MWT	2-Minute Walk test
AAC	Augmentative and alternative communication
ABC-2	Aberrant Behavior Checklist 2
AE	Adverse event
[redacted]	[redacted]
ALP	Alkaline phosphatase
ALT	Alanine amino transferase
AMSE	Autism Mental Status Exam
APTT	Activated Partial Thromboplastin
AS	Angelman syndrome
ASD	Autism Spectrum Disorder
AST	Aspartate transaminase
AUC	Area under (concentration-time) curve to last time point
AUC_{inf}	Area under (concentration-time) curve from time zero extrapolated to infinite time
AUC_{0-24}	Area under (concentration-time) curve from time 0 to 24 hours at steady state
AUC_{tau}	AUC from time zero to the end of the dosing interval
bHLH	Basic helix–loop–helix
BID	Twice daily
BP	Blood pressure
BPI-SF	Behavior Problems Inventory-Short Form
bpm	Beats per minute
BR	Bilirubin
BSID-4	Bayley Scales of Infant Development version 4
BUN	Blood urea nitrogen
CAS	Chemical Abstracts Service
CBC	Complete Blood Count
CBD	Cannabidiol
CDKL5	Cyclin-dependent kinase-like 5
CdLS	Cornelia de Lange syndrome
CFR	Code of Federal Regulations
CGI-I	Clinical Global Impression Scale – Improvement
CGI-S	Clinical Global Impression – Severity
cGP	Cyclic Glycine-Proline
[redacted]	[redacted]
CLss/F	Apparent total body clearance after oral administration
CL_{tv}	True population value for clearance of the drug
C_{max}	The maximum observed blood concentration
CMP	Comprehensive metabolic panel
CoRDS	Coordination of Rare Diseases
COVID-19	Coronavirus disease 2019
CSHQ	Child Sleep Habits Questionnaire

C_{ss}	Concentration at steady-state
C_t	Concentration at the last measurable time point
C_{tv}	True population value for clearance of the drug
C_τ	Concentration at the end of the dosing interval
$^{\circ}C$	Degree(s) centigrade
CVA	Costovertebral angle tenderness
DBP	Diastolic blood pressure
dL	Deciliter
DNA	Deoxyribonucleic acid
DSM-5	Diagnostic and Statistical Manual of Mental Disorders, 5th Edition
DSMC	Data Safety Monitoring Committee
DQ	Development quotient
EC	Expressive communication
ECG	Electrocardiogram
eCRF	Electronic case report form
EDC	Electronic Data Capture System
eGFR	Estimated glomerular filtration rate
EL	Expressive language
EOT	End of Treatment
FDA	Food and Drug Administration
FM	Fine motor
FR	Fluid reasoning
FSIQ	Full scale intellectual quotient
g	Gram
GCP	Good clinical practice
GERD	Gastroesophageal reflux disease
GGT	Gamma-glutamyl transferase
GI	Gastrointestinal
GIHQ	Gastrointestinal Health Questionnaire
GM	Gross motor
GMP	Good manufacturing practice
GRAS	Generally Recognized as Safe
GSV	Growth scale values
Hb	Hemoglobin
HbA1C	Glycated (or glycosylated) hemoglobin
Hct	Hematocrit
HDPE	High-density polyethylene
hr, hrs	Hour, hours
HPF	High power field
HTN	Hypertension
IB	Investigator’s brochure
ICH	International Council on Harmonisation
ICMJE	International Committee of Medical Journal Editors
ICND	Impact of Childhood Neurological Disability Scale

IGF-1	Insulin-Like Growth Factor 1
IND	Investigational new drug
IMP	Investigational medicinal product
IQ	Intelligence quotient
IRB	Institutional review board
ITT	Intent to treat
IUD	Intrauterine device
[redacted]	[redacted]
kg	Kilogram
KN	Knowledge
L	Liter
m	Meter
MAO	Monoamine oxidase
MB-CDI	MacArthur-Bates Communicative Development Inventory
MCH	Mean cell hemoglobin
MCHC	Mean cell hemoglobin concentration
MCV	Mean cell volume
MDMA	Methylenedioxymethamphetamine
MedDRA	Medical Dictionary of Regulatory Activities
mg	Milligram
MIS-C	Multisystem Inflammatory Syndrome in Children
mL	Milliliter
mmHG	Millimeter of mercury
mmol/L	Millimole per liter
ms	Millisecond
N	Number of participants
NCA	Non-compartmental analysis
NDD	Neurodevelopmental disorder
NGAL	Neutrophil gelatinase-associated lipocalin
NHANES	National Health and Nutrition Examination Survey
NOAEL	No adverse effect level
NVIQ	Nonverbal IQ
ORCA	Observer-Reported Communication Ability
OTC	Over-the-counter
PHRF	Pitt Hopkins Research Foundation
PK	Pharmacokinetic
PLT	Platelets
PMS	Phelan-McDermid syndrome
popPK	Population pharmacokinetics
PP	Per protocol
PTHS	Pitt-Hopkins syndrome
QI-Disability	Quality of Life Inventory-Disability
QR	Quantitative reasoning
QTcF	Fridericia's correction factor for QT

RBC	Red blood cell count
RDW, RDW CV	Red cell distribution width
RL	Receptive language
rpm	Respirations per minute
SAE	Serious adverse event
SAER	Serious adverse event report
SAP	Statistical analysis plan
SARS-CoV-2	Severe acute respiratory syndrome coronavirus
SB5	Stanford-Binet Intelligence Scales version 5
SBP	Systolic blood pressure
SCQ	Social Communication Questionnaire
SUSAR	Suspected Unexpected Serious Adverse Events
$t_{1/2}$	Terminal elimination half-life
T_3	Triiodothyronine
T_4	Thyroxine
TCF4	Transcription Factor 4
TEAE	Treatment emergent adverse event
THC	Tetrahydrocannabinol
T_{max}	Time to maximal concentration
TSH	Thyroid stimulating hormone
UA	Urinalysis
UK	United Kingdom
ULN	Upper limit of normal
US	United States
VIQ	Verbal intelligence quotient
VR	Visual reception
VS	Visual-spatial
WBC	White blood cell count
WM	Working memory
$WT_{centered}$	Population value for the weight in the adult population corresponding to the derived CL_{TV}

2. PROTOCOL APPROVAL

Sponsor Statement

This study protocol was subject to critical review and has been approved by the following persons:

[redacted]

I approve this document
31 October 2023 | 1:04 PM PDT

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3. PROTOCOL SYNOPSIS

Protocol Neu-2591-PTHS-001 Phase 2: Pediatric Safety, Tolerability, and Pharmacokinetic Study	
Study Protocol Title	An Open-Label Study of the Safety, Tolerability, and Pharmacokinetics of Oral NNZ-2591 in Pitt-Hopkins Syndrome
Sponsor	Neuren Pharmaceuticals Limited
Investigational Product Research Number	NNZ-2591
Generic Name	Cyclo-L-Glycyl-2-Allyl Proline (cG-2-AllylP)
Protocol Number	Neu-2591-PTHS-001
NCT Number	NCT05025332
IND Number	155461
Clinical Phase (Trial Type)	Phase 2
Treatment Indication	Pitt-Hopkins syndrome (PTHS)
Primary Objective	• To investigate the safety, tolerability, and pharmacokinetics (PK) of treatment with NNZ-2591 Oral Solution, 50mg/mL Investigational Medicinal Product (IMP) in children and adolescents with PTHS
Secondary Objectives	• To investigate measures of efficacy during treatment with NNZ-2591 Oral Solution, 50 mg/mL IMP in children and adolescents with PTHS
Trial Design	Neu-2591-PTHS-001 is an open-label study of the safety, tolerability, and PK of NNZ-2591 Oral Solution, 50mg/mL in male and female children and adolescents with PTHS. Participants receive treatment of orally administered NNZ-2591 for a total of 13 weeks. <u>Overall Design</u> There are a total of 17 study visits over the duration of the study, 5 in-clinic visits, and 12 remote/in-home visits (see Table 1). Remote visits between the Investigator or designee at the clinical site and the participant at home are conducted via telemedicine or video facilities

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	as appropriate. In-home visit refers to a visit made to the participant's home by a visiting nurse. The study will commence with an approximately 4 to 6 week Screening and Baseline period. During this Screening/Baseline period, participants will be assessed for study eligibility. Rescreening of participants is allowable under certain circumstances with the approval of the Sponsor and Medical Monitor. During the Screening/Baseline period data will also be collected to establish the participant's baseline characteristics and symptom severity based on assessments collected during the 4 to 6 week period. There are two in-clinic visits and one remote/in-home visit during the Screening and Baseline period (Visits 1, 2, 3). Once eligibility is confirmed, participants will be dosed at a starting dose of 4 mg/kg twice-daily (BID) and up-titrated to a target dose of 12 mg/kg BID of orally administered NNZ-2591. The mg/kg dosing is based on the participant's weight at Baseline (Visit 3). Participants will receive treatment with NNZ-2591 for a total of 13 weeks. During the treatment period there are three in-clinic visits: Week 2 (Visit 5), Week 6 (Visit 9) and Week 13 (Visit 16). There is a remote visit by the Investigator via telemedicine and a same-day in-home visit by a visiting nurse at Week 1, Week 4, Week 8, and Week 10. Additionally, there are in-home visits by a visiting nurse at Week 3, Week 5, Week 7, Week 9, Week 11, and Week 12. All participants will have a combined remote Follow-Up visit with the Investigator via telemedicine and an in-person home visit by a visiting nurse approximately 2 weeks after the end of treatment (Week 15, Visit 17). For all visits designated as in-clinic, an in-person visit is preferred. All requests for off-site assessments due to extenuating circumstances, such as related to the Coronavirus-disease (COVID-19) health emergency, must be approved by the Sponsor or Medical Monitor in advance. <u>Enrollment Groups</u> For enrollment, the participants will be divided into three groups by age: 13–17-year-olds (Group 1), 8–12-year-olds (Group 2), and 3–7-year-olds (Group 3). Enrollment will commence in the oldest age group (Group 1). After at least three participants in Group 1 have received two weeks of treatment with the IMP at the starting dose (4 mg/kg BID), safety and tolerability data will be reviewed by the Data Safety Monitoring Committee (DSMC). If tolerability and safety data are deemed acceptable, enrollment for Group 2 will proceed and

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	dosing will begin at the starting dose. When at least three participants in Group 2 have received the first two weeks of treatment at the starting dose, safety and tolerability data will be reviewed by the DSMC. If tolerability and safety data for Group 2 are deemed acceptable, enrollment for Group 3 will commence and dosing will begin at the starting dose.
COVID-19	As a result of the COVID-19 public health emergency, this protocol includes provision for alternative methods of performing safety and efficacy assessments in accordance with the FDA *Guidance on Conduct of Clinical Trials of Medical Products during the COVID-19 Public Health Emergency* January 27, 2021.[*] Provision is made for off-site assessments where necessary, and all efforts have been made to ensure the alternative processes are consistent as much as possible with in-clinic visits. For visits that are designated as in-clinic, an actual in-clinic visit is preferred. However, an in-home/remote visit may be conducted for a scheduled in-clinic visit with the prior approval of the Sponsor and Medical Monitor. Clinical investigators are to document the reason for any contingency measures implemented. If at any time in the study, the participant or his or her caregiver tests positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or becomes ill with symptoms of COVID-19, the family should be instructed to contact the site as soon as possible. Once informed, the Investigator should contact the Medical Monitor.
Study Population	Approximately 10-20 male and female participants between the ages of 3 and 17 years are anticipated to complete the study. The participants will be categorized by age in three groups: 13 to 17-year-olds (Group 1), 8 to 12-year-olds (Group 2), and 3 to 7-year-olds (Group 3). Enrollment will be balanced to the extent possible by sex and age with a minimum of 3 males and 3 females across all age groups, and a minimum of 3 participants in each age group.
Inclusion Criteria	To be eligible for this study, participants must meet all of the inclusion criteria and none of the exclusion criteria. Inclusion criteria include: 1. Clinical diagnosis of PTHS with a documented disease-causing genetic etiology for the disorder. 2. Males or females ages 3 to 17 years at screening, inclusive.

[*] FDA Guidance on Conduct of Clinical Trials of Medical Products During the COVID-19 Public Health Emergency: Guidance for Industry, Investigators, and Institutional Review Boards (Center for Biologics Evaluation and Research; Center for Devices and Radiological Health; Center for Drug Evaluation and Research; Office of the Commissioner; Office of Clinical Policy and Programs; Office of Clinical Policy; Office of Good Clinical Practice) (2021).

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	3. Body weight of 12 kg or higher at Screening. Weight measurements may be repeated during the Screening period in consultation with the Medical Monitor. 4. Participants with a Clinical Global Impression – Severity (CGI-S) score of 4 or greater at the Screening visit. 5. Not actively undergoing regression or loss of skills, defined as no persistent loss of previously acquired developmental skills for a period within 3 months of the Screening visit. a. Previously acquired skills include any skill established and used on a daily basis for at least 3 months. b. Variations in use of skills that is part of a general pattern of ups and downs, as determined by the investigator, are not counted as regression. c. Skills may include language, non-verbal communication, fine motor skills, ambulation (including gait, coordination, independence of walking/standing), social skills, play, self-help. 6. Each participant must be able to swallow the IMP provided as a liquid solution. 7. The participant's caregiver(s) must have sufficient English language skills to complete caregiver assessments in English.
Exclusion Criteria	To be eligible for this study, participants must meet all of the inclusion criteria and none of the exclusion criteria. Exclusion criteria include: ***Laboratory and Screening Values*** 1. Body weight < 12 kg at Screening. Weight measurements may be repeated during the Screening period in consultation with the Medical Monitor. 2. HbA1c values above 7% at the Screening visit. 3. Clinically significant abnormalities (as determined by the Investigator) in safety laboratory values, or vital signs, as measured at the Screening visit. Laboratory testing may be repeated during the Screening period in consultation with the Medical Monitor. 4. Positive pregnancy test at the Screening visit. 5. QTcF Exclusions (any of the following): a. Screening QTcF interval of >450 ms assessed using Fridericia's correction factor based on 12-lead electrocardiogram (ECG) obtained as a continuous ECG of at least 50 seconds. b. Hypokalemia (Serum potassium at the Screening visit <3.0 mmol/L or mEq/L). Serum potassium may be repeated during the Screening period with the agreement of the Medical Monitor.

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c. QTcF prolongation that is currently controlled with medication, in which normal QTcF intervals can only be achieved with medication.

6. Any other clinically significant finding (as determined by the Investigator) on ECG at the Screening visit that would increase the likelihood of a severe arrhythmia.

COVID-19

7. Positive for SARS-CoV-2 at Screening or Baseline.
8. Previous COVID-19 infection within the last 12 months that required admission to the hospital.
9. Previous COVID-19 infection involving multi-organ systems, resulting in Multisystem Inflammatory Syndrome in Children (MIS-C) or with clinically significant sequelae.
10. Previous COVID-19 infection associated with acute kidney injury (AKI) or renal manifestations (e.g. proteinuria, hematuria, echogenic kidneys).

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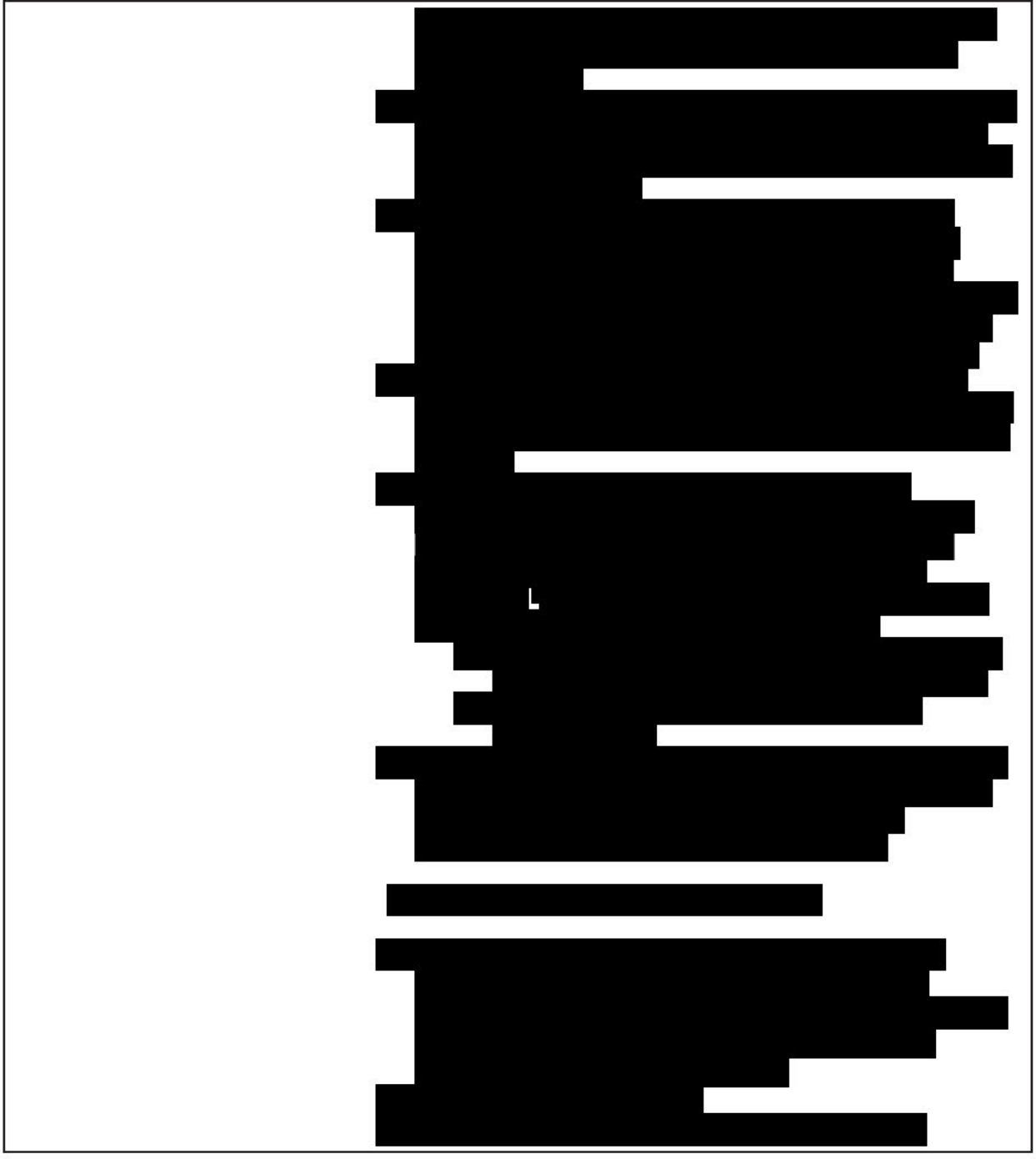

Protocol Neu-2591-PTHS-001 Phase 2: Pediatric Safety, Tolerability, and Pharmacokinetic Study

[redacted]

Concomitant Treatments

28. Current treatment or treatment within the two weeks prior to the Screening visit with MAO inhibitors, d-cycloserine, oxytocin, carbetocin, tricyclic antidepressants, or bupropion.
29. Current treatment with more than 3 allowable psychotropic medications.
    a. Includes medications used to treat problems with sleep onset and sleep continuity except melatonin. Melatonin for difficulties with sleep onset is permissible and is not included in the count of psychotropic medications.
    b. The use of anti-epileptics for other indications such as the treatment of mood disorders counts toward the limit of permitted psychotropic medications.
    c. Anti-epileptic medications used for treatment of seizures does not count toward the limit of psychotropic medications.
30. Current treatment with insulin or treatment with insulin within the last 12 weeks prior to commencement of IMP.
31. Current treatment with insulin-like growth factor 1 (IGF-1) or treatment within 6 months of commencement of the IMP.
32. Current treatment with growth hormone or treatment within 12 months of commencement of the IMP.
33. Nonpharmacologic somatic treatment (e.g., a ketogenic diet or vagal nerve stimulation) whose dosing regimen has not been stable at least 4 weeks prior to the Screening visit. If the treatment was discontinued, the discontinuation must have occurred no fewer than 2 weeks before the Screening visit.
34. Participants planning to start a new behavioral, educational, or cognitive therapy during the period of the study or those who have begun a new behavioral or cognitive therapy regimen within 4 weeks prior to the Screening visit.
    a. Note: changes to an on-going treatment regimen that are due to school schedules or are otherwise seasonally related are not exclusionary as long as changes are consistent with their typical type and pattern of behavioral treatment.

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35. Seizure medication whose dosing regimen is not stable 8 weeks prior to commencing IMP. Adjustments for changes in weight or age are allowable. If the treatment was discontinued, the discontinuation must have occurred no fewer than 2 weeks before the Screening visit.
36. Psychotropic medications or any other medication used for a chronic illness (not including antibiotics, pain relievers, anti-diarrheals, and laxatives) whose doses and dosing regimen have not been stable for at least 4 weeks prior to the Screening visit. If the treatment was discontinued, the discontinuation must have occurred no fewer than 2 weeks before the Screening visit.
    a. This includes nutraceutical treatments and cannabinoids (tetrahydrocannabinol [THC] and cannabidiol [CBD]) which are allowable but also must meet the stability conditions above.
    b. Pain relievers, laxatives, and anti-diarrheals may be used as needed.
    c. Standard of care immunizations and vaccinations (including COVID-19) are allowed and are not subject to the above stability conditions.
    d. The Investigator should consult with the medical monitor if a participant is being treated with ocular toxic, hepatotoxic, nephrotoxic or antibiotic medications.
    e. If the Investigator is unsure if a medication is permitted, the Medical Monitor should confirm if the medication is allowed and will provide written documentation to support the use of the medication, if allowed.

Regression of Skills

37. Actively undergoing regression or loss of skills defined as a persistent loss of previously acquired developmental skills within 3 months of the Screening visit.
    a. Previously acquired skills include any skill established and used on a daily basis for at least 3 months.
    b. Variations in use of skills that is part of a general pattern of ups and downs (as determined by the Investigator) are not counted as regression.
    c. Skills may include language, non-verbal communication, fine motor skills, ambulation (including gait, coordination, independence of walking/standing), social skills, play, self-help.

Seizures

38. Seizure profile that is not stable 8 weeks prior to commencing IMP. The overall pattern of seizure activity for the participant should be consistent with their typical pattern of seizures and be stable within the 8-week period in terms of type, intensity, duration, and frequency as determined by the clinical Investigator.

Medical Conditions

39. Currently pregnant, lactating or breastfeeding.
40. Current clinically significant (as determined by the Investigator) cardiovascular, gastrointestinal, respiratory, or endocrine disease or clinically significant organ impairment. The Investigator may consult with the Medical Monitor as needed if he/she is uncertain if symptoms are exclusionary.
41. Current clinically significant (as determined by the Investigator) hypo- or hyperthyroidism. If the participant has hypo- or hyperthyroidism that is controlled with medication, the Investigator should consult with the Medical Monitor to confirm if the participant is eligible.
42. Type 1 or Type 2 diabetes mellitus requiring insulin (whether well controlled or uncontrolled), or uncontrolled Type 1 or Type 2 diabetes.
43. Has planned surgery during the study.
44. History of, or current, cerebrovascular disease or brain trauma.
45. History of, or current catatonia or catatonia-like symptoms.
46. History of, or current, malignancy.
47. Current major or persistent depressive disorder (including bipolar depression).
48. Significant uncorrected hearing impairment.

Other Criteria

49. Allergy to strawberry.
50. The participant is unable to take the IMP orally and swallow the IMP provided as a liquid solution. Administration of the IMP by nasogastric tube, feeding tube, thickened liquid, or gastrostomy tube is not allowed.
51. Has participated in another interventional clinical study within 30 days prior to the Screening visit.
52. Prior enrollment in this clinical trial.
    a. Individuals who leave the study during the Screening period and have not entered the study may be able to rescreen pending review and approval of the Sponsor and Medical Monitor.
    b. Individuals who were deemed eligible and entered the study (enrolled) who discontinue the study may not re-enroll in the study.

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53. The participant is judged by the Investigator or the Medical Monitor to be inappropriate for the study for any reason.

Investigational Product

NNZ-2591 Oral Solution, 50mg/mL will be administered open-label at the target dose of 12 mg/kg BID. The mg/kg dosing is based on the participant's weight at Baseline (Visit 3). Participants will be up-titrated as outlined in the table below.

Dosing Schedule

Week 1 and Week 2	Week 3	Week 4 and Week 5	Week 6	Week 7 and Week 8	Week 9	Week 10 to Week 13
4 mg/kg BID (8 mg/kg daily)	4 mg/kg BID (8 mg/kg daily)[1]	8 mg/kg BID (16 mg/kg daily)	8 mg/kg BID (16 mg/kg daily)[1]	12 mg/kg BID (24 mg/kg daily)	12 mg/kg BID (24 mg/kg daily)	12 mg/kg BID (24 mg/kg daily)
	DSMC Reviews Data Prior To Up-titration [2]		**DSMC Reviews Data Prior To Up-titration [2]**		**DSMC Reviews Data After 2 Weeks on 12 mg/kg to Confirm Safety[2]**	

BID = twice daily; DSMC = Data Safety Monitoring Committee
[1]Participants will stay on their current dose until the data is reviewed by the DSMC.
[2]Expected time for DSMC review will be one week.

The dose titration schedule may be modified based on updated pharmacokinetic modelling analyses including data from the study.

The IMP will be administered at a starting dose of 4 mg/kg BID for a total of approximately three weeks. After two weeks of dosing at the staring dose, the data will be reviewed by the DSMC, and the participants will stay on the starting dose while the data are reviewed. If safety and tolerability are deemed acceptable, participants will be up-titrated to 8 mg/kg BID and be dosed for a total of approximately three additional weeks. After two weeks of dosing at 8 mg/kg, the data will be reviewed by the DSMC, and the participants will stay on the 8 mg/kg dose while the data are reviewed. If safety and tolerability are deemed acceptable, participants will be up-titrated to12 mg/kg BID and remain on this dose for seven weeks, The DSMC will determine if dose escalation can proceed based on pre-specified stopping rules detailed in the body of the protocol. The total administration period for NNZ-2591 will be 13 weeks. For staggered enrollment, the

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	participants will be divided into three groups by age: 13 to 17-year-olds (Group 1), 8 to 12-year-olds (Group 2), and 3 to 7-year-olds (Group 3). The DSMC will review and approve the start of enrollment in the younger age groups. NNZ-2591 is provided as a ready-to-use strawberry flavored liquid for oral administration. The IMP should not be mixed with water, liquid thickeners, or food for administration. The dose can be taken from a cup or dosing syringe. Doses should be given on an empty stomach (2 hours after the previous meal or snack and 30 minutes prior to the next). The first dose of IMP will be administered in the clinic after all Baseline assessments are completed, or, if the Investigator judges that it is too late in the day, on the following day. The participant should be monitored for at least three hours after the first dose. If the first dose is administered the day following Baseline, vital signs should be collected before the dose is administered. Day 1 of dosing is defined by the date when the first dose is taken. At this visit, the site will provide IMP for the caregiver to dispense at home. Enough IMP will be provided to cover the interval until the next in-clinic visit when the caregiver will be re-supplied from the site. The protocol includes procedures for dose titration and dose adjustments, if any are necessary (Section 7.2), and pre-specified stopping criteria (i.e., discontinuation of IMP) consistent with the exclusion criteria, including QTcF, renal, ocular, and hepatic, exclusion criteria (Section 9.4.5).
Endpoints	Primary Endpoints Safety: The incidence of AEs and SAEs from commencement of IMP administration through to the time of the last study visit will be evaluated. Vital signs, laboratory measures, urine output history, physical exam results (neurological, ophthalmologic, and general), [redacted], and ECGs will also be evaluated from screening/baseline through the end of the study. Tolerability will be assessed based on caregiver reports and the Caregiver Diary, required dosing adjustments or discontinuations, dosing compliance, and AEs related to participant experience. Pharmacokinetics: Pharmacokinetic blood samples will be collected according to the age appropriate sampling schedule for each participant (See Table 2 and Table 3). Pharmacokinetic concentrations and exposure parameters for NNZ-2591 will be derived by sparse non-compartmental analysis

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(NCA), and may include maximum concentration (C_{max}), area under the concentration-time curve from time 0 to the end of the dosing period (AUC_{tau}) and other parameters.

