CHEST™ Online Supplement

e-Appendix 7.

200325

ADDITIONAL SUPPLEMENT TO FLUID RESPONSIVENESS EVALUATION IN SEPSIS-ASSOCIATED HYPOTENSION (FRESH)

This supplement contains the following items:

Item	Document Title
Original FRESH Protocol	Evaluation of fluid volume in patients with sepsis and refractory hypotension_Clinical Protocol_Rev G
Original FRESH Protocol Appendix	Clinical Protocol Appendix A rev G
Final FRESH Protocol	Evaluation of fluid volume in patients with sepsis and refractory hypotension_Clinical Protocol_Rev H
Summary of Protocol Changes	Summary of Protocol Changes Rev A
Original Statistical Analysis Plan (SAP)	FRESH Study SAP_Rev. A
Addendum to SAP	FRESH_SAP Addendum 1 Rev. A
Summary of SAP Changes	Summary of SAP Changes Rev A

EVALUATION OF FLUID VOLUME IN PATIENTS WITH SEPSIS AND REFRACTORY HYPOTENSION

Protocol Number:	PRO-0001
Date/Version:	September 19, 2016 / Version G
Study Product:	Noninvasive Starling™ SV
Sponsor:	Cheetah Medical, Inc.™ 1320 Centre Street Newton, MA 02459

This clinical investigation is performed in accordance with applicable guidelines, standards and regulations. This report template is based on ISO 14155:2011 Clinical Investigation Plan (annex A).

1. CONTENTS

INVESTIGATOR'S STATEMENT AND SIGNATURE

The signing of this protocol by the Principal Investigator signifies that the contents have been laid down in full agreement and that the study will be conducted according to this protocol, its amendments, the clinical trial agreement and the applicable regulatory requirements.

Investigator approval:
I have read this protocol and agree that it contains all the necessary information required to conduct the study, and I agree to conduct it as described. I understand that this study will not be initiated without appropriate Institutional Review Board (IRB) approval and that the administrative requirements of the governing body will be fully complied with.

Principal Investigator's Signature	Date
Principal Investigator's Printed Name	
Site Name	Site #

2. LIST OF ABBREVIATIONS

AE	Adverse Event
AKI	Acute kidney injury
BP	Blood pressure
CPR	Cardiopulmonary resuscitation
CRF	Case report form
CV	Curriculum vitae
eCRF	Electronic case report form
EDC	Electronic data capture
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICC	Intraclass correlation
ICF	Informed consent form
ICU	Intensive Care Unit
IRB	Institutional Review Board
ITT	Intention to treat
IV	Intravenous
L/min	Liter per minute
mITT	Modified intent to treat
PLR	Passive leg raise
PPV	Pulse Pressure Variation
RRT	Renal replacement therapy
SAE	Serious adverse event
SV	Stroke volume
SVI	Stroke volume index
SVV	Stroke volume variation
TFC	Thoracic fluid content
TPRI	Total peripheral resistance index
VET	Ventricular ejection time

3. SYNOPSIS

Title:	Evaluation of fluid volume in patients with sepsis and refractory hypotension
Short Title:	**FRESH** (Fluid Responsiveness Evaluation in Sepsis-associated Hypotension) Fluid Trial
Investigational Device:	Noninvasive Starling™ SV
Objective:	To assess the mean difference in fluid balance and associated patient outcomes, based on a dynamic assessment of fluid responsiveness in septic patients with refractory hypotension in an ICU setting.
Design:	Multi-center randomized study comparing dynamic assessment of fluid responsiveness utilizing Starling SV compared to a control group. Subjects will be randomized in a 2:1 treatment to control group ratio to increase power for sub-analysis by patient population. Patients randomized to the Starling SV arm will have treatment guided by a dynamic assessment of fluid responsiveness (measured by a change in stroke volume index > 10%) as assessed by passive leg raise (PLR). Patients randomized to the control group will receive standard of care treatment. The use of dynamic fluid assessment (i.e. SVV, PPV, or SV change) to determine fluid responsiveness is prohibited.
Primary Endpoint:	Mean difference in fluid balance (L) at 72 hours or ICU discharge, whichever occurs first, between the two treatment groups
Secondary Endpoints	1. Length of ICU stay (days) until subject is medically ready for discharge 2. Number of hours free from ventilator use 3. Number of hours free from vasopressor use 4. Changes in serum creatinine levels from baseline 5. Requirement for renal replacement therapy (RRT) 6. Mean volume of fluid between the two treatment groups 7. Incidence of Adverse Events (AE) 8. Number of ICU readmissions 9. 30 day in-hospital mortality rate 10. Discharge location 11. Mean difference in fluid balance at ICU discharge
Planned Enrollment:	Approximately 210 subjects will be involved in the analysis population

Population:	Patients with sepsis who exhibit refractory hypotension (MAP < 65)following initial fluid resuscitation (1L of fluid)
Follow-up Schedule:	Hemodynamic data collection will occur for a 72 hour period starting at the time of study enrollment. Subject outcome data will be collected until the time of hospital discharge.
Number of Sites:	Between 7-10 clinical sites located in the US
Sub Analysis:	Primary and secondary endpoints will also be evaluated by the following secondary analysis: • Patient disease state • Patients determined to be fluid responsive vs non-fluid responsive • AKI measures in patients determined to increase serum creatinine levels from baseline • Enrollment time frame Additional sub-analysis will be run as appropriate.
Inclusion Criteria:	1. Diagnosis of sepsis, as exhibited by 2 or more of the following SIRS criteria and a known or presumed infection at time of screening: o Temperature of > 38 C or < 36 C o Heart rate of > 90/min o Respiratory rate of > 20/min or PaCO2 < 32 mm Hg (4.3 kPA) o White blood cell count > 12000/mm^3 or < 4000/mm^3 or >10% immature bands 2. Refractory hypotension (MAP <65) despite initial fluid resuscitation (1L of treatment fluid) 3. Patient enrolled in study within 24 hours of arrival to the hospital 4. Anticipated ICU admission 5. Able to provide signed informed consent or consent can be obtained from the patient's authorized representative
Exclusion Criteria:	1. Primary diagnosis of: acute cerebral vascular event, acute coronary syndrome, acute pulmonary edema, status asthmaticus, major cardiac arrhythmia, drug overdose, or injury from burn or trauma 2. Known aortic insufficiency, or aortic abnormalities 3. Hemodynamic instability due to active hemorrhage (e.g. gastrointestinal bleeding / coagulopathy / trauma) 4. Patient has received >3 liters of IV fluid prior to study randomization 5. Requires immediate surgery 6. Patient transferred to the ICU from another hospital unit 7. Do not attempt resuscitation (DNAR or DNR) order 8. Advanced directives restricting implementation of the resuscitation protocol

	9. Contraindication to blood transfusion 10. Attending clinician deems aggressive resuscitation unsuitable 11. Transferred from another in-hospital setting 12. Not able to commence treatment protocol within 1 hour after randomization 13. Known intraventricular heart defect, such as VSD or ASD 14. Use of additional hemodynamic monitoring involving SVV, PPV, or SV change to determine fluid responsiveness 15. Seizure in the last 24 hours 16. Prisoner 17. Pregnancy 18. Age <18 19. Known allergy to sensor material or gel 20. Inability or contraindication to doing a passive leg raise with both extremities, such as inability to interrupt venous compression boots 21. Patient has an epidural catheter in place 22. Suspected intra-abdominal hypertension 23. Inability to obtain IV access 24. Patient should be excluded based on the opinion of the Clinician/Investigator
Statistical Design:	Variables will be tabulated using descriptive statistics. Continuous variables will be presented as number, mean, and standard deviation with 95% confidence intervals, as well as medians and ranges. For categorical variables, relative frequencies and 95% confidence intervals will be provided. Any survival analyses performed will utilize the Kaplan-Meier method. All tests for continuous endpoints utilize a two-sided t-test for difference in two independent means at the overall 0.05 level of significance. The study will be conducted under a common protocol. To evaluate differences among investigational sites in the trial, summary tables by site will be presented and compared. Results from the sites will be pooled only if there are no statistical differences between the sites (95% confidence). If variables are found to differ by investigational site, then the variable and/or study site may be identified for special consideration in subsequent analyses.
Sponsor/ Manufacturer:	Cheetah Medical, Inc 1320 Centre Street Newton, MA 02459

4. INTRODUCTION

4.1 Background

Hemodynamic optimization of critically ill patients is a goal for clinicians in order to afford the patient the best possible outcomes. Being able to precisely and rapidly determine patient fluid responsiveness provides the bedside physician and nursing staff the information needed to make critical decisions in regards to the patient's fluid status and management of additional fluids and medications. Patient management in the ICU is complex and multiple studies and meta-analyses have shown that clinicians struggle to diagnose which patients will benefit from fluid administration (i.e. the administration of intravenous fluids will increase cardiac output).[1] The findings show that up to 50% of patients in the ICU will not benefit from fluid administration.[2] Studies have also shown that excessive fluid administration and fluid retention leads to poorer outcomes.[3-5] Well-timed decision making with accurate information is critical and challenging particularly in the setting of a patient with sepsis.

As fluid management and cardiac output determination are linked to better decision-making and improved outcomes in ICU, the use of a dynamic assessment of fluid responsiveness becomes a key tool for patient management. The Noninvasive Starling SV device, which is FDA cleared and CE marked, is currently in clinical use and can be used to provide a dynamic assessment of fluid responsiveness. This study will show the clinical utility of initiating an assessment of patient fluid responsiveness prior to the administration of treatment fluid.

5. STUDY JUSTIFICATION

This study is designed to highlight the benefits of treatment guided by dynamic measures using the Starling SV within the population of patients diagnosed with sepsis and refractory hypotension. To confirm this, treatment and outcome data will be compared between patients randomized to receive treatment guided by a dynamic assessment of fluid responsiveness, compared to the current standard of care treatment.

6. DEVICE

6.1 Device Description

Cheetah Medical's Noninvasive Starling SV is a portable, non-invasive, cardiac output detector system. The Starling SV system measures the cardiac output by employing electrical bioreactance. Bioreactance is a measure of the electrical characteristics of a volume of tissue and fluid. In the case of cardiac output measurements, the relevant tissue includes the heart and the immediate surrounding volume of the thorax. The relevant fluid is blood.

Cheetah Medical's Starling SV electrode is a double electrode sensor. Within each sensor, one electrode is used to transmit a high frequency sine wave into the body, while the resulting voltage is measured at the adjacent electrode. Four electrodes are placed at specific areas of the thorax, the impedance to the current flow calculated, and the electrical bioreactance waveform constructed. This information is used to determine cardiac output. The Starling SV device also measures and displays associated hemodynamic parameters based on calculations of measurements already incorporated into the Starling SV device. These parameters are: Cardiac Index (CI), Ventricular Ejection Time (VET), Total Peripheral Resistance Index (TPRI), Stroke Volume Index (SVI), Stroke Volume Variation (SVV), Cardiac Power (CP), Cardiac Power Index (CPI) and Thoracic Fluid Content (TFC). Fluid responsiveness is indicated by a change in SV of >10% in response to a fluid challenge.[6]

6.2 Device Accountability

The Starling SV system (monitor and electrode disposables) will be supplied by Cheetah Medical to each investigational site.

6.3 Device Indication for Use

The Starling SV with NIBP and SpO2 functionalities is a portable, hemodynamic monitoring non-invasive Cardiac Output monitoring device that monitors and displays a patient's Cardiac Output (CO) in Ltr/Min with a Non Invasive Blood Pressure (NIBP) function that non-invasively measures and displays blood pressure (diastolic, systolic and mean arterial pressure) and heart rate and with a SpO2 function that non-invasively measures and displays blood oxygen saturation (SpO2). The device displays associated hemodynamic parameters based on measurements or calculations of measurements already incorporated into the Starling SV. These parameters are:

- Cardiac Index (CI),
- Stroke Volume (SV),
- Stroke Volume Index (SVI),
- Stroke Volume Variation (SVV),
- Heart Rate (HR),
- Ventricular Ejection Time (VET),
- Total Peripheral Resistance (TPR),
- Total Peripheral Resistance Index (TPRI),
- Cardiac Power (CP),
- Cardiac Power Index (CPI),
- Blood Oxygenation (SPO2)
- Oxygen Delivery Index (DO2I),
- Electrical impedance of the chest cavity (Z0),
- Thoracic Fluid Content (TFC),
- Thoracic Fluid Content change from preset time period (TFCd) and
- Thoracic Fluid Content from baseline (TFCd0).
- Changes in SV, CO and other hemodynamic parameters which are derived by Bioreactance® as a result of posture

The Starling SV with NIBP and SpO2 functionalities is intended for use within hospitals and other healthcare facilities (e.g., outpatient clinics) that provide patient care).

6.4 Device Procedure(s)/Training

All sites will receive device training on the Cheetah Starling SV system prior to study initiation.

6.5 Fluid Assessment-Passive Leg Raise (PLR)

The passive leg raise (PLR) shall be followed according to the following guidelines:

- Select PLR Test from main menu on Starling SV system
- With patient in semi-recumbent position (30-45°), obtain three minutes of monitoring for average baseline SVI
- With patient legs raised to 45 degree angle, obtain three minutes of monitoring for peak SVI value
- Fluid responsiveness is indicated by a Δ SVI $\geq$ 10%

7. INVESTIGATIONAL DESIGN

7.1 Study Objective

The objective of this study is to assess the mean difference in fluid balance at 72 hours or ICU discharge, whichever occurs first, and associated patient outcomes, based on a dynamic assessment of fluid responsiveness in septic patients with refractory hypotension in an ICU setting.

7.2 Study Design

This study is a prospective, multi-center, randomized trial comparing the mean difference in fluid balance and associated patient outcomes between a treatment guided by a dynamic assessment of fluid responsiveness and a standard of care control group not using dynamic fluid assessment. Between seven and ten sites will be used to enroll 210 subjects with the diagnosis of sepsis and refractory hypotension and in whom the Starling SV system can be attached. Subjects will be randomized in a 2:1 treatment to control group ratio. In the treatment group, a dynamic assessment of fluid responsiveness will be performed at every clinical decision point to treat hypoperfusion or impending hypoperfusion for the first 72 hours of study enrollment. Examples of a clinical decision point include a MAP of < 65, SBP < 90 or blood pressure that is rapidly trending lower, low urine output, or any other clinical indication to administer/alter fluid bolus or pressors. Additionally, an assessment of fluid responsiveness will be performed every 12 hours if no clinical assessments have taken place during this time period. Fluid responsiveness will be assessed using a passive leg raise (PLR) to guide corresponding treatment (**Appendix A**). Continuous data will be collected from the Starling SV system during the entire treatment period. In the control group, no required therapeutic protocol will be used for patient treatment, and is determined per the discretion of the physician and hospital standards. The use of dynamic fluid assessment (i.e. SVV, PPV, or SV change) to determine fluid responsiveness within the control group is prohibited.

7.3 Study Endpoints

7.3.1 Primary Endpoint

The primary endpoint is an evaluation of the difference (L) between the two treatment groups mean fluid balance at 72 hours or ICU discharge, whichever occurs first. The following hypothesis will be tested:

$$Ha: \mu c - \mu_T \neq 0$$

where μt and μc is the average fluid balance in the Starling SV and control groups, respectively.

7.3.2 Secondary Endpoints

Secondary endpoints to be evaluated:

1. Length of ICU stay (days) until subject is medically ready for discharge
2. Number of hours free from ventilator use
3. Number of hours free from vasopressor use
4. Changes in serum creatinine levels from baseline
5. Requirement for renal replacement therapy (RRT)
6. Mean volume of fluid between the two treatment groups
7. Incidence of Adverse Events (AE)
8. Number of ICU readmissions
9. 30 day in-hospital Mortality rate
10. Discharge location
11. Mean difference in fluid balance at ICU discharge

7.4 Study Duration

Estimated Study Start:	Q3 2016
Estimated Enrollment Completion:	Q2 2017
Estimated Final Report:	Q2 2017

8. SUBJECT POPULATION AND SELECTION

8.1 Subject Population

Adult subjects with the diagnosis of sepsis and refractory hypotension will be considered as a candidate for the study. A maximum of 210 subjects enrolled and in the analysis population will be included in the study. Subjects will be considered to be enrolled when they or their designated representative sign the informed consent form (ICF).

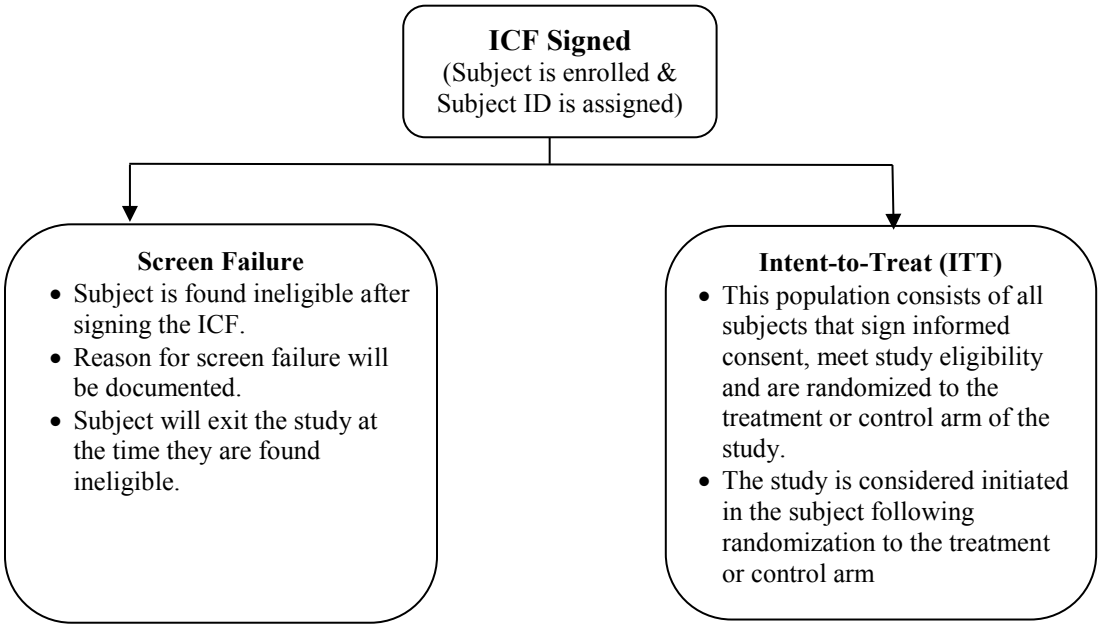
Subjects who are enrolled will be assigned a subject ID. Subjects that sign the ICF, but are found ineligible will be considered screen failures and will be exited from the study at that time.

Enrolled subjects who meet study eligibility will be included in the analysis populations and are further described in **Section 12**.

