A Phase III Double-Blind, Randomized, Placebo Controlled, Multi-Center Clinical Study to Evaluate the Efficacy and Safety of Intravenous L-citrulline for the Prevention of Clinical Sequelae of Acute Lung Injury induced by Cardiopulmonary Bypass in Pediatric Subjects Undergoing Surgery for Congenital Heart Defects (Protocol CIT-003-01)

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Protocol Approval

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Protocol Title: A Phase III Double-Blind, Randomized, Placebo Controlled, Multi-Center Clinical Study to Evaluate the Efficacy and Safety of Intravenous L-citrulline for the Prevention of Clinical Sequelae of Acute Lung Injury Induced by Cardiopulmonary Bypass in Pediatric Subjects Undergoing Surgery for Congenital Heart Defects

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These signatures constitute approval of this protocol and an assurance that this study will be conducted according to all requirements of this protocol.

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Table of Contents

List of Abbreviations and Definition of Terms

%	=	per cent
+/-	=	plus/minus
ABG(s)	=	arterial blood gas(es)
ACT	=	activated clotting time
AE(s)	=	adverse event(s)
ALI	=	acute lung injury
ALT	=	alanine aminotransferase
ANOVA	=	analysis of variance
AP	=	alkaline phosphatase
API	=	active pharmaceutical ingredient
ASD	=	atrial septal defect
ASL	=	argininosuccinate lyase
ASS	=	argininosuccinate synthetase
AST	=	aspartate aminotransferase
AV	=	atrioventricular
AVSD	=	atrioventricular septal defect
BPAP	=	bilevel positive airway pressure
BP	=	blood pressure
BDRM	=	blind data review meeting
BUN	=	blood urea nitrogen
°C	=	degrees Celsius
Ca	=	calcium
CBC	=	complete blood count
cc(s)	=	cubic centimeter(s)
CFR	=	Code of Federal Regulations
cGCP	=	current Good Clinical Practice
cGMP	=	cyclic guanosine monophosphate
Cl	=	chloride

CPAP	=	continuous positive airway pressure
CPB	=	cardiopulmonary bypass
CPMP	=	Committee for Proprietary Medicinal Products
CPSI	=	carbamoyl phosphate synthetase I
CRA(s)	=	Clinical Research Associate(s)
CRF	=	case report form
CRO	=	contract research organization
CVP	=	central venous pressure
DSMB	=	data safety monitoring board
eCRF	=	electronic case report form
EDC	=	electronic data capture
EU	=	European Union
°F	=	degrees Fahrenheit
FAS	=	full analysis set
FDA	=	Food and Drug Administration
g/m^2	=	grams per square meter
HCO_3	=	bicarbonate
hr(s)	=	hour(s)
ICF	=	informed consent form
ICH	=	International Conference on Harmonisation
ICU	=	intensive care unit
IEC	=	Independent Ethics Committee
IMP	=	investigational medicinal product
iNO	=	inhaled nitric oxide
IRB	=	Institutional Review Board
ITT	=	intent-to-treat
i.v.	=	intravenous
IWRS	=	interactive web response system
K	=	potassium

L-	=	levorotatory
LOCF	=	last observation carried forward
LDH	=	lactate dehydrogenase
LFT(s)	=	liver function test(s)
MAP	=	mean arterial (blood) pressure
MedDRA	=	Medical Dictionary for Regulatory Activities
mFAS	=	modified full analysis set
Mg	=	magnesium
mg/kg	=	milligram(s) per kilogram(s)
mg/kg/hr	=	milligram(s) per kilogram(s) per hour
mg/mL	=	milligram(s) per milliliter
mL	=	milliliter(s)
mm	=	millimeter(s)
N	=	number of subjects in the dataset
Na	=	sodium
NO	=	nitric oxide
NOS	=	nitric oxide synthase
O_2	=	oxygen
OR	=	operating room
OTC	=	ornithine transcarbamylase
PA	=	pulmonary artery
$PaCO_2$	=	arterial carbon dioxide partial pressure
PaO_2	=	arterial oxygen pressure
PAP	=	pulmonary artery pressure
pH	=	hydrogen ion concentration
PICU	=	pediatric intensive care unit
PK	=	pharmacokinetics
PP	=	per protocol analysis set
PT	=	preferred term

PVT	=	pulmonary vascular tone
QA	=	quality assurance
Qhr	=	every hour
RBC	=	red blood cell
SAE	=	serious adverse event
SAF	=	safety analysis set
SaO_2	=	arterial oxygen percent saturation
SAP	=	statistical analysis plan
SAS	=	Statistical Analysis System
sem	=	standard error of the mean
SOC	=	system organ class
SOP(s)	=	standard operating procedure(s)
SUSAR(s)	=	suspected unexpected serious adverse reaction(s)
TPN	=	total parenteral nutrition
µmol/L	=	micromole(s) per liter
US	=	United States
vs	=	versus
VSD	=	ventricular septal defect
WBC	=	white blood cell
WHO	=	World Health Organization
WMA	=	World Medical Association

Protocol Synopsis

Protocol Number	CIT-003-01
Protocol Title:	A Phase III Double-Blind, Randomized, Placebo Controlled, Multi-Center Clinical Study to Evaluate the Efficacy and Safety of Intravenous L-citrulline for the Prevention of Clinical Sequelae of Acute Lung Injury induced by Cardiopulmonary Bypass in Pediatric Subjects Undergoing Surgery for Congenital Heart Defects.
Investigational Medicinal Product	L-citrulline
Number of Study Sites	Approximately 28 investigative sites in the U.S., Europe and Israel
Phase	III
Time Schedule	Study Start: First subject signs informed consent Study End: Last subject last visit
Coordinating Investigator:	Frederick E. Barr, MD
Objectives:	Primary: The primary objective of the study will be to determine if intravenous (i.v.) L-citrulline delivery given perioperatively reduces clinical sequelae of acute cardiopulmonary bypass-induced lung injury in pediatric subjects undergoing repair of congenital heart defects as evidenced by the reduction of post-operative need for mechanical ventilation and inotrope therapy. Secondary: ▪ To evaluate the safety of L-citrulline compared with placebo ▪ To evaluate the effect of L-citrulline on post-operative hemodynamic improvement ▪ To evaluate the effect of L-citrulline on the usage and length of time on vasodilators ▪ To evaluate the effect of L-citrulline on length of hospitalization ▪ To evaluate the effect of L-citrulline on the duration of

	chest tube placement ▪ To compare the plasma levels of L-citrulline between the two treatment groups
Study Design:	This is a randomized, double-blind, placebo controlled, multi-center study that will compare the efficacy and safety of L-citrulline versus placebo in subjects undergoing surgery for congenital heart defects. Eligible subjects undergoing repair of a large unrestrictive ventricular septal defect (VSD), a partial or complete atrioventricular septal defect (AVSD), or an ostium primum atrial septal defect (primum ASD) will be eligible for enrollment in this study. Each enrolled subject will be randomized to receive either L-citrulline or placebo throughout all administrations in the study. Subjects will receive an L-citrulline bolus of 150 mg/kg or placebo at the initiation of cardiopulmonary bypass, the addition of L-citrulline at a concentration of 200 µmol/L or placebo given as a bolus during cardiopulmonary bypass, an L-citrulline bolus of 20 mg/kg or placebo 30 minutes after decannulation from cardiopulmonary bypass, followed immediately by a 9 mg/kg/hr continuous L-citrulline infusion or placebo for up to 48 hours. The study drug or placebo infusion will be discontinued once invasive arterial blood pressure monitoring is discontinued or at 48 hours, whichever comes first. Subjects will be followed until Day 28 or discharge from the hospital, whichever comes first. For subjects discharged prior to Day 28, a final assessment via telephone will be conducted at Day 28.
Number of Subjects	Approximately 450 subjects will be screened, with 190 randomized to either L-citrulline or placebo in a 1:1 ratio.
Study Treatments:	Eligible subjects will be randomized in a 1:1 fashion to either L-citrulline or placebo and receive the following: ▪ Bolus of 150 mg/kg at the initiation of cardiopulmonary bypass, but after removal of any crystalloid base; • Addition of study medication at a concentration of 200 µmol/L given as a bolus during bypass. This may be administered as a contemporaneous one-time bolus or multiple administrations to compensate for fluids containing L-citrulline that may be removed from the patient during the course of the operation and thus to

	maintain the concentration of 200 µmol/L; ▪ Bolus of 20 mg/kg 30 minutes after decannulation from cardiopulmonary bypass; ▪ 9 mg/kg/hr continuous infusion for up to 48 hours.
Study Duration	The study will consist of an initial screening phase, followed by enrollment of eligible subjects to the treatment phase on Day 0 (Day of Surgery) and will end once study medication is discontinued: at 48 hours or upon removal of the arterial line. Subjects will be followed until Day 28 or discharge from the hospital, whichever comes first. For subjects discharged prior to Day 28, a final assessment via telephone will be conducted at Day 28. The study is planned to be conducted over an 11-month enrollment period.
Subject Selection:	Inclusion Criteria: • Subjects or parents or legal guardian of the subject who are willing and able to sign consent • Male and female subjects aged ≤18 years of age • Infants, children and adolescents undergoing cardiopulmonary bypass for repair of a large unrestrictive VSD, an ostium primum ASD or a partial or complete AVSD • Pre-operative echocardiogram which confirms the cardiovascular anatomy and defect to be surgically repaired Exclusion Criteria: • Evidence of pulmonary artery or vein abnormalities on the pre-operative echocardiogram that will not be addressed surgically. Specific abnormalities excluded include the following: ▪ Significant pulmonary artery narrowing not amenable to surgical correction ▪ Previous pulmonary artery stent placement ▪ Significant left-sided atrioventricular (AV) valve regurgitation not amenable to surgical correction ▪ Pulmonary venous return abnormalities not amenable to surgical correction ▪ Pulmonary vein stenosis not amenable to surgical correction • Preoperative requirement for mechanical ventilation or

	intravenous inotrope support • Presence of fixed or idiopathic pulmonary hypertension (i.e. Eisenmenger's Syndrome) prior to surgical repair • Pre-operative use of medications to treat pulmonary hypertension • Pregnancy; Sexually active females of child-bearing potential must be willing to practice an acceptable method of birth control for the duration of study participation (e.g. oral contraceptive, hormonal implant, intra-uterine device) • Participation in another clinical trial within 30 days of Screening or while participating in the current study, including the 28 days of follow-up post study drug administration. • Any condition which, in the opinion of the investigator, might interfere with the study objectives
Endpoints:	Primary Efficacy Endpoint: A composite variable consisting of the longer of either (1) length of time on mechanical ventilation or (2) length of inotrope use. The definition of mechanical ventilation shall include invasive and non-invasive mechanical ventilation including bilevel positive airway pressure (BPAP) or continuous positive airway pressure (CPAP). Secondary Efficacy Endpoints: ▪ Length of time on mechanical ventilation ▪ Length of time on positive pressure ventilation ▪ Length of time of inotrope use ▪ Inotrope score calculated each hour post-operatively from the time of separation from bypass until the completion of study drug. Additionally, the total inotrope score over time until Day 28 or hospital discharge will be derived. ▪ Hemodynamic improvement (heart rate, systemic arterial blood pressure, oxygen saturation, and central venous pressure) ▪ Length of time of intubation ▪ Length of PICU stay

	▪ Length of time on vasodilators ▪ Length of hospitalization ▪ Thoracotomy output; duration and volume of chest tube drainage ▪ Plasma concentrations of L-citrulline
Safety Assessments	▪ Adverse events ▪ Incidence of refractory hypotension ▪ Laboratory values
Statistical Methods:	Continuous variables will be summarized with means, standard deviations, medians, lower and upper quartiles, minimums and maximums. Frequencies and percentages will be used to summarize categorical variables. For time-to-event analyses censoring information will be used. No other imputation of missing values (e.g., no last observation carried forward (LOCF)) will be used, unless stated otherwise. Analysis sets: *Safety Analysis Set (SAF)*: All randomized subjects who received surgery and study medication (independent of whether it is L-citrulline or placebo) will be valid for the SAF. The SAF will be used for the evaluation of the safety assessments. *Full Analysis Set (FAS)*: The FAS includes all subjects who received surgery. The FAS serves as the primary efficacy analysis set. *Modified Full Analysis Set (mFAS)*: The mFAS includes all subjects who received surgery and study medication (independent of whether it is L-citrulline or placebo). The mFAS serves as a sensitivity population for the efficacy analyses. *Per-protocol Analysis Set (PP)*: The PP includes all subjects included in the SAF who had no significant protocol deviations and completed through Day 28 or until discharge from the hospital (end of study follow-up). The PP will only be analyzed for main efficacy outcome measures.

	ANALYSIS OF PRIMARY EFFICACY ENDPOINT: Hypothesis to be tested: H0: Composite endpoint within the L-citrulline group is equal to that of the placebo group H1: Composite endpoint within the L-citrulline group is unequal to that of the placebo group H0 will be tested using a Wilcoxon-rank-sum test. The level of significance is 1% (two-sided). 95% confidence intervals using Hodges-Lehman method will be additionally calculated. Survival analyses using Kaplan-Meier estimates and plots, log-rank test and Cox-regression modeling will serve as sensitivity analyses. Additional sensitivity analyses will be performed. ANALYSIS OF SECONDARY EFFICACY ENDPOINTS: No formal statistical hypotheses are specified for the secondary endpoint variables. Nonetheless statistical testing methods will be applied to compare treatment groups. A significance level of 5% (two-sided) will be used for such analyses. Secondary efficacy endpoints will be analyzed using the FAS, mFAS and the PP. *Length of time on mechanical ventilation* and *length of time on positive pressure ventilation:* The same analyses as described for the primary endpoint will be applied. *Length of Time of Inotrope Use:* The same analyses as described for the primary endpoint will be applied. *Inotrope Score:* The total inotrope score at each hour post-operatively from the time of separation from bypass, until the completion of study drug will be calculated. A Wilcoxon-rank-sum test, an ANOVA and a repeated measures analysis of variance will be applied. Additionally, the total inotrope score over time until Day 28 or hospital discharge will be derived. *Hemodynamic Improvement:* The absolute changes in hemodynamic parameters from baseline at hours 1, 2, 4, 12, 24, and 48 will be compared

	between groups using an analysis of variance (ANOVA) with a fixed effect for treatment group and baseline level. *Length of Time of Intubation:* For the length of time of intubation, the same analyses as described for the primary endpoint will be applied *Length of PICU stay:* For the length of PICU stay, the same analyses as described for the primary endpoint will be applied. *Length of Time on Vasodilators:* For the length of time on vasodilators the same analyses as described for the primary endpoint will be applied. *Length of Hospitalization:* For the length of hospitalization, the same analyses as described for the primary endpoint will be applied. *Thoracotomy Output: Duration and Volume of Chest Tube Drainage:* For the total postoperative duration of chest tube drainage, the same analyses as described for the primary endpoint will be applied. The total amount of chest tube drainage in milliliters (mL), added up during each duration of chest tube placement, will be summarized using descriptive measures and compared between groups using an ANOVA with a fixed effect for treatment group. *L-citrulline Plasma Levels:* L-citrulline blood levels will be obtained on each patient at set times, and plotted and examined to confirm that they are above the threshold level of 100 µmol/L. ANALYSIS OF SAFETY ENDPOINTS: Safety endpoints will be analyzed using the SAF. *Adverse Events:* Adverse events (AEs) will be summarized. No statistical testing procedures will be applied to the analysis of AEs. *Incidence of Refractory Hypotension:* The number of subjects with any refractory hypotension from

	end of surgery until 48 hours will be compared between groups using Fishers' exact test. Additionally, a logistic regression analysis will be applied including factor treatment and site. *Laboratory Values:* The absolute values and the absolute and percentage changes from baseline will be tabulated using summary tables describing descriptive measurements for all observed time points. No statistical testing procedures will be applied to the analysis of laboratory values.
Sample Size Estimation	A sample size of 92 patients in each group will have 90% power to detect a difference between L-citrulline and placebo on the primary endpoint, using a Wilcoxon-rank-sum test with a 0.01 two-sided significance level. To allow for subjects being enrolled but not assigned to the full analysis set, an overall N=95 subjects per group will be enrolled.
Interim Analysis:	One interim analysis is planned to be carried out when the first 95 consecutively randomized full analysis set (FAS)-patients (i.e. 50% of the planned patients) will have undertaken their Follow up visit (Day 28 or Hospital Discharge).

Schedule of Study Assessments and Procedures

	Pre-Treatment		Treatment						Follow-Up	
	Screen	Baseline Day of Surgery	Surgical Observation	Post-Operative Surgical Observation					Day 28 or Hospital Discharge	Day 28 Phone Assessment
	Days -14 to 0	Day 0 Pre-Op	Day 0 Intra-Op	Day 0- 0 hrs (30 min post decannulation)	Day 0 6 hrs	Day 0 12 hrs	Day 1 24 hrs	Day 2 48 hrs		
Informed Consent/Assent	x									
Eligibility Assessment	x									
Demographic Data	x									
Medical History	x									
Physical Examination (Pre-op)	x									
Body Weight and Height	x	x[3]								
Inotrope & other concomitant medications, incl. vasodilators	x	x	x	x →						
Blood draw for serum electrolytes, BUN, and creatinine	x[1]		x[5]				x[15]	x[15]	x[16]	
CBC with differential	x[1]		x[5]				x[15]	x[15]	x[16]	
Blood Sampling for Coagulation: ACT	x[1]		x[5]				x[15]	x[15]		
LFTs	x[1]							x[15]	x[16]	
Urinalysis	x[2]						x[2]			
Urine pregnancy test for females of child-bearing potential		x								
Randomization		x								

	Pre-Treatment		Treatment						Follow-Up	
	Screen	Baseline Day of Surgery	Surgical Observation	Post-Operative Surgical Observation					Day 28 or Hospital Discharge	Day 28 Phone Assessment
	Days -14 to 0	Day 0 Pre-Op	Day 0 Intra-Op	Day 0- 0 hrs (30 min post decannulation)	Day 0 6 hrs	Day 0 12 hrs	Day 1 24 hrs	Day 2 48 hrs		
Pharmacy Prep of Study Drug		x								
Plasma L-citrulline Collection		x[4]	x[5]	x[10]; x[12]	x[14]	x[14]	x[15]	x[15]	x[16]	
Adverse Events		x	x	x →						x
Cardiopulmonary Bypass Data			x[6]							
Hemodynamic Monitoring			x[7]	x →						
ABGs			x[8]	x →						
Thoracotomy Output				x →						
Administration of Study Drug			x[9]	x[11] →						
Hypotension Assessment				x →						
Ventilator Settings				x[13] →						
Phone Assessment										x[17]

[1]Results of laboratory tests done as standard of care for pre-op testing are acceptable and may be recorded as screening values, provided the tests were performed within 14 days of Day 0. If any of the required tests were not done as part of the pre-op testing, these must be completed within 14 days of Day 0.

[2]Urinalysis (not required, but results should be recorded if test is performed).

[3] Weight only

[4] Collect 1.0 mL of blood for plasma L-citrulline levels (***prior to surgery***). All L-citrulline plasma samples must be processed within four hours of being drawn, stored at -20°F (-28.8°C) or below, and sent on dry ice to the central lab.

[5] Blood collected for intra-op laboratory values and plasma L-citrulline levels (***10 minutes after study drug administered***); (1.0 mL for plasma L-citrulline). All L-citrulline plasma samples must be processed within four hours of being drawn, stored at -20°F (-28.8°C) or below, and sent on dry ice to the central lab.

[6] Cardiopulmonary bypass data include: Equipment type, cardioplegia solution type, composition of isovolumetric exchange fluid for ultrafiltration and/or modified ultrafiltration, exchange volumes in and out, documentation that L-citrulline or placebo was added to the filtration and hemoconcentration fluids, cardiopulmonary bypass (CPB) start and end times and decannulation time.

[7] Hemodynamic monitoring includes heart rate, systemic BP, O_2 saturation, CVP, and PAP measurements (if data is available). **Baseline values are first recorded in the operating room (OR)** by Anesthesia. Results will be *monitored* during surgery according to the Institution's standard of care, but results will only be *recorded* approximately every 15 minutes. Standard of care will continue to be followed from time of post-op admission to the PICU through 48 hours. Results at 1, 2, 4, 12, 24, and 48 hours *from baseline (in OR)* must be obtained and recorded.

[8] Arterial Blood Gas (ABG) includes PaO_2, $PaCO_2$, HCO_3, and pH. ABGs will be drawn according to the Institution's standard of care. Baseline results are first recorded in the OR by Anesthesia. All results obtained during surgery, and from the time of post-op admission to the PICU until removal of the arterial line or through 48 hours (whichever occurs first), will be recorded.

[9] Begin bolus of intravenous (i.v.) L-citrulline at 150 mg/kg or placebo at initiation of CPB & add L-citrulline at a concentration of 200 µmol/L or placebo given as a bolus during bypass. The 200 µmol/L dose may be administered as a one-time bolus or multiple administrations, to maintain the concentration at 200 µmol/L.

[10] Collect 1.0 mL of blood for plasma L-citrulline levels (***30 minutes after decannulation from CPB in OR; prior to bolus)***. All L-citrulline plasma samples must be processed within four hours of being drawn, stored at -20°F (-28.8°C) or below, and sent on dry ice to the central lab.

[11] Bolus of 20 mg/kg of L-citrulline or placebo 30 minutes after decannulation from CPB immediately followed by a continuous infusion of L-citrulline at 9 mg/kg/hr or placebo

[12] Collect 1.0 mL of blood for plasma L-citrulline levels (***5 minutes after bolus***). All L-citrulline plasma samples must be

processed within four hours of being drawn, stored at -20°F (-28.8°C) or below, and sent on dry ice to the central lab.

[13] Recorded approximately every hour (Qhr) (+/- 10 minutes) from time of separation from CPB until discontinuation of mechanical ventilation

[14] Collect 1.0 mL of blood for plasma L-citrulline levels (***at 6 hours and 12 hours post-operatively; +/- 1 hr. If continuous i.v. L-citrulline infusion discontinued before 6 or 12 hour timepoint, collect blood within +/- 1 hr of end of infusion.)***. All samples must be processed within four hours of being drawn, stored at -20°F (-28.8°C) or below, and sent on dry ice to the central lab.

[15] Blood collected for post-op laboratory values and plasma L-citrulline levels (***24 hours and 48 hours post-operatively; +/- 1 hr. If continuous i.v. L-citrulline infusion discontinued before 24 or 48 hour timepoint, collect blood within +/- 1 hr of end of infusion.)***; (1.0 mL for plasma L-citrulline). All samples must be processed within four hours of being drawn, stored at -20°F (28.8°C) or below, and sent on dry ice to the central lab.

[16] An attempt should be made to obtain final labs on Day 28 or prior to discharge from the hospital. If lab results within 24 hours are available, these may be captured. If results are not available within 24 hours, then record the results of the labs obtained closest to discharge from the hospital. These labs must include LFTs. If no i.v. access is available on Day 28 or the day of discharge, venipuncture is not required since results of the labs obtained closest to discharge may be used.

[17] A phone assessment will be performed on Day 28 ***only*** for those patients discharged prior to Day 28

1 Introduction

1.1 Condition to be studied

Congenital heart disease is the most common of the major congenital anomalies, and represents a significant global health problem. Twenty-eight percent of all major congenital anomalies consist of heart defects. In a systematic review of the 8 most common congenital heart defect subtypes performed by van der Linde et al. (van der Linde, Konings et al. 2011) the authors determined ventricular septal defect (VSD) to be the most common heart defect (34% of all cases examined), followed by atrial septal defect (ASD) (13%). Prevalence was comparable for Europe and North America, with approximately 2.7 and 2.4 children, respectively, per 1000 live births presenting with a VSD and approximately 1.6 and 1.7 children, respectively, presenting with an ASD.

L-citrulline is being developed for prevention of clinical sequelae of acute lung injury induced by cardiopulmonary bypass (CPB) in pediatric patients undergoing surgery for congenital heart defects (CHD). It is well recognized that cardiopulmonary bypass causes a systemic inflammatory response characterized clinically by acute compromise of cardiovascular and pulmonary function (Apostolakis et al. 2010; Huffmyer and Groves 2015). However, for a number of medical and physiological reasons, pediatric patients subjected to CPB during surgical repair of congenital heart defects are more susceptible to this cascade and at greater medical risk therefrom than adult patients (Kozik and Tweddell 2006; Shure 2010; Shekerdemian 2009). Reduction of key manifestations of acute CPB-induced lung injury, namely the post-operative need for mechanical ventilation and for inotrope therapy, will provide evidence of the intended clinical effect.

1.1.1 Cardiopulmonary Bypass-Induced Injury

A number of factors place the lung at risk for injury during CPB. Chief among these is surface activation of neutrophils and other leukocytes, complement, and cytokines (pro- and anti-inflammatory) *inter* alia, and an associated systemic inflammatory cascade (Apostolakis et al. 2010; Shure 2010). The degree to which the lung is damaged by the inflammatory response mediated by contact activation of leukocytes during extracorporeal circulation can vary in severity from microscopic changes of no clinical consequence to a capillary leak syndrome, or, in the worst case, to acute respiratory failure.

