CLINICAL STUDY PROTOCOL

A Phase 1, Randomized, Double-Blind, Placebo-Controlled Single Ascending Dose Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of ST-2427 Intravenous Infusion in Healthy Subjects/Development of a Potent and Highly Selective NaV1.7 Inhibitor for the Treatment of Acute Pain with the Goal of Reducing Opioid Use and Preventing Opioid Use Disorder

PROTOCOL NUMBER:	ST-2427-CS-001/[redacted] 5UG3 DA049599-01
SPONSOR:	SiteOne Therapeutics
SPONSOR CONTACT:	[redacted]
MEDICAL MONITOR:	[redacted]
PROTOCOL DATE:	10 July 2020

AMENDMENT 1:	23 November 2020
AMENDMENT 2:	18 December 2020
AMENDMENT 3:	25 June 2021

STATEMENT OF COMPLIANCE

This document contains confidential information. It is intended solely for the use of the Principal Investigator, co-Investigators, staff, appropriate Institutional Review Boards or Ethical Committees, and other required regulatory bodies.

INVESTIGATOR'S AGREEMENT

I have received and read the Investigator's Brochure for ST-2427. I have read the protocol for ST-2427-CS-001/[redacted] 5UG3 DA049599 01 and agree to conduct the study as outlined. I agree to maintain the confidentiality of all information received or developed in connection with this protocol.

[redacted]

Signature of Investigator

30 JUN 2021

Date

PROCEDURES IN CASE OF EMERGENCY

Role in Study	Name	Address and Telephone Number
Clinical Study Leader	[redacted]	[redacted]
Responsible Investigator	[redacted]	
Drug Safety Investigator	[redacted]	
24-Hour Emergency Contact	[redacted]	

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialist Term	Explanation
AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ANCOVA	Analysis of Covariance
AUC	Area Under the Curve
BID	Twice Daily
BP	Blood Pressure
BUN	Blood Urea Nitrogen
CL	Clearance of drug from circulation
Cmax	Maximum drug concentration in circulation
CNS	Central Nervous System
Ctrough	Lowest drug concentration in circulation between repeat doses
CRF	Case Report Form
DBP	Diastolic Blood Pressure
DMC	Data Monitoring Committee
ECG	Electrocardiogram
GCP	Good Clinical Practice
HBV, HCV	Hepatitis B, Hepatitis C
HIV	Human Immune Deficiency
HR	Heart Rate
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee
IRB	Institutional Review Board
NOAEL	No Observed Adverse Event Level
OAE	Other Significant Adverse Event
PI	Principal Investigator The investigator who leads the study conduct at an individual study center. Every study center has a principal investigator.
PK	Pharmacokinetics
Q12H	Every 12 hours
QT	Cardiac QT interval

Abbreviation or Specialist Term	**Explanation**
QTcF	QT interval corrected for heart rate using the Fredericia method
SAD	Single Ascending Dose
SAE	Serious Adverse Event
SBP	Systolic Blood Pressure
Vd	Volume of distribution of drug
Vss	Volume of distribution of drug at steady state
WBC	White Blood Cell

1. SYNOPSIS

Name of Sponsor/Company: SiteOne Therapeutics, Inc.		
Name of Investigational Product: ST-2427 for Intravenous (IV) Infusion		
Name of Active Ingredient: ST-2427		
Protocol Number: ST-2427-CS-001/[redacted] 5UG3 DA049599-01	**Phase:** Phase 1	**Country:** USA
Title of Study: A Phase 1, Randomized, Double-Blind, Placebo-Controlled Single Ascending Dose Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of ST-2427 IV Infusion in Healthy Subjects/Development of a Potent and Highly Selective NaV1.7 Inhibitor for the Treatment of Acute Pain with the Goal of Reducing Opioid Use and Preventing Opioid Use Disorder		
Study Center(s): Altasciences Clinical Kansas, Inc., 10103 Metcalf Ave. Overland Park, KS, USA 66212		
Principal Investigator: [redacted]		
Studied Period (years): Estimated date first patient enrolled: April 2021 Estimated date last patient completed: December 2021		**Phase of development:** Phase 1
Objectives: **Primary:** To evaluate the safety and tolerability of ST-2427 intravenous (IV) Infusion. **Secondary:** To measure the pharmacokinetics (PK) of ST-2427 IV Infusion. **Study Design:** This randomized, double-blind, placebo controlled, study will be conducted to evaluate the safety, tolerability, and pharmacokinetics of ST-2427. Subjects will be randomized to receive a single dose of ST-2427 or placebo in a Single Ascending Dose (SAD) design. A total of 30 subjects will be enrolled. Subjects will be randomized in a 4:2 ratio of ST-2427 to placebo. Study drug will be blinded to all subjects and Investigators.		
Methodology: Subjects will be enrolled into the following sequential cohorts and randomized 4:2 to receive ST-2427 or placebo: • Cohort 1: 6 subjects will receive a single administration of placebo or ST-2427 5 mg by IV infusion over approximately 1 hour. • Cohort 2: 6 subjects will receive a single administration of placebo or ST-2427 10 mg by IV infusion over approximately 1 hour. • Cohort 3: 6 subjects will receive a single administration of placebo or ST-2427 15 mg by IV infusion over approximately 1 hour.		

