**Statistical Analysis Plan**
**for**
**Final Analysis**

**Version 2.0**

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|---|---|
| Study: | 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 |
| Study-ID: | CIT-003-01 |
| Sponsor / Contact: | Asklepion Pharmaceuticals, LLC<br>729 E. Pratt Street; Suite 360<br>Baltimore, MD 21202 |
| Evaluation: | FGK Clinical Research GmbH |
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**Revision history**

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| 1.0 | O.Boehm | 17 Nov 2017 | Final version 1.0 |
| 2.0 | V. Voelkl | 01 Oct 2018 | Final version 2.0<br>• PF ratio and 28-day mortality added as exploratory endpoints (according to EU Study Protocol)<br>• Definition of FAS, mFAS, and SAF clarified<br>• Sensitivity analyses added for the primary efficacy variable and for secondary efficacy variables 'Length of time on mechanical ventilation', and 'Length of time of inotrope use'<br>• Numbering of TLG corrected in SAP Appendix A<br>• SAP appendix B replaced by external sample size reestimation document describing procedure |

## Table of Contents

**LIST OF ABBREVIATIONS** ..... **4**
**1 GENERAL** ..... **5**
1.1 Study Design ..... 5
1.2 Objectives ..... 5
1.3 Sample Size Calculation ..... 6
1.4 Interim Analysis ..... 6
1.5 Visit Terminology ..... 7
**2 EFFICACY AND SAFETY VARIABLES** ..... **8**
2.1 Primary Efficacy Variable ..... 8
2.2 Secondary Efficacy Variables ..... 8
2.3 Secondary Safety Variables ..... 8
2.4 Exploratory Variables ..... 8
2.5 Other Relevant Variables ..... 8
**3 STATISTICAL ANALYSIS SETS** ..... **9**
3.1 Safety Analysis Set ..... 9
3.2 Full Analysis Set ..... 9
3.3 Modified Full Analysis Set ..... 9
3.4 Per-Protocol Set ..... 9
3.5 Assignment of Analysis Sets to Analysis ..... 9
**4 STATISTICAL EVALUATION** ..... **10**
4.1 Dispositions of Subjects and Analysis Sets ..... 10
4.2 Demographics and Other Covariates ..... 10
4.3 Study Drug Administration and Intra-operative Details ..... 11
4.4 Efficacy Analysis ..... 11
4.4.1 Analysis of Primary Efficacy Variable ..... 11
4.4.2 Analysis of Secondary Efficacy Variables ..... 12
4.5 Safety Analysis ..... 16
4.6 Analysis of Exploratory Variables ..... 19
4.7 Analysis of Other Relevant Variables ..... 19
4.8 Missing Values ..... 20
4.9 Data Base Closure and Data Review ..... 20
4.10 Miscellaneous ..... 21
**5 CHANGES FROM PROTOCOL** ..... **21**
**6 LITERATURE** ..... **23**
**7 APPENDICES** ..... **23**
**8 SIGNATURES** ..... **24**

## List of Abbreviations

In the following abbreviations are listed as used within this statistical analysis plan or which might occur within the tables, listings and graphs outputs:

| | |
|---|---|
| ABG | Arterial blood gases |
| ACT | Activated clotting time |
| AE | Adverse event |
| ANOVA | Analysis of variance |
| ASD | Atrial septal defect |
| ATC | Anatomical therapeutic chemical classification |
| AVSD | Atrioventricular septal defect |
| BDR | Blind data review meeting |
| BMI | Body mass index |
| BPAP | Bilevel (biphasic) positive airway pressure |
| CBC | Complete blood count |
| CI | Confidence interval |
| CPAP | Continuous positive airway pressure |
| CPB | Cardiopulmonary bypass |
| CRF | Case report form |
| CS | Clinically significant |
| EC | Exclusion criteria |
| ECG | Echocardiogram |
| ET | Early termination |
| FAS | Full analysis set |
| FDA | Food and Drug Administration |
| H0 | Null hypothesis |
| H1 | Alternative hypothesis |
| IC | Inclusion criteria |
| ICF | Informed consent form |
| ITT | Intention-to-treat |
| LOCF | Last observation carried forward |
| MedDRA | Medical dictionary for regulatory activities |
| mFAS | Modified full analysis set |
| N | Number of subjects |
| NCS | Not clinically significant |
| OR | Odds ratio |
| PICU | Pediatric intensive care unit |
| PP | Per-protocol analysis set |
| PT | Preferred term |
| PTAE | Pre-treatment adverse event |
| RD | Risk difference |
| RR | Risk ratio |
| SAE | Serious adverse event |
| SAF | Safety analysis set |
| SAP | Statistical analysis plan |
| SD | Standard deviation |
| SOC | System organ class |
| TAE | Treatment adverse event |
| TLG | Tables, listings, graphs |
| VSD | Ventricular septal defect |
| WHO-DD | World Health Organization drug dictionary |

