CLINICAL TRIAL PROTOCOL

An Open-label, Single-dose, Randomized, Two-way, Two-period Crossover Study to Compare the Pharmacokinetics and Bioavailability of a Novel Furosemide Regimen Administered Subcutaneously Versus the Same Dose (80 mg) Administered Intravenously in Subjects with Chronic Heart Failure

Protocol Number:	SQI-01-01
Version:	Amendment 4
IND Number:	150023
Clinical Phase:	1/2
Investigational Drug:	Furosemide Injection for Subcutaneous Infusion
Indication:	Chronic Heart Failure
Sponsor:	SQ Innovation, Inc. 20 Mall Road, Suite 220 Burlington, MA 01803
Medical Monitor:	Kimberly Bennett, MD, MPH Nuventra 2525 Meridian Parkway, Suite 280 Durham, NC 27713
Principal Investigator:	Bruce G. Rankin, DO, CPI, FACOFP Medical Director DeLand Clinical Research Unit Accel Research Sites 860 Peachwood Drive DeLand, Florida 32720 386-785-2380
Clinical Laboratory:	LabCorp 5610 W. LaSalle Street Tampa, Florida 33507
Protocol Date:	7 October 2020 (Original, 4 March 2020)

Statement of GCP Compliance

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki in its most recent version, clinical research guidelines established by the Code of Federal Regulations (CFR), the International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines, and all applicable local regulations and laws. Essential documents will be archived in accordance with applicable regulations.

Confidentiality Statement

The information contained in this document and all information provided related to Furosemide Injection for Subcutaneous Infusion ("Study Drug") are the confidential and proprietary information of SQ Innovation,

PROTOCOL APPROVAL PAGE

Study Title:	An Open-label, Single-dose, Randomized, Two-way, Two-period Crossover Study to Compare the Pharmacokinetics and Bioavailability of a Novel Furosemide Regimen Administered Subcutaneously Versus the Same Dose (80 mg) Administered Intravenously in Subjects with Chronic Heart Failure
Protocol Number:	SQI-01-01
Version:	Amendment 4, (Original, 4 March, 2020)
Date of Issue:	7 October 2020
Sponsor Name and Address:	SQ Innovation, Inc. 20 Mall Road, Suite 220 Burlington, MA 01803

I have read and approve this protocol and agree on its content. It is confirmed that the information and guidance given in this protocol complies with scientific principles, the guidelines of Good Clinical Practices, the Declaration of Helsinki in the latest relevant version, and the applicable legal and regulatory requirements.

Sponsor Signatory:

Pieter Muntendam, MD
President and CEO
SQ Innovation, Inc.

(Date)

INVESTIGATOR PROTOCOL AGREEMENT

Protocol Title:	An Open-label, Single-dose, Randomized, Two-way, Two-period Crossover Study to Compare the Pharmacokinetics and Bioavailability of a Novel Furosemide Regimen Administered Subcutaneously Versus the Same Dose (80 mg) Administered Intravenously in Subjects with Chronic Heart Failure	
Protocol Number:	SQI-01-01	Amendment 4, 7 October 2020 (Original Protocol, 4 March, 2020)

By my signature, I

- Confirm that my staff and I have carefully read and understand this protocol and the Investigator's Brochure and are thoroughly familiar with the appropriate use of the investigational drug and methods of administration described herein.
- Agree to comply with the conduct and terms of the study specified herein and with any other study procedures provided by the Sponsor.
- Agree to comply with US Food and Drug Administration (FDA) regulations, the International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) guidelines, the Declaration of Helsinki, and all applicable rules, regulations, and federal, state, and local laws relating to the conduct of clinical studies and the protection of human subjects.
- Agree not to implement changes to the protocol without agreement from the Sponsor and prior written approval (where required) from the Institutional Review Board (IRB) or Independent Ethics Committee (IEC), except when necessary to eliminate an immediate hazard to the subjects.
- Agree to onsite monitoring of the electronic case report forms (eCRFs) and source documents by the Sponsor or designee and to audit by the Sponsor or designee and appropriate regulatory authorities, including, but not limited to, the FDA and IRB/IEC inspectors.
- Agree to supervise the conduct of the study and maintain responsibility for training and supervising all personnel who have delegated responsibilities under my leadership. The protocol and other important study materials will be available to study staff throughout the conduct of the study.

_____ _____

Investigator's Signature Date

_____

Print Name

SUMMARY OF AMENDMENT CHANGES

Date of Revision	Version	Summary of Changes
4 March 2020	Original	None
9 Apr 2020	Amendment 1	1. Exclusion criteria were added to minimize subjects that might have or introduce the Coronavirus Disease 2019 (COVID-19) pandemic. 2. Follow-up Visit changed to a Follow-up Phone Call. 3. Added sections describing steps related to this study to mitigate the COVID-19 spread (Section 3.3), and any clinical study reporting of COVID-19 cases (Section 8.1.5).
June 30, 2020	Amendment 2	1. Added device risks to Section 1.2.3. 2. Clarified device training for site staff in Section 3.2. 3. Removed Exclusion 17 in Section 4.2, Exclusion Criteria. 4. Clarified drug and device accountability in Section 5.5. 5. Removed Appendix 2, Quick Reference Guide (original Appendix 2) and adjusted Appendix numbering. 6. Clarified description of study drug selection in Pain Scale Assessment in Appendix 2. 7. Updated typographical errors.
3 September, 2020	Amendment 3	1. Removal of reference to investigational Infusor for use in study and replace with FDA cleared Medfusion (v6) infusion pump (multiple locations). 2. Addition of VEKLURY® as an additional Captisol containing product approved by the FDA in Section 1.1.2.2. 3. Removal of Infusor information from Section 1.1.3. 4. Expansion and update of study related risks as requested by FDA and introduction of risks related to use of the FDA cleared infusion pump and infusion set in Section 1.1.3 5. Replacement of Device (Infusor) Instructions with a section regarding Operating Instructions for the FDA cleared infusion pump and set (Section 3.2).

		6. Modified "A washout period of 7 days" to "A washout period of a minimum of 7 days" in Section 3.6. 7. Replaced information on placement of the Infusor with information on placement of infusion set cannula in Section 5.1.1. 8. Change title of Section 5.2 from 'Dosage" to "Dosage and Administration" and inserted a section pertaining to use of the Medfusion 3500 (v6) pump and the selection of the infusion set. 9. Added language about preparing the syringe for use with the Medfusion 3500 (v6) syringe pump to Section 5.4.4. 10. Replaced language pertaining to interruption of infusion with the Infusor with language pertaining to the use of the FDA cleared pump including, under certain circumstances, of the option for individuals to reschedule the treatment with the pump in case of interruption of infusion for certain reasons in Section 6.4. 11. Correction of an error in Section 7.9.1. referencing need for blood samples in the opposite arm of where the subcutaneous infusion is administered. 12. Expanding the instructions in Section 8.1.1. to include the need to record device- (infusion pump/system) related adverse events. 13. Added Section 8.1.7 pertaining to recording and reporting adverse events related to the infusion pump and system. 14. Corrected an error in Appendix 2 regarding pain assessment for maximum pain during infusion. 15. Added Appendix 4: Medfusion 3500 (v6) Operator's manual 16. Corrected typographical errors.
07 October, 2020	Amendment 4	17. Corrected an error pertaining to the day of COVID-19 testing from Day -8 (minus 8) to Day 8 (admission for 2nd treatment) 18. In Section 5.2 Dosage and Administration removed reference to the programming using the PharmaGuard applicaion and replaced this with the delivery parameters to be set prior to use of the MedFusion pump.

SYNOPSIS

PROTOCOL TITLE	An Open-label, Single-dose, Randomized, Two-way, Two-period Crossover Study to Compare the Pharmacokinetics and Bioavailability of a Novel Furosemide Regimen Administered Subcutaneously Versus the Same Dose (80 mg) Administered Intravenously in Subjects with Chronic Heart Failure
PROTOCOL NUMBER	SQI-01-01
PHASE	1/2
INVESTIGATIONAL PRODUCT	Investigational subcutaneous furosemide formulation at 30 mg/mL at pH 7.4 (range 7.0 to 7.8), intended for slow subcutaneous infusion using a biphasic delivery profile. 80 mg furosemide to be administered by subcutaneous infusion over 5 hours using a biphasic delivery profile of 30 mg in first hour and 12.5 mg/hour for 4 hours. Subcutaneous doses will be administered using a commercial precision syringe infusion pump.
REFERENCE PRODUCT	Furosemide Injection, USP (Hospira, Inc., Lake Forest, IL, USA), administered by intravenous (IV) bolus over 2 minutes. It contains furosemide 10 mg/mL in solution at alkaline pH of 8.0 to 9.3 and is marketed for IV and intramuscular (IM) injection.
STUDY OBJECTIVES	The primary objective of the study is: • To estimate the absolute bioavailability of furosemide administered by continuous subcutaneous infusion compared with an equivalent dose of furosemide administered by IV bolus administration. The secondary objectives of the study are: • To characterize the pharmacokinetics (PK) of furosemide administered by continuous subcutaneous infusion using a biphasic delivery profile. • To characterize the pharmacodynamics (diuresis and natriuresis) of furosemide after administration by continuous subcutaneous infusion or IV. • To characterize the tolerability of administration of furosemide by continuous subcutaneous infusion and by IV.
STUDY ENDPOINTS	The primary endpoint is relative absolute bioavailability following 5-hour subcutaneous infusion based on a comparison of AUC (subcutaneous: IV) of furosemide. The secondary endpoints are: • PK parameters over the timeframe of 24 hours, including, but not limited to, maximum plasma concentration (Cmax), time to Cmax (Tmax), area under the concentration versus time curve (AUC) from time 0 to the last measurable plasma concentration (AUClast) and to infinity (AUCinf), half-life ($t_{½}$), apparent systemic clearance and volume of distribution (subcutaneous only), and systemic clearance and volume of distribution (IV only). • Urine volume and sodium concentration in urine collected over 8 hours and 24 hours post-dose. • Infusion site pain (IV and subcutaneous administration) measured using subject reported scales, and presence of erythema and edema (subcutaneous

	only) both recorded through photography and assessed by Principal Investigator (PI)/designee-reported scales.
STUDY DESIGN	This study will be an open-label, single-dose, randomized, two-way, two-period crossover study in 20 adult subjects previously diagnosed with mild to moderate heart failure (New York Health Association [NYHA] class II/III) being treated with oral furosemide therapy at a dose of ≥40 mg/day. Each subject will complete Screening, Admission, Treatment, and Follow-up Phone Call phases. The Screening Phase will be conducted on an outpatient basis between 28 and 3 days prior to admission. Subjects will be instructed to reduce sodium intake (target <2 g sodium/day) starting 3 days prior to each of the clinical research unit (CRU) admissions. Admission (Day -1) consists of CRU admission and final qualification assessments. The Treatment Phase will comprise 2 crossover periods separated by a 7-day outpatient fluid re-equilibration washout. Subjects will discontinue oral furosemide at least 24 hours prior to administration of study drug for each Crossover Period (e.g., no oral furosemide should be administered after 10pm the night prior to CRU admission). Subjects will be randomly assigned in a 1:1 ratio to 1 of 2 treatment sequences (AB or BA) to receive both subcutaneous furosemide (Treatment A; Test) and IV furosemide (Treatment B; Reference) in Crossover Periods (i.e., subcutaneous followed by IV or vice versa). Subjects will remain domiciled in the CRU for each treatment Period during the Treatment Phase through 24 hours after administration of study drug, after which time they will be discharged from the CRU if safety parameters are acceptable to the Investigator. Oral furosemide therapy will be re-initiated at discharge after Treatment 1 (i.e., during the 7-day fluid re-equilibration washout) and after Treatment 2 (i.e., between discharge and Follow-up Phone Call). The Follow-up Phone Call Phase will occur 7 days (± 1) after discharge from the CRU following Treatment 2, completing subjects' study participation.
STUDY & PARTICIPANT DURATION	The study is targeted to last 2 months, from the first subject first visit to the last subject last visit. Individual subject duration is not expected to exceed 45 days (from Screening to Follow-up Phone Call).
NUMBER OF PLANNED SUBJECTS	20 total subjects are planned. Subjects who withdraw/are withdrawn or subjects with major protocol deviations may be replaced, upon consultation with the Sponsor.
PARTICIPATING COUNTRIES	This study will take place in the US.
STUDY POPULATION	Adult subjects previously diagnosed with mild to moderate heart failure (NYHA class II/III) being treated concomitantly with oral furosemide therapy at a dose of ≥40 mg/day.