The diagnosis of sepsis is defined as a known or presumed infection and the presence of 2 or more SIRS criteria as detailed in **Section 8.2**. Refractory hypotension is defined as the presence of hypotension following initial resuscitation with 1L of fluid. After obtaining ICF and confirming that the inclusion and exclusion criteria are met, the Cheetah sensors will be placed and connected to the Starling SV monitor. Useable readings will be confirmed prior to initiating the study data collection period.

A diagram outlining the overall subject enrollment plan is shown below:

Figure 1: Subject Flow Diagram



8.2 Inclusion Criteria

Subjects must meet the following inclusion criteria:

1. Diagnosis of sepsis, as exhibited by 2 or more of the following SIRS criteria and a known or presumed infection at the time of screening:
   - Temperature of > 38 C or < 36 C
   - Heart rate of > 90/min
   - Respiratory rate of > 20/min or PaCO2 < 32 mm Hg (4.3 kPA)

- White blood cell count > 12000/mm^3 or < 4000/mm^3 or >10% immature bands

2. Refractory hypotension (MAP < 65) despite initial fluid resuscitation (1L of treatment fluid)
3. Patient enrolled in study within 24 hours of arrival to the hospital
4. Anticipated ICU admission
5. Able to provide signed informed consent or consent can be obtained from the patient's authorized representative

8.3 Exclusion Criteria

Subjects must not have any of the following exclusion criteria:

1. Primary diagnosis of: acute cerebral vascular event, acute coronary syndrome, acute pulmonary edema, status asthmaticus, major cardiac arrhythmia, drug overdose, or injury from burn or trauma
2. Known aortic insufficiency, or aortic abnormalities
3. Hemodynamic instability due to active hemorrhage, (e.g. gastrointestinal bleeding/coagulopathy/trauma)
4. Patient has received >3 liters of IV fluid prior to study randomization
5. Requires immediate surgery
6. Patient transferred to the ICU from another hospital unit
7. Do not attempt resuscitation (DNAR or DNR) order
8. Advanced directives restricting implementation of the resuscitation protocol
9. Contraindication to blood transfusion
10. Attending clinician deems aggressive resuscitation unsuitable
11. Transferred from another in-hospital setting
12. Not able to commence treatment protocol within 1 hour after randomization
13. Known intraventricular heart defect, such as VSD or ASD
14. Use of additional hemodynamic monitoring involving SVV, PPV or SV changes to determine fluid responsiveness
15. Seizure in the last 24 hours
16. Prisoner
17. Pregnancy
18. Age <18
19. Known allergy to sensor material or gel
20. Inability or contraindication to doing a passive leg raise with both extremities, such as inability to interrupt venous compression boots
21. Patient has an epidural catheter in place
22. Suspected intra-abdominal hypertension
23. Inability to obtain IV access
24. Patient should be excluded based on the opinion of the Clinician/Investigator

8.4 Subject Screening

All subjects diagnosed with sepsis and refractory hypotension will be considered potential candidates. All subjects screened for the study will be captured on the site's Screening Log. The log will capture the subject's gender, age, date of screening and the reason(s) for study exclusion.

8.5 Informed Consent Procedures

Written informed consent on the approved IRB informed consent form must be obtained for all subjects who are potential study candidates before any study specific tests or procedures are performed. Due to the high likelihood of the subject's critical clinical state and therefore the possibility of their inability to provide informed written consent, the subject's designated representative will be acceptable to provide written informed consent.

The general process for obtaining informed consent shall:

- Ensure that the principal investigator or his/her authorized designee conducts the informed consent process;
- Include all aspects of the clinical investigation that are relevant to the subject's decision to participate throughout the clinical investigation;
- Avoid any coercion or undue improper influence on, or inducement of, the subject to participate;
- Not waive or appear to waive the subject's legal rights;
- Use native non-technical language that is understandable to the subject;
- Provide ample time for the subject, or designee, to read and understand the informed consent form and to consider participation in the clinical investigation;
- Include personally dated signatures of the subject and the principal investigator or an authorized designee responsible for conducting the informed consent process;
- Provide the subject, or designee, with a copy of the signed and dated informed consent form and any other written information required per Site's Institutional Policy (i.e. additional HIPAA language).

8.6 Subject Randomization

Subjects will be randomized to either the treatment or control arm of the study once the decision has been made to admit the patient to the ICU.. Any patients that are enrolled but are not admitted to the ICU, or who receive > 3L of treatment fluid prior to randomization will be considered a screen failure and will be exited from the study at this time.

Subjects will be randomized in a 2:1 treatment to control ratio to increase power for sub-analysis by patient population. Additionally, subject randomization will be stratified to ensure even randomization between the three time windows of enrollment (0-6 hours, 6-12 hours, 12-24 hours)

8.7 Subject Discontinuation/Withdrawal Criteria

Once the subject has been enrolled in the study, she/he may withdraw her/his consent to participate in the study at any time without prejudice. Participation in this clinical investigation is entirely voluntary. Likewise, there may be a reason identified by the Investigator that deems the subject no longer suitable for the study. In either case, the Investigator will contact Cheetah

Medical to discuss the circumstances for discontinuation/withdrawal. Discontinuation or withdrawal may occur for any of the reasons listed below (this list is not all inclusive).

1. Subject is uncooperative with compliance of required study tests and/or medical management
2. Investigator determines that subject has developed a condition in which continued participation in the study is considered potentially harmful to the subject
3. Subject withdraws their consent
4. Adverse event which is considered intolerable by the patient, their LAR of the Principal Investigator
5. Subject has a significant protocol violation
6. Subject incorrectly enrolled in the study
7. Cheetah Medical terminates the study

8.7.1 Subjects Lost to Follow-Up

Subjects may be considered lost to follow-up for the following reasons:

- During data analysis, data indicates there is unacceptable bias in the measurements
- Data collection is interrupted prior to 72 hour data collection period of ICU stay
- Severe agitation and/or cardiopulmonary resuscitations resulted in poor quality data

9. STUDY PROCEDURES

9.1 Baseline Assessments

After written informed consent is obtained, baseline assessments will be made to further determine the subject's eligibility for the study. Data collected will include:

- Demographics
- Age
- Medical history
- SOFA assessment
- Physical exam, limited
- Hemoglobin and blood gas (per chart review, if available)
- Serum creatinine level
- Lactate level (per chart review, if available)
- Urine sample (to be frozen for possible future testing)
- Current medications

9.2 Enrollment Procedure

Assuming the subject meets all study eligibility criteria, the investigator, or designees, will place the Cheetah device (Starling SV) on the subject. The device will be evaluated for obtaining the optimum expected hemodynamic readings before the study is begun according to the Starling SV Instructions for Use (IFU). This is done to confirm that data collected from the monitoring devices will be evaluable. It is recommended that approximately 15-20 minutes are allowed for the device to autocalibrate and achieve a stable baseline prior to beginning any measurements at the initiation of the study.

If the Starling SV does not provide the expected hemodynamic recordings, it will be adjusted or replaced as needed until it provides the expected recordings. If it is determined that the device will not provide the expected recording, then the subject will be withdrawn from the study.

9.3 Data Collection Procedure

When the Starling SV is determined to be providing the expected waveforms and recordings, the study device data collection period will begin. Recorded information will be collected and stored by the device. After the data collection period has ended the data will be downloaded and used for analysis.

9.3.1 Hemodynamic Collection Methods

Starling SV: Following the Instructions for Use, the Starling SV device will be connected to the subject by placing four of the system's sensors on the subject's chest: left and right mid-clavicle and left and right mid-last ribs. The device will undergo 1 minute of autocalibration after which measurements will begin to be obtained. Data from the device is available as a visible read-out on the monitor screen and stored internally. At the conclusion of the data collection period, the data will be downloaded and provided to the sponsor or designee.

9.3.2 Fluid Assessment

A fluid assessment will be initiated at the following time points:

- Baseline (at study enrollment)
- At every clinical decision point for the first 72 hours of study enrollment. Examples of a clinical decision point include the decision to give additional fluid volume, or escalate vasopressors.
- MAP <65 during first 72 hours of study enrollment

A fluid assessment will be administered using a passive leg raise according to **Section 6.5**.

9.3.3 Additional Data Collection

Other data points and associated times will be collected in addition to the analysis of the continuous data from the Starling SV system. Types of data points for collection are:

- Medical history
- Medications
- Volume of treatment fluid administered
- Urine output (to be recorded every 12 hours)
- Respiratory status
- Serum Creatinine level (every 24 hours +/- 4 hours , for 72 hour monitoring period and at 30 day discharge)
- Lactate level (per chart review, if available)
- Significant clinical events not previously identified for study data collection, e.g. cardiac arrest, CPR, intubation, extubation, placement of catheters (intravenous, arterial, urinary, etc), etc.

- Changes in mechanical ventilator status parameters

9.4 Schedule of Assessments

Each subject will follow a schedule of assessments and events from which data will be collected (Table 1).

Table 1: Schedule of Assessments

Assessment/Event	Pre-Consent	Consent	Baseline	Enrollment-72 hours	72 hours-Discharge
Subject Identification	X				
Determine Eligibility	X				
Informed Consent		X			
Medical History			X	X	X
Physical Exam, limited, and lab values			X		
Urine Sample			X		
Serum Creatinine			X	X	X (chart review)
Medications, specific			X	X	X
Volume resuscitation fluid			X	X	
Respiratory Status (intubated, ventilated)			X	X	X
Starling SV System			Attached	Continuous hemodynamic recordings	
Fluid Assessment				X	
Adverse events				X (+48 hours)	

10. SAFETY/DEVICE ASSESSMENT

10.1 Adverse Events

Subjects will be carefully monitored during the study for possible device related adverse events (AEs). The investigator or designee will determine AE occurrences and collect the required data. It is anticipated that the occurrence of device related adverse events will be a low number. All device related adverse events will be reviewed not only by the site investigator, but also by the sponsor's medical officer.

Serious Adverse events will be collected during the 72 hour (**+ 48 hours**) treatment period in the following areas: Cardiac, CNS, pulmonary, renal, and skin disorders.

10.1.1 Device Related Adverse Event (AE)

Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory finding) in subjects, users or other persons occurring as a result of the study device

10.1.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that**:**

- Results in death
- Led to serious deterioration in the health of the subject, that either resulted in:
    - a life-threatening illness or injury, or
    - a permanent impairment of a body structure or a body function, or
    - in-patient or prolonged hospitalization, or
    - medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,
- Led to fetal distress, fetal death or a congenital abnormality or birth defect

10.2 Reporting Procedures

10.2.1 General Reporting Requirements

All device related adverse events must be recorded on the Adverse Event eCRF by the investigator (or designee). The report should include: start date of the adverse event, treatment, resolution, and assessment of both the seriousness and the relationship to the investigational device.

The following criteria must also be adhered to by the Investigator:

- Completion of separate device related Adverse Event forms to document each event
- The forms must be signed by the Investigator, and
- Supplying to the Sponsor (or designee), upon Sponsor's request, with any additional information related to the safety reporting of a particular event

10.2.2 Reporting Requirements of SAEs

All serious adverse events must be reported by the investigator by submitting the Adverse Event eCRF within 24 hours of learning of the adverse event.

The Investigator shall:

a) Report to the IRB serious adverse events per the institutional requirements for timing and amount of support information,
b) Supply the Sponsor (or designee), upon Sponsor's request, with any additional information related to the safety reporting of a particular event.

10.2.3 Sponsor Reporting

All adverse events will be reported to the applicable regulatory authority as required per applicable regulatory authority.

Cheetah Medical, or their designee is responsible for the classification and reporting of adverse events and applicable regulatory requirements.

11. STUDY MONITORING

The study will be monitored according to Cheetah Medical's monitoring procedures. A study specific monitoring plan will be developed to ensure protocol compliance and applicable regulatory requirements.

Clinical monitors will be designated by Cheetah Medical. Monitors will verify subject data and ensure compliance with GCP, clinical protocol and other study requirements to be utilized for the study.

11.1 Monitor Training

All Cheetah Medical designated monitors will be trained appropriately to monitor study progress including but not limited to the protocol and CRFs.

11.2 Site/Investigator Training

Cheetah Medical will be responsible for providing training to the investigator and appropriate clinical site personnel.

11.2.1 Protocol Training

Training on study protocol requirements will be provided for the entire study team at the initiation of the study. It is ultimately the responsibility of the Investigator to ensure all clinical site personnel participating in this study are adequately trained.

To ensure uniform data collection and protocol compliance, Cheetah Medical and/or designee will perform study initiation visits to review the clinical protocol, techniques for the identification of eligible subjects, instructions on in-hospital data collection, and methods for soliciting data from alternative sources.

11.2.2 Device Training

This procedure may only be performed by qualified investigators, familiar with the Cheetah Medical system. Training on the Starling SV system will be performed and documented for the site Investigator who is responsible for using the device, by the study Sponsor, Cheetah Medical.

11.3 Site Monitoring

The Sponsor or designee may conduct periodic compliance assessments at various study sites. The Sponsor or designee may request access to all study records including source documentation for inspection and photocopying during a compliance assessment. The Investigator and research coordinator must be available to respond to reasonable requests and queries made during the compliance assessment process.

11.4 Regulatory Agency Inspection

In the event that an investigator is contacted by a regulatory agency regarding this study, the investigator will notify the Sponsor or its designee immediately. The investigator and research coordinator must be available to respond to reasonable requests and queries made during the inspection process. The investigator must provide the Sponsor or designee with copies of all correspondence that may affect review of the current trial. The Sponsor may provide needed assistance in responding to regulatory audits.

12. STATISTICAL CONSIDERATIONS

12.1 Sample Size Estimation

Subjects will be randomly assigned in a 2:1 ratio to treatment with Starling SV or to treatment with standard of care. The primary effectiveness endpoint for this study is the difference between the two treatment groups in mean fluid balance at ICU discharge.

Minimum enrollment (N_{min}) in the study will be set at 120 subjects (80 Starling SV and 40 control) to power at 80% for possible demonstration of superiority for the secondary endpoint of creatinine levels as a measurement of change from baseline. The secondary endpoint for change in creatinine levels at 120 evaluable subjects displays 80% power at a two-sided alpha level of 0.05 to demonstrate superiority under an assumption of a treatment effect of -1.4 mg/dL with a standard deviation of 2.5 mg/dL.

Under an assumption of a treatment effect of -2 L with a standard deviation of 3 L, the sample size of 120 evaluable subjects provides 92.7% power in a test of superiority for the primary effectiveness endpoint.

The trial will incorporate a sample size re-estimation (SSR) at the time of the interim look based on promise for superiority in the key secondary endpoint. Methods for the SSR have been described in detail in previous literature. The maximum enrollment being considered for this trial is 210 total enrolled (N_{max} = 1.75 times N_{min}).

An interim analysis incorporating a SSR will take place when 120 subjects have been enrolled and completed endpoint evaluations. The interim analysis will be performed by an independent statistician. It is assumed that the acute endpoint assessments will be fully informed at the time of the interim analysis.

At this point, the primary study endpoint will be tested as final, and will not incorporate an alpha spend.
The conditional power to claim superiority (CP_{sup}) on the key secondary endpoint for change in creatinine levels will be computed. The conditional power (CP_{sup}) will be calculated based on the interim results of the key secondary endpoint, with the specific interest of demonstrating superiority. CP_{sup} is defined as the approach that quantifies the statistical power to yield an answer different from that seen at the interim analysis. The data will be partitioned into three

zones based on CP_{sup} unfavorable zone ($CP_{sup}<50\%$), promising zone ($50\% \leq CP_{sup}<80\%$), and favorable zone ($CP_{sup} \geq 80\%$).

12.2 Analysis Populations

12.2.1 All Enrolled

Any subject who has signed informed consent will be included in the all enrolled population. Should a subject be considered a screen failure, the reason for failure will be documented, and the subject will be exited from the study. Adverse events will be reported for all enrolled subjects through the time of study exit.

12.2.2 Intent-to-Treat (ITT)

The intent-to-treat population (ITT) will consist of any subjects that have signed the ICF, meet study eligibility criteria, and are randomized to the treatment or control arm of the study.

12.2.3 Modified Intent-to-Treat (mITT)

Subjects will be included in the Modified Intent-to-Treat group if they sign the ICF, meet study eligibility criteria, are randomized, and in the treatment group receive monitoring for the first 72 hours of study enrollment or until ICU discharge, whichever occurs first. The mITT population represents the Primary and Secondary Analysis population for this study.

12.2.4 Per-Protocol (PP)

Subjects will be included in the Per-protocol group if they sign the ICF, are randomized and have the assigned procedure completed, meet critical study eligibility criteria and have no major protocol deviations (defined in the Statistical Analysis Plan), and, in the treatment group, receive monitoring for the first 72 hours of study enrollment. The PP population represents a subgroup analysis population for this study.

12.3 Primary Endpoint Analysis

The primary endpoint is an evaluation of the difference between the two treatment groups in mean fluid balance (L) at 72 hours or ICU discharge, whichever occurs first. The following hypothesis will be tested:

$$H_{o:}\ \mu c - \mu_T = 0$$
$$Ha:\ \mu c - \mu_T \neq 0$$

where μt and μc is the average fluid balance in the Starling SV and control groups, respectively. Statistical summaries will include means, medians, and standard deviation, as well as 95% confidence intervals. A t-test for superiority at a significance level of 0.025 will be performed to test the hypothesis that μt is significantly less than μc. If it can be established that the difference is greater than 0 points (i.e. $\mu C > \mu T$), superiority will also be claimed.

12.4 Secondary and Exploratory Endpoints

Secondary objectives that have been pre-specified to be included in the formal statistical analysis will be tested in sequential fashion using the Holms step-down procedure for type-I error rate correction if and only if the primary objective passes according to the criteria outlined above. At the conclusion of the trial, the key secondary endpoints will be ordered sequentially from most significant to least significant. The endpoints will then be tested in order at adjusted levels of significance (i.e. E1: 0.05/k, E2: 0.05/k-1; E3: 0.05/k-2, where k=number of specified endpoints). Once an endpoint fails, all following endpoints will not be tested. Following is a list of key secondary endpoints:

Following is a list of key secondary endpoints for formal testing:

- Length of ICU stay (days) until subject is medically ready for discharge
- Number of days free from ventilator use (30 day period)
- Number of days free from vasopressor use
- Changes in serum creatinine levels from baseline
- Requirement for renal replacement therapy (RRT)

Additional secondary endpoint have also been identified for testing, but will not be included in the formal statistical testing procedures for key secondary endpoints:

- Incidence of Adverse Events (AE)
- Number of ICU readmissions
- Mortality rate
- Mean volume of treatment fluid
- Discharge location
- Mean difference in fluid balance at ICU discharge

Additional sub-analyses have been planned for the primary and key secondary endpoints. Primary and secondary endpoints will also be evaluated by the following subgroup analysis:

- Patient disease state
- Patients determined to be fluid responsive vs non-fluid responsive
- AKI measures in patients determined to increase serum creatinine levels from baseline vs decrease serum creatinine levels from baseline
- Enrollment time frame

12.5 Baseline Data Summary

Baseline data including subject demographics, clinical history, risk factors, and pre-procedure patient characteristics will be summarized using descriptive statistics (e.g., mean, median, standard deviation, 25th percentile, 75th percentile, minimum and maximum) for continuous variables and frequency tables or percentages for categorical variables.

