PROTOCOL

Pharmacokinetics of oral L-citrulline in infants at high risk of developing pulmonary hypertension associated with Bronchopulmonary Dysplasia

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LIST OF ABBREVIATIONS

ASL	arginine succinate lyase
ASS	argininosuccinate synthetase
BPD	bronchopulmonary dysplasia
eNOS	endothelial nitric oxide synthase
FDA	Food and Drug Administration
ICF	Informed Consent Form
iNO	Inhaled nitric oxide
iNOS	inducible nitric oxide synthase
IRB	Institutional Review Board
MELAS	mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes
NO	nitric oxide
NOS	nitric oxide synthase
nNOS	neuronal nitric oxide synthase
PAECs	Pulmonary arterial endothelial cells
PH	pulmonary hypertension
VLBW	very low birth weight

1.0 Background and Introduction

1.1 Introduction

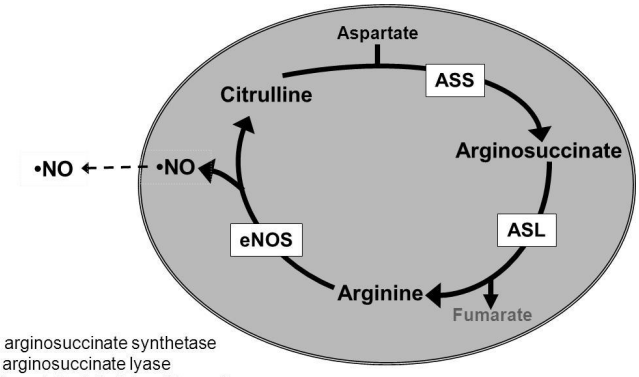
Bronchopulmonary dysplasia (BPD) is a chronic lung disease that affects up to 35% of very low birth weight infants (VLBW < 1500 g) (1, 2). Based on the current numbers of VLBW infants born annually in the U.S., between 5,000-10,000 neonates will develop BPD each year (3). It is estimated that 8-42% of infants with BPD will develop pulmonary hypertension

(PH)(4-8). Moreover, it has been known since the 1980's that echocardiographic evidence of PH in infants with BPD is associated with up to 40% mortality (9, 10).

Treatment options to ameliorate PH in infants with BPD (BPD-PH) are limited. There have been no randomized clinical trials of any therapy in infants with BPD-PH. The standard care for the management of BPD-PH is to attempt to resolve the underlying lung disorder and the judicious use of oxygen as a potent pulmonary vasodilator (11, 12). Using this management approach, which has not changed since the 1980's, the survival rates for infants with BPD-PH in the 2000's has been reported to be 64% at 6 months and 53% at 2 years after diagnosis of PH (13). The lack of improvement in outcomes for the past 3 decades has led to the widespread agreement that novel and effective therapies are desperately needed for infants with BPD-PH.

1.2 Nitric oxide metabolism

Nitric oxide (NO) is a potent vasodilator synthesized from the amino acid substrate, L-arginine, by one of three NO synthase (NOS) isoforms: neuronal NOS (nNOS), inducible NOS (iNOS), and endothelial NOS (eNOS). L-citrulline provides an intracellular source for L-arginine via a two-step biosynthetic pathway, involving the co-substrate, aspartate and the enzymes argininosuccinate synthetase (ASS) and arginine succinate lyase (ASL). Through this recycling pathway, L-citrulline serves as both a substrate for arginine and as the end product when arginine is converted to NO by NOS. Thus, L-citrulline provides an approach to deliver bioavailable L-arginine to synthesize NO. A simplified diagram of the L-citrulline-L-arginine-NO pathway is shown below:



1.3Nitric oxide and Pulmonary Hypertension

Evidence that impaired NO signaling plays a pivotal role in the pathobiology of PH in humans is now well established (14-17). There is evidence that inhaled NO (iNO) may be beneficial for the treatment of infants with BPD-PH. In particular, iNO has been shown to function as an acute pulmonary vasodilator in infants with BPD-PH (18, 19). There is also some data showing clinical improvement when iNO is used long term, for 8-90 days (20). Use of iNO is expensive and cumbersome especially when used long-term. The responsiveness to iNO

suggests that other agents that improve NO bioavailability might have beneficial effects in infants with BPD-PH.

