# Rare Diseases Clinical Research Network
# Urea Cycle Disorders Consortium

# Manipulating the Gut Microbiome in Urea Cycle Disorders



**Nicholas Ah Mew**
**Children's National Medical Center**
**202-476-5863**
**nahmew@childrensnational.org**

## Participating Institutions/Investigators Table

**Principal Investigator: Nicholas Ah Mew, MD**

Contact: Lillian Assatourian
Institution: Children's National Medical Center
Address: 111 Michigan Avenue, NW
Washington, DC
20010
Phone: 202-476-6137 (Lillian)
202-476-5863 (Dr. Ah Mew)
Fax: 202-476-2390
Email: lassatouri@childrensnational.org (Lillian)
nahmew@childrensnational.org (Dr. Ah Mew)

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**Co-Principal Investigator: Marshall Summar, MD**

Institution: Children's National Medical Center
Address: 111 Michigan Avenue, NW
Washington, DC
20010
Phone: 202-476-5291
Fax: 202-476-5566
Email: msummar@childrensnational.org

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**Data Management and Coordinating Center Principal Investigator: Jeffery Krischer, Ph.D.**

Contact: Amy Holbert
Institution/ Department: Data Management and Coordinating Center (DMCC); Pediatrics Epidemiology Center; University of South Florida
Address: 3650 Spectrum Blvd; Suite 100; Tampa, FL 33612
Phone: (813) 396-9567
Fax: (813) 910-5967
Email: amy.holbert@epi.usf.edu

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# 1. Protocol Synopsis

Interventional Synopsis

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|---|---|
| **Protocol Number:** | 5115 |
| **Protocol Title:** | Manipulating the Gut Microbiome in Urea Cycle Disorders |
| **Study Chair:** | Nicholas Ah Mew, MD |
| **Study Co-Chair:** | Marshall Summar, MD |
| **Statistician:** | Robert McCarter, ScD |
| **Consortium:** | Urea Cycle Disorders Consortium |
| **Participating Sites:** | Children's National Medical Center |
| **Activation Date:** | Pending |
| **Current Status:** | Pre-enrollment |
| **Sample Size:** | Group 1: 8 healthy adult subjects<br>Group 2: 8 adult subjects with urea cycle disorders |
| **Target Enrollment Period:** | Group 1: 1 year<br>Group 2: 2 years |
| **Study Phase** | I/II |
| **Study Design:** | Open-label, cross-over |
| **Primary Study Objective:** | The objective is to determine if acetohydroxamic acid (AHA) can prevent hydrolysis of urea by inhibiting the bacterial urease of gut flora of both healthy control adults as well as adults with urea cycle disorders |
| **Secondary Study Objective(s):** | Determine if the proposed dose of acetohydroxamic acid is safe |
| **Study Population and Main Eligibility/ Exclusion Criteria:** | A subject may be included in the study if all of the following criteria are met:<br><br>For Group 1 (healthy adults):<br>- Ages 18-60 years<br>- Compliant with receiving medications orally and intravenously<br>- Compliant with providing blood and urine samples<br><br>For Group 2 (adult UCD patients):<br>- Ages 18-60 years<br>- Compliant with receiving medications orally and intravenously<br>- Compliant with providing blood and urine samples<br>- Established diagnosis of CPSD, OTCD, ASSD or ASLD as follows:<br>o Diagnosis of CPS I deficiency, defined as decreased (less than 20 % of control) CPS I enzyme activity in liver or an identified pathogenic mutation<br>o Diagnosis of OTC deficiency, defined as the identification of a pathogenic mutation, linkage analysis in an affected family, less than 20% of control of OTC activity in the liver, or elevated urinary orotate (greater than 20 uM/mM) in a random sample or following allopurinol loading with absence of argininosuccinic acid<br>o Diagnosis of AS deficiency (Citrullinemia), defined as a greater than or equal to 10-fold elevation of citrulline in plasma, decreased AS enzyme activity in cultured skin fibroblasts or other appropriate tissue, or identification of a pathogenic mutation in the AS gene<br>o Diagnosis of AL deficiency (Argininosuccinic Aciduria, |

| | |
|---|---|
| | ASA), defined as the presence of argininosuccinic acid in the blood or urine, decreased AL enzyme activity in cultured skin fibroblasts or other appropriate tissue, or identification of a pathogenic mutation in the AL gene<br><br>A subject may not be included in the study if any one of the following criteria is met:<br><br>For both Group 1 and Group 2:<br>- Current or prior *Helicobacter pylori* infection<br>- Chronic gastrointestinal illness (e.g., inflammatory bowel disease)<br>- Chronic renal failure<br>- Taking probiotic medications within a week of study start date<br>- Currently pregnant or lactating. Documentation of a negative pregnancy test within a week prior to testing is required, unless pre-menarchal or menopausal, experiencing menses that week, or other circumstances which preclude pregnancy (e.g. hysterectomy).<br>- Presence of acute infection at the time of inclusion<br>- Participation in any other clinical interventional trial or received experimental medication within the last 30 days<br>- 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 |
| **Treatment** | |
| **Agent-** | Acetohydroxamic Acid (AHA, Lithostat) |
| **Dosage, schedule, route of administration-** | Single dose, 60 mg/kg PO |
| **Safety Issues-** | Bone marrow depression (leukopenia, anemia, and thrombocytopenia) has occurred in experimental animals receiving large doses of AHA. However, only a single dose will be administered in this study, and is not likely to result in this adverse effect. |
| **Primary Outcome Measures:** | Difference in the time-averaged level of blood $^{13}CO_2$ after a single bolus of intravenous [$^{13}$C]-Urea, measured on two occasions: once at baseline, and once 2-hr after an oral bolus of AHA |
| **Secondary Outcome Measures:** | Differences in blood [$^{13}$C]-Urea, Urea, Ammonia, Glutamine |
| **Safety Variables** | Hemoglobin, White blood count, platelets, AST, ALT, Bilirubin, Creatinine |
| **Statistical Considerations (sample size and analysis plan):** | Longitudinal linear regression models will assess and compare time averaged mean changes in blood $^{13}CO_2$, and blood levels of [$^{13}$C]-urea, ammonia and glutamine.<br>A sample size of 8 crossover participants provides 90% power to detect a 20% relative difference in the time-averaged level of $^{13}CO_2$ in treatment with AHA versus non-AHA under a 2-tailed type I error of 0.05. |
| **Sponsors (federal, state, foundation and industry support):** | Rare Disorders Clinical Research Network, National Institutes of Health, National Institute of Child and Human Development<br>Mission Pharmacal |

## *1.1 Overview*

This project will study the efficacy and safety of the pharmacologic blockade of urease in the nitrogen salvage pathway of intestinal microbes in subjects with partial urea cycle disorders. Additional trapping of ammonia as excretable urea may result in improved nitrogen excretion and reduced ammonia levels.

Urea cycle disorders (UCDs) are a group of disorders resulting from a complete or partial deficiency of one of the 6 enzymes or 2 transporters that comprise the urea cycle, the essential biochemical pathway which converts toxic ammonia into urea. These disorders have as a common feature, a reduced or complete inability to convert ammonia into urea, thereby resulting in high ammonia levels, or hyperammonemia. If untreated, hyperammonemia may result acutely in lethargy and coma, and chronically in intellectual disability. Current treatment for hyperammonemia is suboptimal, thus the search for new treatments is critical.

The urease inhibitor, acetohydroxamic acid (AHA, Lithostat®, Mission Pharmacal), is an FDA-approved product for another indication- the treatment of struvite nephrolithiasis in chronic urinary tract infections in both adults and children[1,2].

It is known that many urea-splitting bacteria also exist in the gut, and that in healthy individuals, approximately 15-30% of blood urea is degraded via gut bacteria into ammonia[3], which returns to the liver via the portal vein, only to be recycled into urea. This percentage of degraded urea may even be greater in patients with urea cycle disorders, who are on a low protein-diet[4] and whose gastrointestinal contents thus likely have lower nitrogen content, promoting bacterial recycling of nitrogen from available urea. Additionally, urea hydrolysis has been shown to be greatest in infants[5], precisely the age at which hyperammonemic episodes are the most frequent in UCD patients.

We intend to study if AHA can inhibit gut bacteria degradation of urea, thereby reducing the quantity of ammonia returning to the liver. We intend to investigate this by studying subjects on two occasions at least 3 days apart:

On the first occasion, subjects will receive an intravenous dose of $^{13}$C-urea. Following the intravenous bolus of $^{13}$C-urea, over the subsequent 4 hours, we will collect several sequential measurements of blood and urine biomarkers from an IV catheter placed in the other arm. The intent is to obtain baseline $^{13}CO_2$ kinetics in the subject.

On the second occasion, subjects will first receive an oral dose of AHA approximately 1 hour prior to the intravenous $^{13}$C-urea dose. Similar sequential measurements of blood and urine biomarkers will be performed. The intent is to observe a reduction in $^{13}CO_2$ when AHA is administered.

We intend to initially study a cohort of unaffected adult subjects. If successful, we will study adults with partial urea cycle disorders.

This study will be conducted in the Clinical Research Center (CRC) of the Clinical and Translational Research Institute (CTSI) of Children's National Medical Center (CNMC).

### 2. Specific Aims (Hypothesis and Objectives)

The specific aim is to investigate whether a single oral dose of AHA can inhibit intestinal bacterial urease in healthy subjects as well as in patients with partial UCDs, and whether it is safe. We propose a study with two components: the first without AHA, and the second after a single dose of AHA, 60mg/kg/d. We will evaluate whether AHA will inhibit bacterial urease, by measuring $^{13}$C-labeled urea in blood and urine samples, following an intravenous load of $^{13}$C-urea. Blood and urine $^{13}$C-urea, plasma ammonia, plasma glutamine and blood urea will also be measured.

## 3. Background

It is well known that gut commensal bacteria modulate nutrient absorption, mucosal barrier fortification, xenobiotic metabolism, angiogenesis, and intestinal maturation. The gut microbiome also interacts strongly with the host in determining metabolic phenotypes. Microbial metabolism and co-metabolism has been described for various nutrients and metabolic products including vitamins, lipids, fatty acids, organic acids and amino acids. Gut microbial metabolism also affects urea cycle function more directly by contributing to portal ammonia via exogenous and endogenous pathways that include: 1) microbial breakdown of dietary protein and 2) the enzymatic hydrolysis of urea by urease to produce ammonia and carbonic acid. A number of gut dwelling bacteria produce urease including: *Proteus* species, *Helicobacter pylori*, and *Klebsiella*. Urea is transported from the blood to the intestinal lumen where it is hydrolyzed to ammonia by microbial urease. Studies in healthy human subjects indicate that up to 30% of liver-synthesized urea may enter the intestine where it can be hydrolyzed by microbial urease to produce ammonia. It is estimated that 15-30% of the nitrogen derived from microbial urea hydrolysis is incorporated back into urea by the liver. This process of recycling of urea is known as urea nitrogen salvage (UNS). UNS may be dependent on protein intake as well as microbial populations. For example, subjects, such as UCD patients, on a low protein diet showed greater UNS compared to controls. Secondary effects of gut bacteria are evident in urea cycle enzyme activity. Germ-free mice have significantly lower Ornithine Transcarbamylase (OTC) activity, and decreased urea cycle intermediates ornithine and arginine. In addition, liver slices from germ-free mice synthesize less urea compared to their conventional controls. Overall gut microbes contribute up to 50% or more of the portal blood ammonia pool.

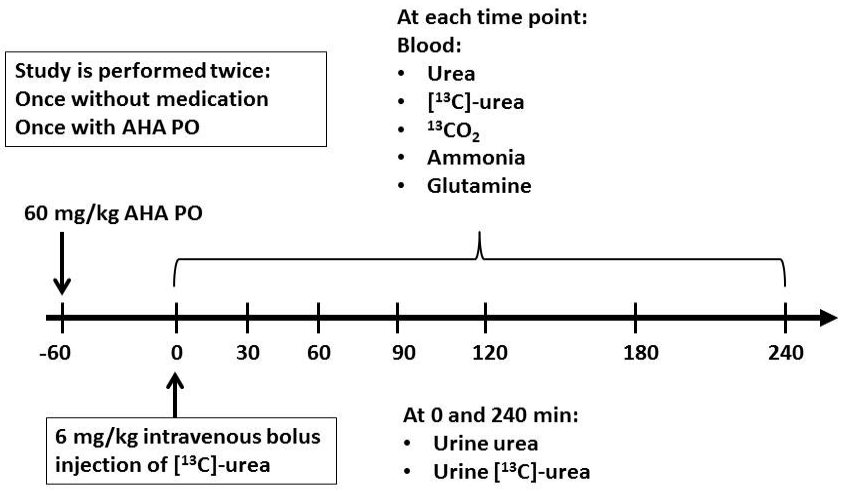
Currently, the only approved clinical use of the drug for blockade of urease in bladder/renal proteus infections associated with renal struvite calculi (Lithostat, Mission Pharmacal, San Antonio, TX). The prescribing information indicates a recommended AHA dosing of 10-15 mg/kg/d (and 10 mg/kg/d in children), administered at 6-8 hour intervals when the stomach is empty. Children have tolerated a dose of 10 mg/kg/ satisfactorily for periods up to one year.