Efficacy:

- **Overall Improvement:**
  - PTHS-specific Clinical Global Impression Scale–Improvement (CGI-I), Overall Improvement Scores; clinician-assessed
  - PTHS-specific Clinical Global Impression Scale – Domain Improvement Scores; clinician-assessed
  - Caregiver Global Impression Change Scores; caregiver-assessed
- **PTHS Symptom Severity**:
  - PTHS-specific Clinical Global Impression Scale–Severity (CGI-S)–Overall; clinician-assessed
  - PTHS-specific Clinical Global Impression Scale–Severity (CGI-S)– Domain; clinician-assessed
  - Caregiver Top 3 Concerns Likert Scale Scores; caregiver-assessed
- **Communication**:
  - MacArthur-Bates Communicative Development Inventory (MB-CDI); caregiver-assessed
  - Observer-Reported Communication Ability Measure (ORCA); caregiver-assessed
- **Maladaptive Behavior**:
  - Aberrant Behavior Checklist-2; caregiver-assessed
  - Behavioral Problems Inventory-Short Form (BPI-SF); caregiver-assessed
- **Sleep**: Child Sleep Habits Questionnaire (CSHQ) Scores; caregiver-assessed
- **GI**: Gastrointestinal Health Questionnaire (GIHQ); caregiver-assessed
- **Adaptive Behavior**: Vineland Adaptive Behavior Scales-3, Comprehensive Interview version; clinician-assessed
- **Motor:**
  - Bayley Scales of Infant Development 4 (BSID-4) motor scale, clinician-assessed (for participants who are assessed on the BSID-4)
  - Modified two-minute walk test; clinician-assessed
- **Quality of Life (Child and Caregiver)**
  - Quality of Life Inventory-Disability (QI-Disability); caregiver-assessed
  - Impact of Childhood Neurological Disability (ICND)-Overall quality of life rating; caregiver-assessed

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	Biomarkers and Microbiome Samples Blood and stool samples will be collected for analysis of biomarkers and gut microbiome. The analysis will be detailed in a separate analysis plan but will not include DNA sequencing. Samples will be collected at Screening (Visit 1), Baseline (Visit 3, before dosing) and at the End of Treatment (EOT) visit, or upon early termination.
Sample Size Estimation	Sample sizes were estimated for this open-label study using within-participant change as the measure of improvement. Given the limited data from interventional trials in these patient populations, and that this is the first clinical study of NNZ-2591 IMP in this population, treatment change estimates were based on CGI-I data from completed clinical trials in other rare, neurodevelopmental disorders. Based on the information provided in Glaze et al. 2019 (Double-blind, randomized, placebo-controlled study of trofinetide in pediatric Rett syndrome), the standard deviation of the CGI-I is approximately 0.7 at a single timepoint.[1] For a sample size of 10 participants, a two-sided significance level of 0.05, and a Wilcoxon signed-rank test, the study will be able to detect a difference of 0.87 units of change in the CGI-I at a power of 0.90. For a power of 0.80, 0.85, and 0.90, the detectable difference would be 0.75, 0.80, and 0.87 or larger, respectively. The effect size for a power of 0.80, 0.85, and 0.90 is 1.07, 1.14, and 1.24, respectively. If the observed standard deviation is larger, the detectable differences would also be larger. For example, if the standard deviation is 1.5, the detectable difference for a power of 0.80, 0.85, and 0.90 is 1.61, 1.71, and 1.86 or larger, respectively. For a sample size of 20 participants, a two-sided significance level of 0.05, and a Wilcoxon signed-rank test, the study will be able to detect a difference of 0.55 units of change in the CGI-I at a power of 0.90. For a power of 0.80, 0.85, and 0.90, the detectable difference would be 0.48, 0.51, and 0.55 or larger, respectively.
Statistical Methods	Safety Adverse events and other safety data will be summarized as frequencies and percentages, described in terms of severity and potential attribution. Objective safety assessments will be summarized over time on study; shift tables at time points of interest may be generated. Details of the safety analyses will be specified in the SAP. Efficacy Efficacy will be assessed by looking at within-participant changes from the Baseline period to the EOT. The participant's baseline characteristics and symptom severity will be established based on assessments collected during the 4 to 6 week Screening/Baseline period. Baseline values will be based on the average values of visits 1, 2, and3 for assessments collected at more than one visit. A Wilcoxon

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	signed rank test will be used to test for improvement and a significance level will be computed. Potentially influential factors will be considered and addressed with a linear regression model. This model may include covariates such as age, sex, NVIQ or NVIQ equivalent, genotype, history of regression (yes/no), ASD diagnosis yes/no with no more than two covariates included in any model. Descriptive statistics and corresponding graphics will be used to present the results. The efficacy analysis details will be specified in the SAP. <u>Pharmacokinetics:</u> NNZ-2591 PK concentrations will be listed by participant and time point for each dose level with actual time they were acquired. The concentrations will be summarized by time, dose level and age group. For the concentrations measured at trough points dose, relationship will be examined statistically with age group and/or body weight of participant as covariate. Overall exposure parameters for NNZ-2591 such as C_{max} and AUC_{tau} may be derived by sparse non-compartmental analysis (NCA). Additionally, PK concentrations of NNZ-2591 measured in pediatric participants will be used to refine a population PK model developed from adult NNZ-2591 PK data and extrapolated to the pediatric population. After refinement, complete profiles for each participant will be simulated using range of variability of model parameters and individual covariate effects. A standard NCA approach will be used for simulated profiles to calculate parameters C_{max}, AUC_{tau}, etc. for characterization of NNZ-2591 exposure in pediatric participants. The PK analysis details will be specified in the PK Statistical Analysis Plan (PK SAP). Biomarkers and Microbiome: Biomarker analysis will be detailed in the SAP and will not include DNA sequencing. Microbiome analysis will be described in a separate analysis plan.
Trial Sites	Approximately 4-7 sites in the US.

4. SCHEDULE OF ASSESSMENTS

Table 1 Schedule of Assessments for Neu-2591-PTHS

Period	Screening/ Baseline[a] 4 to 6 weeks		Baseline	Treatment Period													Follow-up[b]
Visit Week	-4 to -6	Visit 1 +2 wks	0	1	2	3	4	5	6	7	8	9	10	11	12	13/ EOT[b]	15
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Visit window (days)	±2[a]	±2[a,c]	±1[a]	±1	±2	±1	±1	±1	±2	±1	±1	±1	±1	±1	±1	-3	+4
Type of Visit	Clinic[d]	Remote and In-Home[e,f]	Clinic[d]	Remote and In-Home[e,f]	Clinic[d]	In-Home[f]	Remote and In-Home[e,f]	In-home[f]	Clinic[d]	In-Home[f]	Remote and In-Home[e,f]	In-Home[f]	Remote and In-Home[e,f]	In-Home[f]	In-Home[f]	Clinic[d]	Remote and In-Home[e,f]
Informed consent (mandatory)	X																
Consent for reuse of leftover Biomarker and PK samples (optional)	X																
Inclusion/exclusion criteria	X		X														
Medical history	X	X	X														
Confirm documented PTHS diagnosis and genotype	X																
PTHS history and exam	X																
Confirm comorbid psychiatric disorders by DSM-5	X																
Rapid SARS-CoV-2 test	X		X													X	
Autism Mental Status Exam	X															X	
Recent clinical history				X	X		X		X		X		X			X	X

Period	Screening/ Baseline[a] 4 to 6 weeks		Baseline	Treatment Period													Follow-up[b]
Visit Week	-4 to -6	Visit 1 +2 wks	0	1	2	3	4	5	6	7	8	9	10	11	12	13/ EOT[b]	15
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Visit window (days)	±2[a]	±2[a,c]	±1[a]	±1	±2	±1	±1	±1	±2	±1	±1	±1	±1	±1	±1	-3	+4
Type of Visit	Clinic[d]	Remote and In-Home[e,f]	Clinic[d]	Remote and In-Home[e,f]	Clinic[d]	In-Home[f]	Remote and In-Home[e,f]	In-home[f]	Clinic[d]	In-Home[f]	Remote and In-Home[e,f]	In-Home[f]	Remote and In-Home[e,f]	In-Home[f]	In-Home[f]	Clinic[d]	Remote and In-Home[e,f]
Physical examination[g]	X		X		X		X[h]		X		X[h]		X[h]			X	X[h]
Targeted Physical Exam[f,g]		N		N		N	N	N		N	N	N	N	N	N		N
Neurological examination	X		X		X		X[h]		X		X[h]		X[h]			X	X[h]
[redacted]																	
Ophthalmic examination[i]	X															X	
Vital signs	X	N	X	N	X	N	N	N	X	N	N	N	N	N	N	X	N
Height/length	X		X													X	
Weight	X	N	X	N	X	N	N	N	X	N	N	N	N	N	N	X	N
Review for AEs/SAEs[j]				X, N	X	N	X, N	N	X	N	X, N	N	X, N	N	N	X	X, N
Caregiver Diary[k]	X	X	X	X, N	X	N	X	N	X	N	X	N	X	N	N	X	X, N
IMP administration[c]			X	Twice daily													
Review dosing compliance documentation in diary[l]				X, N	X	N	X, N	N	X	N	X, N	N	X, N	N	N	X	
12-lead ECG	X		X		X				X							X	N
CBC with differential	X		X		X		N		X		N		N			X	N
Coagulation[m]	X		X		X		N		X		N		N			X	N
Comprehensive metabolic panel[n]	X		X	N	X	N	N	N	X	N	N	N	N	N	N	X	N
Urinalysis[o]	X		X	N	X	N	N	N	X	N	N	N	N	N	N	X	N
Urine screen for drugs of abuse[o]	X																

Period	Screening/ Baseline[a] 4 to 6 weeks		Baseline	Treatment Period													Follow-up[b]
Visit Week	-4 to -6	Visit 1 +2 wks	0	1	2	3	4	5	6	7	8	9	10	11	12	13/ EOT[b]	15
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Visit window (days)	±2[a]	±2[a,c]	±1[a]	±1	±2	±1	±1	±1	±2	±1	±1	±1	±1	±1	±1	-3	+4
Type of Visit	Clinic[d]	Remote and In-Home[e,f]	Clinic[d]	Remote and In-Home[e,f]	Clinic[d]	In-Home[f]	Remote and In-Home[e,f]	In-home[f]	Clinic[d]	In-Home[f]	Remote and In-Home[e,f]	In-Home[f]	Remote and In-Home[e,f]	In-Home[f]	In-Home[f]	Clinic[d]	Remote and In-Home[e,f]
TSH, Free T3, Free T4	X															X	
HbA1c	X															X	
Serum pregnancy test[p]	X								X							X	
Blood samples for PK[q]					X				X							X	
Blood sample for biomarkers[r]	X		X													X	
Stool sample for microbiome	X		X													X	
CGI-I									X							X	X
CGI-S	X		X						X							X	X
Stanford-Binet Intelligence Scales version 5 (SB5) or Bayley Scales of Infant Development ver 4[r]	X															X	
MB-CDI			X													X	
ORCA			X													X	
Caregiver Top 3 Concerns	X	X	X						X							X	X
ABC-2	X	X	X						X							X	X
BPI-SF			X													X	
Caregiver Global Impression of Change																X	
QI-Disability			X													X	

Period	Screening/ Baseline[a] 4 to 6 weeks		Baseline	Treatment Period													Follow-up[b]
Visit Week	-4 to -6	Visit 1 +2 wks	0	1	2	3	4	5	6	7	8	9	10	11	12	13/ EOT[b]	15
Visit Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Visit window (days)	±2[a]	±2[a,c]	±1[a]	±1	±2	±1	±1	±1	±2	±1	±1	±1	±1	±1	±1	-3	+4
Type of Visit	Clinic[d]	Remote and In-Home[e,f]	Clinic[d]	Remote and In-Home[e,f]	Clinic[d]	In-Home[f]	Remote and In-Home[e,f]	In-home[f]	Clinic[d]	In-Home[f]	Remote and In-Home[e,f]	In-Home[f]	Remote and In-Home[e,f]	In-Home[f]	In-Home[f]	Clinic[d]	Remote and In-Home[e,f]
ICND-Overall quality of life rating			X													X	
CSHQ	X	X	X						X							X	X
Vineland Adaptive Behavior Scales-3			X													X	
GIHQ	X	X	X						X							X	X
Modified two-minute walk test	X		X													X	
Study Exit Form																	X[s]

X = Conducted by site personnel or caregiver as applicable
N = Conducted by visiting nurse
Abbreviations: ABC-2 = Aberrant Behavior Checklist-2; AE = adverse event; BPI-SF = Behavior Problems Inventory-Short Form; CBC = Complete Blood Count; CGI-I = Clinical Global Impression Scale-Improvement; CGI-S = Clinical Global Impression Scale-Severity; CMP = Comprehensive Metabolic Panel; CSHQ = Child Sleep Habits Questionnaire; DSM-5 = Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition; ECG = electrocardiogram; EOT = end of treatment; GIHQ = GI Health Questionnaire; ICND = Impact of Childhood Neurological Disability; IMP = Investigational Medicinal Product; MB-CDI = MacArthur-Bates Communicative Development Inventory; ORCA = Observer-Reported Communication Assessment; PK = pharmacokinetic; PTHS = Pitt-Hopkins syndrome; QI-Disability = Quality of Life Inventory-Disability; SAE = serious adverse events; SB5 = Stanford Binet Intelligence Scales ver 5; T3 = triiodothyronine; T4 = thyroxine; TSH = thyroid stimulating hormone.

[a] No assessments can be conducted until after informed consent has been given by the caregiver or legally authorized representative. Training for the caregivers on all caregiver completed assessments must be done before the caregiver/rater may complete the assessments. The Screening/Baseline period will be conducted over 4 to 6 weeks. The second visit during the Screening period (Visit 2) should be conducted 2 weeks ± 1 day after the Screening visit (Visit 1). The participant will then remain in Screening for an additional 2 to 4 weeks. The caregiver

diary must be completed daily during the entire Screening/Baseline period whether it is 4 weeks or 6 weeks. The Screening visit (Visit 1) and Baseline visit (Visit 3) may be conducted over three consecutive days. It is preferrable that ECGs, safety labs and biomarker sample collection are conducted on the same day. All assessments must be completed within the window for the visit (visit day ± 2 days). For Visit 3, all assessments must be completed before administration of the IMP.

[b] The End of Treatment visit is conducted when the participant is still on IMP treatment. For early termination, in addition to study completers, participants who have received IMP for longer than 2 weeks and discontinue prematurely any time after Visit 5 should return to the investigational site for final safety and efficacy assessments as scheduled for the EOT visit (Visit 16) and complete the study exit form.

[c] At Visit 3, the first dose of IMP will be administered in the clinic after all Baseline assessments are completed, or, if the Investigator judges that it is too late in the day, on the following day. The participant should be monitored for at least three hours post first dose. If the first dose is administered the day following Baseline, vital signs (except height) should be collected before the dose is administered. Day 1 of dosing is defined by the date when the first dose is taken. At the Baseline visit, the site will dispense an adequate supply of IMP for dispensing by the caregiver at home. Dosing at the end of Baseline counts as Day 1 whether one or two doses are given. Dosing may not exceed 91 days including Day 1, and all efficacy assessments for the EOT visit must be completed on or before Day 91 of treatment.

[d] A clinic visit is preferred but an off-site visit may be conducted for a scheduled in-clinic visit in extenuating circumstances due to the COVID-19 health crisis. All requests for off-site visit assessments due to extenuating circumstances must be approved in advance by the Sponsor or Medical Monitor. The in-clinic visits may be conducted over three consecutive days. All assessments including caregiver-completed assessments must be done within the window for the visit.

[e] This is a combined remote and in-person nurse visit. The remote visit is conducted by the site via telemedicine or video facilities as appropriate. Any caregiver-completed assessments should be completed the same day of the visit or no earlier than the day before the visit (except the Caregiver Diary which is completed daily) and reviewed by the Investigator. A modified version of the physical and neurological exam will be done at the remote visits based on the guidance documents provided to the Investigator. At the Week 4, the site should confirm with the caregiver that the child has started the new dose if they were approved for dose titration. These combination remote/in-home visits will be conducted simultaneously whenever possible, or they will ideally be conducted on the same day. If that is not possible, the telemedicine visit and the nurse visit may be conducted over two consecutive days. A combined remote visit may be conducted in-clinic by the study staff if agreed upon by the Investigator and the caregiver.See Section 9.1 and Section 9.2.

[f] In-home visits will be conducted by a visiting nurse. At every nurse visit, the nurse will collect urine, vital signs including weight, and blood samples for a comprehensive metabolic panel (CMP). The nurse will also conduct a targeted physical exam assessing general appearance, abdominal/CVA tenderness, and edema. A suprapubic exam (bladder palpation) to assess for urinary retention may be performed. The visiting nurse will also review the dosing diary, query oral intake, and do a review of tolerability. Blood samples for CBC with differential, and coagulation will be collected at Weeks 4, 8, 10 and Week 15 (indicated by “N” in the Schedule of Assessments). At the post-treatment follow-up visit at Week 15, the visiting nurse will also collect ECG, in addition to the above assessments. At Week 7, the site will also have a phone call with the caregiver to confirm that the child has started the new dose if they were approved for dose titration. The site will confirm the visiting nurse has

been informed so the dosing schedule can be reviewed with the family during the in-home visit. A nurse-only in-home visit may be conducted in-clinic by the study staff if agreed upon by the Investigator and the caregiver. See Section 9.1and 9.2.

[g] The in-clinic physical exam will include an abdominal exam and an assessment of CVA. A suprapubic exam (bladder palpation) to assess for urinary retention may be performed. General awareness will be captured as part of the neurological exam. For in-home nursing visits, the nurse will do a Targeted Physical exam assessing general appearance (e.g., alertness), an abdominal/CVA, and edema. A suprapubic exam (bladder palpation) to assess for urinary retention may be performed.

[h] A modified version of the neurological and physical exam will be done for the remote visit based on the guidance documents provided to the Investigator.

[redacted]

[j] The visiting nurse will query any changes in tolerability during the in-home visit. Any changes will be noted and provided to the Investigator. The Investigator will also query changes in tolerability and note any changes in the remote assessment. The Investigator is responsible for determining and reporting any changes that qualify as Adverse Events.

[k] The caregiver fills out the diary daily from the first day of screening through two weeks after the EOT Visit. X denotes review of the Caregiver Diary including the daily summary log, medication log, non-medicinal treatments log, seizure log and new/changes in symptoms log. All visits: The Investigator (or appropriately credentialed designee) is responsible for reviewing for ALL visits. In-home visits: At the in-home nurse visit, the nurse will review the Caregiver Diary to flag and check any issues that could be reported as AEs. The nurse will notify the site about any urgent issues the day of the visit and note anything else in the source documents. The nurse does not sign the diary. The source documents and the diary will be sent to the site. The Investigator (or appropriately credentialed designee) will review the diary and source documents collected at the in-home visit.

Note: There are two rows for Caregiver Diary review procedures (to emphasize the importance of thorough and complete dosing documentation): this row (Caregiver Diary) denotes that the caregiver fills out the diary daily and a designated clinician reviews it for safety/tolerability; a second row (Review dosing compliance documentation in diary, see footnote “l”) is intended to ensure that the caregiver is administering IMP/documenting dose properly in the Caregiver Diary.

[l] The daily dosing log in the Caregiver Diary will be reviewed at all post-baseline visits through EOT. If any doses are missed or mis-dosed, this should be queried with the caregiver and appropriate feedback given to the family. This feedback should be documented in the source documents. At the in-home nurse visit, the nurse will review the caregiver diary to check any issues and provide feedback to the family at the visit. The nurse will notify the site about any urgent issues the day of the visit and note anything else in the source documents. The nurse does not sign the daily dosing log/summary page. The source documents and the diary will be sent to the site. The Investigator (or appropriately credentialed delegee) will review the diary and source documents collected at the in-home visit.

[m] Coagulation includes prothrombin time, activated partial thromboplastin (aPTT), and international normalized ratio (INR).

[n] A Comprehensive Metabolic Panel will be assessed every post-enrollment visit (see Section 9.5.2). If abnormal findings are observed on renal or liver function tests, additional tests as is clinically indicated may be conducted as described in Sections 9.5.2.1 and 9.5.2.3).

[o] Both urinalysis and a urine screen for drugs of abuse will be evaluated at Screening (See Section 9.5.2).

[p] For female participants who have reached menarche.

[q] See Table 2 and Table 3 for PK blood collection times.

[r] The Bayley is done for participants who cannot attain a basal score on SB5 or for whom the SB5 is developmentally inappropriate.

[s] The study exit form should also be completed if the participant terminates the study early.

Table 2 Pharmacokinetic Blood Sampling Schedule (Participants Ages 7 to 17 Years Old)

Week	Visit No.	Treatment	Timing of Sample (relative to start of dosing)[a]	Sample Time Point
2	5		Pre dose (-120 to -15 minutes)[b]	1
			1-3 hours post dose[c]	2
			4-7 hours post dose[c]	3
6	9	Mid-treatment	Pre dose (-120 to -15 minutes) [b]	4
			1-3 hours post dose[c]	5
			4-7 hours post dose[c]	6
13	16	End of Treatment	Pre dose (-120 to -15 minutes)[b]	7
			1-3 hours post dose[c]	8
			4-7 hours post dose[c]	9

[a] All post-dose windows are determined from the first dose of the visit (morning dose) and all PK samples must be collected before the second dose (PM dose).

[b] Pre dose counts as both T0 and 12 hours post dose from previous evening (near trough). The previous evening's dose should be taken in the evening as late as possible, preferably before bedtime.

[c] Post-dose sample collection can be +/- 15 minutes of the target time and must be at least 30 minutes from the previous sample (samples can be no closer than 30 minutes apart). The actual collection time must be recorded.

Table 3 Pharmacokinetic Blood Sampling Schedule (Subects Ages 3 to <7 Years Old)

Week	Visit No.	Treatment	Collection Window	Timing of Sample (relative to start of dosing)[a]	Sample Time Point	Comment
2	5			Pre dose (-120 to -15 minutes)[b]	1	
			A	1-3 hour post dose[c]	2	Sample 2 can be taken at either collection window A or B[d]
			OR	OR		
			B	4-7 hours post dose[c]		
6	9	Mid-treatment		Pre dose (-120 to -15 minutes) [b]	3	
			A	1-3 hours post dose[c]	4	Sample 4 can be taken at either collection window A or B[d]
			OR	OR		
			B	4-7 hours post dose[c]		
13	16	End of Treatment		Pre dose (-120 to -15 minutes)[b]	5	
			A	1-3 hours post dose[c]	6	Sample 6 can be taken at either collection window A or B[d]
			OR	OR		
			B	4-7 hours post dose[c]		

[a] All post-dose windows are determined from the first dose of the visit (morning dose) and all PK samples must be collected before the second dose (PM dose).

[b] Pre dose counts as both T0 and 12 hours post dose from previous evening (near trough). The previous evening's dose should be taken in the evening as late as possible, preferably before bedtime.

[c] Post-dose sample collection can be +/- 15 minutes of the target time and must be at least 30 minutes from the previous sample (samples can be no closer than 30 minutes apart). The actual collection time must be recorded.

[d] For the 3 to <7 year-old age range, two sampling timepoints are taken at each visit. The post-dose sample may be taken at either collection window A or collection window B. Ideally, it is better that the second sample is not taken at the same collection window at each visit. So, an attempt should be made to vary the collection time of the second sample as is feasible. For example, if on visit 5 the second sample is taken in collection window B, at visit 9 or 16, the second sample should ideally be taken in collection window A.

5. INTRODUCTION

5.1 Pitt-Hopkins Syndrome

Pitt-Hopkins syndrome (PTHS) is a rare, neurodevelopmental disorder characterized by developmental delays, moderate to profound intellectual disability, absent/limited speech, distinctive facial dysmorphology, and breathing problems such as episodic hyperventilation and/or breath-holding while awake. Other common symptoms may include repetitive behaviors and social communication impairments, constipation, severe near-sightedness (myopia), sleep disturbances, and seizures.[2, 3]

Individuals with PTHS have distinct facial features that are important elements of the diagnostic criteria, including a square face with narrow forehead, thickened helix, wide nasal bridge, beaked nose characterized by down-turned nasal tip, flared nasal alae and wide nasal tip, full cheeks, wide mouth, full lips and a cupid bow upper lip.[3-7] Myopia is observed in the majority of patients (48-88%) and strabismus and astigmatism are also observed.[4, 6-11]

Individuals with PTHS demonstrate severe developmental delay in motor milestones including delayed ambulation and delayed, or ultimately absent, speech.[8] While moderate to profound intellectual disability has been reported across the literature, most individuals have severe to profound cognitive impairments. Mental age equivalents for development level that have been reported as between 3.5 to 36 months for individuals between 32 months and 32 years old.[9, 12] The majority of individuals (68%) have absent or limited speech (less than 5 words) and all have impaired speech.[4, 6, 8, 9] While between 46-70% of individuals are reported to be independently ambulatory, the majority of individuals have gait abnormalities including a wide-based ataxic gait, as well as impaired motor coordination and hypotonia.[4, 6, 8-11, 13, 14] Adaptive function for individuals with PTHS is low with developmental equivalent scores of 20 or less.[12]

Breathing abnormalities are a characteristic feature and affect 45%-86% of individuals with PTHS, in particular hyperventilation or hyperventilation with apnea (46%) when awake.[3, 6-11, 15] GI problems are also highly prevalent, and can be severe, with around 35% needing corrective treatment.[13] Chronic constipation, with an onset as early as infancy,[8, 9] is particularly common (67%-85% of patients).[6-11, 15]

Seizures present another challenge for 30%-51% of individuals with PTHS.[4, 6, 8-11, 13, 15] The range of seizure types includes generalized tonic-clonic, atonic, focal onset, and infantile spasms.[6, 8, 10, 16-18] Additionally, between 18%-42% of individuals with PTHS experience some type of sleep disturbance, typically insomnia or night awakenings.[6, 8]

Individuals with PTHS face a range of behavioral challenges.[3, 4, 6-8, 10] Compared to Angelman Syndrome (AS) and Cornelia de Lange syndrome (CdLS), other genetically based disorders with similar early signs and features, the behavioral phenotype of PTHS is particularly characterized by the following: 1) a high level of Autism Spectrum Disorder (ASD) symptomatology 2) social communication difficulties, 3) atypical sensory processing, and 4) high levels of physical aggression and self-injury.[13] Aggression and aggressive behavior are observed in 40%-54% of individuals,[8, 11-13] and in 38% of individuals, this has included destruction of property.[13] Between 60-71% of individuals demonstrate self-injurious behaviors.[6, 9, 11] Anxiety (81%) and agitation (88%) are also common symptoms.[6]

Up to 95% of individuals met the cut off score for possible ASD on the Social Communication Questionnaire (SCQ) screener for ASD.[13] Individuals have also been shown to score high on the Autism Diagnostic Interview measure.[12] On the SCQ, they demonstrated more problems with communication and reciprocal social interaction than individuals with AS. They also showed lower sociability with both

familiar and unfamiliar people compared to individuals with AS, and with familiar people compared to individuals with CdLS.[13] Individuals with PTHS also have atypical sensory processing. In response to behaviors in social contexts, the majority of individuals are hyporesponsive, but are hyperresponsive to sensory input.[13]

Stereotypic behaviors are highly prevalent (54%-94%)[3, 6, 8, 10, 12, 13] and include object stereotypy,[3, 13] body stereotypies such as body rocking (54%-78%) and hand and finger stereotypies (48-85%) including clapping, flapping (up to 80%), hand-wringing/hand-washing (45%), hand mouthing or biting (54%), finger movements (54%), and wrist movements (45%).[6-9, 12, 13] Head shaking and head banging have also been reported.[6, 9]

5.2 Current Treatments for Pitt-Hopkins Syndrome

No methods exist to prevent the onset of PTHS, nor is there any cure or approved medication for treatment for PTHS.