Pulmonary injury manifests in several ways and may involve both parenchymal and vascular lung tissues. Parenchymal effects of CPB are reflected in alterations in pulmonary compliance, most commonly related to an increase in lung water. The impact of this on the patient is a

requirement for increased ventilatory support and a diminished ability of the lungs to perform their function in gas exchange. Vascular effects are manifested by changes in pulmonary vascular resistance, which in turn affect the function of the right ventricle. This condition constitutes, in effect, pulmonary arterial hypertension. The lungs are in a unique position in the circulation and may thus be vulnerable to different mechanisms of injury. Circulating leukocytes that elaborate inflammatory mediators following contact with surfaces in CPB apparatus or by direct damage by CPB equipment account for only part of the inflammatory damage that may occur in the lung (Clark 2006). The lung is also an important source of inflammatory cells, as well as being a target for damage by those same cells. The consequences of the mechanical and inflammatory effects on the lung are decreased functional residual capacity, diminished compliance, and impaired gas exchange. These changes are ultimately associated with increased pulmonary vascular resistance and pulmonary artery pressure.

Inflammation and mechanical factors are not the only factors causing impaired pulmonary function related to CPB. When patients are placed on bypass, the lungs undergo a sudden and significant decrease in perfusion via the pulmonary artery. During total bypass, the lungs receive only nutrient flow from the bronchial arterial circulation. This ischemic effect of CPB is added to its inflammatory effect to produce clinical pulmonary dysfunction. It seems that low-flow CPB produces worse pulmonary injury than does circulatory arrest, which suggests that the interaction between the inflammatory and ischemic components is complex. Both the inflammatory and ischemic factors damage the pulmonary endothelium (http://tele.med.ru/book/cardiac_anesthesia/es_home.htm 2001).

Acute CPB-induced lung injury leads to significant cardiopulmonary problems. The inflammatory response leads to constriction of the pulmonary and systemic vasculature. The constriction leads to increased right ventricular and left ventricular workload. The inflammatory response also leads to pulmonary edema and deterioration in lung compliance and postoperative lung function. The standard treatments for these postoperative complications include mechanical ventilation until the lung function returns to normal and inotropic support until pulmonary and systemic vascular tone returns to normal, eventually decreasing the right and left ventricular workload. Mechanical ventilation and inotropic support are therapies that can thus serve as effective biomarkers of acute CPB-induced lung injury. Additionally, prolonged mechanical ventilation can in turn often lead to other morbidities including ventilator associated lung injury, ventilator associated pneumonia (VAP), central line associated blood stream infections (CLABSI), and even more prolonged intensive care unit stays. Prevention of acute CPB-associated lung injury and its sequelae is therefore a desirable therapeutic goal.

1.1.2 Pediatric CHD Patients

Children undergoing surgery for congenital heart defects are especially susceptible to developing CPB-induced acute lung injury due to age dependent differences in the inflammatory response, and the elevated sensitivity of their immature organ systems to injury as well as distinct differences between pediatric and adult CPB (Kozik and Twedell 2006). Neonates and infants are especially affected as the relatively large extracorporeal circuit size, the blood prime and the need for increased flow rates result in greater exposure of blood to the foreign surface (Schure AY et al. 2010).

For congenital cardiac surgery, the extracorporeal circuit must be adjusted to a wide range of age groups and size variations, from 1.5kg premature infants to >100kg adolescents or adults. Infants and children have smaller circulating blood volumes, higher oxygen consumption rates and, often, highly reactive pulmonary vascular beds. In addition, neonates and infants have labile thermoregulation and immature organ systems with multiple implications for ischemic tolerance and inflammatory response. Many complex repairs require a bloodless operative field, which can be difficult to achieve in the presence of intra or extra cardiac shunts, aortopulmonary collaterals, or otherwise increased pulmonary venous return (Schure AY et al. 2010).

With the possible exception of secundum ASD cases, in which recovery is relatively and consistently robust, there is so far, no known biomarker or patient characteristic among pediatric CHD patients linked with the probability of clinically significant CPB-induced ALI. It appears to occur in approximately 1/3 of pediatric CHD surgeries (Russell et al. 1998).

The functional and structural status of the pulmonary vascular bed plays a pivotal role in the presentation and outcome of children with congenital cardiovascular disease. However, it is in the immediate postoperative period that these pediatric patients are most vulnerable to CPB-induced acute lung injury. CPB-induced acute lung injury represents a complex interplay between the preoperative condition of the patient (importantly age at repair, type of lesion and presence of a syndrome) and the inevitable disruption in the endocrine and vasoactive peptide milieu that results from cardiac surgery. Important factors leading to enhanced vasoconstriction are cardiopulmonary bypass, hypothermia and circulatory arrest with some degree of associated ischemia. Residual cardiac lesions and the sequelae of the stress response, hypoxia, metabolic and respiratory acidosis may all contribute additional imbalances that favor pulmonary vasoconstriction. Many of the manifestations of acute lung injury associated with CPB can be explained wholly or in part by endothelial dysfunction, which provides at once a possible unifying hypothesis as well as a potential therapeutic target. Acute lung injury associated with CPB may also lead to important adverse cardiac sequelae. The

inflammatory response after surgery for CHD is commonly associated with abnormal ventricular– vascular interaction, with systemic vasoconstriction and elevated afterload, as well as with myocardial injury with impaired systolic and diastolic function. In a proportion of patients, these haemodynamic manifestations can lead to the serious consequence of low cardiac output (Shekerdemian 2009).

The serious pulmonary and cardiac sequelae of acute lung injury are clinically important to outcome. These sequelae are risk factors for prolonged intensive care stay, and death. Lability of pulmonary vascular tone is common in neonates and infants after surgery for CHD. This can be most problematic after biventricular repairs in patients who had preoperative unrestricted pulmonary flow (large septal defects, common arterial trunk) or pulmonary venous hypertension (obstructed anomalous pulmonary venous drainage). Instability of the pulmonary vascular resistance is also common after palliative surgery in patients with a functionally univentricular circulation, including Norwood-type operations, a systemic-to-pulmonary artery shunt, or a pulmonary artery band. While many therapeutic interventions optimize systemic oxygen delivery through their direct influences on the myocardium and systemic vasculature, manipulation of the pulmonary vascular tone can play an important role in optimizing the circulation of children undergoing surgery for heart disease (Shekerdemian 2009).

CPB-induced acute lung injury and in its most severe form, a pulmonary hypertensive crisis, often requires an aggressive combination of therapies for right ventricular failure and careful management of inotropes and vasopressors (e.g., milrinone, epinephrine). Furthermore, fluids need to be prudently balanced, and sinus rhythm and atrioventricular synchrony need to be supervised and maintained. The challenge is to find the optimal preload to avoid the detrimental effects of ventricular interdependence. The general treatment of acidosis and hypoxia includes oxygenation, normocapnia, analgesia, sedation, and muscle relaxation, all of which require aggressive utilization of mechanical ventilation. If a postoperative pulmonary hypertensive crisis is not improved by general therapies mentioned above, inhaled nitric oxide (iNO) is often administered. Finally, because either mechanical ventilation or inotrope usage or both precludes discharge from intensive care, CPB induced lung injury can result in prolonged utilization of intensive care services.

In summary, the consequences of CPB-induced lung injury lead to:

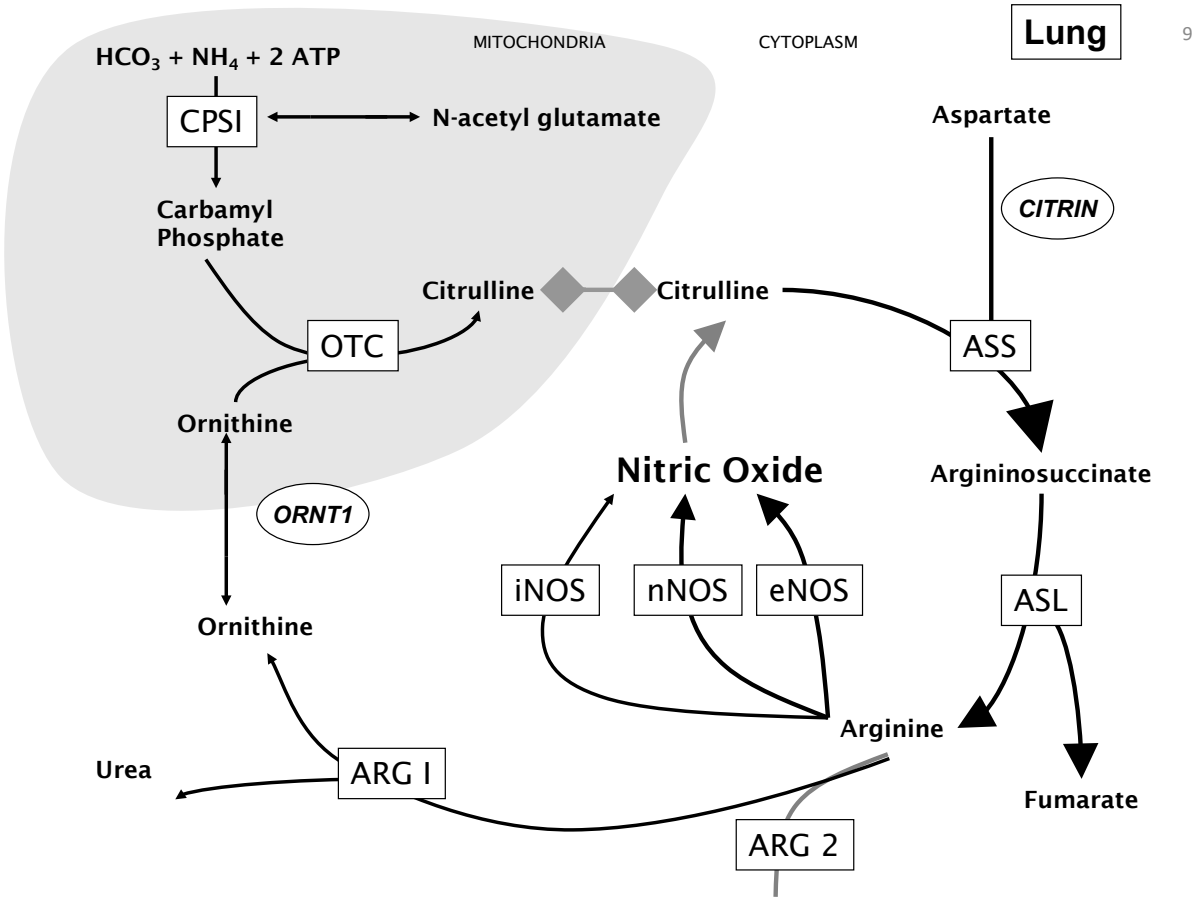
1. Extended requirement for mechanical ventilation
2. Extended requirement for inotrope therapy
3. Extended requirement for intensive care

1.2 L-citrulline

Citrulline is a naturally occurring amino acid and the first intermediate in the urea cycle. Most medical personnel think of the urea cycle as a hepatic metabolic pathway that serves to convert nitrogen waste from protein breakdown into urea for excretion. It is not generally appreciated that key elements of the urea cycle exist in other tissues where, for example, in lung, it is ultimately responsible for the production of NO under precise metabolic control, as shown in Figure 1. The shift in function of the urea cycle comes about because the enzymes catalyzing the first two steps of the hepatic urea cycle (carbamoyl phosphate synthetase I (CPSI) and ornithine transcarbamylase (OTC), which lead to the production of citrulline, are expressed only in the liver and gut. In the liver and gut, newly synthesized citrulline is transported from the mitochondria to the cytoplasm. In contrast, other tissues such as lung are dependent upon exogenous circulating citrulline since they cannot synthesize it endogenously. Following either endogenous synthesis or transport into a non-hepatic cell, argininosuccinate synthetase (ASS) combines citrulline with aspartate to form argininosuccinate. Subsequently, argininosuccinate lyase (ASL) cleaves fumarate off from argininosuccinate to form arginine. Arginine is the precursor for NO. Nitric oxide is synthesized by several different isoforms of the enzyme NO synthase (NOS), including constitutive and inducible forms. Unlike CPSI and OTC, the remaining enzymes of the pathway, including ASS, ASL and NOS, are distributed throughout the body, including the pulmonary vascular endothelium (Summar 1998).

Endogenous NO plays a critical role in the regulation of pulmonary vascular tone. It is a potent vasodilator and is produced by vascular endothelial cells in response to many different stimuli. Nitric oxide diffuses from the vascular endothelial cell to the vascular smooth muscle cell where it activates guanylate cyclase, leading to increased intracellular levels of cyclic guanosine monophosphate (cGMP). Increased cGMP, in turn, leads to relaxation of the vascular smooth muscle cells and increased blood flow. The extremely short half-life of NO allows for very tight vasoregulation. The pathophysiology of increased postoperative pulmonary artery pressure is thought to involve CPB induced pulmonary vascular endothelial cell dysfunction.

Figure 1: Schematic description of the urea cycle in lung



Source: Personal communication from Marshall Summar, M.D., Ph.D., Children's National Medical Center Wash DC).

Citrulline is thus a precursor to arginine and NO. New substrate supply for NO generation comes entirely from the production of citrulline as part of the normal urea cycle function. Once citrulline is generated by the hepatic urea cycle, it crosses mitochondrial and cellular membranes easily and therefore is also transported to other organs in the body. In the pulmonary vascular endothelium, citrulline can then be converted into arginine and subsequently into NO. Because the majority of circulating arginine is from urea cycle synthesis and not dietary sources, citrulline availability is critical to maintaining adequate arginine supply for NO production.

It has been demonstrated in multiple observational and clinical studies that plasma levels of citrulline and arginine drop precipitously and do not recover for up to 48 hours after CPB for congenital cardiac surgery.

A study conducted by Barr et al. (Barr, Beverly et al. 2003) revealed that CPB significantly decreases several urea cycle intermediates and NO metabolites after repair of unrestrictive VSD and atrioventricular septal defect (AVSD). Both citrulline and arginine levels were decreased in the postoperative period. Unlike citrulline, arginine was not decreased immediately after surgery, but was significantly decreased at 12, 24, and 48 hours after surgery. The slight lag in the drop in arginine levels compared with citrulline levels may reflect continued synthesis of arginine from precursors available before surgery. Citrulline and arginine levels continued to decline at 48 hours after surgery. Also, levels of NO metabolites decreased throughout the postoperative period. Patients undergoing these specific cardiac procedures are therefore at risk for elevated postoperative pulmonary vascular resistance, especially if they are physiologically stressed with hypoxia. An increase in the availability of NO and its precursors could decrease the risk for this postoperative complication.

In a study of 10 infants undergoing CPB for repair of congenital defects, supplementation of the NO precursor L-arginine was shown to partially ameliorate pulmonary endothelial dysfunction (Schulze-Neick, Penny et al. 1999). However, intravenous (i.v.) L-arginine has been shown to reduce both pulmonary and systemic pressures. This global response would not be tolerated in patients after cardiac surgery, who are already prone to low cardiac output states. The maintenance of high plasma arginine concentrations is also problematic because of poor bioavailability and swift metabolism by intestinal and cytosolic arginase. In contrast administration of L-citrulline is more effective in maintaining plasma L-arginine concentrations than administration of arginine in healthy volunteers (Haeberle, McCandless et al. 2014). L-citrulline has no recognized toxicity and is used as replacement therapy for children with urea cycle defects.

In summary, due to its intracellular and intercellular transport mechanisms, citrulline is the ultimate substrate for endogenous production of NO. Because the majority of circulating arginine is from urea cycle synthesis and not dietary sources, citrulline availability is critical to maintaining adequate arginine supply for NO production. L-citrulline supplementation is therefore a useful method of increasing arginine and NO synthesis and maintaining plasma arginine, citrulline, and NO metabolite levels in the postoperative period (Barr, Beverly et al. 2003; Smith, Canter et al. 2006; Barr, Tirona et al. 2007).

1.3 Non-clinical data

(For further information please see the current version of the Investigator Brochure)

A study performed in pulmonary hypertension in newborn piglets showed that pulmonary arterial pressure and pulmonary vascular resistance were significantly lower in hypoxic animals treated with L-citrulline compared with untreated hypoxic animals ($p < 0.001$). The authors suggested that L-citrulline may benefit neonates exposed to prolonged periods of hypoxia from cardiac or pulmonary causes (Ananthakrishnan, Barr et al. 2009).

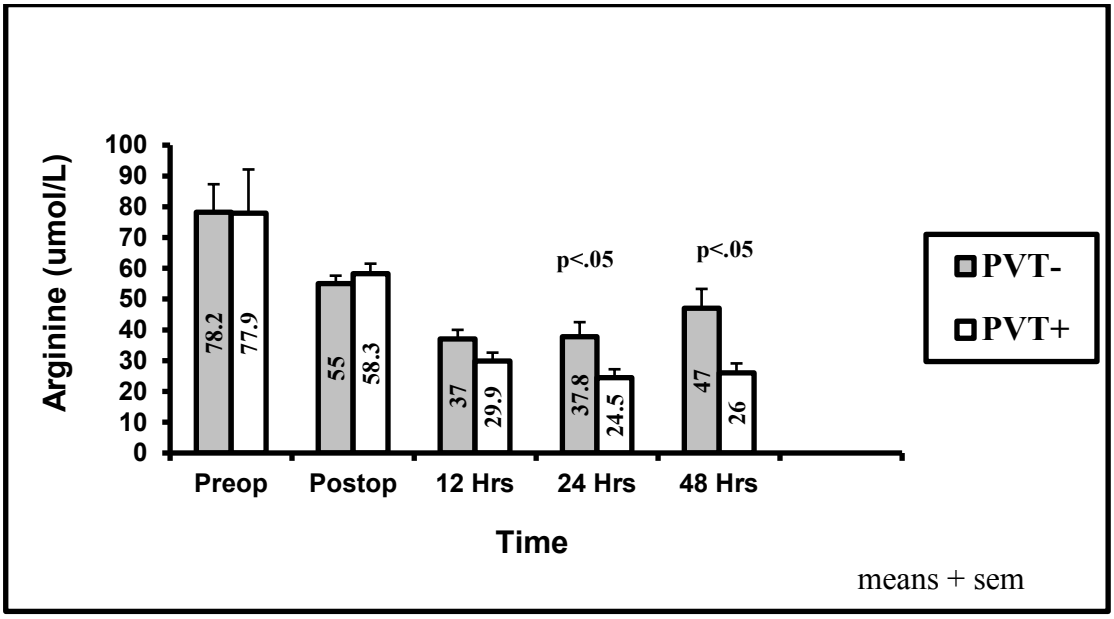
1.4 Clinical data

(For further information please see the current version of the Investigator Brochure)

A study conducted by Barr et al. (Barr, Beverly et al. 2003) investigated the prevalence of increased postoperative pulmonary vascular tone in infants and children undergoing cardiac surgery for correction of congenital heart defects. Of 169 patients, 56 (33.1%) developed clinical evidence of increased postoperative pulmonary vascular tone (PVT+). Many of these patients required clinical intervention including sedation, paralysis, and hyperventilation. Cardiopulmonary bypass caused a significant decrease in mean citrulline and arginine levels at all postoperative time points compared to preoperative levels. Plasma NO metabolite levels were also depressed immediately after surgery but showed a partial rebound at 12 and 24 hours before returning to preoperative levels at 48 hours.

In patients who subsequently developed increased postoperative PVT (PVT+), a particular decrease in plasma arginine levels was noted compared to patients without increased PVT (PVT-). Similar observations were not noted for citrulline and NO metabolites (Figure 2). The authors concluded that decreased availability of NO precursors may contribute to the increased risk of postoperative pulmonary hypertension.

Figure 2: Reduced Post-Operative Arginine Levels in PVT+ Group



In a pilot study, Smith, Canter et al. 2006 investigated whether oral citrulline supplementation could increase plasma citrulline concentrations and decrease the risk of postoperative pulmonary hypertension. Forty children, undergoing CPB and at risk for pulmonary hypertension, were randomized to receive 5 perioperative doses (1.9 g/m^2 per dose) of either oral citrulline or placebo. The 1st dose was administered immediately prior to CPB and the 2nd dose immediately on arrival in the pediatric intensive care unit (PICU) after surgery, followed by another 3 doses every 12 hours.

Median citrulline concentrations were significantly higher in the citrulline group versus the placebo group immediately postoperatively (36 µmol/L vs 26 µmol/L, P=0.012) and at 12 hours postoperatively (37 µmol/L vs 20 µmol/L, P=0.015). Mean systemic blood pressure was not different between the oral citrulline and placebo groups.

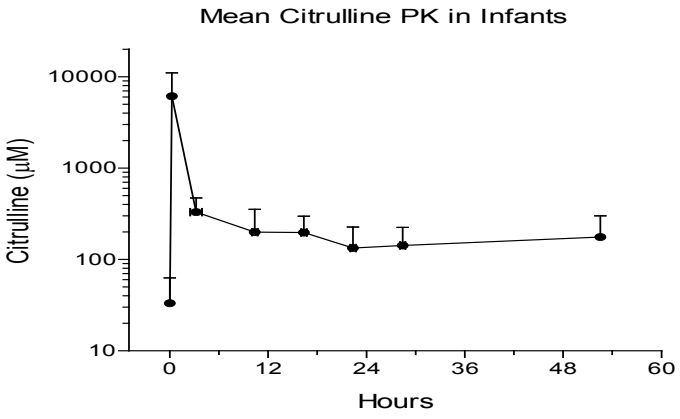
The most significant result was that postoperative pulmonary hypertension developed in 9 patients, 6 of 20 (30%) in the placebo group and 3 of 20 (15%) in the citrulline group (P=0.451), all of whom had plasma citrulline concentrations less than the age-specific norms. In a contingency analysis that reached statistical significance, all of the children developing postoperative pulmonary hypertension had a plasma citrulline level <37 µmol/L. Conversely, none of the children with citrulline levels ≥37 µmol/L developed pulmonary hypertension. This result implicated low citrulline levels as a strong risk factor and possible causal element in the

development of postoperative pulmonary hypertension. Therefore, more aggressive citrulline supplementation, resulting in consistent citrulline concentrations in excess of 37 µmol/L, could potentially prevent postoperative pulmonary hypertension.

In a Phase I/II clinical trial (Barr, Tirona et al. 2007) determined the pharmacokinetics (PK) and safety profile of 3 doses of i.v. L-citrulline in children undergoing CPB. The dose escalation design used three concentrations of i.v. L-citrulline: 50, 100, & 150 mg/kg. Each patient was given two doses of i.v. L-citrulline: the first in the operating room immediately after initiation of CPB and the second 4 hours later in the critical care unit. The overall goal was to achieve a sustained citrulline level of 80 to 100 µmol/L up to 4 hours after the initial dose. The 4-hour time point was selected to allow for the surgical procedure to be completed and the patient to return to the PICU postoperatively before further dosing.

The results showed that 150 mg/kg was the optimal dose, as it resulted in 4 hour levels close to the 100 µmol/L target. However, from PK modeling it was also determined that the ½ life of the bolus doses of i.v. L-citrulline was approximately 60-90 minutes and that it would require at least Q4hr dosing to achieve a consistently increased citrulline level, which is impractical even in an intensive care unit (ICU) setting. The PK modelling suggested a sustained citrulline level of approximately 100 µmol/L could be achieved by a bolus dose of 150 mg/kg of i.v. L-citrulline given at the beginning of surgery after initiation of CPB, followed 4 hours later by a continuous infusion of 9 mg/kg/hr. In a second phase of the study, 9 patients were enrolled and treated with this continuous infusion protocol. Using this regimen, plasma arginine, citrulline, and NO metabolite levels were well maintained (Figure 3).

Figure 3: Mean Citrulline PK in Infants with Continuous Infusion Protocol (Barr, Tirona et al. 2007)



Based on the data from the PK study, a pilot randomized placebo-controlled Phase II/III trial of i.v. L-citrulline was performed at Vanderbilt (Vanderbilt Pilot Study SA3 [NCT00335244], May 2006-July 2008). Based on the previous results, this study utilized a protocol of an i.v. L-citrulline bolus of 150 mg/kg during CPB followed postoperatively 4 hours later by a 9 mg/kg/hr continuous infusion for 48 hours.

Patients undergoing one of 5 surgical procedures (VSD, ASD, arterial switch procedure, bidirectional Glenn procedure, or Fontan procedure) were eligible for enrollment. A total of 77 patients were enrolled in this study. Serial arterial blood samples were obtained at 7 perioperative time points.