Enteral AHA has previously been administered to patients with hyperammonemia due to hepatic encephalopathy in two published reports[6,7] A single dose of oral 750 mg in adult patients was insufficient to result in any change in ammonia after 90 minutes in cirrhotic subjects free of *H. Pylori*[7]. However, in encephalopathy cirrhotic subjects administered AHA 20-60 mg/kg via naso-gastric tube, a statistically significant decreased in blood ammonia was observed[6], with the effect most striking in subjects receiving a dose of 40-60 mg/kg/d.

This proposal would introduce a novel therapeutic approach for patients with UCD using a low-toxicity compound that may be particularly effective in patients with partial UCDs. There are two potential ways this approach may lower blood ammonia or excess nitrogen. The first is through blockage of new ammonia formation from urease. The second path may be increased consumption of residual dietary nitrogen by the gut flora in the absence of available nitrogen released through urease. Both effects may decrease the amount of nitrogen entering the portal vein. This new therapy will not interfere with other therapies, but would be complementary to them and may potentiate the nitrogen trapping effects of arginine and citrulline by preventing the degradation of the urea produced by their administration.

# 4. Study Design and Methods

This pilot crossover study will first evaluate the impact of AHA versus non-AHA primarily on $^{13}CO_2$ and secondarily on other biomarkers in healthy adults before applying the same crossover design to UCD subjects. A randomization table will determine if AHA or non-AHA will be performed first.
The figure below illustrates our study design:




Subjects will be required to fast for a minimum of 4 hours prior to beginning the study. Food consumption will not be permitted during the study; however subjects will be allowed to drink water or pedialyte throughout the study. Subjects will be observed and

various biomarkers will be collected throughout the 4-6 hours that they are studied. 5 mL of blood will be collected at each of the 7 time points shown above. Administration of [$^{13}$C]-urea will be completed through an intravenous catheter. Blood draws will be done through the placement of a Heplock intravenous catheter on the opposite arm from where [$^{13}$C]-urea was first administered. If there is not adequate vascular access, subjects will have a PICC (peripherally inserted central catheter) line placed instead.

Recruitment and enrollment: See Section 4.3, 8.4-5
Follow-up: Subjects will be followed throughout the duration of their participation.
Data collection: See Sections 5.9, 6, 7

## *4.1 Inclusion Criteria*

For healthy adults:
- Ages 18-60 years
- Compliant with receiving medications orally and intravenously
- Compliant with providing blood and urine samples

For UCD subjects:
- Ages 18-60 years
- Compliant with receiving medications orally and intravenously
- Compliant with providing blood and urine samples
- Participants should be diagnosed with one of the following 4 urea cycle disorders:
    - Carbamylphosphate synthetase I deficiency - decreased (less than 20 % of control) CPS I enzyme activity in liver or an identified pathogenic mutation
    - Ornithine Transcarbamylase deficiency- identification of a pathogenic mutation, linkage analysis in an affected family, less than 20% of control of OTC activity in the liver, or elevated urinary orotate (greater than 20 μM/mM) following allopurinol loading with absence of argininosuccinic acid
    - Argininosuccinic acid synthetase deficiency (Citrullinemia) - greater than or equal to 10-fold elevation of citrulline in plasma, decreased AS enzyme activity in cultured skin fibroblasts or other appropriate tissue, or identification of a pathogenic mutation in the AS gene
    - Argininosuccinic acid lyase deficiency - presence of argininosuccinic acid in the blood or urine, decreased AL enzyme activity in cultured skin fibroblasts or other appropriate tissue, or identification of a pathogenic mutation in the AL gene

## *4.2 Exclusion Criteria*

For healthy and UCD Patients:
- Current or prior Helicobacter Pylori infection
- Chronic gastrointestinal illness (e.g., inflammatory bowel disease)
- Chronic renal failure
- Taking probiotic medications within the week prior to the study

- Pregnant or lactating. Documentation of a negative pregnancy test within a week prior to testing is required, unless pre-menarchal or menopausal, experiencing menses that week, or other circumstances which preclude pregnancy (e.g. hysterectomy).
- Presence of acute infection at the time of inclusion
- Participation in any other clinical interventional trial or received experimental medication within the last 30 days
- 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

## *4.3 Recruitment of Participants*

The institution conducting this study, Children's National Medical Center (CNMC), is a nation-wide referral center for patients with urea cycle disorders and other inborn errors of metabolism. Additionally, CNMC is the lead site of the Urea Cycle Disorders Consortium (UCDC), part of the Rare Diseases Clinical Research Network (RDCRN). UCDC members will refer potential participants to this trial. In addition, the RDCRN-UCDC has established a contact registry that informs registrants of clinical trials. We are in close contact with the National Urea Cycle Disorders Foundation (NUCDF), a lay organization dedicated to the promotion of treatment and research to improve the health of patients with urea cycle disorders. This organization helps with patient recruitment for these studies. Our professional relationship with each of these families is very strong and trusting. We have enrolled some of these patients previously in research studies. We anticipate no problem in accommodating the stipulated patient cohort (maximum of 8 individuals total for the current project. Thus, we hope to be able to recruit a sufficient number of participants (see power calculation and statistical considerations) for this trial both locally and nationally.

The Washington DC area is comprised of a large diverse metropolitan population. We do not anticipate difficulty with recruitment of 8 healthy adult control subjects.

## *4.4 Retention strategies*

As the study involves only 2 days of participation, we anticipate retention to be near 100%.

## *4.5 Data Elements*

In addition to the elements described in Table 1, the collected data elements will include age, sex, race, and ethnicity, as required by the NIH, as well as UCD diagnosis and method of diagnosis, if relevant.

### *4.6 Schedule of Events*

Subjects will be studied twice: once without and once with a single dose of acetohydroxamic acid, with the two study days at least 3-days apart.

**Table 1. Laboratory investigations (each study day)**

| | | Time point (min) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | **Baseline** | **0** | **30** | **60** | **90** | **120** | **180** | **240** |
| **Blood [$^{13}$C]-urea** | | **X** | **X** | **X** | **X** | **X** | **X** | **X** |
| **Blood urea** | | **X** | **X** | **X** | **X** | **X** | **X** | **X** |
| **Plasma glutamine** | | **X** | **X** | **X** | **X** | **X** | **X** | **X** |
| **Ammonia** | | **X** | **X** | **X** | **X** | **X** | **X** | **X** |
| **Blood $^{13}CO_2$** | | **X** | **X** | **X** | **X** | **X** | **X** | **X** |
| | | | | | | | | |
| **Urine [$^{13}$C]-urea** | | **X** | | | | | | **X** |
| **Urine urea** | | **X** | | | | | | **X** |
| | | | | | | | | |
| ***Safety labs:*** | | | | | | | | |
| **Hemoglobin** | **X** | | | | | | | **X** |
| **White blood count** | **X** | | | | | | | **X** |
| **Platelets** | **X** | | | | | | | **X** |
| | | | | | | | | |
| **AST** | **X** | | | | | | | **X** |
| **ALT** | **X** | | | | | | | **X** |
| **Bilirubin** | **X** | | | | | | | **X** |
| **Creatinine** | **X** | | | | | | | **X** |

'Baseline' indicates at a time 0 on days when AHA is not administered or at a time prior to AHA administration on the days where AHA is administered. In addition, some of the blood and urine samples will be saved at the Clinical and Translational Science Institute at CNMC for up to 3 years after the conclusion of the study to test for the metabolism of AHA, after which the samples will be discarded.

# 5. Safety Monitoring

## *5.0 Data and Safety Monitoring Plan*

The study protocol will be reviewed and approved by the National Institutes of Health (NIH) before submission to individual center IRBs for approval. Participant enrollment may only begin with IRB approved consent forms.

Adverse events are to be collected and reported to the Study Chair following each completed study, or in the case of a laboratory abnormality, when the abnormal laboratory result is identified. The Study Chair will also review the study adverse event data after completed enrollment of the 8 healthy subjects and 8 subjects with UCD.

The study protocol will be reviewed and approved by the Data Safety and Monitoring Board (DSMB) of the Rare Disorders Clinical Research Consortium of the National Institutes of Health (NIH) before submission to individual center IRB's for approval. Participant enrollment may only begin with IRB approved consent forms.

This is an interventional pilot study that meets the federal definition of more than minimal risk.

## *5.1 Study Oversight*

The Study Chair has primary oversight responsibility of this clinical trial. The NIH appointed Data Safety Monitoring Board (DSMB) has oversight responsibility of the Data Safety Monitoring Plan (DSMP) for this clinical trial. The DSMB will review accrual, patterns and frequencies of all adverse events, protocol compliance every 6 months. The DSMB makes recommendations to the NIH regarding the continuation status of the protocol.

Each site's Primary Investigator and their research team (co-Investigators, research nurses, clinical trial coordinators, and data managers) are responsible for identifying adverse events. Aggregate report- detailed by severity, attribution (expected or unexpected), and relationship to the study drug/study procedures – will be available from the DMCC for site review. Adverse events will be reviewed by the research team at the end of the study. A separate report detailing protocol compliance will also be available from the DMCC for site review on a monthly basis. The research team will then evaluate whether the protocol or informed consent document requires revision based on the reports.

## *5.2 Definitions and Standards*

The Rare Diseases Clinical Research Network defines an adverse event as: "…an unfavorable and unintended sign, symptom or disease associated with a participant's participation in a Rare Diseases Clinical Research Network study."

Serious adverse events include those events that: "result in death; are life-threatening; require inpatient hospitalization or prolongation of existing hospitalization; create persistent or significant disability/incapacity, or a congenital anomaly/birth defects."

An unexpected adverse event is defined as any adverse experience…the specificity or severity of which is not consistent with the risks of information described in the protocol. 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

All reported adverse events will be classified using the Common Terminology Criteria for Adverse Events (CTCAE), developed and maintained by CTEP at National Cancer Institute.

## *5.3 Expected/Known Risks/Discomforts/Adverse Events*

A definition of expected adverse events associated with study intervention and procedures are outlined below.

Study Drug/Intervention:
AHA:
Acetohydroxamic acid has been an FDA approved medication for the treatment of struvite nephrolithiasis for over 25 years. According to the prescribing information, about 150 patients, including children, have been treated, most for periods of more than 1 year.

Well controlled, long-term animal studies that identify the carcinogenic potential of AHA treatment have not been conducted. Acetamide, a metabolite of AHA, has been shown to cause hepatocellular carcinoma in rats at oral doses 1,500 times the human dose. AHA is cytotoxic and was positive for mutagenicity in the Ames test.

The pharmacokinetics of AHA in man are well known: 36-65% of the oral dose of AHA is excreted unchanged in the urine. The plasma half-life of AHA is approximately 5-10 hours in subjects with normal renal function.

According to the AHA (Lithostat) prescribing information, adverse reactions have occurred in up to thirty percent (30%) of the patients receiving AHA. In some instances, the reactions were symptomatic: in others, only changes in laboratory parameters were noted. Adverse reactions seem to be more prevalent in patients with pre-existing thrombophlebitis and/or in patients with advanced degrees of renal insufficiency.

The following reactions have been reported:

- 30% - Mild headaches during the first 48 hours of treatment, responsive to standard therapy
- Tremulousness and nervousness
- 20% - Gastrointestinal symptoms (nausea, vomiting, anorexia)
- 15% - laboratory findings characteristic of a hemolytic anemia
- Non-pruritic, macular rash on the upper extremities and face, usually concomitantly with alcoholic beverages, with spontaneous resolution
- Superficial phlebitis

Acute toxicity has not occurred. Milder overdosages resulting in hemolysis have occurred in an occasional patient with reduced renal function after several weeks or months of continuous treatment.