No abbreviations of units and of laboratory and blood gas values are included in this list.

# 1 General

This Statistical Analysis Plan (SAP) was defined by the Sponsor and the responsible Statistician without knowledge of the randomization code. It is based upon the global Study Protocol (version 4.3.1 of August 11, 2017) and the EU Study Protocol (version 2.0 of July 12, 2018) and contains detailed description of the statistical methods described therein.

The SAP describes prospectively the analyses to be performed on study data. It was finalized prior to data base lock and subsequent unblinding.

## 1.1 Study Design

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 cardiopulmonary bypass (CPB) in pediatric subjects undergoing surgery for congenital heart defects. Children undergoing repair of cardiac defects that include a large unrestrictive ventricular septal defect (VSD) or a partial or complete atrioventricular septal defect (AVSD) or an ostium primum atrial septal defect (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. 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;

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.

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.

## 1.2 Objectives

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.

The primary efficacy parameter 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 shall include invasive mechanical ventilation or non-invasive mechanical ventilation including bilevel (biphasic) positive airway pressure (BPAP) or continuous positive airway pressure (CPAP). The definition of inotrope use includes medications which are considered within the derivation of the total inotrope score (dopamine, dobutamine, milrinone, epinephrine, phenylephrine, norepinephrine).

The following hypothesis will 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

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.

### 1.3 Sample Size Calculation

Sample size was calculated with nQuery 7.0. Assuming a standard deviation of 50 and mean values of 44 for placebo and 14 for the L-citrulline group, the effect size is estimated 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 assumptions concerning standard deviation and mean values are based on results of the preceding study CIT-002-01.

To allow for subjects being enrolled but not assigned to the full analysis set (FAS), an overall N=95 subjects per group will be enrolled. A sample size re-estimation will be done within the framework of an interim analysis (see section 1.4).

### 1.4 Interim Analysis

One interim analysis is planned to be carried out when the first 95 consecutively randomized 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 (See external sample size reestimation document). During this interim analysis, only a sample size re-estimation will be carried out. 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 size estimate. Only the re-estimated variance values will be used for the sample re-calculation, while the assumed mean values will not be re-estimated but kept. Only the results of the sample size re-estimation will be provided to Asklepion Pharmaceuticals.

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 but 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 Food and Drug Administration (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 and mean values will be assessed, no alpha adjustment of statistical analyses is required.

### 1.5 Visit Terminology

The notation displayed in table 1 will be used for table, listing, and graph (TLG) presentation of visits.

**Table 1: Visit terminology**

| Notation in protocol | Notation used for TLG presentation | Notation for treatment phases |
|---|---|---|
| Screening visit (Day -14 to Day 0) | Screening | Pre-Treatment |
| Baseline visit (Day of Surgery, Pre-operation) | Day 0 – Pre-Op | |
| | Baseline[1] | |
| Day 0, Surgery (Intra-operative) | Day 0 – Intra-Op | Treatment |
| Day 0, Post-surgery, 0 hours | Day 0 – 0 hours | |
| Day 0, Post-surgery, 6 hours | Day 0 – 6 hours | |
| Day 0, Post-surgery, 12 hours | Day 0 – 12 hours | |
| Day 1, Post-surgery, 24 hours | Day 1 – 24 hours | |
| Day 2, Post-surgery, 48 hours | Day 2 – 48 hours | |
| Follow-Up Visit (Day 28/ Hospital Discharge) | Day 28/ Discharge | Follow-up |
| Follow-Up Phone Assessment[2] | Day 28 - Phone call | |

[1]Baseline will be defined as the most recent non-missing value prior to surgery.
[2] Patients discharged from hospital prior to Day 28 will be contacted by phone on Day 28 to assess their survival status.