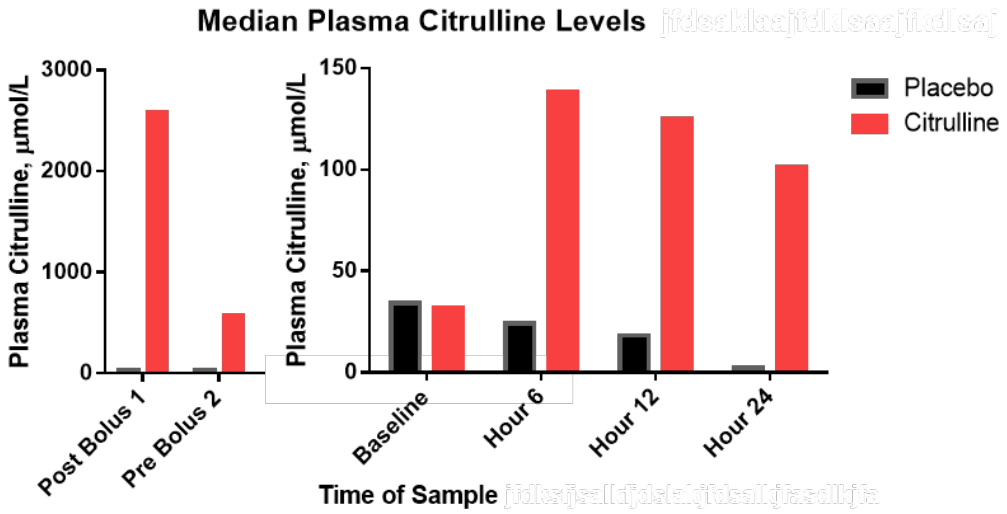
Most patients who received i.v. L-citrulline failed to reach the predicted target threshold of 100 µmol/L. Of the 37 patients who were treated with i.v. L-citrulline, only 1/3 had a sustained plasma citrulline level > 100 µmol/L. Upon investigation, it was found that the duration of hemofiltration had been significantly extended during CPB to maintain the hematocrit, plasma lactate, and plasma potassium within a tightly controlled target range. This modified hemofiltration protocol effectively removed a majority of the i.v. L-citrulline delivered at the time of the preoperative bolus. Due to this finding, the design of a subsequent efficacy trial was revised to take into account L-citrulline removal by hemofiltration during CPB.

The recent Phase IB single-blind, randomized, placebo-controlled study **CIT-002-01** used a revised protocol to determine the PK and safety of i.v. L-citrulline in children undergoing CPB for surgical repair of congenital heart defects. The primary objective of the study was to obtain and maintain a perioperative plasma level of citrulline >100 µmol/L. Twenty-two children (age range 3 months to 5.5 years) with an ASD, a VSD, or a partial or complete AVSD were enrolled and treated. The study is completed and the full clinical study report is available.

Subjects were randomized to receive either L-citrulline or placebo during their congenital heart defect procedures as follows: An i.v. L-citrulline bolus of 150 mg/kg or placebo at the initiation of CPB, followed by the addition of L-citrulline at a concentration of 200 µmol/L or placebo to the filtration or hemoconcentration replacement fluid used during CPB. An L-citrulline bolus of 20 mg/kg or placebo was administered 30 minutes after decannulation from CPB, immediately followed by a 9-mg/kg/hr continuous infusion of L-citrulline or placebo for 48 hours. Blood samples were collected at 7 perioperative time points: baseline (prior to surgical incision, used for comparison to post-surgery time points), 10 minutes after initial bolus (post-bolus #1), after separation from CPB just prior to 2nd bolus (pre-bolus #2), 6h, 12h, 24h, and 48h after pre-bolus #2.

The revised dosing protocol achieved plasma citrulline levels consistently above the target level of 100 µmol/L as shown below in Figure 4.

Figure 4: Median Plasma Citrulline Levels at Indicated Time Points



Patients receiving i.v. L-citrulline showed a reduced duration of mechanical ventilation as shown in Figure 5 and Figure 6. The duration of postoperative invasive mechanical ventilation was derived as the time in hours from separation from CPB until endotracheal extubation. The mean duration of invasive mechanical ventilation was clearly longer in the placebo group (37 hours) than in the citrulline group (5 hours). Kaplan-Meier survival analyses confirmed this reduced duration as seen in Figure 5 with re-intubation time included and ventilation time censored and a log rank p-value of 0.0498, and in Figure 6 with reintubation time included and zero ventilation time not censored and a log rank p-value of 0.0089.

igure 5: Kaplan-Meier Survival Analysis of the Duration of Invasive Mechanical Ventilation (Including Re-intubation Time): Censored

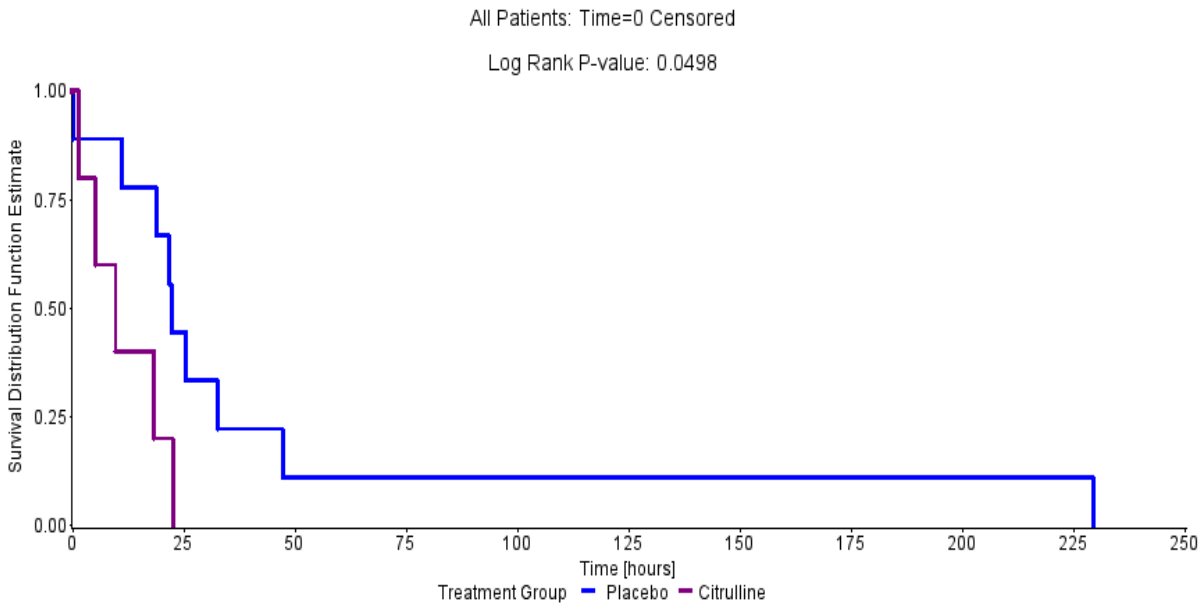
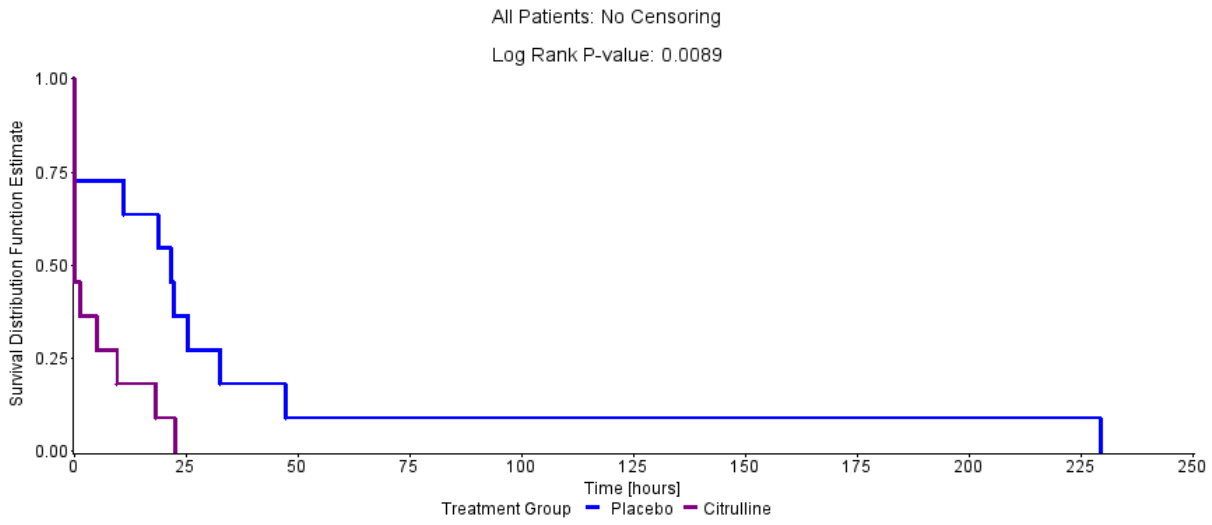


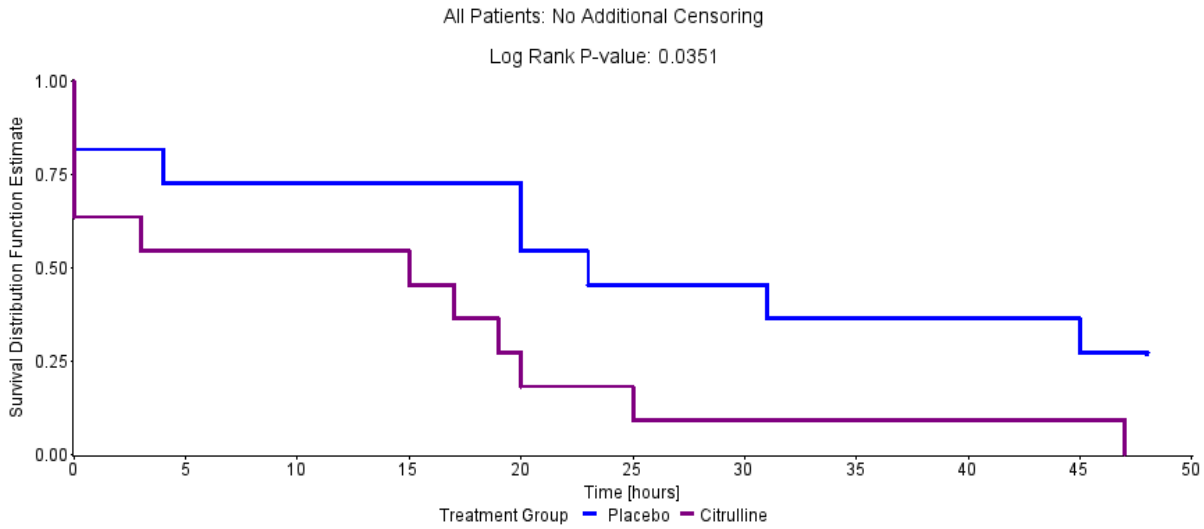
Figure 6: Kaplan-Meier Survival Analysis of the Duration of Invasive Mechanical Ventilation (Including Re-intubation Time): Not Censored



Some differences were noted in clinical practice among sites with regard to mechanical ventilation. One site tended to extubate patients in the operating room without recording a time of extubation for such patients. For purposes of analysis, the duration of postoperative mechanical ventilation was set at zero for such patients. When these patients were stratified by treatment, it was shown that all 6 patients (100%) receiving i.v. L-citrulline had been extubated in the operating room, in comparison to only 2 of 6 patients (33%) in the placebo group. Although the numbers are small, these data achieved borderline significance. The results suggest that children receiving citrulline appeared better clinically following cardiopulmonary bypass than did children receiving placebo.

As with the length of time of mechanical ventilation, the duration of inotrope therapy (dopamine, dobutamine, milrinone, epinephrine, phenylephrine, or norepinephrine) was also recorded. Length of time on inotropes was documented from the time of first use after surgery until completion of the study medication at 48 hours. Patients still receiving inotropes at hour 48 were censored. There was a marked difference between the two treatment groups as shown in Figure 7 with censored patients still using inotropes at hour 48, with no additional censoring with a log rank p-value of 0.0351.

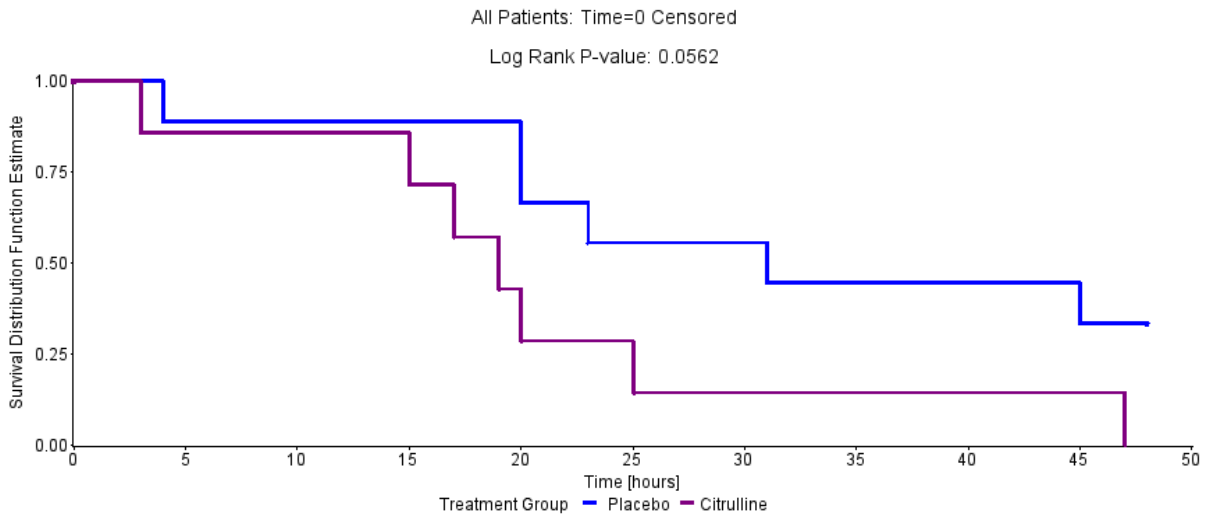
Figure 7: Kaplan-Meier Survival Analysis of the Length of Time on Inotropes: No additional Censoring



Note: Time on inotropes is censored at 48 hours if patient was still receiving inotropes at hour 48.

A second analysis also censored patients without inotrope use; i.e., inotrope time = 0 (Figure 8), which demonstrated a log rank p-value of 0.562.

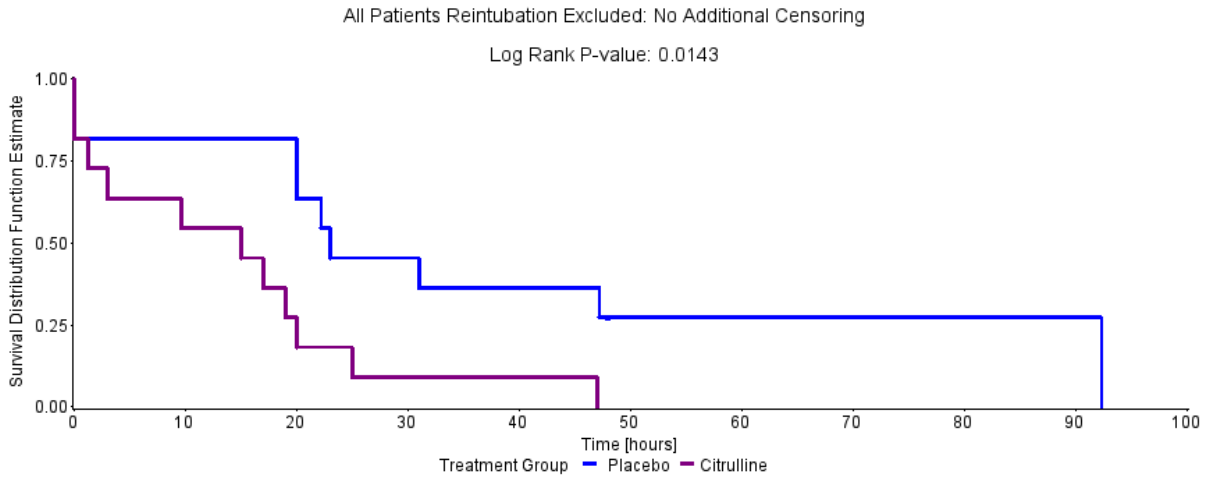
Figure 8: Kaplan-Meier Survival Analysis of the Length of Time on Inotropes: Censored



Note: Time on inotropes is censored at 48 hours if patient was still receiving inotropes at hour 48.

Cessation of mechanical ventilation and of inotrope therapy are the two principal determinants of readiness for discharge from the intensive care unit. A composite variable comprising (for each subject) of the longer of the two parameters - duration of mechanical ventilation or of inotrope therapy - can serve as an effective and accurate surrogate for the duration of intensive care unit stay. Since inotrope use was only documented until Hour 48 after surgery (end of study medication treatment), patients with inotrope use continuing until Hour 48 and with mechanical ventilation duration of ≤ 48 hours were censored at this time point. If mechanical ventilation was continued beyond the 48-hour time point, the duration of mechanical ventilation was used in the analysis. Kaplan-Meier plots of the composite endpoint are presented in Figure 9, with re-intubation times excluded. One (1) patient in the placebo group was re-intubated. This patient was excluded from this analysis; however, results including this patient were comparable. In this analysis, patients with inotrope use still ongoing at 48 hours, and mechanical ventilation of less than 48 hours, were censored. Figure 9 shows the difference in the composite variable when L-citrulline and placebo groups are compared. Citrulline-treated patients required statistically significantly less mechanical ventilation and inotropes than placebo-treated patients. Thus, as assessed by the composite surrogate marker variable, patients receiving study drug were ready for discharge from the PICU sooner than patients receiving placebo. In addition to indicating shorter PICU time, shorter mechanical ventilation time lowers the added risk of physical injury.

Figure 9: Kaplan-Meier Survival Analysis of the Longer Duration of Duration of Postoperative Mechanical Ventilation and the Length of Time on Inotropes: Re-Intubation Time Excluded



In summary, the data from study CIT-002-01 show that the revised dosing protocol achieved the PK endpoint. Further, despite the extremely small sample size of the study, CIT-002-01 indicates that intravenous L-citrulline prevents compromised cardiopulmonary function caused by CPB in pediatric patients undergoing surgery for ASD, VSD, or AVSD. The effect is robust and is evident even in centers practicing early extubation as shown in the strong preferential early extubation of citrulline patients in comparison to the control population. Discharge from the ICU requires cessation of both mechanical ventilation and inotrope therapy. Intravenous L-citrulline shortens the duration of each of these variables, resulting in earlier readiness of patients for discharge from the ICU. Thus, CIT-002-01 indicates that i.v. L-citrulline treatment can play a beneficial role in preventing clinical sequelae of CPB-induced lung injury.

1.5 Safety of L-citrulline

L-citrulline is a naturally occurring amino acid. It is produced by, and normally present in, the human body. Typical circulating levels of citrulline in adults are 16-55 µmol/L. In children <12 years of age, the levels are 16-32 µmol/L. Oral citrulline has been used for years as a food supplement to enhance energy and prevent muscle fatigue and has been found safe in doses of 2-15 g, which is a multiple of the administered dose in the L-citrulline trials conducted to date (http://examine.com/supplements/citrulline/ #summary_full) (Moinard, Nicolis et al.

2008).

No SAEs occurred in the oral citrulline study reported by Smith, Canter et al. 2006. Four adverse reports were submitted to the Institutional Review Board (IRB) per protocol on patients who developed hypotension secondary to surgical complications and required further surgical or medical support. These cases were reviewed and determined to not be related to study drug administration.

In the first report of the use of L-citrulline in humans, L-citrulline was found to be both safe and well tolerated (Barr, Tirona et al. 2007). One patient required advanced life support following a junctional rhythm postoperatively and profound bradycardia consistent with complete heart block. The patient recovered fully. The Data Safety Monitoring Board (DSMB) reviewed the event and deemed it not likely related to L-citrulline.

Serious AEs (SAEs) under L-citrulline treatment in the Vanderbilt pilot study included postoperative bleeding (2 patients), and obstructive airway disease (1 patient). Although these events occurred in 3 out of 35 L-citrulline patients only, these types of adverse events (AEs) will be monitored in future studies.

Safety data from the completed CIT-002-01 study have shown that of the patients randomized and treated, all patients (100%) in the i.v. L-citrulline group and 73% of patients in the placebo group experienced at least one AE. AEs that were judged to be related to study treatment were reported in the placebo group (2 patients, 18%), but not in the active treatment group. No AE leading to death and no other SAE was reported in this study. No AE leading to treatment discontinuation was reported.

The safety of administering even higher doses of citrulline than those being used in this study has been demonstrated in children with urea cycle dysfunction. A previous study has shown that children with carbamyl phosphate synthetase and OTC deficiencies have benefited from citrulline supplementation at doses of 100–170 mg/kg/d (Singh, Rhead et al. 2005). Additionally, in children who are genetically unable to produce citrulline, bolus administrations of citrulline at 400-500 mg/kg are used routinely, again without observed negative consequences (personal communication from Marshall Summar, M.D., Ph.D., Children's National Medical Center Wash DC).

Based on the available published, pre-clinical and clinical data, the only potential adverse event would be transient systemic hypotension. This was seen in 2 out of 11 patients in the L-citrulline group and 1 out of 11 patients in the placebo group in the CIT-002 trial.

1.6 Rationale for the study

Asklepion intends to develop L-citrulline for the prevention of clinical sequelae of acute lung injury induced by CPB in pediatric subjects undergoing surgery for congenital heart defects. Cardiopulmonary bypass causes a transient systemic inflammatory response leading to clinical manifestations of acute compromise of cardiovascular and pulmonary function requiring extended mechanical ventilation and use of inotropes (Kozik and Twedell 2006).

Pediatric patients undergoing congenital heart defects surgery are most vulnerable to experiencing lung injury sequelae during the immediate postoperative period. CPB-induced acute lung injury represents a complex interplay between the preoperative condition of the patient (importantly age at repair, type of lesion and clinical manifestations) and the inevitable disruption in the endocrine and vasoactive peptide milieu that results from cardiac surgery. L-citrulline, with its mechanism of action, is intended as a preventive treatment to improve restoration and maintenance of normal vasodilation, thus improving clinical status and reducing the need for-and exposure to-mechanical ventilation and inotrope treatment.

The reduction of key manifestations of acute CPB-induced lung injury, namely the post-operative need for mechanical ventilation and for inotrope therapy will provide evidence for L-citrulline's intended clinical effect.

2 Study Objectives

2.1 Primary Objective

The primary objective of the study is to determine if L-citrulline delivery given perioperatively reduces clinical sequelae of acute CPB-induced lung injury in pediatric subjects undergoing repair of congenital heart defects, as evidenced by the reduction of post-operative need for mechanical ventilation and inotrope therapy.

2.2 Secondary Objective(s)

Secondary objectives are to evaluate the effect of L-citrulline compared with placebo regarding

- safety and tolerability
- hemodynamic improvement post-operatively
- the usage and length of time on vasodilators
- length of hospitalization

- the duration of chest tube placement
- the plasma concentrations of citrulline

3 Study Endpoints

3.1 Primary Endpoint

A composite variable consisting of the longer of either (1) length of time on mechanical ventilation or (2) length of inotrope use.

The definition of mechanical ventilation shall include invasive mechanical ventilation or non-invasive mechanical ventilation including bi-level (biphasic) positive airway pressure (BPAP) or continuous positive airway pressure (CPAP).

3.2 Secondary Endpoints

3.2.1 Secondary Efficacy Endpoints

- Length of time on mechanical ventilation
- Length of time on positive pressure ventilation
- Length of time of inotrope use
- Inotrope score calculated each hour post-operatively from the time of separation from bypass until the completion of study drug; additionally, the total inotrope score over time until Day 28 or hospital discharge will be derived
- Hemodynamic improvement (heart rate, systemic arterial blood pressure, oxygen saturation, and central venous pressure)
- Thoracotomy output; duration and volume of chest tube drainage
- Length of time of intubation
- Length of PICU stay
- Length of time on vasodilators
- Length of hospitalization
- Plasma concentrations of citrulline

3.2.2 Secondary Safety Endpoints

- Occurrence of adverse and serious adverse events
- Incidence of refractory hypotension
- Change from baseline in laboratory values (serum electrolytes, blood urea nitrogen [BUN], creatinine, complete blood count [CBC] with differential, activated clotting time [ACT] & liver enzymes)

4 Investigational Plan

4.1 Overall Study Design and Plan

This is a multicenter Phase III double-blind, randomized, placebo controlled study to evaluate the efficacy and safety of L-citrulline for the prevention of clinical sequelae of acute lung injury induced by CPB in pediatric subjects undergoing surgery for congenital heart defects.

Children undergoing repair of cardiac defects that include a large unrestrictive VSD or a partial or complete AVSD or an ostium primum ASD will be eligible for enrollment in this study.