KEY INCLUSION & EXCLUSION CRITERIA	**Inclusion Criteria:** Subjects will be considered for inclusion only if they meet **all** of the following criteria: 1. An Institutional Review Board (IRB)-approved informed consent is signed and dated prior to any study-related activities. 2. Male and female subjects ≥18 and ≤80 years of age, with body weight <130 kg and body mass index (BMI) <38 kg/m^2. 3. Females will be non-pregnant, non-lactating, or post-menopausal, or surgically sterile (e.g., tubal ligation, hysterectomy), 4. Females of childbearing potential will use TWO of the following forms of contraception: intrauterine device (IUD), IUD with spermicide, female condom with spermicide, contraceptive sponge with spermicide, an intravaginal system, diaphragm with spermicide, cervical cap with spermicide, a male sexual partner who agrees to use a male condom with spermicide, a sterile sexual partner. 5. History of at least 3 months treated heart failure (NYHA class II/III) with presence of symptoms of chronic volume overload requiring ongoing treatment with oral furosemide at a dose of ≥40 mg per day for at least 30 days prior to Day -1. 6. Agrees to abstain from using alcohol, caffeine-containing products, and tobacco-/nicotine-containing products while in residence at the CRU. 7. Able to participate in the study in the opinion of the Investigator. 8. Has the ability to understand the requirements of the study and is willing to comply with all study procedures. **Exclusion Criteria:** Subjects will be excluded from participation if they meet **any** of the following criteria: 1. Acute Decompensated Heart Failure (ADHF) or recent history of hospitalization for heart failure in the last 4 weeks. 2. Worsening of signs or symptoms of heart failure in the 2 weeks prior to the Screening, or those expected to require IV loop diuretics or inpatient treatment for heart failure during the study. 3. Systolic blood pressure (SBP) <90 mmHg. 4. Temperature ≥38°C (oral or equivalent) or sepsis or active infection requiring IV anti-microbial treatment. 5. Serum sodium <130 mEq/L and serum potassium <3.5 mEq/L. 6. Significant other cardiac abnormalities which may interfere with study participation or study assessments. 7. Current or planned treatment during the study with any IV therapies, including inotropic agents, vasopressors, levosimendan, nesiritide or analogues; or mechanical support (intra-aortic balloon pump, endotracheal intubation, mechanical ventilation, or any ventricular assist device). 8. Subject is cachectic. 9. Diagnosed with Type I diabetes mellitus or Type II diabetes requiring insulin therapy. 10. Presence or need for urinary catheterization, urinary tract abnormality, or disorder interfering with urination. 11. Impaired renal function, defined as an estimated glomerular filtration rate (eGFR) on admission <45 mL/min/1.73 m^2, calculated using the simplified Modification of Diet in Renal Disease (sMDRD) equation. 12. Indication of moderate-to-severe hepatic dysfunctions as determined by the Investigator. 13. Administration of IV radiographic contrast agent within 72 hours prior to Screening or acute contrast-induced nephropathy at the time of Screening. 14. Major surgery within 30 days prior to Screening.

	15. Administration of an investigational drug or implantation of investigational device, or participation in another interventional trial, within 30 days prior to Screening. 16. Any surgical or medical condition, which in the opinion of the Investigator may pose an undue risk to the subject, interfere with participation in the study, or which may affect the integrity of the study data. 17. Positive test for hepatitis B surface antigen (HBsAg), hepatitis C (HCV), or human immunodeficiency virus (HIV) at Screening. 18. Any positive urine drug screen at Screening or clinic admission. 19. Concomitant use of any drugs known to interact with furosemide. 20. History of alcohol abuse within 6 months prior to Screening and/or signs or symptoms of alcoholism, as determined by the Investigator. 21. Any positive alcohol test on admission to the CRU. 22. History of severe allergic or hypersensitivity reactions to furosemide. 23. Donation of greater than 100 mL of either whole blood or plasma within 30 days prior to study drug administration. 24. Been informed of possible COVID-19 exposure in past 4 weeks, or recent onset of signs or symptoms of possible COVID-19 infection, including cough, shortness of breath, or temperature ≥ 38°C. 25. Traveled via airplane or cruise ship within the last 14 days.
STATISTICAL ANALYSIS	**Analysis Populations:** The Safety Population will consist of all subjects who receive at least 1 dose of study drug. The PK Population will consist of all subjects who receive at least 1 dose of study drug and have at least one furosemide PK concentration. The Analysis Population includes all subjects in the PK Population with sufficient concentration-time data to calculate the PK profile for at least one treatment and without a protocol deviation that significantly affects the PK profile. **PK Analysis** Plasma samples will be collected as follows: **For IV furosemide:** Plasma samples will be collected pre-dose, and at 2 minutes (immediately after the IV bolus injection is complete), 5, 15, 30, 60, 120, and 180 minutes, and at 4, 6, 8, 12, 16, and 24 hours after the start of infusion. **For subcutaneous furosemide:** Plasma samples will be collected pre-dose and at 30, 60, 90, 120, 180, 240, 300 (immediately after completion of the infusion), 305, 315, 330, and 345 minutes, and at 6, 7, 8, 10, 12, 14, 16, and 24 hours after the start of infusion. PK parameters will be derived using noncompartmental methods. Compartmental modeling of the PK data may be conducted if necessary. Furosemide concentrations will be summarized using descriptive statistics (including sample size [N], mean, standard deviation [SD], coefficient of variation [CV%], median, minimum, and maximum) for each treatment. Derived plasma PK descriptive statistics will be tabulated by dosing group and summary statistics. Descriptive statistics for PK parameters will include the arithmetic mean (all parameters) and geometric mean (for Cmax, AUClast, and AUCinf, only), CV%, SD of the arithmetic mean, median, minimum, maximum, and N.

	Bioavailability Analysis The pharmacokinetic parameters (Cmax, AUClast and AUCinf) of the Analysis Population will be assessed using a linear repeated measures mixed-effect model appropriate for a 2-period crossover design with treatment and period as fixed effects. A heterogeneous-compound symmetry covariance matrix will be used to allow for unequal treatment variances and to model the correlation between the 2 treatment measurements within each subject. The Kenward-Roger method will be used to calculate the denominator degrees of freedom for the fixed effects. A log transformation will be applied to the Cmax, AUClast and AUCinf data. Back transformed summary statistics will be reported for the pharmacokinetic parameters. The ninety (90%) confidence intervals (CIs), based on the t-distribution, will be generated from the above mixed-effect model for the least-square geometric mean ratios for Cmax, AUClast and AUCinf to compare subcutaneous (Test) to IV (Reference) administration. **Pharmacodynamic Analysis** The pharmacodynamic variables (urine volume and sodium concentration) will be assessed using a linear repeated measures mixed-effect model appropriate for a 2-period crossover design with treatment and period as fixed effects. BMI and eGFR may be included as covariates in the model. A heterogeneous-compound symmetry covariance matrix will be used to allow for unequal treatment variances and to model the correlation between the 2 treatment measurements within each subject. The Kenward-Roger method will be used to calculate the denominator degrees of freedom for the fixed effects. The least-squares mean difference between the treatment groups, 90% CIs and p-value will be calculated. **Safety Analysis** All safety data will be listed by subject. The Safety Population will be used to create the following summaries: Treatment-emergent adverse events (TEAEs) will be summarized for each treatment by system organ class, preferred term, severity, and relationship to study drug. Observed values and changes from baseline for clinical laboratory test data, safety electrocardiograms (ECGs), physical examination results, and vital signs will be summarized using appropriate descriptive statistics.

SCHEDULE OF ASSESSMENTS

Table 1-1. Time and Events Schedule

Study Period	Screening		Admission	Treatment Period 1 [a]			Washout [b]		Treatment Period 2 [a]			Follow-up Phone Call /ET[c]
Overall Study Day	--28 to -3	-2 to -1	-1	1 (Pre-dose)	1 to 2 (0 – 24h)	2 (>24h)	2 to 7	8	9 (Pre-dose)	9 to 10 (0 – 24h)	10 (>24h)	17 ± 1
Informed consent	X											
Confirmation of eligibility	X		X					X				
Medical history & baseline demographics	X		X [d]									
Prior medication assessment	X		X [d]									
Full physical examination [e]	X											
Brief physical examination [e]			X					X				
Height measurement	X											
Weight measurement	X		X	X		X		X	X		X	
BMI	X		X					X				
12-lead electrocardiogram	X		X	X		X		X	X		X	
Vital signs [f]	X		X	X	X	X		X	X	X	X	
Hematology, Urinalysis [g]	X		X			X		X			X	
Serum chemistry [g]	X		X	X	X			X	X	X		
Pregnancy test [h]	X		X					X				
FSH test [i]	X											
Serology (HIV, HCV, HBsAg)	X											
Urine drug screen; alcohol screen	X		X					X				
Reduced sodium diet [j]		X	X	X	X	X	X	X	X	X	X	
Required fluid intake					X					X		
Clinic admission			X					X				
Randomization [k]			X									
Discontinue furosemide [l]			X					X				
Re-initiate furosemide [l]						X					X	
Study drug administration [m]					X					X		

Study Period	Screening		Admission	Treatment Period 1 [a]			Washout [b]		Treatment Period 2 [a]			Follow-up Phone Call /ET[c]
Overall Study Day	--28 to -3	-2 to -1	-1	1 (Pre-dose)	1 to 2 (0 – 24h)	2 (>24h)	2 to 7	8	9 (Pre-dose)	9 to 10 (0 – 24h)	10 (>24h)	17 ± 1
Pharmacokinetic sampling [n]				X	X					X	X	
Urine collections with urine volume and sodium on pooled samples [o]					X					X		
Local tolerance assessment [p]					X					X		
Adverse events assessments			Continuous						Continuous			X
Concomitant medications			Continuous						Continuous			X
Clinic discharge						X					X	
Follow-up Phone Call												X

BMI = body mass index; ET = early termination; FSH = follicle-stimulating hormone; HBsAg = Hepatitis B surface antigen; HCV = hepatitis C; HIV = human immunodeficiency virus

a. For treatment sequence AB, Treatment 1 will be Treatment A (investigational furosemide for subcutaneous injection) and Treatment 2 will be Treatment B (reference drug; Furosemide Injection, USP); for treatment sequence BA, Treatment 1 will be Treatment B and Treatment 2 will be Treatment A.
b. Administration of each dose should be separated by 7 days to allow for treatment washout and fluid re-equilibration.
c. End of study assessments should be attempted in the event of early termination (ET) including vital signs, serum chemistry, hematology, urinalysis, ECG, and adverse event assessments. If the subject discontinues prior to completion of PK sampling, a blood sample for pharmacokinetic (PK) analysis should be collected as soon as possible after the decision to withdraw has been made, unless the subject withdraws consent.
d. Confirm and update medical history and prior medication history, if needed. Note the time and dosage of the last oral furosemide dose.
e. Physical examinations should be conducted as described in Section 7.5. The full physical examination should include evaluation of skin, ENT (ears, nose, and throat), head, eyes, lungs/chest, heart, abdomen, musculoskeletal, extremities, neurologic, and lymphatic. The brief physical examination should only include skin, head, lungs/chest, heart, abdomen, and musculoskeletal systems. Additional examinations may be conducted as clinically indicated.
f. Vital signs (blood pressure, pulse, respiratory rate, and body temperature) should be taken after the subject has rested in the semi-recumbent position for at least 5 minutes. On dose administration days, vitals will be taken prior to dosing and at 4 hours after dosing (within 10 minutes prior to PK sampling if timepoints coincide). The exact time of collection should be recorded
g. Blood and urine will be collected for hematology, serum chemistry, and urinalysis at Screening, CRU admission, and CRU discharge. In addition, blood will be drawn for serum chemistry at pre-dose and 24 hours after dose administrations. The exact time of collection should be recorded.
h. Blood (serum) pregnancy test at Screening, and urine pregnancy test at all other timepoints for females of childbearing potential.
i. FSH test to confirm post-menopausal status only.
j. Subjects will be instructed to avoid salty foods (target <2 g sodium/day) within 3 days prior to each CRU admission. Healthy meals (target <2 g sodium/day) will be provided while subjects are domiciled in the CRU, and subjects will not have access to high sodium snacks/meals.
k. Subjects will be randomized to treatment sequence AB or BA as described in Section 3.5.

l. Subjects should discontinue their prescribed oral furosemide regimen at least 24 hours prior to administration of study drug, i.e., no oral furosemide after 10pm on the night prior to check-in into the CRU. Previously prescribed oral furosemide therapy should be re-initiated at clinic discharge, after completion of assessments for each treatment period.
m. Treatment A (furosemide injection for subcutaneous administration; 80 mg) will be administered by subcutaneous infusion over 5 hours using a biphasic delivery profile of 30 mg in first hour and 12.5 mg/hour for 4 hours; administration will be performed using a commercial precision syringe infusion pump and an infusion set qualified for the study by the sponsor. Treatment B (Furosemide Injection USP; 80 mg), concentration 10 mg/mL will be administered by IV bolus over 2 minutes. Detailed instructions for administration are provided in Section 5.1.
n. Blood samples for analysis of furosemide concentrations for PK will be collected at pre-dose and at the timepoints detailed in Section 7.9.1 for Treatment A and for Treatment B. Actual time of collection must be recorded. PK blood draws should adhere to specified time windows in Section 7.9.2.
o. Spontaneous urine samples should be collected and pooled for the following time intervals post-initiation of furosemide administration: 0 to 1 hour; 1 to 2 hours; 2 to 4 hours; 4 to 6 hours; 6 to 8 hours; 8 to 10 hours; 10 to 12 hours; 12 to 18 hours; and 18 to 24 hours. Subjects should attempt to void prior to study drug administration. Urine samples will be analyzed for urinary sodium as described in Section 7.10.
p. Local tolerance should be assessed for pain on a 0 to 10-point numerical rating scale for all treatments. Pain assessments should be collected upon start of treatment, for the maximum pain during infusion, and upon removal. In addition, for subcutaneous infusion only, the skin should be photographed and evaluated for erythema or edema (scored on 0 to 4-point scales, according to Section 7.13.2) upon removal, at 6 hours and at 24 hours after the start of infusion.