There is a small risk that the blood ammonia level will rise during the study. This unlikely possibility, would mostly likely result from toxicity of the AHA to the liver.

13C Urea:
Urea, and by extension, its stable isotope [$^{13}$C]-urea, is a substance regarded by the FDA to be generally regarded as safe (GRAS). Administration of a small amount of stable isotopic urea should not pose a risk to subjects with urea cycle disorders, as the amount of ammonia generated by its hemolysis would represent only a small fraction of the total body ammonia.

There is a potential risk of contamination of the drug or isotope used in the study. However, a sterile and pyrogen-free preparation of the isotope is used, and the drug is manufactured via Current Good Manufacturing Practices (CGMP).

Study Procedures:
Venipuncture: The placement of the two Heplock intravenous catheters for blood sampling and [$^{13}$C]-urea administration could result in a bruise or infection at the site of insertion. The vein in which the needle has been inserted to draw blood may become sore and red. A temporary "black and blue mark" may develop, and rarely fainting may occur. If a PICC line is inserted in the arm (by an interventional radiologist) intravenous sedation is given and involves risks of apnea or aspiration. There is also risk of vessel perforation and infection of the central line catheter.

There is a risk of loss of confidentiality, which could affect insurability and result in stigmatization.

## *5.4 Reporting Timeline*

- Within **24 hours** (of learning of the event), investigators must report any reportable Serious Adverse Event (SAE) that:
  - Is considered life-threatening/disabling or results in death of subject

  -OR-
  - Is Unexpected/Unanticipated
- Investigators must report all other reportable SAEs within **5 working days** (of learning of the event).
- All other (suspected) reportable AEs must be reported to the RDCRN within **20 working days** of the notification of the event or of the site becoming aware of the event.

Local institutional reporting requirements to IRBs, any GCRC oversight committee and the FDA, if appropriate, remain the responsibility of the treating physician and the Study Chair.

## *5.5 RDCRN Adverse Event Data Management System (AEDAMS)*

Upon entry of a serious adverse event, the DMCC created Adverse Event Data Management System (AEDAMS) will immediately notify the Study Chair, site PIs, the Medical Review Officer, and any additional agencies of any reported adverse events via email.

Serious adverse events: The NIH appointed Medical Review Officer (MRO) determines causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the adverse event. The MRO may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event. A back-up notification system is in place so that any delays in review by the MRO beyond a specified period of time are forwarded to a secondary reviewer. The Adverse Event Data Management System (AEDAMS) maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study.

Non-serious expected adverse events: Except those listed above as immediately reportable, non-serious expected adverse events that are reported to or observed by the investigator or a member of his research team will be submitted to the DMCC in a timely fashion (within 20 working days). The events will be presented in tabular form and given to the MRO and RDCRN DSMB on a bi-annual basis. Local site investigators are also required to fulfill all reporting requirements of their local institutions.

The DMCC will post aggregate reports of all adverse events (serious/not serious and expected, unexpected) for site investigators and IRBs.

## *5.6 Study Discontinuation*

The NIH, RDCRN DSMB and local IRB's (at their local site) have the authority to stop or suspend this trial at any time. This study may be suspended or closed if:

- Accrual has been met
- The study objectives have been met
- The Study Chair / Study Investigators believe it is not safe for the study to continue
- The RDCRN DSMB suspends or closes the trial
- The NIH suspends or closes the trial
- The FDA suspends or closes the trial

## *5.7 Subject Discontinuation*

An intent-to-treat approach will be used. All data acquired prior to termination for the reasons outlined below will be included in the primary analysis unless patient withdraws

consent. Every effort will be made to conduct a final study visit with the participant and participants will be followed clinically until, if applicable, all adverse events resolve.

- Withdrawal by the participant
- Withdrawal by the investigator
- Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease (e.g.-mental status change, large pleural effusion).

### *5.8 Data Quality and Monitoring Measures*

As much as possible data quality is assessed at the data entry point using intelligent on-line data entry via visual basic designed screen forms. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. 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. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency.

- Data Monitoring: The RDCRN DMCC identifies missing or unclear data and generates a data query to the consortium administrator contact.
- Data Delinquency Tracking: The Data Management and Coordinating Center will monitor data delinquency on an ongoing basis.

## 6. Statistical Considerations

Linear longitudinal regression models will be developed to estimate the change in the time-averaged levels of blood $^{13}CO_2$ (primary endpoint), as well as blood levels of [$^{13}$C]-urea, ammonia and glutamine after AHA versus after non-AHA. When we evaluate the effect of AHA on each metabolite, the model will control for each metabolite's pretreatment level and will adjust variance estimates to account for the correlation between measurements on the same person as well as to take account of the random effect on the intercept. We will consider a relative AHA versus non-AHA difference in the time-averaged level of $^{13}CO_2$ of at least 20% as a signal that AHA can effectively reduce the impact of gut bacteria on urease activity. The presence of a clear effectiveness signal in healthy adults will be required before the crossover study can be implemented in adults with UCD.

Pilot data indicates that the expected level $^{13}CO_2$ in the absence of AHA treatment is 0.041% with a conservative precision of +/- 0.0075%. Therefore, using the sampsi procedure in Stata 12, a sample size of 8 crossover participants provides 90% power in 2-tailed testing at an alpha of 0.05 to detect a 20% relative (0.041% versus 0.033%)

difference in the time-averaged level of $^{13}CO_2$ on AHA versus non-AHA in either healthy or UCD affected adults.

# 7. Data Management

All study data will be collected via systems created in collaboration with the RDCRN Data Management and Coordinating Center and will comply with all applicable guidelines regarding patient confidentiality and data integrity. Data will be entered online, and submitted to the DMCC-managed UCDC RDCRN portal.

Blood and urine samples are to be collected on each study day and sent to the Children's Hospital of Philadelphia for stable-isotope analysis of $^{13}CO_2$ and $^{13}$C-urea. As the number of enrollees is small, samples will be tracked via courier and email communication between CNMC and CHOP.

Samples may be kept for up to 3 years after the completion of the study, after which they will be discarded.

## *7.1 Registration*

Registration of participants on this protocol will employ an interactive data system in which the clinical site will attest to the participant's eligibility as per protocol criteria and obtain appropriate informed consent. IRB approval for the protocol must be on file at the DMCC before accrual can occur from the clinical site.

The DMCC will use a system of coded identifiers to protect participant confidentiality and safety. Each participant enrolled will be assigned a local identifier by the enrollment site. This number can be a combination of the site identifier (location code) and a serial accession number. Only the registering site will have access to the linkage between this number and the personal identifier of the subject. When the participant is registered to participate in the study, using the DMCC provided web-based registration system, the system will assign a participant ID number. Thus each participant will have two codes: the local one that can be used by the registering site to obtain personal identifiers and a second code assigned by the DMCC. For all data transfers to the DMCC both numbers will be required to uniquely identify the subject. In this fashion, it is possible to protect against data keying errors, digit transposition or other mistakes when identifying a participant for data entry since the numbers should match to properly identify the participant. In this fashion, no personal identifiers would be accessible to the DMCC.

## *7.2 Data Entry*

Data collection for this study will be accomplished with online electronic case report forms. Using encrypted communication links, on-line forms will be developed that contain the requisite data fields.

## *7.3 Data Quality Control*

As much as possible data quality is assessed at the data entry point. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. 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. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency. In addition to those described above, we propose to build these checks into the initial tables and cross tabulations that should reveal any remaining data quality issues.

## *7.4 Laboratory data flow*

This study intends to collect a small number of samples on a small number of subjects. Some samples will be shipped via courier to Children's Hospital of Philadelphia. Given the trivial number of samples exchanged, online courier tracking and email exchange will be sufficient to ensure sending and receipt of samples.

## *7.5 Study Records Retention*

Study records will be maintained for at least three years from the date that the grant federal financial report (FFR) is submitted to the NIH.

## *7.6 Protocol Deviations*

A protocol deviation is any noncompliance with the clinical study protocol, Good Clinical Practice, or Manual of Procedures requirements. The noncompliance may be on the part of the subject, the investigator, or study staff. As a result of deviations, corrective actions are to be developed by the study staff and implemented promptly.

All deviations from the protocol must be addressed in study subject source documents and promptly reported to the DMCC, Study Chair, and the local IRB, according to their requirements.

# 8. Human Subjects

## *8.1 GCP Statement*

This clinical trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with Good Clinical Practice and all applicable regulatory requirements.

## 8.2 Benefits

### *8.2.1 Potential Benefits of the Proposed Research to Subjects and Others*

Subjects will be compensated for their participation in the study, and will receive $500 for completion of the study ($250 per day).

Additionally, if the proposed studies show that the use of AHA is effective in reducing hyperammonemia and increasing urea excretion in patients with urea cycle disorders, these patients can be treated for their deficiency long-term with the use of this new specific therapeutic agent pending regulatory approval. Thus, there can be an important direct benefit for these participants. If the proposed study and the underlying hypothesis are correct this study could improve the medical condition of some or all of the patients also prevent life-threatening events in those who respond. At this stage, the patients will have the option to be treated under a different specific IND obtained by their local physician or enroll in a long-term study if one is available. Thus, this significant potential benefit balances the relatively low risks of the study.

### *8.2.2 Importance of the Knowledge to Be Gained*

The study will provide the first reliable information of whether AHA could potentially reduce the hyperammonemia associated with urea cycle disorders. Such knowledge could provide an additional treatment for hyperammonemia in these conditions. In addition, the information gained could also be relevant to better understanding the regulation of the urea cycle which may impact also treatment of hyperammonemia due to liver disease.

## *8.3 Recruitment*

Children's National is a nation-wide referral center for patients with urea cycle disorders, and the lead site for the Urea Cycle Disorders Consortium. In addition, to recruiting patients with urea cycle disorders, our recently funded RDCRC-UCD has a centralized registry, where patients are invited to enroll in clinical trials. We are in close contact with the National Urea Cycle Disorders Foundation, a lay organization dedicated to the promotion of treatment and research to improve the health of patients with urea cycle disorders. The NUCDF will help with patient recruitment for these studies.

*Inclusion of women and minorities*

The urea cycle disorders studied in this trial involve both genders equally and all ethnic groups are affected to the best of our knowledge. Thus, there will not be exclusion of either gender or any ethnic group. The investigators of this study plan to make a special effort to enroll subjects from various ethnic groups in proportions consistent with their proportion in the general populations. The Washington D.C. metropolitan areas have large minority groups reflected also by their proportions in the patients seen at Children's National. We believe that the study will have adequate representation by women and minority groups.

The cohort of unaffected control subjects will be adults only, as participation in this trial will not confer any direct benefit to unaffected children.

## *8.4 Written Informed Consent*

Written informed consent will be obtained from each participant before any study-specific procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained. The participant's willingness to participate in the study will be documented in writing in a consent form, which will be signed by the participant with the date of that signature indicated. The investigator will keep the original consent forms and signed copies will be given to the participants. It will also be explained to the participants that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. Written and/or oral information about the study in a language understandable by the participant will be given to all participants.

## *8.5 Process of Consent*

Healthy adult control subjects will be recruited from the Washington DC metropolitan area. The study may be advertised on the campus of some local universities.

Participants with UCDC will be recruited from the metabolism clinics of Children's National, or through the Urea Cycle Disorders Consortium. Alternatively, patients will be notified of the study by a collaborating lay organization, the National Urea Cycle Disorders Foundation.

Informed consent will be obtained by one of the investigators of the study before the study can begin. The participants will receive a copy of the consent before the formal informed consent process takes place at the clinic or at the CRC. Consent forms will be signed by all participants (and legal guardian when appropriate) and the consent process will be documented in the patient's chart. The Research Subject Advocates of the CRCs will be present during some administrations of informed consent in this trial participating sites in order to assure that the informed consent process is conducted according to the highest ethical standards.

### *8.6 Certificate of Confidentiality*

To help protect participant privacy, a Letter of Confidentiality has been obtained from the National Institutes of Health (NIH). With this Certificate, the researchers cannot be forced to disclose information that may identify a study participant, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify a participant, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

Even with the Certificate of Confidentiality, the investigators continue to have ethical and legal obligations to report child abuse or neglect and to prevent an individual from carrying out any threats to do serious harm to themselves or others. If keeping information private would immediately put the study participant or someone else in danger, the investigators would release information to protect the participant or another person.