12.6 Subject Accountability and Missing Data

Subjects enrolled in this study are followed for a short time period, and missing data in this study is expected to occur at a low rate. All efforts will be used to prevent missing data. These efforts include, but are not limited to, complete site training and regular monitoring to help to minimize missing data.

Subjects without the minimum amount of monitoring [i.e. throughout first 72 hours of study enrollment,] and measurements specified in the protocol will be considered as missing in the final analysis. The impact of missing data on the study will be evaluated via sensitivity analyses if more than 5% of subjects do not have evaluable data for the primary outcome. In the final report, the number and proportion of subjects eligible for and compliant with each analysis population will be presented.

13. DATA HANDLING AND RECORD KEEPING

Each participating site will maintain appropriate medical and research records for this trial, in compliance with regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in a manufacturer-sponsored study, each site will permit authorized representatives of the Sponsor(s), the Sponsor's designee, and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

Source data are all information, original records of clinical findings, observations, or other activities in a study necessary for the reconstruction and evaluation of the trial.

13.1 Clinical Data Collection

Electronic Case Report Forms (eCRFs) will be used to collect all subject data during the course of the study and will be entered into an electronic database. eCRFs must be fully completed for each subject and signed by the Investigator when complete.

Good Clinical Practice Guidelines require that Investigators maintain information in the study subject's medical records that corroborate data collected on the eCRFs. In order to comply with these regulatory requirements, the following information should be maintained:

- Medical history/physical condition of the study subject before involvement in the study sufficient to verify protocol entry criteria.
- Dated and signed notes on the day of entry into the study including the study investigator, study name, subject number assigned and a statement that consent was obtained.
- Dated and signed notes from each study subject visit with reference to the eCRFs for further information, if appropriate (for specific results of procedures and exams).
- Information related to adverse events.
- Study subject's condition upon completion of or withdrawal from the study.
- Discharge summaries/procedure reports.

13.2 Data Reporting

All site Investigators or designated individuals shall be responsible for recording all study data on the eCRFs. The Investigator is required to electronically sign the eCRFs to verify that he/she has reviewed and agrees with the recorded data. All protocol deviations shall be documented and a justification for any missed assessments shall be provided on the Protocol Deviation eCRF.

Completed eCRFs will be verified by the monitor at the investigational sites or by remote monitoring as at regular intervals throughout the study, as outlined in the Monitoring Plan. The Investigator will allow the monitor and/or representative of the Sponsor, and any regulatory body to review and inspect the study files, subject eCRFs, subject medical records and other related study documents as required.

13.3 Data Review

eCRFs will be reviewed for completeness and clarity. Missing or unclear data will be investigated by the monitor and will be retrieved, clarified and entered by study personnel as necessary throughout the study. Cheetah Medical, or designee, may request additional documentation from the Investigator such as physician notes or physician written summaries when adverse events are observed and reported. Documentation provided will also be used for the adjudication of specified adverse events by the Independent Safety Physician.

Development of the data collection system for the study will be performed by Cheetah Medical, or designee. Cheetah Medical, or designee will also be responsible for the quality control of the database and confirming the overall integrity of the data.

13.4 Investigator Records

Investigators will maintain complete, accurate and current study records. The following records must be maintained in designated study files:

- Clinical protocol and all amendments
- Signed Clinical Trial Research Agreement
- Institutional Review Board (IRB) Approval Letter(s)
- IRB approved informed consent(s) (including any revisions)
- CV for all Investigators, signed and dated
- Investigator(s) medical license
- Financial Disclosure Form for all Investigators
- Correspondence relating to this study
- Correspondence with the IRB
- IRB membership list and/or assurance number
- Delegation of Authority log
- Device Instructions for Use
- Printed copy of blank set of CRFs
- Subject Log
- Site Visit Log (e.g. for Monitor sign-in)
- Site Training records

- Investigational Device Accountability Logs
- Reports (includes Adverse Event reports and final reports from Investigator and Sponsor)
- Copy of all IRB approved subject-related materials and/or study advertising materials

The following records must be maintained for each subject enrolled in the study:

- Signed subject consent forms
- Copy of final completed CRFs
- Record of any adverse events with supporting documentation
- Reports, progress notes, physician and/or nursing notes, and subject office files
- Records pertaining to subject deaths throughout the course of the study

13.5 Data Retention

Study documents should be retained for a minimum of 5 years. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the Sponsor, if applicable. It is the responsibility of the Sponsor to inform the Investigator when these documents no longer need to be retained.

Table 2: Responsibilities for Preparing and Submitting Reports

Type of Report	Prepared by Investigator for	Time of Notification
Enrollment Notification Form	Sponsor	Within 24 hours of procedure
Electronic Case Report Forms	Sponsor	Within 10 working days
Serious/ Device Related Adverse Event	Sponsor and IRB (as required)	Within 24 hours of knowledge or as required by IRB
Subject Death	Sponsor and IRB (as required)	Within 24 hours of knowledge
Subject Withdrawal	Sponsor	Within 24 hours of knowledge
Withdrawal of IRB Approval	Sponsor	Within 24 hours of knowledge
Protocol Deviations	Sponsor and IRB	Within 5 working days of occurrence or knowledge
Informed Consent Not Obtained	Sponsor and IRB	Within 24 hours of knowledge or as required by IRB
Final summary report	Sponsor and IRB and Regulatory Authority (as required)	Within 3 months of study completion

13.6 Investigator Reports

Each year an annual summary report shall be prepared by the Investigator which provides a summary of the number of subjects treated to date as well as other pertinent clinical information associated with the investigational procedure. The annual report is required to be provided to the IRB and the Sponsor or designee.

Upon completion and/or termination of the study a final report shall be prepared. This report will contain a critical evaluation of all data collected during the course of the investigation at each institution. The Sponsor or its designee is responsible for preparing this compilation to Investigators for submittal as a final report to their reviewing IRB.

14. QUALITY CONTROL AND ASSURANCE

14.1 Site and Investigator Selection

The Sponsor selects qualified investigators with appropriate experience with this patient population at health care facilities with adequate resources to participate in this study. Investigational sites will be selected using combined current assessments of site and investigator qualifications.

14.2 Protocol Deviations

An investigator is not allowed to deviate from the Protocol if the deviation affects subject's rights, safety and wellbeing, or the scientific integrity of the clinical investigation. Under emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the Sponsor and the IRB.

A protocol deviation is a failure to comply with the requirements specified within this clinical study protocol. Examples of protocol deviations may include enrollment of a study subject who does not meet all of the inclusion/exclusion criteria specified in the protocol and missed study visits without documentation. Each investigator shall conduct this clinical study in accordance with this clinical study protocol, regulatory body regulations, Good Clinical Practices.

All deviations are reviewed and assessed for their impact on subject safety by the Sponsor or designee. The PI and study staff is responsible for knowing and adhering to their IRB reporting requirements.

The protocol deviations for this protocol consist of, but not limited to the following:

- Failure to obtain subject's informed consent prior to any study-related activities;
- Subject did not meet the inclusion and/or exclusion criteria and were enrolled;
- Failure to conduct protocol required clinical test or assessment;
- Failure to complete protocol required assessments within the required time frame
- Failure to report serious adverse events according to protocol requirements.

In the event of any deviation from the protocol, the Investigator will be notified of the site's non-compliance. Corrective actions will be required, if necessary. Continued protocol deviations despite re-education of the study site personnel or persistent protocol deviation may result in termination of the site's study participation. Subjects enrolled at these sites will continue to be followed per the clinical protocol.

14.2.1 Protocol Deviation Process

Investigators must report protocol deviations to the Sponsor within 5 working days of investigational site knowledge of the deviation by entering data into the eCRF. Any protocol deviations that affect the rights, safety or well-being of the subject or the scientific integrity of the clinical investigation, including those which occur under emergency circumstances must be reported within 24 hours to the Sponsor and IRB, if required by the IRB, or national regulations.

14.3 Corrective/Preventive Action

Deviations from clinical protocol requirements will be reviewed and evaluated on an ongoing basis and, as necessary, appropriate corrective actions will be put into place to bring a site into compliance.

14.4 Study Audit(s)

Audits may be performed as deemed necessary by Cheetah Medical, in a manner consistent with applicable procedure.

14.5 Study Registration

A description of this trial will be available on www.clinicaltrials.gov, the U.S. approved clinical trial registry site.

15. ETHICS/PROTECTION OF HUMAN SUBJECTS

15.1 Statements of Compliance

This study will be performed in accordance with Good Clinical Practice Guidelines, the Code of Federal Regulations Title 21 CFR Parts 50, 54, and 56.

The clinical investigation shall not begin until the required approval/favorable opinion from the Institutional Review Board (IRB) has been obtained including approval of the Informed Consent Form to be used with subjects.

Any additional requirements imposed by the IRB shall be followed.

15.2 Institutional Review Board

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents by an appropriate Institutional Review Board (IRB) prior to subject enrollment. Any amendments to the protocol or changes to the informed consent document must also be approved before they are placed into use. The Investigator should notify the IRB of deviations from the protocol and SAEs occurring at the site in accordance with local procedures. The Investigator is responsible for continued study related communication with the IRB, including submission of study report and SAE notifications as per local regulatory requirements.

15.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects (and their families, as applicable). Consent forms describing in detail the study interventions/ products, study procedures, and risks are given to the subject and written documentation of informed consent is required prior to starting any study-specific tests or intervention /administering of study product.

Consent forms will be approved by the IRB and the subject will be asked to read and review the document. Upon reviewing the document, the site investigator or designated study personnel will explain the research study to the subject and answer any questions that may arise. The subject (or their authorized legal representative) will sign the informed consent document prior to any procedures being done specifically for the study. The subject should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate, unless study timeframes do not allow for such discussions. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Modifications to the study informed consent must have approval from the Sponsor and the EC as required.

15.4 Subject Confidentiality

Confidentiality of data shall be observed by all parties involved at all times throughout the clinical investigation. All data shall be secured against unauthorized access. Sites will maintain subject privacy in accordance with local and national regulations and institutional requirements including all applicable provisions of the Health Insurance Portability and Accountability Act (HIPAA) and its current regulations.

Subjects must be identified only by their assigned study number and initials on all CRFs and other records and documents submitted to the Sponsor, the monitor, and other authorized parties. The Investigator should maintain a Subject Identification List with complete identification information (name, address, contact number, informed consent version number) on each subject. Documents not required to be submitted to the Sponsor such as subject written informed consent form, should be maintained by the Investigator in strict confidence.

The privacy of each subject and confidentiality of his/her information shall be preserved in reports and when publishing any data. The principal investigator or institution shall provide direct access to source data during and after the clinical investigation for monitoring, audits, IRB review and regulatory authority inspections. As required, the principal investigator or institution shall obtain permission for direct access to source documents from the subject, hospital administration and national regulatory authorities before starting the clinical investigation.

16. PROTOCOL AMENDMENTS

The clinical protocol, eCRFs, ICF and other subject information, or other clinical investigation documents shall be amended as needed throughout the clinical investigation, and a justification statement shall be included with each amended section of a document. Proposed amendments to the protocol shall be agreed upon between the Sponsor and Principal Investigator, or the Coordinating Investigator. The amendments to the protocol and the subject's informed consent form shall be notified to, or approved by, the IRB as required. For non-substantial changes [e.g. minor logistical or administrative changes, change of monitor(s), telephone numbers, renewal of insurance] not affecting the rights, safety and well-being of human subjects or not related to the clinical investigation objectives or endpoints, a simple notification to the IRB can be sufficient. The version number and date of amendments shall be documented.

17. TERMINATION OF STUDY OR STUDY SITE PARTICIPATION

The Sponsor may terminate the study at any time. If the study is terminated prior to the completion of expected enrollment for any reason, all participating centers will be notified within five working days. All subjects already enrolled will continue to be followed for the planned course of study described in this protocol. The study shall be terminated following the last enrolled subject.

The Sponsor reserves the right to terminate study site participation and remove appropriate study materials at any time. Specific instances that may precipitate such termination include but are not limited to the following:

- Failure to meet minimum subject enrollment requirements
- Failure to comply with protocol specified procedures and documentation
- Failure to comply with regulatory regulations
- Failure to comply with Good Clinical Practice

The site Investigator may also discontinue study participation with suitable written notice to the Sponsor.

18. PUBLICATION POLICY

Specifics of the publication policy will be outlined in the Clinical Trial Research Agreement.

19. BIBLIOGRAPHY

1. Marik P and Cavallazzi R. Does central venous pressure predict fluid responsiveness? An updated meta-analysis and a plea for some common sense. Crit Care Med 2013; 41: 1774-1778.

2. Michard F and Teboul JL. Predicting fluid responsiveness in ICU patients: a critical analysis of the evidence. Chest. 2002; 121:2000-2008.

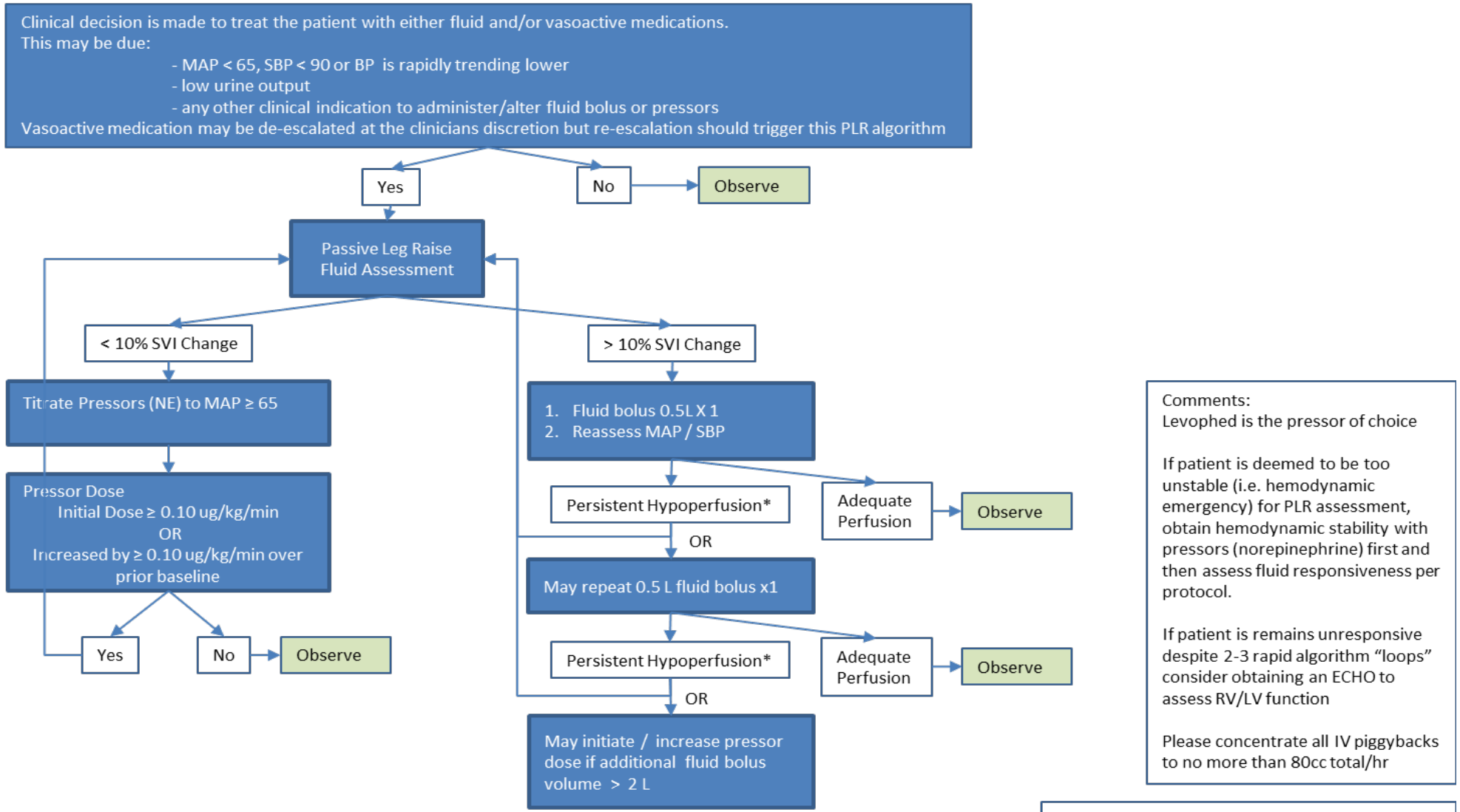
3. Kelm DJ, et al. Fluid overload in patients with severe sepsis and septic shock treated with early goal-directed therapy is associated with increased acute need for fluid-related medical interventions and hospital death. Shock 2015; 43:68-73.

4. Boyd et al. Vasopressin in Septic Shock Trial (VASST). Critical Care Medicine 2011; 39:259-265.

5. Vincent et al. Sepsis in European ICU: Results of the SOAP study. British Journal of Anesthesia 2006; 113: 740-747.

6. Monnet X, Teboul JL. Passive leg raising. Intensive Care Med 2008, 34: 659-63.

APPENDIX A- TREATMENT ALGORITHM



Comments:
Levophed is the pressor of choice

If patient is deemed to be too unstable (i.e. hemodynamic emergency) for PLR assessment, obtain hemodynamic stability with pressors (norepinephrine) first and then assess fluid responsiveness per protocol.

If patient is remains unresponsive despite 2-3 rapid algorithm "loops" consider obtaining an ECHO to assess RV/LV function

Please concentrate all IV piggybacks to no more than 80cc total/hr

*Persistent hypoperfusion may be measured by:
MAP<65
Persistent hyperlactemia
Cryptic shock 2 < lactate < 4

Appendix A
Clinical Protocol Rev G

EVALUATION OF FLUID VOLUME IN PATIENTS WITH SEPSIS AND REFRACTORY HYPOTENSION

Protocol Number:	PRO-0001
Date/Version:	October 30, 2017 / Version H
Study Product:	Noninvasive Starling™ SV
Sponsor:	Cheetah Medical, Inc.™ 1320 Centre Street Newton, MA 02459

This clinical investigation is performed in accordance with applicable guidelines, standards and regulations. This report template is based on ISO 14155:2011 Clinical Investigation Plan (annex A).

1. CONTENTS

INVESTIGATOR'S STATEMENT AND SIGNATURE

The signing of this protocol by the Principal Investigator signifies that the contents have been laid down in full agreement and that the study will be conducted according to this protocol, its amendments, the clinical trial agreement and the applicable regulatory requirements.