- Cohort 4: 6 subjects will receive a single administration of placebo or ST-2427 22 mg by IV infusion over approximately 1 hour.
- Cohort 5: 6 subjects will receive a single administration of placebo or ST-2427 33 mg by IV infusion over approximately 1 hour.

Sentinel dosing will be used in Cohort 1 in the SAD wherein the first 2 subjects (one ST-2427, one placebo) will be randomly chosen to receive study drug and be followed for up to 2 days before any other subject is dosed in that cohort. Dosing of the remaining 4 subjects (3 ST-2427, 1 placebo) in the cohort will commence after safety review by the Principal Investigator. This method of sentinel dosing of 2 subjects on the first day, followed by dosing of all remaining subjects will be used for the assessment of safety and risk mitigation. Subjects will return to the clinic for the Follow-Up visit on Day 8 ± 2 days.

C. General

Blood samples will be collected at pre-defined time points for evaluation of clinical laboratory tests (Table 4), and the PK of ST-2427. The total blood volume to be collected from each subject in this study is approximately 65.2 mL during participation in the SAD (Appendix C).

Safety and tolerability of each regimen will be assessed by adverse event (AE) monitoring, clinical laboratory tests, vital signs, body weight, electrocardiograms (ECGs), and physical examinations. Enrollment into Cohorts 2 through 5 will commence after all subjects in the prior cohort have completed the Follow-Up Visit, and safety and PK data from all prior cohorts have been reviewed by a Data Monitoring Committee (DMC) consisting of the Principal Investigator, a medical representative from the Sponsor, and a PK expert, and no clinical concerns are identified from evaluation of safety. The DMC will additionally examine all available clinical and PK data to determine the dose of ST-2427 to be administered in the next cohort.

[redacted]

In addition to the above subject level stopping criteria, if 2 subjects randomized to ST-2427 in a given cohort experience any of the above drug-related events, will lead to stopping the study/cohort, will define the maximum tolerated dose, and will result in cessation of further subject exposures in that cohort, and prevent further dose escalation in a subsequent cohort. [redacted]

Sample Size Justification:

Descriptive, non-inferential statistics are planned for the primary endpoint analyses. Based on clinical judgement, a sample size of 30 (20 randomized to ST-2427, and 10 randomized to placebo) is expected to be adequate to identify major treatment effects.

Number of Patients (planned):

Approximately 30 subjects

Diagnosis and Main Criteria for Inclusion:

Inclusion Criteria

Only subjects who meet the following criteria will be eligible for inclusion:

1. Healthy adult males and/or females (of non-childbearing potential), 18 to 55 years of age (inclusive) at the time of screening.
2. Body mass index (BMI) within 18.0 to 35.0 kg/m2, inclusive (minimum weight of at least 50.0 kg at Screening).
3. Medically healthy without clinically significant abnormalities at the screening visit, including physical examination and vital signs within the following ranges: heart rate 50 to 100 bpm, systolic blood pressure 100 to 149 mmHg; diastolic 70 to 94 mmHg.
4. The mean QTcF interval duration ≤450 msec for males and ≤470 msec for females measured from the triplicate ECGs taken at least 1 minute apart with QT wave corrected for heart rate (HR) using Fredericia's method.
5. Hemoglobin/hematocrit, white blood cell (WBC) count, and platelet count equal to or greater than the lower limit of normal range of the reference laboratory (may be confirmed upon repeat testing without Sponsor approval).
6. Non-smokers (including tobacco, e-cigarettes, or marijuana), and no use of any tobacco product for at least 1 month prior to admission in the study.
7. Willing and able to provide written informed consent.
8. Willing and able to comply with all study assessments and adhere to the protocol schedule.
9. Have suitable venous access for blood sampling, as determined by an Investigator at screening.
10. If female, be of non-childbearing potential (e.g. post-menopausal as demonstrated by follicle stimulating hormone (>40 mIU/mL), amenorrhea for at least one year, or surgically sterilized by tubal ligation, hysterectomy, bilateral oophorectomy and/or salpingectomy). Site personnel's review of the subject's medical records, medical examination, or medical history interview is acceptable evidence of female sterilization, verbal confirmation is adequate.
11. If male, willing not to donate sperm from the time of first study drug administration until 90 days after the final administration of study drug. If male and not intending to engage in sexual intercourse over the duration of the study, willing to agree to abstinence at screening. If male and engaging in sexual intercourse, willing to use a double barrier method of contraception (condom and spermicide). The latter criterion applies to all males (and/or female partners) including males who are surgically sterile and must be followed from the time of first study drug administration until 90 days after the final administration of study drug.

Exclusion Criteria

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

1. History or presence of significant cardiovascular (including arrhythmia and ventricular tachyphylaxis), pulmonary, hepatic, renal, hematological, gastrointestinal, endocrine, immunologic, dermatologic or neurological disease, including any acute illness or surgery within the past 3 months determined by an Investigator to be clinically relevant.
2. Creatinine, blood urea nitrogen (BUN), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) equal to 1.5 × upper limit of normal for the reference laboratory (may be confirmed upon repeat testing).
3. History of orthostatic reactions.
4. Orthostatic reaction at screening defined as drop in systolic blood pressure by ≥20 mmHg or drop in systolic blood pressure to <90 mmHg on standing for 3 minutes from the supine position.
5. History of seizure disorders, except for non-complex febrile seizures in childhood with absence of non-febrile seizures in parents and siblings.
6. Positive urine drug/alcohol testing at Screening or Day –2.
7. Positive test results for HIV-1/HIV-2 Antibodies, Hepatitis B surface Antigen (HBsAg) or Hepatitis C Antibody (HCVAb).
8. Positive test results for COVID-19 (PCR or Antibodies)
9. History of substance abuse or alcohol abuse (defined as greater than 2 standard drinks per day) within the previous 2 years.
10. Use of any prescription medication or any over-the-counter medication, including herbal products and vitamins within 14 days or 5 half-lives (whichever is longer) prior to randomization.
11. Documented hypersensitivity reaction or anaphylaxis to any medication.
12. Blood donation (excluding plasma donation) of approximately 500 mL within 56 days prior to Screening, or receipt of a blood transfusion within 1 year of Screening.
13. Plasma donation within seven days prior to screening.
14. Dosed in another investigational clinical trial within 30 days prior to Screening.
15. Any condition or prior therapy, e.g., seizures, or head trauma, that may lead to CNS effects during the study.
16. Documented or self-reported history of orthostatic hypotension or symptoms of hypotension such as dizziness, syncope, or blurred vision upon standing.
17. Any condition which is associated with increased brain permeability, e.g., cerebral ischemia, brain trauma, multiple sclerosis, brain tumors, brain infection.

Investigational Product, Dosage and Mode of Administration:

In all cohorts, study drug (ST-2427 or placebo) will be infused via IV over 1 hour. Subjects will be randomized 4:2 to receive a single dose of ST-2427 or placebo.

Subjects randomized to ST-2427 in Cohort 1 will receive 5 mg ST-2427 as a single IV infusion over one hour. The starting dose of 5 mg will be administered to all subjects in Cohort 1 regardless of body weight.