Department of Health and Human Services (DHHS) personnel may request identifying information for purposes of performing audits, carrying out investigations of DHHS grant recipients, or evaluating DHHS funded research projects.

## 9. References

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3. Jackson AA, Danielsen MS, Boyes S. A noninvasive method for measuring urea kinetics with a single dose of [15N15N]urea in free-living humans. The Journal of nutrition 1993;123:2129-36.
4. Danielsen M, Jackson AA. Limits of adaptation to a diet low in protein in normal man: urea kinetics. Clin Sci (Lond) 1992;83:103-8.
5. Steinbrecher HA, Griffiths DM, Jackson AA. Urea production in normal breast-fed infants measured with primed/intermittent oral doses of [15N, 15N]urea. Acta Paediatr 1996;85:656-62.

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7. Zullo A, Rinaldi V, Hassan C, et al. Helicobacter pylori and plasma ammonia levels in cirrhotics: role of urease inhibition by acetohydroxamic acid. Italian journal of gastroenterology and hepatology 1998;30:405-9.

# 10. Appendices and RDCRN Informed Consent Templates

## 10.1 Appendix

**Drug Information:**

**Acetohydroxamic acid (Lithostat)**

**Pharmacology and Pharmaceutical Information**:

Acetohydroxamic acid (AHA) is a stable, synthetic compound derived from hydroxylamine and ethyl acetate. It is a urease inhibitor that reversibly inhibits bacterial enzyme urease, thereby inhibiting the hydrolysis of urea and production of ammonia in urine infected with urea-splitting organisms. AHA is available for oral administration as 250 mg white, round tablets and manufactured by Mission Pharmacal. The intact drug is stored at controlled room temperature (20–25°C)

**Treatment Information:**

All subjects (healthy adults and UCD patients) will receive a single oral dose of 60 mg/kg acetohydroxamic acid during one of the treatment periods, based on the randomization assignment determining whether treatment occurs in the first or the second treatment cycle; doses will be rounded “up” or “down” to the nearest 250 mg to coincide with the available dosage form since the tablets cannot be scored. Patients will be instructed to fast for 4 hours prior to the study; subsequently, the $^{13}$C-Urea tracer will be administered 60 minutes after the ingestion of the acetohydroxamic acid dose.

**$^{13}$C-Urea**

$^{13}$C-Urea is a stable isotope produced by Cambridge Isotope Laboratories, Inc., Tewksbury, MA. The GMP grade pharmaceutical product is provided to Children’s’ National Medical Center as a sterile bulk powder.

**Treatment Information:**

All subjects (healthy adults and UCD patients) will receive a single IV infusion of $^{13}$C-Urea administered over 10-15 minutes, during both treatment cycles. The infusion will be prepared at a dose of 6 mg/kg and infused via a peripheral IV.

Preparation of $^{13}$C-Urea for Intravenous Use

Per USP 797 guidelines, the preparation of a compounded sterile product (CSP) prepared from a powder and intended for intravenous use, represents a high-risk level condition/preparation. USP 797 regulations require a filtration sterilization process utilizing a 0.22 micron negatively charged filter for such high-risk procedures; the use of the specific sterilizing filter ensures sterility by minimizing bacterial particulates and endotoxins that may be associated with the use of compounding products that are not intended for sterile routes of administration. Additionally, the USP 797 regulations state that for a sterilized high-risk level preparation, in the absence of a sterility test, the storage period cannot exceed 24 hours when stored at controlled room temperature. Therefore, subject-specific infusions are prepared on the day of treatment. Per USP 797 regulations, preparations compounded as single dose/single use and not prepared in groups of more than 25 identical individual single-dose packages are exempt from additional bacterial endotoxin testing.

Procedure for preparing IV $^{13}$C-Urea Infusion

***All compounding of a CSP will be performed in USP 797 compliant hood following aseptic procedures***

1. Weigh out appropriate amount of C-13 Urease from bulk container
2. Mix measured powder with 100 mL of 0.9% sodium chloride
3. Swirl to ensure powder is completely in solution
4. Draw up solution into syringe
5. Attach 0.2 micron disk filter* to syringe
6. Attach fluid dispensing connector to other end of filter
7. Attach new receiving syringe on other end of fluid dispensing connector
8. Pass solution through sterilizing disk filter into receiving syringe
9. Remove syringe containing sterilized solution and visually examine solution for the presence of particulate matter
10. All CSPs prepared in this manner will have a beyond-use date (BUD) of 24 hours when stored at room temperature

*Mustang E Membrane Acrodisc Capsule disposable single use 0.2 micron filter is used to remove bacteria and particulates, as well as, endotoxins. The manufacturer (Pall Corporation) confirmed that this is the most appropriate filter to fulfill this sterilization process.

**Storage**

Both the acetohydroxamic acid tablets and $^{13}$C-urea powder is stored in the investigational Drug Service (IDS) pharmacy. The IDS pharmacy is a temperature controlled, limited access area restricted to pharmacy personnel only. Both products are stored at controlled room temperature (20–25°C).

**Accountability**

A drug accountability record will be maintained for both the acetohydroxamic acid tablets and $^{13}C$-urea powder to reflect the day of treatment, the subject/patient's initials and study ID number, the dose dispensed, and the lot number of the product dispensed.

### 10.2 Informed Consent for Healthy Volunteers

CHILDREN'S NATIONAL MEDICAL CENTER
Center for Genetics
111 Michigan Avenue, NW
Washington, DC 20010
(202) 476-5000

Consent/Parental Permission to Participate in a Clinical Research Study and Authorization to Use Protected Health Information

| | |
|---|---|
| TITLE OF STUDY: | Manipulating the Gut Microbiome |
| PRINCIPAL INVESTIGATOR: | Nicholas Ah Mew, MD, Genetic Medicine |
| | Marshall Summar, MD, Genetic Medicine |

Throughout this document, "You" always refers to the person who takes part in the study.

Introduction

We are inviting you to be part of a research study at Children's National Medical Center (Children's National). Before you decide if you would like to participate, we want you to know why we are doing the study. We also want you to know about any risks (anything unexpected that might happen) and what you will be expected to do in the study.

This form gives you information about the study. Your study doctor or a member of the research team will talk to you about the study and answer all of your questions. We encourage you to discuss this study with your family and anyone else you trust before making your decision. You must sign this form if you agree to take part in the study. We will give you a signed copy of this form to keep.

Your participation in this research is voluntary.

You should only take part in this study if you want to volunteer. You should not feel that there is any pressure to take part in the study to please the study doctor or the research staff. You are free to participate in this research or withdraw at any time. If you withdraw, your information will remain confidential. You will not have to complete the study and we will follow up with you as needed.

The Principal Investigator may remove you from the study if you cannot complete the study procedures.
You will be told of any findings that are discovered while you are doing the research study.

There will be no penalty or loss of benefits to which you are otherwise entitled if you decide not to be in the study or withdraw from the study later.

This means that:
You do not have to join the study.
You may change your mind and stop being in the study at any time.
We will tell you if we make any important changes to the study or if there are any important new findings so that you can decide if you still want to be in the study.

If you are an employee or a medical or graduate student in training at Children's National, your decision to participate or not participate will not affect your employment or academic standing.

Why is this research study being done?

Purpose of the study

You are being asked to take part in a research study. The purpose of this study is to evaluate the drug, Lithostat (AHA or acetohydroxamic acid). We want to see if this drug is safe and effective for patients with a rare disease called urea cycle disorder (UCD). You are being asked to participate in this study because it will help find better treatments for patients with UCDs. Individuals with UCD cannot remove ammonia, a waste product, from the blood. We hope that this drug will decrease the production of ammonia to help patients with UCDs. About 16 people will take part in this study.

You are being asked to be in the study because you met the proper criteria for eligibility and ineligibility after being asked a questionnaire.

Lithostat is approved by the Food and Drug Administration for kidney stones. We now use other medications to help treat UCDs, but think that Lithostat will also be able to help patients with UCDs. Lithostat is an Investigational New Drug by the Food and Drug Administration (experimental drug). It has been tested in adults and children in the past to help with kidney stones and was successful. We want to see if using Lithostat can be a useful addition to current treatments for urea cycle disorders in adults and children.

The study will involve up to about 16 participants at 1 site nationally. All 16 participants will be recruited at Children's National.

Dr. Nicholas Ah Mew is the person responsible for this research study at Children's National. He is called the Principal Investigator.

The NIH is paying for this research to be done. A drug company, Mission Pharmacal, is providing the Lithostat free of charge.

What will happen in this research study?

Phone screening and consent:
If you decide to be part of this study, we will look at your medical records and collect your medical history. We will ask you several questions about your health, current medical conditions and medications to see if you are eligible to participate in this study. You will be informed if you are not eligible to participate in this study based off of the questions asked. Females: you will be asked to take a pregnancy test to make sure that you are not pregnant prior to starting the study. There are no direct benefits to participating in this study; however, your participation could help create new therapies for patients with urea cycle disorders. Consent will be confirmed in person the day of or up to a week before the start of the study. There are two study days. The study days must be at least 3 days apart and will take about 4-6 hours on each day. You will not know which day you are receiving the drug.

Study day (without drug):
We will have you fast for 4 hours prior to your appointment. When you come in that morning, we will take a blood sample (one venipuncture and then the IV catheter remains in place for the next 4 hours) and a urine sample. Safety labs are included at the start of the study. The blood test will take 5 mL/1 tsp. .

You will then be given a single dose of $^{13}$C-urea through a different IV which will be inserted into the opposite arm and removed after administration of the 13C-urea. 13C-urea is a tracer that we can see throughout your body in your blood and urine for the tests that we will do. The tracer is for research purposes. There is no known risk of using this tracer as it exists in nature and in the human body. Urea is considered by the FDA to be generally recognized as safe (GRAS) in multiple food products.

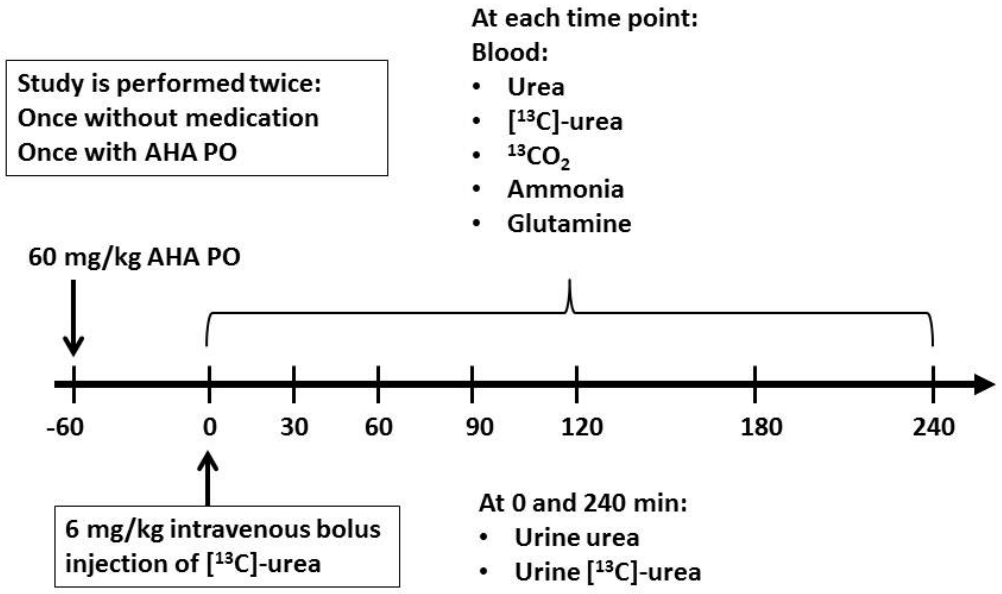
We will observe you for 4 hours after you get the tracer. During this time, the original IV catheter will remain in place so that we can take 6 more blood samples (each will be 5 mL/1 tsp.). Another urine sample will be needed at the end of the 4 hours. We will also measure $^{13}$C urea levels in your blood and urine and $^{13}CO_2$ levels in your blood. Safety labs are included at the end of the 4 hours. You will not be able to eat during the study, but can drink water and/or pedialyte if necessary. This will conclude the study day without the drug.

Study day (with drug):
The next appointment will have to be at least 3 days after the first one. Again, you will need to fast for 4 hours prior to your appointment. You will receive the experimental drug on this day. Safety labs will be collected prior to you receiving the Lithostat (experimental drug). We will give you one dose of the experimental drug, Lithostat. Lithostat has been approved by the FDA and used in patients with kidney stones for over 25 years. 1 hour after you take the Lithostat, we will take a blood sample (one venipuncture and then the

IV catheter remains in place for the next 4 hours) and a urine sample. The blood test will take 5 mL/1 tsp..