Administering L-arginine, the amino acid substrate for NO synthesis, has been studied as a therapy to improve NO signaling and ameliorate PH. We, and other investigators, have shown that limited bioavailability of L-arginine contributes to vascular dysfunction in PH (21-23). Unfortunately, results with L-arginine supplementation have not been consistent, with some studies showing no benefit from either acute (24) or prolonged (25) L-arginine supplementation in animals or humans with PH. In addition, the feasibility and logic of chronic oral L-arginine supplementation are questionable, because the presence of arginase in gut bacteria, intestinal epithelial cells, and hepatocytes dictates that orally administered L-arginine will be largely catabolized to ornithine and urea. This catabolic loss of L-arginine necessitates the administration of massive L-arginine doses to achieve increases in circulating levels that are therapeutically effective (26). These large doses are often poorly tolerated and patient compliance can be difficult to maintain (27). Thus, alternative means of increasing NO production merit investigation.

1.4 Citrulline and Nitric Oxide

Unlike arginine, L-citrulline is not subject to presystemic breakdown in the gut. Instead, L-citrulline is produced and released into the circulation by enterocytes. Adult humans who receive oral L-citrulline achieve dramatic elevations in circulating L-citrulline, with minimal urinary losses (28-30). The bioavailability of oral L-citrulline has also been demonstrated in newborn piglets (31).

Pulmonary arterial endothelial cells (PAECs) express ASS, ASL, and eNOS and can, therefore, use L-citrulline to produce NO (32). In studies with piglet PAECs, we showed that L-citrulline treatment increases NO production in PAECs cultured under both normoxic and hypoxic conditions (33). Moreover, in our newborn piglet model of chronic hypoxia-induced pulmonary hypertension, L-citrulline supplementation was shown to increase plasma and tissue concentrations of citrulline, improve pulmonary vascular NO production, and ameliorate the development of pulmonary hypertension (31). Thus, the presence of the enzymes needed to produce L-arginine-NO from L-citrulline in PAECs affords the opportunity to use L-citrulline supplementation to increase NO bioavailability in the pulmonary vasculature and ameliorate pulmonary hypertension. Additional rationale to evaluate the impact on pulmonary hypertension from oral L-citrulline supplementation is provided by a recent study showing that plasma L-citrulline levels were lower in infants with BPD-PH than in infants with BPD and no PH (34).

1.5 Oral L-citrulline supplementation and previous human and animal studies

Long-term treatment options are needed for infants and children with chronic conditions, such as those with BPD. Intravenous therapies are difficult to maintain and are fraught with complications when used long-term. Infants and children with certain types of urea cycle defects receive oral L-citrulline replacement therapy for decades and show no evidence of toxicity from its use (35, 36). A study recently performed in children, ages 4 to 16 years, with

mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes (MELAS) syndrome reported 5 fold increases in plasma L-citrulline (increase from 21 to 156 μM) and 3 fold increases in NO synthesis with the administration of oral L-citrulline (dose of 10 g/m^2 body surface area/day if weight >20 kg and 500 mg/kg/dose if weight < 20 kg) every 4 hours for 48 hours (37). Of note, no adverse effects were found indicating that oral L-citrulline doses up to 3 g/kg/d (500 mg/kg/dose given every 4 hours) are safe and tolerated in infants and children (37). However, the pharmacokinetics of oral L-citrulline has not been evaluated in infants or children.