Investigator approval:
I have read this protocol and agree that it contains all the necessary information required to conduct the study, and I agree to conduct it as described. I understand that this study will not be initiated without appropriate Institutional Review Board (IRB) approval and that the administrative requirements of the governing body will be fully complied with.

Principal Investigator's Signature	Date
Principal Investigator's Printed Name	
Site Name	Site #

2. LIST OF ABBREVIATIONS

AE	Adverse Event
AKI	Acute kidney injury
BP	Blood pressure
CPR	Cardiopulmonary resuscitation
CRF	Case report form
CV	Curriculum vitae
eCRF	Electronic case report form
EDC	Electronic data capture
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICC	Intraclass correlation
ICF	Informed consent form
ICU	Intensive Care Unit
IRB	Institutional Review Board
ITT	Intention to treat
IV	Intravenous
L/min	Liter per minute
mITT	Modified intent to treat
PLR	Passive leg raise
PPV	Pulse Pressure Variation
RRT	Renal replacement therapy
SAE	Serious adverse event
SV	Stroke volume
SVI	Stroke volume index
SVV	Stroke volume variation
TFC	Thoracic fluid content
TPRI	Total peripheral resistance index
VET	Ventricular ejection time

3. SYNOPSIS

Title:	Evaluation of fluid volume in patients with sepsis and refractory hypotension
Short Title:	**FRESH** (Fluid Responsiveness Evaluation in Sepsis-associated Hypotension) Fluid Trial
Investigational Device:	Noninvasive Starling™ SV
Objective:	To assess the mean difference in fluid balance and associated patient outcomes, based on a dynamic assessment of fluid responsiveness in septic patients with refractory hypotension in an ICU setting.
Design:	Multi-center randomized study comparing dynamic assessment of fluid responsiveness utilizing Starling SV compared to a control group. Subjects will be randomized in a 2:1 treatment to control group ratio to increase power for sub-analysis by patient population. Patients randomized to the Starling SV arm will have treatment guided by a dynamic assessment of fluid responsiveness (measured by a change in stroke volume index > +10%) as assessed by passive leg raise (PLR). Patients randomized to the control group will receive standard of care treatment. The use of dynamic fluid assessment (i.e. SVV, PPV, or SV change) to determine fluid responsiveness is prohibited.
Primary Endpoint:	Mean difference in fluid balance (L) at 72 hours or ICU discharge, whichever occurs first, between the two treatment groups
Secondary Endpoints	1. Length of ICU stay (days) until subject is medically ready for discharge 2. Number of hours free from ventilator use 3. Requirement for mechanical ventilation 4. Number of hours free from vasopressor use 5. Changes in serum creatinine levels from baseline 6. Requirement for renal replacement therapy (RRT) 7. Mean volume of fluid between the two treatment groups 8. Incidence of Major Adverse Cardiac Event (MACE) 9. Incidence of Adverse Events (AE) 10. Number of ICU readmissions 11. 30 day in-hospital mortality rate 12. Discharge location 13. Mean difference in fluid balance at ICU discharge

Planned Enrollment:	Approximately 210 subjects will be involved in the analysis population
Population:	Patients with sepsis who exhibit refractory hypotension (MAP < 65, or require treatment with vasopressors to maintain a MAP > 65) following initial fluid resuscitation (1L of fluid).
Follow-up Schedule:	Hemodynamic data collection will occur for a 72 hour period starting at the time of study enrollment. Subject outcome data will be collected until the time of hospital discharge.
Number of Sites:	Between 7-15 clinical sites located in the US and 1-3 sites located in the UK
Sub Analysis:	Primary and secondary endpoints will also be evaluated by the following secondary analysis: • Patient disease state • Patients determined to be fluid responsive vs non-fluid responsive • AKI measures in patients determined to increase serum creatinine levels from baseline • Enrollment time frame Additional sub-analysis will be run as appropriate.
Inclusion Criteria:	1. Diagnosis of sepsis, as exhibited by 2 or more of the following SIRS criteria and a known or presumed infection at time of screening: o Temperature of > 38 C or < 36 C o Heart rate of > 90/min o Respiratory rate of > 20/min or PaCO2 < 32 mm Hg (4.3 kPA) o White blood cell count > 12000/mm^3 or < 4000/mm^3 or >10% immature bands 2. Refractory hypotension (either one single reading of MAP <65 exhibited during the evaluation period, or requiring treatment with vasopressors to maintain a MAP > 65) despite initial fluid resuscitation (1L of treatment fluid) 3. Patient enrolled in study within 24 hours of arrival to the hospital 4. Anticipated ICU admission. Patients may be maintained on another unit (such as within the ER or a step down unit) during the 72 hour monitoring period if the treatment algorithm is adhered to during this time period. 5. Able to provide signed informed consent or consent can be obtained from the patient's authorized representative
Exclusion Criteria:	1. Primary diagnosis of: acute cerebral vascular event, acute coronary syndrome, acute pulmonary edema, status asthmaticus, major cardiac arrhythmia, drug overdose, or injury from burn or trauma 2. Known aortic insufficiency, or aortic abnormalities

	3. Hemodynamic instability due to active hemorrhage (e.g. gastrointestinal bleeding / coagulopathy / trauma) 4. Patient has received >3 liters of IV fluid prior to study randomization 5. Requires immediate surgery 6. Patient transferred to the ICU from another hospital unit 7. Do not attempt resuscitation (DNAR or DNR) order 8. Advanced directives restricting implementation of the resuscitation protocol 9. Contraindication to blood transfusion 10. Attending clinician deems aggressive resuscitation unsuitable 11. Transferred from another in-hospital setting 12. Not able to commence treatment protocol within 1 hour after randomization 13. Known intraventricular heart defect, such as VSD or ASD 14. Use of additional hemodynamic monitoring involving SVV, PPV, or SV change to determine fluid responsiveness 15. Seizure in the last 24 hours 16. Prisoner 17. Pregnancy 18. Age <18 19. Known allergy to sensor material or gel 20. Inability or contraindication to doing a passive leg raise with both extremities, such as inability to interrupt venous compression boots 21. Patient has an epidural catheter in place 22. Suspected intra-abdominal hypertension 23. Inability to obtain IV access 24. Diabetic ketoacidosis 25. Hyper-osmolarity syndrome 26. Patient treatment uncouples from the treatment algorithm 27. Patient should be excluded based on the opinion of the Clinician/Investigator
Statistical Design:	Variables will be tabulated using descriptive statistics. Continuous variables will be presented as number, mean, and standard deviation with 95% confidence intervals, as well as medians and ranges. For categorical variables, relative frequencies and 95% confidence intervals will be provided. Any survival analyses performed will utilize the Kaplan-Meier method. All tests for continuous endpoints utilize a two-sided t-test for difference in two independent means at the overall 0.05 level of significance. The study will be conducted under a common protocol. To evaluate differences among investigational sites in the trial, summary tables by site will be presented and compared.

	Results from the sites will be pooled only if there are no statistical differences between the sites (95% confidence). If variables are found to differ by investigational site, then the variable and/or study site may be identified for special consideration in subsequent analyses.
Sponsor/ Manufacturer:	Cheetah Medical, Inc 1320 Centre Street Newton, MA 02459

4. INTRODUCTION

4.1 Background

Hemodynamic optimization of critically ill patients is a goal for clinicians in order to afford the patient the best possible outcomes. Being able to precisely and rapidly determine patient fluid responsiveness provides the bedside physician and nursing staff the information needed to make critical decisions in regards to the patient's fluid status and management of additional fluids and medications. Patient management in the ICU is complex and multiple studies and meta-analyses have shown that clinicians struggle to diagnose which patients will benefit from fluid administration (i.e. the administration of intravenous fluids will increase cardiac output).[1] The findings show that up to 50% of patients in the ICU will not benefit from fluid administration.[2] Studies have also shown that excessive fluid administration and fluid retention leads to poorer outcomes.[3-5] Well-timed decision making with accurate information is critical and challenging particularly in the setting of a patient with sepsis.

As fluid management and cardiac output determination are linked to better decision-making and improved outcomes in ICU, the use of a dynamic assessment of fluid responsiveness becomes a key tool for patient management. The Noninvasive Starling SV device, which is FDA cleared and CE marked, is currently in clinical use and can be used to provide a dynamic assessment of fluid responsiveness. This study will show the clinical utility of initiating an assessment of patient fluid responsiveness prior to the administration of treatment fluid.

5. STUDY JUSTIFICATION

This study is designed to highlight the benefits of treatment guided by dynamic measures using the Starling SV within the population of patients diagnosed with sepsis and refractory hypotension. To confirm this, treatment and outcome data will be compared between patients randomized to receive treatment guided by a dynamic assessment of fluid responsiveness, compared to the current standard of care treatment.

6. DEVICE

6.1 Device Description

Cheetah Medical's Noninvasive Starling SV is a portable, non-invasive, cardiac output detector system. The Starling SV system measures the cardiac output by employing electrical bioreactance. Bioreactance is a measure of the electrical characteristics of a volume of tissue and fluid. In the case of cardiac output measurements, the relevant tissue includes the heart and the immediate surrounding volume of the thorax. The relevant fluid is blood.

Cheetah Medical's Starling SV electrode is a double electrode sensor. Within each sensor, one electrode is used to transmit a high frequency sine wave into the body, while the resulting voltage is measured at the adjacent electrode. Four electrodes are placed at specific areas of the thorax, the impedance to the current flow calculated, and the electrical bioreactance waveform constructed. This information is used to determine cardiac output. The Starling SV device also measures and displays associated hemodynamic parameters based on calculations of measurements already incorporated into the Starling SV device. These parameters are: Cardiac Index (CI), Ventricular Ejection Time (VET), Total Peripheral Resistance Index (TPRI), Stroke Volume Index (SVI), Stroke Volume Variation (SVV), Cardiac Power (CP), Cardiac Power Index (CPI) and Thoracic Fluid Content (TFC). Fluid responsiveness is indicated by a change in SV of > +10% in response to a fluid challenge.[6]

6.2 Device Accountability

The Starling SV system (monitor and electrode disposables) will be supplied by Cheetah Medical to each investigational site.

6.3 Device Indication for Use

The Starling SV with NIBP and SpO2 functionalities is a portable, hemodynamic monitoring non-invasive Cardiac Output monitoring device that monitors and displays a patient's Cardiac Output (CO) in Ltr/Min with a Non Invasive Blood Pressure (NIBP) function that non-invasively measures and displays blood pressure (diastolic, systolic and mean arterial pressure) and heart rate and with a SpO2 function that non-invasively measures and displays blood oxygen saturation (SpO2). The device displays associated hemodynamic parameters based on measurements or calculations of measurements already incorporated into the Starling SV. These parameters are:

- Cardiac Index (CI),
- Stroke Volume (SV),
- Stroke Volume Index (SVI),
- Stroke Volume Variation (SVV),
- Heart Rate (HR),
- Ventricular Ejection Time (VET),
- Total Peripheral Resistance (TPR),
- Total Peripheral Resistance Index (TPRI),
- Cardiac Power (CP),
- Cardiac Power Index (CPI),
- Blood Oxygenation (SPO2)
- Oxygen Delivery Index (DO2I),

- Electrical impedance of the chest cavity (Z0),
- Thoracic Fluid Content (TFC),
- Thoracic Fluid Content change from preset time period (TFCd) and
- Thoracic Fluid Content from baseline (TFCd0).
- Changes in SV, CO and other hemodynamic parameters which are derived by Bioreactance® as a result of posture

The Starling SV with NIBP and SpO2 functionalities is intended for use within hospitals and other healthcare facilities (e.g., outpatient clinics) that provide patient care).

6.4 Device Procedure(s)/Training

All sites will receive device training on the Cheetah Starling SV system prior to study initiation.

6.5 Fluid Assessment-Passive Leg Raise (PLR)

The passive leg raise (PLR) shall be followed according to the following guidelines:

- Select PLR Test from main menu on Starling SV system
- With patient in semi-recumbent position (30-45°), obtain three minutes of monitoring for average baseline SVI
- With patient legs raised to 45 degree angle, obtain three minutes of monitoring for peak SVI value
- Fluid responsiveness is indicated by a Δ SVI $\geq$ +10%

7. INVESTIGATIONAL DESIGN

7.1 Study Objective

The objective of this study is to assess the mean difference in fluid balance at 72 hours or ICU discharge, whichever occurs first, and associated patient outcomes, based on a dynamic assessment of fluid responsiveness in septic patients with refractory hypotension in an ICU setting.

7.2 Study Design

This study is a prospective, multi-center, randomized trial comparing the mean difference in fluid balance and associated patient outcomes between a treatment guided by a dynamic assessment of fluid responsiveness and a standard of care control group not using dynamic fluid assessment. Between seven and fifteen sites will be used to enroll 210 subjects with the diagnosis of sepsis and refractory hypotension and in whom the Starling SV system can be attached. Subjects will be randomized in a 2:1 treatment to control group ratio. In the treatment group, a dynamic assessment of fluid responsiveness will be performed at every clinical decision point to treat hypoperfusion or impending hypoperfusion for the first 72 hours of study enrollment. Examples of a clinical decision point include a MAP of < 65, SBP < 90 or blood pressure that is rapidly trending lower, low urine output, or any other clinical indication to administer/alter fluid bolus or pressors. Additionally, an assessment of fluid responsiveness will be performed every 12 hours if no clinical assessments have taken place during this time period.

Fluid responsiveness will be assessed using a passive leg raise (PLR) to guide corresponding treatment (**Appendix A**). Continuous data will be collected from the Starling SV system during the entire treatment period. In the control group, no required therapeutic protocol will be used for patient treatment, and is determined per the discretion of the physician and hospital standards. The use of dynamic fluid assessment (i.e. SVV, PPV, or SV change) to determine fluid responsiveness within the control group is prohibited.

7.3 Study Endpoints

7.3.1 Primary Endpoint

The primary endpoint is an evaluation of the difference (L) between the two treatment groups mean fluid balance at 72 hours or ICU discharge, whichever occurs first. The following hypothesis will be tested:

$$Ha: \mu c - \mu_T \neq 0$$

where μt and μc is the average fluid balance in the Starling SV and control groups, respectively.

7.3.2 Secondary Endpoints

Secondary endpoints to be evaluated:

1. Length of ICU stay (days) until subject is medically ready for discharge
2. Number of hours free from ventilator use
3. Requirement for mechanical ventilation
4.
5. Number of hours free from vasopressor use
6. Changes in serum creatinine levels from baseline
7. Requirement for renal replacement therapy (RRT)
8. Mean volume of fluid between the two treatment groups
9. Incidence of Major Adverse Cardiac Event (MACE)
10. Incidence of Adverse Events (AE)
11. Number of ICU readmissions
12. 30 day in-hospital Mortality rate
13. Discharge location
14. Mean difference in fluid balance at ICU discharge

7.4 Study Duration

Estimated Study Start:	Q3 2016
Estimated Enrollment Completion:	Q2 2018
Estimated Final Report:	Q3 2018

8. SUBJECT POPULATION AND SELECTION

8.1 Subject Population

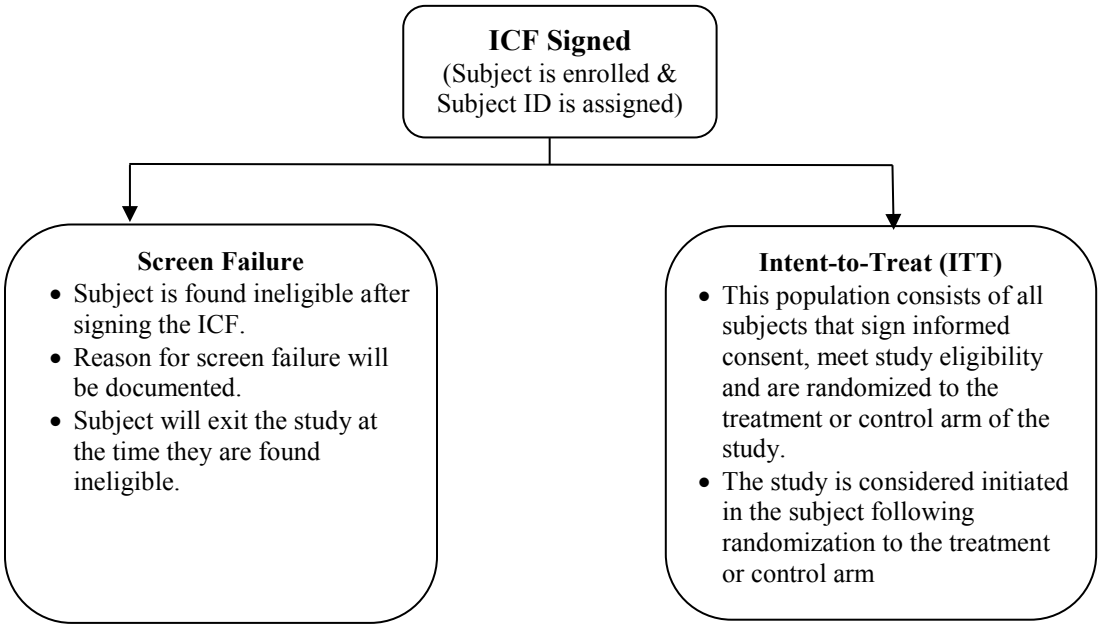
Adult subjects with the diagnosis of sepsis and refractory hypotension will be considered as a candidate for the study. A maximum of 210 subjects enrolled and in the analysis population will be included in the study. Subjects will be considered to be enrolled when they or their designated representative sign the informed consent form (ICF).

Subjects who are enrolled will be assigned a subject ID. Subjects that sign the ICF, but are found ineligible will be considered screen failures and will be exited from the study at that time. Enrolled subjects who meet study eligibility will be included in the analysis populations and are further described in **Section 12**.

The diagnosis of sepsis is defined as a known or presumed infection and the presence of 2 or more SIRS criteria as detailed in **Section 8.2**. Refractory hypotension is defined as the presence of hypotension following initial resuscitation with 1L of fluid. After obtaining ICF and confirming that the inclusion and exclusion criteria are met, the Cheetah sensors will be placed and connected to the Starling SV monitor. Useable readings will be confirmed prior to initiating the study data collection period.