You will then be given a single dose of $^{13}$C-urea through a different IV which will be inserted into the opposite arm and removed after administration of the 13C-urea. We will observe you for 4 hours after you get the tracer. During this time, the IV catheter will remain in place so that we can take 6 more blood samples (each will be 5 mL/1 tsp). Another urine sample will be needed at the end of the 4 hours. We will alsomeasure$^{13}$C urea levels in your blood and urine and $^{13}CO_2$ levels in your blood. Safety labs are included at the end of the 4 hours. You will not be able to eat during the study, but can drink water and/or pedialyte if necessary. This will conclude the study day with the drug.

The studies will be conducted at the Clinical Research Center at Children's National Medical Center in Washington, DC. Below is a timeline of what was just explained above:




Below is a table that gives you more information about what we will be measuring in your blood and urine during the study:

| | Time point (min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Baseline | 0 | 30 | 60 | 90 | 120 | 180 | 240 |
| Blood [$^{13}$C]-urea | | X | X | X | X | X | X | X |
| Blood urea | | X | X | X | X | X | X | X |
| Plasma glutamine | | X | X | X | X | X | X | X |
| Ammonia | | X | X | X | X | X | X | X |
| Blood $^{13}CO_2$ | | X | X | X | X | X | X | X |
| | | | | | | | | |
| Urine [$^{13}$C]-urea | | X | | | | | | X |

| | Time point (min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Baseline | 0 | 30 | 60 | 90 | 120 | 180 | 240 |
| Urine urea | | X | | | | | | X |
| | | | | | | | | |
| *Safety labs:* | | | | | | | | |
| Hemoglobin | X | | | | | | | X |
| White blood count | X | | | | | | | X |
| Platelets | X | | | | | | | X |
| | | | | | | | | |
| AST | X | | | | | | | X |
| ALT | X | | | | | | | X |
| Bilirubin | X | | | | | | | X |
| Creatinine | X | | | | | | | X |

'Baseline' indicates at a time 0 on days when Lithostat is not administered or at a time prior to Lithostat administration on the days where it is administered.

Is it O.K. to take other medications, dietary supplements, or alternative medicines while I am in this research study?

There are many drugs (prescription and over-the-counter) and dietary supplements (including what are sometimes called "complementary" or "alternative" remedies) that might interact with the experimental study drug(s). Tell the study doctor about all of the medications and supplements you are currently taking. The study doctor will review all of these with you and decide if you can participate in the study. Also, you should not take any new medications or dietary supplements without discussing it with the study doctor first.

How long will my participation in the research study last?

You will have one appointment to determine if you are eligible to participate in the study. There will be two study days if you are eligible, which must be at least 3 days apart. You should tell us if you decide to drop out of the study.

-There are any unexpected side effects
-Your study doctor and/or the Sponsor of the study think it is in your best interest

What are the risks and possible discomforts from being in this research study?

Lithostat (acetohydroxamic acid/AHA)
According to the Lithostat prescribing information, adverse reactions have occurred in up to 30/100 of the patients receiving Lithostat. In some cases, the reactions produced symptoms: in others, only changes in laboratory measurements were noted. Adverse reactions seem to be more common in patients with pre-existing thrombophlebitis and/or in patients with advanced kidney problems.

Lithostat has been an FDA approved medication for the treatment of kidney stones for over 25 years. According to the prescribing information, about 150 patients, including children, have been treated, most for periods of more than 1 year.

The following reactions have been reported:
~30/100 - Mild headaches during the first 48 hours of treatment, responsive to usual therapy
Tremulousness and nervousness
~20/100 - Gastrointestinal symptoms (nausea, vomiting, anorexia)
~15/100 - laboratory findings characteristic of a hemolytic anemia (low iron from breaking down blood cells)
Non-itchy, macular (red and bumpy) rash on the upper body and face, usually associated with alcoholic beverages, improved on its own
Superficial phlebitis (inflamed vein)

Acute toxicity has not occurred. Milder overdosages resulting in hemolysis (destruction of red blood cells) have occurred in an occasional patient with reduced kidney function after several weeks or months of continuous treatment. This is very unlikely to occur since you will only receive a single dose of the medication.
We do not know any information about the carcinogenic (cancer causing) potential of Lithostat since many people haven't studied it. Acetamide is a chemical produced when Lithostat is broken down. Acetamide has been shown to cause hepatocellular carcinoma (liver cancer) in rats at oral doses 1,500 times the human dose. Lithostat is cytotoxic (toxic to living cells) and can produce mutations in DNA.

There is a small risk that the blood ammonia level will rise during the study. This unlikely possibility, would mostly likely result from toxicity of Lithostat to the liver. We will monitor this throughout the study.

[$^{13}$C]-urea is a substance regarded by the FDA to be generally regarded as safe (GRAS). Taking a small amount of this drug should not pose a risk to subjects with urea cycle disorders. The amount of ammonia generated by it would be very small compared to the ammonia already in the body. There is no known risk of using stable isotopes (the $^{13}$C-urea) as they exist in nature and in the human body. Urea is considered by the FDA to be generally recognized as safe (GRAS) in multiple food products.
There is a potential risk of contamination of the drug or isotope used in the study. However, a very careful sterile and pyrogen-free preparation of the isotope is used, and the drug is manufactured via Current Good Manufacturing Practices (CGMP).

Study Procedures:
Venipuncture: The vein in which the needle has been inserted to draw blood may become sore and red. A temporary "black and blue mark" may develop, and rarely fainting may occur. The placement of the Heplock intravenous (IV) catheter for blood sampling could result in a bruise or infection at the site of insertion and might cause discomfort.

There is a risk of loss of confidentiality, which could affect insurability and result in stigmatization. We will do our best to keep this information safe and private for your protection.

We do not know if you will have all, some, or none of the possible risks, side effects, or discomforts. You should tell the study doctor, Dr. Nicholas Ah Mew, about any illness, side effect or discomfort that you have right away, even if it is not one of the things listed, or even if you think it is not related to the study.
We will look for side effects when we examine you. The study doctor may have to lower the amount or stop giving the drugs if the side effects are serious.
There may be risks we don't know about.

For female participants: Are there additional risks if I get pregnant or breastfeed my baby?

The Lithostat used in this research study may have an effect on an unborn baby. You should not become pregnant or breast feed your baby while you are participating on this study. If you are sexually active and are at risk of getting pregnant, you and your male partner(s) must use an effective method to avoid pregnancy or you must not have sex.

The study doctor will talk to you about acceptable methods to avoid pregnancy while you are taking part in this study. You will have to use the chosen method to avoid pregnancy or abstain (not have sexual intercourse) during your participation.

Natural family planning methods (such as the rhythm method) will not be a permissible means of avoiding pregnancy during study participation. If you have questions about this or want to change your method to avoid pregnancy, please ask the study doctor. <u>If you become pregnant during the research study, please tell the study doctor immediately</u>.

If you are breastfeeding a baby, the drugs used in this research could pass into the breast milk. You should not breastfeed your baby while you are participating in this study. You may need to continue to avoid breastfeeding even after your participation in the study is over. Talk to the study doctor about the length of time you need to avoid breastfeeding.

For male participants: Are there additional risks if I father a baby?

The Lithostat used in this research study can damage sperm. You should not father a child while you are participating on this study because the Lithostat may indirectly affect an unborn child. If you are sexually active and are at risk of causing a pregnancy, you and your female partner(s) must use an effective method to avoid pregnancy or you must not have sex. The study doctor will talk to you about acceptable methods to avoid pregnancy while you are taking part in this study. You will have to use the chosen method to avoid pregnancy or abstain (not have sexual intercourse) during your participation.

Natural family planning methods (such as the rhythm method) will not be a permissible means of avoiding pregnancy while you are taking part in this study. If you have questions about this or want to change your method to avoid pregnancy, please ask the study doctor. If your partner becomes pregnant during the research study, please tell the study doctor immediately.

What are the possible benefits from being in this research study?

You will not benefit directly from participating in this research study. The things we learn from you taking part might help other people with urea cycle disorders in the future by providing them with more treatment options.

Alternatives: What other choices do I have if I don't want to take part in the study?

If you do not want to participate in the study, there are no other choices except not to take part.

Will it cost me anything to take part in the study?

There are no costs to you or your insurance company to participate in this study. Children's National Medical Center will give you the medicine used in this study at no cost to you. You will not be charged for anything else we do that is part of the study.

Compensation: Will I be paid for taking part in this study?

You may receive up to a total of $500 after finishing this research study. The payment will be made in installments: [$250 for visit one, $250 for visit two]. You will be given a voucher to exchange for cash at the Children's National cashier office each time you complete these visits. We will also pay for your transportation to and parking at Children's National when you are here to take part in the study.

The Internal Revenue Service requires that any monetary payments totaling $600 or more in a calendar year must be reported for tax purposes.

ClinicalTrials.gov

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

How will you protect my privacy if I take part in this study?
Who will see the information that I give you?

We will keep your study records confidential. However, certain people may need to see your study records. By law, anyone who looks at your records must keep them completely confidential.
If you decide to take part in this study, only the people working on the study will know your name. They will keep this information [e.g., so that we can contact you to schedule future appointments; in case we have to find you later to let you know of any new information that may affect your health]. Your personal information will not be given to anyone unless we get your permission in writing or if the law requires it. This information will also only be given for regular hospital care, payment, and hospital management activities. We will make every effort to keep your information private, but no one's privacy can be totally guaranteed.
Your medical record is confidential but, just like any medical record, there are some exceptions under state and federal law.
There are some government agencies or other groups that may check records that identify you without your permission. They might review the records of this study and your medical records to make sure we are following the law and protecting the participants in the study. The agencies or groups who might see these records are: the research team, the Office for Human Research Protections, Department of Health and Human Services (DHHS), Food and Drug Administration, the sponsor (NIH Rare Diseases Clinical Research Network, Mission Pharmacal), Data Management and Coordinating Center (Tampa, FL), National Institute of Child Health and Human Development (NICHD) Data Safety and Monitoring Board (DSMB), and the Institutional Review Board of Children's National Medical Center (the ethics board and their staff that reviewed and approved this research study).
The results of this research may be presented at meetings or in publications. You will not be personally identified.

Transfer of Data to DMCC and Federal data repository

The clinical information collected for this study will be stored in a computer database at the Data Management and Coordinating Center at the University of South Florida in Tampa, FL and also sent to a Federal data repository. A data repository provides a way for researchers to store the information collected during the research study for future research studies. The data management center uses several layers of protection for the clinical data stored in its computer database. It meets all of the local and federal security requirements for research datacenters. Your information is stored only using a study ID.

Certificate of Confidentiality

Sometimes people tell us some very personal information about themselves when they participate in a study and it becomes part of their research record. To help us protect your privacy, the investigators have obtained a Certificate of Confidentiality from the U.S. Department of Health and Human Services (DHHS).

With this Certificate, the investigators cannot be forced (for example, by a court order or subpoena) to give information that may identify you in any federal, state or local civil or

criminal court, or in any administrative, legislative, or other proceedings. At sometime, however, DHHS might request this information as part of a review of the study records and medical records to make sure we are following the law and protecting the people in the study, and to make sure our results are correct. If this happens, we are required to give DHHS all of the information they request for their review.

It is important that you know that a Certificate of Confidentiality does not stop you or a member of your family from voluntarily giving information to others about yourself or your taking part in this research. You should also know that if an insurer or employer learns about your participation and you give them permission to receive research information about you, we cannot use the Certificate of Confidentiality to keep your information private from them. This means that you must also actively protect your own privacy.

Finally, it is important that you know that we are not prevented from taking steps to prevent serious harm to you or to others. For example, if you or anyone else might be in danger, we will have to report this to the authorities and get emergency help if it is needed.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of Federally funded projects or for information that must be disclosed in order to meet the requirements of the federal National Institutes of Health (NIH)

Genetic Information Nondiscrimination Act (GINA)

A Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:
Health insurance companies may not request your genetic information that we get from this research
Health insurance companies may not use your genetic information when deciding whether to insure you or the amount of money they will charge you.
Employers may not use your genetic information that we get from this research when deciding to hire, promote, or fire you.
This Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

What happens if I get hurt or sick because of taking part in this research study?