# 2 Efficacy and Safety Variables

## 2.1 Primary Efficacy Variable

The primary efficacy parameter for this study 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 shall include invasive mechanical ventilation or non-invasive mechanical ventilation including BPAP or CPAP. The definition of inotrope use includes medications which are considered within the derivation of the total inotrope score (dopamine, dobutamine, milrinone, epinephrine, phenylephrine, norepinephrine).

## 2.2 Secondary Efficacy Variables

The following secondary efficacy parameters will be analyzed:

- Length of time on mechanical ventilation
- Length of time on positive pressure ventilation
- Length of time of inotrope use
- Inotrope score
- Hemodynamic improvement
- Length of time of intubation
- Length of pediatric intensive care unit (PICU) stay
- Length of time on vasodilators
- Length of hospitalization
- Thoracotomy output - duration and volume of chest tube drainage
- L-citrulline plasma levels

## 2.3 Secondary Safety Variables

- Occurrence of adverse and serious adverse events
- Incidence of refractory hypotension
- Change from Baseline in laboratory values

## 2.4 Exploratory Variables

- PF ratio
- 28-day mortality

## 2.5 Other Relevant Variables

- Arterial blood gas (ABG)

# 3 Statistical Analysis Sets

## 3.1 Safety Analysis Set

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

## 3.2 Full Analysis Set

The full analysis set (FAS) includes all subjects who underwent surgery including undergoing CPB. 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.

## 3.3 Modified Full Analysis Set

The modified full analysis set (mFAS) includes all subjects who underwent surgery including undergoing CPB 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.

## 3.4 Per-Protocol Set

The per-protocol set (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). Protocol deviations will be identified and classified for each subject during a blind data review meeting. For the PP all subjects will be assigned to the treatment actually received.

## 3.5 Assignment of Analysis Sets to Analysis

The FAS serves as the primary efficacy analysis set. All efficacy analyses are based on the FAS. The mFAS and the PP serve as sensitivity populations for the efficacy analyses.

The SAF will be used for the evaluation of the safety assessments.

# 4 Statistical Evaluation

Continuous variables will be summarized by mean, standard deviation, median, lower and upper quartiles, minimum and maximum. Frequencies and percentages will be used to summarize categorical variables.

Analyses will be performed by visit and treatment group, 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. Handling of missing values is described in detail in the following sections. If no method is described, no imputation of missing values will be applied.

Any parameter describing a “duration” or “length” is calculated as stop date/time - start date/time + 1.

## 4.1 Dispositions of Subjects and Analysis Sets

Disposition of subjects and analysis sets

The disposition of subjects and analysis sets, subjects per visit, subjects per site, subject eligibility (informed consent signed, subject failed inclusion and/or exclusion criteria), and subject status (discontinuation and reasons for early study drug and study discontinuation, hospital discharge prior to Day 28) will be tabulated.

No inferential assessments will be performed on disposition data.

## 4.2 Demographics and Other Covariates

Demographic data

Demographic data (age, gender, race, ethnicity, body height, body weight, body mass index [BMI; calculated: ($kg/m^2$)]) will be tabulated by treatment group. Age, body weight and BMI will be additionally stratified by visit.

Menarche and pregnancy test

The proportion of female patients who reached the age of menarche will be presented. For females with childbearing potential conduction of pregnancy test will be tabulated. For females who conducted a pregnancy test the test results will be tabulated.

Medical history

The proportion of subjects with medical history conditions or events will be tabulated.

Concomitant medication

Concomitant medications should be recorded starting from the date of informed consent until end of follow-up. Inotropes and vasoactive medications are captured separately and will be analyzed separately. Medications will be coded by the World Health Organization drug dictionary (WHO-DD).

Concomitant medication will be tabulated by Anatomic Group (Anatomical therapeutic chemical classification [ATC] level 1), ATC level 4, and WHO-DD preferred term.

Echocardiography

The results of the transthoracic echocardiogram specifying the congenital cardiac anomaly (AVSD / ASD / VSD) will be tabulated.