A diagram outlining the overall subject enrollment plan is shown below:

Figure 1: Subject Flow Diagram



8.2 Inclusion Criteria

Subjects must meet the following inclusion criteria:

1. Diagnosis of sepsis, as exhibited by 2 or more of the following SIRS criteria and a known or presumed infection at the time of screening:
   - Temperature of > 38 C or < 36 C
   - Heart rate of > 90/min
   - Respiratory rate of > 20/min or PaCO2 < 32 mm Hg (4.3 kPA)
   - White blood cell count > 12000/mm^3 or < 4000/mm^3 or >10% immature bands
2. Refractory hypotension (either one single reading of MAP <65 exhibited during the evaluation period, or requiring treatment with vasopressors to maintain a MAP > 65) despite initial fluid resuscitation (1L of treatment fluid)
3. Patient enrolled in study within 24 hours of arrival to the hospital
4. Anticipated ICU admission. Patients may be maintained on another unit (such as within the ER or a step down unit) during the 72 hour monitoring period if the treatment algorithm is adhered to during this time period.
5. Able to provide signed informed consent or consent can be obtained from the patient's authorized representative

8.3 Exclusion Criteria

Subjects must not have any of the following exclusion criteria:

1. Primary diagnosis of: acute cerebral vascular event, acute coronary syndrome, acute pulmonary edema, status asthmaticus, major cardiac arrhythmia, drug overdose, or injury from burn or trauma
2. Known aortic insufficiency, or aortic abnormalities
3. Hemodynamic instability due to active hemorrhage, (e.g. gastrointestinal bleeding/coagulopathy/trauma)
4. Patient has received >3 liters of IV fluid prior to study randomization
5. Requires immediate surgery
6. Patient transferred to the ICU from another hospital unit
7. Do not attempt resuscitation (DNAR or DNR) order
8. Advanced directives restricting implementation of the resuscitation protocol
9. Contraindication to blood transfusion
10. Attending clinician deems aggressive resuscitation unsuitable
11. Transferred from another in-hospital setting
12. Not able to commence treatment protocol within 1 hour after randomization
13. Known intraventricular heart defect, such as VSD or ASD
14. Use of additional hemodynamic monitoring involving SVV, PPV or SV changes to determine fluid responsiveness
15. Seizure in the last 24 hours
16. Prisoner
17. Pregnancy

18. Age <18
19. Known allergy to sensor material or gel
20. Inability or contraindication to doing a passive leg raise with both extremities, such as inability to interrupt venous compression boots
21. Patient has an epidural catheter in place
22. Suspected intra-abdominal hypertension
23. Inability to obtain IV access
24. Diabetic ketoacidosis
25. Hyper-osmolarity syndrome
26. Patient treatment uncouples from the treatment algorithm

27. Patient should be excluded based on the opinion of the Clinician/Investigator

8.4 Subject Screening

All subjects diagnosed with sepsis and refractory hypotension will be considered potential candidates. All subjects screened for the study will be captured on the site's Screening Log. The log will capture the subject's gender, age, date of screening and the reason(s) for study exclusion.

8.5 Informed Consent Procedures

Written informed consent on the approved IRB informed consent form must be obtained for all subjects who are potential study candidates before any study specific tests or procedures are performed. Due to the high likelihood of the subject's critical clinical state and therefore the possibility of their inability to provide informed written consent, the subject's designated representative will be acceptable to provide written informed consent.

The general process for obtaining informed consent shall:

- Ensure that the principal investigator or his/her authorized designee conducts the informed consent process;
- Include all aspects of the clinical investigation that are relevant to the subject's decision to participate throughout the clinical investigation;
- Avoid any coercion or undue improper influence on, or inducement of, the subject to participate;
- Not waive or appear to waive the subject's legal rights;
- Use native non-technical language that is understandable to the subject;
- Provide ample time for the subject, or designee, to read and understand the informed consent form and to consider participation in the clinical investigation;
- Include personally dated signatures of the subject and the principal investigator or an authorized designee responsible for conducting the informed consent process;
- Provide the subject, or designee, with a copy of the signed and dated informed consent form and any other written information required per Site's Institutional Policy (i.e. additional HIPAA language).

8.6 Subject Randomization

Subjects will be randomized to either the treatment or control arm of the study once the decision has been made to admit the patient to the ICU. Any patients that are enrolled but are not admitted to the ICU, or who receive > 3L of treatment fluid prior to randomization will be considered a screen failure and will be exited from the study at this time.

Subjects will be randomized in a 2:1 treatment to control ratio to increase power for sub-analysis by patient population. Additionally, subject randomization will be stratified to ensure even randomization between the three time windows of enrollment (0-6 hours, 6-12 hours, 12-24 hours)

8.7 Subject Discontinuation/Withdrawal Criteria

Once the subject has been enrolled in the study, she/he may withdraw her/his consent to participate in the study at any time without prejudice. Participation in this clinical investigation is entirely voluntary. Likewise, there may be a reason identified by the Investigator that deems the subject no longer suitable for the study. In either case, the Investigator will contact Cheetah Medical to discuss the circumstances for discontinuation/withdrawal. Discontinuation or withdrawal may occur for any of the reasons listed below (this list is not all inclusive).

1. Subject is uncooperative with compliance of required study tests and/or medical management
2. Investigator determines that subject has developed a condition in which continued participation in the study is considered potentially harmful to the subject
3. Subject withdraws their consent
4. Adverse event which is considered intolerable by the patient, their LAR of the Principal Investigator
5. Subject has a significant protocol violation
6. Subject incorrectly enrolled in the study
7. Cheetah Medical terminates the study

8.7.1 Subjects Lost to Follow-Up

Subjects may be considered lost to follow-up for the following reasons:

- During data analysis, data indicates there is unacceptable bias in the measurements
- Data collection is interrupted prior to 72 hour data collection period of ICU stay
- Severe agitation and/or cardiopulmonary resuscitations resulted in poor quality data

9. STUDY PROCEDURES

9.1 Baseline Assessments

After written informed consent is obtained, baseline assessments will be made to further determine the subject's eligibility for the study. Data collected will include:

- Demographics
- Age
- Medical history

- SOFA assessment
- Physical exam, limited
- Hemoglobin and blood gas (per chart review, if available)
- Serum creatinine level
- Lactate level (per chart review, if available)
- Urine sample (to be frozen for possible future testing)
- Optional plasma serum collection (two, 5ml samples to be frozen for possible future testing)
- Current medications

9.2 Enrollment Procedure

Assuming the subject meets all study eligibility criteria, the investigator, or designees, will place the Cheetah device (Starling SV) on the subject. The device will be evaluated for obtaining the optimum expected hemodynamic readings before the study is begun according to the Starling SV Instructions for Use (IFU). This is done to confirm that data collected from the monitoring devices will be evaluable. It is recommended that approximately 15-20 minutes are allowed for the device to autocalibrate and achieve a stable baseline prior to beginning any measurements at the initiation of the study.

If the Starling SV does not provide the expected hemodynamic recordings, it will be adjusted or replaced as needed until it provides the expected recordings. If it is determined that the device will not provide the expected recording, then the subject will be withdrawn from the study.

9.3 Data Collection Procedure

When the Starling SV is determined to be providing the expected waveforms and recordings, the study device data collection period will begin. Recorded information will be collected and stored by the device. After the data collection period has ended the data will be downloaded and used for analysis.

9.3.1 Hemodynamic Collection Methods

Starling SV: Following the Instructions for Use, the Starling SV device will be connected to the subject by placing four of the system's sensors on the subject's chest: left and right mid-clavicle and left and right mid-last ribs. The device will undergo 1 minute of autocalibration after which measurements will begin to be obtained. Data from the device is available as a visible read-out on the monitor screen and stored internally. At the conclusion of the data collection period, the data will be downloaded and provided to the sponsor or designee.

9.3.2 Fluid Assessment

A fluid assessment will be initiated at the following time points:

- Baseline (at study enrollment)
- At every clinical decision point for the first 72 hours of study enrollment. Examples of a clinical decision point include the decision to give additional fluid volume, or escalate vasopressors.

- MAP <65 during first 72 hours of study enrollment

A fluid assessment will be administered using a passive leg raise according to **Section 6.5**.

9.3.3 Additional Data Collection

Other data points and associated times will be collected in addition to the analysis of the continuous data from the Starling SV system. Types of data points for collection are:

- Medical history
- Medications
- Volume of treatment fluid administered
- Urine output (to be recorded every 12 hours)
- Respiratory status
- Serum Creatinine level (every 24 hours +/- 4 hours , for 72 hour monitoring period and at 30 day discharge)
- Lactate level (per chart review, if available)
- Significant clinical events not previously identified for study data collection, e.g. cardiac arrest, CPR, intubation, extubation, placement of catheters (intravenous, arterial, urinary, etc), etc.
- Changes in mechanical ventilator status parameters

9.4 Schedule of Assessments

Each subject will follow a schedule of assessments and events from which data will be collected (Table 1).

Table 1: Schedule of Assessments

Assessment/Event	Pre-Consent	Consent	Baseline	Enrollment-72 hours	72 hours-Discharge
Subject Identification	X				
Determine Eligibility	X				
Informed Consent		X			
Medical History			X	X	X
Physical Exam, limited, and lab values			X		
Urine Sample			X		
Optional plasma serum collection (two, 5ml samples to be frozen for possible future testing)			X		
Serum Creatinine			X	X	X (chart review)
Medications, specific			X	X	X
Volume resuscitation fluid			X	X	
Respiratory Status			X	X	X

Assessment/Event	Pre-Consent	Consent	Baseline	Enrollment-72 hours	72 hours-Discharge
(intubated, ventilated)					
Starling SV System			Attached	Continuous hemodynamic recordings	
Fluid Assessment				X	
Adverse events				X (+48 hours)	

10. SAFETY/DEVICE ASSESSMENT

10.1 Adverse Events

Subjects will be carefully monitored during the study for possible device related adverse events (AEs). The investigator or designee will determine AE occurrences and collect the required data. It is anticipated that the occurrence of device related adverse events will be a low number. All device related adverse events will be reviewed not only by the site investigator, but also by the sponsor's medical officer.

Serious Adverse events will be collected during the 72 hour (**+ 48 hours**) treatment period in the following areas: Cardiac, CNS, pulmonary, renal, and skin disorders.

10.1.1 Device Related Adverse Event (AE)

Any untoward medical occurrence, unintended disease or injury or any untoward clinical signs (including an abnormal laboratory finding) in subjects, users or other persons occurring as a result of the study device

10.1.2 Serious Adverse Events

A serious adverse event (SAE) is any untoward medical occurrence that**:**

- Results in death
- Led to serious deterioration in the health of the subject, that either resulted in:
  - a life-threatening illness or injury, or
  - a permanent impairment of a body structure or a body function, or
  - in-patient or prolonged hospitalization, or
  - medical or surgical intervention to prevent life-threatening illness or injury or permanent impairment to a body structure or a body function,
- Led to fetal distress, fetal death or a congenital abnormality or birth defect

10.2 Reporting Procedures

10.2.1 General Reporting Requirements

All device related adverse events must be recorded on the Adverse Event eCRF by the investigator (or designee). The report should include: start date of the adverse event, treatment,

resolution, and assessment of both the seriousness and the relationship to the investigational device.

The following criteria must also be adhered to by the Investigator:

- Completion of separate device related Adverse Event forms to document each event
- The forms must be signed by the Investigator, and
- Supplying to the Sponsor (or designee), upon Sponsor's request, with any additional information related to the safety reporting of a particular event

10.2.2 Reporting Requirements of SAEs

All serious adverse events must be reported by the investigator by submitting the Adverse Event eCRF within 24 hours of learning of the adverse event.

The Investigator shall:

a) Report to the IRB serious adverse events per the institutional requirements for timing and amount of support information,
b) Supply the Sponsor (or designee), upon Sponsor's request, with any additional information related to the safety reporting of a particular event.

10.2.3 Sponsor Reporting

All adverse events will be reported to the applicable regulatory authority as required per applicable regulatory authority.

Cheetah Medical, or their designee is responsible for the classification and reporting of adverse events and applicable regulatory requirements.

11. STUDY MONITORING

The study will be monitored according to Cheetah Medical's monitoring procedures. A study specific monitoring plan will be developed to ensure protocol compliance and applicable regulatory requirements.

Clinical monitors will be designated by Cheetah Medical. Monitors will verify subject data and ensure compliance with GCP, clinical protocol and other study requirements to be utilized for the study.

11.1 Monitor Training

All Cheetah Medical designated monitors will be trained appropriately to monitor study progress including but not limited to the protocol and CRFs.

11.2 Site/Investigator Training

Cheetah Medical will be responsible for providing training to the investigator and appropriate clinical site personnel.

11.2.1 Protocol Training

Training on study protocol requirements will be provided for the entire study team at the initiation of the study. It is ultimately the responsibility of the Investigator to ensure all clinical site personnel participating in this study are adequately trained.

To ensure uniform data collection and protocol compliance, Cheetah Medical and/or designee will perform study initiation visits to review the clinical protocol, techniques for the identification of eligible subjects, instructions on in-hospital data collection, and methods for soliciting data from alternative sources.

11.2.2 Device Training

This procedure may only be performed by qualified investigators, familiar with the Cheetah Medical system. Training on the Starling SV system will be performed and documented for the site Investigator who is responsible for using the device, by the study Sponsor, Cheetah Medical.

11.3 Site Monitoring

The Sponsor or designee may conduct periodic compliance assessments at various study sites. The Sponsor or designee may request access to all study records including source documentation for inspection and photocopying during a compliance assessment. The Investigator and research coordinator must be available to respond to reasonable requests and queries made during the compliance assessment process.

11.4 Regulatory Agency Inspection

In the event that an investigator is contacted by a regulatory agency regarding this study, the investigator will notify the Sponsor or its designee immediately. The investigator and research coordinator must be available to respond to reasonable requests and queries made during the inspection process. The investigator must provide the Sponsor or designee with copies of all correspondence that may affect review of the current trial. The Sponsor may provide needed assistance in responding to regulatory audits.

12. STATISTICAL CONSIDERATIONS

12.1 Sample Size Estimation

Subjects will be randomly assigned in a 2:1 ratio to treatment with Starling SV or to treatment with standard of care. The primary effectiveness endpoint for this study is the difference between the two treatment groups in mean fluid balance at ICU discharge.

Minimum enrollment (N_{min}) in the study will be set at 120 subjects (80 Starling SV and 40 control) to power at 80% for possible demonstration of superiority for the secondary endpoint of creatinine levels as a measurement of change from baseline. The secondary endpoint for change in creatinine levels at 120 evaluable subjects displays 80% power at a two-sided alpha level of

0.05 to demonstrate superiority under an assumption of a treatment effect of -1.4 mg/dL with a standard deviation of 2.5 mg/dL.

Under an assumption of a treatment effect of -2 L with a standard deviation of 3 L, the sample size of 120 evaluable subjects provides 92.7% power in a test of superiority for the primary effectiveness endpoint.

The trial will incorporate a sample size re-estimation (SSR) at the time of the interim look based on promise for superiority in the key secondary endpoint. Methods for the SSR have been described in detail in previous literature. The maximum enrollment being considered for this trial is 210 total enrolled (N_{max} = 1.75 times N_{min}).

An interim analysis incorporating a SSR will take place when 120 subjects have been enrolled and completed endpoint evaluations. The interim analysis will be performed by an independent statistician. It is assumed that the acute endpoint assessments will be fully informed at the time of the interim analysis.

At this point, the primary study endpoint will be tested as final, and will not incorporate an alpha spend.
The conditional power to claim superiority (CP_{sup}) on the key secondary endpoint for change in creatinine levels will be computed. The conditional power (CP_{sup}) will be calculated based on the interim results of the key secondary endpoint, with the specific interest of demonstrating superiority. CP_{sup} is defined as the approach that quantifies the statistical power to yield an answer different from that seen at the interim analysis. The data will be partitioned into three zones based on CP_{sup} unfavorable zone ($CP_{sup}<50\%$), promising zone ($50\% \leq CP_{sup}<80\%$), and favorable zone ($CP_{sup} \geq 80\%$).

12.2 Analysis Populations

12.2.1 All Enrolled

Any subject who has signed informed consent will be included in the all enrolled population. Should a subject be considered a screen failure, the reason for failure will be documented, and the subject will be exited from the study. Adverse events will be reported for all enrolled subjects through the time of study exit.

12.2.2 Intent-to-Treat (ITT)

The intent-to-treat population (ITT) will consist of any subjects that have signed the ICF, meet study eligibility criteria, and are randomized to the treatment or control arm of the study.

12.2.3 Modified Intent-to-Treat (mITT)

Subjects will be included in the Modified Intent-to-Treat group if they sign the ICF, meet study eligibility criteria, are randomized, and in the treatment group receive monitoring for the first 72 hours of study enrollment or until ICU discharge, whichever occurs first. The mITT population represents the Primary and Secondary Analysis population for this study.

12.2.4 Per-Protocol (PP)

Subjects will be included in the Per-protocol group if they sign the ICF, are randomized and have the assigned procedure completed, meet critical study eligibility criteria and have no major protocol deviations (defined in the Statistical Analysis Plan), and, in the treatment group, receive monitoring for the first 72 hours of study enrollment. The PP population represents a subgroup analysis population for this study.

12.3 Primary Endpoint Analysis

The primary endpoint is an evaluation of the difference between the two treatment groups in mean fluid balance (L) at 72 hours or ICU discharge, whichever occurs first. The following hypothesis will be tested:

$$H_{o:}\ \mu c - \mu_T = 0$$
$$Ha:\ \mu c - \mu_T \neq 0$$

where μt and μc is the average fluid balance in the Starling SV and control groups, respectively. Statistical summaries will include means, medians, and standard deviation, as well as 95% confidence intervals. A t-test for superiority at a significance level of 0.025 will be performed to test the hypothesis that μt is significantly less than μc. If it can be established that the difference is greater than 0 points (i.e. $\mu C > \mu T$), superiority will also be claimed.

12.4 Secondary and Exploratory Endpoints

Secondary objectives that have been pre-specified to be included in the formal statistical analysis will be tested in sequential fashion using the Holms step-down procedure for type-I error rate correction if and only if the primary objective passes according to the criteria outlined above. At the conclusion of the trial, the key secondary endpoints will be ordered sequentially from most significant to least significant. The endpoints will then be tested in order at adjusted levels of significance (i.e. E1: 0.05/k, E2: 0.05/k-1; E3: 0.05/k-2, where k=number of specified endpoints). Once an endpoint fails, all following endpoints will not be tested. Following is a list of key secondary endpoints:

Following is a list of key secondary endpoints for formal testing:

- Length of ICU stay (days) until subject is medically ready for discharge
- Number of days free from ventilator use (30 day period)
- Requirement for mechanical ventilation
- Number of days free from vasopressor use
- Changes in serum creatinine levels from baseline
- Requirement for renal replacement therapy (RRT)

Additional secondary endpoint have also been identified for testing, but will not be included in the formal statistical testing procedures for key secondary endpoints:

- Incidence of Adverse Events (AE)

- Number of ICU readmissions
- Mortality rate
- Mean volume of treatment fluid
- Incidence of Major Adverse Cardiac Event (MACE)
- Discharge location
- Mean difference in fluid balance at ICU discharge

Additional sub-analyses have been planned for the primary and key secondary endpoints. Primary and secondary endpoints will also be evaluated by the following subgroup analysis:

- Patient disease state
- Patients determined to be fluid responsive vs non-fluid responsive
- AKI measures in patients determined to increase serum creatinine levels from baseline vs decrease serum creatinine levels from baseline
- Enrollment time frame

12.5 Baseline Data Summary

Baseline data including subject demographics, clinical history, risk factors, and pre-procedure patient characteristics will be summarized using descriptive statistics (e.g., mean, median, standard deviation, 25th percentile, 75th percentile, minimum and maximum) for continuous variables and frequency tables or percentages for categorical variables.