- 190 subjects will be randomized in a 1:1 fashion to either L-citrulline or placebo. L-citrulline treatment will include: 1) an L-citrulline bolus of 150 mg/kg or placebo at the initiation of CPB, but after removal of any crystalloid base; 2) the addition of L-citrulline at a concentration of 200 µmol/L or placebo given as a bolus during bypass. This may be administered as a one-time bolus or multiple administrations to compensate for fluids containing L-citrulline that may be removed from the patient during the course of the operation and thus to maintain the concentration of 200 µmol/L; and 3) an L-citrulline bolus of 20 mg/kg or placebo 30 minutes after decannulation from CPB immediately followed by a 9 mg/kg/hr continuous infusion of L-citrulline or placebo for 48 hours (see Section 4.4.1 for further details). The study drug or placebo infusion will be discontinued once invasive arterial blood pressure monitoring is discontinued or at 48 hours, whichever comes first. Subjects will be followed until discharge from the hospital, and a final assessment via telephone will be conducted at Day 28.

4.2 Discussion of Study Design

This study will examine patients who develop significant sequelae of CPB-induced lung injury

in the absence of confounding co-morbidities. Consequently, secundum ASDs are excluded because they are often clinically insignificant and patients with secundum ASDs rarely develop significant clinical sequelae of CPB-induced lung injury. Inclusion of the very common secundum ASDs would thus dilute study results. Similarly, single ventricle defect patients are excluded because of their high incidence of co-morbidities that would prolong the use of positive pressure ventilation and inotrope usage by confounding mechanisms other than CPB-induced lung injury.

A primary concern in designing a study to confirm that perioperative administration of L-citrulline to CPB patients does indeed provide a substantive clinical benefit is to construct an endpoint or endpoints that are reliable and consistent.

The following factors underlie the determination of the appropriate primary endpoint for the proposed trial: there is consistent pathophysiology among pediatric congenital heart defect patients receiving CPB: pulmonary endothelial insult and disruption => CPB-induced acute lung injury => increased vascular resistance and elevated vascular pressure => various morbidities and risks, including congestive heart failure => prolonged ICU stays with continued positive pressure ventilation and inotrope therapy.

Each patient will be randomized to receive either L-citrulline or placebo for the duration of the study. Randomization will be performed using a previously generated randomization scheme, and stratified by site to ensure, to the extent possible, equal randomization into both treatment arms.

The study is double-blinded for all investigational staff who make determinations related to the study, with the sole exception of the hospital or site pharmacist who will be single-blind in order to properly prepare the individual subject's medication (see Section 4.4.7).

4.3 Selection of Study Population

4.3.1 Number of Planned Subjects

Approximately 450 subjects will be screened to obtain 190 patients randomized to either placebo or L-citrulline in a 1:1 ratio. A total of approximately 190 subjects are anticipated to complete the study.

The estimated enrollment period is 11 months. The total trial period for a patient is up to 42 days (up to 2 weeks screening, 2 days treatment, up to 28 days follow-up). The trial end is defined as "last patient out" (LPO).

4.3.2 Inclusion Criteria

To be eligible for study entry subjects must satisfy all of the following criteria:

- Subjects, parents, or legal guardian of the subject who are willing and able to sign informed consent
- Male and female subjects aged ≤18 years of age (females of child-bearing potential must be willing to practice an acceptable form of birth control; i.e. oral contraceptive, hormonal implant; etc.)
- Infants, children and adolescents undergoing CPB for repair of a large unrestrictive VSD, an ostium primum ASD, or a partial or complete AVSD
- Pre-operative echocardiogram which confirms the cardiovascular anatomy and defect to be surgically repaired

4.3.3 Exclusion Criteria

Subjects will be excluded from the study if one or more of the following statements are applicable:

- Evidence of pulmonary artery or vein abnormalities on the pre-operative echocardiogram that will not be addressed surgically. Specific abnormalities excluded include the following:
    - Significant pulmonary artery narrowing not amenable to surgical correction
    - Previous pulmonary artery stent placement
    - Significant left sided AV valve regurgitation not amenable to surgical correction
    - Pulmonary venous return abnormalities not amenable to surgical correction
    - Pulmonary vein stenosis not amenable to surgical correction
- Preoperative requirement for mechanical ventilation or i.v. inotrope support
- Presence of fixed or idiopathic pulmonary hypertension (i.e. Eisenmenger's Syndrome) prior to surgical repair
- Pre-operative use of medications to treat pulmonary hypertension
- Pregnancy; Sexually active females of child-bearing potential must be willing to practice an acceptable method of birth control for the duration of study participation

(e.g. oral contraceptive, hormonal implant, intra-uterine device)

- Participation in another clinical trial within 30 days of Screening or while participating in the current study, including the 28 days of follow-up post study drug administration.
- Any condition which, in the opinion of the investigator, might interfere with the study objectives

4.3.4 Removal of Subjects from Therapy or Assessments

The person(s) providing consent is free to withdraw his/her consent regarding participation in this study at any time, for any reason, specified or unspecified and without penalty or loss of benefits to which the subject is otherwise entitled.

Subjects may be withdrawn from the study by the Investigator at any time due to an AE or for any other reason if it is in the opinion of the Investigator that continued participation in the study is no longer in the best interest of the subject. These reasons include, but are not limited to the following:

- Adverse drug reaction or AE or any other reason for which, in the opinion of the Investigator, subject's continued participation in the study is not in the best interest of the subject
- Serious protocol violation(s), such as determination of fulfilment of exclusion criteria during surgery only
- Lack of response of subject or subject's parents/legal guardian to the attempts of the investigational site to contact him/her (lost to follow-up)

The reason(s) for withdrawal must be documented in the case report form (CRF).

For subjects withdrawing or being withdrawn from the study, it will be encouraged to have the same final evaluations completed as those for subjects completing the study according to this protocol, particularly safety evaluations.

For subjects who are lost to follow-up, reasonable efforts will be made to contact the parents or legal guardians of subjects, or the subjects themselves if they provided their own consent. These efforts must be documented in the subject's file.

Subjects who have been randomized and who withdraw or are withdrawn for any reason will not be replaced.

The sponsor has the right to terminate the study at any time in case of SAEs or if special circumstances concerning the investigational medicinal product (IMP) or the company itself occur, making further treatment of subjects impossible. In this event, the investigator(s) will be informed of the reason for study termination.

4.4 Investigational Medicinal Products

4.4.1 Investigational Medicinal Products Administered

The main objective of the dosing schedule, as outlined below, is to achieve a minimum systemic target plasma trough level concentration of 100 µmol/L of L-citrulline to provide the most beneficial effect as demonstrated in the previous phase 2 study. As a general guiding principal during bypass, once dosing has begun, all fluids entering the circuit must contain 200 µmol/L of L-citrulline in order to achieve and maintain the trough level of 100 µmol/L of L-citrulline. Therefore, if fluids are being added or removed, it is crucial to take this into consideration when timing the administration of the required doses. The following section describes the administration of L-citrulline or placebo, while taking into account the above objective.

- The first (initial) dose administered will be an L-citrulline bolus of 150 mg/kg or placebo, administered at the initiation of CPB, but after removal of any crystalloid base.
    - If fluid is added to the priming circuit, but removed prior to initiation of bypass, then the initial 150 mg/kg bolus dose should be added after the crystalloid based fluids are removed and at the initiation of bypass.
    - If fluid is added to the priming circuit, but not removed, then the initial 150 mg/kg dose should be added to the circulating prime used for the bypass.
- The second dose administered consists of L-citrulline at a concentration of 200 µmol/L or placebo, given as a bolus during bypass. This may be administered as a one-time bolus or multiple administrations, to maintain the concentration at 200 µmol/L. The total dose will be dependent upon the total amount of any volume administered and removed during CPB. This includes the crystalloid portion of the cardioplegia so as not to dilute the loading dose; it is significantly important when using a hemoconcentrator.
- The third dose administered will be a bolus of 20 mg/kg of L-citrulline or placebo, administered 30 minutes after decannulation from CPB.

- The above third dose is immediately followed by a 9 mg/kg/hr continuous infusion of L-citrulline or placebo for up to 48 hours. The study drug or placebo infusion will be discontinued once invasive arterial blood pressure monitoring is discontinued or at 48 hours, whichever comes first.

Placebo will be matched for volume using suitable fluid recognized as standard of care for such procedure (e.g. normal saline or Plasmalyte without L-citrulline added).

All study medications will be prepared and provided by each site's investigational pharmacy. A pharmacy manual will be provided to the hospital or institution Investigational Pharmacist. The manual will contain detailed instructions for the preparation of the investigational product. The manual will include specific directions on randomization procedures, preparation of study drug or placebo and labeling of product for administration. The label should contain subject information (e.g. subject initials, subject identification number), study number, volume of product to be administered, and the date of administration. However, the label may not contain any information that identifies the actual product being administered.

All study medications will be administered by the Investigator or by medically trained personnel under his/her supervision (e.g., the anesthesiologist, perfusionist, PICU personnel, etc.).

Doses will be given by a central i.v. catheter that will be emplaced after induction of anesthesia or via the bypass circuit. Separate i.v. access is not required for the study drug. The study drug is isotonic and can run through either a peripheral i.v. or a central venous catheter at the discretion of the clinical staff. The study drug is an amino acid (or placebo) and thus, for compatibility purposes, the drug product is treated like parenteral nutrition. Additionally, it is compatible with fluids used for filtration or hemoconcentration during CPB.

No dose adjustments will be allowed; however, study drug may be discontinued as noted above.

4.4.2 Identity of Investigational Medicinal Products

The L-citrulline drug product is a sterile, non-pyrogenic injectable clear solution intended for intravenous administration. It is supplied as a 10 mL, single use vial containing 500 mg L-citrulline in 10 mL of acidified water. The product is packaged in a clear glass vial (13 mm neck) with a grey stopper and blue flip-off cap/aluminum seal. The L-citrulline drug product is manufactured aseptically at a concentration of 50 mg/mL using fully synthetic drug substance dissolved in water for injection. The resulting solution pH is adjusted to near pH 3, sterile filtered and filled.

Study drug should be stored as instructed: Store at 2-8ºC (36-46ºF); do not freeze.

The drug product is manufactured and imported according to current Good Manufacturing Practice and the relevant regulatory requirements.

4.4.3 Packaging and Labeling

L-citrulline study drug comes in a clear glass vial (13 mm neck) with a grey stopper and blue flip-off cap/aluminum seal. Boxes of 25 vials are supplied to the study centers.

The batch number, strength, storage conditions, and federally required language stating that the product is “for investigational use only” or “for clinical trial use only” will be printed on the outer package. A Material Safety Data Sheet (MSDS) will be provided with the shipment.

All packaging and labeling operations will be performed according to the current Good Manufacturing Practice for Medicinal Products and the relevant regulatory requirements.

4.4.4 Method of Assigning Subjects to Treatment Groups

Screened subjects will be assigned a unique 5-digit number, with the first two digits identifying the site and the next three digits identifying the subject, e.g. 01-001. At each site, the investigator will assign numbers to subjects in ascending order (e.g. 001, 002, 003).

Eligible subjects will be randomized to a treatment (placebo or L-citrulline) in a 1:1 ratio. Randomization will be performed per study site via an interactive web response system (IWRS). The randomization schedule will be generated using a validated randomization program. A unique randomization number will be assigned to each randomized subject. Randomization assignment will be stratified at each site to ensure balance of the number of randomized subjects to both treatment groups.

4.4.5 Selection of Doses in the Study

Initial supplementation trials utilized oral citrulline at a dose of 1.9 g/m^2 per dose, administered before CPB, postoperatively upon admission to the intensive care unit and every 12 hours at hour 12, 24 and 36. Oral citrulline was well tolerated with no evidence of significant AEs. In addition, it was noted that patients who had a 12-hour plasma citrulline level >37 µmol/L (the upper range of normal levels) did not develop increased PVT. Unfortunately, not all patients receiving oral citrulline reached these levels (Smith, Canter et al. 2006).

In a dose escalation study targeting a sustained plasma level of approximately 100 µmol/L, it was noted that L-citrulline has a fairly short half-life, thus requiring a classic bolus and continuous infusion drug delivery protocol (Barr, Tirona et al. 2007). A subsequent study (CIT-002-01) confirmed that the following L-citrulline administration would result in sustained plasma L-citrulline levels of approximately 100 µmol/L:

- An L-citrulline bolus of 150 mg/kg or placebo at the initiation of CPB
- The addition of L-citrulline at a concentration of 200 µmol/L or placebo to both the bypass circuit priming fluid as well as to the hemofiltration fluid utilized during CPB
- A bolus of L-citrulline of 20 mg/kg or placebo, 30 minutes after decannulation from CPB, immediately followed by initiation of a 9 mg/kg/hr continuous infusion of L-citrulline or placebo for 48 hours

This treatment regimen, with clarification added for the dose(s) given during bypass, will also be used in the current study.

4.4.6 Drug Accountability

The Pharmacist at the study site is responsible for keeping accurate drug accountability records throughout the study regarding the receipt of study drug, the preparation of study drug and dispensation to subjects, and the reconciliation of all used and unused study drug.

All empty and partially used vials should be retained by the pharmacy in order for study drug accountability to be performed. The total amount of study drug prepared for administration to the patient will be calculated by the investigational pharmacist, with verification of the calculations performed by a second qualified person (e.g. pharmacy technician). Total volume of study drug administered to the patient will be documented in the source documents and in the electronic case report forms (eCRFs).

A Drug Preparation Log as well as a Drug Dispensing and Accountability Log must be kept current and must contain the following information:

- Batch number
- Subject number for whom the drug was prepared
- Date on which drug was dispensed
- Quantity of the drug administered

4.4.7 Blinding

Randomization will take place via an IWRS system. The site's investigational pharmacist will be responsible for obtaining the randomization assignment. Therefore, the site pharmacist will only be aware of the treatment assigned to each individual subject. The pharmacist has no other role in the study other than preparing the study product. Study drug or placebo will be prepared and labeled with the appropriate subject identifiers; however, no information that would reveal the actual contents of the dose to be administered (active vs. placebo) will be included on the label. Study drug will be provided in either syringes or bags and mask-labeled. Placebo is also mask-labeled and provided in either syringes or bags. The bags are the same size, shape, and fluid clarity, and hence masked to both investigators and staff (if other than the investigator) administering the drug. Therefore, the investigator and staff administering the product will remain blinded as to its contents. Only the pharmacist and the unblinded monitor responsible for performing drug accountability (a different monitor than the person performing routine data monitoring) will be aware of the treatment assignment.

Data may be unblinded at the request of the DSMB to the study Medical Monitor in the event that a safety issue arises for which they determine there is a need to review the subject's treatment assignment. If data is unblinded for any reason, the DSMB will not share the treatment assignment with the Sponsor, unless there is a medical or ethical reason to do so.

The randomization assignment should not be revealed before the study has been completed and the database has been cleaned and closed. The study will be unblinded using a study specific unblinding procedure.

In rare emergencies, such as the investigator feels adequate treatment of an AE requires knowledge of study treatment, unblinding of an individual subject may be necessary. In case of an emergency, the treatment administered to the subjects can be revealed using the unblinding function of the IWRS.

In such events, the Investigator must make every attempt to inform the study Medical Monitor before breaking the blind or as soon as possible after unblinding has been performed (within 24 hours after the emergency unblinding). The date, time and reason for breaking the code, the treatment administered (placebo or L-citrulline), and the sponsor's representative who was informed of the unblinding, will be documented within the eCRF/IWRS.

4.4.8 Prior and Concomitant Therapy

Any medication taken by a subject during their hospitalization and the reason for use will be recorded on the case report form. These include medications taken before, during and after

surgery. Concomitant therapy includes, but is not limited to, inotropic or vasoactive medications, CPB replacement fluids, blood products and perfusion fluids.

Administration of total parenteral nutrition (TPN) will be at the discretion of the clinical staff in the PICU and should not be affected by study participation. Any substance that is incompatible with TPN should not be infused with L-citrulline, since TPN also contains amino acids. A "Y" site may be utilized for the infusion at the discretion of the Principal Investigator.

Subjects can be treated with iNO if the therapy is indicated at the discretion of the Principal Investigator. However, prophylactic treatment with NO is not permitted. Any use of iNO will be tracked.

Preoperative invasive mechanical ventilation or i.v. inotrope support, as well as concomitant use of any pulmonary hypertension medications, is not allowed and renders a subject ineligible for treatment (see Section 4.3.3).

Rescue medications, including use of iNO, after surgery will be permitted. Use of any rescue medications will be recorded and their use will be tracked as an outcome.

4.4.9 Treatment Compliance

The administration of the study medication will be performed by the Investigator or Sub-Investigator or medically trained personnel and will be documented accordingly.

5 Study Procedures

5.1.1 Pre-treatment

5.1.1.1 Screening Visit (Day -14 to Day 0)

A series of screening evaluations will be performed in order to determine whether prospective study participants meet the selection criteria for the trial.

- The Investigator or designated study personnel will inform each prospective subject's family of the nature of the study, explain the potential risks, and obtain written informed consent prior to performing any study related procedures.
- Assessment for eligibility against the inclusion and exclusion criteria
- Demographic data (age, sex, race and ethnicity)

- Screening evaluations will consist of medical history documentation, including documentation of previous echocardiogram confirming cardiovascular anatomy and the defect to be surgically repaired, and a physical examination, including body weight and height
- Blood draw*:
  - serum electrolytes (sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and chloride (Cl)), BUN, and creatinine
  - CBC with differential
  - ACT
  - liver function tests (LFTs), including bilirubin (total), alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH)

  * Results of laboratory tests done as standard of care for pre-op testing are acceptable and may be recorded as screening values, provided the tests were performed within 14 days of Day 0. If any of the required tests were not done as part of the pre-op testing, these must be completed within 14 days of Day 0.
- Urinalysis (not required, but results should be recorded if test is performed)
  - Visual: Color, Clarity/Turbidity
  - Chemical: pH, Specific gravity, glucose, ketones, nitrites
  - Microscopic: RBCs, WBCs, epithelial cells, casts, bacteria
- Concomitant medications

5.1.1.2 Baseline Visit (Day of Surgery, Pre-operation)

The following evaluations will be performed on the day of surgery, prior to the operation:

- Body weight for calculating study drug dosage
- Inotrope and other concomitant medication documentation
- Urine pregnancy test for females of child-bearing potential
- Randomization
- Preparation of study drug by the hospital pharmacy
  - Blood draw (prior to surgery; in the operating room [OR], after induction of

anesthesia); for: plasma L-citrulline (1.0 mL)

- Adverse events documentation (pre-treatment)

5.1.2 Treatment Period

5.1.2.1 Day 0, Surgery (Intra-operative)

The following evaluations will be performed in the OR, during surgery:

- Collection of CPB data, including the following:
  - Equipment type
  - Cardioplegia solution type
  - Composition of isovolumetric exchange fluid for ultrafiltration and/or modified ultrafiltration
  - Exchange volumes in and out
  - Documentation of L-citrulline or placebo administration
  - CPB start and end times and decannulation time

- Hemodynamic monitoring, including the following:
  - Heart rate
  - Systemic blood pressure (BP)
  - O_2 saturation
  - Central Venous Pressure (CVP) measurements
  - Pulmonary Artery Pressure (PAP) measurements (if PA line is placed)

    Baseline values are first recorded in the OR by Anesthesia. Results will be *monitored* during surgery according to the Institution's standard of care, but results will only be *recorded* approximately every 15 minutes.

- Arterial Blood Gases (ABGs)
  - PaO_2, $PaCO_2$, HCO_3, and pH measurements

    Baseline values are first recorded in the OR by Anesthesia. All results obtained after induction of anesthesia and during surgery will be recorded.

- Bolus of L-citrulline at 150 mg/kg or placebo at initiation of CPB; addition of L-citrulline at a concentration of 200 µmol/L or placebo given as a bolus during bypass. This may be administered as a one-time bolus or multiple administrations to compensate for fluids containing L-citrulline that may be removed from the patient

during the course of the operation and thus to maintain the concentration of 200 µmol/L. The total dose will be dependent upon the total amount of any volume administered and removed during CPB.

- Blood draw (10 minutes after study drug is administered):
  - serum electrolytes, BUN, and creatinine
  - CBC with differential
  - ACT
  - plasma L-citrulline (1.0 mL)
- Inotrope and other concomitant medication documentation
- Adverse events documentation

5.1.2.2 Day 0, post-surgery, 0 hours (30 minutes post CPB decannulation)

The following evaluations will be performed 30 minutes post decannulation:

- Blood draw for:
  - plasma L-citrulline (***prior to bolus***); 1.0 mL
- Bolus of L-citrulline at 20 mg/kg or placebo 30 minutes after decannulation from CPB immediately followed by a continuous infusion of L-citrulline at 9 mg/kg/hr or placebo for up to 48 hours. The study drug or placebo infusion will be discontinued once invasive arterial blood pressure monitoring is discontinued or at 48 hours, whichever comes first.
- Hemodynamic monitoring including:
  - Heart rate
  - Systemic BP
  - O_2 saturation
  - CVP measurements
  - PAP measurements (if PA line is placed)

    Results will be recorded according to the Institution's standard of care from time of post-op admission to the PICU through 48 hours. Results at 1, 2, 4, 12, 24, and 48 hours *from baseline (in OR)* must be obtained and recorded.
- ABGs
  - PaO_2, $PaCO_2$, HCO_3, and pH measurements

    ABGs will be drawn according to the Institution's standard of care. All results

obtained from the time of post-op admission to the PICU until removal of the arterial line or through 48 hours (whichever occurs first) will be recorded.

- Thoracotomy output measurements (for the duration of chest tube placement)
- Blood draw for:
  - plasma L-citrulline (***5 minutes after bolus***); 1.0 mL
- Inotrope and other concomitant medication documentation
- Documentation of ventilator settings. Recorded approximately every hour (+/- 10 minutes) from time of separation from CPB until end of mechanical ventilation or 28 days (whichever occurs first)
- Hypotension assessment (Refractory hypotension is defined as a 20% drop of mean arterial blood pressure [MAP] below specific age-related criteria (Haque and Zaritsky (2007) for greater than 30 minutes) (See below)
  - Infants: MAP of 40
  - Age 1 year: MAP of 40
  - Age 2 years: MAP of 44
  - Age 3 years: MAP of 47
  - Age 4 years: MAP of 50
  - Age 5 years: MAP of 52
  - Age 6 years: MAP of 53
  - Age 7 years: MAP of 52
  - Age 8 years: MAP of 54
  - Age 9 years: MAP of 55
  - Age 10 years: MAP of 56
  - Age 11 years: MAP of 57
  - Age 12 years: MAP of 58
  - Age 13 years: MAP of 59
  - Age 14 years: MAP of 61
  - Age 15 years: MAP of 62
  - Age 16 years: MAP of 63
  - Age 17-18 years: MAP of 64
- Adverse events documentation

5.1.2.3 Day 0, post-surgery, 6 hours

The following evaluations will be performed:

- Continuous infusion of L-citrulline at 9 mg/kg/hr or placebo for up to 48 hours. The

study drug or placebo infusion will be discontinued once invasive arterial blood pressure monitoring is discontinued or at 48 hours, whichever comes first.

- Hemodynamic monitoring including:
  - Heart rate
  - Systemic BP
  - O_2 saturation
  - CVP measurements
  - PAP measurements

    Results will be recorded according to the Institution's standard of care from time of post-op admission to the PICU through 48 hours. Results at 1, 2, 4, 12, 24, and 48 hours *from baseline (in OR)* must be obtained and recorded.
- ABGs
  - PaO_2, $PaCO_2$, HCO_3, and pH measurements
- ABGs will be drawn according to the Institution's standard of care. All results obtained from the time of post-op admission to the PICU until removal of the arterial line or through 48 hours (whichever occurs first) will be recorded. Thoracotomy output measurements (for the duration of chest tube placement)
- Blood draw for:
  - plasma L-citrulline (6 hrs; +/-1 hour; collect at end of infusion if infusion discontinued prior to 6 hours); 1.0 mL
- Inotrope and other concomitant medication documentation
- Documentation of ventilator settings. Recorded approximately every hour (+/- 10 minutes) from time of separation from CPB until end of mechanical ventilation or 28 days (whichever occurs first)
- Hypotension assessment (Refractory hypotension is defined as a 20% drop of MAP below specific age-related criteria for greater than 30 minutes) (See Section 5.1.2.2)
- Adverse events documentation

5.1.2.4 Day 0, post-surgery, 12 hours

The following evaluations will be performed:

- Continuous infusion of L-citrulline at 9 mg/kg/hr or placebo for up to 48 hours. The study drug or placebo infusion will be discontinued once invasive arterial blood

pressure monitoring is discontinued or at 48 hours, whichever comes first.

