Clinical Protocol Title: Short-Term Outcome of N-Carbamylglutamate in the Treatment of Acute Hyperammonemia.

Study Chair: Nicholas Ah Mew, MD
Director, Inherited Metabolic Disorders
Children's National Research Institute
Associate Professor of Pediatrics
The George Washington University
111 Michigan Ave, NW
Washington, DC 20010
nahmew@childrensnational.org
202-476-5863

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Sponsor: National Institutes of Health (NIH), Recordati Rare Diseases

Short-Term Outcome of N-Carbamylglutamate in the Treatment of Acute Hyperammonemia

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Study Chair: Nicholas Ah Mew, MD
Director, Inherited Metabolic Disorders
Children's National Research Institute
Associate Professor of Pediatrics
The George Washington University

Signature: _____ _____
Date

Site PI: _____

Site: _____

Signature: _____ _____
Date

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LIST OF PARTICIPATING INSTITUTIONS

Boston Children's Hospital
Gerard T. Berry, MD
Institution: Boston Children's Hospital
Address: Department of Pediatric Endocrinology
300 Longwood Ave., # FEGAN10
Boston, MA 02115
Phone: 857-218-4636
Fax: 617-730-4874

University Hospitals of Cleveland Medical Center
Laura Konczal, MD
Institution: University Hospitals of Cleveland Medical Center
Address: Center for Human Genetics
11100 Euclid Avenue
Lakeside 1500
Cleveland, OH 44106-6055
Phone: 216-844-8567
Fax: 216-844-7497

The Children's Hospital of Colorado
Shawn McCandless, MD
Institution: The Children's Hospital of Colorado
Address: 13123 E. 16th Ave, B300
Aurora, CO 80045
Phone: 303-724-2310
Fax: 720-777-7322

University of California Los Angeles
Derek Wong, MD
Institution: University of California Los Angeles
Address: Division of Genetics
10833 Le Conte Avenue, CHS 32-231
Los Angeles, CA 90095
Phone: 310-206-6581
Fax: 310-206-8616

The Children's Hospital of Philadelphia (CHOP)
Can Ficicioglu, MD, PhD
Institution: The Children's Hospital of Philadelphia
Address: Division of Human Genetics and Metabolism
3501 Civic Center Blvd, #9054
Philadelphia, PA 19104
Phone: 215-590-3376
Fax: 267-425-0121

Lucile Packard Children's Hospital at Stanford University
Gregory Enns, MD
Institution: Lucile Packard Children's Hospital at Stanford University
Address: Pediatric Genetics
730 Welch Rd, 2nd Floor
Palo Alto, CA 94304
Phone: 650-721-5804
Fax: 650-498-4555

Children's National Hospital
Nicholas Ah Mew, MD
Institution: Children's National Hospital
Address: Division of Genetics and Metabolism
111 Michigan Avenue NW,
Washington, DC 20010
Phone: 202-476-5863
Fax: 202-476-5650

Children's Hospital Pittsburgh
Gerard Vockley, MD, PhD
Institution: Children's Hospital Pittsburgh
Address: 4401 Penn Avenue
Suite 1200
Pittsburgh, PA 15224
Phone: 412-692-7746
Fax: 412-692-7816

Mount Sinai Hospital
George Diaz, MD
Institution: Mount Sinai Hospital
Address: 1428 Madison Avenue
1st Floor, Room AB1-12
New York, NY 10029
Phone: 212-241-6947

LIST OF CENTRAL ENTITIES

Study Principal Investigator
Nicholas Ah Mew, MD
Institution: Children's National Hospital
Address: Division of Genetics and Metabolism
111 Michigan Avenue NW
Washington, DC 20010
Phone: 202-476-5863
Fax: 202-476-5650

Project Manager
Katie Rice, MPH, CCRP
Institution: Children's National Hospital
Address: Children's National Research Institute
111 Michigan Avenue NW
Washington, DC 20010
Phone: 202-476-6191
Fax: 202-476-5650

Study Biostatistician **Robert J. McCarter, ScD**	
Institution:	Children's National Hospital
Address:	Center for Translational Science 111 Michigan Avenue NW Washington, DC 20010
Phone:	202-476-3140
Fax:	202- 476-3425
Pager:	202-259-4567
Central Pharmacy **Henry Choi, PharmD**	
Institution:	Children's National Hospital
Address:	Pharmacy Department 111 Michigan Avenue NW Washington, DC 20010
Phone:	202-476-2088
Fax:	202-476-6984
Pager:	202-259-4132
Medical Monitor **Barbara Burton, MD**	
Institution:	Lurie Children's Hospital of Chicago
Address:	225 E Chicago Avenue Box MC 59 Chicago, IL 60611
Phone:	312-227-6120
Cell:	312-919-2056

LIST OF DATA AND SAFETY MONITORING BOARD (DSMB) MEMBERS

DSMB Chair Joan Conry, MD
Institution: Children's National Hospital
Address: Center for Translational Science
111 Michigan Avenue NW
Washington, DC 20010
Phone: 202-476-2120

John Van Den Anker, MD, PhD
Institution: Children's National Hospital
Address: Center for Translational Science
111 Michigan Avenue NW
Washington, DC 20010
Phone: 202-476-4875

An Massaro, MD
Institution: Children's National Hospital
Address: Center for Neurosciences Research
111 Michigan Avenue NW
Washington, DC 20010
Phone: 202-476-5225

Tomas Silber, MD
Institution: Children's National Hospital
Address: Department of Adolescent and Young Adult Medicine
111 Michigan Avenue NW
Washington, DC 20010
Phone: 202-476-3066

Samuel Simmens, PhD, MA
Institution: The George Washington University
Address: Department of Epidemiology and Biostatistics
School of Public Health and Health Services
The George Washington University
2100-W Pennsylvania Ave, NW
8th Floor
Washington, DC 20037
Phone: 202-994-7887

LIST OF ABBREVIATIONS

AE – Adverse Event
ALT – Alanine Aminotransferase
AST – Aspartate Aminotransferase
BCH – Boston Children's Hospital
CBC – Complete Blood Count
CFR – Code of Federal Regulations
CHOP – Children's Hospital of Philadelphia
CPS1 – Carbamyl Phosphate Synthetase 1
CPSD – Carbamyl Phosphate Synthetase deficiency
CRA – Clinical Research Associates
CRP – Central Research Pharmacy
CTCAE – Common Terminology Criteria for Adverse Events
DSMB – Data Safety Monitoring Board
EKG – Electrocardiogram
eCRF – electronic Case Report Form
FDA – Food and Drug Administration
FSS – Functional Status Scale
GCMS – Gas Chromatography-Mass Spectrometry
G-tube – Gastrostomy tube
HA - Hyperammonemia
HHS – Department of Health and Human Services
HHS – Hyperinsulinism Hyperammonemia Syndrome
HIPAA – Health Insurance Portability and Accountability Act
ICH – International Conference on Harmonization
INR – International Normalized Ratio
IRB – Institutional Review Board
MMA – Methylmalonic Acidemia
NAG – N-acetylglutamate
NAGS – N-acetylglutamate synthase
NCG – N-carbamyl-L-glutamate, N-carbamylglutamate
NG-tube – Nasogastric tube
NIH – National Institutes of Health
PA – Propionic Acidemia
PHI – Protected Health Information
PI – Primary Investigator
PID – Participant Identifier
PLBO – Placebo
PTT – Partial Thromboplastin Time
REDCap – Research Electronic Data Capture
SAE – Serious Adverse Event
SOP – Standard Operating Procedures
STD – Standard Therapy
TCHC – The Children's Hospital of Colorado
StGr – Study Group
UCD – Urea Cycle Disorder
UCLA – University of California Los Angeles
UHCMC – University Hospitals of Cleveland Medical Center

STUDY SYNOPSIS AND SCHEMA

Protocol Number:	2894
Protocol Title:	N-carbamylglutamate in the Treatment of Hyperammonemia: Accelerating the Resolution of Hyperammonemia and Clinical Recovery and Safety
Study Chair:	Nicholas Ah Mew, MD
Statistician:	Robert McCarter, ScD
Consortium:	N-carbamylglutamate Consortium (NCGC)
Participating Sites:	Boston Children's Hospital (BCH), University Hospitals of Cleveland Medical Center (UHCMC), The Children's Hospital of Colorado (CHCO), the University of California Los Angeles (UCLA), Children's Hospital of Philadelphia (CHOP), Lucile Packard Children's Hospital at Stanford (SU), Children's National Hospital (Children's National), Children's Hospital Pittsburgh (UPMC), and Mount Sinai Hospital (MSSM).
Activation Date:	July 2nd, 2012
Sample Size:	36 patients experiencing approximately 114 hyperammonemia episodes across diagnoses (with approval to enroll up to 80 patients to allow for dropouts, losses to follow-up, and lower than expected incidence of hyperammonemic episodes)
Target Enrollment Period:	5 years
Study Design:	Phase II
Primary Study Objective:	The overall objective of this project is to determine whether treatment of acute hyperammonemia with N-carbamyl-L-glutamate (N-carbamylglutamate, NCG, Carglumic acid, Carbaglu) in propionic acidemia (PA), methylmalonic acidemia (MMA), late-onset CPS1 deficiency (CPSD) and late-onset Ornithine transcarbamylase deficiency (OTCD) accelerates the resolution of hyperammonemia.
Study Population and Main Eligibility/ Exclusion Criteria:	Inclusion Criteria o Aged older than 1 week with an established diagnosis of CPSD or OTCD (as follows): ▪ Diagnosed with late-onset CPSD confirmed by detection of pathogenic mutation(s), and/or decreased (<20% of control) CPS enzyme activity in liver ***OR*** ▪ Diagnosed with late-onset OTCD by detection of pathogenic OTC mutation, OR decreased (<20% of control) OTC enzyme activity in liver OR elevated urinary orotate (greater than 20 μM/mM creatinine) following allopurinol loading with absence of argininosuccinic acid ▪ ***AND:*** Subject or subject's first-degree relative had initial plasma ammonia level ≥100mcmol/L >1 week of age ***OR*** o An established diagnosis of PA or MMA (as follows): ▪ Diagnosed with PA by semi-quantitative urine organic acid analysis, defined as presence of elevated methylcitric acid and normal

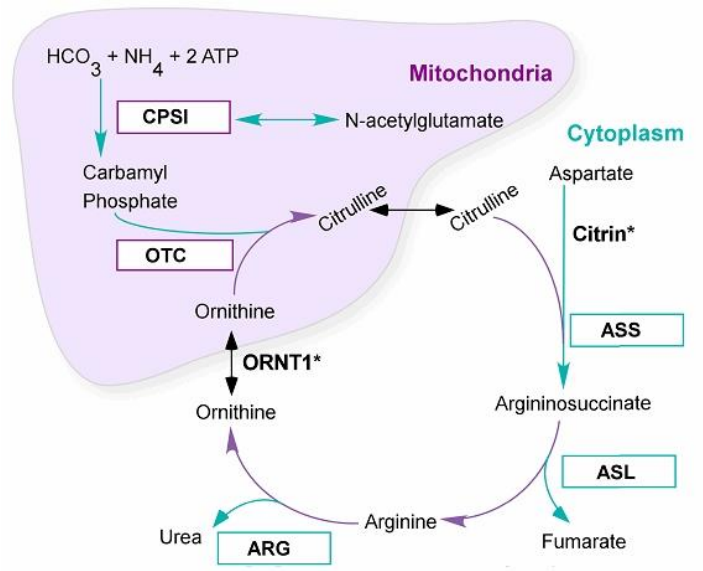
	methylmalonic acid levels and no evidence of biotin-related disorders in the organic acid analysis ***OR*** ▪ Diagnosed with MMA by semi-quantitative urine organic acid analysis, defined as elevation of methylmalonic acid and no evidence of vitamin B12 dependent disorder on plasma amino acid analysis (B12 dependency is defined by documented B12 responsiveness) ▪ ***AND:*** Subject or subject's first-degree relative had plasma ammonia level at any time ≥100 mcmol/L o Able to receive medications orally, by nasogastric (NG)-tube or by Gastric (G)-tube o No concomitant illness which would preclude safe participation as judged by the investigator o If post-menarcheal must have a negative pregnancy test prior to administration of study drug at each episode o Signed informed consent by the subject or the subject's legally acceptable representative Exclusion criteria o Administration of NCG within 7 days of participation in the study o Use of any other investigational drug, biologic, or therapy o Planned participation in any other clinical trial o Diagnosis of any medical condition causing hyperammonemia which is not PA, MMA, CPSD, OTCD o Any clinical or laboratory abnormality or medical condition that, at the discretion of the investigator, may put the subject at an additional risk by participating in this study o Had a liver transplant o Is pregnant o Is not expected to be compliant with the study Patients enrolled in the now-closed long-term outcomes study being conducted in parallel are eligible to enroll in this study provided that all other inclusion and exclusion criteria are satisfied.
Primary Outcome Measures:	Composite of the shortest of the time to reach an ammonia level <50 mcmol/L or time to discharge from the hospital after the first dose of NCG. Safety of NCG treatment as measured by serious adverse events, electrocardiogram test and laboratory blood tests
Statistical Considerations (sample size and analysis plan):	Based on 2-tailed testing assuming a type 1 error of 5% and using comparisons of treatment effects across episodes, a sample size of 36 patients, experiencing an average of <4 episodes each (total 114 episodes) would provide 80% power to detect hazard ratio at least as large as 1.7 favoring a higher average probability of the composite outcome in the NCG group. A sample size of 80 will allow for drop outs, losses to follow-up, and lower than expected incidence of hyperammonemic episodes.
Sponsors (federal, state, foundation and industry support):	National Institutes of Health (NIH), Recordati Rare Diseases

1 INTRODUCTION

1.1 Background

1.1.1 The Urea Cycle

The urea cycle consists of several biochemical steps required for the conversion of ammonia into urea (Figure). The first and rate-limiting step of the urea cycle is catalyzed by the mitochondrial enzyme, carbamyl phosphate synthetase (CPS1), which converts ammonia, bicarbonate, and ATP into carbamyl phosphate. Subsequently, the enzyme ornithine transcarbamylase (OTC) conjugates ornithine and carbamyl phosphate to form citrulline, which then continues through the rest of the urea cycle. CPS1 has as an essential allosteric enzyme, N-acetylglutamate, produced from the enzyme N-acetylglutamate synthase.