Children's National Medical Center cannot promise that the risks we have told you about or other unknown problems will not happen. If you think that you are hurt, sick, or otherwise harmed because of something to do with the study, please call the Principal Investigator, Dr. Nicholas Ah Mew, at (202)476-5863.

In case of a medical emergency, call 911 or go directly to the hospital. Be sure to tell the Emergency Room personnel and your doctor that you are on this study.
If you have any non-emergency side effects or bad reactions, call the Principal Investigator, Dr. Nicholas Ah Mew, at (202)476-5863 right away.

We will give you any urgent medical care needed because of your participation in this research study if reported in a timely manner. Children's National will seek payment from your health insurance company or other third-party payor for any medical care or services you receive. Children's National has no program to provide you with any additional payments for any injuries.

HEALTH INSURANCE PORTABILITY AND ACCOUNTABILITY

In 1996 the government passed a law known as The Health Insurance Portability and Accountability Act (HIPAA). This privacy law protects your individually identifiable health information (Protected Health Information or PHI). The privacy law requires you to sign an agreement so researchers can use or share your PHI for research purposes. This describes to you how information about you may be used or shared if you are in a research study. It is important that you read this carefully and ask a member of the research team to explain anything you do not understand.

I authorize the Principal Investigator, Dr. Nicholas Ah Mew, and his research staff to create, access, use, and disclose my PHI for the purposes described below.

Protected Health Information that may be used and shared includes:

Information that identifies you such as name, address, telephone number, date of birth, Social Security number, and other details about you
Information that relates to your health or medical condition from your medical records
Information obtained from the study procedures outlined in this consent form, for example: things done to see if you can join the study such as physical exams, blood and urine tests, x-rays and other tests, and any other medical information we learn from you about your health history and family history
Laboratory results obtained on specimens collected from you (blood, urine, tissue)
Questionnaires or surveys you complete
Interviews conducted with you by members of the research team
Audio/ video recordings

The Researchers may use and share my Protected Health Information with:

The Principal Investigator, other Investigators, Study Coordinators, and all administrative staff in charge of doing work for the study;

Government agencies that have the right to see or review your PHI including, but not limited to, the Office of Human Research Protections and the Food and Drug Administration;
Children's National Medical Center Institutional Review Board;
Audit Committee of the Children's National Medical Center Institutional Review Board;
Quality Improvement Program Coordinator and other staff in the Office for the Protection of Human Subjects at Children's National Medical Center.

In addition to the above people and organizations, the Researchers may also use and share my Protected Health Information with:

Laboratories and other people or organizations that look at your health information in connection with this study. The name of the laboratory being used in this study is Children's Hospital of Philadelphia
The Data Safety Monitoring Board (a group of people who examine the medical information during the study)
The Patient Advocate or Research Ombudsman (person who watches out for your best interest)

Also, your primary physician will be contacted if during the course of the study the researcher learns of a medical condition that needs immediate attention.

Should your health information be disclosed to anyone outside of the study, your information may no longer be protected by HIPAA and this Authorization. However, the use of your health information will still be regulated by applicable federal and state laws.

<u>Storage of PHI in a Database:</u>

We would like to store personal health information collected from you in this study in a database for future research. The database is maintained by the Division for Genetics and Metabolism at Children's National.

Please indicate your approval of any or all of the following by initialing next to the statement:

My personal health information may be stored in the above named database for future analysis related to this study.
☐ Yes ☐ No Initials _____

My personal health information may be stored in the above named database for future analysis related to [insert name of specific study].
☐ Yes ☐ No Initials _____

My personal health information may be stored in the above named database. Researchers may contact me to request my authorization for future studies that are not related to this study or the disease named above.

☐ Yes ☐ No Initials _____

My personal health information may be stored without any of my identifying information for use in other studies of other diseases.
☐ Yes ☐ No Initials _____

If you agree to participate in this research study, the research team, the research sponsor (when applicable) and the sponsor's representatives may use Personally Unidentified Study Data. The Personally Unidentified Study Data does not include your name, address, telephone, or social security number. Instead, the researcher assigns a code to the Personally Unidentified Study Data. Personally Unidentified Study Data may include your date of birth, initials, and dates you received medical care. Personally Unidentified Study Data may also include the health information used, created, or collected in the research study. The research team or the research sponsor may share the Personally Unidentified Study Data with others to perform additional research, place it into research databases, share it with researchers in the U.S. or other countries, or use it to improve the design of future studies. They may also publish it in scientific journals, or share it with business partners of the sponsor and to file applications with U.S. or foreign government agencies to get approval for new drugs or health care products.

You do not have to sign this Consent/Authorization. If you decide not to sign the Authorization, you will not be allowed to participate in the research study.

After signing the Consent/Authorization, you can change your mind and revoke this Authorization.

If you revoke the Authorization, you will send a written letter to the Principal Investigator to inform him of your decision.
Dr. Nicholas Ah Mew
Children’s National Medical Center
Division of Genetics and Metabolism
111 Michigan Avenue, N.W.
Washington, DC 20010-2970
If you revoke this Authorization, researchers may only use and disclose the PHI that was collected for this research study before you revoked the Authorization.
If you revoke this Authorization, your PHI may still be used and disclosed if you should have an adverse event (unexpected side effect).
If you change your mind and withdraw the Authorization, you will not be allowed to participate in the study.

You will be allowed to review the information collected for this research study.

This Authorization does not expire.

If you have not already received a Notice of Privacy Practices from Children's National Medical Center, you may request a copy and will be given one. If you have any questions

or concerns about your privacy rights, you may contact the Children's Hospital Privacy Officer at 301-572-6348.

Whom can I call if I have questions about this research study?

We want you to ask questions about any part of this research study at anytime,

For questions about the study or the information in this informed consent/parental permission document, call the Principal Investigator, Dr. Nicholas Ah Mew, at 202-476-5863.

Whom can I call if I have questions or concerns about my rights as a research study participant?

The Children's National Office for the Protection of Human Subjects is available to talk with you about:
Your rights as a research participant
Your concerns about the research
A complaint about the research

This is the administration office for the Institutional Review Board, which is a group of doctors, nurses, and non-medical people who review research studies for safety and the protection of people who participate in research. You can call the Office for the Protection of Human Subjects at 301-565-8452.

Children's National has a bilingual (English/Spanish) research participant and family advocate. The advocate, Dr. Tomas Silber, is here to answer your questions or concerns about taking part in this research. Dr. Silber does not work for the doctors who are doing this research and they do not pay him. He is here only to help and protect you during any research.

You may contact Dr. Silber at any time. This can be done before you decide to take part in the research, during the study, or even after you finish the study. You can call Dr. Silber at 202-476-3066 or reach him by e-mail at tsilber@childrensnational.org.

Questions, concerns, or complaints

If you have any questions, concerns or complaints about this study, call Dr. Nicholas Ah Mew at (202)476-5863.

If you have questions about your rights, general questions, complaints, or issues as a person taking part in this study, call the Children's National Medical Center Institutional Review Board at (202)476-3472.

CONSENT

I am the study participant or I am authorized to act on behalf of the participant.
I have read this consent form or had it read to me.
I have been invited to take part in a research study. I was told why the research is being done and how long my participation in the study is expected to last. I was told about what will happen in the study and if there are any procedures or drugs that are experimental.
I was told that taking part in this research is voluntary. I also was told that I can decide not to take part or stop being in it at any time without any penalty to me or any change to the quality of care I receive at Children's National.
I was told about the risks and possible discomforts of taking part in this research study.
I was also informed if there are any possible benefits to me if I am in this study.
I have been given the chance to ask questions about the study, and my questions have been answered. If I have questions later, I can ask one of the people listed in this form.
I agree to take part in this research study.
I will receive a copy of this Informed Consent/Parental Permission form to keep.

Signature of adult participant (18 years of age and older)

Printed Name of Participant: _____

Signature of Participant: _____

Date and Time: ____ a.m. / p.m. (circle one) _____

Signature of language interpreter (if applicable)

Printed Name of Interpreter: _____

Interpreter's Signature: _____

Language: Date and Time: a.m. / p.m.(circle one)

AFFIDAVIT OF PERSON OBTAINING CONSENT / PARENTAL PERMISSION:

I certify that I have explained to the above individual(s) the nature and purpose of the study, potential benefits, and possible risks associated with participation in this study.
I have answered any questions that have been raised.

Printed Name of Person Obtaining Consent: _____

Research Role: _____

Signature: _____

Date and Time: ____a.m. / p.m. (circle one)_____

_____

Signature of Witness (if applicable)
Printed Name of Witness: _____

Witness's Signature: _____

Date and Time: ____a.m. / p.m. (circle one)_____

**10.3 Informed Consent for Urea Cycle Disorder Participants**

CHILDREN'S NATIONAL MEDICAL CENTER
Center for Genetics
111 Michigan Avenue, NW
Washington, DC 20010
(202) 476-5000

Consent/Parental Permission to Participate in a Clinical Research Study and Authorization to Use Protected Health Information

| | |
|---|---|
| TITLE OF STUDY: | Manipulating the Gut Microbiome |
| PRINCIPAL INVESTIGATOR: | Nicholas Ah Mew, MD, Genetic Medicine |
| | Marshall Summar, MD, Genetic Medicine |

Throughout this document, "You" always refers to the person (you or your child) who takes part in the study.

Introduction

We are inviting you to be part of a research study at Children's National Medical Center (Children's National). Before you decide if you would like to participate, we want you to know why we are doing the study. We also want you to know about any risks (anything unexpected that might happen) and what you will be expected to do in the study.

This form gives you information about the study. Your study doctor or a member of the research team will talk to you about the study and answer all of your questions. We encourage you to discuss this study with your family and anyone else you trust before making your decision. You must sign this form if you agree to take part in the study. We will give you a signed copy of this form to keep.

Your participation in this research is voluntary.

You should only take part in this study if you want to volunteer. You should not feel that there is any pressure to take part in the study to please the study doctor or the research staff. You are free to participate in this research or withdraw at any time. If you

withdraw, your information will remain confidential. You will not have to complete the study and we will follow up with you as needed.

The Principal Investigator may remove you from the study if you cannot complete the study procedures.
You will be told of any new findings about your disease that are discovered while you are doing the research study.

There will be no penalty or loss of benefits to which you are otherwise entitled if you decide not to be in the study or withdraw from the study later.

This means that:
You do not have to join the study.
You may change your mind and stop being in the study at any time.
We will tell you if we make any important changes to the study or if there are any important new findings so that you can decide if you still want to be in the study.
If you are an employee or a medical or graduate student in training at Children's National, your decision to participate or not participate will not affect your employment or academic standing.

Why is this research study being done?

Purpose of the study

You are being asked to take part in a research study. The purpose of this study is to evaluate the drug, Lithostat (AHA or acetohydroxamic acid). We want to see if this drug is safe and effective for patients with a rare disease called urea cycle disorder (UCD). You are being asked to participate in this study because it will help find better treatments for patients with UCDs. Individuals with UCD cannot remove ammonia, a waste product, from the blood. We hope that this drug will decrease the production of ammonia to help patients with UCDs. About 16 people will take part in this study.

You are being asked to be in the study because you met the proper criteria for eligibility and ineligibility after being asked a questionnaire.

Lithostat is approved by the Food and Drug Administration for kidney stones. We now use other medications to help treat UCDs, but think that Lithostat will also be able to help patients with UCDs. Lithostat is an Investigational New Drug by the Food and Drug Administration (experimental drug). It has been tested in adults and children in the past to help with kidney stones and was successful. We want to see if using Lithostat can be a useful addition to current treatments for urea cycle disorders in adults and children.

The study will involve up to about 16 participants at 1 site nationally. All 16 participants will be recruited at Children's National.

Dr. Nicholas Ah Mew is the person responsible for this research study at Children's National. He is called the Principal Investigator.

The NIH is paying for this research to be done. A drug company, Mission Pharmacal, is providing the Lithostat free of charge.

What will happen in this research study?

Phone screening and consent:
If you decide to be part of this study, we will look at your medical records and collect your medical history. We will ask you several questions about your health, current medical conditions and medications to see if you are eligible to participate in this study. You will be informed if you are not eligible to participate in this study based off of the questions asked. Females: you will be asked to take a pregnancy test to make sure that you are not pregnant prior to starting the study. There are no direct benefits to participating in this study; however, your participation could help create new therapies for patients with urea cycle disorders. Consent will be confirmed in person the day of or up to a week before the start of the study. There are two study days. The study days must be at least 3 days apart and will take about 4-6 hours on each day. You will not know which day you are receiving the drug.