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ABBREVIATIONS

ADHF	Acute Decompensated Heart Failure
AE	Adverse event
AESI	Adverse events of special interest
AUC0-24	area under the concentration versus time curve from time 0 (pre-dose) to 24 hours post-dose
AUCinf	Area under the concentration versus time curve from time 0 to infinity
AUClast	Area under the concentration versus time curve from time 0 to the last measurable plasma concentration
BMI	Body mass index
CRT	Cardiac resynchronization therapy
CRT-D	Cardiac resynchronization therapy defibrillator
CRF	Case Report Form
Cmax	Maximum plasma concentration
CI	Confidence interval
CL/F	Apparent clearance
COVID-19	Coronavirus disease 2019
CRU	Clinical research unit
CSR	Clinical study report
CV%	Coefficient of Variation
ECG	Electrocardiogram
eCRF	Electronic case report form
eGFR	Estimated glomerular filtration rate
ET	Early termination
FSH	Follicle-Stimulating Hormone
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HCV	Hepatitis C Virus
HIV	Human immunodeficiency virus
ICD	Implantable cardioverter-defibrillator
ICF	Informed consent form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IM	Intramuscular

IRB	Institutional Review Board
IUD	Intrauterine device
IV	Intravenous
N	Sample size
NDA	New Drug Application
NYHA	New York Heart Association
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PKAP	Pharmacokinetic analysis plan
PI	Principal Investigator
SAE	Serious Adverse Events
SAP	Statistical analysis plan
SBECD	Sulfobutylether beta-cyclodextrin
SBP	Systolic blood pressure
SD	Standard deviation
sMDRD	Simplified Modification of Diet in Renal Disease
SQI	SQ Innovation Inc.
t½	Half-life
TEAE	Treatment-emergent adverse event
Tmax	Time to maximum plasma concentration
US	United States
Vz/F	Volume of distribution

1. INTRODUCTION

1.1 Background

Congestion is the primary reason for hospitalization for decompensated heart failure (Gheorghiade et al., 2010). Although the initial phase of congestion is often asymptomatic, patients at some point become increasingly symptomatic and seek medical attention. Unless the patient's clinical condition requires immediate referral, during initial contacts with their health care provider, clinicians will often try to reverse the course by temporarily increasing (doubling) the oral dose of loop diuretics. If this increase in oral dose is ineffective, patients are customarily referred to a hospital setting where intravenous (IV) furosemide is administered in a hospital outpatient clinic, special heart failure diuresis clinic, urgent care center, emergency room, observation unit for short-term stay, or during regular inpatient admission.

During a standard admission for worsening heart failure, a typical heart failure patient loses 8.4 L of urine over 5.8 days, resulting in a weight loss of over 15 pounds over the hospital stay (Miller and Mullan, 2014). The primary reason for requiring IV diuretics is compromised oral bioavailability of the loop diuretic as a result of congestion. Most patients admitted for worsening heart failure only receive diuretic therapy (Fonarow et al., 2007). The clinical dilemma of requiring inpatient care for reversal of congestion is widely recognized and has prompted attempts to develop a method that provides hospital-strength diuresis without the need for IV administration.

This has led to the development of novel drug-device combination products for subcutaneous delivery of buffered loop diuretic formulations (Francis and Alexy, 2018). Indeed, a novel proprietary furosemide formulation (developed by scPharmaceuticals) infused subcutaneously using a 5-hour delivery profile was found to be associated with complete bioavailability and equivalent diuresis (Sica et al., 2018), demonstrating the validity of this approach.

The furosemide formulation (SQIN-01) investigated in this study is the drug constituent component of an investigational drug-device combination. SQIN-01 is formulated with Captisol®, a sulfobutylether beta-cyclodextrin (SBECD) excipient needed to solubilize furosemide at neutral pH. SQIN-01is administered according to the 5-hour delivery profile: 30 mg (1 mL) delivered over the first 60 minutes, followed by 12.5 mg per hour for 4 hours (1.7 mL), for a total volume of 2.7 mL over 5 hours. The device constituent component will not be investigated in this study. An FDA cleared commercial precision syringe infusion pump and infusion set will be used in this study set to the same biphasic delivery profile of the to-be-marketed SQIN-01 drug-device combination product.

The SQIN-01pharmaceutical formulation has not been subject to United States (US) Food and Drug Administration (FDA) review and approval.

Nonclinical experience with SQN01 and Captisol are summarized in Section 1.2.1 and clinical experience with the active ingredient, furosemide, and Captisol are summarized in Section 1.2.2. Additional detail can be found in the Investigator's Brochure.

1.1.1 Nonclinical Experience

Nonclinical experience with furosemide for injection is detailed in the package insert (see Appendix 1)**.**

The toxicology of Captisol was extensively investigated involving mouse, rat, rabbit, dog, and monkey including acute, chronic and reproductive toxicology studies. For further information see the, the Investigator's Brochure.

1.1.2 Clinical Experience

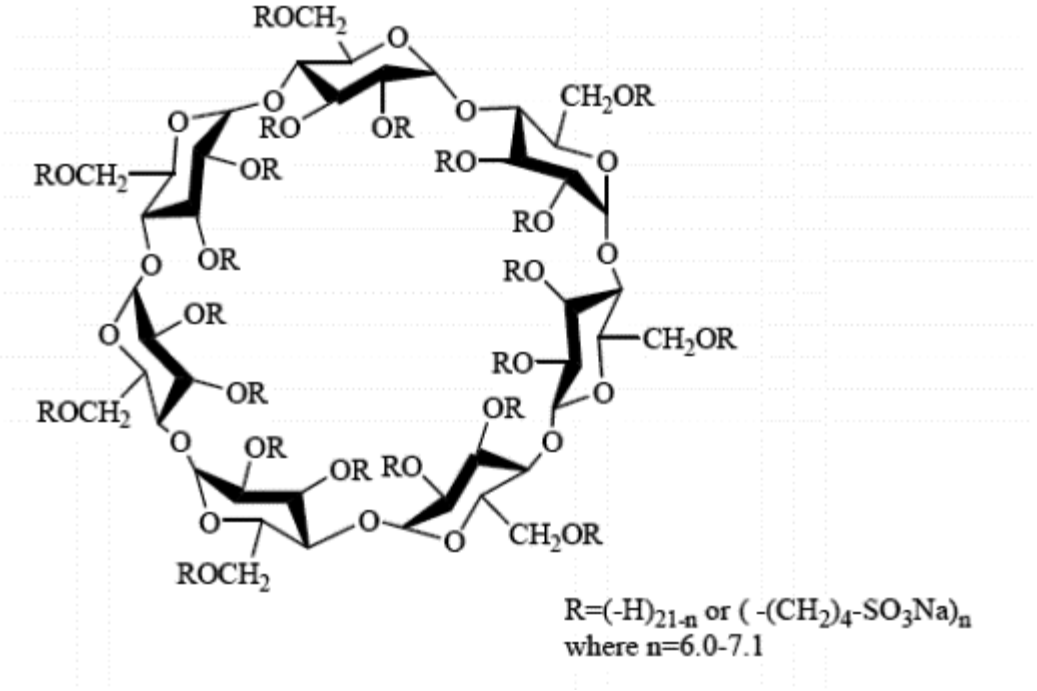
1.1.2.1 Furosemide

Furosemide was first approved in tablet form in July 1966 (NDA: 016273) and as furosemide injection, solution 10 mg/mL on 03/20/1968 for intramuscular (IM) and IV administration (NDA: 016363). Furosemide injection is subject to the USP Monograph Furosemide Injection. Clinical experience with furosemide for injection is described in the package insert (Appendix 1).

1.1.2.2 Captisol

Captisol® (Betadex Sulfobutyl Ether Sodium, NF; also known as a sulfobutylether beta-cyclodextrin [SBECD]) serves as a solubilizing agent. Its chemical structure is shown in Figure 1-1. This solubilizing agent is used in other previously approved parenteral formulations including VFEND®, GEODON®, ABILIFY®, NEXTERONE®, KYPROLIS® CARNEXIV®, ZULRESSO® and VEKLURY® (COVID-19 EUA), with daily SEBECD delivery of 300 mg (ABILIFY) to 20,000 mg (CARNEXIV). ZULRESSO was approved in March of 2019 (New Drug Application [NDA] 211371) and has an average daily SBECD delivery of 5940 (based on a 75 kg body weight). Additionally, there are currently multiple Captisol-enabled pharmaceutical products in clinical development.

Figure 1-1. Chemical Structure of Captisol®



Parenteral products containing SBECD have been commercially available since 2002 (VFEND, NDA 021267, approved 05/24/2002, and GEODON®, NDA 020919, 06/21/2002) and are generally considered safe.

Renal safety of cyclodextrins has been a subject of concern in the past, and it has been proposed that SQIN-01 be contraindicated in patients with anuria, in patients with End Stage chronic kidney disease (glomerular filtration rate, [GFR] <15 mL/min), and in patients with a history of hypersensitivity to furosemide.

1.1.3 Assessment of Potential Risks and Benefits

The investigational product has not been approved for any indication by the FDA or any other regulatory agency. The reference product, Furosemide injection, USP, is approved by the FDA for the treatment of edema associated with congestive heart failure, cirrhosis of the liver and renal disease, including the nephrotic syndrome, in adults and pediatric patients. It is also indicated as an adjunctive therapy in acute pulmonary edema. Approved furosemide products for human use include oral and parenteral products. Parenteral products are approved for IV and IM administration. Following its launch in the 1960s furosemide has become one of the most widely used prescription pharmaceutical products globally and is generally consider safe.

The furosemide formulation used in this study is formulated with a proprietary solubility enhancer call Captisol®. This is a compound belonging to a class called beta-cyclodextrins, which is a derivative of starch. Captisol® and related beta-cyclodextrins are commonly used in pharmaceutical preparations to enhance the solubility of compounds. Captisol® is generally considered safe within the dosage range used in this study and is used in multiple FDA and internationally approved pharmaceutical products.

Use of any pharmaceutical product, invasive procedures and study procedures involving electronic or electromechanical devices carries inherent risks, which may include, biological contamination, chemical contamination, skin trauma, while the use of electrical equipment carries the risk of device explosion or fire, device overheating, and electromagnetic interference.

For specific risks, warnings and precautions carefully read instructions of use and other labeling of the devices and products used in conjunction with the study. All personal using study drug, study supplies and operating study-specific equipment such as the FDA cleared precision infusion pump must be trained by the sponsor or its designee and adhere to all study procedures and instructions. Risks associated with the use of the FDA-cleared commercial infusion pump include:

- Risk of explosion when used in the presence of flammable anesthetics, oxygen or explosive gasses.
- Electroshock
- Electromagnetic interference
- Radio frequency interference
- Risk of physical injury when used in an MR environment
- Dosing error when used syringes and materials not approved and qualified for use with the pump
- Risks associated with unauthorized use or user error

Risks associated with the use of the syringe and infusion include:

- Biological contamination.
- Chemical contamination
- Skin trauma

Risks associated with administration of Furosemide Injection, USP are detailed in the FDA-approved package insert (Appendix 1) and are expected to be comparable to those risks associated with administration of oral furosemide ≥40 mg/day (treatment required by inclusion criteria).

Briefly, these risks include excessive diuresis, electrolyte depletion, and hypokalemia. Increases in blood glucose and alterations in glucose tolerance tests have also been observed, and rarely, precipitation of diabetes mellitus has been reported. In addition, furosemide can cause acute urinary retention related to increased production and retention of urine in patients with severe symptoms of urinary retention. Subjects with Type I or Type II diabetes, with impaired renal function, and with serum sodium and potassium <130 mEq/L and <3.0 mEq/L, respectively, will be excluded from this study, and all patients will be observed for signs and symptoms of fluid or electrolyte imbalance.

Adverse reactions for furosemide (by decreasing severity) include:

- gastrointestinal system reactions (hepatic encephalopathy in patients with hepatocellular insufficiency; pancreatitis; jaundice [intrahepatic cholestatic jaundice]; increased liver enzymes; anorexia; oral and gastric irritation; cramping; diarrhea; constipation; nausea; and vomiting).

- systemic hypersensitivity reactions (severe anaphylactic or anaphylactoid reactions [e.g., with shock]; systemic vasculitis; interstitial nephritis; and necrotizing angiitis).
- hematologic reactions (aplastic anemia; thrombocytopenia; agranulocytosis; hemolytic anemia; leukopenia; anemia; and eosinophilia).
- dermatologic-hypersensitivity reactions (toxic epidermal necrolysis; Stevens-Johnson Syndrome; erythema multiforme; drug rash with eosinophilia and systemic symptoms; acute generalized exanthematous pustulosis; exfoliative dermatitis; bullous pemphigoid; purpura; photosensitivity; rash; pruritus; urticaria).
- cardiovascular reactions (orthostatic hypotension may occur and be aggravated by alcohol, barbiturates or narcotics; increase in cholesterol and triglyceride serum levels).
- other reactions (hyperglycemia; glycosuria; hyperuricemia; muscle spasm; weakness; restlessness; urinary bladder spasm; thrombophlebitis; transient injection site pain following IM injection; and fever).

Risks associated with subcutaneous and IV administration also include potential for infusion site discomfort and reactions. In two clinical studies of furosemide administered by subcutaneous infusion over 5 hours in a total of 26 patients with chronic, stable heart failure (Sica et al., 2018) as well as by IV infusion, study drug administration was well tolerated. Infusion site reactions included 1 subject with very slight erythema (Score 1) during subcutaneous administration, 8 subjects with very slight erythema after completion of infusion and removal of infusion set/adhesive, 1 subject with well-defined erythema during IV administration (Score 2), and 6 subjects with minimal swelling (very slight edema, Score 1) during or following administration.