Physical examination

Physical examination assessments (Normal / Abnormal, not clinically significant / Abnormal, clinically significant / Not done) will be tabulated by body system.

No inferential assessments will be performed on demographics and other covariates.

### 4.3 Study Drug Administration and Intra-operative Details

Study drug administration

Details regarding study drug administration (infusion access, interruption, dosage, and duration) will be tabulated.

Intra-operative details

Details regarding surgery, transfusion, procedural fluids, and cardiopulmonary bypass will be listed.

No inferential assessments will be performed on study drug administration data.

### 4.4 Efficacy Analysis

#### 4.4.1 Analysis of Primary Efficacy Variable

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 shall include invasive mechanical ventilation or non-invasive mechanical ventilation including BPAP or CPAP. The definition of inotrope use includes medications which are considered within the derivation of the total inotrope score (dopamine, dobutamine, milrinone, epinephrine, phenylephrine, norepinephrine).

The 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 minutes from separation from CPB (variable “End time” of parameter “CPB” from section “Post incision” on CRF “Surgery”) until discontinuation of all mechanical ventilation including non-invasive support (variables “Stop Time/Date of last use of non-invasive ventilation” and “Stop Time/Date of last use of invasive ventilation” of parameter “CPB” from section “Post incision” on CRF “Mechanical ventilation”). If invasive or non-invasive mechanical ventilation is stopped but has to be restarted, time will continue to accrue by subtracting the total duration of all ventilation breaks (variables “Stop date/time” and “Restart date/time” from CRF “Ventilation breaks”).

- Length of time on mechanical ventilation (min) =
  (Time mechanical ventilation discontinuation - End time (CPB) + 1) -
  sum of all ventilation breaks with any ventilation break = Restart time - Stop time + 1

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.

Length of time 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 in minutes from separation from CPB (variable “End time” of parameter “CPB” from section “Post incision” on CRF “Surgery”) until last use (last “end date/time” of any medication as documented in section “Inotropes” on CRF “Inotropes and Vasoactive Medications”).

- Length of time of inotrope use (min) =
  Last “end date/time” of any inotrope - End time (CPB) + 1

Any interruptions will discontinue to accrue, 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).

As additional sensitivity analyses, the composite endpoint will be derived with (3) length of time of inotrope use starting on first “start date/time” of inotrope use and (4) length of time of inotrope use starting on first “start date/time” of inotrope use and length of time on mechanical ventilation starting on “start date/time” of mechanical ventilation. For sensitivity analyses (3) and (4) the observed length on mechanical ventilation/ inotropes will be used for subjects who died.

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-Meier estimates and plots, log-rank test (significance level of 5%) and Cox-regression modeling including factor treatment (no further factors to be included) will serve as sensitivity analyses. For subjects who died before discharge from the hospital or before Day 28, respectively, the observed length of time on mechanical ventilation/inotropes will be used. The sensitivity analyses (1) and (2) as described for the primary analysis (subjects who died will (1) be excluded from analysis and (2) be assigned a length of time on inotropes of 28 days) will not be applied for survival analyses. The sensitivity analyses (3) and (4) as described for the primary analysis (composite endpoint will be derived with (3) length of time of inotrope use starting on first “start date/time” of inotrope use and (4) length of time of inotrope starting on first “start date/time” of inotrope use and length of time on mechanical ventilation starting on “start date/time” of mechanical ventilation) will be applied for survival 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

For sensitivity analyses (3) and (4) the first set of analyses (i.e., (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) will be used.

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.

### 4.4.2 Analysis of Secondary Efficacy Variables

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 FAS, mFAS and PP. All secondary analyses are considered exploratory.

Length of time on mechanical ventilation

The same definitions and analyses as described for the primary endpoint will be applied. As sensitivity analysis, length of time on mechanical ventilation will be calculated starting on “start date/time” of mechanical ventilation.

Length of time on positive pressure (non-invasive) ventilation

Positive pressure ventilation describes non-invasive mechanical ventilation. The same definitions (only using non-invasive ventilation stop date/time and non-invasive ventilation breaks: BPAP, CPAP, HFNC, and other non-invasive) 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. As sensitivity analysis, length of time of inotrope use will be calculated starting on first “start date/time” of inotrope use.