The role of oral L-citrulline supplementation has been under studied in infants and children with cardiovascular disease. A pilot phase two clinical trial in children with sickle cell disease showed that oral L-citrulline given twice daily in doses of 0.1 g/kg decreased vascular complications and improved the symptoms of well-being (38). Supplementation with oral L-citrulline using perioperative doses of 1.9 g/m^2 (approximately 0.13 g/kg given every 12 hours for a total of 5 doses), was found to be well tolerated and safe in children, ages 0.3-29 months, undergoing cardiopulmonary bypass at risk for development of postoperative pulmonary hypertension (39). Notably, postoperative pulmonary hypertension did not develop in those children who had naturally elevated levels of citrulline or who achieved plasma citrulline levels greater than 37 μM with the oral L-citrulline supplementation, reflecting an approx. 50-100% increase from their basal L-citrulline levels. In our newborn piglet model we found that the development of chronic hypoxia-induced pulmonary hypertension was ameliorated in piglets treated with an oral dosing strategy that achieved increases in trough plasma L-citrulline that were 50-100% above baseline levels (total daily dosing of 0.26-0.52 g/kg/d) (31, 40). However, piglets treated with a higher oral L-citrulline dose strategy (total daily dosing of 1-1.5 g/kg/d) achieved a 3-5 fold increase in trough plasma L-citrulline and had a more marked reduction in chronic hypoxia-induced pulmonary hypertension than did piglets treated with the lower dose strategy (31). These findings in our newborn pig model provide proof of concept to test the efficacy of using chronic oral L-citrulline to treat human infants at high risk of developing pulmonary hypertension in conditions that are associated with intermittent or persistent hypoxia, such as BPD. In addition, our pre-clinical studies in newborn piglets and the clinical study in infants and children undergoing cardiac bypass surgery suggest targeting a circulating plasma L-citrulline trough that is at least 100%, and perhaps 3-5 fold, above basal levels. Based on published plasma L-citrulline levels of 13-70 μM in infants and children (41), the dose and interval of L-citrulline treatment should target trough L-citrulline plasma levels of approx. 100-150 μM. Moreover, total daily doses of 3 g/kg/d (500 mg/kg/dose given every 4 hours) were recently shown to be safe and tolerated in infants and children (37).

Pharmacokinetic dose-finding, tolerability studies of oral L-citrulline in infants at high risk of developing BPD-PH are needed to determine appropriate dosing strategies (dosing ranges and treatment intervals) before use in large scale efficacy treatment trials for BPD-PH.

Of note, the first phase of this study has now been completed and the data reveals basal plasma L-citrulline levels of 9-36 μM, with a mean of 28 +/- 8 μM for infants in this study (infants at high risk of developing BPD-PH). Therefore, for infants at high risk of developing BPD-PH, the appropriate plasma L-citrulline trough to target is a level that is 50-100% above basal

levels of approx. 9-36 μM. Therefore, the trough L-citrulline plasma level to target for patients in this study has been revised from approx. 100-150 μM to approx. 50-80 μM.

1.6 Hypothesis, what is being proposed, and value provided

The hypothesis is that oral L-citrulline will be well tolerated, without significant adverse effects in infants at high risk of developing pulmonary hypertension (PH) associated with BPD. We propose to first characterize the PK profile of oral L-citrulline in order to define an appropriate dose range and treatment interval for infants at high risk of developing BPD-PH. Then using the doses and intervals generated by the PK profile, with a maximum dose of 3 g/kg/d (37), we propose to evaluate the tolerability and ability to achieve the target trough L-citrulline plasma level of approx.50-80 μM in patients at high risk of developing BPD-PH treated for 72 hours with oral L-citrulline. These studies will provide the data needed to design a full-scale randomized multi-center trial to evaluate the efficacy of oral L-citrulline therapy to ameliorate BPD-PH in human infants, a patient population that has a desperate need of new therapies.

2. Purpose and Objectives

2.1 Specific Goals of this Proposal:

The goal is to develop oral L-citrulline clinically for the treatment of pediatric pulmonary hypertension associated with bronchopulmonary dysplasia (BPD-PH); before pursuing a large scale treatment trial, pharmacokinetic dose-finding, tolerability studies in patients at high risk of developing BPD-PH are warranted.

2.2 Objectives:

2.2.1 Primary Objectives

- To characterize the pharmacokinetic profile of oral L-citrulline in infants at high risk of developing BPD-PH in order to define an appropriate dose range (dose and treatment interval that achieves trough L-citrulline levels of approx. 50-80 μM)
- To evaluate the tolerability and ability to achieve the target L-citrulline plasma level of approx.50-80 μM in patients at high risk of developing BPD-PH treated for 72 hours with oral L-citrulline

2.2.2 Secondary Objective

- To assess the effect of L-citrulline administration on urinary nitric oxide metabolite (nitrite/nitrate) levels in patients at high risk of developing BPD-PH

3. Study Population

3.1 Age of Participants:

32 +/- 1 week postmenstrual age

3.2 Sample Size:

28 participants: 10* participants for pharmacokinetic profile characterization and 18 participants for evaluating the tolerability and ability to achieve target trough plasma levels

- Group 1: 5 participants
- Group 2: 5 participants
- Group 3: 18 participants

***** Note that the total participant size for Groups 1 and 2 has been reduced from the originally proposed total of 18 participants to 10 participants. This change was made based on the single patient PK analysis on the 10 enrolled participants. This analysis was assessed to be sufficient to perform the population PK modeling needed to design the next phase of the study. Both the DSMB and the FDA approved the change in participant size.