Prescribed medications for individuals with PTHS are for the management of specific symptoms such as seizures or GI problems,[3, 8] and do not address the underlying dysfunction of the disorder. GI problems of constipation and GERD are treated using standard treatments such as laxatives, stool softeners and protein pump inhibitors.[3, 8] Seizures are commonly treated with standard antiepileptics such as valproic acid, levetiracetam, lamotrigine, and carbamazepine.[3, 9, 19] There are no systematic recommendations for the use of psychotropic medications to treat behavioral problems, but caregivers report using methylphenidate, clonidine and benzodiazepine for agitation/irritability and anti-psychotics for challenging behaviors.[3, 8] Mood-stabilizing medications and anti-epileptics have been used for treating breathing abnormalities,[8] and melatonin has been used for sleep issues.[3, 8]

Non-medication strategies for the treatment of PTHS are typically multimodal and require the involvement of multiple clinicians with diverse areas of expertise.[2, 3] In centers that specialize in the treatment of individuals with PTHS, the standard of care includes physical, occupational, behavioral and speech therapies, as well as targeted approaches to orthopedic concerns and gastrointestinal dysfunction and seizures, as is reflected in recently published international guidelines.[3] Establishment of an effective long-term care plan that can be implemented across settings, which includes pediatric medical follow-up and a transition plan for adult care, is a recommended approach to the treatment of individuals with PTHS.[2, 3, 20]

5.3 Genetic Basis of Pitt-Hopkins Syndrome

Pitt-Hopkins syndrome is caused by loss of function of the transcription factor 4 (*TCF4*) gene due to heterozygous hypomorphic or null mutations, or up to 12 Mb interstitial deletions on chromosome 18q including the *TCF4* gene, which result in *TCF4* haploinsufficiency.[3, 6, 10, 11, 14, 18, 21, 22] The majority of disease-causing genetic changes are de novo.[2, 3, 11]

TCF4 is expressed in neurons and glial cells and it encodes a basic helix–loop–helix (bHLH) transcription factor that is known to heterodimerize with several other bHLH transcription factors that play important roles in neurogenesis and neuronal migration in the brain, regulating glial cell differentiation, and regulating immune cell function.[14]

TCF4 is highly expressed during embryonic and early postnatal development[18] and has particularly high expression in the hippocampus.[23-25] It is also expressed in adult brain, lymphocytes, fibroblasts, gut, muscle, and myenteric plexus.[18, 21, 26, 27] Recent cognitive and imaging studies have also shown that *TCF4* is important for normal brain function.[25, 28]

Deletions and mutations of the *TCF4* gene disrupt the corresponding protein's ability to control the downstream activity of genes related to nervous system development and function.[14] Crux et al's[29] work on the consequences of functional loss of *TCF4* on dendritic spines in mature neurons showed, with both homo- and heterozygous loss of *TCF4*, a reduction in the number of dendritic spines and changes in their morphology. This work suggested that *TCF4* plays an important role in synaptic plasticity in mature neurons, independent of its developmental function, and functional loss of *TCF4* may contribute to the neurological symptoms in PTHS.

5.4 Scientific Rationale for use of NNZ-2591 in Pitt-Hopkins Syndrome

NNZ-2591 (Cyclo-L-Glycyl-L-2-Allylproline) is a new chemical entity that is a synthetic analogue of cyclic Glycine-Proline (cGP), a metabolite of Insulin-Like Growth Factor 1 (IGF-1) that is present naturally in the brain. IGF-1 signaling pathways appear to be altered as a result of the changes in *TCF4* that occur in individuals with PTHS, in particular the down regulation of genes encoding IGF binding proteins, 3, 4, and 5.[30] Cyclic Glycine-Proline has been reported to regulate binding of IGF-1 to IGF binding protein 3 in the brain and, as a consequence, regulate the bioavailability of IGF-1.[31] This auto-regulatory mechanism maintains homeostasis of IGF-1, increasing bioavailability when IGF-1 is deficient and decreasing bioavailability when IGF-1 levels are excessive. Both cGP and NNZ-2591 also inhibit neuroinflammation which is part of the pathology underlying PTHS. Neuroinflammation contributes to over-activation of microglia that are critical for synaptic development and maintenance. Across numerous animal models, NNZ-2591 normalizes the microglial phenotype which helps restore synaptic function and morphology.

The objective of introducing exogenous NNZ-2591 into the brain is to circumvent the *TCF4* deficiency, mimicking the natural actions of cGP by rescuing the abnormal dendritic morphology and stimulating protein synthesis in excitatory synapses through the following mechanisms:

- Reducing neuroinflammation and pathological glial activation
- Reducing abnormal activation of AKT upstream of mTOR in the PI3K-AKT-mTOR pathway
- Reducing abnormal activation of ERK in the MAPK-ERK signaling pathway
- Restoring normal levels of IGF-1

5.5 Preclinical Data

5.5.1 Overview of Pharmacology and Toxicology Findings

A battery of safety pharmacology and toxicology studies conducted *in vitro* and *in vivo* in mice, rats and dogs has been conducted, providing safety support for the progression of the drug Phase 2 clinical trials. The nonclinical safety studies suggest that NNZ-2591 is unlikely to have adverse effects in humans in the cardiovascular system, nervous system, or respiratory system, nor in skeletal, motor, or reproductive function. NNZ-2591 has been tolerated in repeat-dose toxicology studies at doses up to 2000 mg/kg/day in juvenile rats and 400 mg/kg/day in adult dogs for 14 days and up to 400 mg/kg/day in juvenile rats and 120 mg/kg/day in adult dogs for 13 weeks.

Studies of NNZ-2591 in juvenile animals have demonstrated a favorable safety profile. In a 13-week study in juvenile rats there were no NNZ-2591-related adverse effects [redacted]

In the 14-day study in adult dogs as well as the 13-week studies in adult dogs and juvenile rats, there was a decrease in body weight and food consumption in high dose animals that resolved during the 4-week

recovery period. High dose dogs showed an increase in abnormal feces which again resolved during the recovery period.

In vitro metabolism studies have shown NNZ-2591 to be highly stable to metabolism in human hepatic microsomes, hepatocytes and human plasma. NNZ-2591 has also been shown not to be significantly metabolized by human CYP450 enzymes. *In vivo* studies in rats have shown NNZ-2591 to be excreted primarily (>99%) via the kidney with less than 0.3% in bile and feces. These results confirm that metabolism is not a significant route of elimination.

In vitro cell permeability and in vivo PK studies have shown NNZ-2591 to be approximately 100% orally bioavailable, and NNZ-2591 has demonstrated the ability to cross the blood brain barrier.

5.5.2 Overview of Pitt-Hopkins Mouse Model Findings

NNZ-2591 has shown efficacy in mouse models of the neurodevelopmental disorders Phelan-McDermid, Pitt Hopkins, Prader-Willi and Angelman syndromes. Summaries of these studies are presented in the NNZ-2566 Investigator's Brochure (IB).

Oral administration of NNZ-2591 for 6 weeks has demonstrated the ability to rescue the phenotype of the *TCF4+/-* knockout mouse model of PTHS, while having no impact on wild type, littermate controls. In *TCF4+/-* mice, impairments in analogous syndrome-relevant symptoms of motor function (locomotive activity and hind limb force), repetitive behaviors, measures of daily living, and social interaction were corrected by 6 weeks of treatment with NNZ-2591 at 100 mg/kg twice daily (BID). Learning and memory was additionally rescued at 200 mg/kg BID. These symptom areas are relevant and clinically important to the human phenotype of PTHS.

NNZ-2591 has been assessed in three other animal models of synaptopathies and demonstrated rescue of the phenotype in the measures assessed. Oral administration of NNZ-2591 for 6 weeks has demonstrated the ability to rescue the phenotype of the *Shank3B* knockout mouse model of Phelan McDermid syndrome (PMS), while having no impact on wild type control mice. Improvements were seen in syndrome-relevant symptoms of motor function, repetitive behaviors, social interaction, adaptive behaviors, and memory/learning. In a separate study of the PMS mouse model, 3 weeks of treatment with NNZ-2591 corrected levels of activated ERK and IGF-1 to wild type levels and normalized dendritic spine morphology. In a mouse model of AS, oral administration of NNZ-2591 for 6 weeks demonstrated the ability to rescue the phenotype of the *Ube3a*$^{m-/p+}$ knockout mouse model of AS, while having no impact on wild-type control mice. Improvement was seen in analogous symptoms relevant and clinically important to the human phenotype including cognition, adaptive behaviors, motor function, and seizures. In a mouse model of Fragile X syndrome, 28 days of treatment with NNZ-2591 normalized the behavioral

phenotype of *fmr1* knockout mice and corrected impairment of AKT/ERK activation and abnormal dendritic morphology.

A comprehensive battery of safety, pharmacology, and toxicology studies conducted in mice, rats and dogs has also been conducted, providing support for the progression of the drug into a Phase 2 clinical trial in a pediatric population with PTHS. Summaries of these studies are presented in the NNZ-2591 IB.

5.6 Clinical Data

NNZ-2591 has been studied in 28 healthy male and female adult volunteers. Study Neu-2591-HV-001 was a combined Phase 1 study, with an open-label single dose stage and a double-blind, randomized, multiple ascending dose-escalation stage with 12 participants enrolled in 2 open-label single-dose cohorts and 16 participants enrolled in 2 randomized, double-blind, placebo-controlled multiple dose cohorts. The single ascending dose stage of the Phase 1 study evaluated doses of 12.5 mg/kg and 50 mg/kg. The two cohorts comprising the multiple ascending dose stage of the Phase 1 study evaluated twice-daily dosing at 3 mg/kg for 1 day followed by 6 mg/kg for 6 days (Cohort M1) and 6 mg/kg for 1 day, then 9 mg/kg for 1 day followed by 12 mg/kg for 5 days (Cohort M2).

Twice daily dosing for 5 days at 12 mg/kg following up-titration for 1 day at 6 mg/kg and 1 day at 9 mg/kg was found to be safe and generally well-tolerated. There were no deaths, Serious Adverse Events (SAEs), or severe Treatment Emergent adverse events (TEAEs) reported during the conduct of this study. All adverse events (AEs) were mild or moderate. No clinically significant laboratory, ocular, physical examination, vital sign, or cardiac abnormalities were reported. There was a single discontinuation due to moderate AEs of somnolence and ataxia after the administration of one dose. The participant's two TEAEs resolved after treatment was withdrawn.

The most common TEAEs deemed related to drug were a cluster of apparently related neurological symptoms that included somnolence/drowsiness, blurred vision/difficulty focusing, ataxia/unsteady gait, dizziness, and vertigo. The majority of these TEAEs occurred within 2-3 hours of a participant's first dose. All resolved spontaneously without requiring medical intervention, most within 24 hours from onset. This pattern suggests that these TEAEs are an acute phenomenon temporally correlated with initial T_{max} rather than being associated with cumulative exposure and that participants possibly develop tolerance to these neurological Aes. There were no clinically significant findings on the laboratory tests or electrocardiograms (ECGs), and no renal or hepatic findings were observed.

PK appeared to be linear and predictable, with peak and total exposures (as measured by C_{max} and area under the plasma concentration-time curve [AUC], respectively) appearing to increase in a generally dose proportional manner after both the morning and evening doses.

A summary of the study is provided in the NNZ-2591 IB.

In addition to this study, NNZ-2591 is being assessed in Phase 2 studies in Phelan McDermid syndrome, Angelman Syndrome, and Prader-Willi Syndrome. In these open label studies participants receive BID treatment for a total of 13 weeks. The dose is up-titrated from 4 mg/kg (three weeks of treatment) to 8 mg/kg (three weeks of treatment) to 12 mg/kg (7 weeks of treatment). As of the date of the last annual report to FDA, a total of 16 participants had been dosed across this study, (PTHS-001) and the Angelman Syndrome and Phelan McDermid Syndrome studies. There were no SAEs or Adverse Events of Special Interest (renal, hepatic or ocular) reported. There have been two discontinuations.

6. STUDY RATIONALE AND OBJECTIVES

6.1 Study Rationale

Pitt-Hopkins syndrome (PTHS) is a rare genetic neurodevelopmental disorder caused by the loss of function of the *TCF4* gene, and the resulting reduction in the number and morphology of dendritic spines and the synaptic plasticity in mature neurons.[29] Characterized by developmental delays, moderate to profound intellectual disability, absent/limited speech, distinctive facial dysmorphology, and breathing problems,[2, 3] PTHS causes lifelong difficulties for the individuals affected by the condition and their families. There is currently no approved treatment for PTHS.

In pre-clinical pharmacodynamic studies, NNZ-2591 has demonstrated the ability to improve impaired synaptic structure and signaling as well as rescuing the neurobehavioral phenotype. NNZ-2591 is a synthetic analogue of cGP that inhibits neuroinflammation and restores synaptic function and plasticity. NNZ-2591 has better stability than cGP, readily crosses the blood-brain barrier and has high bioavailability when administered orally. Therefore, the purpose of this study is to investigate the safety, tolerability, and pharmacokinetics (PK) of treatment with oral NNZ-2591 Oral Solution, 50 mg/mL IMP in children and adolescents with PTHS.

6.2 Rationale for Dose Selection and Route of Administration

6.2.0 Rationale for Dose Selection

Selection of the dose schedule of NNZ-2591 IMP that will be utilized in this study is informed by:

- Results of toxicity studies which assessed the effects of oral NNZ-2591 in dogs and juvenile rats with 13 weeks of dosing and a 4-week recovery period. These studies, summarized in the IB, found that the No Adverse Effect Level (NOAEL) of NNZ-2591 was 200 mg/kg/day in juvenile rats and 120 mg/kg/day in adult dogs. At the NOAEL, no NNZ-2591-related clinical signs of toxicity considered adverse, or mortality occurred and no non-reversible effects on the nervous system, skeletal system, renal or reproductive system were observed.

- Pharmacodynamic studies in *Tcf4*$^{+/-}$ mice and wild-type, littermate controls found that 6 weeks of dosing at 100 mg/kg BID and 200 mg/kg BID were effective in reversing cognitive and neurobehavioral deficits analogous to the human PTHS phenotype.

- The safety, tolerability, and PK of NNZ-2591 Oral Solution as determined by the First-in-Human (FIH) Phase 1 study. NNZ-2591 appeared to be safe and generally well-tolerated when administered orally twice daily for 7 days at doses up to 12 mg/kg BID. There were no SAEs. All AEs were mild or moderate. There were no NNZ-2591 related abnormal laboratory results, ECGs, or vital signs and no sentinel safety issues. This study is summarized in the IB.

- The results of allometric scaling of exposure at steady state (AUC_{tau}) in healthy, adult volunteers at 12 mg/kg indicate that exposures for the pediatric population at weights lower than 70 kg are estimated to be lower than those observed for the adult population in the Phase 1 study at 12 mg/kg. Therefore, dosing all children at a maximum of 12 mg/kg BID will not put them at risk of exposures higher than that experienced by healthy adults. At the same time, the predicted exposures are expected to be in the range of what was identified as a potentially efficacious dose from the animal models so there is a prospect of benefit with dosing at the maximum proposed dose of 12 mg/kg BID. A summary of the allometric scaling analysis is presented in Appendix A.

- Data from a parallel PK study in the same background strain of mice utilized in the pharmacodynamic study was conducted to support comparison of exposure between mice and humans. This study, combined with PK data from the Phase 1 study, resulted in an estimate that a dose of approximately 10 mg/kg BID in adults should result in daily exposure equivalent to 100 mg/kg BID in the mouse. The weight-based doses in the Phase 2 study are intended to result in a distribution of exposures equivalent to the 12 mg/kg dose in adults that will provide a reasonable opportunity for efficacy as well as being predicted to be safe and well-tolerated.

In this Phase 2 study, NNZ-2591 Oral Solution, 50 mg/mL will be administered orally BID over the course of 13 weeks. Participants will be up-titrated according to the dosing schedule outlined in Section 7.2. The dose titration schedule may be modified based on updated PK modelling including data from the study.

6.2.1 Rationale for Duration of Dosing

Given the overall safety and tolerability profile of NNZ-2591 in preclinical studies to date, 13 weeks of treatment at this dose is expected to be safe and well-tolerated. NNZ-2591 has been shown to be well-tolerated in animal studies for up to 13 weeks at up to 200 mg/kg/day for rats and 120 mg/kg/day for dogs. In addition, NNZ-2591 Oral Solution appears to exhibit generally linear kinetics with no time-dependent effect on PK parameters and no evidence of accumulation in humans.

A study duration of 13 weeks is expected to provide sufficient time for changes in synaptic plasticity to manifest in behavioral and symptom change. A number of functionally relevant symptoms in PTHS could show a more immediate response as a result of this change in synaptic function. Based on findings in a parallel program of another IGF-1 metabolite analog (trofinetide) in Rett syndrome, it has been observed that improvements in some symptoms can be seen at 4 or 6 weeks of treatment.[1, 32] This includes improvements in symptoms clinicians considered clinically meaningful such as behavior, seizures, ambulation, social interaction, motor skills (hand use) and repetitive behaviors. As such, there is a prospect of benefit with dosing at the maximum proposed dose of 12 mg/kg BID for 7 weeks with a total duration of approximately 13 weeks of treatment in this Phase 2 study in PTHS.

Other symptoms or skills such as communication may need longer than 4 to 6 weeks to manifest, and the treatment period of 13 weeks in this study may allow sufficient time for possible change in more complex symptoms to manifest. So, on the whole, within a 13-week period it is possible for there to be a range of symptoms that could improve and be beneficial to patients. In sum, this approach to dosing maintains a balance between risk and benefit for this first Phase 2 study in the PTHS population.

6.2.2 Rationale for Selection of Efficacy Outcomes

Pitt-Hopkins syndrome is a severely limiting, lifelong disorder in which affected individuals cannot achieve the functional capacity to care for themselves, protect themselves from harm, form normal peer relationships, or achieve independent daily living skills. The constellation of symptoms in PTHS have significant impact on the functionality of affected individuals and their overall quality of life and well-being. The debilitating impairments in cognition, language and social function hinder overall social, emotional, and academic development and independent execution of tasks of daily living. Adaptive behavior is severely impaired with individuals remaining at low developmental and functional levels even as they age.[12] This greatly limits their ability to have any independence in executing activities of daily living rendering them dependent on support throughout their lives. Their severe disability puts them at further risk of adverse medical, developmental, psychosocial, and family outcomes.[8, 33]

The underlying neuropathology and severe neurological and neurodevelopmental impairments also lead to neurobehavioral dysfunction and dysregulation which substantially disrupt daily activities, put individuals at greater risk of harm, and can be potentially life-threatening. Hyperventilation and apnea result in a number of negative impacts on health including swollen abdomen, excessive burping, decreased oxygen saturation, changes in pallor, cyanosis, chronic hypoxia, and finger clubbing.[6, 9, 10] Seizures can be debilitating to cognitive and neurological development. Self-injurious behaviors put individuals with PTHS at risk of physical harm, potentially with long-term cumulative effects which decreases overall quality of life. There is currently no on-going natural history study of PTHS. Cohort studies, and a recent consensus statement paper on diagnosis and treatment[3] have provided detailed information characterizing the clinical manifestations of the disorder and their functional impact. This has provided insights on the symptoms that are central to the disorder and are important to patient functioning and well-being. The Pitt Hopkins Research Foundation (PHRF) maintains a family registry as part of the Coordination of Rare Diseases (CoRDS) Registry at Sanford which collects information on clinical symptoms (https://pitthopkins.org/research/how-to-get-involved-in-research-today/family-registry/). The PHRF also did an assessment of the patient and family experience by conducting a trend analysis of data shared through social networks.[34] In this analysis they identified the most concerning symptoms, most common methods of disease management, and unmet needs.

Together, the output from these initiatives and expert clinical advice has provided guidance into the important domain areas to address with NNZ-2591 treatment that would be most relevant and potentially beneficial to patients with PTHS (see Section 9.5.9). The secondary outcome of efficacy in this study will be assessed by evaluating a range of symptom areas to determine if there is preliminary evidence of efficacy, but also importantly to gather information about the performance of outcome measures and the influence of baseline patient attributes on clinical response.

6.3 Study Objectives and Endpoints

6.3.1 Primary Objective

To investigate the safety, tolerability, and PK of treatment with NNZ-2591 Oral Solution, 50 mg/mL in children and adolescents with PTHS.

6.3.1.1 Primary Endpoints

Safety: Incidence of AEs and SAEs from commencement of IMP administration through to the time of the last study visit will be evaluated. Vital signs, laboratory measures, physical exam results (neurological, ophthalmologic, and general) urine output history, [redacted], and ECGs will also be evaluated from screening/baseline through the end of the study.

Tolerability: Tolerability will be assessed based on caregiver reports and the caregiver diary, required dosing adjustments or discontinuations, dosing compliance and AEs related to participant experience.

Pharmacokinetic:

NNZ-2591 PK concentrations will be listed by participant and time point for each dose level with actual time they were acquired. The concentrations will be summarized by time, dose level and age group. For the concentrations measured at trough points dose relationship will be examined statistically with age group and/or body weight of participant as covariate.

Overall exposure parameters for NNZ-2591 such as C_{max} and AUC_{tau} may be derived by sparse non-compartmental analysis (NCA). Additionally, PK concentrations of NNZ-2591 measured in pediatric

participants will be used to refine a population PK model developed from adult NNZ-2591 PK data and extrapolated to pediatric population. After refinement, complete profiles for each participant will be simulated using a range of variability of model parameters and individual covariate effects. A standard NCA approach will be used for simulated profiles to calculate parameters C_{max}, AUC_{tau}, etc. for characterization of NNZ-2591 exposure in pediatric participants.

6.3.2 Secondary Objectives

To investigate measures of efficacy during treatment with NNZ-2591 Oral Solution, 50 mg/mL in children and adolescents with PTHS.

6.3.2.1 Secondary Endpoints

Within-participant changes will be assessed from the Baseline period to End of Treatment (EOT) using the following measures:

Overall Improvement:

- PTHS-specific Clinical Global Impression Scale–Improvement (CGI-I) Overall Improvement Score; clinician-assessed
- PTHS-specific Clinical Global Impression Scale–Domain Improvement Scores; clinician-assessed
- Caregiver Global Impression of Change Scores; caregiver-assessed

PTHS Symptom Severity:

- Clinical Global Impression Scale–Severity (CGI-S)–Overall Severity Score; clinician-assessed
- Clinical Global Impression Scale–Severity (CGI-S)–Domain Scores; clinician-assessed
- Caregiver Top 3 Concerns Likert Scale Scores; caregiver-assessed

Communication

- MacArthur-Bates Communicative Development Inventory (MB-CDI) Scores; caregiver-assessed
- Observer-Reported Communication Ability Measure (ORCA) Score; caregiver-assessed

Maladaptive Behavior

- Aberrant Behavior Checklist-2 (ABC-2) Scores; caregiver-assessed
- Behavior Problem Inventory-Short Form (BPI-SF) Scores; caregiver-assessed

Sleep: Child Sleep Habits Questionnaire (CSHQ) Scores; caregiver-assessed

GI: Gastrointestinal Health Questionnaire (GIHQ) Scores; caregiver-assessed

Adaptive Behavior: Vineland Adaptive Behavior Scales-3 Scores; clinician-assessed

Motor

- Bayley Scales of Infant Development 4 (BSID-4) motor scale, clinician-assessed (for

participants who are assessed on the BSID-4)
- Modified Two-minute Walk Test scores; clinician-assessed

Quality of Life (Child and Caregiver)

- Quality of Life Inventory-Disability (QI-Disability) Scores; caregiver-assessed
- Impact of Childhood Neurological Disability (ICND) Overall Quality of Life rating; caregiver-assessed

6.3.2.2 Exploratory Endpoints

Blood and stool samples will be collected for an exploratory analysis of biomarkers and gut microbiome. The analysis will be detailed in a separate analysis plan but will not include DNA sequencing. Samples will be collected at Screening (Visit 1) and Baseline (Visit 3, before dosing) and at the EOT visit, or upon early termination.

7. STUDY DESIGN

7.1 Study Design Overview

This is a Phase 2, open-label study of the safety and tolerability of NNZ-2591 Oral Solution, 50 mg/mL in male and female children and adolescents with Pitt-Hopkins syndrome. Approximately 10 to 20 male and female participants between the ages of 3 and 17 years will be enrolled and receive treatment of NNZ-2591 Oral Solution, 50 mg/mL for a total of 13 weeks. Additional participants may be screened, enrolled, and treated to achieve approximately 20 study completers.

There will be a total of 17 study visits, comprising 5 in-clinic visits, and 12 remote telemedicine/in-home nurse visits. The study will commence with an approximately 4 to 6 week Screening and Baseline period. During this Screening/Baseline period, participants will be assessed for study eligibility and data will be collected to establish the participant's baseline characteristics and symptom severity using a variety of assessments (Section 9.2). Two in-clinic visits and one remote/in-home visit (Visits 1, 2, 3) comprise Screening and Baseline.

Once eligibility is confirmed, participants will receive the starting dose of 4 mg/kg and then be up-titrated to the target dose (see Section 7.2). Participants will receive treatment for a total of 13 weeks. During the treatment period, there are three in-clinic visits: Week 2 (Visit 5), Week 6 (Visit 9) and Week 13 (Visit 16). There is a combined remote, telemedicine visit by the site investigator and an in-home visit by a visiting nurse for safety at Week 1 (Visit 4), Week 4 (Visit 7), Week 8 (Visit 11) and Week 10 (Visit 13). These combination remote/in-home visits will be conducted simultaneously whenever possible, or they will be conducted on the same day with the telemedicine visit occurring first. If it is not feasible to conduct these visits simultaneously or on the same day, the telemedicine portion of the visit and the nurse portion of the visit must be conducted on two consecutive days. In-home visits with a visiting nurse will be done at Weeks 3 (Visit 6), 5 (Visit 8), 7 (Visit 10), 9 (Visit 12), 11 (Visit 14), and 12 (Visit 15).