Current understanding of risks associated with subcutaneous administration of the investigational SQIN furosemide formulation are based on inference from a) experience with subcutaneous administration of Furosemide Injection, USP or the novel formulation studied by Sica et al., b) the pre-clinical and clinical safety profile of Captisol containing products and c) the pre-clinical studies conducted with the SQIN furosemide formulation. Based on these combined information sources no particular local or system reactions are anticipated that are different from those of other furosemide products administered subcutaneously.

Risks to the subjects due to study procedures are considered minor. Collecting a blood sample from a vein may cause pain, swelling, bruising, light-headedness, fainting, and very rarely, clot formation, nerve damage, and/or infection at the site of the needle stick. During cannulation, more than one attempt may be needed to insert the cannula in a vein of a subject, and it is possible that bruising and/or inflammation may be experienced at the site of cannulation.

Electrocardiogram (ECG) stickers on subjects' chests and limbs may cause some local irritation and may be uncomfortable to remove, but subjects will be closely monitored to ensure any local irritation does not persist.

Overall, the risks of administration of 80 mg furosemide using this novel formulation are anticipated to be minor, and additional study-related risks are considered minor and acceptable in order to obtain important pharmacokinetics (PK), pharmacodynamics (PD), and safety during this study. Parenteral diuretics are not indicated for participants at the time of the study and participants will not derive a medical benefit from the treatment or participation in the study.

1.2 Study Rationale and Dose Justification

This is an open-label, single-dose, randomized, two-way, two-period crossover study designed to accurately estimate the bioavailability of furosemide administered as 2 different regimens: 80 mg administered subcutaneously over 5 hours using a biphasic delivery profile (30 mg in the first hour and 12.5 mg/h for 4 hours) and 80 mg furosemide injection administered IV over 2 minutes (reference product and regimen). The study is also designed to determine and compare the PK and PD of these regimens.

For the investigational furosemide formulation, the dose of 80 mg was selected based available information regarding diuretic efficiency of furosemide, expert consultation, and published reports on the use this dose (Gilotra et al., 2018, Sica et al., 2018). Administration of 30 mg furosemide over the first 60 minutes followed by 12.5 mg per hour for 4 hours is expected to optimize diuretic efficiency. Furosemide Injection, USP (Hospira, Inc., Lake Forest, IL, USA) is routinely used at doses of 80 mg or greater administered IV over 1 to 2 minutes. For this study, in consultation with the US-FDA (PIND 14378 June 11, 2019 – Q17), a dose of 80 mg has been selected to be administered by IV bolus over 2 minutes. These doses have been selected to address the primary endpoint of estimating the absolute bioavailability of furosemide administered by 5-hour subcutaneous infusion when compared to furosemide administered by IV bolus.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 Objectives

2.1.1 Primary Objective

The primary objective of the study is to estimate the absolute bioavailability of furosemide administered by continuous subcutaneous infusion compared with an equivalent dose of furosemide administered by IV bolus administration.

2.1.2 Secondary Objective

The secondary objectives of the study are:

- To characterize the PK of furosemide administered by 5-hour subcutaneous infusion using a biphasic delivery profile.
- To characterize the PD (diuresis and natriuresis) of furosemide after administration by 5-hour subcutaneous infusion or IV.
- To characterize the tolerability of administration of furosemide by 5-hour subcutaneous infusion and by IV.

2.2 Endpoints

2.2.1 Primary Endpoint

The primary endpoint is the relative absolute bioavailability following 5-hour subcutaneous infusion based on a comparison of AUC of furosemide resulting from each administration (subcutaneous:IV).

2.2.2 Secondary Endpoints

The secondary endpoints are:

- PK parameters over the timeframe of 24 hours, including, but not limited to, maximum plasma concentration (Cmax), time to Cmax (Tmax), area under the concentration versus time curve (AUC) from time 0 (pre-dose) to 24 hours post-dose (AUC0-24), AUC from time 0 to the last measurable plasma concentration (AUClast) and to infinity (AUCinf), half-life (t½), apparent systemic clearance and volume of distribution (subcutaneous only), and systemic clearance and volume of distribution (IV only).
- Urine volume and sodium concentration in urine collected over 8 hours and 24 hours post-dose.
- Infusion site pain/discomfort (IV and subcutaneous administration) measured using patient reported scales, and presence of erythema and edema both recorded through photography and assessed by Principal Investigator (PI)/designee-reported scales.

3. INVESTIGATIONAL PLAN

3.1 Overall Study Design

This study will be an open-label, single-dose, randomized, two-way, two-period crossover study in 20 adult subjects previously diagnosed with mild to moderate heart failure (New York Health Association [NYHA] class II/III) being treated concomitantly with oral furosemide therapy at a dose of ≥40 mg/day.

Each subject will complete Screening, Admission, Treatment, and Follow-up Phone Call phases. The Screening Phase will be conducted on an outpatient basis between 28 and 3 days prior to Admission. The Treatment Phase will comprise 2 crossover treatment periods separated by a 7-day outpatient fluid re-equilibration and washout.

Prior to admission to the clinical research unit (CRU), subjects will be instructed to avoid high salt foods (target <2 g sodium) starting on Day -3 as described in Section 6.1. Subjects should discontinue oral furosemide at least 24 hours prior to administration of study drug (e.g., no oral furosemide should be administered after 10 pm the night prior to CRU admission), as described in Section 6.2.

Subjects will be admitted to the CRU on Day -1, and final qualification assessments will be completed. Subjects will be randomly assigned in a 1:1 ratio to 1 of 2 treatment sequences (AB or BA) to receive subcutaneous furosemide (Treatment A; Test) and IV furosemide (Treatment B; Reference). Subjects randomized to sequence AB will receive subcutaneous furosemide for

Treatment Period 1 and IV furosemide for Treatment Period 2, with treatments administered vice versa for sequence BA.

Study treatment based on the subject's assigned treatment sequence will be administered beginning in the morning of Day 1. Furosemide subcutaneous infusion will be administered by a qualified FDA cleared commercial precision syringe infusion pump as 30 mg delivered over the first 60 minutes (total 1 mL), followed by 12.5 mg per hour for 4 hours (total 1.7 mL), for a total concentration and volume administered over 5 hours of 80 mg and 2.7 mL, respectively. Furosemide Injection, USP (10 mg/mL) will be administered as an IV bolus over 2 minutes, such that the total amount of furosemide administered is 80 mg. PK, PD, and safety assessments will be collected over 24 hours, as detailed in the Schedule of Assessments (Table 1-1).

Subjects will remain domiciled in the CRU through 24 hours after administration of study drug. Subjects will be discharged from the CRU after all Day 2 study procedures and assessments have been completed, provided that safety parameters are acceptable to the Investigator. After the conclusion of Treatment 1 and prior to discharge, the PI or qualified designee will provide individualized instructions to each subject on how to safely resume their previously prescribed oral furosemide therapy.

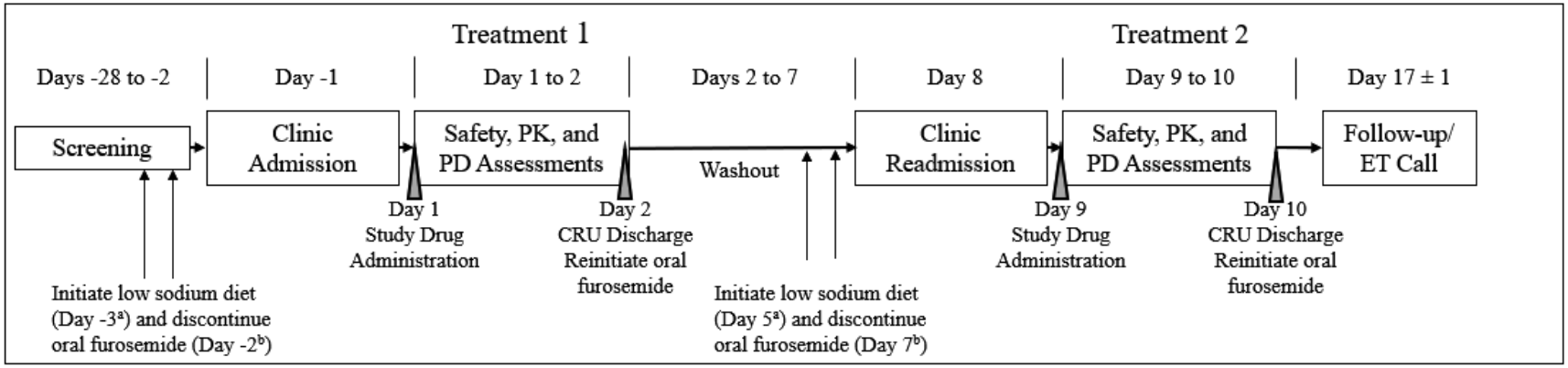
Subjects will repeat the same procedures for Treatment Period 2 as for Treatment Period 1. Subjects will again be instructed to avoid high salt foods (target a <2 g sodium/day) starting 3 days prior to CRU admission and will discontinue oral furosemide at least 24 hours prior to administration of study drug (e.g., no oral furosemide should be administered after 10 pm the night prior to CRU admission). Subjects will be admitted to the CRU on the day before dosing in Treatment Period 2 (Day 8), and eligibility will be reconfirmed. subcutaneous or IV furosemide will then be administered on Day 9, according the subject's randomization sequence, with PK, PD, and safety assessments collected over 24 hours, as detailed in the Schedule of Assessments Table 1-1.

After completion of study assessments, a Follow-up Phone Call day and time will be scheduled, and the subject will be discharged if safety parameters are acceptable to the Investigator. Prior to discharge, the PI or qualified designee will provide individualized instructions to each subject on how to safely resume their previously prescribed oral furosemide therapy.

The Follow-up Phone Call will occur 7 days (± 1) after discharge from the CRU following Treatment Period 2, completing subjects' study participation.

The study schematic is provided in Figure 3-1. The Schedule of Assessments is provided in Table 1-1.

Figure 3-1. Study Schematic



[a] Subjects should begin avoiding salty foods (target <2 g sodium/day) at any time starting 3 days prior to clinic research unit (CRU) admission (Day -3 or Day 5).

[b] Oral furosemide should be discontinued at least 24 hours prior to study drug administration (e.g., no oral furosemide should be administered after 10 pm the night prior to CRU admission [Day -2 or Day 7]). Oral furosemide should be re-initiated at CRU discharge on Day 2 and Day 10.

3.2 Infusion Pump Operating Instructions

Only site staff who have undergone documented infusion pump training specific to the use of the study custom infusion program and qualified infusion set may perform pump-related operations. The staff will handle all aspects of operation of the infusion pump and the infusion set. The study participant will be instructed not to touch the pump, the infusion set or interfere with the subcutaneous infusion. Written operating instructions for the site staff will be provided in the study procedures manual. Each infusion pump will be labeled with a unique identification number.

3.3 Coronavirus Disease Spread Mitigation

As described in the FDA Guidance, Conducting Clinical Trials on Medical Products During COVID-19 Pandemic (March, 2020), steps will be implemented for this study to reduce the risk of infection and transmission of the disease.

- Masks and gloves will be worn by site staff interacting with subjects in person, regardless of their position. Frequent handwashing will be mandated.
- Subjects will be required to wear masks at all times except for eating, drinking, and sleeping.
- Subjects will be separated from other subjects by a minimum of 6 feet for the entirety of their presence in the site.
- During the washout, subjects will be instructed to restrict travel and stay at home as per the local guidelines in effect at the time of the subject's washout period. During the subject's washout period, masks will be provided and subjects will be requested to wear masks when traveling outside their home.
- As part of exclusion criteria, direct exposure of subjects to confirmed COVID-19 patients will be assessed on Day -1 and Day 8. Non-febrile temperature will be confirmed.
- If quick-testing of COVID-19 becomes available for use, testing may be incorporated prior to clinical admission on Day-1 and Day 8. Information concerning the COVID-19 testing will be included into the informed consent, which will be resubmitted to the IRB. Subjects will reconsent prior to the use. If applicable, the clinical study report (CSR) will contain information regarding the testing description and results.

Description of clinical study reporting related to COVID-19 is provided in Section 8.1.5.

3.4 Study Stopping Criteria

The availability of any new adverse safety information related to the investigational product may result in stopping the trial. The Investigator, Sponsor, or the IRB/IEC may take such actions. If the trial is terminated for safety reasons, subjects will be notified immediately and assured that appropriate treatment and follow-up will be available. If the Investigator terminates the trial, he/she will inform the Sponsor, subjects, and IRB/IEC about the reason for such action. If the Sponsor terminates the trial, the Sponsor will inform the Investigator, the IRB/IEC, and the subjects of the reason for such an action. Similar notifications will be sent by the IRB if the Sponsor takes such an action.

3.5 Randomization and Blinding

Subjects will be randomized to one of two treatment sequences per the randomization code after final qualification assessments have been completed for the first dosing period. The study will be conducted as an open-label trial without blinding.

3.6 Trial Period and Duration of Subject Participation

The study is targeted to last 2 months, from the first subject first visit to the last subject last visit.

Individual subject duration is not expected to exceed 45 days (from Screening to Follow-up Phone Call) and will include:

- Screening
- Two admission/treatment periods of approximately 48 hours each
- A washout period of a minimum of 7 days
- A Follow-up Phone Call scheduled on Day 17 (± 1 day) after discharge from Treatment Period 2.

3.7 End of Study Definition

The end of the study for each subject will be defined as completion of all described assessments, up to and including the Follow-up Phone Call. In the event of ET, assessments described in Section 4.5 for Early Termination should be attempted, unless subject withdraws consent.