3.3 Inclusion Criteria:

1. Infants born prematurely at ≤28 weeks gestation requiring invasive (mechanical ventilation) or non-invasive positive pressure support (nasal continuous positive airway pressure, high flow nasal cannula ≥1 lpm) at 32 +/- 1 weeks postmenstrual age
2. Tolerating at least one-half of full volume oral/gavage tube feedings (using 120 ml/kg/d as full volume oral/gavage tube feedings)
3. The continuous need for some form of respiratory support (supplemental oxygen and/or flow, or mechanical ventilation) for the prior 14 days
4. Hemoglobin ≥ 10 mg/dL

3.4 Exclusion Criteria:

1. Known major fetal anomaly or chromosomal aneupleudy
2. Clinical evidence of congenital heart disease (except patent ductus arteriosus, atrial septal defect, or ventricular septal defect [PDA, ASD, VSD])
3. Urine output < 1 ml/kg/hr
4. History of or known to have liver failure
5. History of or known to have necrotizing enterocolitis
6. History of or known to have significant feeding intolerance beyond the first week of life
7. Presence of any acute illness defined by fever >100.4F, vomiting, or diarrhea
8. Hemoglobin < 10 mg/dL
9. NICU cases determined to be futile (anticipated death prior to hospital discharge)
10. Multiple births

4. Design

Study to Assess Pharmacokinetics of L-Citrulline in infants at high risk of developing pulmonary hypertension associated with Bronchopulmonary Dysplasia

5. Study Procedures

5.1 Recruitment/Participant Identification Process:

Participants will be recruited while they are hospitalized at the Neonatal Intensive Care Nursery either at Primary Children's Hospital or University of Utah Hospital or Intermountain Medical Center Hospital. The study team will review patient medical records to determine eligibility.

5.2 Informed Consent:

5.2.1 Description of location(s) where consent will be obtained:

University of Utah Hospital Neonatal Intensive Care Nursery
Primary Children’s Hospital Neonatal Intensive Care Nursery
Intermountain Medical Center Neonatal Intensive Care Nursery

5.2.2 Description of the consent process(es), including the timing of consent:

Parents/guardians with children fitting the eligibility criteria will be approached by the PI or study coordinator and introduced to the study. During that time the study procedures and participant involvement will be thoroughly discussed, questions encouraged, and full understanding of the protocol ensured. Parents/guardians will be encouraged to take the consent form home to discuss with others before making any decision to sign the consent form. Consent from the parent/guardian and assent if

applicable will be obtained before any L-citrulline wil be administered and before any sample will be collected.

5.3 Procedures:

Consent from the parent/guardian will be obtained.

The study drug, L-citrulline, will be provided by Asklepion Pharmaceuticals.

5.3.1 Single-dose pharmacokinetic studies: Participants will be enrolled into the two groups, Group 1 (which will consist of 5 participants) and Group 2 (which will consist of 5 participants) in an alternating basis. Both Group 1 and Group 2 participants will receive a single, 150 mg/kg dose of oral L-citrulline. The L-citrulline will be provided by Asklepion Pharmaceuticals. The L-citrulline will be procured in powder form and will be solubilized in sterile water to achieve a concentration of 50 mg/ml. Therefore, 3 ml/kg of the solubilized L-citrulline (50 mg/ml) will be administered to the participant.

To assess pharmacokinetic parameters, the study team will collect three, 0.3-1.0 ml blood draws (whole blood) from infants who receive the single dose of 150 mg/kg oral L-citrulline. Blood sampling volumes will be limited to a maximum of 1 ml/kg total over a 24-48 hour period. If the participant is not nipple feeding, the dose will be delivered via the participant's indwelling gavage feeding tube.