All participants will also have a remote post-treatment visit with the Investigator via telemedicine and an in-person home visit by a visiting nurse approximately 2 weeks after the end of treatment (Week 15, Visit 17).

For all visits designated as in-clinic, an in-person visit is preferred. Off-site assessments may be allowed due to extenuating circumstances, such as those related to the COVID-19 health emergency. All requests for off-site assessments must be approved in advance by the Sponsor or Medical Monitor.

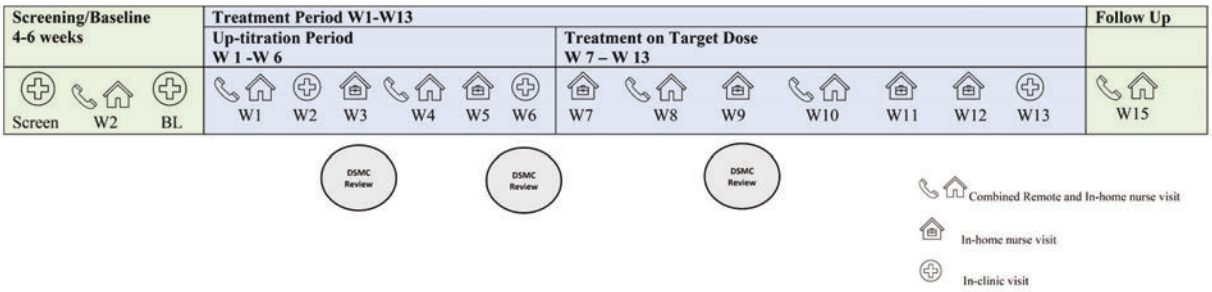
Participants will be divided into three groups by age:

- 13 to 17 years old (Group 1)
- 8 to 12 years old (Group 2)
- 3 to 7 years old (Group 3)

Enrollment will commence with the oldest age group and will proceed as follows. After at least three participants in Group 1 have received two weeks of treatment at the starting dose, the Data Safety Monitoring Committee (DSMC) will review data on safety, and tolerability. If tolerability and safety for those participants during the specified period is deemed acceptable, enrollment for Group 2 will proceed and dosing will begin at the starting dose. When at least three participants in Group 2 have received the first two weeks of treatment, the DSMC will review data on safety, and tolerability. If tolerability and safety for Group 2 is deemed acceptable, enrollment for Group 3 will commence and dosing will begin at the starting dose.

7.1.1 Study Design

Figure 1 Neu-2591-PTHS-001 Study Design Diagram



7.2 Dose-levels and Titration

The total administration period for NNZ-2591 Oral Solution, 50 mg/mL IMP will be 13 weeks. As outlined below in Table 4,the IMP will be administered open-label at a starting dose of 4 mg/kg BID for 2 weeks. Following DSMC review and approval of each dose titration, participants will be up-titrated to 8 mg/kg BID for 2 weeks, and then to the 12 mg/kg BID dose. The participant will then continue on the assigned dose (or highest dose tolerated) for the remainder of the 13 weeks. The process for DSMC review of dose escalation is described in Section 7.2.2.

Table 4 Dose Titration Schedule

Week 1 and Week 2	Week 3	Week 4 and Week 5	Week 6	Week 7 and Week 8	Week 9	Week 10 to Week 13
4 mg/kg BID (8 mg/kg daily)	4 mg/kg BID (8 mg/kg daily)[1]	8 mg/kg BID (16 mg/kg daily)	8 mg/kg BID (16 mg/kg daily)[1]	12 mg/kg BID (24 mg/kg daily)	12 mg/kg BID (24 mg/kg daily)	12 mg/kg BID (24 mg/kg daily)
	DSMC Reviews Data Prior to Up-Titration[2]		**DSMC Reviews Data Prior to Up-Titration[2]**		**DSMC Reviews Data After 2 weeks on 12 mg/kg to Confirm Safety[2]**	

BID = twice daily; DSMC = Data Safety Monitoring Committee
[1]Participants will stay on their current dose until the data is reviewed by the Data Safety Monitoring Committee (DSMC)
[2]Expected time for DSMC review will be one week.

The dose titration schedule may be modified based on updated PK modelling including data from the study.

7.2.1 Adjustment of the Dose for Tolerability

The dose for each participant is based on the participant's weight at Baseline (Visit 3). The dose will not be increased or decreased if the participant's weight at a postbaseline visit changes. Within the up-titration period, if a participant is deemed not to be tolerating the current dose within the 2-week period for that dose, and they have not met any of the stopping criteria (Section 9.4.5), they can be down-titrated to the next lowest dose in the titration schedule. The Investigator must attempt to increase the dose as soon as it is possible based on the clinical situation. The aim is to return to the originally assigned dose. If

the originally assigned dose cannot be reached or the participant is again unable to tolerate that dose, the Investigator will continue treatment on the highest dose that the participant can tolerate. The process for DSMC review of dose escalation is described in section 7.2.2.

7.2.2 Dose Escalation Review by the DSMC

Data from two weeks of dosing at each dose in the dose escalation will be reviewed by the DSMC before participants are allowed to be administered the next highest dose. Participants will stay on their current dose until the DSMC has reviewed the data and approved the participants to escalate to the next dose.

After eligibility is confirmed, the participant will be administered NNZ-2591 IMP open label at a starting dose of 4 mg/kg BID for a total of approximately 3 weeks. After 2 weeks of dosing at 4 mg/kg BID, the DSMC will review the safety data. The participant will remain on the starting dose during the DSMC review. If the DSMC determines that tolerability and safety during the 2-week dosing period are acceptable, the participant will escalate to the next dose of 8 mg/kg BID and be dosed for a total of approximately 3 additional weeks at that dose. After 2 weeks of dosing at 8 mg/kg BID, the DSMC will review the safety data. If the DSMC determines that tolerability and safety during the 2-week dosing period on 8 mg/kg BID are acceptable, the participant will then escalate to the target dose of 12 mg/kg BID and be dosed for 2 weeks. After 2 weeks of dosing at 12 mg/kg BID, the DSMC will review the safety data to determine if the participant can continue dosing at 12 mg/kg BID for the remainder of the 13 weeks.

The DSMC will determine if dose escalation can proceed based on pre-specified stopping rules summarized for individual participants in Section 9.4.5. Note that early cessation of IMP treatment does not necessarily mean the participant is withdrawn from the study. With any early cessation, all evaluations and tests scheduled during the study must be conducted if possible.

The site will inform the caregiver of the up-titration decisions as soon as possible after the site has received the official notification of the DSMC decision. The site will verify with the caregiver that instructions to up-titrate to 8 mg/kg BID have been followed at the Week 4 visit (Visit 7), and will verify with the caregiver that instructions to up-titrate to 12 mg/kg BID have been followed at the Week 7 visit (Visit 10). If the DSMC has not yet approved the participant to escalate to the next dose by the time of the relevant visit, the site should contact the caregiver within 24 hours of receiving the decision from the DSMC. If the participant is not approved to titrate to the next dose, the site will contact the caregiver to discuss appropriate follow-up as is clinically indicated. At each visit, the clinician or nurse should check the dose when reviewing the caregiver diary.

7.3 Treatment Assignment

This is an open-label study in which all participants will be assigned to NNZ-2591 Oral Solution, 50mg/mL and receive 13 weeks of treatment. All participants will be titrated to the dose of 12 mg/kg BID or the highest tolerated dose based on the Dosing Schedule in Section 7.2. The dose titration schedule may be modified based on updated PK modelling including data from the study.

Participants who are unable to tolerate the study treatment may be discontinued from the study.

If a participant meets all eligibility criteria and written informed consent has been provided by a legally authorized representative, the participant will be enrolled in the study.

7.4 Study Timeline

The study duration is 19 weeks from Screening to Follow-Up at Week 15 (+4 days).

7.5 COVID-19

As a result of the Coronavirus disease (COVID-19) public health emergency, this protocol includes provision for alternative methods of performing safety and efficacy assessments in accordance with the *FDA Guidance on Conduct of Clinical Trials of Medical Products during the COVID-19 Public Health Emergency*.[**] Provision is made for off-site assessments where necessary and all efforts have been made to ensure the alternative processes are consistent as much as possible with in-clinic visits. For visits that are designated as in-clinic, an actual in-clinic visit is preferred. However, an off-site visit may be conducted for a scheduled in-clinic visit in extenuating circumstances due to the COVID-19 health crisis with the prior approval of the Sponsor and Medical Monitor. Clinical investigators are to document the reason for any contingency measures implemented. If at any time in the study, the participant or his or her caregiver tests positive for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or becomes ill with symptoms of COVID-19, the family should be instructed to contact the site as soon as possible. Once informed, the Investigator should contact the Medical Monitor.

8. PARTICIPANT POPULATION

Approximately 10 to 20 male and female participants between the ages of 3 and 17 years, inclusive, are planned to complete the study, balanced to the extent possible by age and the number of males and females.

The participants will be categorized by age in three groups: 13 to 17-year-olds (Group 1), 8 to 12-year-olds (Group 2), and 3 to 7-year-olds (Group 3). Enrollment will be balanced to the extent possible by sex and age with a minimum of 3 males and 3 females across all age groups, and a minimum of 3 participants in each age group.

Enrollment will proceed by age group according to the following process. Enrollment will commence first in the oldest age group (Group 1). After at least three participants in Group 1 have received two weeks of treatment at the starting dose, safety, and tolerability data will be reviewed by the DSMC. If tolerability and safety for those participants during that period is deemed acceptable, enrollment for Group 2 will proceed and dosing will commence at the starting dose. When at least three participants in Group 2 have received two weeks of treatment at the starting dose, safety and tolerability data will be reviewed by the DSMC. If tolerability and safety for Group 2 is deemed acceptable, enrollment for Group 3 will commence and dosing will begin at the starting dose. Participants in the younger age groups may be screened and enter the 4 to 6 week Screening period before DSMC review, but they will not be enrolled or dosed until the DSMC has reviewed and approved the initiation of dosing in the younger age group.

[**] FDA Guidance on Conduct of Clinical Trials of Medical Products During the COVID-19 Public Health Emergency: Guidance for Industry, Investigators, and Institutional Review Boards (Center for Biologics Evaluation and Research; Center for Devices and Radiological Health; Center for Drug Evaluation and Research; Office of the Commissioner; Office of Clinical Policy and Programs; Office of Clinical Policy; Office of Good Clinical Practice) (2021).

8.1 Inclusion Criteria

To be eligible for this study, participants must meet all of the inclusion criteria and none of the exclusion criteria. Inclusion criteria include:

1. Clinical diagnosis of PTHS with a documented disease-causing genetic etiology for the disorder.
2. Males or females aged 3 to 17 years at Screening, inclusive.
3. Body weight of 12 kg or higher at Screening. Weight measurements may be repeated during the Screening period in consultation with the Medical Monitor.
4. Participants with a Clinical Global Impression – Severity (CGI-S) score of 4 or greater at the Screening visit.
5. Not currently actively undergoing regression or loss of skills defined as no persistent loss of previously acquired developmental skills for a period within 3 months of the Screening visit.
    a. Previously acquired skills include any skill established and used on a daily basis for at least 3 months.
    b. Variations in use of skills that is part of a general pattern of ups and downs, as determined by the investigator, are not counted as regression.
    c. Skills may include language, non-verbal communication, fine motor skills, ambulation (including gait, coordination, independence of walking/standing), social skills, play, self-help.
6. Each participant must be able to swallow the IMP provided as a liquid solution.
7. The participant's caregiver(s) must have sufficient English language skills to complete caregiver assessments in English.

8.2 Exclusion Criteria

To be eligible for this study, participants must meet all of the inclusion criteria and none of the exclusion criteria. Exclusion criteria include:

Laboratory and Screening Values

1. Body weight < 12 kg at Screening. Weight may be repeated during the Screening period in consultation with the Medical Monitor.
2. HbA1c values above 7% at Screening.
3. Clinically significant abnormalities in safety laboratory tests (as determined by the Investigator) in safety laboratory values, vital signs, as measured at the Screening visit. Laboratory testing may be repeated during the Screening period in consultation with the Medical Monitor.
4. Positive pregnancy test at the Screening visit.
5. QTcF Exclusions (any of the following):
    a. Screening QTcF interval of > 450 ms assessed using Fridericia's correction factor based on 12-lead ECG obtained as a continuous ECG of at least 50 seconds.
    b. Hypokalemia (Serum potassium at the Screening visit < 3.0 mmol/L or mEq/L). Serum potassium may be repeated during the Screening period with the agreement of the Medical Monitor.
    c. QTcF prolongation that is currently controlled with medication, in which normal QTcF intervals can only be achieved with medication.
6. Any other clinically significant finding (as determined by the Investigator) on ECG at the Screening visit that would increase the likelihood of a severe arrhythmia.

COVID-19

7. Positive for SARS-CoV-2 at Screening or Baseline.
8. Previous COVID-19 infection within the last 12 months that required admission to the hospital.
9. Previous COVID-19 infection involving multi-organ systems, resulting in Multisystem Inflammatory Syndrome in Children (MIS-C) or with clinically significant sequelae.
10. Previous COVID-19 infection associated with acute kidney injury (AKI) or renal manifestations (e.g. proteinuria, hematuria, echogenic kidneys).

[redacted]

21. [redacted]

Concomitant Treatments

28. Current treatment or treatment within the two weeks prior to the Screening visit with MAO inhibitors, d-cycloserine, oxytocin, carbetocin, tricyclic antidepressants, or bupropion.
29. Current treatment with more than 3 allowable psychotropic medications.
    a. Includes medications used to treat problems with sleep onset and sleep continuity except melatonin. Melatonin for difficulties with sleep onset is permissible and is not included in the count of psychotropic medications.
    b. The use of anti-epileptics for other indications such as the treatment of mood disorders counts towards the limit of permitted psychotropic medications.
    c. Anti-epileptic medications used for treatment of seizures does not count towards the limit of psychotropic medications.
30. Current treatment with insulin or treatment within the last 12 weeks prior to commencement of IMP.
31. Current treatment with IGF-1 or treatment within 6 months of commencement of the IMP.
32. Current treatment with growth hormone or treatment within 12 months of commencement of the IMP.
33. Non-pharmacologic somatic treatment (e.g., a ketogenic diet or vagal nerve stimulation) whose dosing regimen has not been stable at least 4 weeks prior to the Screening visit. If the treatment was discontinued, the discontinuation must have occurred no fewer than 2 weeks before the Screening visit.
34. Participants planning to start a new behavioral, educational, or cognitive therapy during the period of the study or those who have begun a new behavioral or cognitive therapy regimen within 4 weeks prior to the Screening visit.
    a. Note: changes to an on-going treatment regimen that are due to school schedules or are otherwise seasonally related are not exclusionary as long as they are consistent with their typical type and pattern of behavioral treatment.
35. Seizure medication whose dosing regimen is not stable 8 weeks prior to commencing IMP. Adjustments for changes in weight or age are allowable. If the treatment was discontinued, the discontinuation must have occurred no fewer than 2 weeks before the Screening visit.
36. Psychotropic medications or any other medication used for a chronic illness (not including

antibiotics, pain relievers, anti-diarrheals, and laxatives) whose doses and dosing regimen have not been stable for at least 4 weeks prior to the Screening visit. If the treatment was discontinued, the discontinuation must have occurred no fewer than 2 weeks prior to the Screening visit.

a. This includes nutraceutical treatments and cannabinoids (tetrahydrocannabinol [THC] and cannabidiol [CBD]) which are allowable but also must meet the stability conditions above.
b. Pain relievers and laxatives may be used as needed.
c. Standard of care immunizations and vaccinations (including COVID-19) are allowed and are not subject to the above stability conditions.
d. The Investigator should consult with the medical monitor if a participant is being treated with ocular toxic, hepatotoxic, nephrotoxic or antibiotic medications.
e. If the Investigator is unsure if a medication is permitted, the Medical Monitor should confirm if the medication is allowed and will provide written documentation to support the use of the medication, if allowed.

Regression of Skills

37. Actively undergoing regression or loss of skills defined as a persistent loss of previously acquired developmental skills within 3 months of the Screening visit.
    a. Previously acquired skills include any skill established and used on a daily basis for at least 3 months.
    b. Variations in use of skills that is part of a general pattern of ups and downs, as determined by the investigator, are not counted as regression.
    c. Skills may include language, non-verbal communication, fine motor skills, ambulation (including gait, coordination, independence of walking/standing), social skills, play, self-help.

Seizures

38. Seizure profile that is not stable 8 weeks prior to commencing IMP. The overall pattern of seizure activity for the participant should be consistent with their typical pattern of seizures and be stable within the 8-week period in terms of type, intensity, duration, and frequency as determined by the clinical Investigator.

Medical Conditions

39. Currently pregnant, lactating, or breastfeeding.
40. Current clinically significant (as determined by the Investigator) cardiovascular, gastrointestinal, respiratory, or endocrine disease, or clinically significant organ impairment. The Investigator may consult with the Medical Monitor as needed if he/she is uncertain if symptoms are exclusionary.
41. Current clinically significant (as determined by the Investigator) hypo- or hyperthyroidism. If the participant has hypo- or hyperthyroidism that is controlled with medication, the Investigator should consult with the Medical Monitor to confirm if the participant is eligible.
42. Type 1 or Type 2 diabetes mellitus requiring insulin (whether well controlled or uncontrolled), or uncontrolled Type 1 or Type 2 diabetes.
43. Has planned surgery during the study.
44. History of, or current, cerebrovascular disease or brain trauma.
45. History of, or current catatonia or catatonia-like symptoms.
46. History of, or current, malignancy.

47. Current major or persistent depressive disorder (including bipolar depression).
48. Significant uncorrected hearing impairment.

Other Criteria

49. Allergy to strawberry.
50. Participant is unable to take the IMP orally and swallow the IMP provided as a liquid solution. Administration of the IMP by nasogastric tube, feeding tube, thickened liquid, or gastrostomy tube is not allowed.
51. Has participated in another interventional clinical study within 30 days prior to the Screening visit.
52. Prior enrollment in this clinical trial.
    a. Individuals who leave the study during the Screening period and have not entered the study may be able to rescreen pending review and approval of the Sponsor and Medical Monitor.
    b. Individuals who were deemed eligible and entered the study (enrolled) who discontinue the study may not re-enroll in the study.
53. The participant is judged by the Investigator or the Medical Monitor to be inappropriate for the study for any reason.

8.3 Abnormal Laboratory Findings

Where Screening safety laboratory tests (blood, urine) are found to be outside the reference range provided by the laboratory and the Investigator believes the results to be of clinical significance, the participant may be asked to return to the investigational site or have a nurse visit to allow for repeat sampling. If the abnormal finding has not reverted to normal or is regarded as being clinically significant, the participant will not be entered into the study but will be referred for further investigation, as appropriate. Throughout the study, abnormal or out of range values should be reviewed by the Investigator who should make a determination if the values are clinically significant.

8.4 Withdrawal Criteria

A participant's legally authorized representative is free to withdraw consent and discontinue participation in the study at any time, without prejudice to further treatment, according to standard clinical practice. A participant's participation in the study may also be discontinued at any time at the discretion of the Investigator.

If the study treatment or observations are discontinued in any participant, the reason will be recorded on the e-CRF and the Sponsor notified promptly.

8.5 Risks and Benefits

NNZ-2591 Oral Solution has been given to 28 healthy volunteers to date and was well tolerated. All reported AEs were mild or moderate and resolved during the trial. There were no SAEs. There were no clinically significant findings from safety laboratory tests, vital signs, or cardiac tests. The most common TEAEs deemed related to drug were a cluster of apparently related neurological symptoms that included somnolence/drowsiness, blurred vision/difficulty focusing, ataxia/unsteady gait, dizziness, and vertigo. The majority of these TEAEs occurred within 2 to 3 hours of initial dosing. All resolved spontaneously without requiring medical intervention. There were no clinically significant findings on the laboratory tests or ECGs and no laboratory renal or hepatic laboratory findings were observed. [redacted]

[redacted]

Potential risks associated with the performance of the PTHS assessments include fatigue, frustration, and provocation of maladaptive behaviors related to frustration in the participants. Mild irritation or discomfort may be experienced from blood draws and ECG leads.

Participants may receive no direct benefit from participation in this study. However, participation will help the investigators better understand the safety of NNZ-2591 in people with PTHS. Information obtained from this study may help people with PTHS in the future. It might improve the way people with PTHS are treated and help them better manage their symptoms. Previous scientific information obtained from animal studies indicates that the signs and symptoms of PTHS might be reduced following administration of NNZ-2591. It is possible that participants will experience this same benefit following administration of NNZ-2591 IMP.

9. STUDY PROCEDURES

9.1 Overview of Study Visits

The Screening/Baseline period will be conducted over 4 to 6 weeks. The second visit during the Screening/Baseline period (Visit 2) should be conducted 2 weeks ± 1 day after the Screening visit (Visit 1) . The participant will then remain in Screening for an additional 2 to 4 weeks. The caregiver diary must be completed daily during the entire Screening/Baseline period whether it is 4 weeks or 6 weeks. The Screening visit (Visit 1) and Baseline visit (Visit 3) may be conducted over three consecutive days. It is preferrable that ECGs, safety labs and biomarker sample collection are conducted on the same day. No assessments can be conducted until informed consent has been given by the caregiver or legally authorized representative. Also, training for the caregivers on all caregiver completed assessments must be completed before the caregiver rater may complete the assessments. All assessments must be completed within the window for the visit, as shown in the Schedule of Assessments in Section 4. For Visit 3, all assessments must be completed before administration of the IMP.

The in-clinic visits will be conducted in person at the study site. As described below, safety and efficacy assessments will be completed by site personnel. The caregiver will also complete caregiver assessments the day of the visit or the day before the visit as described below (except the caregiver diary which is completed daily), and these will be reviewed by site personnel. In-clinic visits will be done at Screening (Visit 1), Baseline (Visit 3), Week 2 (Visit 5), Week 6 (Visit 9), and Week 13 (EOT, Visit 16). For all visits designated as in-clinic (Visits 1, 3, 5, 9 and 16), an in-person visit is preferred. Off-site assessments may be allowed due to extenuating circumstances due to the COVID-19 health emergency. All requests for off-site assessments must be approved in advance by the Sponsor or Medical Monitor.

The combined remote telemedicine/in-home visit will be done during Screening/Baseline at Visit 2, and post-enrollment at Week 1 (Visit 4), Week 4 (Visit 7), Week 8 (Visit 11), Week 10 (Visit 13) and Week 15 (Visit 17). The combined remote visit is conducted by the site via telemedicine or video facilities, and by a visiting nurse who will conduct assessments at the participant's home. These combination remote/in-home visits will be conducted simultaneously whenever possible, or they will ideally be conducted on the same day. If not possible, the telemedicine visit and the nurse visit may be conducted on separate days. In this case, the telemedicine portion and in-home nurse portion must be conducted over two consecutive days. During Screening (Visit 2), the Investigator will review the medical history to determine if there are any changes in symptoms from the Screening visit (Visit 1). At post-enrollment visits, the Investigator will do a review of AE/SAEs and tolerability and conduct a recent clinical history. A modified version of

the physical and neurological exam will be completed by the Investigator at the telemedicine visits based on the guidance documents provided to the Investigator. During the in-home component of the visit at Screening (Visit 2), the nurse will collect vital signs, weight, do a Targeted Physical Exam assessing general appearance, abdominal/costovertebral angel tenderness (CVA), and edema, and query oral intake. The nurse will review the caregiver diary during the visit and provide the caregiver with a new section of the diary. Post-enrollment, at the in-home component of the combined visit, the nurse will collect urine, vital signs, weight, and blood samples for a comprehensive metabolic panel (CMP), complete blood count (CBC) with differential, and coagulation (except Visit 1, when only CMP is collected). The nurse will conduct a Targeted Physical Exam assessing: general appearance; the abdomen and the CVA region for tenderness; the extremities and peri-orbital region for edema. A suprapubic exam (bladder palpation) to assess for urinary retention may be performed. The visiting nurse will also review the caregiver diary, provide the caregiver with a new diary section, query oral intake, and a review tolerability. At the post-treatment follow-up visit at Week 15, the visiting nurse will also collect a 12-lead ECG, in addition to the above assessments.

In-home only visits with a visiting nurse will be done at Weeks 3 (Visit 6), 5 (Visit 8), 7 (Visit 10), 9 (Visit 12), 11 (Visit 14), and 12 (Visit 15). At these visits, the nurse will collect urine, vital signs, weight, and a blood sample for the CMP. The nurse will also conduct a Targeted Physical Exam assessing general appearance, abdominal/CVA, and edema. A suprapubic exam (bladder palpation) to assess for urinary retention may be performed. The visiting nurse will review the caregiver diary, query oral intake, and review tolerability.

A combined remote visit or nurse-only in-home visit may be conducted in-clinic by the study staff if agreed upon by the Investigator and the caregiver. The site should inform the nursing service of such a change. If an unscheduled nurse visit is needed for follow-up (e.g. repeat labs or other assessments), this can be requested by the site with the nursing service. The site should inform the Medical Monitor if they require an unscheduled nursing visit or will conduct a remote visit in the clinic.

The nurse will send a report for all in-home nurse visits to the Investigator along with a copy of the completed Caregiver Diary. The Investigator is responsible for reviewing the Caregiver Diary and determining if any issues should be reported as an adverse event or require follow up. The visiting nurse will escalate any issues related to potential AE/SAEs, stopping criteria, major dosing issues or issues of potential safety concern to the Investigator the same day. Any changes noted will be provided to the Investigator who will be responsible for determining and reporting AE/SAEs.

9.2 Study Procedures by Visit

9.2.1 Visit 1 Screening/Baseline (in-clinic) – Week -4 (± 2 days)

The following assessments will be conducted and data collected during Visit 1:

- Eligibility
  - Informed consent (mandatory)
  - Consent for reuse of leftover Biomarker and PK samples (optional)
  - Inclusion/exclusion criteria
  - Medical history
  - Confirm documented PTHS diagnosis and genotype
  - PTHS history and exam
  - Confirm co-morbid psychiatric disorders by Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5)
  - Autism Mental Status Exam

  - Rapid SARS-CoV-2 Test

- Safety
  - Physical and neurological examination
  - [redacted]
  - [redacted]
  - Vital signs
  - Height/length
  - Weight
  - 12-lead ECG
  - CBC with differential
  - Coagulation
  - CMP
  - Urinalysis (including screen for drugs of abuse)
  - TSH, Free T3, Free T4
  - HbA1c
  - Serum pregnancy test (females who have reached menarche only)
- Review the instructions for the Caregiver Diary and completing the clinical section for seizure types and breathing events
- Biomarkers
  - Blood sample for biomarkers
  - Stool sample for microbiome analysis
- Efficacy
  - CGI-S
  - SB5 or Bayley-4 (Bayley-4 is done for participants who cannot attain basal on the SB5)
  - Caregiver Top 3 Concerns
  - ABC-2
  - CSHQ
  - GIHQ
  - Modified two-minute walk test

The screening visit (Visit 1) may be conducted over three consecutive days. Eligibility assessments as outlined above should be done on the first day of the visit. It is preferrable that ECGs, safety labs and biomarker sample collection are conducted on the same day. No assessments can be conducted until after informed consent has been given by the participant's legally authorized representative. Also, training for the caregivers on all caregiver-completed assessments must be completed before the caregiver rater may complete the assessments. All assessments including caregiver completed assessments must be completed within the window for the visit (visit day ± 2 days).