- Hemodynamic monitoring including:
  - Heart rate
  - Systemic BP
  - O_2 saturation
  - CVP measurements
  - PAP measurements

    Results will be recorded according to the Institution's standard of care from time of post-op admission to the PICU through 48 hours. Results at 1, 2, 4, 12, 24, and 48 hours *from baseline (in OR)* must be obtained and recorded.
- ABGs
  - PaO_2, $PaCO_2$, HCO_3, and pH measurements

    ABGs will be drawn according to the Institution's standard of care. All results obtained from the time of post-op admission to the PICU until removal of the arterial line or through 48 hours (whichever occurs first) will be recorded.
- Thoracotomy output measurements (for the duration of chest tube placement)
- Blood draw for:
  - plasma L-citrulline (12 hrs; +/-1 hour; collect at end of infusion if infusion discontinued prior to 12 hours); 1.0 mL
- Inotrope and other concomitant medication documentation
- Documentation of ventilator settings. Recorded approximately every hour (+/- 10 minutes) from time of separation from CPB until end of mechanical ventilation or 28 days (whichever occurs first)
- Hypotension assessment (Refractory hypotension is defined as a 20% drop of MAP below specific age-related criteria for greater than 30 minutes) (See Section 5.1.2.2)
- Adverse events documentation

5.1.2.5 Day 1, post-surgery, 24 hours

The following evaluations will be performed:

- Continuous infusion of L-citrulline at 9 mg/kg/hr or placebo for up to 48 hours. The study drug or placebo infusion will be discontinued once invasive arterial blood

pressure monitoring is discontinued or at 48 hours, whichever comes first.

- Hemodynamic monitoring including:
  - Heart rate
  - Systemic BP
  - O_2 saturation
  - CVP measurements
  - PAP measurements

    Results will be recorded according to the Institution's standard of care from time of post-op admission to the PICU through 48 hours. Results at 1, 2, 4, 12, 24, and 48 hours *from baseline (in OR)* must be obtained and recorded.
- ABGs
  - PaO_2, $PaCO_2$, HCO_3, and pH measurements
- ABGs will be drawn according to the Institution's standard of care. All results obtained from the time of post-op admission to the PICU until removal of the arterial line or through 48 hours (whichever occurs first) will be recorded. Thoracotomy output measurements (for the duration of chest tube placement)
- Blood draw for:
  - serum electrolytes, BUN, and creatinine
  - CBC with differential
  - ACT
  - plasma L-citrulline (24 hrs; +/-1 hour. Collect at end of infusion if infusion discontinued prior to 24 hours); 1.0 mL
- Urinalysis (not required, but results should be recorded if test is performed
- Inotrope and other concomitant medication documentation
- Documentation of ventilator settings. Recorded approximately every hour (+/- 10 minutes) from time of separation from CPB until end of mechanical ventilation or 28 days (whichever occurs first)
- Hypotension assessment (Refractory hypotension is defined as a 20% drop of MAP below specific age-related criteria for greater than 30 minutes) (See Section 5.1.2.2)
- Adverse events documentation

5.1.2.6 Day 2, post-surgery, 48 hours

The following evaluations will be performed:

- Continuous infusion of L-citrulline at 9 mg/kg/hr or placebo for up to 48 hours. The study drug or placebo infusion will be discontinued once invasive arterial blood pressure monitoring is discontinued or at 48 hours, whichever comes first.
- Hemodynamic monitoring including:
  - Heart rate
  - Systemic BP
  - O_2 saturation
  - CVP measurements
  - PAP measurements

  Results will be recorded according to the Institution's standard of care from time of post-op admission to the PICU through 48 hours. Results at 1, 2, 4, 12, 24, and 48 hours *from baseline (in OR)* must be obtained and recorded.
- ABGs
  - PaO_2, $PaCO_2$, HCO_3, and pH measurements

  ABGs will be drawn according to the Institution's standard of care. All results obtained from the time of post-op admission to the PICU until removal of the arterial line or through 48 hours (whichever occurs first) will be recorded.
- Thoracotomy output measurements (for the duration of chest tube placement)
- Blood draw for:
  - serum electrolytes, BUN, and creatinine
  - CBC with differential
  - ACT
  - LFTs
  - plasma L-citrulline (48 hrs; +/-1 hour; collect at end of infusion if infusion discontinued prior to 48 hours); 1.0 mL
- Inotrope and other concomitant medication documentation
- Documentation of ventilator settings. Recorded approximately every hour (+/- 10 minutes) from time of separation from CPB until end of mechanical ventilation or 28 days (whichever occurs first)

- Hypotension assessment (Refractory hypotension is defined as a 20% drop of MAP below specific age-related criteria for greater than 30 minutes) (See Section 5.1.2.2)
- Adverse events documentation

5.1.2.7 Follow-Up Visit [Day 28 (if still in hospital) or upon Hospital Discharge]

The following evaluations will be performed:

- Thoracotomy output measurements (for the duration of chest tube placement)
- Blood draw* for:
  - serum electrolytes, BUN, and creatinine
  - CBC with differential
  - LFTs
  - Plasma L-citrulline (1.0 mL)

  * (An attempt should be made to obtain final laboratory samples on Day 28 or prior to discharge from hospital. If laboratory results within 24 hours are available, these may be captured. If results are not available within 24 hours, then record the results of the labs obtained closest to discharge from the hospital. These labs must include LFTs. If no i.v. access is available on Day 28 or the day of discharge, venipuncture is not required since results of the labs obtained closest to discharge may be used.
- Inotrope and other concomitant medication documentation
- Documentation of ventilator settings. Recorded approximately every hour (+/- 10 minutes) from time of separation from CPB until end of mechanical ventilation or 28 days (whichever occurs first)
- Adverse events documentation

Patients withdrawing from the study prior to Day 28 or hospital discharge will be encouraged to complete all follow-up assessments.

5.1.2.8 Follow-Up Phone Assessment (only for patients discharged prior to Day 28)

Patients discharged prior to Day 28 will have their follow-up visit procedures completed upon discharge from the hospital (see section 5.1.2.7). Therefore, in order to assess their survival status at Day 28, patients (or parents, dependent upon patient age) will be contacted by phone on Day 28 to assess their status.

5.1.3 Duration of Treatment

The total duration of treatment will be the treatment time during CPB plus 48 hours from time of decannulation after CPB onwards.

6 Variables Assessed

6.1 Screening, Baseline, and Surgery Assessments

6.1.1 Demographics

Relevant demographic data include age, sex, race and ethnicity.

6.1.2 Echocardiography

The results of a transthoracic echocardiogram will be required to document the presence of a congenital heart defect and to determine eligibility for the study. The results of the echocardiogram must be documented in the patient's medical history so that eligibility can be confirmed.

6.1.3 Physical Examination

A complete physical examination, including height and weight, will be performed at screening.

6.1.4 Cardiopulmonary Bypass Assessments

The following data on CPB will be documented in the OR: Equipment type, cardioplegia solution type, composition of isovolumetric exchange fluid for ultrafiltration and/or modified ultrafiltration, exchange volumes in and out, documentation that L-citrulline or placebo was added to the filtration and hemoconcentration fluids, CPB start and end times and decannulation time.

6.2 Efficacy and Safety Measurements Assessed

6.2.1 Efficacy Assessments

6.2.1.1 Mechanical Ventilation

The definition of mechanical ventilation includes invasive mechanical ventilation or non-invasive mechanical ventilation including BPAP or CPAP. Length of time on mechanical

ventilation will be assessed from the time of separation from CPB until mechanical ventilation is discontinued.

6.2.1.2 Inotropes

Inotropes are defined as dopamine, dobutamine, milrinone, epinephrine, phenylephrine, and norepinephrine. Inotrope use will be assessed from the start of CPB until the subject is discharged from the hospital or at Day 28.

Total Inotrope Score = Dopamine + Dobutamine + 10*Milrinone + 100*Epinephrine +100*Phenylephrine + 100*Norepinephrine.

6.2.1.3 Hemodynamic Parameters

Hemodynamic evaluations include heart rate, systemic arterial blood pressure, oxygen saturation, and CVP. If a PA catheter is placed for clinical reasons, PAP will be recorded. Hemodynamic parameters will be assessed prior to anesthesia for CPB and repeatedly until 48 hrs post-operation (Day 2).

6.2.1.4 Plasma Citrulline Concentration

L-citrulline treatment in this study is designed to maintain a sustained therapeutic plasma citrulline level above the target threshold of 100 µmol/L from the initiation of CPB, throughout surgery, and for up to 48 hours after surgery. Plasma citrulline concentrations will be assessed in both treatment groups to determine the number of patients who reach the therapeutic sustained target plasma citrulline level of ≥100 µmol/L.

Blood collection for assessment of plasma citrulline concentrations will be taken prior to surgery, during surgery, 30 minutes post-decannulation after CPB (prior to bolus and again 5 minutes after bolus), at the specified post-operative time points (6h, 12h, 24h, 48h), and at hospital discharge or Day 28, whichever occurs first.

All plasma citrulline samples will be sent to a central laboratory for analysis.

6.2.1.5 Thoracotomy Output/Chest Tube Drainage

The thoracotomy output is defined as the total volume of chest tube drainage recorded in cc prior to discontinuation of chest intubation.

The duration of chest intubation as well as the volume of chest tube drainage will be recorded from 30 minutes post decannulation after CPB until chest tube is removed.

6.2.1.6 Vasodilators

Use of vasodilators and infusion dose will be recorded. Examples of vasodilators include nitroprusside, nitroglycerin, and nicardipine

Use of vasodilators will be recorded from first use following separation from bypass, until the subject is discharged from the hospital or at Day 28.

6.2.1.7 Hospitalization

Hospitalization will be documented from the time of hospital admission until Day 28 or discharge, whichever occurs earlier. Days and hours in the PICU will be indicated.

6.2.2 Safety Assessments

6.2.2.1 Adverse Events

Adverse Event Definition

An adverse event (AE) is any event, side effect, or other untoward medical occurrence, (including dosing errors) that may be present during treatment with a medical product or intervention and may or may not be related to that product or intervention. An AE can, therefore, be any unfavorable and unintended sign (e.g., a clinically significant abnormal laboratory finding), symptom or disease temporally associated with the use of a medicinal product or intervention, whether or not the event could be considered related to use of the product or intervention.

Clinical adverse events are illnesses, signs, or symptoms that have appeared or worsened during the study or during the follow-up period. These may include the following: 1) the exacerbation of a pre-existing illness, 2) an increase in the frequency or severity of a pre-existing event or condition, 3) any condition detected or diagnosed after study drug administration even though it may have been present prior to the start of the study or 4) continuous persistent disease or symptoms present at baseline that worsen following the start of the study. Laboratory AEs are clinically significant abnormal values obtained on laboratory tests during the acute and follow-up period.

Any medical condition or clinically significant laboratory abnormality with an onset date before the date the Informed Consent is signed is considered to be pre-existing and should be documented on the Medical History case report form (CRF.) All AEs from the time the Informed Consent is signed, whether believed to be study-related or not, must be recorded on the appropriate CRF.

It is the responsibility of the investigator to document all AEs that occur during the study. Occurrence of AEs will be assessed through observation by Investigational staff and reported on the appropriate page of the CRF.

Serious Adverse Event Definition

A Serious Adverse Event (SAE) is any untoward medical occurrence or effect that, at any dose, results in any of the following outcomes:

- Death
- Life-threatening (subject at immediate risk of death)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in congenital anomaly/birth defect
- Results in a persistent or significant disability or incapacity
- An important medical event that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Documentation and Assessment of AE's and SAE's

Every adverse event, whether serious or not, must be recorded as soon as is reasonably possible in the patient's CRF. The following must be included:

- date of onset (or exacerbation in the case of worsening of an existing AE)
- frequency
- duration of effect

Additionally, the Principal Investigator (PI) must assess every adverse event in the CRF for severity and causality and must define the action taken (defined below).

Assessment of Severity (not to be confused with "seriousness")

Each AE/SAE will be assigned a category by the investigator as follows:

Mild — An AE/SAE that is easily tolerated by the subject, causes minimal discomfort and does not interfere with everyday activities.

Moderate	An AE/SAE that is sufficiently discomforting to interfere with normal everyday activities; intervention may be needed.
Severe	An AE/SAE that prevents normal everyday activities; treatment or other intervention usually needed.

If there is a change in severity of an AE/SAE, it must be recorded as a separate event.

Assessment of Causality

Every effort will be made by the investigator to assess the relationship of the AE, if any, to the IMP. Causality should be assessed using the categories defined below:

Unrelated	Clinical event where evidence exists that the symptom is definitely related to etiology other than the study treatment (e.g., trauma secondary to a motor vehicle accident or a symptom suggestive of another illness which is not accepted to be a possible reaction to the study treatment).
Unlikely	Clinical event with a temporal relationship to IMP administration makes a causal connection improbable, but that could reasonably be explained by underlying disease or other drugs or chemicals.
Possible	Clinical events with a reasonable temporal relationship to IMP administration, but that could also be explained by concurrent disease or other drugs or chemicals that have a possible temporal relationship to the study treatment; however, a potential alternative etiology exists which may be responsible for the symptom (e.g., fever or irritability when other symptoms are present that suggest another etiology, for example: upper respiratory infection).
Probable	Clinical events with temporal relation to the study treatment, and a potential alternative etiology is not apparent (e.g., fever or irritability when no other symptoms suggestive of an illness are present) with a reasonable time relationship to IMP administration, and is unlikely to be attributed to concurrent disease or other drugs or chemicals.
Definite	Clinical events with plausible temporal relationship to IMP administration, and that cannot be explained by concurrent disease or other drugs or chemicals; events which have a timely relation to the study and no alternative etiology is present. They must have occurred within a reasonable temporal sequence of the treatment, must be reasonably explained and must follow a known pattern of response.

Action Taken

The investigator will describe the action taken in the appropriate section of the CRF, as follows:

- None
- IMP stopped
- IMP temporarily interrupted
- Concomitant medication
- Other, specify

"Expected" Adverse Events

"Expected" adverse events are those AE's that are associated with the condition or the treatment and are listed as such in the trial protocol and/or the Investigator Brochure, whether or not they occur in every case.

Congenital heart surgery and events associated with the surgery are considered "expected" in many participants as part of the underlying disease of study and therefore not necessarily reportable to the IRB as adverse events. A compilation of these AE's is in **Table 1**.

Table 1: Adverse Events Associated with Pediatric Congenital Heart Defect Surgery

Surgical Bleeding > 10 cc/kg/hr x 2 hours Chest re-exploration **Respiratory:** Reintubation Pleural effusion requiring chest tube Pneumothorax Pulmonary embolus **Cardiac** Ventricular tachycardia Supraventricular tachycardia Junctional ectopic tachycardia Atrial fibrillation Cardioversion **ID:** Fever > 38.5 Nosocomial blood stream infection (utilizing NNIS criteria) Nosocomial pneumonia (utilizing NNIS criteria) Nosocomial urinary tract infection (utilizing NNIS criteria) Surgical wound infection (utilizing NNIS criteria) **Hematologic** WBC<5 PLT <50K	HCT < 20 INR >2.0 **Electrolyte** K <2.5 (not due to lasix) Na <125 iCa<4.0 Mg < 1.5 Glucose <50 **Neurologic** IVH Seizures **Renal** BUN >40 Creat > 1.5 Need for CVVH or dialysis **Medications** Epi bolus in non code situation Atropine bolus in non code situation Vasopressin infusion Amiodarone infusion **General** PCCU readmission

Based on available data with L-citrulline treatment, there are no anticipated risks or side effects. The only potential AE would be transient systemic arterial hypotension.

Unexpected Adverse Events

An unexpected adverse event is any adverse event that has not been previously described in available risk information (included in the protocol or the investigator's brochure).

Reporting of Adverse Events

All AEs occurring during the study must be documented on the relevant CRF pages. Non-serious AE's will only be recorded in the CRF. Serious AE's will be recorded in the CRF and

also reported to the CRO. SAEs must be reported by the investigator if it occurs during the clinical study or within 30 days of the patient receiving the IMP, whether or not the SAE is considered to be related to the IMP.
SAEs must be reported within 24 hours to the following:

Edgar Fenzl
FGK Clinical Research GmbH
Heimeranstr. 35
80339 Munich
Germany
Toll-free phone: 1-888-270-3895
P: + 49 (0) 89 893 119-0
F: + 49 (0) 89 893 119-180
Email: edgar.fenzl@fgk-pv.com
Cc: safety@fgk-cro.com

The investigator should not wait to receive additional information to document the event before notification of an SAE, although additional information may be requested. Where applicable, information from relevant laboratory results, hospital case records, and autopsy reports should be obtained.

SAEs must be followed until resolution or death, including those still ongoing at the Follow-up visit.

The CRO Medical Monitor must, in turn, forward the SAE report to the Asklepion Chief Medical Officer (CMO) as soon as is reasonably possible, but in any case, within 24 hours of receipt from the site. The CRO Medical Monitor or designee will notify the participating Institutional Review Board(s) (IRBs)/Ethics Committee(s), and CMO will notify the DSMB as appropriate.

SUSAR (Suspected Unexpected Serious Adverse Reaction)

A SUSAR is a serious adverse event that is suspected to be at least possibly related to the investigational drug (per the definitions of Causality in this section) and Unexpected (per the preceding definition of expectedness).

6.2.2.2 Clinical Laboratory Variables

The laboratory analyses will be performed at the local hospital/institution laboratories. Reference ranges will be supplied by all appropriate laboratory facilities and used by the investigator to assess the laboratory data for clinical significance and pathological changes. The following laboratory tests will be performed:

Hematology

Hemoglobin, hematocrit, red blood cell (RBC) count, white blood cell (WBC) count (total and differential), platelet count

Serum Electrolytes, BUN, and creatinine

Serum electrolytes (Na, K, Ca, Mg and Cl), BUN, and creatinine

Liver function tests

Bilirubin (total), AP, AST, ALT, and LDH

Coagulation parameter

ACT

A total amount of 16-19 mL of blood will be taken from each subject during the study for both clinical laboratory testing and citrulline plasma concentration determinations.

Urinalysis

Visual: Color, Clarity/Turbidity

Chemical: pH, Specific gravity, glucose, ketones, nitrites

Microscopic: RBCs, WBCs, epithelial cells, casts, bacteria

6.3 Appropriateness of Measurements

All efficacy and safety assessments are widely used and generally recognized as reliable, accurate and relevant to the disease condition.

7 Statistical Methods

7.1 Statistical and Analytical Plans

All aspects of statistical analyses will be detailed within a statistical analysis plan (SAP), which will be finalized prior to both data base lock and subsequent unblinding.

Continuous variables will be summarized by means, standard deviations, medians, lower and upper quartiles, minimums and maximums. Frequencies and percentages will be used to summarize categorical variables.

Analyses will be performed by visit/ time point, if not stated otherwise. Baseline will be defined

as the most recent non-missing value prior to surgery.

For time to event analyses censoring information will be used for statistical analyses. No other imputation of missing values (e.g., last observation carried forward [LOCF]) will be used, if not stated otherwise.

7.1.1 Datasets Analyzed

Safety Analysis Set (SAF)

All randomized subjects who received surgery and study medication (independent of whether it is L-citrulline or placebo) will be valid for the SAF. Within the SAF a subject will be considered for the treatment actually received and not for the treatment assigned by randomization, if different. The SAF will be used for the evaluation of the safety assessments.

Full Analysis Set (FAS)

The FAS includes all subjects who underwent surgery. Within the FAS a subject will be considered for the treatment assigned by randomization and not for the treatment actually received, if different, i.e. following the intent-to-treat (ITT) principle. The FAS will be used for the evaluation of the efficacy assessments. The FAS serves as the primary efficacy analysis set.

Modified Full Analysis Set (mFAS)

The mFAS includes all subjects who underwent surgery and received study medication (independent of whether it is L-citrulline or placebo). Within the mFAS, a subject will be considered for the treatment assigned by randomization and not for the treatment actually received, if different, i.e. following the ITT principle. The mFAS will be used for the evaluation of the efficacy assessments. The mFAS serves as a sensitivity population for the efficacy analyses.

Per-protocol Analysis Set (PP)

The PP includes all subjects included in the SAF who had no significant protocol deviations and completed through Day 28 or until discharge from the hospital (end of study follow-up). For the PP, all subjects will be assigned to the treatment actually received. The PP will only be analyzed for main efficacy outcome measures.

L-citrulline Plasma Levels

L-citrulline blood levels will be obtained on each patient at set times, and plotted and examined to confirm that they are ≥ the threshold level of 100 µmol/L.

Blind data review meeting (BDRM)

Subjects will be assigned to the SAF, FAS, mFAS, and PP during a blind data review meeting (BDRM). Further details on the analysis sets, criteria for the PP, and the DRM will be specified within the SAP.

7.1.2 Handling of Missing Data

For laboratory data, concentrations below the limit of quantification will be set to zero.

Missing safety data or safety assessments and missing plasma levels will not be estimated nor imputed. All safety summaries and analyses will be based on observed data.

Censoring procedures will be applied for all time variables as described within the sections below. Further specifications on handling of missing data are added below, as applicable.

7.1.3 Demographic and Other Baseline Characteristics

Baseline data, including demographic data will be analyzed descriptively. Patient disposition, including discontinuation and reasons for discontinuation, and subjects' exposure will be tabulated in detail. Concomitant medication will be coded according to World Health Organization (WHO) drug dictionary and tabulated accordingly.

7.1.4 Efficacy Variables

Analysis of Primary Efficacy Endpoint

The primary efficacy endpoint is a composite variable consisting of the longer of either (1) length of time on mechanical ventilation or (2) length of time of inotrope use.

The definition of mechanical ventilation includes invasive mechanical ventilation or non-invasive mechanical ventilation including BPAP or CPAP. Length of time on mechanical ventilation will be measured until the subject is discharged from the hospital or at Day 28. The length will be derived as the time in hours from separation from CPB until discontinuation of all mechanical ventilation including non-invasive support. If invasive or non-invasive mechanical ventilation is stopped but has to be restarted, time will continue to accrue. If a subject did not receive any mechanical ventilation the length is set to 0. For subjects who died before discharge from the hospital or before Day 28, respectively, the observed length of time on mechanical ventilation will be used. As sensitivity analyses subjects who died will (1) be excluded from analysis and (2) be assigned a length of time on mechanical ventilation of 28 days.

The definition of inotrope use includes medications which are considered within the derivation of the total inotrope score (dopamine, dobutamine, milrinone, epinephrine, phenylephrine, norepinephrine). Length of inotrope use will be measured until the subject is discharged from the hospital or until Day 28, whichever occurs first. The length will be derived as the time of first use until last use (in hours). Any duration of re-use will continue to accrue. If a subject did not use any inotropes the length is set to 0. For subjects who died before discharge from the hospital or before Day 28, respectively, the observed length on inotropes will be used. As sensitivity analyses subjects who died will (1) be excluded from analysis and (2) be assigned a length of time on inotropes of 28 days.

Hypothesis to be tested:

H0: Composite endpoint within the L-citrulline group is equal to that of the placebo group

H1: Composite endpoint within the L-citrulline group is unequal to that of the placebo group

H0 will be tested using a Wilcoxon-rank-sum test.

The level of significance is 1% (two-sided).

The FAS will be the primary analysis set for the analysis on the composite endpoint. Analyses using the mFAS and the PP serve as sensitivity analyses.

Two-sided 95% confidence intervals using Hodges-Lehman method will be additionally calculated. Survival analyses using Kaplan-Meyer estimates and plots, log-rank test and Cox-regression modeling will serve as sensitivity analyses.

For survival analyses, different sets of analyses will be done:

(1) The composite variable for subjects without any mechanical ventilation and without any use of inotropes is set to 0, observations will not be censored

(2) The composite variable for subjects without any mechanical ventilation and without any use of inotropes is set to 0, observations will be censored

(3) Subjects without any mechanical ventilation and without any use of inotropes will be deleted from analysis

Subjects who receive mechanical ventilation or are still using inotropes on the last day of observation (which can be Day 28, day of early discontinuation, or day of death) will be censored for survival analyses.

Analysis of Secondary Efficacy Endpoints

No formal statistical hypotheses are specified for the secondary endpoint variables. Nonetheless statistical testing methods will be applied to compare treatment groups. A significance level of 5% (two-sided) will be used for such analyses. Secondary efficacy endpoints will be analyzed using the FAS, mFAS and the PP.

Length of Time on Mechanical Ventilation

The same definitions and analyses as described for the primary endpoint will be applied.

Length of Time on Positive Pressure Ventilation

The same definitions and analyses as described for the primary endpoint will be applied.

Length of Time of Inotrope Use

The same definitions and analyses as described for the primary endpoint will be applied.

Inotrope Score

The total inotrope score at each hour post-operatively from the time of separation from bypass, until the completion of study drug will be calculated as:

Total Inotrope Score = Dopamine + Dobutamine + 10*Milrinone + 100*Epinephrine +100*Phenylephrine + 100*Norepinephrine.

The total inotrope score will be summarized at each post-operative hour and analyzed using a Wilcoxon-Rank sum test and an analysis of variance (ANOVA) with a fixed effect for treatment group. Also, a repeated measures analysis of variance will be used to compare total inotrope score between placebo and L-citrulline over time.