- For Group 1: one blood draw will be pre-dose and 2 blood draws will be post-dose. The pre-dose blood draw will occur 24-48 hours prior to administering the dose of oral L-citrulline and the two post-dose draws will occur at 1 hours (+/- 10 minutes) and 2.5 hours (+/- 10 minutes) post-dose.
- For group 2: one blood draw will be pre-dose and 2 blood draws will be post-dose. The pre-dose blood draw will occur 24-48 hours prior to administering the dose of oral L-citrulline and the two post-dose draws will occur at 15 minutes (+/- 10 minutes) and 3 hours (+/- 10 minutes) post-dose

These sampling times are designed to estimate the population PK parameters of a typical infant using scaling and population PK analysis of available oral PK profiles in adults (28).

The single dose pharmacokinetic studies have been completed. PK data from 10 subjects (5 in group 1 and 5 in group 2) were analyzed and assessed as being sufficient to provide the information needed to design the next phase, the steady state pharmacokinetic studies.

5.3.2 Steady-state pharmacokinetic studies:

To evaluate the tolerability and ability to achieve target trough L-citrulline levels of 50-80 μM, an additional group of 18 infants (group 3) will be given oral L-citrulline doses at intervals over a total of 72 hours. The L-citrulline will be provided by Asklepion Pharmaceuticals. If the participant is not nipple feeding, the dose will be

delivered via the participant's indwelling gavage feeding tube. The dose and interval of L-citrulline are based on results from the studies that assessed pharmacokinetic parameters using a maximum daily dose of 3 g/kg/d. The choice of the maximum daily dose is based on a recent study that showed good tolerability and reported no adverse effects in children with mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes (MELAS syndrome) treated with oral l-citrulline at a dose of 3 g/kg/d (500 mg/kg/dose given every 4 hours) (37).

Based on results from the single dose pharmacokinetic studies, the infants in group 3 will be given oral L-citrulline doses of 60 mg/kg every 6 hours +/- 30 minutes (4 times a day) for 72 hours (total of 12 doses). This is a total daily dose of 240 mg/kg/day.

The L-citrulline will be provided by Asklepion Pharmaceuticals. The L-citrulline will be procured in powder form and will be solubilized in sterile water to achieve a concentration of 50 mg/ml. Therefore, 1.2 ml/kg of solubilized L-citrulline (50 mg/ml) will be administered to the participant every 6 hours +/- 30 minutes (4 times a day) for 72 hours. This is a total daily volume of 4.8 ml/kg/day.

For group 3, the study will collect two, 0.3-1.0 ml blood draws (whole blood) and two, 1 ml urine samples. Blood sampling volumes will be limited to a maximum of 1 ml/kg total over a 24 hour period. One blood draw will occur between 10 minutes and 6 hours prior to administering the first dose of oral L-citrulline and the second blood draw will occur 10 to 30 minutes prior to administering the last dose of oral L-citrulline. These sampling times are designed to assess baseline and trough L-citrulline plasma levels. One urine sample will be collected on the day prior to administering the first dose of oral L-citrulline and the second urine sample will be collected between 4 and 8 hours following the last dose of oral L-citrulline. These urine collection times are designed to assess baseline levels of nitric oxide metabolites (nitrite/nitrate) in the urine and assess whether there is an increase in levels of nitric oxide metabolites in the urine in response to 72 hours of L-citrulline dosing.

5.3.3 Blood Samples

Blood samples will be processed and the plasma harvested into labeled tubes which are stored in a designated freezer section at -80 degrees C, where they will be collected by study personnel. For each of the blood samples we will test for plasma citrulline and arginine levels. Analysis will be done by ARUP.

5.3.4 Urine samples

Each 1 ml urine samples will be aliquoted into 2 separate tubes (0.5 ml urine per tube), then stored in a designated freezer section at -80 degrees C, where they will be collected by study personnel. One of the aliquoted urine samples will be tested for NO metabolites (nitrites/nitrates) and the second aliquoted urine sample will be tested for Cr (to normalize the sample). Urine nitrite/nitrate analysis will be done in Dr. Fike's laboratory. Urine Cr analysis will be done by ARUP.