1.1.2 Carbamyl Phosphate Synthetase I Deficiency (CPSD)

Carbamyl phosphate synthetase I is the first and rate-limiting step of the urea cycle and converts ammonia, bicarbonate, and adenosine triphosphate (ATP) into carbamyl phosphate. Mutations which completely abolish CPS activity, eliminate flux through this step, and lead to hyperammonemia. However, mutations resulting in reduced but not abolished CPS enzyme function may result in an attenuated form of hyperammonemia, characterized by a later presentation, plasma ammonia better amenable to dietary and pharmacotherapy or fewer admissions for hyperammonemia. Nevertheless, episodes of hyperammonemia may still be life-threatening, and if untreated, may result in coma or death. Clinical features of hyperammonemia may also include encephalopathy, brain edema, tachypnea, lethargy, vomiting, hypotonia, and hypothermia.

1.1.3 Ornithine Transcarbamylase Deficiency (OTCD)

Ornithine transcarbamylase (OTC) conjugates carbamyl phosphate and ornithine to form citrulline. Deficiency of the OTC enzyme results in hyperammonemia and may result in encephalopathy, brain edema, tachypnea, lethargy, vomiting, hypotonia, hypothermia, and if untreated, coma and/or death. The OTC gene is located on the X-chromosome, thus males have only one copy of the OTC gene, whereas females have two copies, but due to lyonization have only one active copy per cell. The X-chromosome in each cell that is inactivated is

determined randomly and prenatally, at an early embryological stage. A partial deficiency of OTC may be observed in hemizygous males only if a mutation in the OTC gene is hypomorphic. However, in affected females, a partial deficiency is typically only observed with mutations that entirely abolish OTC activity. This is because due to lyonization, affected females have two populations of hepatocytes – those that express the mutant OTC allele, and those that express the normal allele.

1.1.4 Propionic Acidemia (PA)

Propionic acidemia (PA) is caused by a complete or partial deficiency of the enzyme, propionyl-CoA carboxylase, or a defect in the utilization or salvage of its cofactor, biotin. In the neonatal period, this condition can present with lethargy, poor feeding, vomiting, ketoacidosis, and hyperammonemia, and may result in death if not treated appropriately. Ammonia can reach extremely high levels, approaching those seen in patients with urea cycle disorders (ref).

1.1.5 Methylmalonic Acidemia (MMA)

Methylmalonic acidemia/aciduria (MMA) is caused by a complete or partial deficiency of the enzyme methylmalonyl-CoA mutase, a defect in the transport or synthesis of its cofactor, adenosyl-cobalamin, or deficiency of the enzyme methylmalonyl-CoA epimerase. In the neonatal period, the disease can present with lethargy, vomiting, hypotonia, hypothermia, respiratory distress, severe ketoacidosis, and hyperammonemia, and can result in death if not appropriately treated. Hyperammonemia also occurs in methylmalonic acidemia, in particular in the neonatal period, and like in PA, can approach levels seen in patients with urea cycle disorders.

1.1.6 Mechanism of Hyperammonemia in PA and MMA

Hyperammonemia in PA and MMA are thought to result from the inhibition of the N-acetylglutamate (NAG) synthase reaction. This may be either the result of direct competitive inhibition of the NAG synthase reaction by the organic acid, from decreased availability of acetyl-Coenzyme A or from Coenzyme A depletion (ref). Any of these mechanism lead to decreased mitochondrial concentration of NAG. Because NAG is the essential allosteric cofactor to carbamylphosphate synthase (CPS1) reaction (the first step of the urea cycle), a decrease in NAG concentration leads to decreased flux through the CPS1 reaction and results in hyperammonemia.

1.1.7 Current Therapies for Hyperammonemia

Very few drugs to treat hyperammonemia have been developed and brought to market. Dietary protein restriction and administration of alternate pathway acylating agents are the current primary treatments, but they frequently fail to avert brain damage. Current alternate pathway therapies remove excess nitrogen stoichiometrically, eliminating a fixed number of nitrogen atoms (1 or 2) for every molecule of drug administered, reactions, which require the adequate liver function to be effective.

Orthotopic liver transplantation cures hyperammonemia, but organ availability has been limited and the procedure is highly invasive and requires life-long immunosuppression.

Since hyperammonemia is critical in the pathophysiology of screenable disorders such as urea cycle defects and possibly organic acidemias, which are currently, or will be in the near future detectable by expanded newborn screening, developing new innovative treatments for these disorders is essential if early diagnosis is to change the natural course of these conditions.

1.2 Study Rationale

As described above, current drug therapy for inherited hyperammonemia relies mainly on the activation of alternate pathways for nitrogen elimination. In contrast, N-carbamylglutamate (NCG), a stable analog of N-acetylglutamate, catalytically stimulates the urea cycle, thus converting many more molecules of ammonia to urea with a relatively small dose of the drug. This represents a paradigm shift in the medical treatment of hyperammonemia.

A drug that could repair or stimulate a dysfunctional urea cycle would have several advantages over current therapy. First, it would intervene through the normal pathway of ureagenesis, which is catalytic rather than stoichiometric. Second, it may not need to replace current treatment but would be complementary to it. Third, the ability to stimulate residual ureagenesis could benefit many conditions that cause hyperammonemia, not just a single disorder. Enhancement of residual ureagenesis capacity in patients with hyperammonemia would markedly alter the natural disease course resulting in a manageable phenotype and better clinical outcome.

Our recent studies show that ureagenesis is augmented by NCG in healthy individuals and in more than half of all individuals with inborn errors that were tested.

The eligibility for the studies in this study specifically includes severe PA and MMA and “milder” CPSD and OTCD, which may appear paradoxical. However, the rationale for these selection criteria is based on the different mechanism(s) of action in these disorders. Significant hyperammonemia in PA or MMA is usually seen only in the most severely affected patients who present as newborns. Patients with hypomorphic alleles have milder disease and are unlikely to have serious hyperammonemia.[72] In severely affected patients with PA and MMA, NCG works to alleviate deficiency of NAG created by the inhibition of NAGS by the mitochondrial accumulation of propionyl-CoA, hence, the more profound the NAG deficiency, the more robust the NCG effect. In CPSD and OTCD however, the putative effect of NCG relies on stimulating residual CPS1 or OTC activity. Indeed, when these enzymes are totally absent, no amount of NCG would help, hence, the need to enroll patients with partial rather than complete enzyme deficiencies.

If the proposed studies show that the use of NCG is effective in enhancing or completely restoring ureagenesis in patients with PA, MMA, CPSD and/or OTCD, they can be treated long-term with this therapeutic agent either during acute episodes or chronically. Carbaglu is FDA-approved (03-2010). Thus, responsive participants could receive this medication off-label if their physician obtains a single patient IND. There can be an important direct benefit for these participants. If the underlying hypothesis is correct, this study could improve the medical condition of some or all of the patients, also preventing life-threatening events in those who respond.

The study will provide the first reliable information of whether NCG could potentially reduce the hyperammonemia associated with the 4 disorders to be studied. Such knowledge could provide a new treatment for some patients with hyperammonemia. In addition, the information gained could also be relevant to better understanding the regulation of the urea cycle, which may impact also the treatment of hyperammonemia due to generalized liver disease or other causes.

This emerging concept posits that NCG will be effective as an adjunct treatment for hyperammonemia in several conditions. A few case reports provide anecdotal evidence for the effectiveness of NCG in reversing hyperammonemia in propionic acidemia (PA) and methylmalonic acidemia (MMA) [17-22]. Although these observations may be factually correct, they do not represent credible scientific evidence and are unlikely to be accepted by regulatory agencies. The studies proposed herein, are

placebo-controlled, randomized, and blinded addressing this question using multiple true and surrogate endpoints, increasing scientific reliability.

1.3 Study Treatment: N-carbamyl-L-glutamate (NCG)

N-carbamylglutamate, Carglumic acid, and Carbaglu are synonyms for the study treatment, N-carbamyl-L-glutamate (NCG).

N-carbamyl-L-glutamate (NCG) has recently been found to have virtually curative of a urea cycle defect, N-acetylglutamate synthase (NAGS) deficiency (FDA IND# 68,185). In this disorder, treatment with NCG alone normalizes ureagenesis, blood ammonia, and glutamine levels, allows normal protein tolerance, and restores health. Knowledge from the study of NCG in inborn errors could be applied to acquired hyperammonemia, specifically in patients with propionic acidemia (PA) and methylmalonic acidemia (MMA), to improve neurodevelopmental outcomes and reduce nitrogen load.

1.3.1 Preclinical Profile

Carbaglu® is synthesized by Synth-Innove and was confirmed to activate CPS I in vitro. Labeled NCG injected into mice can be detected in the liver.
Toxicology studies in rats, at doses of up to 1000 mg/kg did not show any neurobehavioral, neurovegetative, neurotoxic or psychotropic effects nor any effects on body temperature. Respiratory function, including respiratory rate, peak respiratory and inspiratory flows, inspiration and expiration time, tidal volume and airway resistance also showed no statistically significant change.

In isolated canine Purkinje fibers, there was no statistically significant effect on action potential parameters, and no dearly depolarization was observed at any concentration. Additionally NCG at concentrations up to 0.1 mM had no effect on action potential duration.

Toxicology studies on the cardiovascular system in dogs showed no change in blood pressure, heart rate and cardiac conduction times including QT interval and QTc after administration of up to 1000 mg/kg of Carbaglu.

Carbaglu was administered enterally once up to 2806 mg/kg or intravenously up to 238.6mg/kg, in Sprague-Dawley rats, and no mortality was observed.

No clastogenic activity was found in a study with human lymphocytes, and the micronucleus test in rats was negative. The impurity hydantoin-5-propionic acid demonstrated clastogenic activity at concentrations > 2500 µg/ml. The quality profile of Carbaglu is less than 0.1% hydantoin-5-propionic acid. NCG does not seem to affect male or female fertility.

1.3.1.1 Pediatric Animal Studies

In newborn rats, NCG given at 2000 mg/kg/day was lethal to all pups between 2-14 days. At 1000 mg/kg/d, orange colored feces, a slight reduction of body weight gain, and a decreased thymus weight were observed. At doses of <500 mg/kg/d, NCG was well tolerated, and no adverse effects were observed.

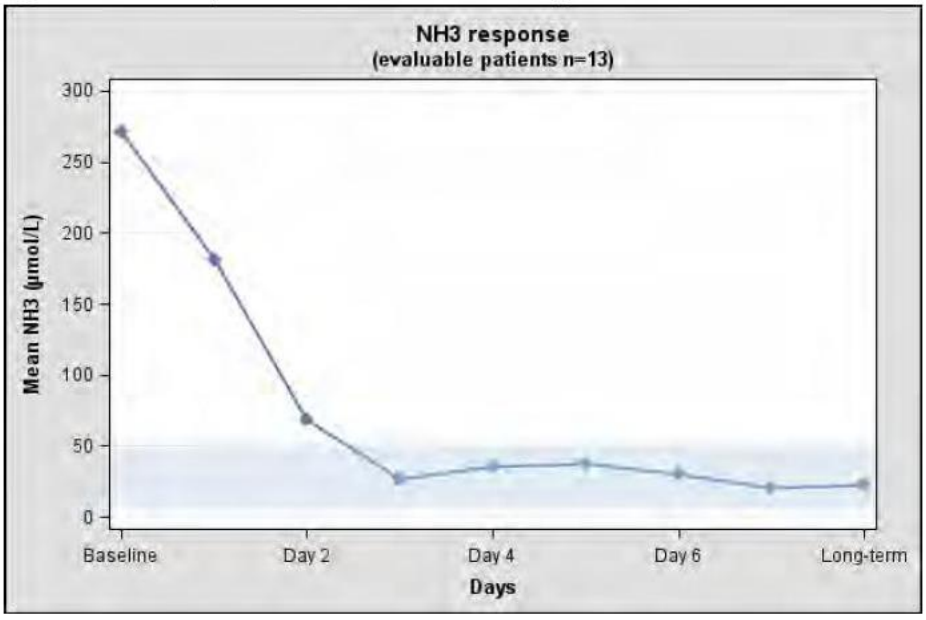
NCG was also administered to 4-week old Sprague-Dawley rats daily for 6 months at doses of 500 or 1000 mg/kg/d. At 1000 mg/kg/d, ptyalism (excess salivation) was the only adverse effect observed.

1.3.2 Clinical Profile

1.3.2.1 Carbaglu in Treatment of NAG Synthase Deficiency

Carbaglu corrects the biochemical defect and normalizes all biochemical parameters, including ammonia, in patients with a primary deficiency of NAG, as observed in NAG Synthase (NAGS) deficiency. Retrospective data from 13 patients with NAGS who were treated for acute hyperammonemia were collected by Orphan Europe. Mean plasma ammonia for these patients at initial presentation, as well as each day following Carbaglu administration, is shown in Figure 1, below:

Figure 1. Mean ammonia vs. time in n=13 subjects with NAGS deficiency receiving Carbaglu



1.3.2.2 Carbaglu in Secondary Deficiencies of NAG

Several publications have pointed out the potential of NCG as a therapeutic adjunct to the treatment of hyperammonemia in secondary deficiencies of NAG (i.e., not resulting from NAGS deficiency). All these reports were uncontrolled case reports or case series[10-13,15,20].

Our first project was initially funded for 34 months from 8/5/2008 through 5/31/2011. The original specific aims were: 1) To determine whether 3-day treatment with NCG improves or restores ureagenesis in patients with NAGS deficiency, Carbamyl phosphate synthase (CPSD), PA, MMA and hyperinsulinism and hyperammonemia syndrome (HHS), and 2) To evaluate the safety of short-term (3-day) treatment with NCG. The accomplishments thus far indicate that 3-day treatment with NCG:

- Normalizes ureagenesis rate, plasma ammonia, glutamine and urea in NAGS deficiency[10,21]
- Enhances ureagenesis and reduces plasma ammonia and glutamine levels in PA[9]
- Improves ureagenesis and decreases plasma ammonia and glutamine in some patients with CPSD
- Improves ureagenesis in some patients with HHS

- Is safe at the prescribed dose of 100/mg/k/d or 2.2 g/m^2/d

In addition, we found that a single dose of NCG rapidly augments ureagenesis rate in healthy adults[8].