12.6 Subject Accountability and Missing Data

Subjects enrolled in this study are followed for a short time period, and missing data in this study is expected to occur at a low rate. All efforts will be used to prevent missing data. These efforts include, but are not limited to, complete site training and regular monitoring to help to minimize missing data.

Subjects without the minimum amount of monitoring [i.e. throughout first 72 hours of study enrollment,] and measurements specified in the protocol will be considered as missing in the final analysis. The impact of missing data on the study will be evaluated via sensitivity analyses if more than 5% of subjects do not have evaluable data for the primary outcome. In the final report, the number and proportion of subjects eligible for and compliant with each analysis population will be presented.

13. DATA HANDLING AND RECORD KEEPING

Each participating site will maintain appropriate medical and research records for this trial, in compliance with regulatory and institutional requirements for the protection of confidentiality of subjects. As part of participating in a manufacturer-sponsored study, each site will permit authorized representatives of the Sponsor(s), the Sponsor's designee, and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

Source data are all information, original records of clinical findings, observations, or other activities in a study necessary for the reconstruction and evaluation of the trial.

13.1 Clinical Data Collection

Electronic Case Report Forms (eCRFs) will be used to collect all subject data during the course of the study and will be entered into an electronic database. eCRFs must be fully completed for each subject and signed by the Investigator when complete.

Good Clinical Practice Guidelines require that Investigators maintain information in the study subject's medical records that corroborate data collected on the eCRFs. In order to comply with these regulatory requirements, the following information should be maintained:

- Medical history/physical condition of the study subject before involvement in the study sufficient to verify protocol entry criteria.
- Dated and signed notes on the day of entry into the study including the study investigator, study name, subject number assigned and a statement that consent was obtained.
- Dated and signed notes from each study subject visit with reference to the eCRFs for further information, if appropriate (for specific results of procedures and exams).
- Information related to adverse events.
- Study subject's condition upon completion of or withdrawal from the study.
- Discharge summaries/procedure reports.

13.2 Data Reporting

All site Investigators or designated individuals shall be responsible for recording all study data on the eCRFs. The Investigator is required to electronically sign the eCRFs to verify that he/she has reviewed and agrees with the recorded data. All protocol deviations shall be documented and a justification for any missed assessments shall be provided on the Protocol Deviation eCRF.

Completed eCRFs will be verified by the monitor at the investigational sites or by remote monitoring as at regular intervals throughout the study, as outlined in the Monitoring Plan. The Investigator will allow the monitor and/or representative of the Sponsor, and any regulatory body to review and inspect the study files, subject eCRFs, subject medical records and other related study documents as required.

13.3 Data Review

eCRFs will be reviewed for completeness and clarity. Missing or unclear data will be investigated by the monitor and will be retrieved, clarified and entered by study personnel as necessary throughout the study. Cheetah Medical, or designee, may request additional documentation from the Investigator such as physician notes or physician written summaries when adverse events are observed and reported. Documentation provided will also be used for the adjudication of specified adverse events by the Independent Safety Physician.

Development of the data collection system for the study will be performed by Cheetah Medical, or designee. Cheetah Medical, or designee will also be responsible for the quality control of the database and confirming the overall integrity of the data.

13.4 Investigator Records

Investigators will maintain complete, accurate and current study records. The following records must be maintained in designated study files:

- Clinical protocol and all amendments
- Signed Clinical Trial Research Agreement
- Institutional Review Board (IRB) Approval Letter(s)
- IRB approved informed consent(s) (including any revisions)
- CV for all Investigators, signed and dated
- Investigator(s) medical license
- Financial Disclosure Form for all Investigators
- Correspondence relating to this study
- Correspondence with the IRB
- IRB membership list and/or assurance number
- Delegation of Authority log
- Device Instructions for Use
- Printed copy of blank set of CRFs
- Subject Log
- Site Visit Log (e.g. for Monitor sign-in)
- Site Training records
- Investigational Device Accountability Logs
- Reports (includes Adverse Event reports and final reports from Investigator and Sponsor)
- Copy of all IRB approved subject-related materials and/or study advertising materials

The following records must be maintained for each subject enrolled in the study:

- Signed subject consent forms
- Copy of final completed CRFs
- Record of any adverse events with supporting documentation
- Reports, progress notes, physician and/or nursing notes, and subject office files
- Records pertaining to subject deaths throughout the course of the study

13.5 Data Retention

Study documents should be retained for a minimum of 5 years. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the Sponsor, if applicable. It is the responsibility of the Sponsor to inform the Investigator when these documents no longer need to be retained.

Table 2: Responsibilities for Preparing and Submitting Reports

Type of Report	Prepared by Investigator for	Time of Notification
Enrollment Notification Form	Sponsor	Within 24 hours of procedure
Electronic Case Report Forms	Sponsor	Within 10 working days
Serious/ Device Related Adverse Event	Sponsor and IRB (as required)	Within 24 hours of knowledge or as required by IRB
Subject Death	Sponsor and IRB (as required)	Within 24 hours of knowledge
Subject Withdrawal	Sponsor	Within 24 hours of knowledge
Withdrawal of IRB Approval	Sponsor	Within 24 hours of knowledge
Protocol Deviations	Sponsor and IRB	Within 5 working days of occurrence or knowledge
Informed Consent Not Obtained	Sponsor and IRB	Within 24 hours of knowledge or as required by IRB
Final summary report	Sponsor and IRB and Regulatory Authority (as required)	Within 3 months of study completion

13.6 Investigator Reports

Each year an annual summary report shall be prepared by the Investigator which provides a summary of the number of subjects treated to date as well as other pertinent clinical information associated with the investigational procedure. The annual report is required to be provided to the IRB and the Sponsor or designee.

Upon completion and/or termination of the study a final report shall be prepared. This report will contain a critical evaluation of all data collected during the course of the investigation at each institution. The Sponsor or its designee is responsible for preparing this compilation to Investigators for submittal as a final report to their reviewing IRB.

14. QUALITY CONTROL AND ASSURANCE

14.1 Site and Investigator Selection

The Sponsor selects qualified investigators with appropriate experience with this patient population at health care facilities with adequate resources to participate in this study. Investigational sites will be selected using combined current assessments of site and investigator qualifications.

14.2 Protocol Deviations

An investigator is not allowed to deviate from the Protocol if the deviation affects subject's rights, safety and wellbeing, or the scientific integrity of the clinical investigation. Under

emergency circumstances, deviations from the protocol to protect the rights, safety and well-being of human subjects may proceed without prior approval of the Sponsor and the IRB.

A protocol deviation is a failure to comply with the requirements specified within this clinical study protocol. Examples of protocol deviations may include enrollment of a study subject who does not meet all of the inclusion/exclusion criteria specified in the protocol and missed study visits without documentation. Each investigator shall conduct this clinical study in accordance with this clinical study protocol, regulatory body regulations, Good Clinical Practices.

All deviations are reviewed and assessed for their impact on subject safety by the Sponsor or designee. The PI and study staff is responsible for knowing and adhering to their IRB reporting requirements.

The protocol deviations for this protocol consist of, but not limited to the following:

- Failure to obtain subject's informed consent prior to any study-related activities;
- Subject did not meet the inclusion and/or exclusion criteria and were enrolled;
- Failure to conduct protocol required clinical test or assessment;
- Failure to complete protocol required assessments within the required time frame
- Failure to report serious adverse events according to protocol requirements.

In the event of any deviation from the protocol, the Investigator will be notified of the site's non-compliance. Corrective actions will be required, if necessary. Continued protocol deviations despite re-education of the study site personnel or persistent protocol deviation may result in termination of the site's study participation. Subjects enrolled at these sites will continue to be followed per the clinical protocol.

14.2.1 Protocol Deviation Process

Investigators must report protocol deviations to the Sponsor within 5 working days of investigational site knowledge of the deviation by entering data into the eCRF. Any protocol deviations that affect the rights, safety or well-being of the subject or the scientific integrity of the clinical investigation, including those which occur under emergency circumstances must be reported within 24 hours to the Sponsor and IRB, if required by the IRB, or national regulations.

14.3 Corrective/Preventive Action

Deviations from clinical protocol requirements will be reviewed and evaluated on an ongoing basis and, as necessary, appropriate corrective actions will be put into place to bring a site into compliance.

14.4 Study Audit(s)

Audits may be performed as deemed necessary by Cheetah Medical, in a manner consistent with applicable procedure.

14.5 Study Registration

A description of this trial will be available on www.clinicaltrials.gov, the U.S. approved clinical trial registry site.

15. ETHICS/PROTECTION OF HUMAN SUBJECTS

15.1 Statements of Compliance

This study will be performed in accordance with Good Clinical Practice Guidelines, the Code of Federal Regulations Title 21 CFR Parts 50, 54, and 56.

The clinical investigation shall not begin until the required approval/favorable opinion from the Institutional Review Board (IRB) has been obtained including approval of the Informed Consent Form to be used with subjects.

Any additional requirements imposed by the IRB shall be followed.

15.2 Institutional Review Board

Each participating institution must provide for the review and approval of this protocol and the associated informed consent documents by an appropriate Institutional Review Board (IRB) prior to subject enrollment. Any amendments to the protocol or changes to the informed consent document must also be approved before they are placed into use. The Investigator should notify the IRB of deviations from the protocol and SAEs occurring at the site in accordance with local procedures. The Investigator is responsible for continued study related communication with the IRB, including submission of study report and SAE notifications as per local regulatory requirements.

15.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects (and their families, as applicable). Consent forms describing in detail the study interventions/ products, study procedures, and risks are given to the subject and written documentation of informed consent is required prior to starting any study-specific tests or intervention /administering of study product.

Consent forms will be approved by the IRB and the subject will be asked to read and review the document. Upon reviewing the document, the site investigator or designated study personnel will explain the research study to the subject and answer any questions that may arise. The subject (or their authorized legal representative) will sign the informed consent document prior to any procedures being done specifically for the study. The subject should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate, unless study timeframes do not allow for such discussions. The subjects may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the subjects for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

Modifications to the study informed consent must have approval from the Sponsor and the EC as required.

15.4 Subject Confidentiality

Confidentiality of data shall be observed by all parties involved at all times throughout the clinical investigation. All data shall be secured against unauthorized access. Sites will maintain subject privacy in accordance with local and national regulations and institutional requirements including all applicable provisions of the Health Insurance Portability and Accountability Act (HIPAA) and its current regulations.

Subjects must be identified only by their assigned study number and initials on all CRFs and other records and documents submitted to the Sponsor, the monitor, and other authorized parties. The Investigator should maintain a Subject Identification List with complete identification information (name, address, contact number, informed consent version number) on each subject. Documents not required to be submitted to the Sponsor such as subject written informed consent form, should be maintained by the Investigator in strict confidence.

The privacy of each subject and confidentiality of his/her information shall be preserved in reports and when publishing any data. The principal investigator or institution shall provide direct access to source data during and after the clinical investigation for monitoring, audits, IRB review and regulatory authority inspections. As required, the principal investigator or institution shall obtain permission for direct access to source documents from the subject, hospital administration and national regulatory authorities before starting the clinical investigation.

16. PROTOCOL AMENDMENTS

The clinical protocol, eCRFs, ICF and other subject information, or other clinical investigation documents shall be amended as needed throughout the clinical investigation, and a justification statement shall be included with each amended section of a document. Proposed amendments to the protocol shall be agreed upon between the Sponsor and Principal Investigator, or the Coordinating Investigator. The amendments to the protocol and the subject's informed consent form shall be notified to, or approved by, the IRB as required. For non-substantial changes [e.g. minor logistical or administrative changes, change of monitor(s), telephone numbers, renewal of insurance] not affecting the rights, safety and well-being of human subjects or not related to the clinical investigation objectives or endpoints, a simple notification to the IRB can be sufficient. The version number and date of amendments shall be documented.

17. TERMINATION OF STUDY OR STUDY SITE PARTICIPATION

The Sponsor may terminate the study at any time. If the study is terminated prior to the completion of expected enrollment for any reason, all participating centers will be notified within five working days. All subjects already enrolled will continue to be followed for the planned course of study described in this protocol. The study shall be terminated following the last enrolled subject.

The Sponsor reserves the right to terminate study site participation and remove appropriate study materials at any time. Specific instances that may precipitate such termination include but are not limited to the following:

- Failure to meet minimum subject enrollment requirements
- Failure to comply with protocol specified procedures and documentation
- Failure to comply with regulatory regulations
- Failure to comply with Good Clinical Practice

The site Investigator may also discontinue study participation with suitable written notice to the Sponsor.

18. PUBLICATION POLICY

Specifics of the publication policy will be outlined in the Clinical Trial Research Agreement.

19. BIBLIOGRAPHY

1. Marik P and Cavallazzi R. Does central venous pressure predict fluid responsiveness? An updated meta-analysis and a plea for some common sense. Crit Care Med 2013; 41: 1774-1778.

2. Michard F and Teboul JL. Predicting fluid responsiveness in ICU patients: a critical analysis of the evidence. Chest. 2002; 121:2000-2008.

3. Kelm DJ, et al. Fluid overload in patients with severe sepsis and septic shock treated with early goal-directed therapy is associated with increased acute need for fluid-related medical interventions and hospital death. Shock 2015; 43:68-73.

4. Boyd et al. Vasopressin in Septic Shock Trial (VASST). Critical Care Medicine 2011; 39:259-265.

5. Vincent et al. Sepsis in European ICU: Results of the SOAP study. British Journal of Anesthesia 2006; 113: 740-747.

6. Monnet X, Teboul JL. Passive leg raising. Intensive Care Med 2008, 34: 659-63.

APPENDIX A- TREATMENT ALGORITHM

EVALUATION OF FLUID/VOLUME IN PATIENTS WITH SEPSIS AND REFRACTORY HYPOTENSION

Protocol Number: PRO-0001

Protocol Release Log

Protocol Version	Effective Date
Rev G [1]	September 19, 2016
Rev H	October 30, 2017
[1] Study sites enrolled patients under Rev G and Rev H of clinical protocol; Rev A-F were internal	

Summary of Changes from Protocol G to Protocol H

Page/Section	Changes
7/3 Synopsis: Secondary Endpoints **13/7.3.2** Secondary Endpoints **24-25/12.4** Secondary and Exploratory Endpoints	Added two secondary endpoints: *3. Requirement for mechanical ventilation* *8. Incidence of Major Adverse Cardiac Event (MACE)*
8/3 Synopsis: Population	Further clarification of study population: *Patients with sepsis who exhibit refractory hypotension (MAP < 65, or require treatment with vasopressors to maintain a MAP > 65) following initial fluid resuscitation (1L of fluid).*
8/3 Synopsis: Number of Sites **12/7.2** Study Design	Expanded US sites from 10 to 15 and added UK sites: *Between 7-15 clinical sites located in the US and 1-3 sites located in the UK* *Between seven and fifteen sites will be used to enroll 210 subjects with the diagnosis of sepsis and refractory hypotension and in whom the Starling SV system can be attached.*
8/3 Synopsis: Inclusion Criteria **15/8.2** Inclusion Criteria **9/3** Synopsis: Exclusion Criteria **16/8.3** Exclusion Criteria	Added/Clarified Inclusion and Exclusion Criteria: Inclusion Criteria *2. Refractory hypotension (either one single reading of MAP <65 exhibited during the evaluation period, or requiring treatment with vasopressors to maintain a MAP > 65) despite initial fluid resuscitation (1L of treatment fluid)* *4. Anticipated ICU admission. Patients may be maintained on another unit (such as within the ER or a step down unit) during the 72 hour monitoring period if the treatment algorithm is adhered to during this time period.* Exclusion Criteria *24. Diabetic ketoacidosis* *25. Hyper-osmolarity syndrome*

	26. Patient treatment uncouples from the treatment algorithm
12/6.5 Fluid Assessment-Passive Leg Raise (PLR)	Clarification that fluid responsiveness is reflected by a positive value *Fluid responsiveness is indicated by a Δ SVI ≥ +10%*
13/7.4 Study Duration	Changes to the study timeline: *Estimated Enrollment Completion: Q2 2018* *Estimated Final Report: Q3 2018*
18/9.1 Baseline Assessment **19/9.4** Schedule of Assessment	The addition of a serum sample *Optional plasma serum collection (two, 5ml samples to be frozen for possible future testing)*

STATISTICAL ANALYSIS PLAN

Protocol Title (Number):
Evaluation of Fluid Volume in Patients with Sepsis and Refractory Hypotension
(FRESH Study)
PRO-0001

Sponsor: Cheetah Medical, Inc.

June 28, 2018
Boston Biomedical Associates, LLC (BBA)
100 Crowley Drive, Suite 216
Marlborough, MA 01752

TABLE OF CONTENTS

1 ABBREVIATIONS

Abbreviation	Definition
AE	Adverse Event
AKI	Acute Kidney Injury
BBA	Boston Biomedical Associates, LLC
CEC	Clinical Events Committee
CP_{sup}	Conditional Power to Claim Superiority
CRF	Case Report Forms
CSR	Clinical Study Report
DMC	Data Monitoring Committee
FDA	United States Food and Drug Administration
FRESH	Fluid Responsiveness Evaluation in Sepsis-associated Hypotension
ICU	Intensive Care Unit
IRB	Institutional Review Board
ITT	Intent-To-Treat Population
MITT	Modified Intent-To-Treat Population
PLR	Passive Leg Raise
PP	Per-Protocol Population
RRT	Renal Replacement Therapy
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SOP	Standard Operating Procedure
SSR	Sample Size Re-estimation
SV	Stroke Volume
SVI	Stroke Volume Index
TEAE	Treatment Emergent Adverse Event

2 SUMMARY

TITLE	Evaluation of fluid volume in patients with sepsis and refractory hypotension.
PREFACE	This Statistical Analysis Plan (SAP) describes the planned analysis and reporting for Cheetah Medical, Inc. protocol PRO-0001 *(Fluid Responsiveness Evaluation in Sepsis-associated Hypotension [FRESH] Fluid Trial)*. This study is being completed to assess the safety and efficacy in using Starling SV for dynamic assessment of fluid responsiveness in the treatment of septic patients with refractory hypotension. The following documents were reviewed in preparation of this SAP: • Clinical Research Protocol PRO-0001, issued 30OCT2017 • Case report forms (CRFs) issued 01DEC2017 for Protocol PRO-0001
PURPOSE	The purpose of this SAP is to outline the planned analyses in support of the Clinical Study Report (CSR) for protocol PRO-0001. Exploratory analyses not necessarily identified in this SAP may be performed to support the clinical development program. Any post-hoc, or unplanned, analyses not identified in this SAP will be clearly identified in the respective CSR or manuscript.
STUDY OBJECTIVES	The objective of this study is to assess the mean difference in fluid balance at 72 hours or ICU discharge, whichever occurs first, and associated patient outcomes, based on a dynamic assessment of fluid responsiveness in septic patients with refractory hypotension in an ICU setting.
STUDY ENDPOINTS	**Primary:** Fluid balance (L) at 72 hours or ICU discharge, whichever occurs first **Secondary:** 1. Changes in serum creatinine level from baseline to 72 hours (key secondary endpoint) 2. Requirement for renal replacement therapy (RRT) 3. Requirement for ventilator use 4. Length of ICU stay (days) until subject is medically ready for discharge 5. Number of hours with ventilator use (30-day period) 6. Number of hours with vasopressor use **Not for Formal Testing:** 1. Incidence of Adverse Events (AE) 2. Number of ICU readmissions 3. 30 day in-hospital Mortality rate 4. Volume of Treatment Fluid 5. Incidence of MACE 6. Discharge location 7. Mean difference in fluid balance at ICU discharge
STUDY DESIGN	This study is a prospective, multi-center, randomized trial comparing the mean difference in fluid balance and associated patient outcomes between a treatment guided by a dynamic assessment of fluid responsiveness and a standard of care control group not using dynamic fluid assessment.