5.3.5 Additional data

Additional data collected will include demographics; vital signs including weight, length, heart rate, respiratory rate, temperature, and blood pressure; dietary/nutrition information; concomitant medications. For the Group 1 and Group 2 participants (single dose PK studies), the blood pressure will be monitored at least 3 times after the participant receives the study drug. For the Group 3 participants (steady state PK studies), the blood pressure will be monitored at least 4 times daily for 4 days (72 hours when the participant is receiving the study drug and 12 hours after last dose). The heart rate, respiratory rate, temperature, and blood pressure will be monitored for 12 hours following the last dose of L-citrulline.

5.3.6 Participant monitoring

All participants will be monitored for clinical evidence of necrotizing enterocolitis, gastrointestinal bleeding, sepsis, feeding intolerance (including vomiting and diarrhea), electrolyte disturbances, hemodynamics, alteration of hematological parameters and death. Any electrolytes, hematologic laboratories, and eye examinations that are ordered as part of standard of care by the participants clinical care team will be monitored. Hypotensive subjects (defined as a decrease in mean arterial blood pressure of more than 25% baseline) will be treated with pharmacological/volume supplementation interventions at the discretion of the attending physician. Treatment unresponsive hypotensive subjects will be withdrawn from the study. Subjects who develop significant feeding intolerance will be withdrawn from the study. Infants who are made NPO for a time period long enough that they miss any doses of L-citrulline will be withdrawn from the study.

6. Statistical Methods, Data Analysis and Interpretation

After the first 3 participants are enrolled in the single-dose PK study (3 participants enrolled in either Group 1 or Group 2), and after the first 3 participants are enrolled in the steady-state pharmacokinetic study (3 participants enrolled in Group 3), an initial PK analysis will be performed and the data will be reviewed by the DSMB to assess for safety to continue the single dose PK study and to continue the steady-state pharmacokinetic study.

Throughout the study, participants who are withdrawn from treatment due to adverse events will be replaced for PK analysis.

Data collected from this study will be examined to ensure that all participants in the analysis meet inclusion criteria. Summary statistical analysis will be conducted to describe participant demographics, citrulline dosing, and participant PK profiles. Outliers in the measured citrulline levels that may influence the estimation of PK parameters will be evaluated. A population model will be developed to describe the citrulline PK and population variability.

The non-linear mixed effect modeling software NONMEM® version 7.3.0 (ICON Development Solutions, Ellicott City, MD, USA) will be used to develop models. Perl-speaks-

NONMEM (PsN) 4.4.0, Pirana 2.9.0, and R packages (version 3.2.1) will be used to facilitate, evaluate, and visualize the model development.

The model development process would include two major steps: base model structure evaluation and covariate screening. The development of a base structural model will include the evaluation of compartment numbers, baseline effects, absorption and elimination kinetics. The stochastic models describing between-subject variability in PK parameters and intra-subject variability in observations will be also examined.

Potential covariates that are predictive of citrulline PK variability will be screened using the stepwise covariate model (scm) building tool of PsN. The continuous covariates to be examined will include, but not be limited to, body weight, length, postnatal age, gestational age, etc. Categorical covariates to be examined will include, but not be limited to, sex, race, ethnicity, and concomitant medication (yes/no). The predefined parameter-covariate association for continuous covariates will be included as a power relation, while categorical covariates will be linearly included. Both stepwise forward selection and backward elimination will be incorporated for covariate searching. Changes in OFV will be considered significant at $p<0.05$ (χ2-distribution, 1 df, $\Delta OFV > 3.84$) during forward selection, and $p<0.01$ (1 df, $\Delta OFV >6.63$) during backward elimination. In addition to the aforementioned statistical criteria, the following standard diagnostic plots will also be used to visually assess the final covariate model fit: observed concentrations versus population- and individual-predicted concentrations, conditional weighted residuals versus time and population-predicted concentrations. To assess the model stability, a nonparametric bootstrap analysis will be conducted. The visual predictive check (VPC) will be performed to further evaluate the final model performance.

Once the final population PK model is determined, an optimal dosing plan will be developed using the model to achieve target plasma trough concentrations of 50-80 μM in patients at high risk of developing BPD-PH. Statistical comparison between actual trough concentrations and model predicted concentrations will be conducted. Model prediction capability will be evaluated and refinement will be performed.