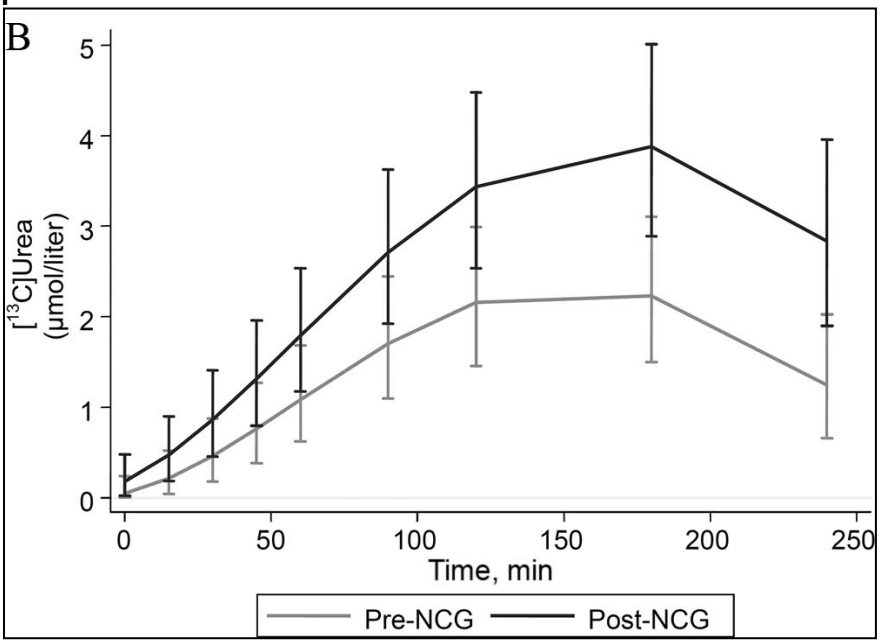
1.3.2.3 Carbaglu in PA and MMA

As described above, limited case reports have suggested the use of NCG in PA and MMA[9-14].

We applied the same study design as described above to investigate the effect of NCG in severe PA and MMA. These patients usually present as newborns and have marked and recurrent hyperammonemia. As of the time of this reporting, we completed studies of 7 patients with PA and 1 patient with MMA. We specifically wanted to determine whether NCG reduces plasma levels of ammonia and glutamine and increases ureagenesis rate, even though plasma glutamine levels are typically not elevated in PA.

Results from longitudinal mixed effects linear regression (Figure 2) show that peak [13C]urea increased following 3 days of NCG treatment (from 2.2 μM to 3.8 μM p<0.0005). There were corresponding decreases in mean plasma ammonia (59 to 43 μM p<0.0005) and glutamine (552 to 331 μM p<0.0005). This study demonstrated conclusively that NCG enhances ureagenesis and reduces plasma ammonia and glutamine concentrations in patients with PA. We obtained similar results in the patient with MMA, thus, this protocol represents a systematic approach to validating these findings a controlled clinical trial.

Figure 2. [13C]-urea before (pre-NCG) and after (post-NCG) a 3-d NCG treatment in 7 patients with PA[9]



1.3.3 Carbaglu Formulation and Dosage

Chemical Composition: N-carbamoyl-L-glutamic acid (NCG)

Study drug will be prepared for administration according to the package insert. Briefly, study drug tablets will be dispersed in water and ingested or administered immediately via nasogastric or gastrostomy tube.

The daily dose will be 150 mg/kg/ day or 3.3 g/m2/day for patients >15 kg. The doses are to be divided into 2 equal doses and administered orally or enterally by nasogastric or gastrostomy tube. Standard care will prevail when choosing the mode of drug administration.

1.3.4 Safety and Side Effects of Carbaglu

The safety of Carbaglu for the treatment of hyperammonemia due to NAGS deficiency was evaluated in a retrospective review of 23 patients (14 males and 9 females) (Carbaglu [prescribing information]. Orphan Europe; 2010). The most common adverse reactions (occurring in ≥13% of patients), regardless of causality, were infections, vomiting, abdominal pain, pyrexia, tonsilitis, anemia, ear infection, diarrhea, nasopharyngitis, and headache.

2 OBJECTIVES

To determine whether NCG treatment of acute hyperammonemia in severe, neonatal-onset PA and MMA, partial CPSD, and OTCD is efficacious and whether it is safe.

2.1 Assess Efficacy of NCG Treatment

The objective of this study is to assess whether NCG is efficacious in treating hyperammonemia and improving the following outcomes:

- The primary efficacy outcome of this study is the resolution of the ammonia levels or earlier discharge following the hyperammonemia episode(s).
- The secondary efficacy outcomes of this study include the Functional Status Scale, measured daily during each hyperammonemic episode, and the length of hospitalization.

This goal will be realized by randomizing each hyperammonemic episode from every subject to NCG plus standard treatment (NCG-STD) versus placebo plus standard treatment (PLBO-STD) and subsequently gauging response with the primary outcome of plasma ammonia levels, in addition to the Functional Status Scale and the length of hospitalization.

2.2 Safety

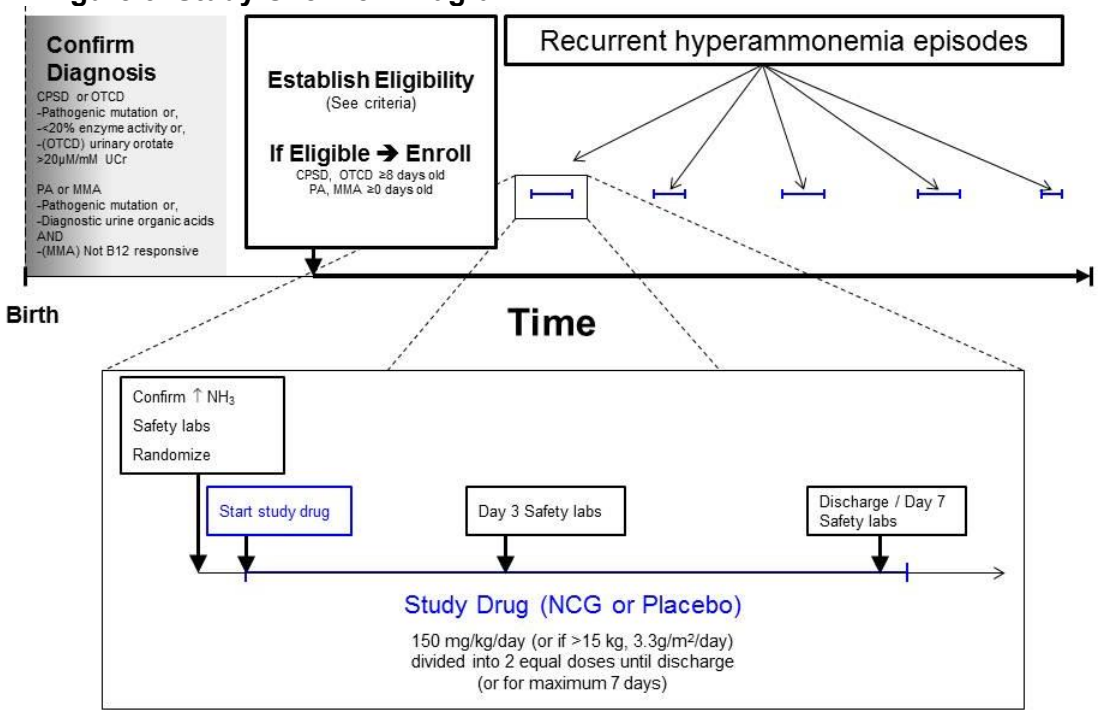
The primary safety outcome of the study will be the rate of Serious Adverse Events (SAEs) during study episodes defined in this study as death or substantial prolongation of hospitalization, as patients are hospitalized as part of having a qualifying hyperammonemia episode. Safety tests consisting of complete blood count (CBC), liver and kidney function tests, coagulation profile (PTT/INR) will be performed before treatment, on the third day of treatment, just prior to episode treatment completion of NCG and thirty days (up to six months) after the last study dose. Patients in a hyperammonemia crisis frequently experience transient anemia once appropriately hydrated during an emergency room visit. In order to monitor the transient anemia, in addition to collecting CBC's, if a patient receives a blood transfusion during hospitalization this information will also be collected. An EKG test will be performed before treatment and between days 3-5 of treatment or before discharge if earlier.

3 STUDY DESIGN

3.1 Overview of Study Design

This will be a double-blinded, placebo controlled randomized clinical trial to evaluate the efficacy of NCG in the treatment of two organic acidemias (severe PA and MMA), and two urea-cycle disorders (late-onset CPSD and OTCD). Figure 3 summarizes the design of the proposed trial.

Figure 3. Study Overview Diagram



Protocol Overview

- **Eligibility** – Briefly, subjects (a) aged >1 week with verified diagnosis of CPSD or OTCD or (b) subjects of any age with verified diagnosis of PA or MMA and ammonia level ≥70mcmol/L. All subjects (or a first-degree relative) must have had a recorded plasma ammonia ≥100 mcmol/L and must have presented initially at >1 week of age for CPSD or OTCD. Subjects must not be pregnant. A complete listing of eligibility criteria is included in Section 4 (Study Population).
- **Enrollment** – Informed consent from the participant or one parent or legal guardian.
- **Randomization** – At each hyperammonemic episode, participants will be randomized to either placebo plus standard therapy (PLBO-STD) or NCG plus standard therapy (NCG-STD) at a dose of 150 mg/kg/ day or 3.3 g/m2/day for patients >15 kg, divided into 2 equal doses and administered enterally by nasogastric or gastrostomy tube or by oral syringe.
- **Study Treatment** – The NCG/PLBO dose will be administered for metabolic decompensation for 7 days or until discharge, whichever is shorter.
- **Concomitant Standard Treatment** – Participants will continue receiving standard treatment which may include intravenous glucose (and insulin if required), L-carnitine dosing and/or dialysis. Ammonia scavengers may only be administered in patients with urea cycle disorders.

- **Laboratory Measurements** – Plasma ammonia testing should be performed as clinically indicated, including checking the level at admission and at least every 6-12 hours for the first 48 hours and every day thereafter if the previous level was ≥50 mcmol/L, for a total of 7 days or until discharge, whichever is shorter. Plasma amino acids should also be checked as clinically indicated at admission and at least every 2 days thereafter, if ammonia levels are measured. Safety laboratory assessments including complete blood cell count, liver and kidney function tests, amylase and lipase testing, as well as a coagulation profile (if clinically indicated) will be done at the time of initial admission, at 3-5 days, at 6-7 days or at discharge, whichever is shorter.
- **Electrocardiogram (EKG) Test** – At each episode, participants will have an EKG at admission and repeated on day 3-5 of treatment (48 hours following the initial drug administration, before discharge if earlier) to check for cardiac toxicity.
- **Functional Scale Score (FSS)** – Determined for each episode at admission and every day of hospitalization for a total of 7 days or until discharge, whichever is shorter.
- **Recurrent Episodes** – Each subsequent admission for metabolic decompensation will follow the same above procedure but has to occur at least 7 days after the last discharge to guarantee study drug wash out before the next administration should patient have received study drug.

4 STUDY POPULATION

Patients enrolled in the long-term outcomes study being conducted in parallel are eligible to enroll in this study when the long-term outcomes study closes, provided that all other inclusion and exclusion criteria are satisfied.

4.1 Inclusion Criteria

- Aged older than 1 week with an established diagnosis of CPSD or OTCD (as follows):
  - Diagnosed with late-onset CPSD confirmed by detection of pathogenic mutation(s), and/or decreased (<20% of control) CPS enzyme activity in liver ***OR***
  - Diagnosed with late-onset OTCD by detection of pathogenic OTC mutation, OR decreased (<20% of control) OTC enzyme activity in liver OR elevated urinary orotate (greater than 20 μM/mM) following allopurinol loading with absence of argininosuccinic acid
  - ***AND:*** Subject or subject's first-degree relative had plasma ammonia level ≥100mcmol/L >1 week of age

  OR

- An established diagnosis of PA or MMA (as follows):
  - Diagnosed with PA by semi-quantitative urine organic acid analysis, defined as presence of elevated methylcitric acid and normal methylmalonic acid levels and no evidence of biotin-related disorders in the organic acid analysis ***OR***
  - Diagnosed with MMA by semi-quantitative urine organic acid analysis, defined as elevation of methylmalonic acid and no evidence of vitamin B12 dependent disorder on plasma amino acid analysis (B12 dependency is defined by documented B12 responsiveness)
    - ***AND:*** Subject or subject's first-degree relative had plasma ammonia level at any time ≥100mcmol/L
- Able to receive medications orally, by nasogastric (NG)-tube or by gastric (G)-tube
- No concomitant illness which would preclude safe participation as judged by the investigator
- If post-menarcheal must have a negative pregnancy test prior to administration of study drug at each episode
- Signed informed consent by the subject or the subject's legally acceptable representative

4.2 Exclusion Criteria

- Administration of NCG within 7 days of participation in the study
- Use of any other investigational drug, biologic, or therapy.
- Planned participation in any other clinical trial
- Diagnosis of any medical condition causing hyperammonemia that is not PA or MMA. CPSD or OTCD. Other urea cycle disorders will be excluded from this study.
- Any clinical or laboratory abnormality or medical condition that, at the discretion of the investigator, may put the subject at an additional risk by participating in this study
- Has had a liver transplant
- Is not expected to be compliant with this study in terms of returning to site for subsequent episodes of hyperammonemia crises
  Is pregnant

5 STUDY PROCEDURES

5.1 Screening of Potential Subjects

Prior to enrollment, patients must be deemed by the site PI, or his assigned surrogate, to meet eligibility criteria.

5.1.1 Confirmation of diagnosis of PA, MMA, CPSD or OTCD

5.1.1.1 Diagnosis of PA or MMA

Results from the subject's urine organic acid analysis, performed via semi-quantitative methods by gas chromatography–mass spectrometry (GCMS), should be reviewed. Patients who do not meet these laboratory diagnostic criteria will not be enrolled.