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 score will be calculated for each hour post separation from CPB (see section 4.4.1) until 48 hours post separation. If within the time interval of hours x after the separation from CPB any inotrope was given, the total score will be calculated and used for analysis.

Example for a patient's inotrope documentation:

- 12:00: Separation from CPB
- Dopamine: 100 mcg/kg/min from 12:00 to 13:00,
- Dobutamine 100 mcg/kg/min from 13:00 to 13:30, 14:01 to 14:59, and 16:00 to 17:00.

The times of interest at 1, 2, 3, … hrs will be derived as time of separation from CPB + 59, 119, 179, … minutes. I.e., the times of interest and the total inotrope score would then be calculated as follows:

- 1 hrs (12:00-12:59) = 100 (Dopamine (100) documented 12:00-13:00)
- 2 hrs (13:00-13:59) = 200 (Dopamine (100) documented 12:00-13:00
  Dobutamine (100) documented 13:00-13:30 )
- 3 hrs (14:00-14:59) = 100 (Dobutamine (100) documented 14:01-14:59 )
- 4 hrs (15:00-15:59) = 0 (no inotropes documented 15:00-15:59 )
- 5 hrs (16:00-16:59) = 100 (Dobutamine documented 16:00-17:00 )
- 6 hrs (17:00-17:59) = 100 (Dobutamine documented 16:00-17:00 )
- 7 hrs (18:00-18:59) = 0 (no inotropes documented 17:01-18:00 )
- …

The total inotrope score will be imputed to zero after the bypass pump has been removed (separation from CPB). It will also be imputed as zero for patients not using inotropes at all.

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. Additionally, a repeated measures analysis of variance will be used to compare total inotrope score between placebo and L-citrulline over time.

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.

The last non-missing hemodynamic assessment before start of surgery will be used as Baseline level; i.e. "Date and time of assessment" from section "Hemodynamics" on CRF "Hemodynamics" before "Date of surgery" and "Start time (incision time)" from section "Surgery" on CRF "Surgery". Documented assessments as closest to the target hours 1, 2, 4, 12, 24, and 48 using the following analysis time windows will be used for analysis:

- 1 hrs = 1 min to 89 min ( >0 hrs to <1.5 hrs)
- 2 hrs = 90 min to 179 min ( ≥1.5 hrs to <3.0 hrs)
- 4 hrs = 180 min to 479 min ( ≥3.0 hrs to <8.0 hrs)
- 12 hrs = 480 min to 1079 min ( ≥8.0 hrs to <18.0 hrs)
- 24 hrs = 1080 min to 2159 min ( ≥18.0 hrs to <36.0 hrs)
- 48 hrs = 2160 min to 3599 min ( ≥36.0 hrs to <60.0 hrs)

An ANOVA per hour 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. Absolute values and absolute change from Baseline values will be summarized for all observed time points.

Length of time on intubation (invasive ventilation)

The same definitions (only using invasive ventilation stop date/time and invasive ventilation breaks: FVS, PVS and other invasive) and 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. The length will be derived as the number of days from day after surgery (variable “Date of surgery” on CRF “Surgery” + 1) until discharge from PICU (variable “Date transferred to floor” on CRF “Post-operative observation”). If subject has to be readmitted to PICU, time will continue to accrue (variables “Date of readmission” and “Date transferred to floor” (second assessment) from CRF “Post-operative observation”).

- Length of PICU stay (days) =
  (Date transferred to floor - (Date of surgery + 1) + 1) +
  (Date transferred to floor (2$^{nd}$ assessment) - Date of readmission + 1)

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 minutes from separation from CPB (variable “End time” of parameter “CPB” from section “Post incision” on CRF “Surgery”) until last use (last “end date/time” of any medication as documented in sections “Vasodilators” or “Other vasodilators” on CRF “Inotropes and Vasoactive Medications”).

- Length of time of vasodilators (min) =
  Last “end date/time” of any vasodilators - End time (CPB) + 1

Any interruptions will discontinue to accrue, 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. The length will be derived as the number of days from day after surgery (variable “Date of surgery” on CRF “Surgery” + 1) until discharge from the hospital (variable “Hospital Discharge date” on CRF “End of study”).