Study day (without drug):
We will have you fast for 4 hours prior to your appointment. When you come in that morning, we will take a blood sample (one venipuncture and then the IV catheter* remains in place for the next 4 hours) and a urine sample. Safety labs are included at the start of the study. The blood test will take 5 mL/1 tsp.

You will then be given a single dose of $^{13}$C-urea through a different IV which will be inserted into the opposite arm and removed after administration of the 13C-urea. 13C-urea is a tracer that we can see throughout your body in your blood and urine for the tests that we will do. The tracer is for research purposes. There is no known risk of using this tracer as it exists in nature and in the human body. Urea is considered by the FDA to be generally recognized as safe (GRAS) in multiple food products.

We will observe you for 4 hours after you get the tracer. During this time, the original IV catheter will remain in place so that we can take 6 more blood samples (each will be 5 mL/1 tsp.). Another urine sample will be needed at the end of the 4 hours. We will also measure $^{13}$C urea levels in your blood and urine and $^{13}CO_2$ levels in your blood. Safety labs are included at the end of the 4 hours. You will not be able to eat during the study, but can drink water and/or pedialyte if necessary. This will conclude the study day without the drug.

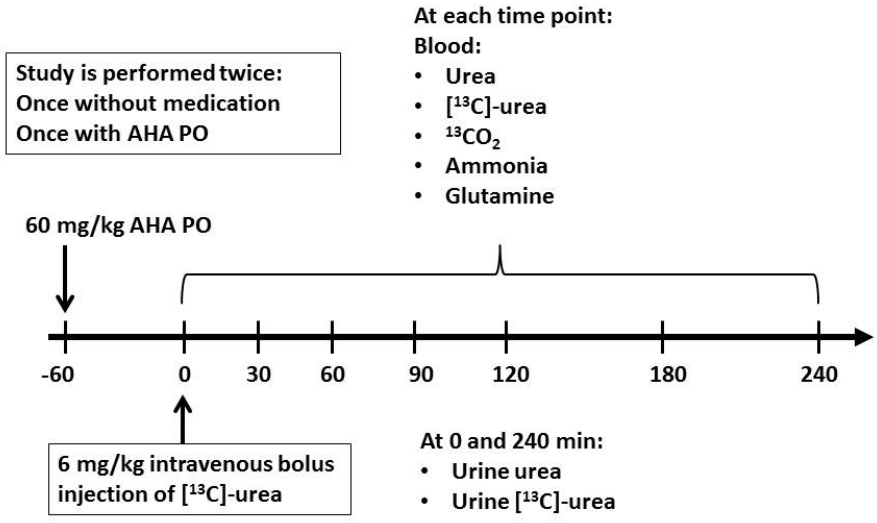
Study day (with drug):
The next appointment will have to be at least 3 days after the first one. Again, you will need to fast for 4 hours prior to your appointment. You will receive the experimental drug on this day. Safety labs will be collected prior to you receiving the Lithostat (experimental

drug). We will give you one dose of the experimental drug, Lithostat. Lithostat has been approved by the FDA and used in patients with kidney stones for over 25 years. 1 hour after you take the Lithostat, we will take a blood sample (one venipuncture and then the IV catheter* remains in place for the next 4 hours) and a urine sample. The blood test will take 5 mL/1 tsp..

You will then be given a single dose of $^{13}$C-urea through a different IV which will be inserted into the opposite arm and removed after administration of the 13C-urea. We will observe you for 4 hours after you get the tracer. During this time, the original IV catheter will remain in place so that we can take 6 more blood samples (each will be 5 mL/1 tsp.). Another urine sample will be needed at the end of the 4 hours. We will alsomeasure$^{13}$C urea levels in your blood and urine and $^{13}CO_2$ levels in your blood. Safety labs are included at the end of the 4 hours. You will not be able to eat during the study, but can drink water or pedialyte if necessary. This will conclude the study day with the drug.

* An intravenous catheter for blood sampling will be inserted in the arm or hand. If there is not adequate vascular access, a PICC (peripherally inserted central catheter) line will be inserted in the arm by an interventional radiologist using conscious sedation (Versed and Fentanyl) and will be maintained for the duration of the study by daily heparin flushes. Note: A PICC line is not required. If the site IRB determines that a PICC line should not be used or should not be used in certain (i.e. young) subjects those restrictions will be described in the site's informed consent forms. The site PI may also decide that neither an intravenous catheter nor PICC line can be used, in which case the subject would not participate in the study until an intravenous catheter or PICC line can be used safely.

The studies will be conducted at the Clinical Research Center at Children's National Medical Center in Washington, DC. Below is a timeline of what was just explained above:



Below is a table that gives you more information about what we will be measuring in your blood and urine during the study:

| | Time point (min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Baseline | 0 | 30 | 60 | 90 | 120 | 180 | 240 |
| Blood [$^{13}$C]-urea | | X | X | X | X | X | X | X |
| Blood urea | | X | X | X | X | X | X | X |
| Plasma glutamine | | X | X | X | X | X | X | X |
| Ammonia | | X | X | X | X | X | X | X |
| Blood $^{13}CO_2$ | | X | X | X | X | X | X | X |
| | | | | | | | | |
| Urine [$^{13}$C]-urea | | X | | | | | | X |
| Urine urea | | X | | | | | | X |
| | | | | | | | | |
| *Safety labs:* | | | | | | | | |
| Hemoglobin | X | | | | | | | X |
| White blood count | X | | | | | | | X |
| Platelets | X | | | | | | | X |
| | | | | | | | | |
| AST | X | | | | | | | X |
| ALT | X | | | | | | | X |
| Bilirubin | X | | | | | | | X |
| Creatinine | X | | | | | | | X |

'Baseline' indicates at a time 0 on days when Lithostat is not administered or at a time prior to Lithostat administration on the days where it is administered.

Is it O.K. to take other medications, dietary supplements, or alternative medicines while I am in this research study?

There are many drugs (prescription and over-the-counter) and dietary supplements (including what are sometimes called "complementary" or "alternative" remedies) that might interact with the experimental study drug(s). Tell the study doctor about all of the medications and supplements you are currently taking. The study doctor will review all of these with you and decide if you can participate in the study. Also, you should not take any new medications or dietary supplements without discussing it with the study doctor first.

How long will my participation in the research study last?

You will have one appointment to determine if you are eligible to participate in the study. There will be two study days if you are eligible, which must be at least 3 days apart. You should tell us if you decide to drop out of the study.

-There are any unexpected side effects
-Your study doctor and/or the Sponsor of the study think it is in your best interest

What are the risks and possible discomforts from being in this research study?

Lithostat (acetohydroxamic acid/AHA)
According to the Lithostat prescribing information, adverse reactions have occurred in up to 30/100 of the patients receiving Lithostat. In some cases, the reactions produced symptoms: in others, only changes in laboratory measurements were noted. Adverse reactions seem to be more common in patients with pre-existing thrombophlebitis and/or in patients with advanced kidney problems.
Lithostat has been an FDA approved medication for the treatment of kidney stones for over 25 years. According to the prescribing information, about 150 patients, including children, have been treated, most for periods of more than 1 year.

The following reactions have been reported:
~30/100 - Mild headaches during the first 48 hours of treatment, responsive to usual therapy
Tremulousness and nervousness
~20/100 - Gastrointestinal symptoms (nausea, vomiting, anorexia)
~15/100 - laboratory findings characteristic of a hemolytic anemia (low iron from breaking down blood cells)
Non-itchy, macular (red and bumpy) rash on the upper body and face, usually associated with alcoholic beverages, improved on its own
Superficial phlebitis (inflamed vein)

Acute toxicity has not occurred. Milder overdosages resulting in hemolysis (destruction of red blood cells) have occurred in an occasional patient with reduced kidney function after several weeks or months of continuous treatment. This is very unlikely to occur since you will only receive a single dose of the medication.
We do not know any information about the carcinogenic (cancer causing) potential of Lithostat since many people haven't studied it. Acetamide is a chemical produced when Lithostat is broken down. Acetamide has been shown to cause hepatocellular carcinoma (liver cancer) in rats at oral doses 1,500 times the human dose. Lithostat is cytotoxic (toxic to living cells) and can produce mutations in DNA.

There is a small risk that the blood ammonia level will rise during the study. This unlikely possibility, would mostly likely result from toxicity of Lithostat to the liver. We will monitor this throughout the study.

[$^{13}$C]-urea is a substance regarded by the FDA to be generally regarded as safe (GRAS). Taking a small amount of this drug should not pose a risk to subjects with urea cycle disorders. The amount of ammonia generated by it would be very small compared to the ammonia already in the body. There is no known risk of using stable isotopes (the $^{13}$C-urea) as they exist in nature and in the human body. Urea is considered by the FDA to be generally recognized as safe (GRAS) in multiple food products.
There is a potential risk of contamination of the drug or isotope used in the study. However, a very careful sterile and pyrogen-free preparation of the isotope is used, and the drug is manufactured via Current Good Manufacturing Practices (CGMP).

Study Procedures:
Venipuncture: The vein in which the needle has been inserted to draw blood may become sore and red. A temporary “black and blue mark” may develop, and rarely fainting may occur. The placement of the Heplock intravenous (IV) catheter for blood sampling could result in a bruise or infection at the site of insertion and might cause discomfort.

If a PICC line is inserted in the arm (by an interventional radiologist) intravenous sedation is given and involves risks of apnea or aspiration. There is also risk of vessel perforation and infection of the central line catheter.

There is a risk of loss of confidentiality, which could affect insurability and result in stigmatization. We will do our best to keep this information safe and private for your protection.

We do not know if you will have all, some, or none of the possible risks, side effects, or discomforts. You should tell the study doctor, Dr. Nicholas Ah Mew, about any illness, side effect or discomfort that you have right away, even if it is not one of the things listed, or even if you think it is not related to the study.
We will look for side effects when we examine you. The study doctor may have to lower the amount or stop giving the drugs if the side effects are serious.
There may be risks we don’t know about.

For female participants: Are there additional risks if I get pregnant or breastfeed my baby?

The Lithostat used in this research study may have an effect on an unborn baby. You should not become pregnant or breast feed your baby while you are participating on this study. If you are sexually active and are at risk of getting pregnant, you and your male partner(s) must use an effective method to avoid pregnancy or you must not have sex.

The study doctor will talk to you about acceptable methods to avoid pregnancy while you are taking part in this study. You will have to use the chosen method to avoid pregnancy or abstain (not have sexual intercourse) during your participation.

Natural family planning methods (such as the rhythm method) will not be a permissible means of avoiding pregnancy during study participation. If you have questions about this or want to change your method to avoid pregnancy, please ask the study doctor. <u>If you become pregnant during the research study, please tell the study doctor immediately</u>.

If you are breastfeeding a baby, the drugs used in this research could pass into the breast milk. You should not breastfeed your baby while you are participating in this study. You may need to continue to avoid breastfeeding even after your participation in the study is over. Talk to the study doctor about the length of time you need to avoid breastfeeding.

For male participants: Are there additional risks if I father a baby?

The Lithostat used in this research study can damage sperm. You should not father a child while you are participating on this study because the Lithostat may indirectly affect an unborn child. If you are sexually active and are at risk of causing a pregnancy, you and your female partner(s) must use an effective method to avoid pregnancy or you must not have sex. The study doctor will talk to you about acceptable methods to avoid pregnancy while you are taking part in this study. You will have to use the chosen method to avoid pregnancy or abstain (not have sexual intercourse) during your participation.

Natural family planning methods (such as the rhythm method) will not be a permissible means of avoiding pregnancy while you are taking part in this study. If you have questions about this or want to change your method to avoid pregnancy, please ask the study doctor. If your partner becomes pregnant during the research study, please tell the study doctor immediately.

What are the possible benefits from being in this research study?

You will not benefit directly from participating in this research study. The things we learn from you taking part might help other people with urea cycle disorders (including you) in the future by providing them with more treatment options.

Alternatives: What other choices do I have if I don't want to take part in the study?

If you do not want to participate in the study, there are no other choices except not to take part.

Will it cost me anything to take part in the study?

There are no costs to you or your insurance company to participate in this study. Children's National Medical Center will give you the medicine used in this study at no cost to you. You will not be charged for anything else we do that is part of the study.

Compensation: Will I be paid for taking part in this study?