Additionally, the total inotrope score over time until Day 28 or hospital discharge will be derived and analyzed using a Wilcoxon-Rank sum test and an ANOVA with a fixed effect for treatment group.

The total inotrope score will be imputed to zero after the bypass pump has been removed. It will also be imputed as zero for patients not using inotropes at all. For any missing scores between two non-missing scores they will be imputed using the most recent non-missing score (the last observation carried forward principle).

Hemodynamic Improvement

Hemodynamic evaluations include heart rate, systemic arterial blood pressure, oxygen

saturation, and central venous pressure. The absolute changes from baseline at hours 1, 2, 4, 12, 24, and 48 will be compared between groups using an ANOVA with a fixed effect for treatment group and baseline level.

An ANOVA adding site and site-by-treatment group interaction will be conducted as a sensitivity analysis. If the p-value for the site-by-treatment group interaction is lower than 0.1 the analysis will also be provided by site. Summary tables describing descriptive measurements will be generated for absolute values and absolute change from baseline values for all observed time points.

Length of time on intubation

The length will be derived as the time in hours from separation from CPB until discontinuation of intubation. Any duration of re-use of intubation will continue to accrue. If a subject did not use any intubation the length is set to 0. As a sensitivity analysis, subjects with no use of intubations will be excluded. For subjects who died before discharge from the hospital or before Day 28, respectively, the observed length of time on intubation will be used. As sensitivity analyses subjects who died will (1) be excluded from analysis and (2) be assigned a length of time on intubation of 28 days.

For the length of time on intubation the same analyses as described for the primary endpoint will be applied.

Length of PICU stay

The length of PICU stay will be calculated as the total number of days postoperative until discharge from PICU. For subjects who died before discharge from PICU or before Day 28, respectively, the observed length of PICU stay will be used. As sensitivity analyses subjects who died will (1) be excluded from analysis and (2) be assigned a length of PICU stay of 28 days.

For the length of PICU stay the same analyses as described for the primary endpoint will be applied.

Length of Time on Vasodilators

Length of time on vasodilators will be measured from first use following separation from bypass, until the subject is discharged from the hospital or at Day 28. The length will be derived as the time in hours from separation from CPB until discontinuation of all vasodilators. Any duration of re-use of vasodilators will continue to accrue. If a subject did not use any vasodilators the length is set to 0. As a sensitivity analysis, subjects with no use of vasodilators

will be excluded. For subjects who died before discharge from the hospital or before Day 28, respectively, the observed length of time on vasodilators will be used. As sensitivity analyses subjects who died will (1) be excluded from analysis and (2) be assigned a length of time on vasodilators of 28 days.

For the length of time on vasodilators the same analyses as described for the primary endpoint will be applied.

Length of Hospitalization

The length of hospitalization will be calculated as the total number of days postoperative until discharge from the hospital. For subjects who died before discharge from the hospital or before Day 28, respectively, the observed length of hospitalization will be used. As sensitivity analyses subjects who died will (1) be excluded from analysis and (2) be assigned a length of hospitalization of 28 days.

For the length of hospitalization, the same analyses as described for the primary endpoint will be applied.

Thoracotomy Output - Duration and Volume of Chest Tube Drainage:

The total postoperative duration (in hours) that the chest tube is used will be calculated as the time from the end of the surgery to the time the chest tube is removed. If an additional chest tube is required or reinserted (until discharge from the hospital or at Day 28) the duration that the additional chest tube was used (from time of insertion to time of removal) will be added to the time the original chest tube was used for the total postoperative duration. If a subject did not use any chest tube the duration is set to 0. As a sensitivity analysis, subjects with no use of chest tube will be excluded. For subjects who died before discharge from the hospital or before Day 28, respectively, the observed duration of chest tube drainage will be used.

For the total postoperative duration, the same analyses as described for the primary endpoint will be applied. Again, as sensitivity analyses subjects who died will (1) be excluded from analysis and (2) be assigned a length of time of chest tube placement of 28 days.

The total amount of chest tube drainage (mL), added up during each duration of chest tube as described above, will be summarized using descriptive measures and compared between groups using an ANOVA with a fixed effect for treatment group.

L-citrulline Plasma Levels

Summary statistics using descriptive measures will be tabulated for plasma L-citrulline levels.

7.1.5 Safety Variables

Safety endpoints will be analyzed using the SAF.

Adverse Events

Pre-treatment adverse events and treatment adverse events will be analyzed separately. AEs will be summarized by system organ class (SOC) and preferred term (PT) according to Medical Dictionary for Regulatory Activities (MedDRA). The number of events, as well as the number and rate of affected subjects will be reported. AEs (SOC and PT) will also be summarized by seriousness, as well as by severity and relationship to study medication. No statistical testing procedures will be applied to the analysis of AEs. No statistical testing procedures will be applied to the analysis of AEs.

Incidence of Refractory Hypotension

Refractory hypotension is defined as a 20% drop in mean MAP below specific age-related criteria for more than 30 minutes. The number of subjects with any refractory hypotension from end of surgery until 48 hours will be compared between groups using Fishers' exact test. Additionally, a logistic regression analysis will be applied including factor treatment and site.

Laboratory Values (CBC with differential, serum creatinine and electrolytes & liver enzymes)

The absolute values, absolute and percentage changes from baseline will be tabulated using summary tables listing descriptive measurements for all observed time points. No statistical testing procedures will be applied to the analysis of laboratory values.

7.2 Initial Determination of Sample Size Estimate

The primary endpoint of this study is a composite variable consisting of the longer of either (1) length of time on mechanical ventilation or (2) length of inotrope use. The primary analysis will be the based on the full analysis set and will be conducted using a Wilcoxon-rank-sum test. As a sensitivity analysis, the primary endpoint variable will also be analyzed using survival analysis methods.

The results for the composite endpoint variable, with re-intubation periods included, from the Phase IB study, CIT-002-01, are shown in Table 2:

Table 2: Phase IB study (CIT-002-01) Composite endpoint results

	Mean (SD)		
	Placebo (N = 11)	**Citrulline (N = 11)**	**Total (N = 22)**
Composite endpoint	44.4 (63.62)	14.3 (14.06)	29.3 (47.54)

SD = standard deviation, N = number of patients.
Data source: Table 14.2.2.5.1.1, CIT-002-01 Clinical Study Report

The sample size calculation was calculated using nQuery 7.0. The common standard deviation (SD) was estimated to be 46.3 using the observed SDs of 64 and 14. Again, using an assumed SD of 50 and expected mean values of 44 for placebo and 14 for the L-citrulline group, the effect size is estimated as being 0.600, which yields a sample size of N=92 per group when applying a two-sided Wilcoxon-rank-sum test with a 0.01 significance level and a power of 90%.

The power is even larger than 90% when applying the Phase IB results of the composite endpoint variable with re-intubation periods excluded. Only one patient within the Placebo group was re-intubated, resulting in a lower mean composite endpoint value of 32 and a SD of 26.26.

To allow for subjects being enrolled but not assigned to the full analysis set, an overall N=95 subjects per group will be enrolled.

7.3 Sample Size Estimation Revision

A sample size re-estimation will be done as soon as the first 95 consecutively randomized full analysis set (FAS)-patients will have undertaken their Follow up visit (Day 28 or Hospital Discharge). (Refer to section 5.1.2.7 for details.)

7.4 Interim Analysis

One interim analysis is planned to be carried out when the first 95 consecutively randomized full analysis set (FAS)-patients (i.e. 50% of the planned patients) will have undertaken their Follow up visit (Day 28 or Hospital Discharge), data required for the analysis of the composite endpoint have been entered into the eCRF, and those data have been cleaned appropriately. Only a sample size re-estimation will be carried out during this interim analysis; no other statistical analyses will be conducted. The sample size re-estimation will be done by an independent, unblinded statistician following the same procedures and criteria as for the initial

sample estimate, as described in section 7.2. Only the results of the sample size re-estimation will be provided to Asklepion.

Only the re-estimated variance values will be used for sample re-calculation, while the assumed values will not be re-estimated but kept as described in Section 7.2.

In the event that the sample size re-estimation leads to a reduction of the sample size due to lower re-estimated variance compared to the assumed variance values, the sample size as currently planned will be kept, and not reduced, to ensure robust estimations for safety and efficacy variables.

If the trial is to be continued with the re-estimated sample size, an appropriate protocol amendment will be submitted to the FDA as soon as feasible.

Final analysis will be based on the population of all randomized patients without taking the sample size re-estimation results into consideration. Due to the fact that only the interim variance will be assessed, no alpha adjustment of statistical analyses is required.

8 Quality Assurance and Quality Control

8.1 Audit and Inspection

Investigator sites and study documentation may be subject to Quality Assurance (QA) audit during the course of the study by the sponsor, CRO or its nominated representative. In addition, inspections may be conducted by regulatory authorities at their discretion. It is important that the investigator(s) and their relevant personnel are available during audits or inspections and that sufficient time is devoted to the process.

8.2 Monitoring

Data for each subject will be recorded on an eCRF. Data collection must be completed for each subject for whom there is an informed consent form (ICF) and is administered IMP.

In accordance with current Good Clinical Practice (cGCP) and International Conference on Harmonisation (ICH) guidelines, the study monitor will carry out source document verification at regular intervals to ensure that the data collected in the eCRF are accurate and reliable. The frequency of monitoring visits will be determined by the rate of subject recruitment.

The following will be reviewed at these visits:

- Compliance with the protocol.
- Consent procedure.
- Source documents.
- AE procedures.
- Storage and accountability of materials.

The monitoring visits also provide the sponsor with the opportunity to ensure the investigator's obligations and all applicable ICH or health authority regulation requirements are being fulfilled.

The investigator must permit the monitor, the IRB/IEC, the sponsor's internal auditors and representatives from regulatory authorities direct access to all study-related documents and pertinent hospital or medical records for confirmation of data contained within the CRFs. Subject confidentiality will be protected at all times.

An electronic medical record may be the source document; however, the study site must provide a standard operating procedure(s) (SOPs) that details review and approval of data entries by the principal, investigator(s).

8.3 Data Management and Coding

The sponsor or CRO will be responsible for activities associated with the data management of this study. This will include setting up a relevant database and data transfer mechanisms, along with appropriate validation of data and resolution of queries. Data generated within this clinical study will be handled according to the relevant SOPs of the data management and biostatistics departments of sponsor or CRO.

For Electronic Data Capture (EDC):

Study sites will enter data directly into an EDC system by completing the eCRF via a secure internet connection. Data entered into the eCRF must be verifiable against source documents at the study site. Data to be recorded directly on the CRF (without prior written or electronic record) will be identified and the CRF will be considered the source document. Any changes to the data entered into an EDC system will be recorded in the audit trail and is Food and Drug Administration (FDA) Code of Federal Regulations (CFR) Title 21 Part 11 compliant.

Data entered into the eCRF will be validated as defined in the Data Validation Plan. Validation includes, but is not limited to, validity checks (for example range checks), consistency checks and customized checks (logical checks between variables to ensure that study data are accurately reported). A majority of edit checks will be triggered during data entry and will

therefore facilitate efficient “point of entry” data cleaning. CRO Clinical Data Management will perform both manual eCRF review and review of additional electronic edit checks to ensure that the data are complete, consistent and reasonable. The electronic edit checks will run continually throughout the course of the study and the issues will be reviewed manually online to determine what action needs to be taken.

Manual queries may be added to the system by Clinical Data Management or Clinical Research Associates (CRAs). Data Managers and CRAs are able to remotely and proactively monitor the subject eCRFs to improve data quality.

CRO or designee will be responsible for:

- eCRF database creation and validation
- eCRF review and data validation

Medical coding will use MedDRA for concomitant diseases and adverse events and WHODrug for therapies.

Prior to the creation of a site data archive, it is necessary to be sure the database for the study in question has been closed. As an EDC system is being utilized for the study, CRO resources will produce the site data archives and deliver these archives to the study sites. Site data archive shall include not only an archive of the data entered, but also a record of the audit trail and any electronically created queries and comments. The site archival material shall be produced separately for each study site and then each set of materials or CDs should then be delivered to the study site for archival.

All study materials will be returned to the sponsor within a pre-agreed time interval from final database approval. The CRO clinical database (SAS), along with the SAS programs, will be stored on the CRO computer for a period of time to be agreed with the sponsor following completion of all activities due to be carried out for sponsor for the study prior to archiving. The archived electronic file will be destroyed after a pre-agreed period of time.

Missing or inconsistent data will be queried for clarification. Subsequent modifications to the database will be documented.

9 Records and Supplies

9.1 Drug Accountability

On receipt of the IMP, the investigator (or pharmacist) will conduct an inventory of the supplies and verify that IMP supplies are received intact and in the correct amounts prior to completing a supplies receipt. The pharmacist will retain a copy of this receipt at the study site and return the original receipt to the unblinded study monitor. The inventory of supplies at each study site may be checked at any time during the study by the unblinded monitor.

It is the responsibility of the unblinded study monitor to ensure that the investigator (or pharmacist) has correctly documented the amount of IMP received and dispensed on the dispensing log that will be provided. A full drug accountability log will be maintained at the study site at all times. The study monitor will also perform an inventory of IMP at the close-out visit to the site. All discrepancies must be accounted for and documented.

9.2 Financing and Insurance

Financing and insurance of this study will be outlined in a separate agreement between CRO and the sponsor.

10 Data and Safety Monitoring Board

The Data and Safety Monitoring Board (DSMB) will act in an advisory capacity to the Coordinating Investigator and Asklepion Pharmaceuticals, LLC to monitor participant safety. The DSMB responsibilities are to:

- Protect the safety of the study participants;
- Review the research protocol and plans for data safety and monitoring;
- Evaluate participant risk versus benefit, and other factors that can affect study safety outcome;
- Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the trial;
- Report to the study sponsor, Asklepion Pharmaceuticals, on the safety and progress of the trial;

- Make recommendations concerning continuation, termination or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study; and
- If applicable, review interim analyses in accordance with stopping rules, which are clearly defined in advance of data analysis and have the approval of the DSMB.

11 Ethics

11.1 Institutional Review Board / Independent Ethics Committee

Before initiation of the study at each investigational site, the protocol, all protocol amendments, the ICF, and any other relevant study documentation will be submitted to the appropriate IRB/IEC. Written approval of the study and all relevant study information must be obtained before the study site can be initiated or the IMP is released to the investigator. Any necessary extensions or renewals of IRB/IEC approval must be obtained; in particular, for changes to the study such as modification of the protocol, the informed consent form, the written information provided to subjects and/or other procedures.

The investigator will report promptly to the IRB/IEC any new information that may adversely affect the safety of the subjects or the conduct of the study. The investigator will submit written summaries of the study status to the IRB/IEC annually, or more frequently if requested by the IRB/IEC. On completion of the study, the sponsor will notify the IRB/IEC that the study has ended.

11.2 Ethical Conduct of the Study

The Guidelines of the World Medical Association (WMA) Declaration of Helsinki in its revised edition (Seoul, 2008), the ICH guidelines for cGCP (CPMP/ICH/135/35), Directive 2001/20/EC for EU studies, and by the FDA CFR (Title 21, § 50, § 54, § 56) for US studies, as well as the demands of national drug and data protection laws and other applicable regulatory requirements will be followed.

11.3 Subject Information and Consent

The informed consent will include all 8 basic required elements (45 CFR 46.1116a) as well as 6 additional elements (45 CFR 46.116b). A waiver of consent will not be sought for this study. The study investigator or coordinator will review all portions of the consent in great detail. Any questions will be addressed prior to asking for a signature. Study staff will emphasize that the

parent or legal guardian may continue to ask questions at any time during the study and he/she may withdraw consent at any time.

Documented informed consent will be obtained from each potential volunteer's parent or legal guardian. Consent must be documented by the volunteer's parents or legal guardian's dated signature on a Consent Form along with the dated signature of the person conducting the consent discussion.

If the volunteer's parent or legal guardian is illiterate, an impartial witness should be present during the entire informed consent reading and discussion. Afterward, the volunteer's parent or legal guardian should sign and date the informed consent, if capable. The impartial witness should also sign and date the informed consent along with the individual who read and discussed the informed consent i.e. study staff personnel.

A copy of the signed and dated consent and assent forms will be given to the volunteer's parent or legal guardian before participation in the trial begins. The original signed consent forms will remain in locked, study files and will be available for review at any time.

The initial informed consent form and any subsequent revised written informed consent form, and written information must receive IRB approval in advance of use. The volunteer's parent or legal guardian will be informed in a timely manner if new information becomes available that may be relevant to the volunteer's parent's or legal guardian's willingness to continue participation in the trial. The communication of this information will be documented.

The investigator is responsible for ensuring that no subject undergoes any study related examination or activity before that subject and/or legal guardian has given written informed consent to participate in the study. The written consent must be given by the subject and/or the legal guardian of the subject, after detailed information about the study has been given and in accordance with any national provisions on the protection of clinical study subjects. The verbal explanation will cover all the elements specified in the written information provided for the subject.

The investigator or designated personnel will inform the subject and/or legal guardian of the subject of the objectives, methods, anticipated benefits and potential risks and inconveniences of the study. The subject's legal guardian should be given every opportunity to ask for clarification of any points he/she does not understand and, if necessary, ask for more information. At the end of the interview, the subject's legal guardian will be given time to consider the study, if this is required, or if the subject's legal guardian requests more time. Subjects and/or legal guardians will be required to sign and date the informed consent form.

After signatures are obtained, the informed consent form will be kept and archived by the investigator in the investigator's study file for possible inspection by regulatory authorities, the IEC, sponsor, and/or CRO personnel.

It should be emphasized to the subject's legal guardian that he/she is at liberty to withdraw from the study at any time, without penalty or loss of benefits to which the subject is otherwise entitled. Subjects' legal guardians who refuse to give written informed consent should not be included. For those subjects whose legal guardians withdraw consent participation in the study should be discontinued.

11.4 Subject Confidentiality (US Studies)

All personal data collected and processed for the purposes of this study should be managed by the investigator and his/her staff with adequate precautions to ensure confidentiality of those data, and in accordance with the Health Insurance Portability and Accountability Act, applicable to national and/or local laws and regulations on personal data protection.

Monitors, auditors, and other authorized agents of the sponsor and/or its designee, the ethics committees approving this research, and the United States (US) FDA, as well as that of any other applicable agency(ies), will be granted direct access to the study subjects' original medical records for verification of clinical study procedures and/or data, without violating the confidentiality of the subjects to the extent permitted by the law and regulations. In any presentations of the results of this study or in publications, the subjects' identity will remain confidential.

Volunteer confidentiality:

The results of the research study may be published by the sponsor, but volunteers' names or identities will not be revealed. Records will remain confidential. In order that confidentiality can be maintained, the principal investigators will keep records in locked cabinets and results of tests will be coded to prevent association with volunteers' names. Volunteers' records will be available to the FDA, study staff, representatives of Asklepion Pharmaceuticals and the IRB.

Serum samples will be collected during this study and stored with code numbers as the only identifiers. Laboratory assays containing data from the study will be labelled only with volunteer code numbers.

12 Reporting and Publication, Including Archiving

Essential documents are those documents that individually and collectively permit evaluation of the study and quality of the data produced. After completion of the study, all documents and data relating to the study will be kept in an orderly manner by the investigator in a secure study file. This file will be available for inspection by the sponsor or its representatives or FDA. Essential documents should be retained for 2 years after the final marketing approval in an ICH region or for at least 2 years after the discontinuation of clinical development of the IMP. It is the responsibility of the sponsor to inform the study site(s) of when these documents no longer need to be retained. The investigator must contact the sponsor before destroying any study related documentation. In addition, all subject medical records and other source documentation will be kept for the maximum time permitted by the hospital, institution or medical practice.

The sponsor must review and approve all results of the study or abstracts for professional meetings prepared by the investigator(s). The sponsor agrees to publish the results of this study; however, publishing may be delayed until the formal filings with government regulatory agencies for market approval are submitted. Published data must not compromise the objectives of the study. Data from individual study sites in multi-center studies may not be published or presented separately.

13 References

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14 Appendices

14.1 Investigator Signature Page

Protocol Title: A Phase III Double-Blind, Randomized, Placebo Controlled, Multi-Center Clinical Study to Evaluate the Efficacy and Safety of Intravenous L-citrulline for the Prevention of Clinical Sequelae of Acute Lung Injury induced by Cardiopulmonary Bypass in Pediatric Subjects Undergoing Surgery for Congenital Heart Defects

Protocol Number: CIT-003-01; Version 4.3.1

Confidentiality and cGCP Compliance Statement

I, the undersigned, have reviewed this protocol, including appendices and I will conduct the study as described in compliance with this protocol, GCP, and relevant ICH guidelines.

Once the protocol has been approved by the IRB/IEC, I will not modify this protocol without obtaining prior approval of Asklepion Pharmaceuticals, LLC and of the IRB/IEC. I will submit the protocol modifications and/or any ICF modifications to Asklepion Pharmaceuticals, LLC and IRB/IEC, and approval will be obtained before any modifications are implemented.

I understand that all information obtained during the conduct of the study with regard to the subjects' state of health will be regarded as confidential. No subjects' names will be disclosed. All subjects will be identified by assigned numbers on all Case Report Forms, laboratory samples or source documents forwarded to the sponsor. Clinical information may be reviewed by the sponsor or its agents or regulatory agencies. Agreement must be obtained from the subject before disclosure of subject information to a third party.

Information developed in this clinical study may be disclosed by Asklepion, to other clinical investigators, regulatory agencies, or other health authority or government agencies as required.

_____ _____
Investigator Signature Date

_____
Printed Name

_____
Institution

14.2 Changes to Protocol

14.2.1 Version 4.0

1. **Page 2; Study Personnel; CRO Personnel: Added clarification regarding responsibilities.**

   Previously Stated:

   US: Clinical Site Monitoring and Data Management
   Veristat LLC

   Europe/Israel: Regulatory Activities, Clinical Site Monitoring, Medical Monitoring/ Safety Reporting, Biostatistics, Medical Writing
   FGK Clinical Research GmbH

   Changed To:

   Veristat LLC
   US: Clinical Site Monitoring/Management
   Global: Data Management

   FGK Clinical Research GmbH
   Europe/Israel: Regulatory Activities, Clinical Site Monitoring/Management
   Global: Medical Monitoring/Safety Reporting; Biostatistics, Medical Writing

2. **Page 13; Synopsis; Study Treatments: Added clarification for initial bolus dose.**

   Previously Stated:

   - Bolus of 150 mg/kg at the initiation of cardiopulmonary bypass

   Changed To:

   - Bolus of 150 mg/kg at the initiation of cardiopulmonary bypass, *but after removal of any crystalloid base;*

3. **Pages 13-14; Synopsis; Study Treatments: Added clarification for second dose.**

   Previously Stated:

   Addition of study medication at a concentration of 200 µmol/L to the filtration or hemoconcentration replacement fluid utilized during cardiopulmonary bypass;

   Changed To:

   Addition of study medication at a concentration of 200 µmol/L *given as a bolus during*

bypass. This may be administered as a one-time bolus or multiple administrations to compensate for fluids containing L-citrulline that may be removed from the patient during the course of the operation and thus to maintain the concentration of 200 µmol/L;

4. **Page 15; Synopsis; Exclusion Criteria; 2nd Bullet: Removed term "invasive" to be consistent with remainder of protocol**.

   Previously Stated:

   Preoperative requirement for invasive mechanical ventilation or intravenous inotrope support

   Changed To:

   Preoperative requirement *for mechanical ventilation* or intravenous inotrope support

5. **Pages 21-23; Schedule of Study Assessments and Procedures; Footnotes 2, 4, 9, 11, 13, and 14: Added clarification regarding storage of plasma L-citrulline.**

   Previously Stated:

   All L-citrulline plasma samples must be processed within four hours of being drawn, stored at -20°F and sent on dry ice to the central lab.

   Changed To:

   All L-citrulline plasma samples must be processed within four hours of being drawn, stored at -20°F *(28.8°C) or below*, and sent on dry ice to the central lab.

6. **Page 22; Schedule of Study Assessments and Procedures; Footnote 6: Added clarification regarding recording of hemodynamic monitoring results.**

   Previously Stated:

   Results will be recorded every 5 minutes during surgery, and then every hour from time of post-op admission to the PICU through 48 hours.

   Changed To:

   Results will be recorded *approximately* every 5 minutes during surgery, and then *approximately* every hour from time of post-op admission to the PICU through 48 hours. *Results at Hours 1, 2, 4, 12, 24, and 48 must be obtained and must be within +/- 10 minutes of the required time point.*

7. **Page 22; Schedule of Study Assessments and Procedures; Footnote 7: Added clarification regarding recording of arterial blood gas results.**

   Previously Stated:

   All results obtained during surgery will be recorded, and then recorded every 2-3 hours from time of post-op admission to the PICU until removal of arterial line or through 48 hours, whichever occurs first.

   Changed To:

   All results obtained during surgery will be recorded, and then recorded every 2-3 hours *(+/- 15 minutes)* from time of post-op admission to the PICU until removal of arterial line or through 48 hours, whichever occurs first.