- Length of hospitalization (days) =
  Hospital Discharge date - (Date of surgery + 1) + 1

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 minutes) that the chest tube will be used will be calculated as the time from the end of the surgery (variables “Date of surgery” and “End time” from section/CRF “Surgery”) to the time the chest tube will be removed (variable “Date/time of removal” from section/CRF “Thoracotomy Output”).

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.

- Length of chest tube drainage (min) =
  (Time removal - End time (surgery) + 1) +
  (Time removal (second assessment) - Time reinsertion + 1)

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), adding up 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

L-citrulline plasma levels will be obtained on each patient at set times. Individual concentration-time curves will be plotted to confirm that they are ≥ the threshold level of 100 µmol/L. In addition, summary statistics using descriptive measures will be tabulated.

### 4.5 Safety Analysis

Safety endpoints will be analyzed using the SAF.

Adverse events

Pre-treatment adverse events (PTAE) and treatment adverse events (TAE) will be analyzed separately. TAE are defined as all adverse events (AE) with time of onset or worsening on or after the time of first drug administration. If unclear due to incomplete start time / date of AE or drug administration, AEs will be assumed as TAEs.

Adverse events (AE) will be summarized by system organ class (SOC) and preferred term (PT) according to Medical Dictionary for Regulatory Activities (MedDRA) version 19.0. The number of events, as well as the number and rate of affected subjects will be reported for each treatment group. AEs will be summarized also by severity, and by relationship to study medication. Separate analyses will be done for serious AEs and AEs leading to death.

Preferred terms of AEs with a proportion of ≥ 5% in either L-citrulline or placebo group will be additionally tabulated in descending order of frequency together with the relative risk (RR), risk difference (RD), and Odds ratio (OR).

SAEs will additionally be tabulated by time of onset after time from the end of the surgery (variables “Date of surgery” and “End time” from section/CRF “Surgery”). The following timeframes will be used:

- ≤ 0 hours: Occurrence from time of first drug administration until and inclusive time of end of the surgery
- >0 to ≤12 hours: Occurrence after time of end of the surgery until and inclusive + 12 hours
- >12 to ≤24 hours: Occurrence after time of end of the surgery + 12 hours until and inclusive + 24 hours
- >24 to ≤36 hours: Occurrence after time of end of the surgery + 24 hours until and inclusive + 36 hours
- >36 to 48 hours: Occurrence after time of end of the surgery + 36 hours until and inclusive + 48 hours
- >48 hours: Occurrence after time of end of the surgery + 48 hours until

If unclear due to incomplete start time / date of AE or drug administration, AEs will be assigned to category >0 to ≤12 hours.

No statistical testing procedures will be applied to the analysis of AEs.

Incidence of refractory hypotension

Refractory hypotension is defined as dropping of mean arterial (blood) pressure below specific age-related criteria (see below) for more than 30 minutes. I.e., 2 or more successive mean arterial blood pressure (MAP) measurements within a time frame of at least 30 minutes have to be below the criteria as listed below.

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 factors treatment and site.

Hypotension assessment (Refractory hypotension is defined as a 20% drop of MAP below specific age-related criteria (Haque and Zaritsky (2007) for greater than 30 minutes):

- 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

Laboratory values

Laboratory values will be analyzed descriptively. For quantitative parameters, absolute values as well as absolute and percentage changes from Baseline will be tabulated using summary statistics for all observed time points. For qualitative variables, frequency tables will be generated for all observed time points.

A conversion to the given standard units will be performed if necessary. Concentrations below the limit of quantification will be set to zero.

All laboratory values will be classified as normal or abnormal according to the laboratories normal ranges. Abnormal values will be indicated as clinically significant or not clinically significant by the investigator. Shift tables (normal / abnormal, not clinically significant / abnormal, clinically significant) will be created illustrating changes with respect to the laboratories normal ranges and clinical significance between Baseline and post Baseline visits Day 1, Day 2, and Day 28/ Discharge.

The following laboratory parameters were determined and will be described for the corresponding visits:

Hematology

Type of all parameters is quantitative.