You may receive up to a total of $500 after finishing this research study. The payment will be made in installments: [$250 for visit one, $250 for visit two]. You will be given a voucher to exchange for cash at the Children's National cashier office each time you complete these visits. We will also pay for your transportation to and parking at Children's National when you are here to take part in the study.

The Internal Revenue Service requires that any monetary payments totaling $600 or more in a calendar year must be reported for tax purposes.

ClinicalTrials.gov

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. law. This Web site will not include information that can identify you. At most, the Web site will include a summary of the results. You can search this Web site at any time.

How will you protect my privacy if I take part in this study?
Who will see the information that I give you?

We will keep your study records confidential. However, certain people may need to see your study records. By law, anyone who looks at your records must keep them completely confidential.
If you decide to take part in this study, only the people working on the study will know your name. They will keep this information [e.g., so that we can contact you to schedule future appointments; in case we have to find you later to let you know of any new information that may affect your health]. Your personal information will not be given to anyone unless we get your permission in writing or if the law requires it. This information will also only be given for regular hospital care, payment, and hospital management activities. We will make every effort to keep your information private, but no one's privacy can be totally guaranteed.
Your medical record is confidential but, just like any medical record, there are some exceptions under state and federal law.
There are some government agencies or other groups that may check records that identify you without your permission. They might review the records of this study and your medical records to make sure we are following the law and protecting the participants in the study. The agencies or groups who might see these records are: the research team, the Office for Human Research Protections, Department of Health and Human Services (DHHS), Food and Drug Administration, the sponsor (NIH Rare Diseases Clinical Research Network, Mission Pharmacal), Data Management and Coordinating Center (Tampa, FL), National Institute of Child Health and Human Development (NICHD) Data Safety and Monitoring Board (DSMB), and the Institutional Review Board of Children's National Medical Center (the ethics board and their staff that reviewed and approved this research study).
The results of this research may be presented at meetings or in publications. You will not be personally identified.

Transfer of Data to DMCC and Federal data repository

The clinical information collected for this study will be stored in a computer database at the Data Management and Coordinating Center at the University of South Florida in Tampa, FL and also sent to a Federal data repository. A data repository provides a way for researchers to store the information collected during the research study for future research studies. The data management center uses several layers of protection for the clinical data stored in its computer database. It meets all of the local and federal security requirements for research datacenters. Your information is stored only using a study ID.

Certificate of Confidentiality

Sometimes people tell us some very personal information about themselves when they participate in a study and it becomes part of their research record. To help us protect your privacy, the investigators have obtained a Certificate of Confidentiality from the U.S. Department of Health and Human Services (DHHS).
With this Certificate, the investigators cannot be forced (for example, by a court order or subpoena) to give information that may identify you in any federal, state or local civil or criminal court, or in any administrative, legislative, or other proceedings. At sometime, however, DHHS might request this information as part of a review of the study records and medical records to make sure we are following the law and protecting the people in the study, and to make sure our results are correct. If this happens, we are required to give DHHS all of the information they request for their review.

It is important that you know that a Certificate of Confidentiality does not stop you or a member of your family from voluntarily giving information to others about yourself or your taking part in this research. You should also know that if an insurer or employer learns about your participation and you give them permission to receive research information about you, we cannot use the Certificate of Confidentiality to keep your information private from them. This means that you must also actively protect your own privacy.

Finally, it is important that you know that we are not prevented from taking steps to prevent serious harm to you or to others. For example, if you or anyone else might be in danger, we will have to report this to the authorities and get emergency help if it is needed.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of Federally funded projects or for information that must be disclosed in order to meet the requirements of the federal National Institutes of Health (NIH)

Genetic Information Nondiscrimination Act (GINA)

A Federal law, called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and most employers to discriminate against you based on your genetic information. This law generally will protect you in the following ways:
Health insurance companies may not request your genetic information that we get from this research
Health insurance companies may not use your genetic information when deciding whether to insure you or the amount of money they will charge you.

Employers may not use your genetic information that we get from this research when deciding to hire, promote, or fire you.
This Federal law does not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

What happens if I get hurt or sick because of taking part in this research study?

Children's National Medical Center cannot promise that the risks we have told you about or other unknown problems will not happen. If you think that you are hurt, sick, or otherwise harmed because of something to do with the study, please call the Principal Investigator, Dr. Nicholas Ah Mew, at (202)476-5863.

In case of a medical emergency, call 911 or go directly to the hospital. Be sure to tell the Emergency Room personnel and your doctor that you are on this study.
If you have any non-emergency side effects or bad reactions, call the Principal Investigator, Dr. Nicholas Ah Mew, at (202)476-5863 right away.

We will give you any urgent medical care needed because of your participation in this research study if reported in a timely manner. Children's National will seek payment from your health insurance company or other third-party payor for any medical care or services you receive. Children's National has no program to provide you with any additional payments for any injuries.

HEALTH INSURANCE PORTABILITY AND ACCOUNTABILITY

In 1996 the government passed a law known as The Health Insurance Portability and Accountability Act (HIPAA). This privacy law protects your individually identifiable health information (Protected Health Information or PHI). The privacy law requires you to sign an agreement so researchers can use or share your PHI for research purposes. This describes to you how information about you may be used or shared if you are in a research study. It is important that you read this carefully and ask a member of the research team to explain anything you do not understand.

I authorize the Principal Investigator, Dr. Nicholas Ah Mew, and his research staff to create, access, use, and disclose my PHI for the purposes described below.

Protected Health Information that may be used and shared includes:

Information that identifies you such as name, address, telephone number, date of birth, Social Security number, and other details about you
Information that relates to your health or medical condition from your medical records
Information obtained from the study procedures outlined in this consent form, for example: things done to see if you can join the study such as physical exams, blood and urine tests, x-rays and other tests, and any other medical information we learn from you about your health history and family history

Laboratory results obtained on specimens collected from you (blood, urine, tissue)
Questionnaires or surveys you complete
Interviews conducted with you by members of the research team
Audio/ video recordings

The Researchers may use and share my Protected Health Information with:

The Principal Investigator, other Investigators, Study Coordinators, and all administrative staff in charge of doing work for the study;
Government agencies that have the right to see or review your PHI including, but not limited to, the Office of Human Research Protections and the Food and Drug Administration;
Children's National Medical Center Institutional Review Board;
Audit Committee of the Children's National Medical Center Institutional Review Board;
Quality Improvement Program Coordinator and other staff in the Office for the Protection of Human Subjects at Children's National Medical Center.

In addition to the above people and organizations, the Researchers may also use and share my Protected Health Information with:

Laboratories and other people or organizations that look at your health information in connection with this study. The name of the laboratory being used in this study is Children’s Hospital of Philadelphia
The Data Safety Monitoring Board (a group of people who examine the medical information during the study)
The Patient Advocate or Research Ombudsman (person who watches out for your best interest)

Also, your primary physician will be contacted if during the course of the study the researcher learns of a medical condition that needs immediate attention.

Should your health information be disclosed to anyone outside of the study, your information may no longer be protected by HIPAA and this Authorization. However, the use of your health information will still be regulated by applicable federal and state laws.

<u>Storage of PHI in a Database:</u>

We would like to store personal health information collected from you in this study in a database for future research. The database is maintained by the Division for Genetics and Metabolism at Children’s National.

Please indicate your approval of any or all of the following by initialing next to the statement:

My personal health information may be stored in the above named database for future analysis related to this study.

☐ Yes ☐ No Initials _____

My personal health information may be stored in the above named database for future analysis related to [insert name of specific study].
☐ Yes ☐ No Initials _____

My personal health information may be stored in the above named database. Researchers may contact me to request my authorization for future studies that are not related to this study or the disease named above.
☐ Yes ☐ No Initials _____

My personal health information may be stored without any of my identifying information for use in other studies of other diseases.
☐ Yes ☐ No Initials _____

If you agree to participate in this research study, the research team, the research sponsor (when applicable) and the sponsor's representatives may use Personally Unidentified Study Data. The Personally Unidentified Study Data does not include your name, address, telephone, or social security number. Instead, the researcher assigns a code to the Personally Unidentified Study Data. Personally Unidentified Study Data may include your date of birth, initials, and dates you received medical care. Personally Unidentified Study Data may also include the health information used, created, or collected in the research study. The research team or the research sponsor may share the Personally Unidentified Study Data with others to perform additional research, place it into research databases, share it with researchers in the U.S. or other countries, or use it to improve the design of future studies. They may also publish it in scientific journals, or share it with business partners of the sponsor and to file applications with U.S. or foreign government agencies to get approval for new drugs or health care products.

You do not have to sign this Consent/Authorization. If you decide not to sign the Authorization, you will not be allowed to participate in the research study.

After signing the Consent/Authorization, you can change your mind and revoke this Authorization.

If you revoke the Authorization, you will send a written letter to the Principal Investigator to inform him of your decision.
Dr. Nicholas Ah Mew
Children’s National Medical Center
Division of Genetics and Metabolism
111 Michigan Avenue, N.W.
Washington, DC 20010-2970
If you revoke this Authorization, researchers may only use and disclose the PHI that was collected for this research study before you revoked the Authorization.
If you revoke this Authorization, your PHI may still be used and disclosed if you should have an adverse event (unexpected side effect).

If you change your mind and withdraw the Authorization, you will not be allowed to participate in the study.

You will be allowed to review the information collected for this research study.

This Authorization does not expire.

If you have not already received a Notice of Privacy Practices from Children's National Medical Center, you may request a copy and will be given one. If you have any questions or concerns about your privacy rights, you may contact the Children's Hospital Privacy Officer at 301-572-6348.

Whom can I call if I have questions about this research study?

We want you to ask questions about any part of this research study at anytime,

For questions about the study or the information in this informed consent/parental permission document, call the Principal Investigator, Dr. Nicholas Ah Mew, at 202-476-5863.

Whom can I call if I have questions or concerns about my rights as a research study participant?

The Children's National Office for the Protection of Human Subjects is available to talk with you about:
Your rights as a research participant
Your concerns about the research
A complaint about the research

This is the administration office for the Institutional Review Board, which is a group of doctors, nurses, and non-medical people who review research studies for safety and the protection of people who participate in research. You can call the Office for the Protection of Human Subjects at 301-565-8452.

Children's National has a bilingual (English/Spanish) research participant and family advocate. The advocate, Dr. Tomas Silber, is here to answer your questions or concerns about taking part in this research. Dr. Silber does not work for the doctors who are doing this research and they do not pay him. He is here only to help and protect you during any research.

You may contact Dr. Silber at any time. This can be done before you decide to take part in the research, during the study, or even after you finish the study. You can call Dr. Silber at 202-476-3066 or reach him by e-mail at tsilber@childrensnational.org.

Questions, concerns, or complaints

If you have any questions, concerns or complaints about this study, call Dr. Nicholas Ah Mew at (202)476-5863.

If you have questions about your rights, general questions, complaints, or issues as a person taking part in this study, call the Children's National Medical Center Institutional Review Board at (202)476-3472.

CONSENT/PARENTAL PERMISSION:

I am the study participant or I am authorized to act on behalf of the participant.
I have read this consent form or had it read to me.
I have been invited to take part in a research study. I was told why the research is being done and how long my participation in the study is expected to last. I was told about what will happen in the study and if there are any procedures or drugs that are experimental.
I was told that taking part in this research is voluntary. I also was told that I can decide not to take part or stop being in it at any time without any penalty to me or any change to the quality of care I receive at Children's National.
I was told about the risks and possible discomforts of taking part in this research study.
I was also informed if there are any possible benefits to me if I am in this study.
I have been given the chance to ask questions about the study, and my questions have been answered. If I have questions later, I can ask one of the people listed in this form.
I agree to take part in this research study.
I will receive a copy of this Informed Consent/Parental Permission form to keep.

Signature of adult participant (18 years of age and older)

Printed Name of Participant: _____

Signature of Participant: _____

Date and Time: ____a.m. / p.m. (circle one)_____

Signature of language interpreter (if applicable)

Printed Name of Interpreter: _____

Interpreter's Signature: _____

Language: Date and Time: a.m. / p.m.(circle one)

AFFIDAVIT OF PERSON OBTAINING CONSENT / PARENTAL PERMISSION:

I certify that I have explained to the above individual(s) the nature and purpose of the study, potential benefits, and possible risks associated with participation in this study.

I have answered any questions that have been raised.

Printed Name of Person Obtaining Consent: _____

Research Role: _____

Signature: _____

Date and Time: ____a.m. / p.m. (circle one)_____