	Subjects will be randomized in a 2:1 treatment to control group ratio to increase power for sub-analysis by patient population. Subject randomization will be stratified by time window of enrollment to ensure even randomization between the three time windows of enrollment (0-6 hours, 6-12 hours, and 12-24 hours). Patients randomized to the Starling SV arm will have treatment guided by a dynamic assessment of fluid responsiveness (measured by a change in stroke volume index > 10%) as assessed by passive leg raise (PLR). Patients randomized to the control group will receive standard of care treatment. The use of dynamic fluid assessment (i.e. SVV, PPV, or SV change) to determine fluid responsiveness within the control group is prohibited.
INTERIM ANALYSES	An interim analysis is planned after 90 subjects have been enrolled and completed the evaluation of the key secondary endpoint of change in serum creatinine at 72 hours. At this analysis, a sample size re-estimation will occur in order to determine promise for superiority in the key secondary endpoint. No annual reports for the FDA are anticipated prior to the end of data collection as this study is not intended to support a regulatory filing. An annual report and project status update may be requested by the local IRB at study sites. No other interim analyses are planned.
FINAL ANALYSES	All final planned analyses identified in this SAP will be completed after the last subject has completed their 30 day follow up.

3 SEQUENCE OF PLANNED ANALYSES

3.1 INTERIM ANALYSES

3.1.1 INTERIM ANALYSES FOR SAMPLE SIZE OF SECONDARY ENDPOINT SUPERIORITY

The trial will incorporate a sample size re-estimation (SSR) at the time of the interim look based on promise for superiority in the key secondary endpoint of change in serum creatinine from baseline to 72 hours. The maximum enrollment being considered for this trial is 210 total enrolled (N_{max} = 1.75 times N_{min} = 120, where the sample size of 120 is determined as noted in Section 5 below).

The interim analysis on the key secondary endpoint incorporating a SSR will take place when 90 subjects have been enrolled and completed the key secondary endpoint evaluation. The interim analysis will be performed by an independent statistician. It is assumed that the acute endpoint assessments will be fully informed at the time of the interim analysis.

There will be no inspection of the primary study endpoint at this interim stage. The primary study endpoint will be tested as final at the formal planned final sample size of 120 patients and will not incorporate an alpha spent. In the event of an SSR due to the key secondary endpoint, the primary endpoint will be tested again at the final sample size. No alpha-adjustment is needed across this potential multiple testing of the primary endpoint since the primary endpoint will need to be met at <u>both</u> N_{min}=120 and, if the sample size is increased to >120, at the final sample size.

After 90 patients have completed the secondary endpoint, the conditional power to claim superiority (CP_{sup}) on the key secondary endpoint for change in creatinine level from baseline to 72 hours will be computed assuming a planned final sample size of 120 patients. The conditional power (CP_{sup}) will be calculated based on the observed interim results of the key secondary endpoint, with the specific interest of demonstrating superiority of the experimental treatment versus the control. The data will be partitioned into three zones based on CP_{sup} unfavorable zone (CP_{sup}<50%), promising zone (50%≤CP_{sup}<80%), and favorable zone (CP_{sup}≥80%). If CP_{sup}<50% or CP_{sup}≥80% the study will continue as is. If 50%≤CP_{sup}<80% then the sample size may be increased (to a maximum of 210 total) to yield 80% CP_{sup} without increasing Type I error, following the methodology of Chen, DeMets and Lan (2004).

3.1.2 ANNUAL REPORTS

As this study is not designed to support regulatory filings, no annual reports are anticipated at this time. Annual project updates may be provided at the request of site IRBs.

3.2 Final Analyses and Reporting

All final, planned, analyses identified in the protocol and in this SAP will be performed only after the last subject has had 30-day follow-up. Key statistics and study results will be made available to Cheetah Medical, Inc. following database lock. Any post-hoc, exploratory analyses completed to support planned study analyses, which were not identified in this SAP, will be documented and reported as necessary. Any results from these unplanned analyses will also be clearly identified as post-hoc analyses.

4 Study Objectives and Endpoints

4.1 Study Objective

The objective of this study is to assess the mean difference in fluid balance at 72 hours or ICU discharge, whichever occurs first, and associated patient outcomes, based on a dynamic assessment of fluid responsiveness in septic patients with refractory hypotension in an ICU setting.

4.2 Study Endpoints

4.2.1 Primary Endpoint Analysis

The primary endpoint analysis is an evaluation of the difference (L) between the two treatment groups' mean fluid balance at 72 hours or ICU discharge, whichever occurs first. The following are the null and alternative hypotheses:

$$H_0: \mu_c - \mu_t = 0$$
$$Ha: \mu_c - \mu_t \neq 0$$

where μ_t and μ_c are the average fluid balance in the Starling SV and control groups, respectively.

4.2.2 Secondary Endpoints

4.2.2.1 For formal testing

Key secondary endpoints for the purposes of formal statistical testing are:

- Changes in serum creatinine level from baseline to 72 hours (key secondary endpoint)
- Requirement for renal replacement therapy (RRT)
- Requirement for ventilator use
- Length of ICU stay (days) until subject is medically ready for discharge
- Number of hours with ventilator use (30-day period)
- Number of hours with vasopressor use

4.2.2.2 Not for formal testing

Additional exploratory secondary endpoints identified for testing, but will not be included in the formal statistical testing procedures for key secondary endpoints are:

- Incidence of Adverse Events (AE)
- Number of ICU readmissions
- Mortality rate
- Volume of treatment fluid
- Incidence of Major Adverse Cardiac Event (MACE)
- Discharge location
- Mean difference in fluid balance at ICU discharge

5 SAMPLE SIZE

Subjects will be randomly assigned in a 2:1 ratio to treatment with Starling SV or to treatment with standard of care, stratified by time window of enrollment (0-6 hours, 6-12 hours, and 12-24 hours). The primary effectiveness endpoint for this study is fluid balance at ICU discharge.

Minimum enrollment (N_{min}) in the study will be set at 120 subjects (80 Starling SV and 40 control) to power at 80% for demonstration of superiority of means for the secondary endpoint of creatinine levels as a measurement of change from baseline at 72 hours. The secondary endpoint for change in creatinine levels at 120 evaluable subjects displays 80% power at a two-sided alpha level of 0.05 to demonstrate superiority of Starling SV under an assumption of an average treatment effect of -1.4 mg/dL with a standard deviation of 2.5 mg/dL.

Under an assumption of an average treatment effect of -2 L with a standard deviation of 3 L, the sample size of 120 evaluable subjects provides 92.7% power in a test of superiority of means for the primary effectiveness endpoint at a two-sided 0.05 level of significance.

The trial will incorporate a sample size re-estimation (SSR) at the time of the interim look (after 90 patients have been evaluated for the key secondary endpoint) based on promise for superiority in the key secondary endpoint. Sample size re-estimation, described in further detail in Section 3.1, will result in a maximum of 210 total evaluable subjects, or no more than 1.75 times the minimum of 120.

6 Analysis Populations

6.1 All Enrolled

Any subject who has signed informed consent will be included in the all enrolled population. Should a subject be considered a screen failure, the reason for failure will be documented and the subject will be exited from the study.

6.2 Intent to Treat Population (ITT)

The intent-to-treat (ITT) population for this study includes subjects that are enrolled in the study, have signed the ICF, meet study eligibility criteria, and have been randomized to either the treatment or control arm of the study.

6.3 Modified Intent to Treat Population (mITT)

Subjects will be included in the Modified Intent-to-Treat (mITT) group if they sign the ICF, meet study eligibility criteria, are randomized, and in the treatment group receive monitoring for the first 72 hours of study enrollment or until ICU discharge, whichever occurs first. The mITT population will be conducted as a supportive analysis population for the primary and secondary endpoints.

6.4 Per-Protocol (PP)

Subjects will be included in the Per-protocol group if they sign the ICF, are randomized and have the assigned procedure completed, meet critical study eligibility criteria and have no major protocol deviations (to be pre-defined as an attachment to the interim and final analyses), and, in the treatment group, receive monitoring for the first 72 hours of study enrollment. The PP population represents the primary analysis population for the primary and secondary endpoints.

6.5 Safety

Subject will be included in the safety group if they are in the ITT analysis population and study treatment was at least attempted. This is the primary analysis population for safety, including adverse events (this is a change from the protocol, where it states that adverse events will be reported on all enrolled patients; it has been since felt that this could underreport the true adverse event rates in patients to whom randomized treatment was attempted). Patients are analyzed under the treatment received.

7 General Issues for Statistical Analysis

7.1 Analysis Software

Analysis data sets, statistical analyses and associated output generated by Boston Biomedical Associates, LLC (BBA) will be generated using SAS® Software version 9.4 or later or R version

3.2.3 or later. BBA Standard Operating Procedures (SOPs) will be followed in the creation and validation of all analysis datasets, tables, listings, figures and analyses.

7.2 Disposition of Subjects and Withdrawals

All subjects who provide written informed consent will be accounted for. The number and percent of subjects in each analysis population will be presented, where the percentage is based on the number of enrolled subjects. The number and percent of PP, mITT and ITT subjects who completed each scheduled assessment will be presented. The frequency and percent of PP, mITT and ITT subjects randomized at each investigational site by treatment arm will be presented. A flow chart and listing will also summarize subject accountability.

The number and percentage of subjects prematurely withdrawing from the study will be presented all enrolled subjects by treatment group overall and by reason for premature withdrawal. Descriptive statistics (mean, standard deviation, median, minimum, maximum) of number of days on study will be presented by treatment group.

7.3 Methods for Withdrawals, Missing Data, and Outliers

All reasonable efforts will be made to obtain complete data for all subjects; however, missing observations will occur due to death, loss to follow-up, withdrawal, non-adherence to the protocol, and possible non-evaluable assessments. Since this is unlikely due to the short follow-up time schedule and the consistent nature of data collection, missing data will not be imputed. However, should more than 5% of subjects be missing evaluable data for the primary endpoint, then as a sensitivity analysis to assess the impact of missing data on the primary endpoint treatment comparisons, treatment group comparisons on the primary endpoint will be performed where missing data are first imputed via multiple imputation linear regression using the FCS method in SAS. The multiple imputation model will use, as covariates in the imputation model, the demographic variables shown in the demographics table shell in Appendix A. A total of 10 complete datasets will be created for the multiple imputation and the primary endpoint treatment comparisons will be carried out within each imputed dataset; results will then be combined across imputed datasets using standard multiple imputation techniques in PROC MIANALYZE to obtain one treatment comparison p-value on the primary endpoint. Tables detailing missing data and analysis populations will be provided in the final report. Should the sensitivity analysis be required, it will also be provided in the final report.

7.4 Protocol Violations

A protocol violation is a failure to comply with the requirements specified within this clinical study protocol. Protocol violations will be summarized in the CSR. This summary will include the number and percent of subjects (overall and by site) with each violation type for the ITT analysis set. Protocol violations, according to section 14.2 of the protocol and the Protocol Deviation CRF, include:

- Failure to obtain subject's informed consent prior to any study-related activities;
- Subject did not meet the inclusion and/or exclusion criteria and were enrolled;
- Failure to conduct protocol required clinical test or assessment;
- Failure to complete protocol required assessments within the required time frame
- Failure to perform assessment or test according to the protocol
- Failure to report serious adverse events according to protocol requirements.

7.5 Multiple Comparisons and Multiplicity

It is recognized that with a multiplicity of tests comes inflation in the chance of a false finding. Therefore, formal statistical testing will only be done for the following secondary endpoints:

- Changes in serum creatinine level from baseline to 72 hours (key secondary endpoint)
- Requirement for renal replacement therapy (RRT)
- Requirement for ventilator use
- Length of ICU stay (days) until subject is medically ready for discharge
- Number of hours with ventilator use (30-day period)
- Number of hours with vasopressor use

These endpoints will be tested in a hierarchical (sequential) manner in the order given above (and testing will only proceed if a significant beneficial treatment effect is detected in the primary endpoint). Specifically, the first secondary endpoint (change in creatinine) will be compared between treatments at a two-sided 0.05 level of significance. If significant in the direction favoring Starling SV, then Starling SV will be considered beneficial on change in serum creatinine level, and the next endpoint (ICU stay) will be compared between treatments at a two-sided 0.05 level of significance; otherwise the statistical testing will stop and Starling SV will not be considered statistically significantly beneficial on any secondary endpoint. If the treatment comparison is able to proceed to this next secondary endpoint of ICU stay, it will be carried out at the two-sided 0.05 level of significance. If the treatment comparison is significant in the direction favoring Starling SV, then Starling SV will be considered beneficial on this endpoint and testing will proceed in a similar manner for the remaining endpoints; otherwise, treatment comparisons will stop and Starling SV will not be considered beneficial on this second secondary endpoint of ICU stay nor on all endpoints after it in the above sequence.

7.6 Assessment of Homogeneity

To evaluate difference among sites in the study, summaries of baseline variables and endpoints by site will be tabulated for the PP analysis set. Comparisons will be made across sites for selected baseline variables. Continuous baseline data will be compared across sites for the PP analysis set using one-way ANOVA; categorical data will be compared across sites using the chi-square test.

To assess consistency in the treatment effect on the primary endpoint and on continuous secondary endpoints across sites, two-way ANOVAs with the main effects of treatment group and site and with the treatment group by site interaction effect will be carried out on the PP and mITT analysis sets. An endpoint with an interaction effect that is not significant at the 0.15 level of significance will be considered as supporting the poolability of data across sites for the treatment comparisons on that endpoint. Otherwise, treatment comparisons within sites will be inspected to determine the direction and magnitude of the interaction; if treatment effect is consistent in direction across sites centers but only differs in magnitude across sites, poolability of sites for the endpoint analysis is also supported. Any site with fewer than six subjects (combined treatment and control) will be combined into a single pooled site for purposes of this analysis.

The above assessment of consistency will be repeated across the following subgroups, with appropriate descriptive statistics being presented for each treatment group within each subgroup category:

- Patient disease state
- Patients determined to be fluid responsive vs non-fluid responsive
- AKI measures in patients determined to increase serum creatinine levels from baseline vs decrease serum creatinine levels from baseline (AKI measures vs. no AKI measures)
- Enrollment time frame (0-6 hours, 6-12 hours, 12-24 hours)
- Sex

Dichotomous secondary endpoint data will similarly be analyzed for poolability using logistic regression analysis.

8 DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

8.1 DEMOGRAPHICS

Demographics will be summarized by treatment group for the PP and mITT analysis sets. There will be no formal statistical comparisons between treatment groups on demographic variables. The continuous variables of age, height (cm), weight (kg), BMI, qSOFA, SOFA, FiO_2, and Glascow Coma Score will be summarized by treatment group using sample size, mean, standard deviation, minimum and maximum. For the categorical variables of sex (male, female), race (white, black or African American, American Indian/Alaska Native, Asian, Native Hawaiian or Other Pacific Islander, other, unknown), ethnicity (Hispanic or Latino, Not Hispanic or Latino, Unknown), known or presumed infection, SIRS criteria exhibited (Temperature >38 C, Temperature <36 C, Heart Rate > 90/min, Respiratory rate >20/min or PaCO2 <32mm, White Blood Cell Count >12000/mm3, White Blood Cell Count <4000/mm^3,White Blood Cell Count

>10% Immature Cells), smoking history (never, current, former), sepsis diagnosis (bacterial, fungal, viral, parasitic, other), Receiving Respiratory Support, and Cardiovascular Score (MAP ≥ 70 mmHg, MAP < 70 mmHg, Dopamine <5 or dobutamine (any dose), Dopamine 5.1-15 or epinephrine ≤0.1 or norepinephrine ≤0.1, Dopamine >15 or epinephrine >0.1 or norepinephrine >0.1), the number and percentage of patients in each category will be presented for each treatment group.

8.2 Prior and Concurrent Medications

A listing will be provided detailing subjects' medications, but no table is planned at this time.

8.3 Baseline Medical History

The medical history of all PP and mITT subjects will be summarized in a table by treatment group. For each condition, the number and percent of subjects who currently have the condition, who have a resolved history of the condition, and who have no prior history will be presented. The list of conditions is captured in the FRESH Medical History CRF.

8.4 Baseline Labs and Vital signs

A table presenting descriptive statistics (sample size, mean, standard deviation, median, min and max) of laboratory variables and vital signs by treatment group at baseline will be provided for the PP and mITT analysis sets. The laboratory variables are hemoglobin, serum creatinine, lactate levels, platelets, and bilirubin, and the arterial gases pH, $PaCO_2$, PaO_2, HCO_3, O_2CT, and O_2SAT. The vital signs are body temperature (C), heart rate (bpm), respiration rate (breaths/min), pulse oximetry (%), systolic blood pressure (mmHg), and diastolic blood pressure (mmHg).

9 Efficacy Analyses

All efficacy analyses will be performed on the PP analysis population (primary) and mITT analysis population (supportive).

9.1 Primary Efficacy Variable

The primary endpoint analysis is an evaluation of the difference between the two treatment groups in mean fluid balance (L) at 72 hours or ICU discharge, whichever occurs first. The following hypothesis will be tested:

$$H_o: \mu_c - \mu_t = 0$$
$$Ha: \mu_c - \mu_t \neq 0$$

where μ_t and μ_c is the average fluid balance in the Starling SV and control groups, respectively. Statistical summaries will include means, medians, quartiles and standard deviation for each

treatment group, as well as 95% confidence interval of the difference between treatment group means. Subjects that enter the study on dialysis, or are on chronic dialysis, are excluded from this endpoint. A two-sample t-test will be used to test the null hypothesis at a two-sided 0.05 level of significance. A significant p-value combined with a lower sample mean for Starling SV than for the control group will indicate statistical superiority of Starling SV over the control with respect to the primary endpoint. Fluid balance will be analyzed under sensitivity analyses not intended for formal hypotheses testing.