- Hemoglobin
- Hematocrit
- Red blood cell (RBC) count
- White blood cell (WBC) count (total and differential)
- Platelet count

Serum Electrolytes, BUN, and creatinine

Type of all parameters is quantitative.

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

Liver function tests

Type of all parameters is quantitative.

- Bilirubin (total)
- AP
- AST
- ALT
- LDH

Coagulation

Type of all parameters is quantitative.

- ACT

Urinalysis

Specific gravity and pH are quantitative, the remaining parameters are qualitative.

- Visual: Color, Clarity/Turbidity
- Chemical: pH, specific gravity, glucose, ketones, nitrites
- Microscopic: RBCs, WBCs, epithelial cells, casts, bacteria

No statistical testing procedures will be applied to the analysis of laboratory values.

### 4.6 Analysis of Exploratory Variables

PF ratio

PF ratio is defined as PaO2 [mmHg] divided by FiO2 in [%]. PaO2 and FiO2 are measured at varying times. Since FiO2 is measured more often, PF ratio will only be calculated for the times when a corresponding PaO2 measurement is available (defined as FiO2 value measured at time of PaO2 value ± 0.5h). As denominator, the FiO2 value measured with minimum time difference will be used. If there is no FiO2 value measured at time of PaO2 ± 0.5h, no PF ratio will be calculated.

The PF ratio will be listed. In addition, the mean PF ratio per patient and visit will be calculated and displayed using basic statistics.

Example:

| ID | Date/time of FiO2 | FiO2 value |
|---|---|---|
| 1 | 2018-01-08T11:57 | 100 |
| 2 | 2018-01-08T13:43 | 80 |
| 3 | 2018-01-08T14:00 | 80 |
| 4 | 2018-01-08T14:43 | 80 |
| 5 | 2018-01-08T15:00 | 70 |
| 6 | 2018-01-08T17:11 | 75 |
| 7 | 2018-01-08T18:00 | 75 |
| 8 | 2018-01-08T19:00 | 75 |
| 9 | 2018-01-08T20:18 | 75 |
| 10 | 2018-01-08T23:33 | 75 |

| Date/time of PaO2 | PaO2 value | PF ratio | ID of matched FiO2 |
|---|---|---|---|
| 2018-01-08T11:15 | 73 | no matching value | |
| 2018-01-08T12:32 | 236 | no matching value | |
| 2018-01-08T14:49 | 267 | 267/80 | 4 |
| 2018-01-08T16:50 | 147 | 147/75 | 6 |
| 2018-01-08T17:59 | 213 | 213/75 | 7 |
| 2018-01-08T19:04 | 273 | 273/75 | 8 |
| 2018-01-08T20:15 | 326 | 326/75 | 9 |
| 2018-01-08T22:27 | 336 | no matching value | |

28-day mortality

The percentage of patients who have died at Day 28 will be presented by treatment group.

### 4.7 Analysis of Other Relevant Variables

Arterial blood gases (ABG)

The number of patients with any low or high values for AGB parameters PaO2, PaCO2, HCO3, pH per 6 hour time window and overall will be evaluated.

6 hour time windows from time of post-op admission to the PICU will be defined as:

- Time of AGB evaluation = Time collected AGB - Time of admission to PICU
- >0 to ≤6 hrs ( >0min to ≤360min )
- >6 to ≤12 hrs ( >360min to ≤720min )
- >12 to ≤18 hrs ( >720min to ≤1080min )
- …
- >42 to ≤48 hrs (>2520min to ≤2880min )
- >48 hrs ( >2880min )

Normal ranges for the AGB parameters are defined as:

- PaO2: <70 mmHg = low; 70-100 mmHg = normal; >100 mmHg = high
- PaCO2: <35 mmHg = low; 35-45 mmHg = normal; >45 mmHg = high
- HCO3: <22 mmol/L = low; 22-26 mmol/L = normal; >26 mmol/L = high
- pH: <7.35 = low; 7.35-7.45 = normal; >7.45 = high

Patients will be categorized per AGB parameter and per time window (and overall) as follows:

- Low: At least one value at one visit was low
- Normal: All values at all visit were normal
- High: At least one value at one visit was high
- Low/High: At least one value at one visit was low, and at least one value at one visit was high

The number of patients for those categories will be tabulated per AGB parameter and per time window (and overall).