5.1.1.1.1 Laboratory Diagnostic Criteria for PA

1) Elevation of methylcitric acid and propionylglycine
2) No elevation in methylmalonic acid
3) No evidence of biotin-related disorders

5.1.1.1.2 Laboratory Diagnostic Criteria for MMA

1) Elevation of methylmalonic acid
2) No elevation of homocysteine, total homocysteine, or homocysteine-cysteine disulfide by plasma amino acid analysis
3) No response to 1 mg IM hydroxocobalamin injections

5.1.1.1.3 Laboratory Diagnostic Criteria for CPSD

- Detection of pathogenic CPS1 mutation(s) **OR**
- Decreased (<20% of control) CPS enzyme activity in liver

5.1.1.1.4 Laboratory Diagnostic Criteria for OTCD

- Detection of pathogenic OTC mutation **OR**
- Decreased (<20% of control) OTC enzyme activity in liver **OR**
- Elevated urinary orotate (greater than 20 μM/mM) following allopurinol loading, with absence of argininosuccinic acid on plasma amino acid analysis

5.1.1.2 Documentation of Hyperammonemia

1) The subject (or subject's first-degree relative) should have a previously documented ammonia level at any time of ≥100 mcmol/L
2) If diagnosed with CPSD or OTCD (as per above), initial hyperammonemia should have occurred >1 week of age

In some cases, a subject may have been diagnosed prenatally, or before the manifestation of symptoms, because of previously-identified affected family member. As a result, the subject may have been treated prospectively and averted any early hyperammonemic events. In such cases, the previously-identified first-degree relative must meet the above criteria instead.

5.2 Informed Consent Procedures

Participants will be recruited from all participating sites and their metabolic services. Ideally, patients will be recruited to this study during periods of clinical and metabolic stability, rather than at the time of presentation of acute illness or hyperammonemia. Whenever possible, the participants (or

guardians) will be given a copy of the consent form for review, several days before the formal informed consent process takes place.

For participants who can provide informed consent:
The licensed physician investigator will meet with the potential study participant to describe the study. Patients will be brought to a private, quiet room where the benefits and risk of study will be explained thoroughly, and any questions will be answered. The investigator will then give the patient time to read the consent form and consider participation. The investigator will return to answer questions, ensure that the patient understands the information provided, and obtain informed consent.

For participants unable to provide informed consent who arrive with parents/guardians:
The site PI will meet with the parent(s) or guardian(s) to describe the study. Parents will be brought to a private, quiet room where the benefits and risks of the study will be explained thoroughly, and any questions will be answered. The investigator will then give the parent(s) time to read the consent form and consider participation. The investigator will return to answer questions, ensure that the parent/guardian understands the information provided, and obtain informed consent.

For participants unable to provide informed consent arriving without parents/guardians:
Some of these patients may be transferred directly from another institution without parents or guardians. Patients' families will be first contacted by phone by clinical personnel to discuss the possibility of participating in a clinical trial. Should they be interested, informed consent will be obtained by the site PI by telephone before the study can begin. The investigator will speak with the parent/guardian by phone, and provide them with a copy of the consent form (e.g., by fax, e-mail). When the parent/guardian receives the consent form, it will be immediately signed and sent/faxed back to the investigator. No research-related activities will occur until a signed hard copy of the consent form has been received by the investigator.

For participants who are being recruited during a period of clinical and metabolic stability:
Whenever possible, patients will be recruited to participate during periods of clinical and metabolic stability. The site PI will meet with these patients (and their families, if applicable) following their routine clinical visits to discuss study participation, to answer questions, to ensure that all information provided is understood, and to obtain informed consent. Patients who are not scheduled for a routine clinical visit within the next 3 months will be contacted by phone by clinical personnel to discuss the possibility of participating in a clinical trial. Should they be interested, informed consent will be obtained by the site PI by telephone. The investigator will speak with the patient or parent/guardian by phone, and provide them with a copy of the consent form (e.g., by fax, e-mail, or post). When the patient or parent/guardian receives the consent form, it will be signed and returned to the investigator. No research-related activities will occur until a signed hard copy (original or facsimile) of the consent form has been received by the investigator.

Informed consent documents will be available in English. If patients or their parents/guardians are only fluent in another language, and the site has a standard operating procedure for informed consent in that language (such as an on-call interpreter and if available abbreviated consent form in respective language) and the site IRB has approved the process for this study in advance, non-English speaking patients, and parents/guardians will also be included.

5.3 Retention Procedures

Every effort will be made to identify patients who have enrolled in this study at their next presentation to the emergency department or another medical facility.

- At the time of enrollment, patient families will be provided with a card, indicating that they are participating in an investigational drug trial, to be presented to hospital staff at

subsequent episodes of illness. They will also be reminded to inform the on-call genetics or metabolism attending physician of their participation in this trial.
- The last note from the treating genetics and/or metabolism physician will indicate in the plan, or in an addendum, that the patient is participating in a clinical investigational drug trial
- The patient charts will be flagged, if possible, indicating that the patient is participating in a clinical investigational drug trial

5.4 Duration of Study and Visit Schedule

5.4.1 Hyperammonemia Episodes

At any time after the initial enrollment, participants may present to the hospital with PA-, MMA-, CPSD- or OTCD- associated symptoms (see Sections 1.1.2-1.1.5). Indicated clinically, the plasma ammonia level should be verified.

- Subjects are not eligible to receive study drug if they have received NCG or study drug any time in the preceding 7 days.
- If plasma ammonia is ≥70 mcmol/L at presentation (confirmed on site) or during a hospitalization for an acute decompensation, participants should receive the study drug. The time from an episode- qualifying ammonia level to randomization should not be longer than a maximum of 4 hours.

5.4.1.1 Random Group Assignment

At each hyperammonemia episode, subjects will be randomized to a treatment arm. Randomization will be performed online using a stored, pre-generated, encrypted randomization schedule created by the CNMC Division of Biostatistics and Study Methodology. To complete the process, the Site Pharmacy will simultaneously receive an automated notification of the assignment to enable them to prepare the correct prescription for the participant. The time from randomization to study treatment administration should be as brief as possible and no longer than a maximum of 6 hours.

5.4.1.2 Concurrent with Randomization

Pre-Treatment

1. Indicated clinically, a blood sample will be drawn for plasma ammonia and amino acid analysis. All patients are expected to have clinically indicated vascular access. If vascular access is inadequate, blood will be drawn by venipuncture. All ammonia levels measured up to 24 hours prior to first treatment administration will be recorded. Ammonia levels measured >24 hours prior to first treatment administration will not be recorded in this study.
2. Laboratory safety assessments: complete blood count (CBC), liver function tests (LFT), renal function test (RFT), amylase and lipase. If indicated clinically, a coagulation profile (PTT and INR) should also be done. A pregnancy test is required (if applicable).
3. Electrocardiogram (EKG) test
4. Functional Status Scale (FSS) assessment

Table 1. Hospitalization Study Procedures

	Day 1					Day 2		Day 3		Day 4		Day 5		Day 6		Day 7		Day 30 (≤6 Months)
Time (hours)	0	Post Dialysis	6	12	18	24	36	48	60	72	84	96	108	120	132	144	156	
Confirm Eligibility	X																	
Pregnancy Test[b]	X																	
Randomization	X																	
Plasma Ammonia Level[c]	X	(X)	X	X	X[d]	X	X	X		X[f]		X[f]		X[f]		X[f]		
Plasma Amino Acids[c]	X	(X)				X				X[g]				X[g]				
Safety Labs[c]	X							X[h]						X				
EKG Test[i]	X							X[i]										
Functional Status Scale[c]	X				X		X		X		X		X		X		X	
Concomitant Medications Log					X		X		X		X		X		X		X	
Adverse Events Log					X		X		X		X		X		X		X	
Study Drug (NCG or PLBO)	X			X		X	X	X	X	X	X	X	X	X	X	X	X	
Treatment Administration Log	X			X		X	X	X	X	X	X	X	X	X	X	X	X	
Blood Transfusion Log	X			X		X	X	X	X	X	X	X	X	X	X	X	X	

NCG=N-carbamylglutamate; PLBO=Placebo

[a] *Suggested timeline to be followed for 7 days, or until discharge if discharged prior to 7 days of drug administration*

[b] *For women of childbearing potential only. A serum pregnancy test will be performed in the event of a positive or equivocal urine pregnancy test result, or if the patient is anuric*

[c] *Allowable windows for day 3 (days 3-5) and day 7 (days 6-7), unless otherwise indicated*

[d] *Plasma ammonia should be drawn as frequently as clinically indicated, for example, every 6 hours during the first 24-hour window if the patient has a central or peripheral venous catheter.*

[e] *Only to be drawn if dialysis is performed. Blood draws should be performed immediately upon termination of hemodialysis or hemofiltration, even if the regular time-point labs have been drawn.*

[f] *Plasma ammonia should be drawn as frequently as clinically indicated, for example, after 48 hours, follow-up ammonia should be obtained if the prior measurement was ≥50 mcmol/L*

[g] *Amino acids should be drawn as frequently as clinically indicated, for example, obtained only if ammonia also obtained (see f)*

[h] *Safety Lab blood draw to be obtained on day 6-7, or if discharged <7d, within 24h prior to discharge*

[i] *EKG should be done before treatment begins and repeated once between Days 3-5 or before discharge*

5.4.1.3 Post-Randomization

Day 1

1. As per their assignment, the participant will be started on NCG (150 mg/kg/day for patients ≤15 kg or 3.3 g/m^2/day for patients >15 kg in two divided doses) OR PLBO (three-quarters tablet/kg/d for patients ≤15 kg or 16.5 tablets/m^2/day for patients >15 kg, in two divided doses) to be administered through a nasogastric tube, or if not available, by oral syringe, as described in Section 6.1. The participant will also continue to receive standard therapy.
2. Following the first drug administration, the subsequent drug administration should be given between 6 and 12 hours after the first dose, at a standard drug administration time for the site. All subsequent doses should be given every 12 hours.
3. Indicated clinically, plasma ammonia testing will be performed every 6 hours following study drug administration if the patient has a central or peripheral catheter. In all other cases, plasma ammonia testing will be performed every 12 hours, as indicated clinically.
4. Clinical monitoring FSS
5. Treatment administration log, concomitant medications log, blood transfusion log, and adverse events log will be completed daily

Day 2

1. NCG/PLBO Rx. & Standard Rx every 12 hours.
2. Plasma ammonia every 12 hours (as indicated clinically)
3. Amino acids (as indicated clinically)
4. Clinical monitoring FSS
5. Treatment administration log, concomitant medications log, blood transfusion log, and adverse events log will be completed daily

Day 3 (if not yet discharged)

1. NCG/PLBO Rx. & Standard Rx every 12 hours.
2. Plasma ammonia (as indicated clinically)
3. Safety labs should be performed between days 3 - 5
4. EKG test should be performed between days 3-5
5. Clinical monitoring FSS
6. Treatment administration log, concomitant medications log, blood transfusion log, and adverse events log will be completed daily

Day 4 (if not yet discharged)

1. NCG/PLBO Rx. & Standard Rx every 12 hours.
2. Plasma ammonia (as indicated clinically)
3. Amino acids (as indicated clinically)
4. Clinical monitoring FSS
5. Treatment administration log, concomitant medications log, blood transfusion log, and adverse events log will be completed daily

Day 5 (if not yet discharged)

1. NCG/PLBO Rx. & Standard Rx every 12 hours.
2. Plasma ammonia (as indicated clinically)
3. Clinical monitoring FSS
4. Treatment administration log, concomitant medications log, blood transfusion log, and adverse events log will be completed daily

Day 6 (if not yet discharged)

1. NCG/PLBO Rx. & Standard Rx every 12 hours.
2. Plasma ammonia (as indicated clinically)
3. Safety labs should be performed between days 6 – 7
4. EKG test should be performed between days 6-7 (if not done already between days 3-5)
5. Amino acids (as indicated clinically)
6. Clinical monitoring FSS
7. Treatment administration log, concomitant medications log, blood transfusion log, and adverse events log will be completed daily

Day 7 (if not yet discharged)

1. NCG/PLBO Rx. & Standard Rx.
2. Plasma ammonia (as indicated clinically)
3. Safety labs if not done on Day 6
4. EKG if not already done
5. Clinical monitoring FSS
6. Treatment administration log, concomitant medications log, blood transfusion log, and adverse events log will be completed daily

Day 8 (if not yet discharged)

1. The final dose of NCG/PLBO Rx. & Standard Rx.
2. Plasma ammonia (as indicated clinically)
3. Treatment administration log, concomitant medications log, blood transfusions log and adverse events log will be completed a final time.

5.4.1.4 Discharge Procedures

If still on NCG/PLBO, the subjects will be discontinued from the study drug at the time of discharge from the hospital.

We will contact subjects or their families to collect any adverse events that occurred in the 72 hours following episode treatment completion.

5.5 Study Assessments

5.5.1 Laboratory Tests

- Plasma ammonia (as indicated clinically)
- Plasma amino acids (as indicated clinically)
- Complete blood cell count
  - White blood cells and differential
  - Platelets
  - Hemoglobin
  - Hematocrit
- Liver function tests
  - ALT (Alanine Aminotransferase)
  - AST (Aspartate Aminotransferase)
  - Alkaline phosphatase
  - Direct bilirubin
  - Total bilirubin
  - Albumin
  - Total protein

- Kidney function tests
  - Blood urea nitrogen
  - Creatinine
- Amylase and lipase
  - Amylase
  - Lipase
- Coagulation profile (as indicated clinically)
  - PTT (Partial Thromboplastin Time)
  - INR (International Normalized Ratio)
- Pregnancy test (if applicable)

5.5.2 Electrocardiogram test (EKG)

QT interval and QTc interval will be collected from each EKG, along with any abnormalities noted in the interpretation

5.5.3 Transfusion Information

Type of transfusion (e.g., packed red blood cells, fresh frozen plasma) and number of units will be collected

5.5.4 Clinical Assessments

The secondary objective of this study is to evaluate the impact of NCG treatment on the functional status scale. During each admission, the functional status scale will be evaluated prior to the start of study drug, as well as every day thereafter, until discharge or until episode treatment completion of the study drug.