4. STUDY POPULATION

The study population will consist of adult subjects previously diagnosed with mild to moderate heart failure (NYHA class II/III) being treated concomitantly with oral furosemide therapy at a dose of ≥40 mg/day.

4.1 Inclusion Criteria

Subjects will be considered for inclusion only if they meet **all** of the following criteria:

1. An IRB-approved informed consent is signed and dated prior to any study-related activities.
2. Male and female subjects ≥18 and ≤ 80 years of age, with body weight <130 kg and body mass index (BMI) <38 kg/m^2.
3. Females must be non-pregnant, non-lactating, or post-menopausal or surgically sterile (e.g., tubal ligation, hysterectomy).
4. Females of childbearing potential will use TWO (2) of the following forms of contraception: intrauterine device (IUD), IUD with spermicide, female condom with spermicide, contraceptive sponge with spermicide, an intravaginal system, diaphragm with spermicide, cervical cap with spermicide, a male sexual partner who agrees to use a male condom with spermicide, or a sterile sexual partner.

5. History of at least 3 months treated heart failure (NYHA class II/III) with presence of symptoms of chronic volume overload requiring ongoing treatment with oral furosemide at a dose of ≥40 mg per day for at least 30 days prior to Day -1.
6. Agrees to abstain from using alcohol, caffeine-containing products, and tobacco-/nicotine-containing products while in residence in the CRU.
7. Able to participate in the study in the opinion of the Investigator.
8. Has the ability to understand the requirements of the study and is willing to comply with all study procedures.

4.2 Exclusion Criteria

Subjects will be excluded from participation if they meet **any** of the following criteria:

1. Acute Decompensated Heart Failure (ADHF) or recent history of hospitalization for heart failure in the last 4 weeks.
2. Worsening of signs or symptoms of heart failure in the 2 weeks prior to the Screening, or those expected to require IV loop diuretics or inpatient treatment for heart failure during the study.
3. Systolic blood pressure (SBP) <90 mmHg.
4. Temperature ≥38°C (oral or equivalent) or sepsis or active infection requiring IV anti-microbial treatment.
5. Serum sodium <130 mEq/L and serum potassium <3.5 mEq/L.
6. Significant other cardiac abnormalities which may interfere with study participation or study assessments.
7. Current or planned treatment during the study with any IV therapies, including inotropic agents, vasopressors, levosimendan, nesiritide or analogues; or mechanical support (intra-aortic balloon pump, endotracheal intubation, mechanical ventilation, or any ventricular assist device).
8. Subject is cachectic.
9. Diagnosed with Type I diabetes mellitus or Type II diabetes requiring insulin therapy.
10. Presence or need for urinary catheterization, urinary tract abnormality, or disorder interfering with urination.
11. Impaired renal function, defined as an estimated glomerular filtration rate (eGFR) on admission <45 mL/min/1.73 m^2, calculated using the simplified Modification of Diet in Renal Disease (sMDRD) equation.
12. Indication of moderate-to-severe hepatic dysfunctions as determined by the Investigator.
13. Administration of IV radiographic contrast agent within 72 hours prior to Screening or acute contrast-induced nephropathy at the time of Screening.
14. Major surgery within 30 days prior to Screening.
15. Administration of an investigational drug or implantation of investigational device, or participation in another interventional trial, within 30 days prior to Screening.
16. Any surgical or medical condition, which in the opinion of the Investigator may pose an undue risk to the subject, interfere with participation in the study, or which may affect the integrity of the study data.

17. Positive test for hepatitis B (HBsAg), hepatitis C (HCV), or human immunodeficiency virus (HIV) at Screening.
18. Any positive urine drug screen at Screening or clinic admission.
19. Concomitant use of any drugs known to interact with furosemide (see the package insert in Appendix 1**)**.
20. History of alcohol abuse within 6 months prior to Screening and/or signs or symptoms of alcoholism, as determined by the Investigator.
21. Any positive alcohol test on admission to the CRU.
22. History of severe allergic or hypersensitivity reactions to furosemide.
23. Donation of greater than 100 mL of either whole blood or plasma within 30 days prior to study drug administration.
24. Been informed of possible COVID-19 exposure in past 4 weeks, or recent onset of signs or symptoms of possible COVID-19 infection, including cough, shortness of breath, or temperature ≥ 38°C.
25. Traveled via airplane or cruise ship within the last 14 days.

4.3 Screen Failures

A Screen Failure will be defined as any study candidate who signs the informed consent but does not qualify for the study. Screen Failures will not have electronic case report forms (eCRFs) completed; however, the study candidate's unique screening number/initials, date screened, and reason(s) for screening failure must be recorded on the Screening Log.

4.4 Removal of Subjects

Subjects will be informed that they have the right to withdraw from the study at any time, without providing a reason, without prejudice to their medical care.

Subjects will be discontinued from the study for the following medical or administrative reasons:

- A treatment-emergent adverse event (TEAE) occurs that represents an unacceptable risk to the subject and when continued participation in the investigational study is not warranted, based on the judgment of the Investigator. The Investigator must follow the subject until the adverse event (AE) resolves or satisfactorily stabilizes;
- The Investigator may discontinue individual subjects from the study at any time if he/she deems it in the best interest of the subject, or if he/she feels the subject is not capable of complying with study directions;
- Sponsor discontinue the subject from the study because of emerging safety information;
- Subject withdraws consent.

Subjects who withdraw consent will be encouraged, but are not required, to share their reason for withdrawing from the study. The primary reason for discontinuation, if provided, must be recorded on the subject's eCRF and, if necessary, on an AE form if the subject is discontinued due to an AE. All subjects discontinued from the study because of an emergent AE will be followed until AE(s) resolution or up to 30 days after the last dose of study drug. At this time, the subject will be referred to their primary physician.

4.5 Early Termination Procedures

Subjects who terminate early from the study or who are removed from the study should undergo the following end of study assessments, if consent is still maintained: vital signs, serum chemistry, hematology, urinalysis, ECG, and AE assessments. If the subject discontinues prior to completion of PK sampling, a blood sample for PK analysis should be collected as soon as possible after the decision to withdraw has been made, unless consent has been withdrawn.

4.6 Replacement of Subjects

Twenty (20) subjects are to be enrolled into the study. Subjects who withdraw/are withdrawn or subjects with major protocol deviations may be replaced, upon consultation with the Sponsor. The decision to replace subjects will be documented. Replacement subjects will be assigned the same randomization sequence (AB or BA) as the subject that they replace, if one has already been assigned.

5. STUDY TREATMENTS

5.1 Description of Study Treatments

5.1.1 Furosemide Injection for Subcutaneous Infusion (Investigational Product)

The investigational furosemide formulation is a Captisol-buffered solution at 30 mg/mL and at pH 7.4 (range: 7.0 to 7.8). Subcutaneous infusion will be performed using a qualified FDA-cleared commercial precision syringe infusion pump, which will deliver 2.7 mL of the furosemide formulation over 5 hours, using a biphasic delivery profile. The infusion set cannula will be placed approximately at 1/3 of an imaginary line between the belly button and the frontal bottom of the rib cage (10^{th} rib).

5.1.2 Furosemide Injection, USP (Reference)

Furosemide Injection, USP (Hospira, Inc., Lake Forest, IL) is provided as a sterile solution intended for IM or IV administration. Each mL contains furosemide 10 mg and sodium chloride sufficient to render the solution isotonic in water for injection. Furosemide Injection, USP contains sodium hydroxide and sodium chloride (hydrochloric acid used for pH adjustment); the pH is 9.0 (range: 8.0 to 9.3).

5.2 Dosage and Administration

Subcutaneous infusion of the investigational furosemide formulation will be administered as 30 mg delivered over the first 60 minutes (total 1 mL), followed by 12.5 mg per hour for 4 hours (total 1.7 mL). The total dosage and volume administered over 5 hours will be 80 mg and 2.7 mL, respectively.

Subcutaneous Infusion will be performed using the Medfusion 3500 (v6) precision infusion pump manufactured by Smith Medical ASD, Inc., St. Paul, MN, USA. The Medfusion was FDA cleared subject to 510(k) K040499. A copy of the Operator Manual (software version 6) is provided in Appendix 4. Each pump used in the study will be qualitied by a third- party contract

lab which will confirm that the pump will meet the requirements for flow accuracy and dose accuracy of the Lasix® Infusor under development by the sponsor. Each pump will be labeled with study identifiers and a unique pump number.

Prior to use, the pumps will be set to deliver 1 mL over the first hour followed by 0.42ml/hr for 4 hours for a total of approximately ~2.7 mL.

Subcutaneous infusion will be performed using a standard FDA-cleared subcutaneous infusion set with a 90-degree steel 27G or 29G cannula.

The pump number will be recorded as part of the study drug administration record.

Furosemide Injection, USP (10 mg/mL) will be administered as an IV bolus over 2 minutes. The total amount of furosemide administered will be 80 mg.

5.3 Study Treatment Compliance

All study treatment will be administered in the CRU under supervision of qualified study staff.

For subcutaneous infusion the following details will be recorded:

- Infusion pump number
- Time of start (start of treatment)
- Time of completion

For IV administration, the start and end time of the bolus administration should be recorded.

5.4 Study Drug Supply, Labeling, Storage, and Preparation

5.4.1 Supply and Retention of Bioavailability Samples

In accordance with 21 CFR §320.38, the study Sponsor/clinical site shall purchase and reserve at the clinical site, a sufficient quantity of both the test article (the investigational subcutaneous formulation of furosemide) and the reference drug (Furosemide Injection) to permit the FDA to perform 5 times all of the release tests required in the application or supplemental application.

Each reserve sample shall be stored in an area segregated from the area where testing is conducted and with access limited to authorized personnel. Each reserve sample shall be retained for a period of at least 5 years following the date on which the application or supplemental application is approved, or, if such application or supplemental application is not approved, at least 5 years following the date of completion of the bioavailability study in which the sample from which the reserve sample was obtained was used.

The clinical site should ***randomly select*** a sufficient quantity of units of the test article and reference standard to conduct the study; the remaining units of the test article and reference standard should be retained as the reserve samples in the original packaging.

5.4.2 Product Package and Labeling

5.4.2.1 Investigational Product Packaging and Labeling

Reserve samples of the test article (investigational furosemide formulation) must be adequately identified so that the reserve sample can be positively identified as having come from the same sample as used in this specific bioavailability study. Packaging labels will include at a minimum the following information:

- Investigational Product statement
- Protocol and lot number
- Storage Conditions
- Manufacturer/Sponsor Identification

5.4.2.2 Reference Drug Product Labeling

The reference standard (Furosemide Injection, USP) will be purchased as a commercial product and will retain FDA-approved drug labeling. Reserve samples of the reference standard shall be adequately identified so that the reserve sample can be positively identified as having come from the same sample as used in this specific bioavailability study. Lot numbers of the commercial product used for treatment and of the product held as reserve samples will be recorded.

5.4.3 Drug Storage

Both the investigational product and Furosemide Injection, USP should be stored at 20 to 25°C (68 to 77°F) and protected from light. Solutions should not be used if discolored or if they contain particulates. Furosemide Injection, USP should be stored in its original packaging and as specified by the FDA-approved label.

Based on available data, the investigational drug product is expected to be stable at room temperature for a minimum of 2 years. The investigational furosemide formulation will have a "use by" dating which may be extended from time to time by the Sponsor as additional stability data becomes available.

5.4.4 Drug Preparation

The study drug will not require preparation. The investigational furosemide formulation for subcutaneous infusion will be provided in clear glass cartridges containing approximately 2.7 mL of furosemide (30 mg/mL). The drug formulation of two cartridges will be aspired using a 5mL syringe to fill the syringe with approximately 3.5mL of investigational drug solution. The overage in used to bleed air from the infusion set prior to placement in accordance with training and instructions.

Furosemide Injection, USP will be purchased by the Sponsor/study site and used in accordance with the FDA-approved label (Appendix 1), except that the dose administered will be a single bolus administration of 80 mg over 2 minutes which is in accordance with routine clinical use. This dose has been selected in consultation with the US-FDA and clinical experts (Appendix 1).

Drug product accountability should be followed, as described in Section 5.5.

5.5 Study Drug Accountability

This section describes study drug-device accountability for both the investigational product (furosemide formulation for subcutaneous administration) and for Furosemide Injection, USP.

Study drug, devices and supplies supplied for this study are intended for use only within the context of this study. It should be stored in a secure place and maintained under adequate security until dispensed for subject use or destroyed according to standard operating procedures at the study site once study procedures have been concluded.

The Investigator, pharmacist, or designee, will verify that study supplies are received intact and in the correct amounts by signing and dating the receipt log or other means of documentation. The person receiving the supplies must verify that the shipment contains all the items noted in the shipment inventory. Any damaged or unusable study items in each shipment will be

documented. The Investigator must notify the Sponsor or designee of any damaged or unusable study product supplied to the site.

The site will maintain a Drug Accountability Log (including, but not limited to, the following: lot number, expiration date, number received, and number dispensed). The site will also maintain subject-specific drug dispensing logs for study drug.

An overall accountability of study drug will be performed and verified throughout the study and at site closeout. Upon completion of the study, copies of the study drug accountability records will be returned to the Sponsor. By signing the Investigator Agreement page of this protocol, the Investigator or named sub-Investigator agrees not to supply study drug to any person(s) not enrolled in the study.

Study drug will be returned to the Sponsor or destroyed according to standard operating procedures of the site once study procedures have been concluded. Documentation of drug shipments returned to the Sponsor or drug destruction will be maintained in the study files.