8. **Page 22; Schedule of Study Assessments and Procedures; Footnote 12: Added clarification regarding recording of ventilator settings.**

   Previously Stated:

   Recorded every hour (Qhr) from time of separation from CPB until discontinuation of mechanical ventilation

   Changed To:

   Recorded *approximately* every hour (Qhr) *(+/- 10 minutes)* from time of separation from CPB until discontinuation of mechanical ventilation

9. **Page 41; Section 4.1; Overall Study Design and Plan; Last Paragraph: Added clarification regarding dosing**

   Previously Stated:

   190 subjects will be randomized in a 1:1 fashion to either L-citrulline or placebo. L-citrulline treatment will include an L-citrulline bolus of 150 mg/kg or placebo at the initiation of CPB, the addition of L-citrulline at a concentration of 200 µmol/L or placebo to the filtration or hemoconcentration replacement fluid utilized during CPB, an L-citrulline bolus of 20 mg/kg or placebo 30 minutes after decannulation from CPB immediately followed by a 9 mg/kg/hr continuous infusion of L-citrulline or placebo for 48 hours.

   Changed To:

   190 subjects will be randomized in a 1:1 fashion to either L-citrulline or placebo. L-citrulline treatment will include*: 1)* an L-citrulline bolus of 150 mg/kg or placebo at the initiation of CPB, *but after removal of any crystalloid base; 2)* the addition of L-citrulline at a

concentration of 200 µmol/L or placebo *given as a bolus during bypass. This may be administered as a one-time bolus or multiple administrations to compensate for fluids containing L-citrulline that may be removed from the patient during the course of the operation and thus to maintain the concentration of 200 µmol/L; and 3)* an L-citrulline bolus of 20 mg/kg or placebo 30 minutes after decannulation from CPB immediately followed by a 9 mg/kg/hr continuous infusion of L-citrulline or placebo for 48 hours *(see Section 4.4.1 for further details).*

10. Pages 44-45; Section 4.4.1; Investigational Medicinal Products Administered: Added clarification regarding dosing

Previously Stated:

An L-citrulline bolus of 150 mg/kg or placebo will be administered at the initiation of CPB; L-citrulline at a concentration of 200 µmol/L or placebo will be added to the filtration and hemoconcentration fluid utilized during CPB; a bolus of 20 mg/kg of L-citrulline or placebo will be administered 30 minutes after decannulation from CPB, immediately followed by a 9 mg/kg/hr continuous infusion of L-citrulline or placebo for 48 hours.

Changed To:

The main objective of the dosing schedule, as outlined below, is to achieve a minimum systemic target plasma trough level concentration of 100 µmol/L of L-citrulline to provide the most beneficial effect as demonstrated in the previous phase 2 study. As a general guiding principal during bypass, once dosing has begun, all fluids entering the circuit must contain 200 µmol/L of L-citrulline in order to achieve and maintain the trough level of 100 µmol/L of L-citrulline. Therefore, if fluids are being added or removed, it is crucial to take this into consideration when timing the administration of the required doses. The following section describes the administration of L-citrulline or placebo, while taking into account the above objective.

- *The first (initial) dose administered will be an L-citrulline bolus of 150 mg/kg or placebo, administered at the initiation of CPB, but after removal of any crystalloid base.*
    - *If fluid is added to the priming circuit, but removed prior to initiation of bypass, then the initial 150 mg/kg bolus dose should be added after the crystalloid based fluids are removed and at the initiation of bypass.*
    - *If fluid is added to the priming circuit, but not removed, then the initial 150 mg/kg dose should be added to the circulating prime used for the bypass.*

- *The second dose administered consists of L-citrulline at a concentration of 200 µmol/L or placebo, given as a bolus during bypass. This may be administered as a one-time bolus or multiple administrations, to maintain the concentration of 200 µmol/L. The total dose will be dependent upon the total amount of any volume administered and removed during CPB. This includes the crystalloid portion of the cardioplegia so as not to dilute the loading dose; it is significantly important when using a hemoconcentrator.*
- *The third dose administered will be a bolus of 20 mg/kg of L-citrulline or placebo, administered 30 minutes after decannulation from CPB.*
- *The above third dose is immediately followed by a 9 mg/kg/hr continuous infusion of L-citrulline or placebo for 48 hours.*

11. Page 46; Section 4.4.2; Identity of Investigational Medicinal Products; 2nd Paragraph: Added clarification regarding storage of investigational product

Previously Stated:

Study drug should be stored as indicated on the package label: Store at room temperature, at 15°C - 30°C (59-86°F). Study drug should not be refrigerated or frozen.

Changed To:

Study drug should be stored as indicated on the package label: *Store at 25°C (77°F) or below; do not freeze.*

12. Page 46; Section 4.4.2; Identity of Investigational Medicinal Products; 3rd Paragraph: Removed statement regarding replacement fluid

Previously Stated:

The L-citrulline bolus and L-citrulline infusion will be provided by each site's investigational pharmacy at a concentration of 50 mg/mL. The filtration or hemoconcentration replacement fluid will be provided as standard fluid (Plasmalyte) with L-citrulline added to achieve an L-citrulline concentration of 200 µmol/L.

Changed To:

The L-citrulline bolus and L-citrulline infusion will be provided by each site's investigational pharmacy at a concentration of 50 mg/mL.

13. Page 47; Section 4.4.4; Method of Assigning Subjects to Treatment Groups: Clarified numbering format

Previously Stated:

Screened subjects will be assigned a unique 4-digit number, with the first two digits identifying the site and the last two digits identifying the subject, e.g. 01-01. At each site, the investigator will assign numbers to subjects in ascending order e.g. 01, 02, 03 etc.

Changed To:

Screened subjects will be assigned a unique *5-digit* number, with the first two digits identifying the site and the *next three* digits identifying the subject, e.g. 01-*001*. At each site, the investigator will assign numbers to subjects in ascending order *(e.g. 001, 002, 003).*

14. Page 52; Section 5.1.2.1; Day 0, Surgery (Intra-Operative); 4th Bullet: Added clarification regarding dosing

Previously Stated:

Bolus of L-citrulline at 150 mg/kg or placebo at initiation of CPB; addition of L-citrulline at a concentration of 200 µmol/L or placebo to the filtration and hemoconcentration fluids utilized during CPB

Changed To:

Bolus of L-citrulline at 150 mg/kg or placebo at initiation of CPB; addition of L-citrulline at a concentration of 200 µmol/L or placebo *given as a bolus during bypass. This may be administered as a one-time bolus or multiple administrations to compensate for fluids containing L-citrulline that may be removed from the patient during the course of the operation and thus to maintain the concentration of 200 µmol/L. The total dose will be dependent upon the total amount of any volume administered and removed during CPB.*

15. Pages 52-59; Section 5.1.2; Treatment Period; All Visits: Added clarification regarding recording of hemodynamic monitoring, arterial blood gases, and ventilator settings

Previously Stated:

1) Hemodynamic Monitoring (Day 0, Surgery (Intra-Operative)): Results will be recorded every 5 minutes after induction of anesthesia and during surgery

   Hemodynamic Monitoring (Day 0, Post-Surgery, 0 Hours through Day 2, Post-Surgery, 48 Hours): Results will be recorded every hour from time of post-op admission to the

PICU through 48 hours.

2) Arterial Blood Gases (Day 0, Post-Surgery, 0 Hours through Day 2, Post-Surgery, 48 Hours):
Results will be recorded every 2-3 hours from time of post-op admission to the PICU until removal of arterial line or through 48 hours, whichever occurs first.

3) Ventilator Settings (Day 0, Post-Surgery, 0 Hours through Follow-Up Visit (Day 28 or Hospital Discharge)): Recorded every hour from time of separation from CPB until end of mechanical ventilation or 28 days (whichever occurs first)

Changed To:

1) Hemodynamic Monitoring (Day 0, Surgery (Intra-Operative)): Results will be recorded *approximately* every 5 minutes after induction of anesthesia and during surgery

   Hemodynamic Monitoring (Day 0, Post-Surgery, 0 Hours through Day 2, Post-Surgery, 48 Hours): Results will be recorded *approximately* every hour from time of post-op admission to the PICU through 48 hours. *Results at Hours 1, 2, 4, 12, 24, and 48 must be obtained and must be within +/- 10 minutes of the required time point.*

2) Arterial Blood Gases (Day 0, Post-Surgery, 0 Hours through Day 2, Post-Surgery, 48 Hours): Results will be recorded every 2-3 hours *(+/- 15 minutes)* from time of post-op admission to the PICU until removal of arterial line or through 48 hours, whichever occurs first.

3) Ventilator Settings (Day 0, Post-Surgery, 0 Hours through Follow-Up Visit (Day 28 or Hospital Discharge)): Recorded *approximately* every hour *(+/- 10 minutes)* from time of separation from CPB until end of mechanical ventilation or 28 days (whichever occurs first)

16. Pages 53-58; Section 5.1.2.2 through 5.1.2.6; Treatment Period; Visits Day 0, post-surgery, 0 hours through Day 2 post-surgery, 48 hours: Added age-related criteria for MAP

Previously Stated (Day 0, post-surgery, 0 hours):

Hypotension assessment (Refractory hypotension is defined as a 20% drop of mean arterial blood pressure [MAP] below specific age-related criteria for greater than 30 minutes)

Changed To:

Hypotension assessment (Refractory hypotension is defined as a 20% drop of mean arterial blood pressure [MAP] below specific age-related criteria *(Haque and Zaritsky*

(2007) for greater than 30 minutes) *(See below)*

- *Infants: MAP of 40*
- *Age 1 year: MAP of 40*
- *Age 2 years: MAP of 44*
- *Age 3 years: MAP of 47*
- *Age 4 years: MAP of 52*
- *Age 6 years: MAP of 53*
- *Age 7 years: MAP of 52*
- *Age 8 years: MAP of 54*
- *Age 9 years: MAP of 55*
- *Age 10 years: MAP of 56*
- *Age 11 years: MAP of 57*
- *Age 12 years: MAP of 58*
- *Age 13 years: MAP of 59*
- *Age 14 years: MAP of 61*
- *Age 15 years: MAP of 62*
- *Age 16 years: MAP of 63*
- *Age 17-18 years: MAP of 64*

Previously Stated (Day 0, post-surgery, 6 hours through Day 2, post-surgery, 48 hours):

Hypotension assessment (Refractory hypotension is defined as a 20% drop of mean arterial blood pressure [MAP] below specific age-related criteria for greater than 30 minutes)

Changed To:

Hypotension assessment (Refractory hypotension is defined as a 20% drop of mean arterial blood pressure [MAP] below specific age-related criteria for greater than 30 minutes) *(See Section 5.1.2.2)*

17. Page 59; Section 5.1.2.8; Follow-Up Phone Assessment: Added clarification regarding assessment

Previously Stated:

Patients discharged prior to Day 28 will have their follow-up visit procedures completed upon discharge form the hospital (see section 5.1.2.7). Therefore, in order to assess their status at Day 28, patients will be contacted by phone on Day 28 to assess their status.

Changed To:

Patients discharged prior to Day 28 will have their follow-up visit procedures completed

upon discharge form the hospital (see section 5.1.2.7). Therefore, in order to assess their *survival* status at Day 28, patients (or parents, dependent upon patient age) will be contacted by phone on Day 28 to assess their status.

18. Page 80; Section 10; Data and Safety Monitoring Board: Added section regarding responsibilities of DSMB

Previously Stated:

N/A

Changed To:

Data and Safety Monitoring Board

The Data and Safety Monitoring Board (DSMB) will act in an advisory capacity to the Coordinating Investigator and Asklepion Pharmaceuticals, LLC to monitor participant safety. The DSMB responsibilities are to:

- *Protect the safety of the study participants;*
- *Review the research protocol and plans for data safety and monitoring;*
- *Evaluate participant risk versus benefit, and other factors that can affect study safety outcome;*
- *Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the trial;*
- *Report to the study sponsor, Asklepion Pharmaceuticals, on the safety and progress of the trial;*
- *Make recommendations concerning continuation, termination or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study; and*
- *If applicable, review interim analyses in accordance with stopping rules, which are clearly defined in advance of data analysis and have the approval of the DSMB.*

14.2.2 Version 4.1

1. **Page 22; Schedule of Study Assessments and Procedures; Footnote 8: Removed reference to filtration and hemoconcentration fluids for 200 µmol/L or placebo dose.**

   Previously Stated:

   Begin bolus of intravenous (i.v.) L-citrulline at 150 mg/kg or placebo at initiation of CPB & add L-citrulline at a concentration of 200 µmol/L or placebo to the filtration and hemoconcentration fluids utilized during CPB.

   Changed To:

   Begin bolus of intravenous (i.v.) L-citrulline at 150 mg/kg or placebo at initiation of CPB & add L-citrulline at a concentration of 200 µmol/L or placebo *given as a bolus during bypass. This may be administered as a one-time bolus or multiple administrations, to maintain the concentration at 200 µmol/L.*

2. **Page 51; Section 5.1.2.1; Day 0, Surgery (Intra-Operative); Collection of CPB Data; Sub-bullet 5: Updated wording regarding documentation of L-citrulline or placebo.**

   Previously Stated:

   Documentation that L-citrulline or placebo was added to the filtration and hemoconcentration fluids

   Changed To:

   Documentation of L-citrulline or placebo administration

14.2.3 Version 4.2

1. **Cover Page: Added EudraCT Number.**

   2016-002427-28

2. **Page 13; Synopsis; Study Design: Added clarification for second dose.**

   Previously Stated:

   …the addition of L-citrulline at a concentration of 200 µmol/L or placebo to the filtration or hemoconcentration fluid utilized during cardiopulmonary bypass,…

   Changed To:

   …the addition of L-citrulline at a concentration of 200 µmol/L or placebo *given as a bolus* during cardiopulmonary bypass,…

3. **Page 15; Synopsis; Subject Selection: Added further clarification regarding females of child-bearing potential. Added new exclusion.**

   Previously Stated:

   - Pregnancy

   Changed To:

   - Pregnancy*; Sexually active females of child-bearing potential must be willing to practice an acceptable method of birth control for the duration of study participation (e.g. oral contraceptive, hormonal implant, intra-uterine device)*
   - *Participation in another clinical trial within 30 days of Screening or while participating in the current study, including the 28 days of follow-up post study drug administration.*

4. **Page 20; Schedule of Study Assessments and Procedures: Moved "x(s)" for laboratory specimens.**

   Moved blood draws for serum electrolytes, BUN, creatinine, CBC with differential, Coagulation: ACT, and LFTs, and Urinalysis from Day 0, Pre-Op to Screen, Day -14 to 0.

5. **Page 20; Schedule of Study Assessments and Procedures: Deleted "x" for laboratory specimens.**

   Deleted blood draw for LFTs at Day 1, 24 hours.

6. **Page 20-21; Schedule of Study Assessments and Procedures: Updated Schedule of Study Assessments and Procedures.**

   Moved "x(s)" and rearranged order of procedures to be consistent with changes made to collection of laboratory specimens.

7. **Page 21; Schedule of Study Assessments and Procedures; Footnotes 1,2,3: Changed timing for blood draws for baseline laboratory values. Added statement regarding acceptability of using pre-op laboratory values for Screening. Also changed order of footnotes to match new placement in Schedule of Assessments and Procedures.**

   Previously Stated:

   [1] Weight only

   [2] Blood collected for baseline laboratory values and plasma L-citrulline levels (***prior to surgery***); 3 mL total (0.5 for plasma L-citrulline). All L-citrulline plasma samples must be processed within four hours of being drawn, stored at -20°F (28.8°C) or below, and sent on dry ice to the central lab.

   [3] Urinalysis not required, but results should be recorded if test is performed

   Changed To:

   [1] Results of laboratory tests done as standard of care for pre-op testing are acceptable and may be recorded as screening values, provided the tests were performed within 14 days of Day 0. If any of the required tests were not done as part of the pre-op testing, these must be completed within 14 days of Day 0.

   [2] Urinalysis not required, but results should be recorded if test was performed.

   [3] Weight only

8. **Page 21-23; Schedule of Study Assessments and Procedures; Footnotes 2, 4, 9, 11, 13, and 14: Changed volume of blood draw for plasma L-citrulline and deleted total volume for blood draw.**

   Previously Stated:

   [2,4,14] Blood collected for … laboratory values and plasma L-citrulline levels ***(…)*****;** 3.0 mL total; (0.5 mL for plasma L-citrulline).

   [9,11,13] Blood collected for plasma L-citrulline levels ***(…)***; 0.5 mL

   Changed To:

[5,15] Blood collected for … laboratory values and plasma L-citrulline levels ***(…)***; (*1.0* mL for plasma L-citrulline).

[4,10,12,14] Collect *1.0* mL of blood for plasma L-citrulline levels…

9. **Page 43-44; Section 4.3.3; Exclusion Criteria; 3rd, 5th, and 6th bullet: Added additional detail to match synopsis. Added further clarification regarding females of child-bearing potential. Added new exclusion.**

   Previously Stated:

   - Presence of fixed or idiopathic pulmonary hypertension prior to surgical repair
   - Pregnancy

   Changed To:

   - Presence of fixed or idiopathic pulmonary hypertension *(i.e. Eisenmenger's Syndrome)* prior to surgical repair
   - Pregnancy; *Females of child-bearing potential must be willing to practice an acceptable method of birth control for the duration of study participation (e.g. oral contraceptive, hormonal implant, intra-uterine device)*
   - *Participation in another clinical trial within 30 days of Screening or while participating in the current study, including the 28 days of follow-up post study drug administration.*

10. **Page 46; Section 4.4.1; Investigational Medicinal Products Administered 3rd bullet: Added additional detail to match synopsis.**

    Previously Stated:

    - The above third dose is immediately followed by a 9 mg/kg/hr continuous infusion of L-citrulline or placebo for 48 hours.

    Changed To:

    - The above third dose is immediately followed by a 9 mg/kg/hr continuous infusion of L-citrulline or placebo for *up to* 48 hours. *The study drug or placebo infusion will be discontinued once invasive arterial blood pressure monitoring is discontinued or at 48 hours, whichever comes first.*

11. Page 48; Section 4.4.5; Selection of Doses in the Study; Last Paragraph: Added clarification regarding dosing regimen.

Previously Stated:

This treatment regimen will also be used in the current study.

Changed To:

This treatment regimen, *with clarification added for the dose(s) given during bypass,* will also be used in the current study.

12. Page 51; Section 5.1.1.1; Screening Visit (Day -14 to Day 0): Added new bullet to allow use of results of laboratory specimens collected for pre-op testing.

Previously Stated:

N/A

Changed To:

- *Blood draw*:*
  - *serum electrolytes (sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and chloride (Cl)), BUN, and creatinine*
  - *CBC with differential*
  - *ACT*
  - *liver function tests (LFTs), including bilirubin (total), alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH)*

  * Results of laboratory tests done as standard of care for pre-op testing are acceptable and may be recorded as screening values, provided the tests were performed within 14 days of Day 0. If any of the required tests were not done as part of the pre-op testing, these must be completed within 14 days of Day 0.
- *Urinalysis (not required, but results should be recorded if test is performed)*
  - *Visual: Color, Clarity/Turbidity*
  - *Chemical: pH, Specific gravity, glucose, ketones, nitrites*
  - *Microscopic: RBCs, WBCs, epithelial cells, casts, bacteria*

13. Page 51; Section 5.1.1.2; Baseline Visit (Day of Surgery, Pre-operation); 6th and 7th bullets: Deleted total volume for blood draw and deleted most Day of Surgery labs. Changed volume for plasma L-citrulline sample. Also deleted Urinalysis (moved to Screening; see above).

Previously Stated:

- Blood draw (prior to surgery; in the operating room [OR], after induction of anesthesia); 3 mL total for:
  - serum electrolytes (sodium (Na), potassium (K), calcium (Ca), magnesium (Mg) and chloride (Cl)), BUN, and creatinine
  - CBC with differential
  - ACT
  - liver function tests (LFTs), including bilirubin (total), alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and lactate dehydrogenase (LDH)
  - plasma L-citrulline (0.5 mL)
- Urinalysis (not required, but results should be recorded if test is performed)
  - Visual: Color, Clarity/Turbidity
  - Chemical: pH, Specific gravity, glucose, ketones, nitrites
  - Microscopic: RBCs, WBCs, epithelial cells, casts, bacteria

Changed To:

- Blood draw (prior to surgery; in the operating room [OR], after induction of anesthesia total for:
  - plasma L-citrulline *(1.0* mL)

14. Page 53; Section 5.1.2.1; Day 0, Surgery (Intra-operative); 5th bullet: Deleted total volume for blood draw and changed volume for plasma L-citrulline sample.

Previously Stated:

- Blood draw (10 minutes after study drug is administered); 3 mL total for:
  - serum electrolytes, BUN, and creatinine
  - CBC with differential
  - ACT

- plasma L-citrulline

Changed To:

- Blood draw (10 minutes after study drug is administered):
  - serum electrolytes, BUN, and creatinine
  - CBC with differential
  - ACT
  - plasma L-citrulline *(1.0 mL)*

15. Page 53-54; Section 5.1.2.2; Day 0, post-surgery, 0 hours (30 minutes post decannulation); 1st, 2nd, 5th, and 6th bullets: Changed volume of blood drawn for plasma L-citrulline sample. Added additional detail to match synopsis. Added clarification regarding thoracotomy outputs.

Previously Stated:

- Blood draw for:
  - plasma L-citrulline (prior to bolus); 0.5 mL
- Bolus of L-citrulline at 20 mg/kg or placebo 30 minutes after decannulation from CPB immediately followed by a continuous infusion of L-citrulline at 9 mg/kg/hr or placebo
- Thoracotomy output measurements
- Blood draw for:
  - plasma L-citrulline (5 minutes after bolus); 0.5 mL

Changed To:

- Blood draw for:
  - plasma L-citrulline (***prior to bolus***); *1.0* mL
- Bolus of L-citrulline at 20 mg/kg or placebo 30 minutes after decannulation from CPB immediately followed by a continuous infusion of L-citrulline at 9 mg/kg/hr or placebo *for up to 48 hours. The study drug or placebo infusion will be discontinued once invasive arterial blood pressure monitoring is discontinued or at 48 hours, whichever comes first.*
- Thoracotomy output measurements *(for the duration of chest tube placement)*
- Blood draw for:
  - plasma L-citrulline (***5 minutes after bolus***); *1.0* mL

16. **Page 54-58; Section 5.1.2.3 through 5.1.2.6; Day 0, post-surgery, 6 and12 hours, Day 1, post-surgery, 24 hours, and Day 2, post-surgery, 48 hours; 1st, 4th, and 5th bullets: Added additional detail to match synopsis. Added clarification regarding thoracotomy outputs. Changed volume of blood drawn for plasma L-citrulline sample.**

    Previously Stated:

    - Continuous infusion of L-citrulline at 9 mg/kg/hr or
    - Thoracotomy output measurements
    - Blood draw for:
        - plasma L-citrulline (6 {12, 24, 48} hrs; +/-1 hour; collect at end of infusion if infusion discontinued prior to 6 {12,24,48} hours); 0.5 mL

    Changed To:

    - Continuous infusion of L-citrulline at 9 mg/kg/hr or placebo *for up to 48 hours. The study drug or placebo infusion will be discontinued once invasive arterial blood pressure monitoring is discontinued or at 48 hours, whichever comes first.*
    - Thoracotomy output measurements *(for the duration of chest tube placement)*
    - Blood draw for:
        - plasma L-citrulline (6 {12, 24, 48} hrs; +/-1 hour; collect at end of infusion if infusion discontinued prior to 6 {12, 24, 48} hours); *1.0* mL

17. **Page 57; Section 5.1.2.5; Day 1, post-surgery, 24 hours: Deleted total blood volume. Deleted LFTs.**

    Previously Stated:

    - Blood draw (3 mL total) for:
        - serum electrolytes, BUN, and creatinine
        - CBC with differential
        - ACT
        - LFTs

    Changed To:

    - Blood draw for:

- serum electrolytes, BUN, and creatinine
- CBC with differential
- ACT

18. **Page 58; Section 5.1.2.6; Day 2, post-surgery, 48 hours: Deleted total blood volume.**

Previously Stated:

- Blood draw (3 mL total) for:

Changed To:

- Blood draw for:

19. **Page 59; Section 5.1.2.7; Follow-Up Up Visit [Day 28 (if still in hospital) or upon Hospital Discharge]: Added thoracotomy output to ensure collection of data if patient still in hospital. Changed volume for plasma L-citrulline sample.**

Previously Stated:

- Blood draw* for:
  - serum electrolytes, BUN, and creatinine*
  - CBC with differential*
  - LFTs*

    * (An attempt should be made to obtain final laboratory samples while i.v. access is available, either on Day 28 or prior to discharge from hospital. If laboratory results within 24 hours are available, these may be captured. If no i.v. access is available, venipuncture is not required unless indicated for clinical reasons.)
  - Plasma L-citrulline (0.5 mL)

Changed To:

- *Thoracotomy output measurements (for the duration of chest tube placement)*
- Blood draw* for:
  - serum electrolytes, BUN, and creatinine
  - CBC with differential
  - LFTs
  - Plasma L-citrulline (*1.0* mL)

* (An attempt should be made to obtain final laboratory samples while i.v. access is available, either on Day 28 or prior to discharge from hospital. If laboratory results within 24 hours are available, these may be captured. If no i.v. access is available, venipuncture is not required unless indicated for clinical reasons.)