5.5.4.1 Pharmacokinetic Assessments (CNMC & CHOP)

**** THIS PROTOCOL SECTION ONLY PERTAINS TO CHILDREN'S NATIONAL HOSPITALAND CHILDREN'S HOSPITAL OF PHILADELPHIA (CHOP) ****

Left over plasma or serum from any sample drawn for clinical laboratory tests will be saved for pharmacokinetic (PK) analysis to be paired with study drug dosage and time of administration. The plasma and/or serum samples will be stored frozen at -20˚C and batch-shipped on dry-ice to Atlanbio Laboratory (Rue Graham Bell, ZI de Brais – BP 40309, 44605 Saint-Nazaire, Cedex, France) for determination of N-carbamylglutamate levels at the end of each patient episode. Since study participants experience a high number of blood draws for clinical tests, no additional blood will be drawn specifically for PK analysis. There will be no schedule of assessments for PK samples. Instead, study staff at CNMC and CHOP sites will be encouraged to collect the left over plasma or serum samples during each hyperammonemia episode. The exact time and date of the blood draw is recorded (and will correspond to the medical record) for each sample. These samples will be stripped of hospital identifiers and will be labeled with the study number and episode number before being shipped to the reference laboratory.

5.5.5 Efficacy Assessments

For the primary outcome, we will evaluate the plasma ammonia trajectory during episodes in which patients receive NCG vs. PLBO. Secondary outcomes will consist of FSS, and length of hospitalizations.

5.5.5.1 Functional Status Scale

The Functional Status Scale (FSS) will be used to assess changes in the subject's global functioning in the hospital setting. The measure will be obtained at baseline (i.e., prior to administration of study drug or placebo at each episode) and then daily during hospitalization up to discharge or for 7 days whichever is shorter. The scale includes 6 domains; mental status, sensory functioning, communication, motor functioning, feeding, and respiratory status. Each domain is rated on a 5-point scale from 1 (normal) to 5 (severe dysfunction), thus the range of overall scores is 6 to 30 (Table 4). The measure was designed to be quantitative, rapid, reliable, minimally dependent on subjective assessments, applicable to as broad an age range as possible, and pertinent to hospitalized patients in as many inpatient environments as possible[22]. All individuals collecting data will be trained in the measure and can include medical professionals such as a nurse, genetic counselor, or physician investigator at the site.

Table 2. The Functional Status Scale (from Pollack et al., 2009)

	Normal (Score = 1)	Mild Dysfunction (Score = 2)	Moderate Dysfunction (Score = 3)	Severe Dysfunction (Score = 4)	Very Severe Dysfunction (Score = 5)
Mental status	Normal sleep/wake periods; appropriate responsiveness	Sleepy but arousable to noise/touch/movement and/or periods of social nonresponsiveness	Lethargic and/or irritable	Minimal arousal to stimuli (stupor)	Unresponsive, coma, and/or vegetative state
Sensory functioning	Intact hearing and vision and responsive to touch	Suspected hearing or vision loss	Not reactive to auditory stimuli or to visual stimuli	Not reactive to auditory stimuli and to visual stimuli	Abnormal responses to pain or touch
Communication	Appropriate noncrying vocalizations, interactive facial expressiveness, or gestures	Diminished vocalization, facial expression, and/or social responsiveness	Absence of attention-getting behavior	No demonstration of discomfort	Absence of communication
Motor functioning	Coordinated body movements, normal muscle control, and awareness of action and reason	1 limb functionally impaired	≥2 limbs functionally impaired	Poor head control	Diffuse spasticity, paralysis, or decerebrate/decorticate posturing
Feeding	All food taken by mouth with age-appropriate help	Nothing by mouth or need for age-inappropriate help with feeding	Oral and tube feedings	Parenteral nutrition with oral or tube feedings	All parenteral nutrition
Respiratory status	Room air and no artificial support or aids	Oxygen treatment and/or suctioning	Tracheostomy	Continuous positive airway pressure treatment for all or part of the day and/or mechanical ventilatory support for part of the day	Mechanical ventilatory support for all of the day and night

5.5.6 Safety Assessments

Safety assessments will consist of serious adverse events, adverse events, EKG tests, and safety laboratory measurements in the NCG vs. the PLBO group.

5.6 Treatment Compliance

Treatment logs will capture the timing and dosage of each occurrence of study drug administration. All treatments will be administered in the hospital so non-compliance with the medication is not a concern.

5.7 Study Termination/Stopping Rules

The study PI and Coordinating Center will seek input from the DSMB regarding the need to stop the study if either of the following events occurs: (a) Life-threatening adverse event that is clearly associated with drug hypersensitivity and/or (b) Unexplained serious illness or death of one of the participants.

The NIH and local IRBs (at their local site) or the site PI has the authority to stop or suspend this trial at any time. The Medical Monitor, DSMB, or FDA may recommend suspending or stopping the trial after a thorough review, due to evidence related to safety or efficacy based on planned interim analyses (see Section 7 on Safety Monitoring and Section 8.3.1 on Interim Analysis).

5.8 Subject Discontinuation

A subject will be discontinued from the study if s/he:

- Is determined not to have PA, MMA, CPSD, or OTCD (as defined in Section 5.1.1.1)
- Voluntarily withdraws or is voluntarily withdrawn from the study by his/her parents

The study drug will also be discontinued if a subject:

- Experiences a serious adverse event, deemed by the Medical Monitor and site PI to be related to or likely related to study drug administration.
- Has received a hepatic transplantation
- Relocates to a location, not within the catchment area of one of the other participating institutions

If a participant drops from the study before its completion, a narrative description of the reason(s) for discontinuation will be recorded on the Study Closeout Form (see Manual of Procedures).

5.9 Blinding

This is a double-blind, placebo-controlled trial performed in children's hospitals across the country. Handling, storage, dispensing, and record keeping of all study medication will follow a Pharmacy Manual, which will be developed by the Central Research Pharmacy at Children's National Hospital. The Pharmacy Manual will define the responsibilities and procedures of the Central Research Pharmacy and of the Site Pharmacies both of which will be unblinded.

5.9.1 Type of Blinding

The participants, their families, caregivers, and all research personnel at the sites, as well as the study chair and co-chair, will be blinded to the identity of the drug (NCG or PLBO). The appearance of active and placebo tablets is nearly identical.

5.9.2 Maintenance of Blinding

- NCG and PBLO will be sent to each site pharmacy by the Central Pharmacy (CNMC).

- Assigned NCG/PLBO doses will be prepared individually for each subject by the Site Pharmacy.
- Identically labeled NCG/PLBO doses prepared by the Site Pharmacy will be dispensed by blinded hospital staff based on a prescription from the blinded study physician.
- An additional supply of NCG/PLBO will be provided as needed based on each site's enrollment rate to provide the doses of study medication for all newly enrolled subjects as well as a small supply to meet emergency needs at each site.
- The individual Site Pharmacy will prepare, blind and dispense unit doses of study medication for each enrolled subject from the drug supply while the subject is hospitalized.
- All unopened medications will be stored at controlled refrigerated temperature (2-8° C; 36-46° F), and opened medications will be stored at controlled room temperature (15-30° C; 59-86° F) in a secure area in the site's research pharmacy to which access will be limited to the research pharmacist and designated assistants. To maintain blinding, only the site pharmacist and designated assistants will have access to the secured area.

5.9.3 Breaking the Blinding

Treatment assignment should not be unblinded unless the Medical Monitor assesses that knowledge of drug assignment is required in order to assess how to treat a safety concern within a patient. The site investigator should contact the Coordinating Center immediately if he/she believes that knowledge of the treatment assignment will affect their decision regarding treatment of a safety concern.

6 STUDY TREATMENTS

6.1 Study Intervention

Carbaglu® 200 mg Dispersible Tablets (N-carbamylglutamate), scored in three places, allowing each tablet to be broken into four 50 mg pieces.

The target dose is 150 mg/kg/d for patients ≤15kg or 3.3 g/m2/day for patients >15kg, divided into two equal doses 12 hours apart. Each dose will be rounded to the nearest 50mg using the dosing nomograms provided. The maximum variance from the calculated target daily dose and the actual daily dose will be 23.5% or less. Study drug will be prepared for administration according to the package insert. The suspension has a slightly acidic taste and a white, cloudy appearance. Participants will either receive PLBO or the study intervention, NCG (Carbaglu®) 150 mg/kg/day for patients ≤15kg or 3.3 g/m2/day for patients >15 kg.

6.2 Control (Placebo) Intervention

The placebo and NCG tablets, which are produced by the same manufacturer and include the same inactive components look and taste similar. When the two tablets are suspended in solution, there is a slight difference in a hue that is only distinguishable when the two solutions are observed side by side.

6.3 Dispensing of Study Medications

- Handling, storage, dispensing, and record keeping of all study medication will follow a Pharmacy Manual, which will be developed by the Central Research Pharmacy (CRP) at the Children's National Hospital. The CRP Pharmacy Manual will define the responsibilities and procedures of the CRP and of the site pharmacies both of which will be unblinded.
- The CRP will receive study medication (NCG) and matched placebo (PLBO) from Orphan Europe.
- Once the CRP and the Coordinating Center at Children's National Hospital determine a site pharmacy is appropriately certified and ready to receive the drug, the CRP will ship an initial quantity of NCG and PLBO to the individual site research pharmacy.
- All doses of NCG/PLBO (as assigned) will be prepared, blinded, and dispensed in unit doses individually for each subject by each site pharmacy while the subject is hospitalized.

6.4 Provisions for Access to Investigational Treatment after Study

The results of the study will be shared with the patient, his/her family, and his/her treating physician. If the study reveals that NCG is effective, the treating physician will have the prerogative for treating the patient off-label with NCG, which is already approved for use in NAGS deficiency, until it is approved by the FDA for additional indications.

6.5 Drug Packaging/Handling/Storage/Accountability

- Oral NCG and matching PLBO will be supplied by Orphan Europe and distributed to each site by the CRP
- The designated research pharmacist, investigator, or coordinator at each participating site will be required to acknowledge receipt of study drug shipments/doses and notify the CRP of any problems with shipments or with the on-site supply of study medication.

- S/he will sign receipts for medication supplies, which will be kept in a secure area in the site's research pharmacy to which access will be limited to the research pharmacist and their designated assistants. To maintain blinding, only the site pharmacist and designated assistants will have access to the secured area.
- Unopened bottles of medications will be stored at controlled refrigerated temperatures (2-8° C; 36-46° F).
- Opened bottles of medications will be stored at controlled room temperature (15-30° C; 59-86° F). The date of first opening a bottle will be recorded on the tablet container.
- Drug accountability logs will be completed by the site pharmacists and the study coordinators.
- The sites will update their monthly supplies logs and expiration dates and the information will be monitored by the central pharmacy.
- Unopened bottles of medications will not be used after the expiration date stated on the tablet container. Opened containers of medications will not be used after 30 days of first opening.
- All unused study medication will be returned by the site to the central pharmacy and be documented in the study database. After reconciliation, the drug will be destroyed or retained and used in animal research.
- At the conclusion of the study or as required for review, supplies of received, dispensed and returned medication will be reconciled to account for all distributed supplies of PLBO/NCG.

6.6 Concomitant Therapy

6.6.1 Therapies Prohibited Before/During the Trial

All investigational drugs, biologics, and therapies are prohibited following enrollment in this trial.

This includes the use of alternative pathway medications, including sodium benzoate, and any medication with phenylacetate as an active metabolite (e.g., Buphenyl, Ammonul, Ravicti) in propionic acidemia, and methylmalonic acidemia; these medications have not been approved for this indication and there is no evidence for their effectiveness in these conditions.

No other changes in the special diet or medications are called for in this protocol.

6.6.2 Therapies Allowed During the Trial

Standard therapy, including but not limited to intravenous fluids, dextrose, intralipids, biotin, hydroxocobalamin, levocarnitine, metronidazole, and metabolic specialty formulas. Hemodialysis or hemofiltration may be administered as determined by the treating physician.

In CPSD and OTCD, FDA approved alternative pathway medications (e.g., Na-benzoate, Na-phenylacetate, Buphenyl, Ammonul, Ravicti) are allowed and are indicated in these conditions.

7 SAFETY MONITORING

The study protocol will be submitted to the FDA under IND # 68,185 and will be reviewed by the study DSMB (Section 7.2) and the Medical Monitor (Section 7.3). Participant enrollment may only begin after final IRB approval.

7.1 Adverse Events (AEs)

7.1.1 Definitions

7.1.1.1 Adverse Event and Suspected Adverse Reaction

As defined in title 21 of the Code of Federal Regulations Part 312, an *adverse event (AE)* is "any untoward medical occurrence associated with the use of a drug, whether or not considered drug related". All adverse events will be classified using version 4.0 of Common Terminology Criteria for Adverse Events (CTCAE), developed and maintained by CTEP at the National Cancer Institute (http://evs.nci.nih.gov/ftp1/CTCAE/About.html).

A suspected adverse reaction is any adverse event for which there is a reasonable possibility that the drug (NCG) caused the adverse event. "Reasonable possibility" means that there is evidence, such as a temporal relationship, to suggest a causal relationship between administration of NCG and the adverse events. It is less certain about causality than *adverse reaction*.

7.1.1.2 Serious Adverse Events

Title 21 of the CFR also provides a definition for serious adverse events (SAEs), described as those events that result in death; or are life-threatening; or require prolonged inpatient hospitalization or prolongation of existing hospitalization, or create persistent or significant disability/incapacity, or a congenital anomaly/birth defects. However, application of this definition is difficult in subjects who, because of their underlying congenital disorder, present with a life-threatening illness, requiring lengthy inpatient hospitalization.

Administration of NCG has not been described to result in serious adverse events (see side effects section 1.3.4), and using the standard definition of SAEs is likely to result in capture of events secondary to the underlying acute metabolic disease, and which would not be helpful in the collection of data pertaining to NCG or its administration. Therefore, for this study, we modify the standard definition and define **serious adverse events** as those events that**:**

- Result in death
- Are life-threatening, that is, places the patient at immediate risk of death from the event as it occurred. Because acutely ill patients with PA or MMA are likely to experience life-threatening events as part of their underlying condition and meeting eligibility criteria, this category will be restricted to events that arise as new events subsequent to administration of NCG, not having started prior to administration of NCG. Life-threatening events resulting from processes, which based on the opinion of the investigator likely began prior to administration of NCG but had not been identified because the diagnostic investigation had not yet been performed, will not be considered serious adverse events.

- Require prolongation of existing hospitalization. This category will be restricted to only complications that clearly extend the hospitalization that was anticipated at randomization to an episode.