5.6 Overdose

The Furosemide Injection, USP package insert (Appendix 1) indicates that the principal signs and symptoms of overdose are dehydration, blood volume reduction, hypotension, electrolyte imbalance, hypokalemia and hypochloremic alkalosis, and are extensions of its diuretic action. Reported IV lethal dose (50%; LD_{50}) for mice, rats, and dogs ranged from 300 to 680 mg/kg.

Treatment of overdosage is supportive and consists of replacement of excessive fluid and electrolyte losses. Serum electrolytes, carbon dioxide level and blood pressure should be determined frequently. Adequate drainage must be assured in subjects with urinary bladder outlet obstruction (such as prostatic hypertrophy). Hemodialysis does not accelerate furosemide elimination.

6. STUDY PROCEDURES

6.1 Diet

6.1.1 Pre-Admission Diet

Starting 3 days prior to each of the CRU admission days, subjects will be instructed to avoid high salt foods. Participants will be provided with an instruction sheet identifying common foods rich in sodium. It is recognized that despite such instructions, subjects may be non-compliant or may inadvertently consume foods that are rich in sodium. The objective of these instructions is to target sodium intake of <2 g sodium/day. Compliance with the diet will not be assessed. Salt intake is not significant to the primary endpoints and pharmacokinetics.

6.1.2 Clinic Diet

Once in the clinic, subjects will be provided with standardized meals targeting 2000 calories (±5%) and <2 g sodium per day. Subjects will be provided a standard breakfast 60 minutes prior to dosing and are to consume the entire meal within 30 minutes. A standardized lunch should be provided at 4 hours after the start of dosing. Beverages provided with lunch will be included as part of the required fluid intake (described in Section 6.2**)**. Dinner will be provided at 10 hours

after the start of dosing. Identical meals will be served on dosing days for each dose period, i.e., the exact same breakfast, lunch, and dinner will be served to minimize variability in response due to food.

6.1.3 Required Fluid Intake

Subjects will be required to maintain fluid intake during the first 8 hours after the start of dose administration. At the start of the infusion or IV injection, subjects will be given 8 oz of fluid (water, fruit juice, or decaffeinated beverages [soda, coffee, tea]) that should be consumed at their own pace but within 2 hours. This process will be repeated every 2 hours (i.e., a total of 4 x 8 oz) until they have consumed 32 oz. After the 8-hour period is complete, subjects may consume fluids ad libitum.

6.2 Discontinuation and Re-Initiation of Oral Furosemide

All subjects will be on stable doses of oral furosemide ≥40 mg/day prior to admission to the CRU. For each Treatment Period, subjects must be medically able to discontinue oral furosemide at least 24 hours prior to administration of study drug (e.g., no oral furosemide should be administered after 10 pm the night prior to CRU admission). The date, time, and dosage of the last oral furosemide dose should be recorded.

After completion of Treatment 1 (i.e., Day 2) and after completion of Treatment 2 (i.e., Day 10) but prior to discharge, the PI or designee will instruct each subject on how to safely resume his/her oral furosemide therapy.

6.3 Randomization

Subjects will be randomized to treatment sequence AB or BA, as described in Section 3.5.

6.4 Interruption of Infusion

In the event of accidental or intentional interruption of the subcutaneous infusion the following details should be recorded: time of interruption, reason and circumstances of interruption.

Accidental interruption or interruption due to malfunction of the infusion pump system or infusion set may not be a cause for termination from the study and the participant may be rescheduled. Details of the incident and resulting deviations will be recorded. In the event interruption occurs after initiation of infusion for reasons other than adverse reactions or participant-related reasons, at the discretion of the principal investigator, the participant may be offered to reschedule the treatment episode which will again follow the procedures of the 2nd treatment

In the event that subjects terminate early from the study due to infusion interruption, ET and subject replacement procedures should be conducted as described in Section 4.5 and 4.6, respectively.

7. STUDY EVALUATIONS

A detailed schedule of procedures and events is provided in Table 1-1. Note that when vital sign assessments and blood sampling coincide, vitals signs should be taken prior to blood draws for PK or other analyses. Blood collected for PK and laboratory assessments should be collected from the arm opposite the study dose administration. AEs will be collected after all other procedures at each specified time are performed, when possible.

7.1 Informed Consent

The Investigator will obtain informed consent from each subject enrolled in the study, in accordance with the Declaration of Helsinki, the current version of ICH guidelines, and the laws and regulations of the country in which the investigation is being conducted. An appropriately constituted IRB will approve the informed consent form (ICF) to be used by the Investigator. Additional details on informed consent procedure are described in Section 13.3.

7.2 Demographic Data

The comprehensive medical history will include: demographic information (including age; sex; ethnicity; both medical and psychiatric conditions; surgical history and any scheduled medical procedures; tobacco, alcohol, caffeine use; illicit drug use; blood donation history for the past 3 months; and participation in other drug or device trials in the past 3 months).

7.3 Medical History

Any findings on the baseline safety assessments at Screening such as clinical laboratory or urinalysis test results, ECG findings, and physical examination will be considered medical history and will be exclusionary if deemed clinically significant by the Investigator or the Medical Monitor.

The medication history will include use of any prescription or non-prescription drugs, including vitamins, herbal and dietary supplements, whether taken internally or applied topically.

7.3.1 Height, Body Weight, and BMI

Height, body weight, and BMI will be recorded at Screening; weight and BMI will be recorded on Day -1 and Day 8 for confirmation of eligibility. Weight will also be recorded prior to and 24 hours after the start of each dose administration. Except for Screening and Admission, weight will be assessed in the morning after subjects have emptied their bladders and bowels, but prior to breakfast. Subjects should be weighed while wearing a single layer of clothing (hospital gown, scrubs, or similar) and should be wearing the same type of clothing each time they are weighed. If multiple scales are in use at the CRU, it is recommended that a given subject should be weighed on the same scale each time they are weighed.

BMI will be calculated using the following formula or the BMI calculator found on the website below:

$$\text{BMI } (kg/m^2) = \text{Weight (in kilograms)}/\text{Height}^2 \text{ (height in meters)}$$

https://www.calculator.net/bmi-calculator.html

7.3.2 Renal Function

eGFR will be calculated using the sMDRD equation (Johnson et al., 2004).

$$GFR\ (mL/min/1.73\ m^2) = 186 \times (S\text{-}Cr)^{1.154} \times age^{0.203} \times 0.742\ (\text{if female}) \times 1.21\ (\text{for black race group})$$

where S-Cr is the subjects serum creatinine.

7.4 Concomitant and Excluded Medications

All subjects will be receiving concomitant treatment with oral furosemide ≥40 mg/day at baseline. Subjects will discontinue oral furosemide treatment at least 24 hours prior to administration of study drug and will re-initiate after CRU discharge, as described in Section 6.2.

Medical treatments that are excluded per the exclusion criteria include: current or planned treatment during the study with any IV therapies, including inotropic agents, vasopressors, levosimendan, nesiritide or analogues; or mechanical support (intra-aortic balloon pump, endotracheal intubation, mechanical ventilation, or any ventricular assist device); administration of IV radiographic contrast agent within 72 hours prior to Screening or acute contrast-induced nephropathy at the time of Screening; and concomitant use of any drugs known to interact with furosemide (see the package insert for furosemide, Appendix 1).

7.5 Physical Examinations

A full physical examination will include assessments of the head, eyes, ears, nose, throat, thyroid, lungs, heart, abdomen (liver and spleen), lymph nodes, skin, extremities, motor function, balance, coordination, and reflexes.

A brief physical examination will include the following body systems: dermatologic, cardiovascular, respiratory, and gastrointestinal. Additional systems may be evaluated at the discretion of the Investigator.

A full physical examination should be conducted at Screening. A brief physical examination should be conducted at each CRU admission on Day -1 and Day 8.

7.6 Urine Drugs of Abuse and Alcohol Tests

Urine screens will be conducted at Screening and at each CRU admission (Day -1, and Day 8) for drugs of abuse (at a minimum amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, and opiates) and alcohol. A positive result at any time will result in subject exclusion from the study.

7.7 Pregnancy and Follicle-Stimulating Hormone Tests

Blood for serum pregnancy tests will be taken for females of childbearing potential at Screening, and urine pregnancy tests will be conducted at each CRU admission (on Day -1 and Day 8).

7.8 Safety Electrocardiogram

A single 12-lead safety ECG will be evaluated at Screening, at each CRU admission (on Day -1 and Day 8), pre-dose (Day 1 and Day 9), and at 24 hours after the start of dose administration (on Day 2 and Day 10). Subjects should be resting for at least 10 minutes in a semi-recumbent position.

7.9 Pharmacokinetic Assessments

7.9.1 Pharmacokinetic Sample Collection

Blood samples for the measurement of furosemide concentrations in plasma should be collected as follows:

For IV furosemide: Plasma samples will be collected pre-dose, and at 2 minutes (immediately after the IV bolus injection is complete), 5, 15, 30, 60, 120, and 180 minutes, and at 4, 6, 8, 12, 16, and 24 hours after the start of infusion.

For subcutaneous furosemide: Plasma samples will be collected pre-dose and at 30, 60, 90, 120, 180, 240, 300 (immediately after completion of the infusion), 305, 315, 330, and 345 minutes, and at 6, 7, 8, 10, 12, 14, 16, and 24 hours after the start of infusion.

Note that if the infusion is prematurely stopped during subcutaneous administration, a PK sample should be collected as soon as practicable.

All blood samples should be collected from the opposite arm of administration of furosemide (IV). The exact time of blood sampling should be recorded in the eCRF.

7.9.2 Pharmacokinetic Time Windows

Collection of PK samples should occur as close to the nominal timepoint as possible. The actual time of PK sample collection will be recorded on the eCRF. PK collections should occur within the following time windows; however, collections that occur outside of the time windows will not be considered to be protocol deviations. Any differences that the PK analyst deems to be impactful to the PK profile will be described in the CSR.

- The 2-minute sample for IV furosemide should be collected immediately after IV bolus injection.
- The 300-minute sample for subcutaneous furosemide is the collection time immediately after completion of the infusion.
- Blood samples from 15 minutes to 60 minutes, inclusive, should be collected within ± 2 minutes of the scheduled (nominal) timepoints.
- The 90- and 120-minute timepoints for IV furosemide and subcutaneous furosemide should be collected within ± 5 minutes of the scheduled (nominal) timepoints.
- All other PK blood draws should be collected within ± 10 minutes of the scheduled (nominal) timepoints.

7.9.3 Pharmacokinetic Sample Processing and Analysis

Details on the blood sampling procedure, including the samples processing, shipment, and storage, will be provided in the study procedures manual.

7.10 Pharmacodynamic Assessments

7.10.1 Urine Sample Collection

Subjects should attempt to void prior to study drug administration.

Spontaneous urine samples should be collected and pooled for the following time intervals post-initiation of furosemide administration: 0 to 1 hour; 1 to 2 hours; 2 to 4 hours; 4 to 6 hours; 6 to 8 hours; 8 to 10 hours; 10 to 12 hours; 12 to 18 hours; and 18 to 24 hours.

7.10.2 Urine Sample Processing and Analysis

Pooled urine samples will be processed as described in the study procedures manual, and aliquots will be analyzed for sodium concentration.

7.11 Vital Signs

All vital signs should be taken after the subject has been in a semi-recumbent position for at least 5 minutes and will include blood pressure, pulse, respiratory rate, and body temperature.

Vital signs should be collected at Screening, at CRU admission (Day -1 and Day 8), and at CRU discharge (Day 2 and Day 10). In addition, vitals will be taken prior to dosing and at 4 hours after dosing on dose administration days (within 10 minutes prior to PK sampling if timepoints coincide). The exact time of collection should be recorded in the eCRF.

If blood samples are scheduled to be collected at the same time as vital signs, then the vital signs should be obtained before the blood sample collection because drawing blood may affect the vital signs.

7.12 Clinical Labs and Disease Screen

Blood and urine will be collected for hematology, serum chemistry, and urinalysis at Screening, at CRU admission, and CRU discharge. In addition, blood will be drawn for serum chemistry at pre-dose and 24 hours after the start of each dose administration. The exact time of collection should be recorded. Serology should be collected at Screening only.

Laboratory tests will be performed by the site or a 3rd party laboratory. Venous blood will be drawn for hematology and serum chemistry tests, and the following tests will be completed:

Hematology/Serum Chemistry: Complete blood count with differential, sodium, potassium, calcium, magnesium, phosphates, total bilirubin, aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transferase, alkaline phosphatase, creatine phosphokinase, urea, creatinine, total protein, albumin

Urinalysis: Specific gravity, pH, protein, glucose, sodium, ketones, bilirubin, urobilinogen, nitrites, blood, and leukocytes, with microscopy of the sediment in case of abnormal dipstick findings

Serology: Approximately 7 mL of blood will be withdrawn into a plain vacutainer for standard serological tests for the presence of HIV infection, hepatitis B viral infection (HBsAg), and hepatitis C viral infection (anti-HCV).

The Investigator or a medically qualified designee will review the results and comment on all abnormal values, identifying those that are not clinically significant as well as those that are clinically significant. Abnormal clinical labs should be reported as AEs if they are clinically significant and be followed up to establish the outcome (see Section 8.1.5).