9.2.2 Visit 2 Screening/Baseline (telemedicine and in-home) – 2 Weeks from Visit 1 (± 1 day)

The Screening/Baseline period will be conducted over 4 to 6 weeks. The second visit during the Screening period (Visit 2) should be conducted 2 weeks ± 1 day after the Screening visit (Visit 1) .

Visit 2 of the Screening/Baseline period will be a remote telemedicine and in-home visit. The assessments to be conducted and data collected are divided accordingly below.

Remote/Telemedicine – Investigator and Appropriate Staff

The following assessments will be conducted and data collected during Visit 2, conducted by the site via telemedicine or video facilities as appropriate. Caregiver assessments must be completed the day of the visit or no earlier than the day before the visit (except the caregiver diary which is completed daily) and reviewed by the Investigator.

- Medical history (any updates since Visit 1)
- Review of the Caregiver Diary (con-meds, seizures, voids, oral intake, breathing events, changes in symptoms)

Caregiver Assessments

The following assessments should be completed by the caregiver. Caregiver assessments must be completed the day of the visit or no earlier than the day before the visit.

- Efficacy
    - Caregiver Top 3 Concerns
    - ABC-2
    - CSHQ
    - GIHQ

In-home Nurse Visit

The following assessments should be conducted, and data collected by the visiting nurse in the participant's home during Visit 2:

- Vital Signs
- Targeted Physical Exam: abdominal, CVA tenderness, general appearance, edema. Exam may include suprapubic exam (bladder palpation) to assess for urinary retention if decreased urine output is noted.
- Weight
- Review of Caregiver Diary

9.2.3 Visit 3 Baseline (in-clinic) – Week 0 (± 2 days)

The following assessments will be conducted and data collected during the Baseline visit (Visit 3). Caregiver assessments must be completed the day of the visit or no earlier than the day before the visit (except the caregiver diary which is completed daily) and reviewed by the Investigator.

- Eligibility
    - Review inclusion/exclusion criteria
    - Medical history (updates from Screening visit)
    - Rapid SARS-CoV-2 test
- Safety
    - Physical and neurological examination
    - Vital signs
    - Height/length
    - Weight
    - 12-lead ECG
    - CBC with differential

  - Coagulation
  - CMP
  - Urinalysis
- Review of the Caregiver Diary (con-meds, seizures, voids, oral intake, breathing events, changes in symptoms)
- Biomarkers
  - Blood sample for biomarkers
  - Stool sample for microbiome
- Efficacy
  - CGI-S
  - Vineland Adaptive Behavior Scales-3
  - MB-CDI
  - ORCA
  - Caregiver Top 3 Concerns
  - ABC-2
  - BPI-SF
  - ICND
  - QI-Disability
  - CSHQ
  - GIHQ
  - Modified two-minute walk test

The Baseline visit (Visit 3) may be conducted over three consecutive days. It is preferrable that ECGs, safety labs and biomarker sample collection are conducted on the same day. All assessments must be completed within the two-day window for the visit (visit day ± 2 days). For Visit 3, all assessments must be completed before administration of the IMP.

- In-clinic administration of first dose
  - The first dose of IMP will be administered in the clinic after all Baseline assessments are completed, or, if the Investigator judges that it is too late in the day, on the following day. The participant should be monitored for at least three hours post the first dose.
  - If the first dose is administered the day following Baseline, vital signs (except height) should be collected before the first dose is administered.
  - The dose can be taken orally from a cup or from a dosing syringe.
  - Doses should be given on an empty stomach (2 hours after the previous meal or snack and 30 minutes prior to the next).
  - The IMP should not be mixed with water, liquid thickeners, or food for administration.
  - Day 1 of dosing is defined by the date when the first dose is taken. At this visit, the site will dispense an adequate supply of IMP for dispensing by the caregiver at home.

9.2.4 Visit 4 Treatment Period (telemedicine and in-home) – Week 1 (± 1 day)

Visit 4 (Week 1) will be a remote telemedicine and in-home visit. The assessments to be conducted and data collected are divided accordingly below.

Remote/Telemedicine – Investigator and Appropriate Site Staff

The following assessments and data will be conducted or collected by the site via telemedicine or video facilities as appropriate during Visit 4. Caregiver-completed assessments should be reviewed by the Investigator.

- Recent Clinical History
- AE/SAE and tolerability review
- Review of the Caregiver Diary (dosing, con-meds, seizures, voids, oral intake, changes in symptoms)

Caregiver Assessments

The following assessments should be completed by the caregiver.

- Caregiver diary

In-home Nurse Visit

The in-home visit will be conducted by a visiting nurse. The nurse will escalate any issues related to AE/SAEs, stopping criteria, major dosing issues or issues of potential safety concern to the Investigator via telephone the same day. Any changes noted will be provided to the Investigator who will be responsible for determining and reporting AE/SAEs.

The following assessments should be conducted, and data collected by the visiting nurse in the participant's home during Visit 4:

- AE/SAE/tolerability query
- Review caregiver diary: concomitant medications, voids, oral intake, and changes in symptoms
- Review Dosing Compliance
- Collect blood for CMP
- Collect urine for urinalysis
- Vital Signs
- Weight
- Targeted Physical Exam: general appearance, abdominal, CVA tenderness**,** check for edema. Exam may include a suprapubic exam (bladder palpation) to assess for urinary retention may be performed if decreased urine output is noted.

9.2.5 Visit 5 Treatment Period (in-clinic) – Week 2 (± 2 days)

Visit 5 (Week 2) will be an in-clinic visit. This visit may be conducted over three consecutive days. The following assessments should be conducted and data collected by site personnel during this visit. Caregiver assessments must be completed the day of the visit or no earlier than the day before the visit (except the Caregiver Diary which is completed daily) and reviewed by the Investigator. All assessments must be completed within the window for the visit (visit day ±2 days).

- Safety
    - Recent clinical history
    - Physical and neurological examination
    - Vital signs
    - Weight
    - Review for AEs/SAEs
    - 12-lead ECG
    - CBC with differential
    - Coagulation
    - CMP

    - Urinalysis
- Caregiver Diary (con-meds, seizures, voids, oral intake, breathing events, changes in symptoms)
- Review Dosing Compliance
- Pharmacokinetics/Pharmacodynamics
    - Blood sampling for PK

9.2.6 Visit 6 Treatment Period (in-home nurse) – Week 3 (±1 day)

The in-home visit at Visit 6 (Week 3) will be conducted by a visiting nurse. The nurse will escalate any issues related to AE/SAEs, stopping criteria, major dosing issues or issues of potential safety concern to the Investigator via telephone the same day. Any changes noted will be provided to the Investigator who will be responsible for determining and reporting AE/SAEs.

The following assessments should be conducted, and data collected by the visiting nurse in the participant's home during Visit 6:

- AE/SAE/tolerability query
- Review caregiver diary: concomitant medications, voids, oral intake, changes in symptoms
- Review Dosing Compliance
- Collect blood for CMP
- Collect urine for urinalysis
- Vital Signs
- Weight
- Targeted Physical Exam: general appearance, abdominal, CVA tenderness, check for edema. Exam may include a suprapubic exam (bladder palpation) to assess for urinary retention if decreased urine output is noted if decreased urine output is noted.

9.2.7 Visit 7 Treatment Period (telemedicine and in-home) – Week 4 (±1 day)

Visit 7 (Week 4) will be a remote telemedicine and in-home visit. The assessments to be conducted and data collected are divided accordingly below.

<u>Remote/Telemedicine – Investigator and Appropriate Site Staff</u>

The following assessments and data will be conducted or collected by the site via telemedicine or video facilities as appropriate during Visit 7. Caregiver-completed assessments should be reviewed by the Investigator.

- Recent Clinical History
- Modified neurological exam
- Modified physical exam
- AE/SAE and tolerability review
- Inform visiting nurse of dose titration, and confirm that dose titration instructions have been followed by the caregiver.
- Review of the Caregiver Diary (con-meds, seizures, voids, oral intake, changes in symptoms)

<u>Caregiver Assessments</u>

The following assessments should be completed by the caregiver.

- Caregiver diary

In-home Nurse Visit

The in-home visit will be conducted by a visiting nurse. The nurse will escalate any issues related to AE/SAEs, stopping criteria, major dosing issues or issues of potential safety concern to the Investigator via telephone the same day. Any changes noted will be provided to the Investigator who will be responsible for determining and reporting AE/SAEs.

The following assessments should be conducted, and data collected by the visiting nurse in the participant's home during Visit 7:

- AE/SAE/tolerability query
- Review caregiver diary: concomitant medications, voids, oral intake, and changes in symptoms
- Review Dosing Compliance
- Collect blood labs
    - CBC with differential
    - Coagulation
    - CMP
- Collect urine for urinalysis
- Vital Signs
- Weight
- Targeted Physical Exam: general appearance, abdominal, CVA tenderness, check for edema. Exam may include a suprapubic exam (bladder palpation) to assess for urinary retention if decreased urine output is noted.

9.2.8 Visit 8 Treatment Period (in-home nurse) – Week 5 (±1 day)

The in-home visit at Week 5 (Week 8) will be conducted by a visiting nurse. The nurse will escalate any issues related to AE/SAEs, stopping criteria, major dosing issues or issues of potential safety concern to the Investigator via telephone the same day. Any changes noted will be provided to the Investigator who will be responsible for determining and reporting AE/SAEs.

The following assessments should be conducted, and data collected by the visiting nurse at the participant's home during Visit 8:

- AE/SAE/tolerability query
- Review caregiver diary: concomitant medications, voids, oral intake, changes in symptoms
- Review Dosing Compliance
- Collect blood for CMP
- Collect urine for urinalysis
- Vital Signs
- Weight
- Targeted Physical Exam: general appearance, abdominal, CVA tenderness, check for edema. Exam may include suprapubic exam (bladder palpation) to assess for urinary retention if decreased urine output is noted.

9.2.9 Visit 9 Treatment Period (in-clinic) – Week 6 (± 2 days)

Visit 9 (Week 6) is an in-clinic visit. The following assessments will be conducted and data collected during Visit 9. This visit may be conducted over three consecutive days. Caregiver assessments must be completed the day of the visit or no earlier than the day before the visit (except the caregiver diary which is completed daily) and reviewed by the Investigator. All assessments must be completed within the window for the visit (visit day ±2 days).

- Safety
  - Recent clinical history
  - Physical and neurological examination
  - Vital signs
  - Weight
  - Review for AEs/SAEs
  - 12-lead ECG
  - CBC with differential
  - Coagulation
  - CMP
  - Urinalysis
  - TSH, Free T3, Free T4
  - Serum Pregnancy Test (for female participants who have reached menarche)
- Caregiver Diary (dosing, con-meds, seizures, voids, oral intake, breathing events, changes in symptoms)
- Review Dosing Compliance
- Pharmacokinetics/Pharmacodynamics
  - Blood sampling for PK
- Efficacy
  - CGI-I
  - CGI-S
  - Caregiver Top 3 Concerns
  - ABC-2
  - CSHQ
  - GIHQ

9.2.10 Visit 10 Treatment Period (in-home nurse) – Week 7 (± 1 day)

The in-home visit at Week 7 (Visit 10) will be conducted by a visiting nurse. The nurse will escalate any issues related to AE/SAEs, stopping criteria, major dosing issues or issues of potential safety concern to the Investigator via telephone the same day. Any changes noted will be provided to the Investigator who will be responsible for determining and reporting AE/SAEs.

At Week 7, the site will also have a phone call with the caregiver to confirm the instructions for the dose titration have been followed. The site will also inform the visiting nurse so the dosing schedule can be reviewed with the family during the in-home visit.

The following assessments should be conducted, and data collected by the visiting nurse in the participant's home during Visit 10:

- AE/SAE/tolerability query
- Review caregiver diary: concomitant medications, voids, oral intake, changes in symptoms

- Review Dosing Compliance
- Collect blood for CMP
- Collect urine for urinalysis
- Vital Signs
- Weight
- Targeted Physical Exam: general appearance, abdominal, CVA tenderness, check for edema. Exam may include a suprapubic exam (bladder palpation) to assess for urinary retention if decreased urine output is noted.

9.2.11 Visit 11 Treatment Period (telemedicine and in-home) – Week 8 (± 1 day)

Visit 11 (Week 8) will be a remote telemedicine and in-home visit. The assessments to be conducted and data collected are divided accordingly below.

Remote/Telemedicine – Investigator and Appropriate Site Staff

The following assessments and data will be conducted or collected by the site via telemedicine or video facilities as appropriate during Visit 11. Caregiver-completed assessments should be reviewed by the Investigator.

- Recent Clinical History
- Modified neurological exam
- Modified physical exam
- AE/SAE and tolerability review
- Review of the Caregiver Diary (dosing, con-meds, seizures, voids, oral intake, changes in symptoms)

Caregiver Assessments

The following assessments should be completed by the caregiver.

- Caregiver diary

In-home Nurse Visit

The in-home visit will be conducted by a visiting nurse. The nurse will escalate any issues related to AE/SAEs, stopping criteria, major dosing issues or issues of potential safety concern to the Investigator via telephone the same day. Any changes noted will be provided to the Investigator who will be responsible for determining and reporting AE/SAEs.

The following assessments should be conducted, and data collected by the visiting nurse in the participant's home during Visit 11:

- AE/SAE/tolerability query
- Review caregiver diary: concomitant medications, voids, oral intake and changes in symptoms
- Review Dosing Compliance
- Collect blood labs
    - CBC with differential
    - Coagulation
    - CMP

- Collect urine for urinalysis
- Vital Signs
- Weight
- Targeted Physical Exam: general appearance, abdominal, CVA tenderness, check for edema. Exam may include a suprapubic exam (bladder palpation) to assess for urinary retention if decreased urine output is noted.

9.2.12 Visit 12 Treatment Period (in-home nurse) – Week 9 (± 1 day)

The in-home visit at Week 9 (Visit 12) will be conducted by a visiting nurse. The nurse will escalate any issues related to AE/SAEs, stopping criteria, major dosing issues or issues of potential safety concern to the Investigator via telephone the same day. Any changes noted will be provided to the Investigator who will be responsible for determining and reporting AE/SAEs.

The following assessments should be conducted, and data collected by the visiting nurse in the participant's home during Visit 12:

- AE/SAE/tolerability query
- Review caregiver diary: concomitant medications, voids, oral intake, changes in symptoms
- Review Dosing Compliance
- Collect blood for CMP
- Collect urine for urinalysis
- Vital Signs
- Weight
- Targeted Physical Exam: general appearance, abdominal, CVA tenderness**,** check for edema. Exam may include a suprapubic exam (bladder palpation) to assess for urinary retention if decreased urine output is noted.

9.2.13 Visit 13 Treatment Period (telemedicine and in-home) – Week 10 (±1 day)

Visit 13 (Week 10) will be a remote telemedicine and in-home visit. The assessments to be conducted and data collected are divided accordingly below.

<u>Remote/Telemedicine – Investigator and Appropriate Site Staff</u>

The following assessments and data will be conducted or collected by the site via telemedicine or video facilities as appropriate during Visit 13. Caregiver-completed assessments should be reviewed by the Investigator.

- Recent Clinical History
- Modified neurological exam
- Modified physical exam
- AE/SAE and tolerability review
- Review of the Caregiver Diary (dosing, con-meds, seizures, voids, oral intake, changes in symptoms)

<u>Caregiver Assessments</u>

The following assessments should be completed by the caregiver.

- Caregiver diary

In-home Nurse Visit

The in-home visit will be conducted by a visiting nurse. The nurse will escalate any issues related to AE/SAEs, stopping criteria, major dosing issues or issues of potential safety concern to the Investigator via telephone the same day. Any changes noted will be provided to the Investigator who will be responsible for determining and reporting AE/SAEs.

The following assessments should be conducted, and data collected by the visiting nurse at the participant's home during Visit 13:

- AE/SAE/tolerability query
- Review caregiver diary: concomitant medications, voids, oral intake, and changes in symptoms
- Review Dosing Compliance
- Collect blood labs
    - CBC with differential
    - Coagulation
    - CMP
- Collect urine for urinalysis
- Vital Signs
- Weight
- Targeted Physical Exam: general appearance, abdominal, CVA tenderness, check for edema. Exam may include a suprapubic exam (bladder palpation) to assess for urinary retention if decreased urine output is noted.

9.2.14 Visit 14 Treatment Period – (in-home nurse) Week 11 (±1 day)

The in-home visit at Week 11 (Visit 14) will be conducted by a visiting nurse. The nurse will escalate any issues related to AE/SAEs, stopping criteria, major dosing issues or issues of potential safety concern to the Investigator via telephone the same day. Any changes noted will be provided to the Investigator who will be responsible for determining and reporting AE/SAEs.

The following assessments should be conducted, and data collected by the visiting nurse in the participant's home during Visit 14:

- AE/SAE/tolerability query
- Review caregiver diary: concomitant medications, voids, oral intake, changes in symptoms
- Review Dosing Compliance
- Collect blood for CMP
- Collect urine for urinalysis
- Vital Signs
- Weight
- Targeted Physical Exam: general appearance, abdominal, CVA tenderness, check for edema. Exam may include a suprapubic exam (bladder palpation) to assess for urinary retention if decreased urine output is noted.

9.2.15 Visit 15 Treatment Period (in-home nurse) – Week 12 (±1 day)

The in-home visit at Week 12 (Visit 15) will be conducted by a visiting nurse. The nurse will escalate any issues related to AE/SAEs, stopping criteria, major dosing issues or issues of potential safety concern to the Investigator via telephone the same day. Any changes noted will be provided to the Investigator who will be responsible for determining and reporting AE/SAEs.

The following assessments should be conducted, and data collected by the visiting nurse in the participant's home during Visit 15:

- AE/SAE/tolerability query
- Review caregiver diary: concomitant medications, voids, oral intake, changes in symptoms
- Review Dosing Compliance
- Collect blood for CMP
- Collect urine for urinalysis
- Vital Signs
- Weight
- Targeted Physical Exam: general appearance, abdominal, CVA tenderness, check for edema. Exam may include a suprapubic exam (bladder palpation) to assess for urinary retention if decreased urine output is noted.

9.2.16 Visit 16 End of Treatment (in-clinic) – Week 13 (-3 days)

The following assessments will be conducted and data collected during the EOT visit (Week 13, Visit 16). The EOT visit is conducted when the participant is still on treatment. Caregiver assessments must be completed the day of the visit or no earlier than the day before the visit (except the caregiver diary which is completed daily) and reviewed by the Investigator.

The EOT visit (Week 13, Visit 16) may be conducted over three consecutive days. It is preferrable that ECGs, safety labs and biomarker sample collection are conducted on the same day. All assessments must be completed within the window for the visit and thus conducted before the participant completes the dosing period (stops dosing).

- Safety
  - Recent clinical history
  - Physical and neurological examination
  - [redacted]
  - Vital signs
  - Height/length
  - Weight
  - Review for AEs/SAEs
  - 12-lead ECG
  - Serum Pregnancy Test (females who have reached menarche)
  - Rapid SARS-CoV-2 test
  - CBC with differential
  - Coagulation
  - CMP Urinalysis
  - TSH, Free T3, Free T4

  - HbA1c

- Review Caregiver Diary (con-meds, seizures, breathing events, changes in symptoms)
- Review Dosing Compliance
- Pharmacokinetics/Pharmacodynamics
  - Blood sampling for PK
- Biomarkers
  - Blood sample for biomarkers
  - Stool sample for microbiome
- Efficacy
  - CGI-I
  - CGI-S
  - SB5 or Bayley-4 (Bayley is completed for participants who cannot achieve a basal on SB5)
  - Vineland Adaptive Behavior Scales-3
  - ASME
  - MB-CDI
  - ORCA
  - Caregiver Top 3 Concerns
  - ABC-2
  - BPI-SF
  - Caregiver Impression of Change
  - ICND
  - QI-Disability
  - CSHQ
  - GIHQ
  - Modified two-minute walk test

9.2.17 Visit 17 Follow-up (telemedicine and in-home) – Week 15 (+ 4 days)

Visit 17 (Week 15, Follow-up) will be a remote and in-home visit. The assessments to be conducted and data collected are divided accordingly below.

Remote/Telemedicine – Investigator and Appropriate Site Staff

The remote visit will be conducted by the site via telemedicine or video facilities as appropriate. Caregiver assessments must be completed the day of the visit or no earlier than the day before the visit (except the caregiver diary which is completed daily) and reviewed by the Investigator.

The Investigator will also query changes in tolerability and note any changes. The Investigator is responsible for determining and reporting any changes that qualify as AEs.

The following assessments will be conducted and data collected or reviewed by site personnel during Follow-up:

- Safety
  - Recent clinical history
  - Physical and neurological examination: A modified version of the physical and neurological exam will be done based on the guidance document provided to the Investigator.
  - Review for AEs/SAEs

- Review Caregiver Diary (dosing, con-meds, seizures, voids, oral intake, breathing events, changes in symptoms)
- Efficacy
    - CGI-I
    - CGI-S
    - Caregiver Top 3 Concerns
    - ABC-2
    - CSHQ
    - GIHQ
- Study Exit Form

In-home Nurse Visit

The in-home visit will be conducted by a visiting nurse who will also query any changes in tolerability during the in-home visit. Any changes will be noted and provided to the Investigator.

The following assessments will be conducted and data collected by the home nurse during Follow-up:

- AE/SAE/tolerability query
- Review caregiver diary: dosing, concomitant medications, voids, oral intake, changes in symptoms
- Collect blood:
    - CMP
    - CBC with differential
    - Coagulation
- Collect urine for urinalysis
- Vital Signs
- Weight
- 12-lead ECG
- Targeted Physical Exam: general appearance, abdominal, CVA tenderness**,** check for edema. Exam may include a suprapubic exam (bladder palpation) to assess for urinary retention if decreased urine output is noted.

9.3 Screening and Baseline Procedures

Screening and Baseline assessments are completed after informed consent during a period of approximately 4 to 6 weeks. During the Screening/Baseline period data will also be collected to establish the participant's baseline characteristics and symptom severity based on assessments collected during the 4 to 6 week period. There are two in-clinic visits and one remote/in-home visit for Screening and Baseline (Visits 1, 2, 3). Screening (Visit 1, in-clinic), Screening Visit 2, (combined remote/in-home nurse visit), Baseline (Visit 3, in-clinic). The second visit during the Screening period (Visit 2) should be conducted 2 weeks ± 1 day after the Screening visit (Visit 1) . The participant will then remain in Screening for an additional 2 to 4 weeks. The caregiver diary must be completed daily during the entire Screening period whether it is 4 weeks or 6 weeks.

During the Screening visit (Visit 1) informed consent to participate in the study will be obtained from the parent or legally authorized representative.

Participants will be evaluated for the diagnosis of PTHS and documented disease-causing genetic abnormality of *TCF4*. If the participant has been genotyped at any time in the past, these will not need to be repeated at Screening, provided the documentation of these tests is available for verification.

A rapid screening test for SARS-CoV-2 will be conducted by the site at Screening and Baseline. At Screening, a complete medical history will be conducted, and a neurological and physician exam will be done. [redacted] Caregivers will complete a 4 to 6 week diary to record seizure frequency, major breathing events, urine frequency (number of voids), any changes in symptoms or oral intake, and any changes in concomitant medications.

Baseline cognitive characteristics will be assessed at Screening using the Stanford-Binet Intelligence Scales version 5 (SB5) scored using the z-deviation method for intellectual disability[38] or the Bayley Scales of Infant Development version 4. The Bayley will be done for participants who cannot attain a basal on the SB5 or for whom the SB5 is developmentally inappropriate.

Presence of a co-morbid psychiatric disorder (e.g., Autism Spectrum Disorder [ASD], bipolar disorders, attention deficit disorder, obsessive-compulsive disorder) will be confirmed by medical history and based on the DSM-5 criteria. The Autism Mental Status Exam (AMSE) will be used as part of the medical history and to characterize ASD symptoms.[35] The AMSE is an 8-item observational assessment that structures the way clinicians observe and document social, communicative and behavioral functioning in people with ASD. Each individual item is scored 0, 1, or 2 and the AMSE yields a total score of 0 to 14. AMSE will be collected at screening to characterize ASD symptoms and at EOT.

The Screening visit (Visit 1) may be conducted over three consecutive days. Eligibility assessments as outlined in Section 9.1.1 including the SARS-CoV-2 rapid test should be done on the first day of the visit. It is preferrable that ECGs, safety labs and biomarker sample collection are conducted on the same day. No assessments can be conducted until informed consent has been given by the participant's legally authorized representative. Also, training for the caregivers on all caregiver completed assessments must be completed before the caregiver rater may complete the assessments. All assessments including caregiver completed assessments must be completed within the window for the visit (visit day ± 2 days).

Information on participants who are screened for the study but found to be ineligible will be recorded on a screening log. The visit date, confirmation of informed consent, demographics and reason for screen fail will be entered into the Electronic Data Capture System (EDC).

The Baseline visit (Visit 3) is completed after eligibility is confirmed and the participant is enrolled. The Baseline visit (Visit 3) may be conducted over three consecutive days. Confirmation of eligibility criteria including the rapid SARS-CoV-2 test and recent clinical history must be done on the first of the three days. It is preferrable that ECGs, safety labs and biomarker sample collection are conducted on the same day. All assessments must be completed within the two-day window for the visit (visit day ± 2 days). For Visit 3, all Baseline assessments must be completed before administration of the IMP.

At the end of the Baseline visit (Visit 3), the first dose of IMP will be administered in the clinic after all Baseline assessments are completed, or, if the Investigator judges that it is too late in the day, on the following day. The participant should be monitored for at least three hours after the first dose. If the first dose is administered the day following Baseline, vital signs (except height) should be collected before the dose is administered. Day 1 of dosing is defined by the date when the first dose is taken. At the Baseline visit, the site will dispense an adequate supply of IMP for dispensing by the caregiver at home.

9.4 Treatment of Participants

9.4.1 Investigational Medicinal Product

Full information regarding the preparation, storage, and administration of NNZ-2591 Oral Solution, 50 mg/mL is provided in the Pharmacy Manual. NNZ-2591 IMP is provided as a ready-to-use strawberry-flavored liquid for oral administration.

9.4.2 Investigational Medicinal Product Administration

NNZ-2591 Oral Solution, 50 mg/mL will be administered open-label at a target dose of 12 mg/kg BID. The total administration period for NNZ-2591 will be 13 weeks. Participants will be up-titrated to the target dose according to the dosing schedule outlined in Section 7.2. The dose titration schedule may be modified based on updated PK modelling including data from the study.

NNZ-2591 Oral Solution, 50 mg/mL, is provided as a ready-to-use strawberry-flavored liquid for oral administration. The IMP should not be mixed with water, liquid thickeners, or food for administration.

Each day there will be two dose administrations, one in morning and one in afternoon or evening. There should be at least 8 hours between doses. Doses should be given on an empty stomach (2 hours after the previous meal or snack and 30 minutes prior to the next). Doses may be taken over a 10-minute period with a follow up of 250 mL of water.