Serious Adverse Events in between episodes

- Result in death
- Are life–threatening
- Require hospitalization (except for elective hospitalizations for e.g. elective procedures and hospitalizations for a hyperammonemic event which are episodes and not SAEs)
- Note: if a hospitalization occurred without an episode-qualifying ammonia level, but later in the course of the hospitalization, the ammonia level rises to a qualifying episode, the participant will be randomized to treatment and the hospitalization will **not** be considered an SAE.
- Cause prolongation of existing hospitalization
- Cause persistent or significant disability/incapacity
- Cause a congenital anomaly/birth defects

7.1.1.3 Expected and Unexpected Adverse Events

An unexpected adverse event or reaction is defined as any adverse experience, the specificity or severity of which is not consistent with the risks described in the protocol (see Section 1.3.4 on Safety and Side Effects of Carbaglu) or in the Carbaglu investigator's brochure. Expected adverse events are those that are identified in the research protocol as having been previously associated with or having the potential to arise as a consequence of participation in the study or are part of the clinical spectrum of the underlying disorder. Common acute and chronic complications of propionic and methylmalonic acidemia include:

- Anorexia
- Vomiting
- Lethargy
- Stupor
- Coma
- Seizures
- Ketosis
- Acidosis
- Hyperventilation
- Hyperglycemia
- Hypoglycemia
- Hypothermia
- Neutropenia; bone marrow suppression
- Electrolytes abnormalities
- Amino acid abnormalities
- Pancreatitis (PA)
- Renal dysfunction (MMA)
- Cardiomyopathy (PA)

7.1.1.4 Relationship to Intervention Assessment

Relationship of NCG to the adverse event or suspected adverse reaction is defined as follows:

- **Unrelated:**
  Adverse event is clearly due to extraneous causes (e.g., underlying disease)

- **Unlikely related (must have 2 of the below):**
  Does not have temporal relationship to NCG administration
  Could readily have occurred due to subject's clinical state
  Could have been due to environment or other interventions
  Does not follow known pattern of response to NCG
  Does not reappear or worsen with reintroduction of NCG

- **Possibly related (must have 2 of below):**
  NCG administration and the occurrence of the AE are reasonably related in time
  Could not readily have occurred due to subject's clinical state
  Could not readily be due to environment or other interventions
  Follows a known pattern of response to NCG

- **Probably related (must have 3 of the below):**
  NCG administration and the occurrence of the AE are reasonably related in time
  Could not readily have occurred due to subject's clinical state
  Could not readily be due to environment or other interventions
  Follows a known pattern of response to NCG

- **Definitely related (must have all 4 of the below**):
  NCG administration and the occurrence of the AE are reasonably related in time
  Could not readily have occurred due to subject's clinical state
  Could not readily be due to environment or other interventions
  Follows a known pattern of response to NCG

7.1.2 Collection and Reporting of Adverse Events

The site study coordinator is responsible for collecting and recording all clinical and laboratory data. Adverse events will be collected on subjects on each day of admission, as well as by phone or e-mail communication with parents when the study coordinator contacts subjects or their families to collect any adverse events that occurred in the 72 hours following episode treatment completion following each admission. The reporting period for new AEs is the period from the start of study drug administration until the patient completes their participation in the study (i.e., until the end of the study period or until discontinuation if early withdrawal occurs). Follow-up reports on AEs should continue up to 30 days after the administration of study treatment if the AE did not resolve.

For each AE, the site investigator will assess severity, and whether the event meets the definition of a serious adverse event (see Section 7.1.1) and will report whether study treatment was interrupted or stopped, as well as the outcome of the AE. If the event is an SAE, both the site PI and the Medical Monitor will assess the relationship to study treatment. As described in 5.7.3, treatment assignment should not be unblinded unless the Medical Monitor assesses that knowledge of drug assignment is required in order to assess how to treat a safety concern within a patient.

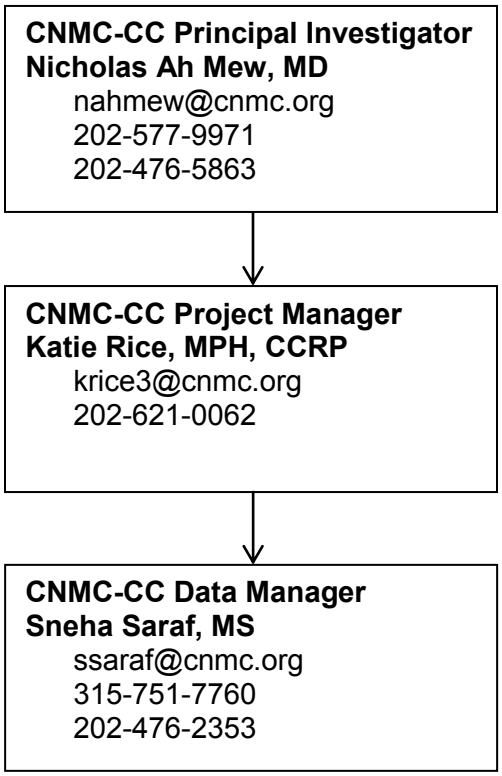
If a non-serious adverse event is unresolved at the time of discharge from the study, the site PI will make a clinical assessment as to whether continued follow-up of the AE is warranted. The

site PI may request input from the Medical Monitor. Resolution is defined as the return to baseline status or stabilization of the condition with the expectation that it will remain chronic.

The CNMC-CC will prepare aggregate reports of all adverse events (serious/not serious and expected, unexpected) for the DSMB as required and for the FDA on an annual basis. Local site investigators are also required to fulfill all reporting requirements to their IRB and to the CNMC-CC.

7.1.2.1 Reporting of Serious Adverse Events

Serious adverse events (SAEs) must be reported by each site PI to the CNMC-CC within 24 hours of learning of the event. The CNMC-CC has a joint phone & email tree for reporting SAEs to the CNMC-CC at any time. Those involved in the phone & email tree are aware that they may expect to receive a phone call or email at any time for an SAE. The site reporting the SAE must attempt to call or email first the CNMC-CC Principal Investigator (PI), followed by the Project Manager (PM), and then the Data Manager (DM) until someone has been reached in real time. It will be the responsibility of the CNMC-CC PI, PM, and DM to have a backup point of contact for instances of sick leave or vacation.



The Medical Monitor (or their delegate) will then be contacted by the CNMC-CC and be informed of the details of the SAE. The CNMC-CC will immediately report unexpected SAEs to the CNMC IRB according to local guidelines.

Sites are required to submit follow-up reports to the initial report as promptly as is feasible. The CNMC-CC will submit a report of the SAE to the FDA within 15 calendar days of the event if the event meets the reporting requirement of a serious and unexpected suspected

adverse reaction (SUSAR). In those cases, the CNMC-CC will also report the event to the DSMB. In all other cases, the CNMC-CC will submit the report as part of periodic reporting to the DSMB and FDA.

The Medical Monitor will review causality (unrelated, not likely related, possibly related, probably related, definitely related) of the serious adverse event. The Medical Monitor may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event.

Additionally, site investigators will be responsible for reporting SAEs to their IRB within the time mandated by the site IRB. The CNMC-CC will inform all sites of any safety updates or changes, and the site investigators will be responsible for notifying their local IRB of these modifications. The CNMC-CC will ensure that all SAEs and any safety updates or changes are reported to the site's IRB by collecting a copy of all IRB-related correspondence and reviewing regulatory submissions during monitoring visits.

7.2 Data and Safety Monitoring Board (DSMB)

A DSMB will be assembled by the investigators. Typically, the Board members represent the following specialties, all of whom will have no formal involvement with the participants or the investigators: a medical doctor with experience in inherited metabolic disorders, a neonatologist, a biostatistician, and the clinical trial coordinator. This Board is responsible for safety and accuracy monitoring of the data entered by the investigators. The DSMB will regularly evaluate trial performance, monitor interim data for safety and effectiveness of study regimens, review any protocol modifications, and advise the investigators regarding early termination or continuation of a study based on the interim monitoring or scientific findings. The DSMB will receive and respond to reports of any serious adverse events (SAEs) and will be immediately notified of fatal or life-threatening events. Based on the review of safety, efficacy, and performance data, the DSMB will make recommendations regarding the conduct of the study. It is expected that the DSMB will meet at least biannually in Washington, D.C., with conference calls as necessary.

7.3 Medical Monitor

A Medical Monitor has been identified by the investigators. The Medical Monitor is responsible for being available by pager to monitor study safety issues and must identify an appropriate delegate to provide coverage whenever he is not available. The Medical Monitor (or designee) will be contacted whenever a critical situation with a patient arises and a decision about unblinding the treatment assignment must be made (i.e., in the event that unblinding the treatment assignment would affect subsequent management of the patient). The Medical Monitor may be unblinded whenever he deems it necessary. The Medical Monitor will also conduct an independent blinded review of all Serious Adverse Events (SAEs) and will contribute to the preparation of all SAE reports. The Medical Monitor will communicate any identified safety concerns to the study PI and Coordinating Center. The Medical Monitor may consult with the DSMB.

8 STATISTICAL ANALYSIS

8.1 Study Aims

8.1.1 Primary Aim

The primary aim is to evaluate the impact of NCG treatment during hyperammonemic events on the resolution of ammonia levels, among patients diagnosed with severe PA or MMA and partial CPSD or OTCD.

8.1.2 Secondary Aim

Secondary analyses will also evaluate the safety of NCG. Safety is a secondary aim because there is already evidence that NCG, which is approved by the FDA for use in the treatment of NAGS, poses no serious risks to patient safety. Therefore, the focus of this research is less on safety than on its effectiveness in treating PA and MMA, which share the common risks associated with hyperammonemia with NAGS. The analysis of safety will focus on three outcomes; these include the level of selected laboratory markers related especially to organ health, the frequency of reported symptoms, and the frequency of reported adverse events by seriousness. The analysis of laboratory markers will compare the time specific and time averaged mean levels of each marker by study group. In addition, we will compare the frequency of symptoms and adverse events, relying mainly on tabulations by a study group of reports by organ system paying most attention to the magnitude of the difference between groups and consistency of the evidence than on formal hypothesis testing.

8.2 Factors for Stratification

Because clinical management practices and referral patterns may vary from site to site, stratification during randomization by the site will ensure that episodes will be balanced across study groups within each site.

8.3 Statistical Considerations

Experience with models evaluating the overall change in ammonia level during episodes following randomization (not divided by treatment group) has demonstrated that the shape of the ammonia curve is more variable and more complex than envisioned during study design. This circumstance has made it difficult to see how to generate a stable model to properly reflect and compare the slope of ammonia decline by treatment group as the primary outcome.

Challenges included the following:

1. The need to introduce curvilinearity in order to model change appropriately; and
2. The need to also rely on piecewise regression models to adequately model bends or points of inflection in the curve.

The latter has proven especially difficult to manage because methods vary in their recommendation regarding the number (1 vs 2) and position (time point of each bend) to define piecewise regression segments. This variability would lead to the need to create a number of alternative models that would affect the interpretation of results with no clear way to prioritize the choice of the final model.

This situation prompted us to choose an alternative composite outcome and modeling strategy based on time to reach an ammonia level of 50 mcmol/L or time to discharge whichever occurs first. This approach avoids the complexity of modeling the correct shape of the ammonia decline curve but provides a rigorous, well-defined, and clinically-relevant outcome for comparison of treatment effects.

The choice of 50 mcmol/L as the target for ammonia decline is based on the extensive experience of metabolic physicians that a patient reaching this level of ammonia is no longer at risk of acute hyperammonemic injury and would be a candidate for discharge in the absence of other complications, which are not hypothesized to be affected by treatment with NCG.

We propose to use Cox Proportional Hazard modeling to implement the primary analysis to compare the average differences in time to reach the composite outcome and allow us to control for differences in severity (ammonia level and or FSS) at episode baseline. This method will also allow us to take account of and adjust statistical testing for the correlation between results of participants who contributed more than one event to the analysis. The comparison of slopes of the ammonia decline curves by treatment group will shift to secondary analysis, which will allow us to focus on consistency of results rather than choosing the precise model for significance testing for the purpose of rejecting the null hypothesis of no treatment effect.

Study analyses will be based on an intention-to-treat paradigm. In analyzing the primary outcome, we will evaluate the difference over time in the probability of reaching an ammonia level of 50 mcmol/L or hospital discharge. For this purpose, we will use a z-test to assess the evidence that the hazard (event) ratio (HR) differs from 1 (no effect) to test for a difference in the probability of the composite outcome over time by the group. We will account for the correlation between repeat episodes per participant by treating participant episodes with the same ID as a cluster, which will adjust standard error estimates for the intraclass correlation between episodes in the same cluster. The primary analysis will be implemented using the sts cox procedure in Stata 14 (StataCorp. *Stata Statistical Software: Release 14*, College Station, TX: StataCorp LP, 2015).

Secondary efficacy outcomes are based on groupwise comparisons of the trajectory (slope) of ammonia decline, FSS, and time-to-hospital-discharge during hyperammonemia episodes. For analysis of the ammonia decline trajectory and FSS analysis, we will implement longitudinal linear hierarchical models. The models will enable us to estimate and compare the slope(s) based on ammonia change trajectory over time, as well as the time, averaged FSS across all episodes treated with NCG vs. PLBO. For time to discharge comparisons, we will rely on the same Cox Regression Analysis described for the primary outcome except the outcome event will be limited to time to discharge. As in the primary analysis, we will adjust model error for the clustering of observations by patient and control for severity based on ammonia level or FSS at admission to each episode. To assess NCG safety we will compare the levels/frequency of laboratory test results, reported symptoms, and adverse events. Secondary analyses will control for indicators of episode severity, e.g. peak ammonia.

8.3.1 Interim Analysis

In addition to the proposed final analysis, we will conduct an interim analysis using the same methodology described above when at least 50% (70 episodes) or when at least 60 episodes have occurred after the study midpoint based on the projected 4 hyperammonemic events per participant have been completed (participant discharged). Allowing for one interim look for both efficacy and futility when half the information becomes available, using an O'Brien-Fleming stopping rule and a Lan-DeMets approach to the use function of the p-value. If conducted exactly at the 50% outcome, the boundary for rejecting the null hypothesis and concluding that Carbaglu is either efficacious or worse that placebo is a z-score of +/-2.9626, corresponding to a p-value <0.0031 at the interim look. The boundary for deciding futility, i.e., that Carbaglu is not expected to be significantly different than placebo is +/-0.3557, corresponding to a p-value of >0.72 at the interim look. With these boundaries, the decision rules at the conclusion of the study will be +/-1.9686 or more extreme to conclude that Carbaglu is either efficacious or worse

than placebo, corresponding to a p-value of <0.049. These values were calculated using East 5.3 (Cytel, Inc.).