9.2 Secondary Efficacy Variables

9.2.1 For Formal Testing Using the Sequential Approach

If a significant beneficial Starling SV effect is found in the primary endpoint, the following secondary endpoints will be compared between treatments in the sequential manner discussed above.

9.2.1.1 Changes in Serum Creatinine Levels from Baseline to 72 hours or ICU discharge

The hypotheses of interest relating to the mean change in serum creatinine level from baseline to 72 hours or ICU discharge, whichever comes first, are as follows:

$$H_0: \mu_t = \mu_c$$
$$H_1: \mu_t \neq \mu_c$$

where μ_t and μ_c represent the mean change in serum creatinine level from baseline to 72 hours in the treatment and control groups, respectively. The treatment groups will be compared with respect to the mean days using an analysis of covariance adjusting for baseline serum creatinine. Subjects that enter the study on dialysis are excluded from this endpoint. Subjects that are put on dialysis within 72 hours, or prior to ICU discharge, will have the final creatinine value prior to dialysis used for the change from baseline calculation. All creatinine values post dialysis treatment are not applicable to this endpoint test. Summary of this endpoint will include the mean, median, quartiles, minimum, maximum and standard deviation of levels for each treatment arm, a 95% confidence interval for the difference in the two treatment arm mean levels, and the p-value from the test described above.

9.2.1.2 Requirement for Renal Replacement Therapy (RRT)

The hypotheses of interest relating to RRT are as follows:

$$H_0: P_t = \mathrm{P}_c$$
$$H_1: P_t \neq \mathrm{P}_c$$

where P_t and P_c represent the proportion of subjects requiring RRT in the treatment and control groups, respectively. Treatments will be compared using the chi-square test. Subjects that enter the study on dialysis, or are on chronic dialysis, are excluded from this endpoint. Summary of this endpoint will include the number and percentage of patients requiring RRT, a 95% confidence interval for the risk difference in the two arm percentages calculated using the normal

approximation to the binomial distribution, and the p-value from the test described above. If the observed event rates are less than 10% in either treatment group, a Fisher's exact test will be used to compare treatments instead of the chi-square test, and the 95% confidence of the risk difference will be based on the Wilson method instead of the normal approximation to the binomial distribution.

9.2.1.3 Requirement for Ventilator Use

The hypotheses of interest relating to ventilator use are as follows:

$$H_0: P_t = P_c$$
$$H_1: P_t \neq P_c$$

where P_t and P_c represent the proportion of subjects requiring ventilator use in the treatment and control groups, respectively. Treatments will be compared using the chi-square test. Subjects that enter the study on ventilation are excluded from this endpoint analysis. Summary of this endpoint will include the number and percentage of patients requiring ventilator use, a 95% confidence interval for the risk difference in the two arm percentages calculated using the normal approximation to the binomial distribution, and the p-value from the test described above. If the observed event rates are less than 10% in either treatment group, a Fisher's exact test will be used to compare treatments instead of the chi-square test, and the 95% confidence of the risk difference will be based on the Wilson method instead of the normal approximation to the binomial distribution.

9.2.1.4 Length of ICU Stay

The distribution of treatments with respect to length of ICU stay (days) will be compared between treatments using the Wilcoxon Rank Sum test given the expected skewness in this variable. Subjects that die while in the ICU will be censored from the analysis. ICU length of stay will be calculated using the earliest of date that the subject is medically ready for discharge when captured, the date of discharge, or the study exit date.

Summary of this endpoint will include the mean, median, quartiles, minimum, maximum and standard deviation of days for each treatment arm, a 95% confidence interval for the difference in the two treatment arm mean days, and the p-value from Wilcoxon Rank Sum test.

9.2.1.5 Number of Hours with Ventilator Use

The hypotheses of interest relating to the number of hours free from ventilator use (within 30 days) are as follows:

$$H_0: \mu_t = \mu_c$$
$$H_1: \mu_t \neq \mu_c$$

where μ_t and μ_c represent the mean number of hours in the treatment and control groups, respectively. The treatment groups will be compared with respect to the mean hours using a two-

sided two-sample t-test. Subjects that enter the study on ventilation are excluded from this endpoint analysis. Summary of this endpoint will include the mean, quartiles, median, minimum, maximum and standard deviation of hours for each treatment arm, a 95% confidence interval for the difference in the two treatment arm mean hours, and the p-value from the test described above.

9.2.1.6 Number of Hours with Vasopressor Use

The hypotheses of interest relating to the number of hours with vasopressor use are as follows:

$$H_0: \mu_t = \mu_c$$
$$H_1: \mu_t \neq \mu_c$$

where μ_t and μ_c represent the mean number of hours in the ICU in the treatment and control groups, respectively. The treatment groups will be compared with respect to the mean hours using a two-sided two-sample t-test. Summary of this endpoint will include the mean, median, quartiles, minimum, maximum and standard deviation of hours for each treatment arm, a 95% confidence interval for the difference in the two treatment arm mean hours, and the p-value from the test described above.

9.2.2 NOT FOR FORMAL TESTING

9.2.2.1 Incidence of Treatment Emergent Adverse Events (AE)

A Treatment Emergent Adverse Event (TEAE) is one that started or worsened in severity during or after randomized treatment was attempted. The number and percentage of patients with incidence of at least one TEAE will be summarized by treatment arm in a table. The summary will include the number and percent of subjects experiencing one or more AEs cumulatively up to 72 hours, one week, and 30 days. Though no formal hypothesis tests are planned, 95% confidence intervals for the difference in rates between the two arms at each time point will be completed and included using the normal approximation to the binomial distribution.

The analysis will be repeated for serious TEAS. In addition, the number and percentage of patients with at least one TEAEs will be presented by severity and relationship to study procedure; patients with more than one TEAE will be categorized according to the maximum severity and maximum relationship experienced.

9.2.2.2 Number of ICU Readmissions

The number of ICU readmissions through 30 days will be summarized by treatment arm in a table. The summary will include the number and percent of subjects with 0, 1, or >1 ICU readmissions. Though no formal hypothesis tests are planned, 95% confidence intervals for the difference in rates between the two arms will be completed and included using the normal approximation to the binomial distribution or Wilson method (if an observed rate is less than 10% in any treatment group).

9.2.2.3 Mortality Rate

The mortality rate will be summarized by treatment arm in a table. The summary will include the number and percent of subjects who died by arm; Kaplan-Meier mortality curves will be presented for each treatment group. Though no formal hypothesis tests are planned, 95% confidence intervals for the difference in rates between the two arms will be completed and included using the normal approximation to the binomial distribution or Wilson method (if an observed rate is less than 10% in any treatment group).

9.2.2.4 Volume of Treatment Fluid

The volume of treatment fluid will be summarized by treatment arm in a table. The summary will include the mean, standard deviation, median, minimum and maximum by arm. Though no formal hypothesis tests are planned, 95% confidence intervals for the difference in means between the two arms will be completed and included.

9.2.2.5 Incidence of Major Adverse Cardiac Event (MACE)

The incidence of MACE through to 72 hours, one week, and 30 days will be summarized by treatment arm in a table. The summary will include the number and percent of subjects by arm by category. Though no formal hypothesis tests are planned, 95% confidence intervals for the difference in rates between the two arms will be completed and included using the normal approximation to the binomial distribution or Wilson method (if an observed rate is less than 10% in any treatment group). Kaplan-Meier curves of time to first component of MACE will be presented by treatment group.

9.2.2.6 Discharge Location

The discharge location will be summarized by treatment arm in a table. The summary will include the number and percent of subjects by arm by category. Though no formal hypothesis tests are planned, 95% confidence intervals for the difference in rates between the two arms will be completed and included using the normal approximation to the binomial distribution or Wilson method (if an observed rate is less than 10% in any treatment group).

9.2.2.7 Fluid Balance at ICU discharge

The fluid balance at ICU discharge will be summarized by treatment arm in a table. The summary will include the mean, standard deviation, median, quartiles, minimum and maximum by arm. Though no formal hypothesis tests are planned, 95% confidence intervals for the difference in means between the two arms will be completed and included.

10 Adverse Events

All adverse events (AEs) will be coded using the standardized Medical Dictionary for Regulatory Activities (MedDRA) central coding dictionary, version 19.1 or greater. Adverse event analyses will be performed on the mITT and Safety analysis populations.

10.1 All Treatment Emergent Adverse Events

The number of TEAEs and the number and percentage of patients with at least one TEAE, with at least one device-related TEAE, with at least one serious TEAE and with at least one device-related TEAE will be presented by treatment group. These summaries will be presented for TEAEs occurring from start of treatment through 72 hours, from 72 hours through Discharge, from Discharge through 30 days, and from the start of treatment through 30 days.

The number of TEAEs and the number and percentage of patients with at least one TEAE will be presented for each treatment group by MedDRA system organ class and preferred term. This analysis will be repeated for serious TEAEs (collected through 72 hours only), device-related TEAEs, and AEs leading to withdrawal from the study.

In addition, the by-treatment number and percentage of patients with at least one TEAEs will be presented by severity and relationship to study procedure within each system organ class and preferred term; patients with more than one TEAE within a given system organ class and preferred term will be categorized according to the maximum severity and maximum relationship experienced.

A listing of all adverse events will include the subject number, AE number, days since index procedure, the AE SOC and PT, the severity of AE, whether the AE is classified as serious (SAE), the relationship of the AE to the investigational device or procedure, the action taken, the outcome, and the adjudication status.

10.2 Laboratory Values and Vital Signs

Descriptive statistics of each laboratory value and vital sign and of the change from baseline for each will be presented at each post-baseline visit at which they are measured. Descriptive statistics to be presented are mean, standard deviation, median, minimum, and maximum.

10.3 Deaths

In addition to the secondary endpoint analysis, should any subjects die during the FRESH study, relevant information will be supplied in a data listing.

11 Reporting Conventions

All reporting will meet the standards of BBA SOP BS002 and its associated work instructions.

Addendum to the Statistical Analysis Plan

Protocol Title (Number):

Evaluation of Fluid Volume in Patients with Sepsis and Refractory Hypotension (FRESH Study)
PRO-0001

Addendum 1 to Rev. A of the SAP

Prepared by:

BBA

July 9, 2018

Change in Key Secondary Endpoint:

The original protocol and statistical analysis plan (SAP) specified changes in serum creatinine from baseline as a key secondary endpoint. Baseline was defined as creatinine level drawn at ~ 6 months prior to hospitalization, obtained by chart review. The goal of this study endpoint was to have a change in creatinine serve as an indicator of acute kidney injury. However, obtaining a true baseline of serum creatinine was inconsistently available within the medical chart in these subjects. Therefore, the pre-defined secondary endpoint of incidence of new Renal Replacement Therapy (RRT) will serve as an indicator of acute kidney injury.

The study was initially powered for the secondary endpoint of change in serum creatinine from baseline (Section 5 in the SAP). It is expected that the rate of required RRT in the control arm will be 25%, and the Starling SV system will reduce requirement for RRT by 60%, for a treatment rate of 10%. The planned sample size of n=120 yields 57.5% power to reject the null hypothesis that the treatment arm is superior to the control arm. While the study was initially underpowered for requirement for the RRT endpoint, it is the best available replacement indicator of acute kidney injury.

Change in Interim Analysis (Section 3.1.1 in SAP):

The trial will incorporate a sample size re-estimation (SSR) at the time of the interim look based on promise for superiority in the new key secondary endpoint of requirement for RRT. The maximum enrollment being considered for this trial is 210 total enrolled (N_{max} = 1.75 times N_{min} = 120, where the sample size of 120 was determined based on serum creatinine as noted in Section 5 of the SAP).

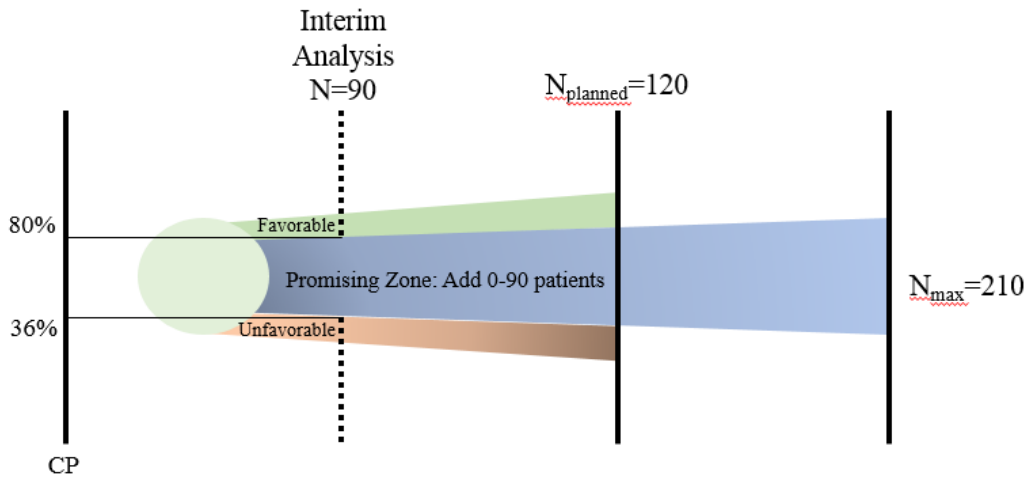
The interim analysis on the key secondary endpoint incorporating a SSR will take place when 90 subjects have been enrolled and completed the key secondary endpoint evaluation. The interim analysis will be performed by an independent statistician.

There will be no inspection of the primary study endpoint at this interim stage. The primary study endpoint will be tested as final at the formal planned final sample size of 120 patients and will not incorporate an alpha spent. In the event of an SSR due to the key secondary endpoint, the primary endpoint will be tested again at the final sample size. No alpha-adjustment is needed across this potential multiple testing of the primary endpoint since the primary endpoint will need to be met at <u>both</u> N_{min}=120 and, if the sample size is increased to >120, at the final sample size.

After 90 patients have completed the secondary endpoint, the conditional power to claim superiority (CP_{sup}) on the key secondary endpoint for requirement of RRT will be computed assuming a planned final sample size of 120 patients. The conditional power (CP_{sup}) will be calculated based on the observed interim results of the key secondary endpoint, with the specific interest of demonstrating superiority of the experimental treatment versus the control. The data will be partitioned into three zones (see Figure 1) based on CP_{sup} unfavorable zone (CP_{sup}<36%), promising zone (36%≤CP_{sup}<80%), and favorable zone (CP_{sup}≥80%). If CP_{sup}<36% or CP_{sup}≥80% the study will continue as is. If 36%≤CP_{sup}<80% then the sample size may be increased (to a maximum of 210 total) to yield 80% CP_{sup} without increasing Type I error, following the methodology of Mehta and Pocock (2011).[1]

[1] Mehta, Cyrus and Pocock, Stuart. *Adaptive increase in sample size when interim results are promising: A practical guide with examples.* Statistics in Medicine. 2011, 30 3267–3284

Figure 1. Promising Zone Overview for FRESH Trial



CP=Conditional Power
Probability of success (statistical significance) at the end of the trial given current data trend

Change in Order of Secondary Endpoints (Section 4.2.2.1 in SAP):

Key secondary endpoints for the purposes of formal statistical testing are:

- Requirement for renal replacement therapy (RRT) (key secondary endpoint)
- Requirement for ventilator use
- Length of ICU stay (days) until subject is medically ready for discharge
- Number of hours with ventilator use (30-day period)
- Number of hours with vasopressor use
- Changes in serum creatinine level from baseline to 72 hours

EVALUATION OF FLUID/VOLUME IN PATIENTS WITH SEPSIS AND REFRACTORY HYPOTENSION

Protocol Number: PRO-0001

SAP Release Log

Document	Effective Date
Statistical Analysis Plan Rev. A	June 28, 2018
Addendum 1 to the SAP Rev. A	July 09, 2018

Summary of Changes Noted in Addendum 1

Page	Changes
2	Change in key secondary endpoint: Change in serum creatinine level from baseline to 72 hours was dropped and incidence of new Renal Replacement Therapy (RRT) was made the key secondary endpoint due to inability to reliably obtain prehospital (~ 6 month prehospital) serum creatinine. Expected rate of RRT was determined and study power was reassessed: *It is expected that the rate of required RRT in the control arm will be 25%, and the Starling SV system will reduce requirement for RRT by 60%, for a treatment rate of 10%. The planned sample size of n=120 yields 57.5% power to reject the null hypothesis that the treatment arm is superior to the control arm. While the study was initially underpowered for requirement for the RRT endpoint, it is the best available replacement indicator of acute kidney injury.*
2	Change to Interim Analysis: Endpoint for Interim Analysis was changed to the new key secondary endpoint. Additionally, reference for conditional power testing updated: *The data will be partitioned into three zones based on CPsup unfavorable zone (CPsup<36%), promising zone (36%≤CPsup<80%), and favorable zone (CPsup≥80%). If CPsup<36% or CPsup≥80% the study will continue as is. If 36%≤CPsup<80% then the sample size may be increased (to a maximum of 210 total) to yield 80% CPsup without increasing Type I error, following the methodology of Mehta and Pocock (2011).*
3	Change in order of secondary endpoints: Ordering of key secondary endpoints was adjusted. Key endpoints were to be tested sequentially and testing stopped if the intervention was not considered statistically significantly beneficial. *•Requirement for renal replacement therapy (RRT) (key secondary endpoint)* *• Requirement for ventilator use* *• Length of ICU stay (days) until subject is medically ready for discharge* *• Number of hours with ventilator use (30-day period)* *• Number of hours with vasopressor use* *• Changes in serum creatinine level from baseline to 72 hours*

BBA
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MEMO TO FILE

Sponsor: Cheetah Medical, Inc.

Study: *FRESH*

Created By: Mark McIlduff

Date: August 28, 2019

Re: SAP approval for the FRESH study

This memo to file serves as a record of the approval process for the SAP and subsequent data analysis in the FRESH study. A request for the timeline of SAP approval resulted from the review of the JAMA manuscript submission.

Rev. H of the clinical protocol was issued on 17Oct2017. SAP drafts were authored by BBA post protocol finalization, with an advanced draft circulated 07Mar2018. A final SAP was executed as Rev. A on 28Jun2018, prior to trial data unblinding to the sponsor or statistician at BBA.

BBA SOPs specify that an SAP must be approved and pre-specify analyses prior to any primary analysis and unblinding of study data. BBA is ISO-13485 certified to the 2016 standards by BSI and follows this process regularly.

Following the Rev. A release, an addendum to the SAP was authored on 09Jul2018 as it became clear through data cleaning that creatinine data was incomplete and would not be appropriate to use as the key secondary endpoint for sample size re-sizing.

The pre-specified sample size re-estimation was performed on 10Jul2018. The primary endpoint analysis was first performed as defined in the protocol and SAP on 13Mar2019.

BBA Signature:

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General Manager
Boston Biomedical Associates, LLC

28 Aug 2019
Date

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