The final population PK model has been determined and the optimal dosing plan has been developed to target plasma trough concentrations of 50-80 μM (trough plasma concentrations that are based on data from the single dose PK study and that are appropriate for infants in this study). The optimal dosing plan is to give oral L-citrulline doses of 60 mg/kg every 6 hours +/- 30 minutes (4 times a day) for 72 hours (total of 12 doses). This is a total daily dose of 240 mg/kg/day.

6.1 Data and Safety Monitoring Board

This study will use a Data and Safety Monitoring Board (DSMB). The plan is to perform an initial PK analysis after the first 3 participants are enrolled in the single dose PK arm and also after the first 3 participants are enrolled in the steady-state pharmacokinetic arm. The data will be reviewed by the DSMB to assess for safety to continue the single-dose pharmacokinetic study and to continue the steady-state pharmacokinetic study**.**

7. Protection of Human Subjects

7.1 Investigator Responsibilities

The investigator is responsible for ensuring that the study is performed in accordance with the protocol, current ICH guidelines on Good Clinical Practice (GCP), and applicable regulatory and country-specific requirements.

Good Clinical Practice is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects.

Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the study data are credible.

7.2 Institutional Review Board and FDA Submissions

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved; a determination will be made regarding whether previously consented participants need to be re-consented.

During the study, the investigator will send the following documents and updates to the FDA for their review and the IRB for their review and approval, where appropriate:

- Protocol amendments (excluding the ones that are purely administrative, with no consequences for subjects, data, or study conduct).
- Revision(s) to ICF and any other written materials to be provided to subjects.
- Summaries of the status of the study at intervals stipulated in guidelines of the IRB and the FDA (at least annually).
- Reports of AEs that are serious, unlisted/unexpected, and associated with the study drug.
- New information that may adversely affect the safety of the subjects or the conduct of the study.
- Deviations from or changes to the protocol to eliminate immediate hazards to the subjects.
- Report of deaths of subjects under the investigator's care.
- Notification if a new investigator is responsible for the study at the site.
- At least once a year, the IRB will be asked to review and reapprove this study, where required. An annual report will be submitted to the FDA.
- At the end of the study, the investigator will notify the IRB and the FDA about the study completion

7.3 Informed Consent Form

Written permission from parents or legal guardians will be required for participation. Parental consent will also be required. After determining that a subject is eligible, the site investigator or designee will approach the parent or legal guardian to offer participation in the study. The parent or legal guardian will be informed about the objectives of the study and the potential risks and benefits of participation. If the parent or legal guardian refuses consent to participate OR refuses permission for their infant to participate, then all clinical management for the infant will be provided by the clinical staff in accordance with institutional practice and judgment.

7.3.1 Child Assent

Subjects who are eligible for this study are under 6 months of age and assent is not applicable.

7.4 Adverse Event Reporting

7.4.1 Assessment of Safety

Safety assessments will consist of monitoring and reporting adverse events (AEs) and serious adverse events (SAEs) per protocol for 48 hours after the last study drug dose. This includes all events of death, and any study specific issue of concern.

7.4.2 Adverse Events

Investigators must report all potential unanticipated problems and events to the IRB. Unanticipated problems (UPs) are defined as *any incident, experience or outcome that meets all of the following criteria:*

- **Unexpected** (unforeseen by the researcher or the research participant) in terms of nature, severity, or frequency, given the research procedures and the subject population being studied;

and

- **Related or probably** related to participation in the research, or if the event or problem probably or definitely affects the safety, rights and welfare of current participants;

and

- Suggests that the research places subjects or others at a **greater risk of harm** (including physical, psychological, economic or social harm) than was previously known or recognized.

The Investigator must consider each problem, event, or new information and decide whether or not it represents a UP, using the criteria above.

In fatal or life-threatening serious adverse events, either unexpected and unrelated, or suspected to be related to the study, notification will be sent to the DSMB Chair, the IRB, and NHLBI program office within 7 calendar days of the PI becoming aware of the event.

In non-fatal, non-life-threatening serious adverse reactions either unexpected and unrelated, or suspected to be related to the study, notification will be sent to the DSMB Chair, the IRB, and NHLBI program office within 14 calendar days of the PI learning of the event.

In both cases, the DSMB chair will review the event, determine if a full DSMB meeting should be held, and will respond to the PI and NHLBI program office with recommendations within 3 business days of notification.