20. Page 68; Section 6.2.2.2; Clinical Laboratory Variables: Adjusted total volume of blood draw needed for clinical laboratory testing.

Previously Stated:

A total amount of 14-17 mL of blood will be taken from each subject during the study for both clinical laboratory testing and citrulline plasma concentration determinations.

Changed To:

A total amount of *16-19* mL of blood will be taken from each subject during the study for both clinical laboratory testing and citrulline plasma concentration determinations.

14.2.4 Version 4.3

1. **Page 3; Study Personnel; CRO Personnel: Deleted Veristat, LLC; Added ResearchPoint Global.**

   Previously Stated:

   Veristat LLC
   118 Turnpike Rd, Suite 200
   Southborough, MA 01772
   P: 508-429-7340
   www.veristat.com

   Changed To:

   ResearchPoint Global
   5301 Southwest Parkway
   Suite 100
   Austin, TX 78735
   Phone: 512-343-1092
   www.researchpointglobal.com

2. **Page 14; Synopsis; Subject Selection; Exclusion Criteria: Clarified exclusion regarding left-sided AV valve regurgitation**

   Previously Stated:

   - Significant left-sided atrioventricular (AV) valve regurgitation

   Changed To:

   - Significant left-sided atrioventricular (AV) valve regurgitation *not amenable to surgical correction*

3. **Page 22; Schedule of Study Assessments and Procedures; Footnote 7: Changed requirement for obtaining hemodynamics to align with Institution's standard of care.**

   Previously Stated:

   …Results will be recorded approximately every 5 minutes during surgery, and then approximately every hour from time of post-op admission to the PICU through 48 hours.

Changed To:

… Results will be recorded, *during surgery and from time of post-op admission to the PICU through 48 hours, according to the Institution's standard of care.*

4. **Page 22; Schedule of Study Assessments and Procedures; Footnote 8: Changed requirement for obtaining ABGs to align with Institution's standard of care.**

Previously Stated:

…Baseline values are first recorded in the OR by Anesthesia. All results obtained during surgery will be recorded, and then recorded every 2-3 hours (+/- 15 minutes) from time of post-op admission to the PICU until removal of arterial line or through 48 hours, whichever occurs first.

Changed To:

…ABGs will be drawn according to the Institutions' standard of care. Baseline results are first recorded in the OR by Anesthesia. All results obtained during surgery, and from the time of post-op admission to the PICU until removal of the arterial line or through 48 hours (whichever occurs first), will be recorded.

5. **Page 28; Section 1.2; L-citrulline: Updated section to clarify urea cycle.**

Previously Stated:

Citrulline is a naturally occurring amino acid and the first intermediate in the urea cycle (Figure 1). The first two steps of the hepatic urea cycle, leading to the production of citrulline, are carried out by carbamoyl phosphate synthetase I (CPSI) and ornithine transcarbamylase (OTC). These two enzymes are limited to the liver and gut. Afterwards, citrulline is transported from the mitochondria to the cytoplasm. There, argininosuccinate …endothelium (Summar 1998).

Changed To:

Citrulline is a naturally occurring amino acid and the first intermediate in the urea cycle. *Most medical personnel think of the urea cycle as a hepatic metabolic pathway that serves to convert nitrogen waste from protein breakdown into urea for excretion. It is not generally appreciated that key elements of the urea cycle exist in other tissues where, for example, in lung, it is ultimately responsible for the production of NO under precise metabolic control, as shown in Figure 1. The shift in function of the urea cycle comes about because the enzymes catalyzing the first two steps of the hepatic urea cycle (carbamoyl phosphate synthetase I (CPSI) and ornithine transcarbamylase (OTC),* which lead to the production of citrulline, *are expressed only in the liver and gut. In the liver and gut, newly synthesized*

citrulline is transported from the mitochondria to the cytoplasm. In contrast, other tissues such as lung are dependent upon exogenous circulating citrulline since they cannot synthesize it endogenously. Following either endogenous synthesis or transport into a non-hepatic cell, argininosuccinate…endothelium (Summar 1998).

6. Page 29; Figure 1; Title and Figure: Changed Title and Figure to be consistent with new information added to Section 1.2

Previously Stated:

Figure 1: Schematic description of the hepatic urea cycle

[See previous figure]

Figure 10: Schematic description of the urea cycle in lung

[See new figure]

7. Page 32-40; Section 1.4; Clinical data: Updated section to reflect results of data from CIT-002-01 final Clinical Study Report.

a. Page 32; 3rd Paragraph:

Previously Stated:

Postoperative pulmonary hypertension developed in 9 patients, 6 of 20 (30%) in the placebo group and 3 of 20 (15%) in the citrulline group (P=0.451), all of whom had plasma citrulline concentrations less than age-specific norms. None of the patients with plasma citrulline level >37 µmol//L developed pulmonary hypertension.

Changed To:

The most significant result was that postoperative pulmonary hypertension developed in 9 patients, 6 of 20 (30%) in the placebo group and 3 of 20 (15%) in the citrulline group (P=0.451), all of whom had plasma citrulline concentrations less than the age-specific norms. *In a contingency analysis that reached statistical significance, all of the children developing postoperative pulmonary hypertension had a* plasma citrulline level <37 µmol/L. *Conversely, none of the children with citrulline levels >37 µmol/L* developed pulmonary hypertension. *This result implicated low citrulline levels as a strong risk factor and possible causal element in the development of postoperative pulmonary hypertension.* Therefore, more aggressive citrulline supplementation, resulting in consistent citrulline concentrations in excess of 37 µmol/L, could *potentially* prevent postoperative pulmonary hypertension.

b. Page 33; 4th Paragraph:

Previously Stated:

It was concluded that elevations in plasma citrulline concentrations above age-specific norms, whether naturally occurring or with citrulline supplementation, were associated with a decreased risk of postoperative pulmonary hypertension. Therefore, more aggressive citrulline supplementation, resulting in consistent citrulline concentrations in excess of 37 µmol/L, could prevent postoperative pulmonary hypertension.

Changed To:

N/A [Deleted first sentence; moved 2nd sentence to last sentence of previous paragraph]

c. Page 34; 2nd Paragraph:

Previously Stated:

… clinical study report is currently being generated.

Changed To:

… *full* clinical study report is *available.*

d. Page 34; 3rd Paragraph:

Previously Stated:

…after end of CPB…

Changed To:

…after *separation from* CPB…

e. Page 35; 1st Paragraph:

Previously Stated:

Patients receiving i.v. L-citrulline showed a reduced duration of mechanical ventilation as shown in Figure 5. The differences in mechanical ventilation were statistically significant by the Wilcoxon rank sum test (p = 0.0222) and by the ANOVA t-test (p = 0.0317).

Changed To:

Patients receiving i.v. L-citrulline showed a reduced duration of mechanical ventilation as shown in Figure 5 *and Figure 6. The duration of postoperative invasive mechanical ventilation was derived as the time in hours from separation from CPB until endotracheal extubation. The mean duration of invasive mechanical ventilation was clearly longer in the*

placebo group (37 hours) than in the citrulline group (5 hours). Kaplan-Meier survival analyses confirmed this reduced duration as seen in Figure 5 with re-intubation time included and ventilation time censored and a log rank p-value of 0.0498, and in Figure 6 with reintubation time included and zero ventilation time not censored and a log rank p-value of 0.0089.

f. Page 36; Figure 5; Title and Figure:

Previously Stated:

Figure 5: Kaplan-Meier Survival Analysis of the Duration of Invasive Mechanical Ventilation from End of Surgery until Last Extubation (Excluding Re-intubation)

[See previous figure]

Changed To:

Figure 5: Kaplan-Meier Survival Analysis of the Duration of Invasive Mechanical Ventilation *(Including Re-intubation Time): Censored*

[See new figure]

g. Page 37; Added new Figure 6:

Previously Stated:

N/A

Changed To:

Figure 6: Kaplan-Meier Survival Analysis of the Duration of Invasive Mechanical Ventilation (Including Re-intubation Time): Not Censored

[See new figure]

h. Page 37; 1st Paragraph:

Previously Stated:

Some differences…such patients (Figure 5). When these patients were stratified by treatment, it was shown that all 6 patients (100%) receiving i.v. L-citrulline had been extubated in the operating room, in comparison to only 2 of 6 patients (33%) in the placebo group.

Changed To:

Some differences…such patients. When these patients were stratified by treatment, it was shown that all 6 patients (100%) receiving i.v. L-citrulline had been extubated in the operating room, in comparison to only 2 of 6 patients (33%) in the placebo group. *Although*

the numbers are small, these data achieved borderline significance. The results suggest that children receiving citrulline appeared better clinically following cardiopulmonary bypass than did children receiving placebo.

i. Page 37; Last Paragraph:

Previously Stated:

As with the length of time of mechanical ventilation, the duration of inotrope therapy (dopamine, dobutamine, milrinone, epinephrine, phenylephrine, or norepinephrine) showed marked differences between the two treatment groups as shown in Figure 6.

Changed To:

As with the length of time of mechanical ventilation, the duration of inotrope therapy (dopamine, dobutamine, milrinone, epinephrine, phenylephrine, or norepinephrine) *was also recorded. Length of time on inotropes was documented from the time of first use after surgery until completion of the study medication at 48 hours. Patients still receiving inotropes at hour 48 were censored. There was a marked difference between the two treatment groups as shown in Figure 7 with censored patients still using inotropes at hour 48, with no additional censoring with a log rank p-value of 0.0351.*

j. Page 38; Figure 6; Title and Figure:

Previously Stated:

Figure 6: Kaplan-Meier survival analysis of the length of time on inotropes

[See previous figure]

Changed To:

Figure 7: Kaplan-Meier Survival Analysis of the Length of Time on Inotropes: *No additional Censoring*

[See new figure]

k. Page 38; Last Paragraph; Added new paragraph:

Previously Stated:

N/A

Changed To:

A second analysis also censored patients without inotrope use; i.e., inotrope time = 0 (Figure 8), which demonstrated a log rank p-value of 0.562.

l. Page 39; Added new figure

Previously Stated:

N/A

Changed To:

Figure 8: Kaplan-Meier Survival Analysis of the Length of Time on Inotropes: Censored

[See new figure]

m. Page 39; 1st Paragraph:

Previously Stated:

Cessation of mechanical ventilation and of inotrope therapy are the two principal determinants of readiness for discharge from the intensive care unit. A composite variable comprising (for each subject) of the longer of the two parameters - duration of mechanical ventilation or of inotrope therapy - can serve as an effective and accurate surrogate for the duration of intensive care unit stay. Figure 7 shows the difference in the composite variable when L-citrulline and placebo groups are compared. Patients receiving study drug showed shorter composite durations of mechanical ventilation and inotrope therapy than did patients receiving placebo. Thus…physical injury.

Changed To:

Cessation of mechanical…stay. *Since inotrope use was only documented until Hour 48 after surgery (end of study medication treatment), patients with inotrope use continuing until Hour 48 and with mechanical ventilation duration of ≤ 48 hours were censored at this time point. If mechanical ventilation was continued beyond the 48-hour time point, the duration of mechanical ventilation was used in the analysis. Kaplan-Meier plots of the composite endpoint are presented in Figure 9, with re-intubation times excluded. One (1) patient in the placebo group was re-intubated. This patient was excluded from this analysis; however, results including this patient were comparable. In this analysis, patients with inotrope use still ongoing at 48 hours, and mechanical ventilation of less than 48 hours, were censored. Figure 9 shows the difference in the composite variable when L-citrulline and placebo groups are compared. Citrulline-treated patients required statistically significantly less mechanical ventilation and inotropes than placebo-treated patients.* Thus…physical injury.

n. Page 40; Figure 7; Title and Figure:

Previously Stated:

Figure 7: Kaplan-Meier survival analysis of the longer duration of duration of postoperative mechanical ventilation and the length of time on inotropes.

[See previous figure]

Changed To:

Figure 9: Kaplan-Meier Survival Analysis of the Longer Duration of Duration of Postoperative Mechanical Ventilation and the Length of Time on Inotropes: *Re-Intubation Time Excluded*

[See new figure]

o. Page 40; 1st Paragraph

Previously Stated:

In summary, the data from study CIT-002-01 show that the revised dosing protocol achieved the PK endpoint. Further, despite the extremely small sample size of the study, CIT-002-01 demonstrated clear treatment-dependent differences in favor of L-citrulline for the duration of mechanical ventilation and inotrope therapy between the treated and control groups. Combined in a composite variable, the results show clinically meaningful therapeutic efficacy for L-citrulline for the time to discharge from the intensive care unit. Thus, CIT-002-01 indicates that i.v. L-citrulline treatment can play a beneficial role in preventing clinical sequelae of CPB-induced lung injury.

Changed To:

In summary…*CIT-002-01 indicates that intravenous L-citrulline prevents compromised cardiopulmonary function caused by CPB in pediatric patients undergoing surgery for ASD, VSD, or AVSD. The effect is robust and is evident even in centers practicing early extubation as shown in the strong preferential early extubation of citrulline patients in comparison to the control population. Discharge from the ICU requires cessation of both mechanical ventilation and inotrope therapy. Intravenous L-citrulline shortens the duration of each of these variables, resulting in earlier readiness of patients for discharge from the ICU.* Thus…injury.

8. Page 41; Section 1.5; Safety of L-citrulline; 4th Paragraph: Updated section to reflect results of data from CIT-002-01 final Clinical Study Report.

Previously Stated:

Safety data…have shown that i.v. L-citrulline administration to 11 patients was safe and no unexpected AEs and /or SAEs occurred under this treatment.

Changed To:

Safety data…have shown *that of the patients randomized and treated, all patients (100%) in the i.v. L-citrulline group and 73% of patients in the placebo group experienced at least one AE. AEs that were judged to be related to study treatment were reported in the placebo group (2 patients, 18%), but not in the active treatment group. No AE leading to death and no other SAE was reported in this study. No AE leading to treatment discontinuation was reported.*

9. **Page 46; Section 4.3.3; Exclusion Criteria: Clarified exclusion criteria for patients with AV valve regurgitation.**

   Previously Stated:

   - Significant left sided AV valve regurgitation

   Changed To:

   - Significant left sided AV valve regurgitation *not amenable to surgical correction*

10. **Page 49; Section 4.4.1; Investigational Medicinal Products Administered; 1st Paragraph: Added new language regarding matching placebo.**

    Previously Stated:

    N/A

    Changed To:

    Placebo will be matched for volume using suitable fluid recognized as standard of care for such procedure (e.g. normal saline or Plasmalyte without L-citrulline added).

    All study medications will be prepared and provided by each site's investigational pharmacy.

11. **Page 49-50; Section 4.4.2; Identity of Investigational Medicinal Products: Updated wording regarding Investigational Medicinal Product; Updated storage conditions.**

    Previously Stated:

    Citrulline is a naturally occurring amino acid. The L-citrulline drug substance as fully synthetic active pharmaceutical ingredient (API) is a white crystalline powder. The L-citrulline drug product is a sterile, non-pyrogenic injectable clear solution in water. The

concentrated solution is supplied to the study sites in heat-sterilized borosilicate glass vials labeled “L-citrulline for Injection”. Each vial contains 500 mg sterile L-citrulline in 10 mL of sterile water. The containers are closed with 13 mm brominated butyl stoppers and light blue, plastic/aluminum flip-off seals.

Study drug should be stored as indicated on the package label: Store at 25°C (77°F) or below; do not freeze.

The L-citrulline bolus and L-citrulline infusion will be provided by each site’s investigational pharmacy at a concentration of 50 mg/mL.

Placebo will be matched for volume using either normal saline or Plasmalyte without L-citrulline added.

The IMP is manufactured and imported according to the current Good Manufacturing Practice and the relevant regulatory requirements.

Changed To:

The L-citrulline drug product is a sterile, non-pyrogenic injectable clear solution intended for intravenous administration. It is supplied as a 10 mL, single use vial containing 500 mg L-citrulline in 10 mL of acidified water. The product is packaged in a clear glass vial (13 mm neck) with a grey stopper and blue flip-off cap/aluminum seal. The L-citrulline drug product is manufactured aseptically at a concentration of 50 mg/mL using fully synthetic drug substance dissolved in water for injection. The resulting solution pH is adjusted to near pH 3, sterile filtered and filled.

Study drug should be stored as instructed: Store at 2-8ºC (36-46ºF); do not freeze.

The *drug product* is manufactured and imported according to current Good Manufacturing Practice and the relevant regulatory requirements.

12. Page 50; Section 4.4.3; Packaging and Labeling: Updated description of packaging.

Previously Stated:

L-citrulline study drug is provided as vials for injection and supplied to the study centers. The study medication vials will be packed and dispatched in containers.

The batch number, strength, storage conditions, and federally required language stating that the product is for investigational use only will be given on the outer package. A batch

release certificate and a Good Manufacturing Practice-conformity statement will be provided, in addition to required language noting investigational use only.

Changed To:

L-citrulline study drug comes in a clear glass vial (13 mm neck) with a grey stopper and blue flip-off cap/aluminum seal. Boxes of 25 vials are supplied to the study centers.

The batch number, strength, storage conditions, and federally required language stating that the product is "for investigational use only" *or "for clinical trial use only" will be printed on the outer package. A Material Safety Data Sheet (MSDS) will be provided with the shipment.*

13. **Page 51; Section 4.4.6; Drug Accountability: Added wording regarding drug accountability (deleted previous language from Section 5.1.2.6), and updated instructions.**

Previously Stated:

N/A

Changed To:

All empty and partially used vials should be retained by the pharmacy in order for study drug accountability to be performed. The total amount of study drug prepared for administration to the patient will be calculated by the investigational pharmacist, with verification of the calculations performed by a second qualified person (e.g. pharmacy technician). Total volume of study drug administered to the patient will be documented in the source documents and in the electronic case report forms (eCRFs).

14. **Page 55**; **Section 5.1.2.1; Day 0, Surgery (Intra-Operative): Changed requirement for obtaining hemodynamics to align with Institution's standard of care.**

Previously Stated:

…Results will be recorded approximately every 5 minutes after induction of anesthesia and during surgery.

Changed To:

…Results will be recorded *according to the Institution's standard of care during surgery.*

15. **Page 56-61; Section 5.1.2.2 through 5.1.2.6; Day 0, post-surgery, 0, 6, and 12 hours, Day 1, post-surgery, 24 hours, and Day 2, post-surgery, 48 hours: Changed requirement for obtaining hemodynamics to align with Institution's standard of care.**

Previously Stated:
Results will be recorded approximately every hour from time of post-op admission to the PICU through 48 hours.

Changed To:

Results will be recorded *according to the Institution's standard of care* from time of post-op admission to the PICU through 48 hours.

16. **Page 56-61; Section 5.1.2.2 through 5.1.2.6; Day 0, post-surgery, 0, 6, and12 hours, Day 1, post-surgery, 24 hours, and Day 2, post-surgery, 48 hours: Changed requirement for obtaining ABGs to align with Institution's standard of care.**

Previously Stated:

Results will be recorded every 2-3 hours (+/- 15 minutes) from time of post-op admission to the PICU until removal of arterial line or through 48 hours, whichever occurs first.

Changed To:

ABGs will be drawn according to the Institution's standard of care. All results obtained from the time of post-op admission to the PICU until removal of the arterial line or through 48 hours (whichever occurs first) will be recorded.

17. **Page 57**; **Section 5.1.2.2; Day 0, post-surgery, 0 hours (30 minutes post CPB decannulation): Corrected MAP criteria for Age 4 years, and added MAP criteria for Age 5 years (missing from previous list).**

Previously Stated:

- Age …
- Age 4 years: MAP of 52
- Age 6 years: MAP of 53
- Age …

Changed To:

- Age …
- Age 4 years: MAP of *50*
- *Age 5 years:* *MAP of 52*

- Age 6 years: MAP of 53
- Age …

18. Page 62; Section 5.1.2.6; Day 2, post-surgery, 48 hours: Removed reference to drug accountability here, and moved to Section 4.4.6. (See #11 above).

Previously Stated:

Any remaining IMP, as well as empty or partially used IMP containers (e.g. bags, syringes), will be returned to the pharmacy and an accountability check will be performed. The total amount of study drug administered to the patient will be calculated by the investigational pharmacist, with verification of the calculations performed by a second qualified person (e.g. pharmacy technician).

Changed To:

N/A

19. Page 80; Section 7.4; Interim Analysis; 2nd Paragraph: Added additional wording regarding sample size re-estimation; Removed wording which stated study will be stopped.

Previously Stated:

In the event that the calculated sample size has already been exceeded, the study will be stopped.

Changed To:

Only the re-estimated variance values will be used for sample re-calculation, while the assumed values will not be re-estimated but kept as described in Section 7.2.

In the event that the sample size re-estimation leads to a reduction of the sample size due to lower re-estimated variance compared to the assumed variance values, the sample size as currently planned will be kept, and not reduced, to ensure robust estimations for safety and efficacy variables.

14.2.5 Version 4.3.1

1. **Page 22; Schedule of Study Assessments and Procedures; Footnote 7: Further clarified instructions for obtaining hemodynamics.**

   Previously Stated (Version 4.3):

   Baseline values are first recorded in the operating room (OR) by Anesthesia. Results will be recorded, during surgery and from time of post-op admission to the PICU through 48 hours, according to the Institution's standard of care. Results at Hours 1, 2, 4, 12, 24, and 48 must be obtained and must be within +/- 10 minutes of the required time point.

   Changed To:

   Baseline values are first recorded in the operating room (OR) *by Anesthesia. Results will be monitored during surgery according to the Institution's standard of care, but results will only be recorded approximately every 15 minutes. Standard of care will continue to be followed from time of post-op admission to the PICU through 48 hours. Results at 1, 2, 4, 12, 24, and 48 hours from baseline (in OR) must be obtained and recorded.*

2. **Page 23; Schedule of Study Assessments and Procedures; Footnote 16: Clarified instructions for obtaining final laboratory sample.**

   Previously Stated:

   An attempt should be made to obtain final labs while i.v. access is available, either on Day 28 or prior to discharge from hospital. If lab results within 24 hours are available, these may be captured. If no i.v. access is available, venipuncture is not required unless indicated for clinical reasons.

   Changed To:

   An attempt should be made to obtain final labs on Day 28 or prior to discharge from the hospital. If lab results within 24 hours are available, these may be captured*. If results are not available within 24 hours, then record the results of the labs obtained closest to discharge from the hospital. These labs must include LFTs.* If no i.v. access is available on Day 28 or the day of discharge, venipuncture is not required *since results of the labs obtained closest to discharge may be used.*

3. **Page 55**; **Section 5.1.2.1; Day 0, Surgery (Intra-Operative): Further clarified instructions for obtaining hemodynamics.**

Previously Stated (Version 4.3):

…Results will be recorded according to the Institution's standard of care during surgery.

Changed To:

Results will be monitored during surgery according to the Institution's standard of care, but results will only be recorded approximately every 15 minutes.

4. **Page 56-61; Section 5.1.2.2 through 5.1.2.6; Day 0, post-surgery, 0, 6, and 12 hours, Day 1, post-surgery, 24 hours, and Day 2, post-surgery, 48 hours: Further clarified instructions for obtaining hemodynamics.**

Previously Stated (Version 4.3):

…Results at Hours 1, 2, 4, 12, 24, and 48 must be obtained and must be within +/- 10 minutes of the required time point.

Changed To:

…Results at 1, 2, 4, 12, 24, and 48 hours from baseline (in OR) must be obtained and recorded.

5. **Page 62; Section 5.1.2.7; Follow-Up Visit [Day 28 (if still in hospital) or upon Hospital Discharge]: Clarified instructions for obtaining final laboratory sample.**

Previously Stated:

- Blood draw* for:

  ….

  * (An attempt should be made to obtain final laboratory samples while i.v. access is available, either on Day 28 or prior to discharge from hospital. If laboratory results within 24 hours are available, these may be captured. If no i.v. access is available, venipuncture is not required unless indicated for clinical reasons.)

Changed To:

- Blood draw* for:

….

** (An attempt should be made to obtain final laboratory samples on Day 28 or prior to discharge from hospital.* If laboratory results within 24 hours are available, these may be captured. *If results are not available within 24 hours, then record the results of the labs obtained closest to discharge from the hospital. These labs must include LFTs.* If no i.v. access is available on Day 28 or the day of discharge, venipuncture is not required *since results of the labs obtained closest to discharge may be used.*