A report of the interim analyses will be provided to the DSMB, which will review and discuss the results considering all evidence available, including magnitude of difference between groups and p-value, as well as results on secondary outcomes and safety to consider whether the results warrant stopping the trial either from persuasive evidence of benefit or harm.

8.4 Rationale for Sample Size and Statistical Power

All sample size and power estimates are based on 2-tailed testing, which assumes a type 1 error of 5% and achieving 80% power to detect an HR difference by treatment group and that there is no censoring as each episode will contribute information to one of the composite outcomes. We used the Cox Regression procedure in PASS (Hintze, J. PASS 11. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com, 2011) to evaluate the composite time to event outcome of the earlier of the time to reach an ammonia level of 50 mcmol/L or time to discharge within hyperammonemic episodes (the unit of randomization and analysis). We evaluated HR estimates above 1, reflecting that the NCG group achieved the higher probability of achieving the composite event. Based on these considerations, a total sample size of N=146 episodes can detect an HR of 1.6, one of N=114 can detect an HR of 1.7, one of N=93 can detect an HR of 1.8 and one of N=78 can detect an HR of 1.9. These sample size estimates account for an inflated R-square as large as 0.05 between the time to event outcome and covariates reflecting baseline severity at the start of an episode. We used the inflated R-square to account for the modest effect of clustering of episodes within a person. Therefore, we consider the originally-targeted sample size of 144 episodes to be more than sufficient to meet the aims of the study. Based on our re-analysis of the time-to-event outcome, an HR of 1.7 is realistic and a sample size of 114 episodes is sufficient to detect that effect. Therefore, the target sample size will be 114 episodes.

9 REGULATORY COMPLIANCE AND SAFETY MONITORING

9.1 Regulatory Compliance

This clinical trial will be conducted in accordance with the protocol, the ICH Harmonised Tripartite Guideline "Note for Guidance on Good Clinical Practice," and the applicable local regulatory requirements.

9.2 Statement of Compliance

The CNMC IRB and all participating site IRBs comply with Good Clinical Practices as defined by the U.S. Food and Drug Administration (FDA) regulations and the International Conference on Harmonization (ICH) guidelines.

Investigator Assurances

- For quality assurance reasons, the Coordinating Center reserves the right to perform site-monitoring visits.
- Monitoring shall ensure that the study is planned, conducted, evaluated, and reported according to this protocol and the applicable SOPs of the Coordinating Center, the ethical principles that have their origin in the Declaration of Helsinki (1996), the requirements of the ICH Harmonised Tripartite Guidelines.
- Monitoring shall also ensure that the documentation of the study is available, complete, organized, and valid.
- The investigator agrees to give the study monitor access to all relevant documents, including source documents, for review. The same applies in the case of an inspection of federal authorities or the relevant IRB.

9.3 Informed Consent

- It is the responsibility of the investigator to ensure that no subject is enrolled in any study-related examination or activity before written informed consent has been obtained.
- Written informed consent will be obtained from subjects or their legal representative(s) in compliance with 21 CFR Part 50 and the ethical principles that have their origin in the Declaration of Helsinki.
- For underage subjects, written informed consent will be obtained from the subject's legal representative(s) (e.g., his/her parent(s) or legal guardian(s)) in compliance with 21 CFR Part 50 and the ethical principles that have their origin in the Declaration of Helsinki.
- If the subject is younger than the age of consent but old enough to provide assent, it is the investigator's responsibility to ensure that the site's assent procedures are followed and that there is documentation of the fact that the assent procedures were followed. The subject and the subject's legal representative(s) will be informed that they are completely free to refuse to enter the study or to withdraw from it at any time and that this will not affect their overall care at the institution.
- If the subject's mental age or psychological state is such that they cannot assent or if there is potential medical benefit to the subject by participating in the study an assent waiver must be signed by a parent or legal representative.
- Subjects, whose legal representative(s) refuse to give written informed consent or withdraw their informed consent, later on, must not be included in the trial or must be excluded from further participation, respectively.
- Before personally dating and signing the informed consent form, the subject's legal representative(s) will be informed in detail by the investigator about all pertinent aspects of the trial according to 21 CFR Part 50 and ICH GCP.

- The subject's legal representative(s) should be given sufficient time to request further details about the trial before signing the informed consent form, in accordance with the ICH GCP Guidelines (1997). Under certain circumstances, when a patient cannot provide informed consent and is not accompanied by parents/guardians, the investigator will speak with the parent/guardian by phone, and provide them with a copy of the consent form (e.g., by fax, e-mail). When the parent/guardian receives the consent form, it will be immediately signed and sent/faxed back to the investigator.
- The receipt of the informed consent from the legal representative(s) must be documented on the appropriate page of the subject's CRF and in the source documents.
- One copy of the consent form signed and dated by the subject's legal representative(s) and by the physician who informed the legal representative(s) will be kept at the study site; a second copy will be provided to the subject's legal representative(s).

9.4 Records Retention and Requirements

- The investigators and the sponsor(s) must archive all essential records and documents including but not limited to CRF, informed consent, and identification codes for subjects and other original records.
- These documents of the study will be retained at least 3 years after study close and data lock.
- The site investigators will be informed by the Coordinating Center (CNMC-CC) when the documents need not be retained any longer.

9.5 Financial Disclosure

Financial disclosure information will be collected per Part 54 of Title 21 of the CFR and ICH E6. In addition, the site investigators must provide to the study PI a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following completion of the study.

9.6 Inclusion of Minorities and Children

The disorders to be investigated affect both genders and all different ethnic groups and we, therefore, expect to have proportional representation among the participants of the trials. These are rare disorders, and we will attempt to recruit all presenting potentially eligible patients. Participants of this study will include neonates, infants, children, adolescents, and adults. There is no upper age limit.

9.7 Confidentiality

By conducting this study, the investigator pledges that he/she will keep all information pertaining to the study strictly confidential, including data generated from this study, except as exempted for regulatory purposes. Blood samples and neuropsychological testing will be collected and diagnostic records will be reviewed. Data recorded will include demographics, clinical summary, diagnostic tests and study results. All research data will be accessible only to the research team and as required by law.

9.7.1 Protection Against Risk

- The NCG drug and placebo will be handled by the respective site investigational drug pharmacy and will be dispensed by that pharmacy.
- The participants and their families will be provided psychological and emotional support to deal with the results of the developmental testing.
- Venipuncture: The vein in which the needle has been inserted to draw blood may become sore and red. A temporary bruise may develop, and rarely fainting may occur.
- Confidentiality is protected to the full extent required by law and applicable local, State and Federal regulations and guidance including HIPAA.

- All medical records, including case report forms, will be kept in locked files accessible only to the health professionals involved in the clinical research or responsible for the participant's care, governmental agencies (e.g., FDA, NIH), and local convening authorities (e.g., IRB) for the purpose of audit regarding scientific validity and/or aspects pertaining to the ethical conduct of human clinical investigation. Identifier data are not released without the parent/participant's knowledge and consent. Electronic databases are user ID/password protected.
- The results of the study will be shared with the participant of the study as soon as they become available.

9.7.2 Health Insurance Portability and Accountability Act (HIPAA)

The Standards for Privacy of Individually Identifiable Health Information ("Privacy Rule") establishes a set of national standards for the protection of certain health information. The U.S. Department of Health and Human Services (HHS) issued the Privacy Rule to implement the requirement of the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

The Privacy Rule standards address the use and disclosure of individuals' health information—called protected health information (PHI) by organizations subject to the Privacy Rule — called "covered entities", as well as standards for individuals' privacy rights to understand and control how their health information is used.

Under HIPAA, Protected health information encompasses 18 identifiers, which must be treated with special care, including information on a patient's medical chart or a patient's test results, contact information, as well as an individual's billing information for medical services rendered. PHI also includes identifiable health information about subjects of clinical research gathered by a researcher who is a covered health care provider. This study does not collect any PHI except dates, and other are considered a limited data set.

For this portion of the study, which involves a minimal data set of PHI for recruitment purposes, only the minimum necessary information required to accomplish the research objectives will be extracted from the electronic medical record and recorded in the research database. Allowing the study team to use the electronic medical record to identify and capture information about all potentially eligible subjects is necessary to meet the research objectives.

9.8 Regulatory Files

Prior to beginning the study, the study Principal Investigator and Site Investigators will be asked to comply with ICH E6 8.2 and Title 21 of the CFR by providing the following essential documents, including but not limited to:

- An original investigator-signed Investigator Agreement page of the protocol.
- An IRB-approved informed consent, samples of site advertisements for recruitment for this study, and any other written information regarding this study that is to be provided to the subject or legal guardians.
- IRB approval of the protocol and amendments.
- Form FDA 1572, fully executed, and all updates on a new fully executed form FDA 1572
- Curriculum vitae (CV) for the site Principal Investigator and each Sub-Investigator listed on Form FDA 1572. Current licensure must be noted on the CV. They will be signed and dated by the site Principal Investigators and Sub-Investigators at study start up, indicating that they are accurate and current.
- Financial disclosure information to allow the sponsor to submit complete and accurate certification or disclosure statements required under Part 54 of Title 21 of the CFR and

ICH E6. In addition, the Investigators must provide to the study PI a commitment to promptly update this information if any relevant changes occur during the course of the investigation and for 1 year following the completion of the study.

- Laboratory certifications and normal ranges for any test or assay being performed.

9.9 Source Data Verification Monitoring

- The study PI will authorize the Coordinating Center (CNMC-CC) to monitor the study according to the relevant standard operating procedures (SOPs) and GCP guidelines as frequently as deemed necessary by the CNMC-CC to verify that data entries into the CRF are correct and that the study is conducted in accordance with this protocol. The first monitoring visit will take place approximately 12 months after the Site Initiation Visit.
- During each monitoring visit, the monitor will check the entries made in the CRFs. The monitor will compare these entries with the source data, e.g., the subject's medical records or laboratory results (source data verification). The case number, informed consent, demographic data, inclusion/exclusion criteria, concomitant diseases, concomitant treatment, and all AEs will be verified.
- The monitor will check all data for plausibility and completeness in collaboration with the site investigator. At the same time, a data check for medical plausibility and conformity with ICH GCP will be performed.

9.10 Modification of the Protocol

Protocol modifications that affect the safety of the subjects or that alter the scope of the investigation, the scientific quality of the study, the experimental design, dosages, assessment variable(s), the number of subjects treated, or the subject selection criteria may be proposed by the study Principal Investigator and the Medical Monitor. Any change to the protocol can be made only in the form of a written amendment to this study protocol. Such amendments must be approved by the DSMB and the institutional IRB of the study PI and the CNMC-CC first. Once approved, these amendments will be circulated to the sites for their IRB approval prior to implementation at each site. The IRB at each site will determine if the changes are such to warrant that the informed consent form must be revised and re-signed by the legal representatives of all subjects enrolled in the trial, or only applied to new subjects in the study.

9.11 Protocol Deviations and Violations

Protocol deviations or violations are any non-adherences to the procedures outlined in this document. After a subject has been enrolled, it is the investigator's responsibility to make any reasonable effort to avoid and, if necessary, correct protocol deviations or violations

Protocol Deviation

A protocol deviation is any change, divergence, or departure from the IRB-approved protocol, which does not affect the subject's rights, safety or well-being, or the completeness, accuracy, and reliability of primary study data.

Protocol Violation

A protocol violation is any change, divergence, or departure from the IRB-approved protocol that may affect the subject's rights, safety, or well-being and/or the completeness, accuracy, and reliability of primary study data.

If any of the following five criteria are met, a protocol violation has occurred (examples provided):

1. **The deviation has harmed or posed a significant or substantive risk of harm to the research subject.**
    - A research subject received the wrong treatment or incorrect dose or expired drug
    - A research subject met withdrawal criteria during the study but was not withdrawn
    - A research subject received an excluded concomitant medication
2. **The deviation compromises the scientific integrity of the data collected for the study.**
    - A research subject was enrolled but does not meet the protocol's eligibility criteria
    - Changing the protocol without prior IRB approval
3. **The deviation is a breach of human subject protection regulations, policies, or procedures on the part of the investigator(s).**
    - Failure to obtain informed consent prior to initiation of study-related procedures
    - Falsifying research or medical records
    - Performing tests or procedures beyond the individual's professional scope or privilege status (credentialing)
4. **The deviation involves a serious or continuing noncompliance with federal, state, local, or institutional human subject protection regulations, policies, or procedures.**
    - Working under an expired professional license or certification
    - Failure to follow federal and/or local regulations, and intramural research or Coordinating Center policies
    - Repeated minor deviations
5. **The deviation is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles.**
    - A breach of confidentiality.
    - Inadequate or improper informed consent procedure

All protocol violations will be reported by the site to the CNMC-CC immediately upon discovery. The CNMC-CC will report to the study PI any violations reported by sites or discovered through monitoring. All protocol violations will be listed and the admissibility of the subjects' data will be reviewed and assessed by the CNMC-CC. In case any emergency or AE occurs that requires a protocol deviation in the particular case, the investigator should contact the Medical Monitor and DSMB as soon as possible to enable a decision on whether the subject's participation in the study may be continued.

10 DATA MANAGEMENT AND PROCEDURES

All study data will be collected via systems created at the CNMC Coordinating Center (CNMC-CC) and will comply with all applicable guidelines regarding patient confidentiality and data integrity.

10.1 Data Collection

Data can be captured either on paper source documentation and then entered into the Research Electronic Data CAPture (REDCap) system or entered directly (https://redcap.vanderbilt.edu). The web-based transmission of data is encrypted and enclosed within a firewall.

Copies of all source records (original consent documents, contact notes, laboratory evaluation, and other diagnostic reports) must be kept in the participants' medical record or in a separate research chart as allowable by institutional guidelines. At a minimum, the participants' medical record and study chart must contain a copy of the informed consent. The participant's medical record must contain the source documents.