Unanticipated problems that are not a serious adverse event (SAE) will be reported to the IRB, DSMB, and NHLBI within 14 days of the PI becoming aware of the problem.

If UP Criteria Are Not Met:

1. Document in the research record how the problem/event/information does not meet the UP Criteria.
2. Summarize the problem/event/information in the next Continuing Review application.

Examples of Potential Unanticipated Problems

- A breach of confidentiality or privacy that involves real or potential risk (e.g. unauthorized use of disclosure of protected health information.)
- Data and safety monitoring reports that indicate that frequency or magnitude of harms or benefits may be different than initially presented to the IRB
- A publication that shows that the risks or potential benefits or the research may be different than initially presented to the IRB
- Change in FDA labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol
- Complaints from participants or others involved in the research that indicate unexpected risks or cannot be resolved by the research team
- Warning or determination letters issued by any funding agency or regulatory body including Office of Human Research Protections (OHRP), Department of Health and Human Services (DHHS), Food and Drug Administration (FDA)

7.4.3 Mandatory FDA Safety Reporting

Initial reporting:

The IND application sponsor investigator must report any suspected adverse reaction or adverse reaction to study treatment that is both serious and unexpected as soon as possible but no later than within **15 calendar days** following the sponsor's initial receipt of the information

Unexpected fatal or life-threatening suspected adverse reactions represent especially important safety information and must be reported to FDA as soon as possible but no later than **7 calendar days** following the sponsor's initial receipt of the information.

- ***Follow-up reporting:*** Any relevant additional information obtained by the sponsor that pertains to a previously submitted IND safety report must be submitted as a Follow-up IND Safety Report. These reports should be submitted without delay, as soon as the information is available but no later than 15 calendar days after the sponsor receives the information.

7.4.4 Deviation Reporting

A **deviation** is ANY departure from the defined procedures and treatment plans as outlined in the protocol version or application version submitted and previously approved by the Institutional Review Board (IRB). Deviations have the potential to place participants at risk and can also undermine the scientific integrity of the study thus jeopardizing the justification for the research.

Deviations are *unplanned* and/or *unintentional* events. Any changes in the IRB-approved research protocol should not be initiated without submission of an amendment for IRB review and approval. If such changed are initiated before IRB approval for the change is given, a deviation has occurred.

Criteria for Reporting Deviations

The University of Utah IRB requires researchers to submit deviations that meet **one or more** of the following criteria:

- **Intended to eliminate apparent immediate hazard** to a research participant (such as changing the dose of a medication due to possible toxicity);

or

- **Caused possible harm** to participants or others, or places them at increased risk of harm - including physical, psychological, economic, or social harm, such as a breach of confidentiality;

or

- **Possible serious or continued non-compliance** (such as a deviation that has happened previously and is now being repeated).
    - **Serious non-compliance** is an act or omission to act that resulted in significant harm (physical, psychological, safety, or privacy) or significantly increased the possibility of harm to the rights and welfare of research participants.
    - **Continuing non-compliance** is a pattern of repeated actions or omissions to act that suggests a future likelihood of reoccurrence and that indicates a deficiency in the ability or willingness to comply with Federal regulations, VHA Handbooks or the policy, requirements, and determinations of the IRB governing human subject research.

Some deviations pose no conceivable threat to participant safety or scientific integrity. For example, when the subject misses a clinic visit and the only available re-schedule date is outside the study visit window but no study procedures or medication doses are missed. In this case, the subject may not incur possible harm from a missed dose or missed procedures meant to maintain or evaluate the subject's safety and welfare. As such, reporting is left to the discretion of the PI within the context of the guidelines above. Though a deviation may not pose a conceivable threat or possible harm, it may represent possible continuing non-compliance.

The Investigator must consider each deviation and decide whether or not it meets the reporting criteria above. If the reporting criteria are met, the report must be submitted promptly, within 10 working days from the time the investigator learns of the deviation.

If criteria are not met,

a. Document in the research record how the deviation does not meet the reporting criteria.
b. Log the deviation such that it can be evaluated in the future if other deviations occur.

Any deviations that do not require a change in protocol or consent documents will be submitted to the FDA in the annual report.

8.0 Study Records Retention

Study documents will be retained for a minimum of 2 years after the end of the study.

9. References

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