For all participants, the total duration of NNZ-2591 treatment will be 91 days. Dosing at the end of Baseline counts as Day 1 whether one or two doses are given. Dosing may not exceed 91 days including Day 1, and all efficacy assessments for the EOT visit must be completed on or before Day 91 of treatment.

The first dose of IMP will be administered in the clinic after all Baseline assessments are completed or, if the Investigator judges that it is too late in the day, on the following day. The participant should be monitored for at least three hours after the first dose. If the first dose is administered the day following Baseline, vital signs (except height) should be collected before the dose is administered. Day 1 of dosing is defined by the date when the first dose is taken. At this visit, the site will provide IMP for the caregiver to dispense at home. Enough IMP will be provided to cover the interval until the next in-clinic visit when the caregiver will be re-supplied from site.

Within the up-titration period, if a participant is deemed not to be tolerating the current dose within the 2-week period, and they have not met any of the stopping criteria below (see Section 9.4.5), they can be down-titrated to the next lower dose in the titration schedule (See Section 7.2).The Investigator must attempt to increase the dose as soon as it is possible based on the clinical situation. The aim is to return to the originally assigned dose. If the originally assigned dose cannot be reached, or the participant is again unable to tolerate that dose, the Investigator will continue treatment on the highest dose the participant can tolerate. Stopping rules for the discontinuation of the administration of IMP are described in Section 9.4.5. Investigators are to discontinue administration of IMP if the participant meets any of the criteria described in Section 9.4.5.

Note that early cessation of IMP does not necessarily mean the participant is withdrawn from the study. With any early cessation, all evaluations and tests scheduled during the study must be conducted if possible.

9.4.3 Treatment Compliance

The timing of administration of the IMP should be at approximately the same time each day. Each day there will be two dose administrations, one in morning and one in afternoon or evening. There should be at least 8 hours between doses. Doses should be taken by mouth either from a cup or via a dosing syringe provided by the pharmacist. Doses should be given on an empty stomach (2 hours after the previous meal or snack and 30 minutes prior to the next). The IMP should not be mixed with water, liquid thickeners, or food for administration. Doses may be taken over a 10-minute period with a follow up of 250 mL of water.

The treatment times will be recorded in the Caregiver Diary and then entered into the eCRF. A Study Monitor will review participant source documents and drug accountability records to assess treatment compliance on an ongoing basis during site visits. Participants who receive ≥ 80% and ≤ 120% of IMP, administered in accordance with the protocol are considered compliant.

9.4.4 Protocol Deviations

This study will be conducted as described in this protocol, except during an emergency situation in which the protection, safety and well-being of the participant requires immediate intervention, based on the judgment of the Investigator (or appropriately qualified designee). In the event of a significant deviation from the protocol, the Investigator or designee must contact the Medical Monitor or Sponsor or their agent at the earliest possible time by telephone. This will allow an early joint decision regarding the participant's continuation in the study. The Investigator will document the outcome of the discussion in the source documents and e-CRF, and the Sponsor likewise will retain a documentary record of the decision. The Sponsor will reserve the right to recommend suspension or discontinued involvement of sites with excessive protocol violations.

9.4.5 Early Discontinuation

Investigational Medicinal Product administration may be stopped early if an AE, tolerability issue or inter-current illness occurs that in the opinion of the Investigator (or designee) necessitates cessation of the IMP. Study drug administration must be ceased for all SAEs considered related to the experimental medication.

Note that early cessation of IMP does not necessarily mean the participant is withdrawn from the study. With any early cessation, all evaluations and tests scheduled during the study must be conducted if possible.

- Requests early discontinuation.
- requires treatment with an excluded medication, as defined in Exclusion Criteria (Section 8.2).
- at any point in the study, a participant becomes ill with symptoms of/or tests positive for COVID-19 infection.
- has a positive pregnancy test or becomes pregnant.
- in the Investigator's opinion, the participant is experiencing a clinically significant deterioration in his or her clinical state.
- experiences a Serious Adverse Event deemed to be related to the IMP.
- experiences a severe adverse event deemed to be related to the IMP.
- has an increase in post-enrollment QTcF interval as defined below (see also Section 9.5.8).
- [redacted]

[redacted]

Definition of QTcF[redacted]

Investigational Medicinal Product must be discontinued at any time during the study in the event any of the following are observed post-enrollment:

QTcF Criteria

- A post-enrollment QTcF duration of ≥ 500 ms confirmed by repeat continuous ECG.
- A clinically significant increase of ≥ 60 ms (confirmed by repeat continuous ECG) compared to the average QTcF interval at Baseline (before dosing) is observed.

[redacted]

Participants who have received IMP for longer than 2 weeks and discontinue prematurely any time after Visit 5 should return to the investigational site for final safety and efficacy assessments as scheduled for the EOT visit (Week 13, Visit 16) and complete the study exit form.

Participants who discontinue from the trial prior to receiving any treatment or who discontinue after having received treatment only up to Visit 5 (Week 2 post-baseline) may be replaced.

Participants with early treatment cessation due to SAEs must be followed-up and provided appropriate medical care until their signs and symptoms have remitted or stabilized, or for the remaining study period (until study end), whichever comes sooner. "Study end" is when all participants have completed the study.

9.4.6 Concomitant Therapy

Investigators are encouraged to follow current international and hospital guidelines and current best medical practice for the management of participants with PTHS. Medications should be used per current recommendations. Concomitant medications permitted in the study are outlined below and are described in the Exclusion Criteria (Section 8.2).

Medications prohibited from the study are outlined below and in the Exclusion Criteria (Section 8.2). Participants should not have their existing medication withdrawn in order to enter the study.

Excluded medications/treatments include:

- Current treatment or treatment within the two weeks prior to Screening with MAO inhibitors, d-cycloserine, oxytocin, carbetocin, tricyclic antidepressants, or bupropion.
- Current treatment with more than 3 allowable psychotropic medications.
    - Includes medications used to treat problems with sleep onset and sleep continuity except melatonin. Melatonin for difficulties with sleep onset is permissible and is not included in the count of psychotropic medications.
    - The use of anti-epileptics for other indications such as the treatment of mood disorders counts towards the limit of permitted psychotropic medications.
    - Anti-epileptic medications used for treatment of seizures does not count towards the limit of psychotropic medications.
- Current treatment with insulin or treatment within the last 12 weeks prior to commencement of IMP.
- Current treatment with IGF-1 or treatment within 6 months of commencement of the IMP.
- Current treatment with growth hormone or treatment within 12 months of commencement of the IMP.

The following medications are allowed but must be at stable doses before the start of the study as follows:

- Non-pharmacologic somatic treatment (e.g., a ketogenic diet or vagal nerve stimulation): dosing regimen must be stable at least 4 weeks prior to Screening. If the treatment was discontinued, the discontinuation must have occurred no fewer than 2 weeks before Screening.
- A new behavioral, educational, or cognitive therapy cannot be started during the period of the study or within 4 weeks prior to Screening. Changes to an ongoing treatment regimen that are due to school schedules or are otherwise seasonally related are not exclusionary as long as they are consistent with the typical type and pattern of behavioral treatment received by the participant.
- Seizure medication: dosing regimen must be stable 8 weeks prior to commencing the IMP. Adjustments for changes in weight or age are allowable. If the treatment was discontinued, the discontinuation must have occurred no fewer than 2 weeks before Screening.
- Psychotropics or any other medication used for a chronic illness (not including antibiotics, pain relievers, anti-diarrheals, and laxatives): dosing regimen must have been stable for at least 4 weeks prior to Screening. If the treatment was discontinued, the discontinuation must have occurred no fewer than 2 weeks before the commencement of IMP.
    - This includes nutraceutical treatments and cannabinoids (THC and CBD) which are allowable but also must meet the stability conditions above.
    - The Investigator should consult with the medical monitor if a participant is being treated with ocular toxic, hepatotoxic medications, nephrotoxic, or antibiotic medications.

The following medications/treatments are permitted and may be adjusted as needed:

- Procedural pain control medication.
- Laxatives and anti-diarrheals.

Standard of care immunizations and vaccinations (including COVID-19) are allowed and are not subject to the above stability conditions.

If the Investigator is unsure if a medication is permitted, the Medical Monitor should confirm if the medication is allowed and will provide written documentation to support the medication's use, if allowed. Concomitant medications will be monitored in a caregiver diary for the approximately 4 to 6 week Screening period to confirm there are no changes from the time of initial screening.

Every effort should be made to maintain stable regimens of concomitant medications and allowed non-medicine-based therapies throughout the course of the study, with the understanding that there will be some changes to non-medicinal treatment regimens that are due to school schedules or are otherwise seasonally related.

Details of concomitant medications will be recorded in the participant notes and in the eCRF.

9.5 Assessments

9.5.1 Vital Signs

Vital signs including weight, blood pressure (SBP and DBP in mmHg), heart rate (bpm), respiratory rate (rpm), and body temperature (°C) will be assessed at the Screening visits (Visits 1 and 2) and at the Baseline visit (Visit 3). Height will be measured at Screening (Visit 1), Baseline (Visit 3) and End of Treatment (Week 13, Visit 16). Blood pressure (SBP and DBP in mmHg), weight, heart rate (bpm), respiratory rate (rpm), and body temperature (°C) will also be assessed at all post-enrollment study visits. If the first dose is administered in clinic the day after the Baseline visit, blood pressure (SBP and DBP in

mmHg), weight, heart rate (bpm), respiratory rate (rpm), and body temperature (°C) should be collected before dosing.

Vital signs will be taken ideally after the participant has been seated for 5 minutes. If this is not feasible, this should be documented in the source notes and the EDC.

The procedures for taking blood pressure will follow best practice from the 2017 American Academy of Pediatrics *Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents* [†††]. The clinician conducting the assessments should be trained in the best practice guidelines including how to select the appropriate cuff size. Manual blood pressure measurements from the right arm will be taken by the Investigator or other appropriately credentialed and experienced individual. Ideally, blood pressure should be taken while the participant is seated. The Investigator should ensure to the best extent possible that the participant's feet are flat on the floor, the back is supported, and the right arm supported at level of the heart.

If the manual blood pressure measurements suggest increased blood pressure, an additional blood pressure measurement should be performed manually later in the same visit. If there is a change in blood pressure from baseline, a repeat measurement should be performed manually later in the same visit. The clinician should also document any factors that may affect the blood pressure measurements such as the participant's state (e.g. excited, agitated), disruptive breathing events, hydration level, if the measurements could not be taken seated, or if the participant was uncooperative. If blood pressure measurements suggest elevation, the Investigator should also conduct a medical review with physical exam, and review safety labs, in particular, serum creatinine, urinalysis, and urine volume. If after at least 2 measurements have been taken at the visit, there is confirmed clinical suspicion of true elevation of blood pressure, or if the participant is agitated or uncooperative and the blood pressure cannot be confirmed, or there is a change in blood pressure from baseline, blood pressure should be re-assessed again within 12-24 hours. If elevated blood pressure is confirmed and meets the stopping criteria below, dosing should be discontinued, and a referral should be made to the child's primary care pediatrician or a kidney specialist as deemed appropriate by the Investigator and the Medical Monitor.

IMP must be discontinued at any time during the study in the event that any of the following values are observed on blood pressure exams based upon repeated evaluation on at least three visits.

- For children ages 3-12 years: ≥ 95th percentile (based on height) + 12 mmHg OR ≥ 140/90 mmHg
- For adolescents ages 13 years and above: ≥ 140/90 mmHg (in case a 12-year-old turns 13 years old during the study)

These must be reported and discussed with the Medical Monitor.

9.5.2 Laboratory Tests

Standard of care laboratory assessments will be undertaken at Screening as well as various time points during treatment and at follow up.

Urine samples should be collected at home ideally as the first void of the morning on the visit day using a sample collection kit provided by the site. Caregivers will be provided instruction on procedures for

[redacted]

collecting the samples to reduce contamination.

- **Complete Blood Count with Differential** (Visits 1, 3, 5, 7, 9, 11, 13, 16, 17): Hemoglobin (Hb), hematocrit (Hct), red cell count (RBC), white blood cell count (WBC), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), platelets (PLT), red cell distribution width (RDW-CV), and leukocyte count (neutrophils, lymphocytes, monocytes, eosinophils, and basophils). If abnormalities are seen, additional tests may be conducted as deemed necessary by the Investigator and Medical Monitor and as described below in the clinical management plan in Sections 9.5.2.1 and 9.5.2.3.

- **Coagulation** (Visits 1, 3, 5, 7, 9, 11, 13, 16, 17): prothrombin time test, International normalized ratio (INR), activated partial thromboplastin (aPTT). If abnormalities are seen, additional tests may be conducted as deemed necessary by the Investigator and Medical Monitor and as described below in the clinical management plan in Sections 9.5.2.1 and 9.5.2.3.

- **Comprehensive Metabolic Profile** (All Visits except Visit 2): amylase, sodium, glucose, potassium, blood urea nitrogen (BUN), enzymatic creatinine, BUN/creatinine ratio, estimated glomerular filtration rate (eGFR), bicarbonate, chloride, total calcium, phosphorus, magnesium, bilirubin (direct and indirect), creatine kinase, alkaline phosphatase (ALP), aspartate transaminase (AST), alanine amino transferase (ALT), gamma-glutamyl transferase (GGT), total protein, albumin, total cholesterol, and random triglycerides. If abnormalities are seen, additional tests may be conducted as deemed necessary by the Investigator and/or Medical Monitor and as described below in the clinical management plan in Sections 9.5.2.1 and 9.5.2.3.

    - eGFR is calculated using the modified Bedside Schwartz formula: eGFR=(0.413*height)/creatinine mg/dL

    - This formula will be used for all participants including those who may age up to 18 years old during the study

- **Urinalysis** (All Visits except Visit 2): with microscopic analysis, glucose, bilirubin, ketones, specific gravity, blood, pH, protein, albumin, urobilinogen, nitrite, and leucocyte esterase. If abnormalities are seen on microscopy, additional tests for culture, sensitivity and protein analysis may be conducted as deemed necessary by the Investigator and/or Medical Monitor, and as described below in the clinical management plan in Section 9.5.2.2.

- Urine will also be tested at Screening, for drugs of abuse, i.e., stimulants (e.g., amphetamine, methamphetamine, methylenedioxymethamphetamine [MDMA], cocaine), hallucinogens (e.g., phencyclidine), narcotics/analgesics/ opiates (e.g., codeine, morphine, methadone, propoxyphene), depressants/sedatives/hypnotics (e.g., barbiturates).

- **TSH, Free T3, Free T4** (Visits 1, 16): free triiodothyronine (T_3), free thyroxine (T_4), thyroid stimulating hormone (TSH).

- **Glycosylated hemoglobin (HbA1c)** (Visits 1, 16)

- **Rapid test for SARS-CoV-2** (Visits, 1, 3, 16). The site may use any test with same day results approved for use for children and adolescents in the US.

- **Serum Pregnancy Test** (Visits 1, 9, 16): Females who have reached menarche will have a serum pregnancy test (β-HCG) at Screening, Visit 9 (Week 6) and at Visit 16 (Week 13, EOT).

A central laboratory will be used for the safety sample analysis. Details on sample collection, handling and shipment are outlined in the Laboratory Manual. The laboratory range reference list is also provided in the Laboratory Manual. Abnormal or out of range values should be reviewed by the Investigator who should make a determination if the values are clinically significant. The laboratory reference list designates normal ranges (for age and sex as applicable), and critical alerts for all blood and urine labs. If abnormal results are observed in any of the above tests, additional tests may be conducted via the central laboratory or the site's local laboratory as deemed appropriate by the Investigator and/or the Medical Monitor.

It is preferable that repeat labs are done through the central laboratory. If not feasible, repeat labs may be collected at the site's laboratory or a local laboratory. Repeat labs should be ordered stat. If repeat labs are done through the central lab, a deidentified copy of the report must be provided in the participant's study file. The reference values for the laboratory test for that lab must also be obtained and filed in the study files.

9.5.2.1 [redacted]

[redacted]

9.5.3 Blood sample for biomarkers and stool sample for microbiome

Blood and stool samples will be collected at Visits 1 (Screening), 3 (Baseline), and 16 (Week 13, EOT) or upon early termination for analysis of biomarkers and gut microbiome. The analysis will be detailed in a separate analysis plan but will not include DNA sequencing.

9.5.4 Medical/Clinical History

A complete medical history will be performed at all Screening and Baseline visits (Visits 1, 2, 3) to document all current medical conditions, and previous major medical events and conditions. For participants who may receive their on-going care at the study site, summary documents from the medical record (such as clinician's summaries) should be available as source documentation of major medical conditions or events (e.g., surgeries) within the last two years. For participants who are not a part of the clinical site's practice, the study team should request families obtain summary medical records from their primary care provider (if available, or another qualified healthcare professional as determined by the Investigator) in preparation of the Screening visit. At each in-clinic and combined remote visit (Visits 4, 5, 7, 9, 11, 13, 16 and 17), a current clinical history will be conducted by the Investigator or appropriately credentialed delegate. This will include review of any changes in urine voids or oral intake.

9.5.5 Physical and Neurological Exam

A physical and neurological exam including a complete, standard review of systems will be undertaken by a physician (or appropriately credentialed delegate) at all in-clinic visits. The physical exam procedures will include the following organ systems: Head, ears, eyes, nose, and throat; Skin; Cardiovascular; Respiratory; Gastrointestinal; Genitourinary (as appropriate or indicated); Musculoskeletal; Allergies. The exam will include an assessment for signs of bladder or kidney pathology including an exam of the abdomen and both flanks, looking for signs of discomfort or distress. Assessment of edema will also be performed. Exam may include suprapubic exam (bladder palpation) to assess for urinary retention if decreased urine output is noted.

A standard neurological exam will be undertaken at all in-clinic visits to include an evaluation of mental status, cranial nerves, motor exam, sensory exam, cerebellar (coordination, gait), reflexes.

A modified version of the physical and neurological exam will be performed by a physician (or appropriately credentialed delegate) via telemedicine at the remote visits [Visits 7 (Week 4), 11 (Week 8), 13 (Week 10), and 17 (Week 15)] based on guidance documents provided to the Investigator. The modified version of the neurological exam will follow recommendations for conducting telemedicine exams from the Child Neurology Society. Additionally, as part of the in-home visit, the nurse will conduct a targeted physical exam including assessment of general appearance, an abdominal and a costovertebral angle tenderness exam, assessment for signs of cystitis, and an assessment of edema. Exam may include suprapubic exam (bladder palpation) to assess for urinary retention if decreased urine output is noted. These are done at Visits 4 (Week 1), 6 (Week 3), 7 (Week 4), 8 (Week 5), 10 (Week 7), 11 (Week 8), 12 (Week 9), 13 (Week 10), 14 (Week 11), 15 (Week 12), and 17 (Week 15).

9.5.6 [redacted]

[redacted]

9.5.7 [redacted]

[redacted]

9.5.8 12-lead ECG and Stopping Criteria for Prolonged QT

Formal assessments of QTcF and cardiac irregularities will be done at Screening (Visit 1), Baseline (Visit 3), Visit 5 (Week 2), Visit 9 (Week 6), EOT (Week 13, Visit 16) and Follow Up (Visit 17). At each designated visit, a continuous ECG of at least 50 seconds will be obtained. Ideally, the participant should be supine for at least 5 minutes before the first ECG reading is taken. If this is not possible, this should be documented in the source and the EDC.

If the clinician determines that an ECG is invalid due poor quality or major artefact during data collection, the ECG should be deleted and reassessed. If the clinician is uncertain if the ECG can be read, duplicate ECGs may be submitted to the central reader. The central reader will read and interpret all readable ECGs

submitted. The clinician will be queried to adjudicate which ECG should be considered the final read and entered into the database.

ECGs will be sent via secure transmission and read by a qualified central reader. The results from the reports from the central reader and any alert flags should be reviewed by the Investigator who will determine if the values are clinically significant and sign the report. Results will be assessed against the ECG screening criteria for QTcF at the Screening visit and against the QTcF stopping criteria throughout the study.

Investigational Medicinal Product must be discontinued at any time during the study in the event that a post-enrollment QTcF duration of ≥ 500 ms confirmed by a repeat continuous ECG, or a clinically significant increase of ≥ 60 ms (confirmed by a repeat continuous ECG) compared to the average QTcF interval at Baseline (Visit 3, before dosing) is observed.

9.5.9 Battery of Pitt-Hopkins Syndrome Assessment Instruments

The battery of PTHS specific measures will evaluate symptom change in clinically meaningful and functionally impactful symptom domains for individuals with PTHS including communication, cognition, motor function, maladaptive behavior, adaptive behaviors, sleep, seizures, breathing and GI issues (see Section 6.3.2). The selection of outcome measures for this study reflects the insights from the published literature, and expert clinical advice.

All assessments will be administered in a standardized manner by trained practitioners. To the extent possible, all efforts should be made to maintain the same caregiver informant or investigator rater (as applicable) across visits for a single participant. If in extenuating circumstances (such as severe illness), the caregiver is unable to complete the rating for a visit, another appropriate rater can be designated to complete the assessment for that visit. This should be documented in the source and the EDC. The site staff should ensure the alternative rater has appropriate knowledge of the participant to complete the rating and is informed of the instructions for completing the assessments.

Caregivers will complete most assessments via an online portal. Paper versions of assessments will also be used and may be made available for online versions in the case of difficulty accessing the electronic portal. These assessments should be completed the day of the visit or no earlier than the day before the visit. No assessments can be conducted until informed consent has been given by the caregiver or legally authorized representative. Also, training for the caregivers on all caregiver completed assessments must be completed before the caregiver rater may complete the assessments.

Overall symptom severity and functional level will be assessed using the following tools:

9.5.9.1 Clinical Global Impression of Severity (CGI-S)

Clinical Global Impression of Severity assessments will be done at Screening (Visit 1), Baseline (Visit 3), Week 6 (Visit 9), EOT (Week 13, Visit 16), and Follow-up (Week 15, Visit 17). The Clinical Global Impression - Severity scale (CGI-S)[36] is a 7-point scale that requires the Investigator to rate the severity of the individual's overall illness at the time of assessment, relative to the Investigator's past experience with participants who have the same diagnosis.[36] Considering total clinical experience, a participant is assessed on severity of illness at the time of rating: 1, normal, not at all impaired; 2, borderline, slightly impaired; 3, mildly impaired; 4, moderately impaired; 5, markedly impaired; 6, severely impaired or 7, among the most extremely impaired ill. Following best practice for neurodevelopmental disorders, the CGI-S will be evaluated using PTHS-specific scoring anchors. The clinicians will provide an overall global severity rating and a severity rating for each of seven domains: language/communication, fine motor functioning/self-help, social initiation/social avoidance, challenging behavior, ambulation/gross motor,

gastrointestinal issues, and autonomic/breathing abnormalities. The clinician rater should assess the participant's current state taking into consideration observations at the visit and symptoms within the last week. To ensure that interpretation of the scoring rubric is normalized among the raters, clinicians administering the CGI-S will be required to complete scoring calibration training.

9.5.9.2 Clinical Global Impression of Improvement (CGI-I)

Clinical Global Impression of Improvement assessments will be made at Visit 9 (Week 6), EOT (Week 13, Visit 16), and Follow-up (Week 15, Visit 17). The CGI-I scale[36] requires the Investigator to rate how much the participant's illness has improved or worsened relative to a baseline state. A 7-point scale is used with the following values: 1, very much improved; 2, much improved; 3, minimally improved; 4, no change; 5, minimally worse; 6, much worse; 7, very much worse. The CGI-I will be evaluated using PTHS-specific scoring anchors. The clinicians will provide an overall global improvement rating and an improvement rating for each of seven domains: language/communication, social initiation/social avoidance, fine motor functioning/self-help challenging behavior, ambulation/gross motor, gastrointestinal issues, and autonomic/breathing abnormalities. The clinician should assess the participant's current state taking into consideration observations at the visit and symptoms within the last week. The rating of change is compared with status at Baseline (Visit 3). To ensure that interpretation of the scoring rubric is normalized among the raters, clinicians administering the CGI-I will be required to complete scoring calibration training.

9.5.9.3 Bayley Scales of Infant Development v4 (BSID4) or Stanford-Binet Intelligence Scales v5 (SB5)

The Stanford-Binet Intelligence Scales version 5 (SB5) assessment will be done at Screening (Visit 1) and EOT (Week 13, Visit 16). SB5 is validated for ages 2 to 85+ and measures both verbal and nonverbal intellectual and cognitive abilities through a set of 10 subtests.[37] General cognitive ability is reported as a full-scale intellectual quotient score (FSIQ). Cognitive ability in five categories are reported in the index scores: fluid reasoning (FR), knowledge (KN), quantitative reasoning (QR), visual-spatial processing (VS), and working memory (WM). Non-verbal (NVIQ) and verbal intelligence quotients (VIQ) are also calculated. Scoring of index scores and IQ will be calculated using the z-deviation method for intellectual disability.[38]

The Bayley Scales of Infant Development version 4 (BSID-4) is conducted for participants who cannot achieve a basal score on SB5or for whom the SB5 is developmentally inappropriate .[39] Assessment will be done at Screening (Visit 1) and End of Treatment (Week 13, Visit 16). The Bayley is a norm-referenced developmental assessment validated for ages 16 days to 42 months. It assesses cognitive ability, motor skills, and behavior and provides standardized scores in five domains: Cognitive, Language, Motor, Social-Emotional, and Adaptive Behavior. Scale scores, age equivalents and growth scale values (GSVs) can also be calculated for the scale subtests: cognition (CG), receptive communication (RC), expressive communication (EC), fine motor (FM), and gross motor (GM). Scaled scores and age equivalents can be calculated for the social emotional (SE) subscale. The Adaptive Behavior subscale will not be collected in this study. The Early Learning Composite score and scaled scores are calculated for children 42 months and younger. Raw scores, age equivalents, and growth scale values are calculated for all participants.

If the SB-5 or the Bayley had previously been administered for the participant within 3 months of Screening, the scores from that administration may be used if it meets the following criteria: 1) assessment was administered by site personnel, or another study site; 2) the site has access to all the scores required to be entered in the EDC; 3) the site has permission to share the scores from the previously administered assessment to the study EDC. Assessments done by the school or other clinics

are not acceptable. Only scores from SB-5 and the Bayley are acceptable; cognitive scores from other assessments cannot be entered in the EDC in lieu of the SB-5 or the Bayley.

9.5.9.4 MacArthur-Bates Communicative Development Inventory (MB-CDI)

The MacArthur-Bates Communicative Development Inventory (MB-CDI) will be completed at Baseline (Visit 3) and EOT (Week 13, Visit 16). This caregiver-completed instrument assesses language development through vocabulary comprehension and production, gestures, and grammar.[40] Designed to assess early language development in infants and toddlers, it can also be used with older, developmentally delayed children to assess developmental progress in language development. The MB-CDI includes three modules based on chronological or developmental age: Words and Gestures (8- to 18- months or developmental equivalent), Words and Sentences (16- to 30-months or developmental equivalent) and the CDI-III, an extension of the MB-CDI used with children 30-37 months or developmental equivalent. The Word and Gestures module will be used for this study in line with the expected cognitive and language development of the study population. It assesses developing abilities in language including production of gestures, and vocabulary comprehension and production which are reported as number of instances and percentile scores.