10.2 Data Processing

Data will be entered into REDCap by site study staff and using data validity and cleaning applications within REDCap, all data will be checked against data specifications. Data exceptions (i.e., invalid values, outliers, etc.) or missing data are flagged and described to give the site coordinator an opportunity to review and make corrections directly in the database. All changes to the database will be logged in the REDCap data audit trail. Nevertheless, an eCRF containing data exceptions can be submitted and is logged. Data exceptions, detected at multiple check levels, are queried and followed. The Coordinating Center will also perform regular data queries.

10.3 Design and Development of REDCap for Study Data Management

We will collect and manage study data using the Research Electronic Data CAPture System (REDCap) which has been used to successfully support more than 700 single and multi-centered clinical research studies at CNMC and The George Washington University as part of the collaboration between these two institutions for the Clinical and Translational Research Institute at Children's National (CTSI-CN). REDCap contains tools that are designed to significantly enhance study management and improve the completeness, quality, security, and integrity of research data. The system provides support for all facets of an investigation including protocol oversight, screening, and enrollment of volunteers, a collection of protocol-driven data, as well as reporting and analysis of interim and final results. REDCap also assists with monitoring patient safety and data quality. At enrollment, REDCap supports the random allocation of participants to study groups. In addition to the study data itself, the database houses information about each protocol including information describing each item of data to be collected, targeted enrollment quotas, eligibility criteria, as well as the detailed schedule of visits with information to be collected at each visit (event-time matrix). This enables the system to help with study management and to help improve study quality. The system provides for Web-based data collection from professionally designed forms that can be accessed for online entry directly into the database. Using the Data Access Groups, REDCap also confines each site to view and edit only their own participant dataset. At entry, data checks are performed and data exceptions are logged and flagged for correction. Corrections are implemented based on data edits applied to online forms by approved personnel. All changes are logged and subjected to the same validity and consistency checking of the original data and are applied directly to stored data.

10.3.1 REDCap Time Event Matrix

A key feature of REDCap are the event grids, which define and help to manage the study protocol, it is used as the fundamental view for both scheduling and management of protocol

events, including access to eCRFs. It provides per protocol, a complete and up-to-date picture of each participant's status regarding expected and completed events by the target date.

10.3.2 REDCap Protocol Management

In terms of protocol management, the event grid also lists and tracks each of the data capture events that must take place to implement the research protocol and it provides monitoring to help assure that each is completed accurately (see Section 10.6) and that the requisite data reside in the database within the designated timeframe. During the encounter, it serves as a list to ensure that events that need to occur are completed according to protocol. It also provides a "just in time" link to the eCRF used to record, view, and or edit the data. Symbols are used to show the status of each event at each time point in the event grid. Initially, the symbol for each form will be red if the form has not been completed, yellow if the form has been completed but data have not been verified, and green when the form has been completed. Visit window data will be used to identify any data collected outside of the allowable timeframes; these will be noted as protocol violations.

10.3.3 REDCap Facilitated Screening Features

REDCap will track study screenees from the point of contact through enrollment. During the screening, REDCap will assign each screenee a unique numeric study Screening ID, which is distinct from any personal identifiers. The screening identifier will be selected from a range dedicated to each site. Screenees are classified as "pending eligibility" while they are being evaluated per eligibility criteria. Subsequently, they are labeled as "eligible" or "ineligible", noting the reasons for ineligibility. Those meeting eligibility criteria are classified as "pending enrollment" while they are being processed and later as "enrolled" or "refused" noting the reasons for their refusal once they have made their decisions. At enrollment, each participant is randomly assigned to a study group and receives a Participant ID (PID), which is separate from the Screening ID. In addition to changes in the subject's recruitment status, the time of such changes is also recorded.

10.3.4 REDCap Randomization

At each episode, online checks ensure all episode eligibility criteria are met, and if so, access is provided to the REDCap randomization button. When pressed, the button implements an online program that accesses a pre-generated, encrypted, randomization schedule created by the CNMC Division of Biostatistics and Study Methodology to make the appropriate treatment assignment for that subject. This procedure will provide a balanced allocation of assignments to each treatment arm over the episodes. Once an assignment is made, to maintain the blind the person enrolling the subject will be notified simply that the site pharmacy is preparing the assigned medication. These procedures will ensure tamper-proof, blinded randomization as well as overall balance in assignment to treatment groups. The Site Pharmacy will simultaneously receive online or fax notification of the assignment and will be requested to prepare the appropriate dosing kit for pick-up or delivery to the floor (depending on site logistics).

10.4 Recruitment and Retention Monitoring

REDCap reports will monitor recruitment overall and by the site. It will warn of recruitment problems by plotting actual vs. expected screens, recruits, and enrollees. Should problems arise, REDCap can help diagnose the source of such problems, and identify possible remedies. It is also possible to compare the demographics of all those eligible to all enrollees. These monitoring reports will be forwarded on a monthly basis to study leadership and will be discussed at the weekly calls. Retention rates at each site will be evaluated on a monthly basis using REDCap reporting modules. Should a

site show an unexpectedly high loss to follow-up, causes of the problem will be reviewed, and possible changes to retention strategies will be evaluated.

10.5 Site Data and Study Responsibilities

Each site will:

- Submit protocol and any protocol amendments or modifications to site IRBs for approval
- Maintain regular communications with the Coordinating Center regarding IRB protocol approvals
- AEs or other study related issues
- Attend regular conference calls to discuss milestones and administrative issues
- Maintain study charts
- Recruit, consent and enroll study participants
- Conduct all study visits in accordance with the protocol and the Manual of Operating Procedures
- Receive, log and account for all study drug from the central pharmacy, and will dispense drug to the study participants and will return unused or expired drug to the central pharmacy
- Enter and verify all study data

10.6 CNMC Coordinating Center Responsibilities

In order to ensure that data are of the highest quality, special attention will be paid to project monitoring. Site performance monitoring will be done by the Project Manager. Monitoring of milestones and data quality will be done both in-house at the CNMC-CC and during on-site monitoring.

The internal component will consist of the CNMC-CC data manager performing a clinical review of the eCRFs. This process will supplement the range checks performed at the time of data entry by the sites and real-time review of adverse events and safety data. The internal component will also include tracking of regulatory documents in an ongoing fashion. If the internal processes indicate a problem at a site, the need for additional training or oversight will be assessed. A plan will be developed to address the needs/issues identified, including addressing the needs via remote methods (teleconferences or video conferences) in order to minimize the associated costs. If problems persist, the CNMC-CC will be updated.

The external component will involve the Project Manager visiting each site at least annually if the site has had an episode within that year. The first visit will be for site initiation. The next visit will occur approximately 12 months later. At each visit s/he will monitor the site's compliance with operational protocols, evaluate the site's data tracking and maintenance procedures, and review all safety and efficacy data points as well as to review all other data in the database against source patient records, in compliance with GCP. This visit provides an opportunity to resolve any outstanding queries and deal with potential challenges or problems related to protocol adherence, and participant recruitment and retention. After each site visit, a monitoring report will be prepared and copies sent to the Study File, the CNMC-CC, and the Executive Committee. A follow-up will be initiated to ensure that any concerns raised at a monitoring visit are resolved.

Additional reporting and site monitoring will be conducted remotely on a regular basis, including the following:

- Site reports that include simple tabulations of the number of admissions, screened patients, eligible patients, ineligible patients, and refusals, by month and cumulatively, as well as the observed and expected number of weeks of follow-up on study
- Subject retention and attrition reports

- Adverse event reports
- Pharmacy and study drug accounting activity reports
- Summaries of the paper-based data captured (e.g., number of forms completed, number of forms expected, and the number of missing pages or fields)
- Summaries of the electronic data captured (e.g., amount of data captured, amount of data expected, and the number of missing fields)
- DSMB reports

The Coordinating Center will also:

- Confirm current contact information for all site personnel and study teams
- Confirm that site delegation logs are up-to-date
- Review IRB approvals and continuing reviews
- Obtain documentation of study drug shipment and receipt and sample shipment and receipt
- Assign database IDs
- Oversee training for data entry, data management, and pharmacy randomization

10.7 Data Security

There are several layers of security that protect the (data) stored in REDCap. The first layer consists of network security, through firewalls, and is designed to limit access to the wide-area network (WAN) to specific computers and authorized users and to deny access to all others. The firewall blocks malware and prevents flooding. The second layer consists of database authentication. This limits access to the database to those individuals passing layer one who has an assigned database account and can enter the correct, suitably complex, the user-defined password for that account. The third layer consists of controlling a user's capabilities, what a user can see and do, based on the user's role in each investigation. Each user needing access to the data for a study must be included on the list of key staff for that specific study in the protocol and must be assigned a study role. If a user is not in the key staff list of the study, s/he will not be granted access to that study's data even if s/he has a valid password associated with another study. Key staff will only be allowed to perform those tasks that are dictated by the person's defined role in the study. For instance, a person who enters data will only be able to add records to specific data files; s/he cannot peruse or make changes to existing records in the database. Only those persons who have clinical responsibilities for the patient would be able to see the patient identifying information.

There are also two other components to security and they are monitoring and system administration. The network is closely monitored and all activity is logged. Both the real-time monitor and the logs are reviewed by eye and by the computer to identify suspicious activity so that it can be dealt with appropriately. Furthermore, all access to research data is audited and all activities are logged so that if a problem occurs it will be possible to identify who was responsible and how it happened. The system and database software is kept up-to-date with the latest security protections released for the operating system and for the database. These measures help us to keep ahead of the threats to security or to close loopholes before they can be more widely exploited. Finally, there is excellent physical security; access to the systems is keypad controlled, there is a backup generator to provide power to keep systems operating in the event of power failure, all computer rooms housing database and web servers are environmentally controlled and protected against flood and fire.

10.8 Data Acquisition and Entry

- Data collection for this study will be accomplished with online electronic case report forms.
- On-line forms will be developed that contain the requisite data fields.
- For each subject enrolled all study-related episodes of the enrollment, diagnostic, application, observation, termination and follow-up phases will be recorded in the CRF.

This CRF must be completed and signed by the investigator for every subject on whose behalf written informed consent was given. This also applies to subjects who fail to complete the study. If a subject is being withdrawn from the study by his/her parent(s)/legal guardian(s), the reason must be stated in the CRF, if the parent(s)/legal guardian(s) are willing to provide such reason. If a subject is withdrawn from the study because of a limiting AE, reasonable efforts should be made to clearly document the outcome.

- All subjects will be identified by their PID.
- Each investigator will be responsible for ensuring that the identification of his/her subjects is possible at any time.
- The investigator will be provided with CRF Completion Guidelines from the Coordinating Center.
- These guidelines will provide instructions on how to complete, correct, and archive final copies of the CRFs.
- Prior to the beginning of the study, the investigator must establish a site delegation log, a list of the individuals with the trial-related duties and of the persons authorized to make entries and data changes in the CRFs. These persons will be listed, including details on their function in the study, their full names, initials, and signatures, and the date of their assignment to (and, if applicable, their demission from) their respective duties on this study.

10.9 Data Editing

A number of data checks and procedures may be applied during data collection that either prevent errors or detect them with the requirement for later review and adjudication. No matter what the data source all data must pass through all predefined data checks and all potential errors must be reviewed and corrected or accepted with a comment using the Data Quality tool in REDCap. Any discrepancies are reported in a data exception feedback report for correction and are monitored to ensure that they are accepted or corrected. Predefined procedures and data checks include:

- Limiting the availability of electronic data entry forms to a defined list of enrollees indexed by assigned PID;
- Implementing validation and consistency checks before data are sent to the database to allow correction but logging of all uncorrected exceptions for required later review;
- Checking for and recording on each form the rate of missing data that can be used to set criteria for subsequent forms processing, e.g. to reject forms with high missing data rates from entering the database;
- Flagging out of range values; and
- Creating a context sensitive comment window that allows subsequently retrievable text to be linked to any form or data item, e.g., to explain an unusual data response.

Web-based, forms allow data to be viewed and edited. Quality assurance checks are applied during data entry and editing. In addition to maintaining a time-stamped audit trail of all logins and transactions, the system has the capability to be proactive, for example in requiring all unflagged data corrections be accompanied by a justification as well as to be adjudicated, reviewed, accepted or rejected, by a designee, e.g. a data manager at the Coordinating Center. Together these options and procedures promote a high degree of completeness, and accuracy of study data.

10.10 Data Lock and Freeze

Study data may be preserved by applying electronic security procedures such as ‘locking’ and ‘freezing’. Locking data (via either partial or final lock) implies a ‘soft lock’ by which approved users can make updates to the database. Freezing data disallows users to update the data and it only allows browsing access. The terms partial lock, final lock, and database freeze are explained below.

Partial Lock:

- May be used to lock subsets of data (by site, by the participant, by demographic strata).
- Is flexible and may be used to:
  - Lock an entire set of participant records at a site;
  - Lock a single participant record; or
  - Lock a single form.
- Only allows a designated user with update privileges to make updates to the locked data.
- May be applied if data are not readily available to the sites during study completion (e.g., biomarker results produced at the end of the study).

Final Lock:

- Locks all data entered for a study.
- Only allows a designated user with update privileges to make updates to the locked data.
- May require entered data to exist in the locked state until the final manuscript is submitted to the Coordinating Center.

Database Freeze:

- Once the CNMC-CC has confirmed the data are satisfactory, the database will be frozen.

10.11 Data Entry Training

Data entry training will be conducted online using the REDCap database. Study coordinators will be trained and certified in the on-line use of the REDCap database. Training sessions will be scheduled and organized by the Coordinating Center.

10.12 Data Quality Assurance

Data quality is assessed at the data entry point using intelligent review and controls during on-line data entry or edits via study forms. Quality assurance (QA) reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Equally important quality assurance measures are the internal checks for reasonableness and consistency that will be implemented via simple tabulations and cross-tabulations that should reveal any remaining data quality issues.

11 PUBLICATIONS AND OTHER RIGHTS

Since this project is conducted in UCDC sites, we will subscribe to the data and research resources sharing plan that have been developed and approved by the Rare Disease Clinical Research Network. According to this plan, datasets will be made available to the scientific community after publication(s) of planned analyses (as set forth in the protocol) of the clinical trial results or no later than 3 years after the final visit of the last participant to a clinical trial site, whichever comes first. Data obtained in this trial will be presented at national and international meetings and will be published in the medical literature with no delays. The investigators are not under any agreement with the provider of the drug to limit or delay the publication of data from this trial. Results from this study will also be posted on www.clinicaltrials.gov.

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