### 4.8 Missing Values

Efficacy endpoints describing length of time

If a subject did not receive any mechanical ventilation / inotropes / positive pressure ventilation / intubation / vasodilators / chest tube, the corresponding length of time is set to 0. For subjects who died before discharge from the hospital or before Day 28, respectively, the observed length of time will be used for analysis of all time variables.

For time to event analyses censoring information will be used for statistical analyses.

No other imputation of missing values will be used.

Efficacy endpoint inotrope score

The total inotrope score will be set to zero after the bypass pump has been removed. It will also be set to zero for patients not using inotropes at all.

Further secondary efficacy endpoints

No imputation of missing values will be applied for the secondary endpoints hemodynamic improvement, volume of chest tube drainage, and L-citrulline plasma levels.

Safety endpoints

For laboratory data, concentrations below the limit of quantification will be set to zero. Missing safety data or safety assessments will not be estimated nor imputed. All safety summaries and analyses will be based on observed data.

No other imputation of missing values will be used.

### 4.9 Data Base Closure and Data Review

The statistical analysis of the clinical observation period will be performed after cleaning and database lock of all data and final unblinding.

A blind data review meeting (BDRM) will be conducted based on all data to check for protocol deviations and to allocate the subjects to the analysis sets FAS, mFAS, SAF, and PP. These evaluations and assessments will be done together and in agreement with the Sponsor, however FGK will provide the Sponsor with the appropriate subject listings. BDRM will be done via WebEx.

Data unblinding on the basis of the randomization listing and the analysis will be done after BDRM has been conducted and BDRM minutes have been signed by both the Sponsor and FGK.

### 4.10 Miscellaneous

For qualitative variables the frequencies (absolute and relative) are calculated. If no further remark is given in the description of the tables following format will be used for all tables with qualitative variables:

| | Y-variable(s) (e.g., treatment group) | | | | | |
|---|---|---|---|---|---|---|
| | Category 1 | | Category 2 | | Total | |
| X-variable(s) | N | % | N | % | N | % |
| category 1 | xx | xx.x | xx | xx.x | xx | xx.x |
| category 2 | xx | xx.x | xx | xx.x | xx | xx.x |
| Missing | xx | xx.x | xx | xx.x | xx | xx.x |
| Total | xx | 100.0 | xx | 100.0 | xx | 100.0 |

For this standard format the description of the tables in Appendix A determines only the X- and Y-variables. If another format of table is described in the details to the tables, the real design will be determined by the technical possibilities within SAS and may not look identical to the provided example. However, all information as displayed will be included.

Quantitative parameters will be described by declaring the mean value, standard deviation, minimum, first quartile, median, third quartile, and maximum. In the description of the tables this will be denoted by „basic statistics“.

The listings are always sorted by treatment group, center, and subject. If a different sorting order should be used for some listings this will be remarked separately. The variables for the special listings are explicitly given in the description of listings. All listings will be presented for the full analysis set, if not stated differently. Indicator variables for further analysis sets, e.g., safety analysis set, will be added to listing “Disposition of subjects and analysis sets”.

The following title will be used for all generated tables, listings, and graphs:

CIT-003-01: L-CITRULLINE Page # of #

<Table/Listing/Graph NNN: Description of contents>

<Subtitle for description of contents - if applicable>

<Analysis set>

The numbering NNN of the tables/listings/graphs will be stated in the detailed description (Appendix A).

Following footnote will be used for all generated tables, listings, and graphs:

Date: <Actual date> Program: <Name of program>

The statistical evaluation will be performed using SAS version 9.3 or higher.

## 5 Changes from Protocol

Length of time of inotrope therapy

According to protocol length of time 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).

Actually the exact start and end date/times of inotropes will be documented in the CRF, therefore the length of time of inotrope use will be derived in minutes.

Additionally, to be consistent to the time of mechanical ventilation, the time of separation from CPB will be used as starting point instead of time of first use.

Also, it is stated “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).” Due to the inotrope documentation as described above the LOCF principle is not to be applied.

## 6 Literature

n/a

## 7 Appendices

- Appendix A: List of generated summary tables, listings, and graphs

## 8 Signatures

| **Statistician:** |
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| **Sponsor:** |
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