9.5.9.5 ORCA (Observer-Reported Communication Ability Measure)

The Observer-Reported Communication Ability Measure (ORCA) will be completed at Baseline (Visit 3) and EOT (Week 13, Visit 16). This caregiver-reported measure was originally developed and validated for Angelman syndrome,[41] to assess the individual's overall communicative ability and was designed to capture communicative abilities in low-verbal and non-verbal populations, so is relevant for PTHS. It was developed based on feedback from a patient-focused listening session with the advocacy community and the Food and Drug Administration following best practices for measure development including concept elicitation interviews with caregivers and communication experts, item generation, assessment of validity and psychometrics, and factor analysis confirming single overall communication ability score.[41-43] The rating scale evaluates the caregiver's observation of the individual's communication ability over the past 30 days.[42] The measure consists of 84 total questions with 70 behavioral items. It assesses communication abilities of individuals across all modalities: gestures, AAC (augmentative and alternative communication) and speech so is not dependent solely on verbal abilities. Twenty-two communication concepts relevant to communicative ability in PTHS are assessed within the categories of expressive communication, receptive communication, and pragmatic communication rendering a total communication score.

9.5.9.6 Caregiver Top 3 Concerns Rating

The Caregiver Top 3 Concerns Rating assessment is a syndrome-specific measure of 3 signs or symptoms of PTHS identified by caregivers at Screening/Baseline as being priority concerns in which they would like to see improvement as a result of treatment. The assessment will be completed at all Screening and Baseline visits (Visits 1, 2, 3), as well as Week 6 (Visit 9), EOT (Visit 16), and Follow-up (Visit 17).

In this assessment, concerns are identified on an individual, per-participant basis. The clinician should review the assessment with the caregiver at the Screening visit, ideally as part of the overall medical and clinical history. Caregivers should be encouraged, in selecting the concerns, to identify distinct, observable symptoms rather than broad ones. And caregivers should be advised to consider symptoms that they feel are important and have an impact on their child's daily functioning and well-being.

Caregivers are asked to identify 3 concerns from among 11 clinically important symptom domains: Language/Communication, Social interest/avoidance, Repetitive Behaviors, Challenging Behaviors, Gross motor skills, Fine motor skills, Gastrointestinal problems, Breathing Disruptions, Sleep, Seizures,

and Self-care skills. Each individual concern identified should be from a different symptom domain, but caregivers can select any domain and choose any symptom of concern within that domain. The same three concerns identified at screening/baseline are carried forward and rated at the follow-up visits. The severity of each concern is scored by caregivers using a 10-point Likert scale or severity. The caregiver should give consideration to symptoms within the last two weeks.

9.5.9.7 Aberrant Behavior Checklist-2 (ABC-2)

The ABC-2 will be completed at all Screening and Baseline visits (Visits 1, 2, 3), as well as Week 6 (Visit 9), EOT (Week 13, Visit 16), and Follow-up (Visit 17). The Aberrant Behavior Checklist-2 (ABC-2)[44] is a caregiver-completed rating scale for assessing problem behaviors that has been validated in a variety of clinical populations and been used as an outcome measure in clinical trials of neurodevelopmental disorders (NDDs) across much of the lifespan.[44] The rater considers the relative frequency of an individual's behaviors and gives a rating of the severity of the problem it presents: 0, not at all a problem; 1, the behavior is a problem but slight in degree; 2, the problem is moderately serious; 3, the problem is severe in degree. The caregiver should give consideration to symptoms within the last two weeks.

The ABC-2 includes 58 items providing five subscale scores: Irritability, Social Withdrawal, Stereotypic Behavior, Hyperactive/Noncompliance, and Inappropriate Speech. These subscales have been shown to have good consistency, reliability, and validity with normative data available for specific populations including children with intellectual disability and ASD.[45]

9.5.9.8 Behavior Problems Inventory -Short form (BPI-SF)

The Behavior Problems Inventory Short Form (BPI-SF) will be completed at Baseline (Visit 3) and at the EOT (Week 13, Visit 16). The BPI-SF is a caregiver-completed rating scale for assessing self-injury, aggressive and stereotyped behavior.[46, 47] Frequency is assessed for all three types of behavior on a five-point scale from 0 (Never), 1 (Monthly), 2 (Weekly), 3 (Daily) and 4 (Hourly). For self-injury and aggression, severity is also assessed on a 3-point scale of mild, moderate, or severe.

9.5.9.9 Caregiver Impression of Change

The Caregiver Impression of Change assessment will be done at the EOT (Week 13, Visit 16). The Caregiver is asked to rate the change in his or her child's overall function and well-being since the Baseline visit (Visit 3), and the change in specific symptom domains. Ratings are made using a 7-point Likert scale:1-Very much improved, 2-Much improved, 3-Improved, 4-Unchanged, 5-Worse, 6-Much worse, 7-Very much worse. For the rating of overall function, the caregiver also identifies the one symptom area or feature that has most influenced his or her rating of the child's overall function and provides a description of symptom changes and the impact of the changes. The assessment of change should be made since the Baseline visit (Visit 3). The caregiver should assess the participant's current state at the visit taking into consideration observations at the visit and symptoms within the last week.

9.5.9.10 Quality of Life Inventory-Disability (QI-Disability)

The Quality of Life Inventory-Disability (QI-Disability) will be completed at Baseline (Visit 3) and EOT (Week 13, Visit 16).[48-50] The QI-Disability[48] is a caregiver-report measure that assesses a child's well-being and quality of life over the past month. Designed to be relevant for children who have an intellectual disability, the QI-Disability includes 32 questions in 6 domains: Social Interaction, Physical Health, Independence, Positive Emotions, Leisure and the Outdoors, and Negative Emotions. The QI-Disability has been validated in individuals ages 5 to 18 with Rett syndrome, Downs syndrome, CDKL5

Deficiency Disorder, Cerebral Palsy, and ASD. Items are rated on a 5-point Likert scale with a 0-100 scaling such that the value of each rating is: never = 0, rarely =25, sometimes = 50, often = 75, and very often is 100. Domain scores are calculated by summing all the scores and dividing by the number of items. The total score is calculated by summing the domain scores and dividing by the number of domains.

9.5.9.11 Impact of Childhood Neurological Disability-Overall Quality of Life Rating (ICND)

The Impact of Childhood Neurologic Disability Scale (ICND) will be administered at Baseline (Visit 3) and at End of Treatment (Week 13, Visit 16). The ICND was developed to evaluate the impact that a child's condition has on the child's and the family's everyday life.[51] The full scale assesses the effect of four conditions or health problems on the child's or the family's life, and evaluates the child's overall quality of life. In this study, only the overall quality of life assessment will be completed. The caregiver will rate overall quality of life of the participant by responding to the following: "Please rate your child's overall 'Quality of Life' on the scale below. Choose the number which you feel is best and circle it". The choices range from 1 ("Poor") to 6 ("Excellent"). The caregiver should consider the child's status in the last month including their current condition. .

9.5.9.12 Child Sleep Habits Questionnaire (CSHQ)

The Child Sleep Habits Questionnaire (CSHQ) will be completed by the participant's caregiver at all Screening and Baseline visits (Visits 1, 2, 3), as well as Week 6 (Visit 9), EOT (Week 13, Visit 16), and Follow-up (Visit 17). The CSHQ gathers information on bedtime and assesses sleep behavior, waking during the night, and morning waking/daytime sleepiness[52] to assess both behavioral and medical sleep disorders. The CSHQ has been used across a range of NDDs including PTHS and has been used as an outcome measure for sleep intervention trials.[53-55] It provides a total score and eight subscale scores: bedtime resistance, sleep onset delay, sleep duration, sleep anxiety, night awakenings, parasomnias, sleep disordered breathing, and daytime sleepiness. The caregiver should consider sleep behaviors within the last week in doing their ratings.

9.5.9.13 Vineland Adaptive Behavior Scales-3

The Vineland Adaptive Behavior Scales Third Edition (Comprehensive Interview version, Vineland-3) will be completed at Baseline (Visit 3) and EOT (Week 13, Visit 16). This standardized measure assesses development and adaptive functioning which correspond to the domains of adaptive functioning used in the DSM-5: Communication, Daily Living Skills, and Socialization.[56] The Vineland will be conducted as an interview by a trained site rater with the caregiver. The measure provides an overall adaptive functioning score, and standardized domain scores in Daily Living Skills, Communication, Socialization and Motor Skills. The Vineland also has a maladaptive behavior domain which will not be completed in this study. V-scale scores and growth scale values (GSV) can also be calculated for the subdomains. The GSV scores on the Vineland have been found to be more sensitive to change than standardized scores for developmentally delayed populations.[57] Raw scores, standardized scores, v-scale scores, age equivalents, and GSV will be calculated for all participants. For participants 3 to 9 years of age, Motor Skills can be calculated; for those older than 9 years old, scores will not be calculated for Motor Skills.

9.5.9.14 GI Health Questionnaire (GIHQ)

The Gastrointestinal Health Questionnaire (GIHQ) will be completed by the participant's caregiver at all Screening and Baseline visits (Visits 1, 2, 3), as well as Week 6 (Visit 9), EOT (Week 13, Visit 16), and Follow-up (Visit 17). The measure was developed for use in NDDs and validated in individuals with Rett syndrome and includes GI symptoms that are relevant for PTHS.[58] The GIHQ assesses the impact of GI

issues on the individual's life over the last two weeks.[58] Items are rated on a 5-point Likert scale as follows: 0= it is never a problem, 1=it is almost never a problem, 2=it is sometimes a problem, 3=it is often a problem, 4=it is almost always a problem. A total score and 9 subtest scores are calculated with the 9 subtests comprising issues in three domains: Gastrointestinal symptoms, Mood/Behavior, and Parent Concerns.

9.5.9.15 Modified 2-Minute Walk Test

The modified 2-Minute Walk test (2MWT)[59] is an assessment of how far an individual can walk during a 2 minute period which assesses endurance and also has relevance for everyday functioning. The 2MWT will be done at Screening (Visit 1), Baseline (Visit 3) and at the EOT (Week 13, Visit 16). For this assessment, the individual with PTHS walks back and forth between two cones on a 20-meter track for two minutes and the total distance covered is measured. The modified 2MWT requires two assessors and one walk assistant. The walk assistant knows the person with PTHS well (e.g., parent, caregiver, therapist) and provides the necessary physical support for the person to keep his/her balance and to maintain the highest possible gait speed.

9.5.10 Caregiver Diary

A Caregiver Diary will be completed daily by the caregiver and reviewed at all visits by the Investigator and by visiting nurse at remote visits. In the diary, the participant's caregiver will record dosing compliance of IMP, any changes in concomitant medication or treatments, urine output measured by number of voids or wet diapers, any changes in symptoms including changes in oral intake, sleep activity, seizure activity, and breathing events. The diary also specifically queries changes in maladaptive behavior or self-injury which may suggest changes in the tendency to self-harm. At the Screening visit, the clinician will review the assessment with the caregiver and assist the caregiver to define the child's typical seizure types, breathing abnormalities, sleep issues or other chronic conditions that are to be tracked in the diary. Any new, emergent types of seizures or breathing abnormalities, or any other new symptoms will also be documented if they occur.

The Investigator must review the caregiver diary at or after each visit. At clinic visits, the Investigator will review the diary and the site will provide a new diary section to the caregiver. At remote visits, the nurse will review the diary in the home noting any potential issues in the nurse report and collect the diary to send to the Investigator along with notes from the visit. The nurse will provide the caregiver with a new diary section for the upcoming week. The nurse will not sign-off on the diary. The Investigator is responsible for reviewing the nurse notes, reviewing the diary, signing the diary, and determining if anything needs to be reported as an Adverse Event.

9.6 Pharmacokinetic Measurements

Pharmacokinetic blood samples will be collected in accordance with the sampling schedule outlined in Table 2 for ages 7 to 17 years old and Table 3 for ages 3 to < 7 years old. The date and actual time (hour and minute) that each sample is collected must be accurately recorded. The site must also ensure that the date and time of the previous evening's dose and the morning and evening dose on the day the sample is collected is accurately collected in the Caregiver Diary.

For the Week 2, 6, and 13 data collection, the previous day's PM dose should be taken in the evening as late as possible, preferably before bedtime. The pre-dose sample should be taken 120 minutes to 15 minutes before the morning dose is administered. For post-dose samples, sample collection can be ± 15 minutes of the target time and must be no closer than 30 minutes from the previous sample (must be

at least 30 minutes from the previous sample). The actual collection time is recorded. The post-dose windows are determined from the first dose of the visit (morning dose) and all PK samples should be collected before the second dose (PM dose). Full details regarding the PK sampling collection, storage and shipping may be found in the Laboratory Manual for the study.

PK blood samples may be collected from a cannula port or via venipuncture. Pre-prepared PK sampling tubes will be provided to each site within the lab visit kits for collection and storage of PK samples. Samples of whole blood will be collected, aliquoted, and will be stored at minus 20°C (or colder) no more than 2 hours following sample collection. Samples will be shipped at designated times on dry ice and using temperature monitoring to the laboratory for storage and analysis. It is essential that accurate records are maintained regarding the date and time each PK sample is obtained, and the date and time each sample is placed in the –20°C freezer. The date and time of the most recent dose of IMP and most recent meal relative to that dose should be recorded in the Caregiver Diary.

Minimum requirements for a participant to be considered evaluable within the PK population are administration of NNZ-2591 per protocol through to the morning dose at Visit 5 (Week 2) as a minimum, *and* collection of PK samples at the specified post-dose time point(s) through to Visit 5 (Week 2) as a minimum.

10. ADVERSE EVENTS

The Investigator and site staff are responsible for detection, recording and reporting of events that meet the criteria and definition of an AE or SAE, as described below. At in-home nurse visits, the nurse will do a query of any changes for potential AE/SAEs and document observations from the targeted physical exam. The nurse will escalate any issues related to AE/SAEs, stopping criteria, major dosing issues, or issues of potential safety concern to the Investigator the same day. Any changes noted will be provided to the Investigator who will be responsible for determining, recording, and reporting any events that qualify as AE/SAEs.

10.1 Definition of Adverse Event

An adverse event is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom or disease temporally associated with the use of a drug, without any judgment about causality.

AEs can include the following:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- Conditions detected or diagnosed after IMP administration even though it may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either IMP or a concurrent medication.

SAEs are a sub-category of AEs that are defined in Section 10.2, below. AEs/SAEs are recorded and reported from the time of first dose of IMP until Follow-up (Visit 17).

Planned hospital admissions for an illness or a disease that existed prior to administration of IMP or enrollment in the clinical study are generally not to be considered AEs. Individuals who have planned surgical procedures that would occur during the study are to be excluded from the study (Exclusion 43). If the participant has a planned hospital admission (non-surgical), the Investigator should consult with the Medical Monitor to ensure this will not cause disruption of IP administration or would not qualify the participant for exclusion.

Note that medical or surgical procedures are not considered AEs (e.g., endoscopy); however, the condition that leads to the procedure is an AE.

10.2 Definition of Serious Adverse Event

An adverse event 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 adverse event, in-patient hospitalization or prolongation of existing hospitalization, a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or 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 participant and may require medical or surgical intervention to prevent the outcomes listed in this definition.

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

10.3 [redacted]

[redacted]

10.4 Adverse Reactions

An adverse reaction means any adverse event caused by drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event. Suspected adverse reactions means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the adverse event.

10.5 Clinical Laboratory Abnormalities and Other Abnormal Assessments as AEs and SAEs

Abnormal laboratory findings (e.g., CBC with differential, liver function, renal function, biochemistry, urinalysis) or other abnormal assessments (e.g., ECGs, vital signs) that are judged by the Investigator as clinically significant changes (new findings or worsening of baseline findings that are clinically impactful) will be recorded as AEs or SAEs if they meet the definition of an AE, as defined in Section 10.1 (Definition of Adverse Event), or SAE, as defined in Section 10.2 (Definition of Serious Adverse Event). Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study and significantly worsen following the start of the study will be reported as AEs or SAEs.

The Investigator will exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

10.6 Time Period, Frequency, and Method of Detecting AEs and SAEs

Any pre-existing conditions or signs and/or symptoms present in a participant prior to the start of the study (i.e., before on-study informed consent obtained) that do not result in the exclusion of the participant from participating in the study, should be recorded as Medical History. In addition, any medical occurrence which is reported after informed consent is obtained, but prior to the participant receiving IMP, should be documented as Medical History. If a baseline sign or symptom worsens following dosing, then this should be recorded as an AE or SAE as appropriate.

All AEs occurring after administration of the dose of IMP and on or before the final visit must be reported. These are referred to as treatment emergent adverse events (TEAEs). All AEs must be recorded irrespective of whether they are considered drug related.

At each visit/assessment in the period defined above, AEs will be evaluated by the Investigator and recorded.

Any AEs already documented at a previous assessment and designated as ongoing, should be reviewed at subsequent visits. If these have resolved, this should be documented.

10.7 Recording of AEs and SAEs

When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory, and diagnostics reports) relevant to the event. The Investigator should then record all relevant information regarding an AE/SAE on the eCRF. It is not acceptable for the Investigator to send photocopies of the participant's medical records to the Sponsor in lieu of completion of the appropriate AE/SAE eCRF pages. However, there may be instances when the Sponsor requests copies of medical records for certain cases. In this instance, all participant identifiers will be removed on copies of the medical records prior to submission to the Sponsor.

The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

10.8 Evaluating AEs and SAEs

10.8.1 Assessment of Intensity

The Investigator will make an assessment of intensity for each AE and SAE reported during the study. The assessment will be based on the Investigator's clinical judgment. The intensity of each AE and SAE recorded in the eCRF should be assigned to one of the following three categories:

Mild:
An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.

Moderate:
An event that is sufficiently discomforting to interfere with normal everyday activities.

Severe:
An event that prevents normal everyday activities.

An AE that is assessed as severe should not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 10.2 Definition of Serious Adverse Event.

10.8.2 Assessment of Causality

The Investigator is obligated to assess the relationship between IMP and the occurrence of each AE/SAE. The Investigator will use clinical judgment to determine the relationship. Alternative causes such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal

relationship of the event to the IMP will be considered and investigated. The Investigator will also consult the Investigator's Brochure in the determination of his/her assessment.

All AEs will be assessed by the Principal Investigator or qualified and authorized delegate as to whether there is a 'reasonable possibility that the drug caused the event' and on that basis be classified as either 'related' or 'not related'.

There may be situations when a SAE has occurred and the Investigator has minimal information to include in the initial report to the Sponsor. However, it is very important that the Investigator always make an assessment of causality for every event prior to transmission of the SAE form to the Sponsor. The Investigator may change his/her opinion of causality in light of follow-up information, amending the SAE form accordingly.

10.8.3 Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each participant and provide further information to the Sponsor on the participant's condition.

All AEs and SAEs documented at a previous visit/contact and are designated as ongoing, will be reviewed at subsequent visits/contacts.

All AEs will be followed until resolution, until the condition stabilizes, or until 30 days after the participant's Visit 17 Follow Up, whichever occurs first. At which time, the appropriate AE/SAE eCRF page(s) will be updated. SAEs and renal AEs that are ongoing at the Follow-up visit (Visit 17) will be followed until resolution, stabilization of participant condition, or study end, whichever occurs first. In such cases, follow-up Serious Adverse Event Report (SAER) [redacted] forms will be completed at the Follow-up visit (Visit 17) as well as completed upon resolution, stabilization, or study end, whichever occurs first. "Study end" is when all participants have completed the study.

The Investigator will ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE or SAE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

The Sponsor may request that the Investigator perform or arrange for the conduct of supplemental measurements and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The Investigator is obligated to assist. If a participant dies during the study or during a recognized follow-up period, the Sponsor will be provided with a copy of the death certificate and any post-mortem findings, including histopathology.

New or updated information will be recorded on the originally completed SAE [redacted] with all changes signed and dated by the Investigator. The updated SAE form should be re-sent to the Sponsor within the time frames outlined below in Section 10.8 Prompt Reporting of the SAEs to the Sponsor.

Any clinical adverse event that is serious (as defined above in Section 10.2) [redacted] occurring during the course of the study, irrespective of the treatment received by the participant, must be reported to the Sponsor and Sponsor's agent within 24 hrs (or sooner if possible) of the Investigator or designee becoming aware of the situation.

10.9 Prompt Reporting of the SAEs to the Sponsor

Once an Investigator or delegate becomes aware that an SAE has occurred in a study participant, she/ he will report the information to the Sponsor's appointed agent within 24 hrs.

10.9.0 Completion and transmission of the SAE reports

[redacted] will be the Medical Monitor for this study.

[redacted]
Email: [redacted]

Serious Adverse Events will be processed by the Pharmacovigilance Team at [redacted] with the support of and review by the Medical Monitor and Neuren's Medical Team.

A serious adverse event report (SAER) form is to be completed for each SAE occurring up to the final study visit, regardless of causality. This includes renal AEs that meet criteria for an SAE. In addition, the SAE is to be recorded on the appropriate section of the eCRF. The SAER must be submitted to the Pharmacovigilance Team at the email below within 24 hours of the time the SAE is first observed.

Submit SAER form via email within 24 hours of the event to: [redacted]

The Medical Monitor or pharmacovigilance department will contact the Investigator should it be necessary to clarify any of the event information. The Investigator should provide follow-up information for the event to the Medical Monitor or pharmacovigilance department as soon as it becomes available. If required, the Investigator should report these events to the local IRB.

The SAE form will always be completed as thoroughly as possible with all available details of the event and signed by the Investigator (or authorized designee). If the Investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before notifying the Medical Monitor of the event and completing the form. The form will be updated when additional information is received.

The Investigator will always provide an assessment of causality at the time of the initial report as described in Section 10.7.2 Assessment of Causality.

Any event that, in the opinion of the Principal Investigator, may be of immediate or potential concern for the participant's health or well-being will be reported to the Medical Monitor.

In accordance with applicable regulations and local laws, the Sponsor will identify and report within the required time frame to regulatory authorities and Investigators all adverse events assessed as related that are determined to be both serious and unexpected (Suspected Unexpected Serious Adverse Events reporting SUSAR). Investigators will be responsible for reporting this safety information to their IRB.

SAEs that are ongoing at the Follow-up visit (Visit 17) will be followed until resolution, stabilization of participant condition, or study end, whichever occurs first. In such cases, follow-up SAER forms will be

completed at the Follow-up visit (Visit 17) as well as completed upon resolution, stabilization, or study end, whichever occurs first. "Study end" is when all participants have completed the study.

All SAE and AE data will be reviewed by the DSMC at scheduled meetings.

10.10 Deaths

A SAER form is to be competed for all deaths. Death will be recorded in the eCRF. Source documentation (e.g., autopsy report, death certificate) to confirm the cause of death will be reviewed by the DSMC.

10.11 [redacted]

[redacted]

[redacted]

10.12 Pregnancy

Pregnant, lactating, or breastfeeding females are to be excluded from this study. In the event of pregnancy, it will be reported on a pregnancy form, and will be followed up to determine outcome, including spontaneous or voluntary termination, details of birth, and the presence or absence of any birth defects or congenital abnormalities. The investigator should report all pregnancies in female clinical trial participants to the Premier Pharmacovigilance Team as soon as possible using the Pregnancy Data Collection Form.

Submit Pregnancy Data Collection form vial email to: [redacted]

It is not expected that there will be sexually active participants in the study population, and as such some of the provisions that follow may have limited applicability based on the age range of study participants and the nature of the disease under study. These provisions are nonetheless included for purposes of completeness.

Participants of childbearing potential must abstain from sexual activity for the duration of the study and for at least 30 days thereafter. If a participant is sexually active or becomes sexually active during the study, she or he must use 2 clinically acceptable methods of contraception (e.g., oral, intrauterine device [IUD], diaphragm plus spermicide, injectable, transdermal or implantable contraception, condom use (male type only)) for the duration of the study and for at least 30 days thereafter.

11. STATISTICAL PLAN

11.0 Definitions

11.0.0 Participant Populations

For this trial the following populations will be defined and used in the analysis:

Intent-to-treat (ITT) Population

The Intent to Treat (ITT) population consists of all participants enrolled into the study.

Per-protocol Population

The PP population is defined as all participants recruited into the trial who received Investigational Medicinal Product and had no major protocol violations.

Safety Population

The safety population will include all participants in the ITT population.

Pharmacokinetic (PK) Population

The PK population will include all participants enrolled in this study who receive the IMP through to the morning dose of Visit 5 (Week 2) as a minimum, and who undergo PK sample collection at the specified post-dose time point(s) through to Visit 5 as a minimum.

11.1 Sample Size Determination

Sample sizes were estimated for this open-label study using within-participant change as the measure of improvement. Given the limited data from interventional trials in these patient populations, and that this is the first clinical study for NNZ-2591 IMP in this population, treatment change estimates were based on Clinical Global Impression Scale of Improvement (CGI-I) data from completed clinical trials in other rare, neurodevelopmental disorders.

Based on the information provided in Glaze et al. 2019 (Double-blind, randomized, placebo-controlled study of trofinetide in pediatric Rett syndrome), the standard deviation of the CGI-I is approximately 0.7 at a single timepoint.[1] For a sample size of 10 participants, a two-sided significance level of 0.05, and a Wilcoxon signed-rank test, the study will be able to detect a difference of 0.87 units of change in the CGI-I at a power of 0.90. For a power of 0.80, 0.85, and 0.90, the detectable difference would be 0.75, 0.80, and 0.87 or larger, respectively. The effect size for a power of 0.80, 0.85, and 0.90 is 1.07, 1.14, and 1.24, respectively. If the observed standard deviation is larger, the detectable differences would also be larger. For example, if the standard deviation is 1.5, the detectable difference for a power of 0.80, 0.85, and 0.90 is 1.61, 1.71, and 1.86 or larger, respectively. For a sample size of 20 participants, a two-sided significance level of 0.05, and a Wilcoxon signed-rank test, the study will be able to detect a difference of 0.55 units of change in the CGI-I at a power of 0.90. For a power of 0.80, 0.85, and 0.90, the detectable difference would be 0.48, 0.51, and 0.55 or larger, respectively.

11.2 Safety Analyses

Safety analyses will be conducted on the Safety Population and based on the safety endpoints listed in Section 6.3.1. Adverse events and other safety data will be summarized as frequencies and percentages, described in terms of severity and potential attribution. Objective safety assessments will be summarized over time on study; shift tables at time points of interest may be generated. The safety analysis details will be specified in the Statistical Analysis Plan (SAP).

11.3 Efficacy Analysis

Efficacy will be assessed by looking at within-participant changes from the Baseline period to the EOT using the efficacy endpoints listed in Section 6.3.2.

The participant's baseline characteristics and symptom severity will be established based on assessments collected during the 4 to 6 week Screening/Baseline period. Baseline values will be based on the average values of visits 1, 2, and 3 for assessments collected at more than one visit. A Wilcoxon signed rank test will be used to test for improvement and an exact significance level will be computed.

Potentially influential factors will be considered and addressed with a linear regression model. This model may include covariates such as age, sex, nonverbal NVIQ or NVIQ equivalent, genotype, history of regression (yes/no), ASD diagnosis (yes/no) with no more than two covariates included in any model. Descriptive statistics and corresponding graphics will be used to present the results. The safety and efficacy analysis details will be specified in the SAP.