7.13 Safety Measures

7.13.1 Adverse Events

Safety will be measured by the frequency and type of AEs reported during the study. AEs should be reported and graded as described in Section 8. AEs should be monitored continuously while the subject is in the CRU and assessed at each return to the clinic. Subjects should be instructed to contact the site staff immediately if they experience any adverse events upon release from the clinic (during the washout period or between the end of study dosing and the Follow-up Phone Call). AEs should be reported beginning with subject signing of the ICF. Any AEs occurring from the signing of the ICF until CRU admission on Day -1 should be recorded as medical history.

7.13.2 Local Tolerance Assessment / Skin Photography

Local tolerance should be assessed for pain on a 0 to 10-point numerical rating scale for all treatments (International Association for the Study of Pain, 2016) (Appendix 2. Assessment of Pain). Pain assessments should be collected upon placement of the infusion set, for the maximum pain during infusion, and upon removal.

In addition, for subcutaneous infusion only, the skin should be photographed and evaluated for erythema/edema on a 0 to 4-point scale (National Academy of Sciences, 1977) (Appendix 3. Assessment of Erythema / Edema Formation) prior to dosing, upon removal, at 6 hours and 24 hours after the start of infusion.

The Sponsor will provide iPads for taking photographs for evaluation of local tolerance at the infusion site. Adhesive labels with identifying information (subject ID, date, timepoint, etc.) should be applied to the skin prior to photography. Additional details on photography procedures will be provided in the Study Procedures Manual.

7.14 Assessment Windows

Unless otherwise indicated (e.g., PK time windows), all scheduled study assessments should occur within ± 10 minutes of scheduled timepoints. The actual start and stop dates and times assessments should be recorded.

7.15 Protocol Deviations

Protocol deviations will be recorded and their impact on the study objects will be assessed.

8. ASSESSMENTS OF SAFETY

AEs should be reported beginning with subject signing of the ICF, with any AEs occurring from the signing of the ICF until CRU admission recorded as medical history. AEs that occur starting with initial CRU check-in through the Follow-up Phone Call visit will be monitored and recorded on an AE eCRF, including the AE's description, start and end date, seriousness, severity, action taken, and relationship to the study drug. If AEs occur, the first concern will be the safety of the subject. All AEs will be followed until resolved or up to 30 days after the last dose of study drug, and the outcome documented on the eCRF. If the AE has not resolved, the subject will be referred to their primary physician.

8.1 Definitions and Criteria

8.1.1 Adverse Events

Per ICH E2A: An AE is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product which does not necessarily have to have a causal relationship with study drug. An AE can therefore be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Medical interventions such as surgeries, diagnostic procedures, and therapeutic procedures are not AEs but the action taken to treat the medical condition. They should be recorded as treatment of the AEs.

Adverse events related to the use of the syringe, infusion or infusion set will be recorded as described in section.

8.1.2 Adverse Events of Special Interest

Adverse Events of Special Interest (AESIs) are serious or non-serious events of scientific and medical concern specific to a Sponsor's product or program, for which ongoing monitoring and rapid communication by the Investigator to the Sponsor can be appropriate. Such an event might warrant further investigation to characterize and understand it.

AESIs will be monitored by the CRU staff with study assessments and should be reported to the Sponsor regardless of seriousness or causality. The AESIs for this study are defined as follows:

- Orthostatic hypotension
- Pain or discomfort associated with the infusion
- Infusion site reactions

8.1.3 Serious Adverse Events

A serious AE (SAE) or reaction is any untoward medical occurrence that at any dose:

- results in death;
- is life-threatening;

  Note: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect, or
- is an important medical event (e.g., intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias; convulsions that do not result in hospitalization; development of drug dependency or drug abuse; or malignancy tumors [histologically different from primary tumor]).

Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Seriousness (not severity) serves as a guide for defining regulatory reporting obligations. An SAE is not necessarily severe; e.g., an overnight hospitalization for a diagnostic procedure must be reported as an SAE even though the occurrence is not medically serious. By the same token, a severe AE is not necessarily serious: nausea of several hours' duration may be rated as severe but may not be considered serious.

8.1.4 Unexpected Drug Reactions

An unexpected adverse drug reaction is a reaction for which the nature or severity is not consistent with the applicable product information (see the package insert for the reference product, Furosemide Injection, USP administered by IV (Appendix 1) and the Investigator's Brochure for the investigational product, furosemide injection administered by subcutaneous infusion).

Until product information is amended, expedited reporting is required for additional occurrences of the reaction. Reports that add significant information on specificity or severity of a known, already documented SAE constitute unexpected events. For example, an event more specific or more severe than described in the product label would be considered "unexpected".

8.1.5 COVID-19 Infection Study Reporting

Any symptom that indicates the presence of COVID-19 will be assessed by the PI and will be recorded on the AE pages of the eCRF if it meets either of the following criteria:

- Requires therapeutic intervention or additional diagnostic tests.
- Leads to discontinuation of study drug.

A listing of the suspected or confirmed cases of COVID-19 will be listed in the CSR.

8.1.6 Abnormal Laboratory Value(s)

Any laboratory abnormality that is new in onset or which has worsened in severity or frequency from the baseline condition and meets one of the following criteria will be recorded on the AE pages of the eCRF:

- Requires therapeutic intervention or additional diagnostic tests.
- Leads to discontinuation of study drug.
- Has accompanying clinical symptoms or signs.
- Is judged by the Investigator as clinically significant.

8.1.7 Adverse Events Related to Infusion System

Any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product attributable to the infusion system, including the syringe, infusion set, or infusion pump will be recorded on the Device Adverse Event Form. Examples of such adverse events are burn injury following explosion of the pump or electric shock when touching or handling the pump. In addition to recording as study Devise Adverse Events these events will be reported on behalf of the Principal Investigator using the MedWatch Voluntary Report procedures (FDA Form 3500) and based on the nature and severity, at the discretion of the Principal Investigator, may also be reported to the manufacturer.

8.2 Assessing Severity and Relationship

8.2.1 Severity of Adverse Events

The severity (mild, moderate, or severe) of each AE/SAE must be assessed by the Investigator, or designee. The following criteria should be considered when assessing severity:

Mild:	The symptom is barely noticeable to the subject and does not influence performance or functioning.
Moderate:	The symptom is of sufficient severity to make the subject uncomfortable and performance of daily activities is influenced. Treatment for the symptom may be needed.
Severe:	The symptom causes severe discomfort. Treatment for the symptom may be necessary.

When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the experience should be noted. If the severity of the category changes over a number of days, those changes should be recorded separately, with distinct onset dates.

8.2.2 Assessing Relatedness to Study Drug

The Investigator will assess each AE for causality based on his/her medical judgment and the observed symptoms associated with the event. Each AE will be assessed as related or unrelated to study drug (furosemide injection by IV or subcutaneous infusion) based on the following criteria:

Not related: No causal relationship exists between the investigational product and the AE, but an obvious alternative cause exists (e.g., the subject's underlying medical condition or concomitant therapy).

Possibly related: A connection with the administration of the investigational product appears unlikely but cannot be ruled out with certainty. An AE may be considered possibly related if or when it meets 2 of the following criteria: (1) it follows a reasonable temporal sequence from administration of the investigational product; (2) it could not readily have been produced by the subject's clinical state, environmental or toxic factors, or other modes of therapy administered to the subject; or (3) it follows a known pattern of response to the investigational product.

Related: There is a reasonable/plausible possibility that the AE may have been caused by the investigational product.

8.3 Reporting Procedures and Requirements

The conduct of the study will comply with all safety reporting requirements by the relevant regulatory authorities and site-specific IRB/IEC safety reporting requirements.

8.3.1 Adverse Event Reporting

AEs occurring from when the subject signs the ICF until the last study event will be recorded. All AEs occurring prior to CRU admission will be recorded in the medical history.

If the Investigator is made aware of an AE in a subject within 30 days after the last scheduled Follow-up Phone Call and considers the event possibly related or related to prior study treatment, the Investigator should report it to the Sponsor's Medical Monitor.

The Investigator should report all AEs occurring after admission to the CRU on the AE page(s) of the eCRF and source documents, regardless of seriousness, severity, and causality. Whenever possible, an AE will be reported using a diagnostic term, (e.g., "common cold" or "upper respiratory infection" rather than "runny nose, cough, mild fever") and should be described with the attributes as previously described.

8.3.2 Adverse Events of Special Interest Reporting

Regardless of seriousness or causality, AEs designated as AESIs (see Section 8.1.2) should be reported to the Sponsor within 24 hours in the same manner as SAEs (see Section 8.3.3).

8.3.3 Serious Adverse Event Reporting

Any SAE occurring after the subject signs informed consent will be reported to the Medical Monitor and Sponsor **within 24 hours** of the Investigator, designee, or site personnel's knowledge of the event, regardless of relationship to study drug. Site personnel will complete the Serious Adverse Event Report Form providing all available SAE related information through a pre-established secure transfer media (ShareFile, or similar) and notification of an SAE event without subject information within 24 hours to:

Name: Kimberly Bennett, MD, MPH

Email: kbennett@nuventra.com

Phone: 919-308-7250

The Medical Monitor will send a confirmation of receipt within 1 business day for any information sent to the Investigator and the Sponsor. If a confirmation of receipt is not received within 1 business day, the documentation should be re-sent.

If the Investigator detects an SAE in a subject after the last scheduled Follow-up Phone Call, and considers the SAE possibly related or related to prior study treatment, the Investigator should report it to the Sponsor's Medical Monitor within 24 hours.

The initial report should include the site name and number and the name of the Investigator, the Subject ID Number, the subject demographic information, the details of study drug administration, and as many details of the SAE as are known, including the date of onset, the severity, the treatment, and the relationship to study drug. If the subject died, the report should include the cause of death and whether the death was related to study drug, as well as the autopsy findings, if available.

Additional or new information that becomes available after the initial report should be reported within 48 hours. The Sponsor, or designee, may request for additional source documentation pertaining to the SAE from the investigational site. If a subject is permanently withdrawn from the study due to a SAE, this information must be included in the initial or follow-up SAE report and in the eCRF.

All SAEs will be followed until satisfactory resolution or until the Investigator deems the event to be chronic or the participant is stable.

The Investigator is responsible for notifying the IRB/IEC, in accordance with local regulations, of any serious and unexpected AEs or new information that may adversely affect the safety of the subjects or the conduct of the study.

The mandatory reporting of safety events to the relevant regulatory authorities will be the responsibility of the Sponsor or designee. The Sponsor, or designee, shall notify the relevant regulatory authorities of any SAEs that are unexpected, serious, and possibly related to the study drug, as soon as possible but no later than 15 calendar days after the Sponsor learns of the event.

8.4 Follow-up of Subjects After Adverse Events and/or Serious Adverse Events

Subjects with AE/SAEs will be given treatment or referred for appropriate medical treatment as clinically indicated to be in the best interest of the subject in the medical opinion of the site physician. The Investigator will provide or arrange for appropriate follow-up (if required) for

subjects and document the course of the subject's condition. Additional medical evaluation and treatment will be arranged as clinically appropriate in the opinion of the site physician. All clinically important abnormal laboratory values occurring during the study will be repeated at appropriate intervals until they return either to baseline or to a level deemed acceptable by the Investigator and the Sponsor's Medical Monitor or the subject will be referred for appropriate medical treatment.

8.5 Pregnancy

Females of childbearing potential will be instructed to inform the Investigator if they become pregnant during the study or within 30 days after administration of study drug. The Investigator will counsel female subjects about the risks of pregnancy and the possible effects on the fetus. To report pregnancies in female subjects, the Investigator must complete a Pregnancy Reporting Form no more than 24 hours after becoming aware of the event. This initial Pregnancy Report should be distributed to a pre-established secure webshare platform, and notification of an event without subject information should be forwarded to the Medical Monitor at:

Name: Kimberly Bennett, MD, MPH

Email: kbennett@nuventra.com

Phone: 919-308-7250

The Medical Monitor will send a confirmation of receipt within 1 business day for any information sent to the Investigator and the Sponsor. The Investigator determines if the report meets the definition of serious event (if any of the serious criteria are fulfilled or if it is a report of spontaneous abortion. Note: Elective abortion is not serious.). If a Pregnancy Report contains events that meet the definition of an SAE, the site completes the necessary SAE forms in addition to the Pregnancy Notification Form.

All neonatal deaths that occur within 30 days of birth without regard to causality should be reported as SAEs.

9. DATA ANALYSIS AND STATISTICAL CONSIDERATIONS

9.1 Hypotheses

No hypothesis is tested in this study.

9.2 Sample Size Assumptions and Calculations

A power analysis was undertaken to estimate the sample size required to demonstrate similarity of urine volume from IV and subcutaneous furosemide, delivered by a commercial precision syringe infusion pump.

The assumptions were as follows:

- The bioavailability of the commercial infusion pump is the same as the IV route (F=1.0)
- The PD effect of furosemide delivered by the subcutaneous route with a continuous infusion is similar to IV infusion of the same dose
- A cross over study design

- Power of at least 80% with $\alpha = 0.05$ in two one-sided tests with 90% confidence interval (CI) between 0.8 and 1.25
- No carry over effects (randomized sequence of treatments)

A literature review identified 3 papers with relevant results (Dormans et al., 1996, Sanjay et al., 2008, Sica et al., 2018). Based on a CV = 37%, a sample size of 18 will allow for the determination of bioequivalence with a statistical power of 81%, using $\alpha = 0.05$ in two one-sided tests with 90% CI between 0.8 and 1.25. This is based on a randomized two-period crossover design in which all subjects receive both IV and subcutaneous infusion of furosemide.