11.4 Pharmacokinetic Analysis

The PK analysis will be based on the endpoints listed in Section 6.3.1. Pharmacokinetic (PK) measures will be collected from all participants at Visits 5 (Week 2), 9 (Week 6), and 16 (Week 13, EOT).

NNZ-2591 PK concentrations will be listed by participant and time point for each dose level with actual time they were acquired. The concentrations will be summarized by time, dose level, and age group. For the concentrations measured at trough points dose relationship will be examined statistically with age group and/or body weight of participant as covariate.

Overall exposure parameters for NNZ-2591 such as C_{max} and AUC_{tau} may be derived by sparse non-compartmental analysis (NCA). Additionally, PK concentrations of NNZ-2591 measured in pediatric participants will be used to refine population PK model developed from adult NNZ-2591 PK data and extrapolated to pediatric population. After refinement, complete profiles for each participant will be simulated using range of variability of model parameters and individual covariate effects. A standard NCA approach will be used for simulated profiles to calculate parameters C_{max}, AUC_{tau}, etc. for characterization of NNZ-2591 exposure in pediatric participants.

The PK analysis details will be specified in the PK SAP.

11.5 Biomarkers and Microbiome

Blood and stool samples will be collected for an exploratory analysis of biomarkers and gut microbiome. The analysis will be detailed in a separate analysis plan but will not include DNA sequencing. Samples will be collected at Screening (Visit 1) and Baseline (Visit 3, before dosing) and at the EOT visit (Week 13, Visit 16), or upon early termination.

12. STUDY DRUG MANAGEMENT

12.0 Identity of Investigational Medicinal Product

NNZ-2591 Oral Solution, 50 mg/mL IMP is supplied in 250 mL white PET PP28 neck bottles with white 28 mm child resistant and tamper evident caps. NNZ-2591 Oral Solution, 50 mg/mL IMP is manufactured under GMP by Quay Pharmaceuticals Ltd. (28 Parkway, Deeside Ind Park, Flintshire CH5 2NS).

- Name of Drug: NNZ-2591
- INN: None
- Chemical Name: Cyclo-L-Glycyl-2-Allyl Proline (cG-2-AllylP)
- Molecular formula: $C_{10}H_{14}N_2O_2$ (net)
- Chemical Abstracts Service (CAS) Registration Number: 847952-38-9
- Physical appearance of formulation: Clear, pink to red, non-viscous, free from visible particulates
- Odor and flavor of formulation: Sweet strawberry odor and flavor

NNZ-2591 Oral Solution, 50 mg/mL IMP is provided as a ready-to-use strawberry-flavored liquid for oral administration. The IMP is stored at 15-25°C in white 250 mL PET PP28 neck bottles with white 28 mm

child resistant and tamper evident white caps. The primary and secondary label text and design complies with U.S. FDA regulatory requirements and local codes of good manufacturing practice for medicinal products, as applicable, and will include the batch number, the manufacturing date of the IMP and the retest date of the product.

12.1 Management of Clinical Supplies

Investigational Medicinal Product, along with the dispensing cups and syringes, will be supplied to the site Pharmacist. The site Pharmacy contact is responsible for IMP management including receipt and correct storage/handling, dose calculation and IMP preparation, IMP accountability, and proper destruction of unused IMP and empty bottles. Full details regarding the preparation, storage, resupply, and stability of NNZ-2591 Oral Solution, 50 mg/mL may be found in the Pharmacy Manual.

NNZ-2591 Oral Solution, 50 mg/mL bottles will be shipped at 15-25°C and should be stored at 15-25°C (temperature monitored), according to the procedures outlined in the Pharmacy Manual. NNZ-2591 Oral Solution, 50 mg/mL must be maintained in a secure area with access restricted to designated study personnel. Investigational site personnel should notify the Study Monitor immediately if IMP bottles are damaged or if temperature excursions occur during storage.

12.1.0 Drug Accountability

The Investigator or designees must maintain an inventory record of the IMP administered to ensure that the investigational drug has been stored as per specifications and administered only to enrolled participants, in accordance with this protocol. Refer to the Pharmacy Manual for accountability procedures.

The IMP is to be prescribed only by the site Principal Investigator or named sub-Investigators and may not be used for any purpose other than that outlined in this protocol.

The Pharmacist and clinical team (as applicable) have been delegated responsibility from the Principal Investigator to maintain accurate records detailing the quantity of IMP kits received, dispensed, used, destroyed, or returned. During the study, the following must be noted in the Treatment Log:

- The study number and initials of the participant to whom IMP is dispensed.
- The date(s) and quantity of IMP dispensed.

Study participants will be instructed to return empty dosing bottles and any unused medication to the clinic. These returns will be accounted for in the clinic and the bottles and accountability form will be provided to the Pharmacy for reconciliation with dispensing and IP tracking logs.

At the end of the study, all unused IMP will be returned to the Sponsor's designee or destroyed by the site according to the Sponsor's instructions, once it has been inventoried and the Study Monitor has reviewed the drug accountability records.

12.2 Overdose

Participants should not exceed the maximum daily doses or take any two doses within an 8-hour interval. If an administered dose is spat out, regurgitated, or vomited, the dose should NOT be re-administered, and this should be documented in the dosing diary or the source documents for the in-clinic dose. There is no specific antidote to NNZ-2591 IMP. In the event of an overdose, appropriate supportive measures should be employed as clinically indicated and the Medical Monitor must be informed immediately.

13. QUALITY ASSURANCE AND DATA MANAGEMENT

13.1 Data Quality Assurance

The following measures will be implemented to ensure accuracy, consistency, completeness, and reliability of data:

- Investigator discussions
- Site initiation training
- Early site visits following enrollment
- Routine site management
- Ongoing site communication and training
- Periodic site monitoring
- Data management quality control checks
- Statistical quality control checks
- Continuous data acquisition and cleaning
- Internal review of data
- Quality control check of the final study report

13.2 Case Report Forms

This study will utilize web-based eCRFs developed through a 21CFR Part 11 compliant platform. Prior to the initiation of the trial, each site will be contacted as to computer availability, hardware specifications, security infrastructure, internet connectivity, etc., to evaluate their capacity to use this type of data capture system. The Investigator and site personnel will be responsible for completing the eCRFs or ensuring the completion of forms to be completed by the caregivers and entered into the EDC via the secure portal for caregiver raters. The Investigator is responsible for the accuracy and reliability of all the information recorded on the eCRFs. All information requested on the eCRFs needs to be supplied, including participant identification data, visit date(s), assessment values, etc., and any omission or discrepancy will require explanation. All information on eCRFs must be traceable to source documentation at the site.

During periodic monitoring visits, the site will make their computer and/or high-speed Internet access available to the Study Monitor so that he or she may verify the data entries with the source documentation.

13.3 Monitoring

A Sponsor's Study Monitor will visit each site in accordance with the Clinical Monitoring Plan set forth for this trial as well as maintain regular telephone and written communication. Monitoring visits will be conducted by representatives of the Sponsor according to the ICH Guideline for Good Clinical Practice (GCP), and applicable regional regulations and guidelines.

The Investigator will agree to the monitors making periodic site visits during the study. The timing of these visits will be agreed by the monitors and the study site personnel.

Monitoring will be on an ongoing basis by site. The assessments are to be captured on a specialized device/computer (e.g., 12-lead ECG) or in an online format or paper format (e.g., clinician and caregiver rating scales) and are to be obtained at the time of testing. It is imperative that the Investigator ensures that all tests are completed at each session, according to the participant's capability. Data collected at the site will be entered into the eCRFs in centralized, password protected EDC system. Checking for completeness and rationality will be undertaken via the EDC at a central location. If discrepancies are noted, the site monitor will be alerted and site follow-up undertaken in order that problems may be resolved immediately. This may require retraining of investigators on the testing process and the importance of competing the testing correctly.

13.4 Auditing

The Sponsor (or Sponsor nominee) may conduct periodic audits of the study processes including, but not limited to, the clinical site and the bioanalytical laboratory. The Investigator must authorize access and make available all study related documents to authorized Sponsor's representatives and regulatory authorities.

The scope of a clinical site audit may include but will not be limited to drug accountability, regulatory documentation, the informed consent process, and comparison of eCRFs against source data. The Investigator agrees to cooperate with audits conducted at a reasonable time and in a reasonable manner.

Regulatory agencies involved with the study may also audit the Investigator during or after the study. The Investigator should contact the Sponsor immediately if this occurs.

13.5 Source Documents

The Investigator will, within reason, prepare and maintain adequate and accurate source documents (e.g., medical records, ECGs, raw data collection forms, and electronic source documents) to record all observations and other pertinent data for each participant enrolled into the study. The Investigator will allow Sponsor representatives and authorized regulatory authorities to access documents pertaining to the study.

13.6 Protocol Deviations

This study will be conducted as described in this protocol, except during an emergency situation in which the protection, safety and well-being of the participant requires immediate intervention, based on the judgment of the Investigator (or appropriately qualified designee). In the event of a significant deviation from the protocol, the Investigator or designee must contact the Sponsor or the Medical Monitor at the earliest possible time by telephone. This will allow an early joint decision regarding the participant's continuation in the study. The Investigator will document the outcome of the discussion in the source documents and the Sponsor likewise will retain a documentary record of the decision. The Sponsor will reserve the right to recommend suspension or discontinued involvement of the site if excessive protocol deviations occur.

13.7 Records Retention

The Investigator must maintain essential study documents (protocol and amendments, completed eCRFs, source documentation, signed informed consent forms, relevant correspondence and approvals, 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 have elapsed since the formal discontinuation of clinical development of the

Investigational Medicinal Product. These documents should be retained for a longer period if required by the applicable regulatory requirements or the clinical site's procedures. Participant identification codes (participant names and corresponding study numbers) will be retained for this same time period and stored separately. Custody of the records may be transferred to another responsible party, acceptable to the Sponsor, which agrees to abide by the retention policies. Written notice of transfer must be submitted to the Sponsor. The Investigator must contact the Sponsors prior to disposing of any study records.

Records retention will follow the Data Protection requirements in the participating country.

14. ETHICS

14.1 Informed Consent

Written informed consent will be obtained from a parent or legally authorized representative of the participant before any study-related procedures are performed. The Investigator has an ethical and legal responsibility to ensure the parent or legally authorized representative of each participant being considered for inclusion in the study is given a full explanation of the study. The Investigator or designee shall inform the parent or legally authorized representative of each participant in writing of all aspects pertaining to the participant's participation in the study including (but not limited to) aims, methods, anticipated benefits, and potential risks. The participant's parent or legally authorized representative will have the opportunity to inquire about details of the study and to decide whether to participate.

A participant's parent or legally authorized representative should understand that they are free to refuse to allow the participant to participate in, and are free to withdraw the participant from, the study at any time without prejudice or loss of medical care to which they are otherwise entitled. They have the ability to withdraw the participant from the study at any time, for any reason.

The parent or legally authorized representative of each participant must personally sign and date a study-specific informed consent form on behalf of the participant to be a participant in the study. The participant's caregiver who will complete the study assessments must also provide written informed consent regarding their participation in the study prior to participating in any study procedures. The form must be countersigned by the site Investigator who conducted the informed consent discussion (or an authorized designee). Authorized designees will be defined according to local center and ethics requirements. Their authorization will be documented on a written informed consent form, which, together with this protocol, will be approved by an IRB.

A separate consent for potential re-use of leftover Biomarker and PK samples in later research, including potential genetic research, is optional.

Each informed consent will include the elements required by U.S. 21 CFR 50 and ICH E6, Section 4.8. The Investigator agrees to obtain approval from the IRB and Sponsor for any written informed consent form proposed for use in the study prior to submission to the IRB.

Each parent or legally authorized representative who provides written informed consent for a participant to participate in the study (by signing and dating of the form) will be given a copy of the signed informed consent form, and assent form if the minor is capable of signing an assent form or providing oral consent as required by local IRB regulations. The originals will be kept in the center's regulatory file and a second copy filed in the participant's medical record as permitted by the institution. The Investigator will inform participants and the parent or legally authorized representative of new information that may be relevant to the participant's willingness to continue participation in the study according to local ethics requirements.

It is important to obtain complete follow-up for all participants enrolled. Every attempt should be made to undertake all protocol specified assessments and complete the eCRF except for those participants who specifically withdraw consent for release of such information.

14.2 Institutional Review Board Approval

The Investigator will not begin the study until the protocol and informed consent form have been approved by the local Institutional Review Board (IRB) or specifically for the site by the central IRB as dictated by institutional requirements. Any amendments to the protocol must also be approved in writing

by the Sponsor, and the local IRB or central IRB for the site prior to implementation by the Investigator, except where necessary to eliminate an immediate hazard to study participants.

Written IRB approval of the protocol and Study Information Forms/Informed Consent Forms will be typed on the Institutional letterhead and will refer to the Study Information Forms/Informed Consent Forms and to the study by title and protocol number given by Neuren Pharmaceuticals on the protocol. A copy of the signed and dated letter of approval will be provided to Neuren Pharmaceuticals prior to study commencement. Any written information and/or advertisements to be used for volunteer recruitment will be approved by the IRB prior to use.

All IRB correspondence including progress reports will be retained on file at the clinical site.

14.3 Additional Protections for Minors

The age-related inclusion criterion for the study protocol will allow enrollment of participants aged 3 to 17 years. Participants aged younger than 18 are considered to be minors and, collectively, they constitute a vulnerable population. Additional regulations are in place for their protection (45 CFR Part 46 Subpart D). Normally, minors cannot legally provide consent to participation in research on their own behalf. Consent must be provided by one or both parents, or a legally authorized representative, while the minor provides “assent” for participation. Assent is the minor’s affirmative agreement to participate in the research.

Since this protocol involves greater than minimal risk but presents the prospect of direct benefit to all participants enrolled, consent shall be obtained from a guardian or at least one parent in accordance with local IRB requirements. Minors will be given the opportunity to assent to participation if and when they are considered capable of doing so by the Investigator and per local IRB requirements.

15. STUDY MANAGEMENT

The Sponsor will appoint the project management team, and an independent DSMC. Project management may be delegated to a Contract Research Organization who will be overseen by the Sponsor.

15.1 Principal Investigators

A Principal Investigator will be appointed at each participating site to personally supervise all aspects of the conduct of the study at their site.

15.2 Data Safety Monitoring Committee

An independent DSMC will monitor the progress of the trial and ensure that the safety of trial participants is not compromised. The DSMC will provide independent affirmation that no safety or tolerability issues are arising in the study through their on-going review of the study data. Specifically, the DSMC will review the clinical safety data prior to any individual dose titration and prior to initiation of subsequent age groups. The DSMC will review safety data and make an adjudication on: 1) the ability for any individual participant to escalate to the next higher dose and 2) the ability for enrollment to begin in the younger age groups (Groups 2 and 3).

The DSMC will minimally consist of a clinical chair, other physicians experienced in clinical trials with pediatric expertise (but not participating in this study), and a pediatric cardiologist. The DSMC Charter details the DSMC processes, duties, and responsibilities as well as the study-wide stopping criteria.

15.3 Publications

All publication rights are delineated in the Clinical Study Agreement and/or other separate agreements with the Investigator and/or Institution, as applicable. ICMJE uniform requirements will be respected, with regard to the composition, authorship and submission of manuscripts derived from this study. A publication committee will be composed that will include the principal investigators (or appropriate designees) as well as Sponsor representatives.

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Appendix A. Allometric scaling to determine phase 2 exposures

To determine the appropriate dose for pediatric participants, the Sponsor performed allometric scaling as discussed with the FDA in the Pre-IND Meeting. The findings from the allometric scaling analysis are summarized below.

The allometric scaling analysis was performed using the pharmacokinetic data from the Phase 1 study of NNZ-2591 in healthy adults (PK Report Neu-2591-HV-001). The purpose of the allometric scaling is to predict exposure in younger and lighter participants based on observed exposures in adult healthy volunteers at steady-state from the completed clinical study (Neu-2591-HV-001) for the dose of 12 mg/kg (cohort M2).

1. Methods for the Allometric Scaling

Steady-state PK parameters were used in the modeling. Median and percentiles were estimated for steady-state clearance CLss/F on Day 7 morning dose for the adult participants in the Neu-2591-HV-001 study to be used as input for allometric scaling. Median and 5th and 95th percentiles for the AUC_{tau} at steady-state for the adult participants were used as a target exposure in children for comparison of the results.

Fi value was already accounted for in the apparent steady state clearance CLss/F. The $WT_{centered}$ value for the study was median body weight for the participants in M1 and M2 cohorts of the study Neu-2591-HV-001 was equal to 73 kg.

Median CLss/F derived for steady-state exposure for Day 7 morning dose was used as CL_{TV}. Since the CLss/F was derived for body weight adjusted doses and hence was estimated as L/hr/kg for the simulations, the value was multiplied by the median weight of 73 kg to represent true value for the population of adult participants in the study Neu-2591-HV-001.

For simulation of exposure for children 3-17 (the planned age group for the Phase 2 study), the Centers for Disease Control and Prevention (CDC) Growth Individual charts with 3rd and 97th percentiles (3rd, 10th, 25th, 50th, 75th, 90th, and 97th) were downloaded from the CDC.gov website. The body weights for boys and girls from 36.5 months (3 years) to 17 years old (203.5 months) inclusive were combined for the input to Phoenix WinNonLin as a csv file. The file contained columns for 3rd, 5th, 10th, 25th, 50th, 75th, 90th, 95th, and 97th percentiles for each month of age and gender individually. Column with gender was added for the combined dataset. Age groupings for the allometric scaling analysis were Group 1 (3 to < 6), Group 2 (6 to <12), and 12-17.

The equation for allometric scaling was implemented in the Data Wizard function of Phoenix WinNonLin using weight for children of different age, gender, and percentile as direct input.

Doses were varied in the range from 11 to 20 mg/kg for final estimate with 1 mg/kg increments. The dose levels 11, 12, 13, 14, … 20 mg/kg were used to calculate the related exposures for different body weight values and compare the resulting AUC_{tau} values with adult results.

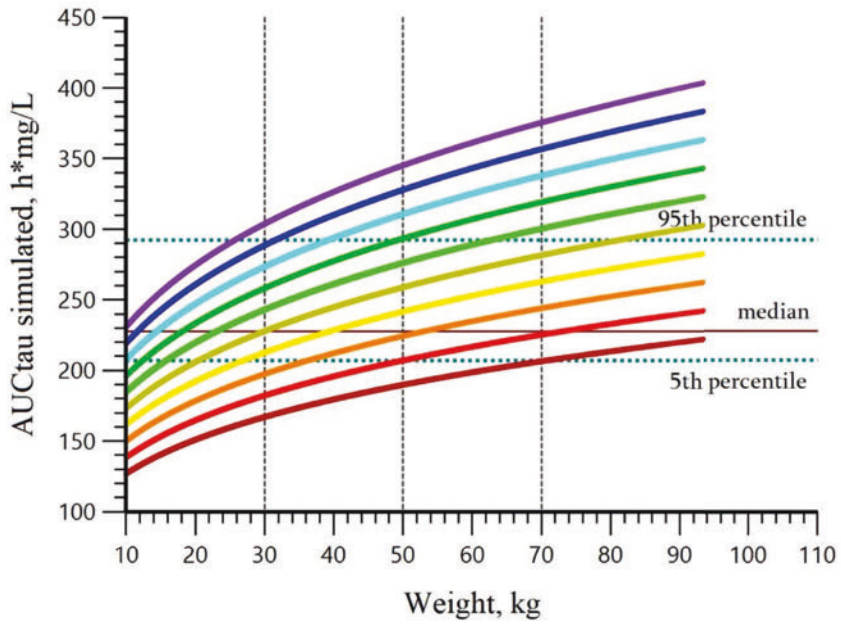
Additionally, a 17.5-mg/kg dose was evaluated for the lowest body weights. Preliminary evaluation of suitable doses was done starting from 3 mg/kg dose levels. The parameters derived for this allometric scaling were AUC_{tau} at steady state to compare with adult exposures.

2. Allometric Scaling Results

Non-compartmental parameters for NNZ-2591 in adult participants from the study Neu-2591-HV-001 were evaluated for dose proportionality of exposure after single, first doses and multiple steady-state doses of NNZ-2591 in healthy adult participants. Evaluation of dose proportionality supported the application of the published allometric scaling formula to steady state exposure parameter AUC_{tau} to be matched to adult exposures using body weight normalized dose in mg/kg and apparent steady state clearance in healthy adult participants. The predicted exposures (steady-state AUC_{tau}) were calculated from adult median CLss/F, estimated for morning and evening 12 mg/kg doses on Day 7 in the study Neu-2591-HV-001, for different dose values and body weights from CDC Growth Chart.

The findings showed that overall the predicted pediatric exposures were lower than adult exposures for the 12mg/kg dose for comparisons of exposure for different dose values versus weight. Combining the dose into 3 age groups resulted in significant overlap of body weight and differences in the dose projected to match adult exposure for 12 mg/kg dose within the group. Hence, body weight-based dosing instead of age-related dosing was originally proposed for the dose selection for children. The combined plots of AUC_{tau} vs weight are as shown in Figure A1.

Figure A2 Predicted AUC_{tau} of NNZ-2591 for different dose values vs weight all ages combined



AUC_{tau}= area under PK concentration-time curve from pre-dose to the end of dosing interval tau; h = hour; mg/L = concentration units milligram/liter

Dark red – 11 mg/kg, red – 12 mg/kg, orange – 13 mg/kg, yellow – 14 mg/kg, dark yellow – 15 mg/kg, light green – 16 mg/kg, dark green – 17 mg/kg, light blue – 18 mg/kg, dark blue – 19 mg/kg, purple – 20 mg/kg.

Brown line – median AUC_{tau} for adult subjects dosed with 12 mg/kg dose of NNZ-2591, dotted teal lines – 5th and 95th percentiles

Source: Neu-2591-HV-001 study adpc dataset, Phoenix 8.3 output for Project file Allometric Neuren.phxproj; CDC Growth chart

Source: Figure 5 in Report Allometric scaling for PK of NNZ-2591 for Pediatric Population with PMS, AS, and PTHS

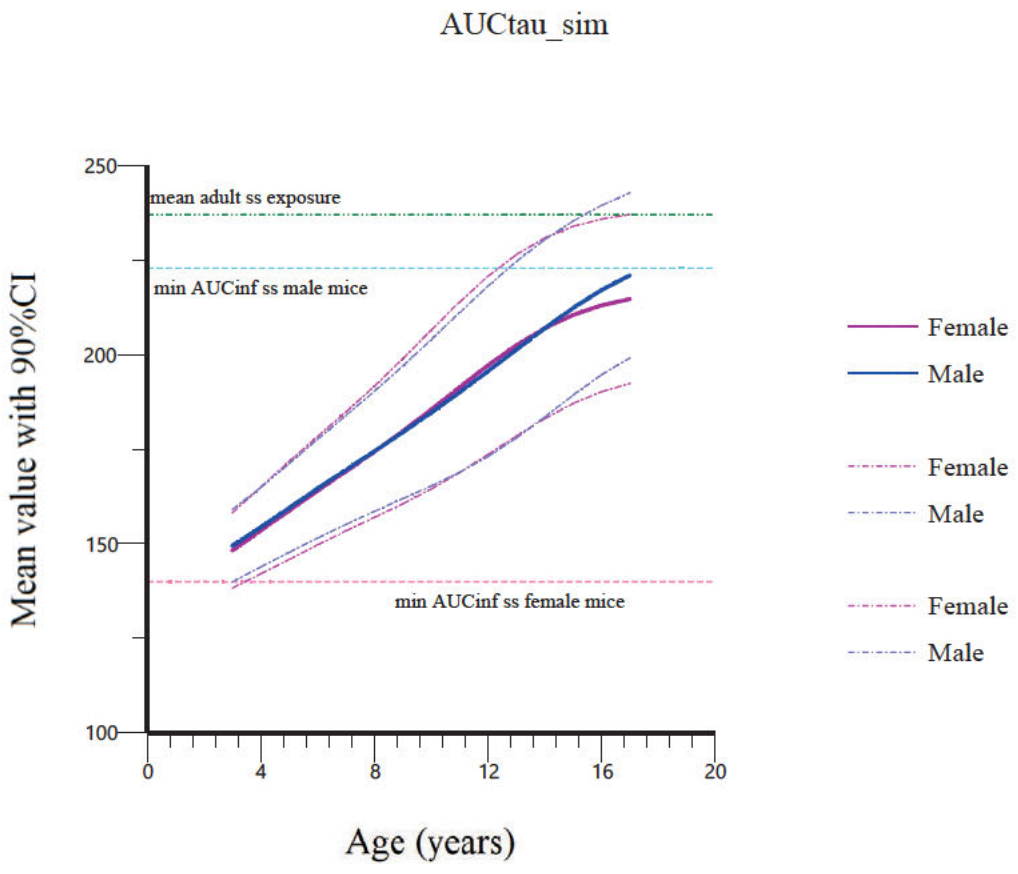
The vertical lines show the potential body weight groups of 30 kg and below, between 30 and 50 kg, between 50 and 70 kg and above 70 kg.

In conclusion, the results of allometric scaling of exposure at steady state (AUC_{tau}) in healthy, adult volunteers at 12 mg/kg indicate that the predicted pediatric exposures were lower than adult exposures for the 12mg/kg dose. The dosing in children with body weights starting from 70 kg was predicted to be the same as in adults.

While a dosing regimen based on body-weight dose bands would be appropriate to provide an exposure-equivalent dose to the 12 mg/kg dose based on allometric scaling, the target dose for the Phase 2 study will be at the fixed mg/kg dose of 12 mg/kg BID. All participants will be up-titrated to the 12 mg/kg BID dose from a starting dose of 4 mg/kg BID, then to 8 mg/kg BID, and then finally to 12 mg/kg BID.

While participants < 70 kg may have lower exposures than those 70 kg and above, the predicted exposures are expected to be in the range of what was identified as a potentially efficacious dose from the animal models (as shown in Figure A2), so there is prospect of benefit with dosing at the maximum proposed dose of 12 mg/kg BID.

Figure A2: Projected exposure AUC_{tau} vs age for the final dose 12 mg/kg BID



Solid lines – mean AUCtau, dash-dot lines – 90% CI. Blue – male, pink – female

AUC_{tau_sim} – simulated AUCtau at steady state for 12 mg/kg BID dosing, h*ng/mL

[a] Source: Neu-2591-HV-001 study adpc dataset, Phoenix 8.3 output for Project file Allometric Neuren.phxproj; CDC Growth chart reported in Figure 5 in the Allometric Scaling Report.

[b] Report 1901515 and Report SOW6, 0621-20173NB_w_in-life_Bioanalytical and Pharmacokinetic Report_Mouse Whole Blood_NNZ-2591_27Aug2020

This more conservative approach, which maintains the planned staggered enrollment of age groups, ensures that each nominal dose is administered to the older group before that dose is administered in the younger pediatric participants. As such the younger age groups receive only nominal doses that have been administered to the older group instead of exposure-equivalent weight-based doses at higher nominal doses. Given that it was estimated from allometric scaling that exposures would be lower for children < 70 kg at 12 mg/kg than for adults at 12 mg/kg, dosing all children at a maximum of 12 mg/kg will not put them at risk of exposures higher than that experienced by healthy adults. So, this approach to the dosing maintains the balance between risk and benefit for this first Phase 2 study in the Angelman syndrome population.