9.3 Analysis Sets

The Safety Population will consist of all randomized subjects who received at least one dose of study drug. The PK Population for furosemide is defined as all subjects receiving at least one dose of study drug and providing at least one measurable plasma concentration. The PK Analysis Population includes all subjects in the PK Population with sufficient concentration-time data to calculate the PK profile for at least one treatment and without a protocol deviation that affects the PK profile, per the discretion of the analyst.

The PD Analysis Population includes all subjects in who received at least one dose of study drug and had measurable sodium concentrations in the urine without a protocol deviation that would affect the bioanalytical results.

9.4 Analysis Plan

There will be a stand-alone statistical analysis plan (SAP). The pharmacokinetic analysis plan (PKAP) will be within the SAP. Summaries of analysis considerations are summarized below and will be detailed in the SAP and PKAP.

9.4.1 Pharmacokinetic Analysis

PK parameters for furosemide will be derived using noncompartmental methods. Compartmental modeling of the PK data may be conducted if necessary. The Analysis Population will be used.

Furosemide concentrations for the PK Population will be summarized using descriptive statistics (including sample size [N], mean, standard deviation [SD], coefficient of variation [CV%], median, minimum, and maximum) for each treatment. Derived plasma PK descriptive statistics will be tabulated by dosing group and summary statistics. Descriptive statistics for PK parameters (Cmax, Tmax, AUClast, AUCinf, λz, $t_{½}$, Vz/F, CL, and CL/F) will include the arithmetic and geometric mean (for Cmax, AUClast, and AUCinf, only), CV%, SD of the arithmetic mean, median, minimum, maximum, and N.

9.4.2 Bioavailability Analysis

The pharmacokinetic parameters (Cmax, AUClast and AUCinf) of the Analysis Population will be assessed using a linear repeated measures mixed-effect model appropriate for a 2-period crossover design with treatment and period as fixed effects. A heterogeneous-compound symmetry covariance matrix will be used to allow for unequal treatment variances and to model the correlation between the 2 treatment measurements within each subject. The Kenward-

Roger method will be used to calculate the denominator degrees of freedom for the fixed effects. A log transformation will be applied to the Cmax, AUClast and AUCinf data. Back transformed summary statistics will be reported for the pharmacokinetic parameters.

The 90% CIs, based on the t-distribution, will be generated from the above mixed-effect model for the least-square geometric mean ratios for Cmax, AUClast and AUCinf to compare subcutaneous (Test) to IV (Reference) Furosemide.

For Tmax, the point estimate and 90% CI for the ratio of median between subcutaneous and IV (IV as a reference) will be provided using the Hodges-Lehmann method of paired samples (Hodges and Lehmann, 1963).

9.4.3 Pharmacodynamic Analysis

The urine volume and sodium concentration in urine collected over 8 hours and 24 hours post-dose will be used to characterize the effects of furosemide on diuresis and natriuresis after subcutaneous or IV administration.

The urine volume and sodium concentration will be tested for departures from normality and appropriate transformations will be applied if necessary.

Summary statistics including mean, standard deviation, minimum and maximum will be reported by treatment group.

The pharmacodynamic variables (urine volume and sodium concentration) will be assessed using a linear repeated measures mixed-effect model appropriate for a 2-period crossover design with treatment and period as fixed effects. BMI and eGFR may be included as covariates in the model. A heterogeneous-compound symmetry covariance matrix will be used to allow for unequal treatment variances and to model the correlation between the 2 treatment measurements within each subject. The Kenward-Roger method will be used to calculate the denominator degrees of freedom for the fixed effects.

The least-squares mean difference between the treatment groups, 90% CIs and p-value will be calculated.

9.4.4 Safety Analysis

All safety data will be listed by subject. TEAEs will be summarized for each treatment by system organ class, preferred term, severity, and relationship to study drug. Observed values and changes from baseline for clinical laboratory test data, safety ECGs, physical examination results, and vital signs will be summarized using appropriate descriptive statistics. The Safety Population will be used.

10. STUDY DOCUMENTATION MANAGEMENT AND MATERIALS

10.1 Study Documentation

The Investigator is required to prepare and maintain adequate and accurate case histories (i.e., source documents and/or Medical Record Supplement) designed to record all observations and other data pertinent to the study for each study participant. This includes accurate documentation

of accountability of study drug. The medical records must contain adequate information to allow for verification of subject identity throughout the study.

eCRFs will be completed for each subject who is enrolled in the study. Subject numbers will be assigned systematically immediately following the execution of written informed consent. A subject Screening/enrollment log, noting reasons for screen failure where applicable, will be maintained for all subjects who are consented.

All information recorded on the eCRFs for this study must be consistent with the subject's source documentation (i.e., source documents and/or Medical Record Supplement). The source documents may include the hospital and/or the physician's chart, X-rays, or laboratory evaluation documentation.

The eCRFs for each subject will be periodically checked against the subject's source documents at the study site by the site monitor. Instances of missing or unclear data will be discussed with appropriate site personnel for resolution. Methods will be detailed in a Study Monitoring Plan.

10.2 Data Handling and Archiving of Study Documents

In accordance with GCP guidelines, all study-related documentation shall be retained by the Investigator for at least the minimum time required by applicable law for the US. At that time, the Investigator will contact the Sponsor regarding further disposition of the study records and comply with the Sponsor's instructions.

The Investigator agrees to adhere to the document retention procedures by signing the study protocol. Examples of essential documents include, but are not limited to:

- IRB/IEC correspondence indicating approval for the study protocol, ICF(s), and all amendments to either of these documents
- All source documents and laboratory records
- ICFs signed by the subject
- Completed Form FDA 1572
- Any other pertinent study document

10.3 Direct Access to Source Data and Documents

The Investigator and his or her institution will permit trial-related monitoring, audits and/or regulatory inspections, providing direct access to source data or documents (original medical files). Monitors, auditors, or inspectors are obliged to maintain the confidentiality of subjects' identities.

10.4 Site Monitoring

Clinical site monitoring will be conducted to ensure that the rights of human subjects are protected, that the study is implemented in accordance with the protocol and/or other operating procedures, and that the quality and integrity of study data and data collection methods are maintained. Additional site monitoring for quality control and assurance is described in Section 12.

10.5 Site Audits

Participation by the Investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable institutional compliance and quality assurance offices. During the conduct of the study, the Sponsor may conduct audits of any data and facility participating in the study. The Investigator and site involved in the study will permit such study-related audits and provide direct access to all study records.

Competent regulatory authorities may also perform inspections either during or after the study. In the event of an inspection by any regulatory authority, the Investigator will promptly notify the Sponsor and will allow Sponsor representatives to be present during the audit, if permitted. The Investigator agrees to cooperate fully with inspections conducted by regulatory authorities and to allow representatives of the regulatory authority access to all study records. The Investigator will forward to the Sponsor a copy of any inspection records received.

11. DATA MANAGEMENT

Data will be entered into the eCRFs by the study site. Queries will be issued for any inconsistencies, omissions, and discrepancies and will be resolved by the appropriate parties. All AEs will be coded using Medical Dictionary for Regulatory Activities in the latest version. Concomitant medications will be coded using the current World Health Organization Drug Dictionary. Data management details will be outlined in a separate data management plan. Following database lock, archive media will be created and forwarded to the Sponsor, or designee, and the clinical site, and the clinical database will be archived.

12. QUALITY CONTROL AND QUALITY ASSURANCE

The Sponsor will appoint a representative who will monitor the study in line with GCP requirements. Prior to initiation of the study, the monitor will review with the site staff the protocol and all study requirements, as well as their responsibilities to satisfy regulatory, ethical, GCP, and Sponsor’s requirements. As outlined in the Study Monitoring Plan, the study will be monitored from time to time to check that the safety and rights of subjects are being protected, and that the study is conducted in accordance with currently approved protocol and any other study agreements. Upon completion of the study, the monitor will conduct site closure activities in accordance with GCP and applicable regulations.

To ensure compliance with GCP and applicable regulatory requirements, the Sponsor may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection. Such audits/inspections can occur at any time during or after completion of the study.

The Investigator and his/her institution agree to allocate his/her time and the time of the study staff to the monitor, auditor, or inspector to discuss findings and any relevant issues.

13. ETHICAL CONSIDERATIONS

13.1 Institutional/Ethical Review

This protocol, including any amendments, and related written ICF and other written materials that will be given to study participants must be approved by an IEC/IRB prior to initiation of the study.

Prior to initiation of the study, the Sponsor will obtain regulatory approval to conduct the study from the appropriate regulatory agency in accordance with applicable regulatory requirements.

13.2 Ethical Conduct of the Study

The study will be conducted in accordance with the ICH guideline on GCP, guiding principles for biomedical research as set in the current version of Declaration of Helsinki, and applicable regulatory requirements, including subject privacy requirements. Plasma samples collected during the study will be managed in line with generally accepted standards for use of human biological material in research.

13.3 Informed Consent

Written informed consent will be obtained from each subject prior to any of the protocol specific procedures.

It is the responsibility of the Investigator to give each subject, before inclusion in the study, full and adequate verbal and written information about the study and their rights, including the right to withdraw from the study at any time. Written information on the study will be provided to the Investigator by the Sponsor and should not be changed by the Investigator without prior discussion with the Sponsor and IEC/IRB approval. The subject should receive sufficient time and opportunity to ask about details of the study and to decide whether to participate or not.

If a potential study participant cannot read or write, impartial witness must be present during the informed consent discussion, and upon subject's verbal consent should sign and personally date the consent form, attesting that information on the study was accurately explained to, and apparently understood by, the subject, and that informed consent was freely given by the subject.

13.4 Confidentiality

The information on individual subjects arising from this study is to be considered confidential and transmitted to the Sponsor only in a form that will not permit identification of the individual. Regulatory and sponsoring agencies may request access to the study records and related medical records of each participating subject, and if requested, the subject's identity will remain confidential to the extent permitted by the applicable laws and regulations. Under informed consent, the subject shall understand that each participant is authorizing access to medical records as required for monitors, auditors, IRB/IECs, and regulatory authorities. All records will be kept in a secure storage area with limited access.

All information supplied to the Investigator by the Sponsor before, during, and after the study is confidential. Such information is to be used solely in connection with the clinical study. The study protocol and any other pertinent study-related materials or records provided are to be

maintained in a confidential manner, reviewed carefully with attention to admonitions and returned to the Sponsor upon request. No part of these materials may be reproduced or transmitted in any form without prior written permission from the Sponsor.

13.5 Conflicts of Interest

The Investigator will be paid by the Sponsor of this study for study-related expenses, but he/she will not profit from results, either positive or negative, with regard to the product being evaluated.

13.6 Finance and Insurance

Financial agreements between all stakeholders will be addressed separately and documented in the Trial Master File.

14. PUBLICATION AND DATA SHARING

The Sponsor will provide the Investigator and IRB/IEC with a full summary of the study results. The Investigator is encouraged to communicate the study results with study participants, as appropriate.

The study information may be registered to ClinicalTrials.gov and may be published in the scientific literature by the Sponsor.

15. REFERENCES

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16. APPENDICES

Appendix 1. Package Insert for Furosemide Injection, USP

Appendix 2. Assessment of Pain

Local tolerance should be assessed for pain on a 0 to 10-point numerical rating scale for all treatments. Pain assessments should be collected upon start of treatment, for the maximum pain during infusion, and upon removal.

Assessment of Pain

Select one: SC Infusion or IV

Subject ID: _____

Timepoint: _____

Treatment Period: _____

For Placement and Removal

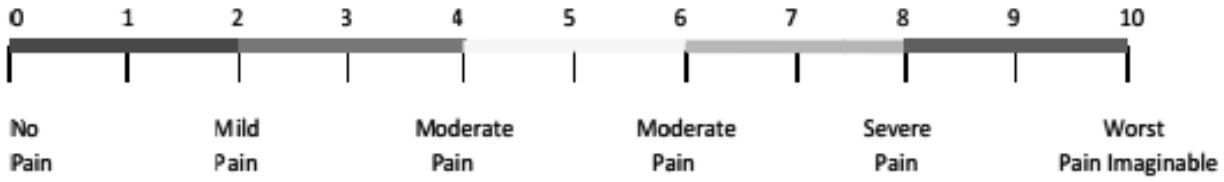
[The question below refers to pain that you may be feeling right now affecting the skin in the area around the site of your dose administration.

On a scale from 0 to 10, with 0 being no pain at all and 10 being the worst pain imaginable, how would you rate your pain RIGHT NOW in the area where you received your dose. (Please circle the number)]

For Max Pain During Infusion

[The question below refers to pain that you may have felt in the area around the site of your dose administration.

On a scale from 0 to 10, with 0 being no pain at all and 10 being the worst pain imaginable, how would you rate the maximum pain you felt in the area where you received your dose while you received the infusion. (Please circle the number)]



Appendix 3. Assessment of Erythema / Edema Formation

For subcutaneous infusion only, the skin should be photographed and evaluated by the Investigator for erythema or edema (scored on 0 to 4-point scales) **prior to dosing, upon removal, at 6 hours and 24 hours after the start of infusion**

Assessment of Erythema/Edema Formation

Subject ID: _____

Timepoint: _____

Treatment Period: _____

Erythema /edema (Circle Response):

0	No response
½	Questionable or faint, indistinct erythema
1	Well-defined erythema
2	Erythema with slight to moderate edema
3	Vesicles (small blisters) or papules (small, circumscribed elevations)
4	Bullous (large blister), spreading, or other severe reaction

Appendix 4. MEDFUSION - Model 3500 – OPERATOR'S MANUAL