The doses of ST-2427 to be administered in all cohorts after Cohort 1 will be approved by the DMC following examination of all available clinical and PK data. In the SAD, the dose increase of

ST-2427 from Cohort 1 onwards is <2-fold from the prior cohort, [redacted] In all cohorts, placebo will be administered as 1-hour IV infusions at matching volumes and times to those administered to subjects randomized to ST-2427 in the same cohort. Details of study drug preparation are provided in the Pharmacy Manual for this study.
Duration of Treatment: Single 1-hour IV infusion
Reference Therapy, Dosage, and Mode of Administration: Placebo (5% Dextrose Injection, USP) will be administered by 1-hour IV infusions at the same volume and rate as used for ST-2427
Criteria for Evaluation: ***Safety:*** Safety measures include incidence and severity of AE's, physical examination [redacted] body weight, ECG, clinical laboratory testing (chemistry, hematology, urinalysis), respiratory rate, body temperature, oxygen saturation, local (injection site) irritation, and QTcF measures. ***Pharmacokinetics:*** Blood and urine samples will be collected at specified times during the study for measurement of ST-2427 concentration using a validated analytical method. The measured concentrations will be used for PK modeling.
Statistical Methods: Treatment effects on safety and tolerability will be evaluated using descriptive statistics (mean, standard deviation, median, minimum, maximum) of the number and proportion of subject experiencing adverse events by system organ class, and preferred term. Prior to dosing on Day 1, three separate measurements of supine BP (including DBP, SBP) and HR will be recorded one minute apart after at least 5 minutes rest. These repeat assessments will be used for analyses of change from baseline for these measures. Cardiodynamic evaluation will be performed to evaluate the treatment effects on heart rate-corrected QT interval using the Fridericia (QTcF) corrections, using concentration-QTc analysis, and on other ECG parameters (heart rate, PR and QRS interval and treatment emergent T and U-wave abnormalities). The statistical analysis will be detailed in a separate SAP. For purposes of monitoring safety, treatment-emergent adverse events (AEs) will be graded using the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers *(FDA 2007)* which is appropriate for healthy subjects. Descriptive statistics will be used to evaluate the treatment effects on clinical laboratory assessments including clinical chemistry, hematology, and urinalysis. PK modeling will be performed using compartmental methods. PK parameters such as, but not limited to C_{max}, T_{max}, Volume of distribution, Elimination half-life, and AUC after the ST-2427 infusion in the SAD. Descriptive statistics will be used to summarize PK parameters, and ST-2427

concentrations in the urine by cohort. In addition, exploratory analyses of the effects of some baseline characteristics (e.g., sex, body weight) on PK parameters may be undertaken.

2. INTRODUCTION

This is a Phase 1, randomized, double-blind, placebo-controlled study in healthy adult males and females of non-child-bearing potential to evaluate the safety, tolerability, and pharmacokinetics (PK) of ST-2427. [redacted] SiteOne Therapeutics, Inc. plans to use the safety, tolerability, and PK findings from this study to inform the doses and study design for Phase 2 clinical studies in subjects with acute post-operative pain.

Approximately 30 subjects, 6 subjects into each of 5 cohorts, will be enrolled in this study at a single clinical site.

Subjects will be randomized 4:2 to receive a single dose of ST-2427 or placebo in a Single Ascending Dose (SAD) design. The study will evaluate 5 dose strengths of ST-2427, infused intravenously (IV) over 1 hour; one dose level in each of 5 cohorts of subjects.

2.1. Indication

SiteOne Therapeutics, Inc. plans to develop ST-2427, a novel, selective, state-independent inhibitor of the voltage-gated sodium channel isoform Nav1.7, for treatment of acute postoperative pain and sparing of opioids. Study ST-2427-CS-001 is the first-in-human study, and aims to evaluate the safety, tolerability, and PK of ST-2427 when administered at increasing dose strengths/durations to healthy volunteers.

An estimated 80% of patients who undergo surgery experience acute postoperative pain that is typically moderate, severe or extreme in nature *(Chou 2016)*, a majority of whom have inadequate pain control which negatively impacts quality of life, recovery of function, the risk of persistent postsurgical pain, and morbidity and mortality due to post-surgical complications *(Apfelbaum 2003, Sharrock 1994, Katz 1996, Kehlet 2006)*. Effective postoperative pain control is essential to enhance patient comfort, earlier mobilization, fewer pulmonary and cardiac complications, reduced risk of deep vein thrombosis, faster recovery, less likelihood of developing neuropathic pain and reduced cost of care *(Ramsay 2000)*.

The American Pain Society, the American Society of Regional Anesthesia and Pain Medicine, and the American Society of Anesthesiologists *(Chou 2016)* recommends the use of opioids, non-steroidal anti-inflammatory drugs (NSAIDs) and/or acetaminophen, gabapentin or pregabalin, ketamine (IV) for management of postoperative pain in conjunction with appropriate

patient education, real-time pain evaluation/scoring, and other analgesia modalities including transcutaneous electrical nerve stimulation.

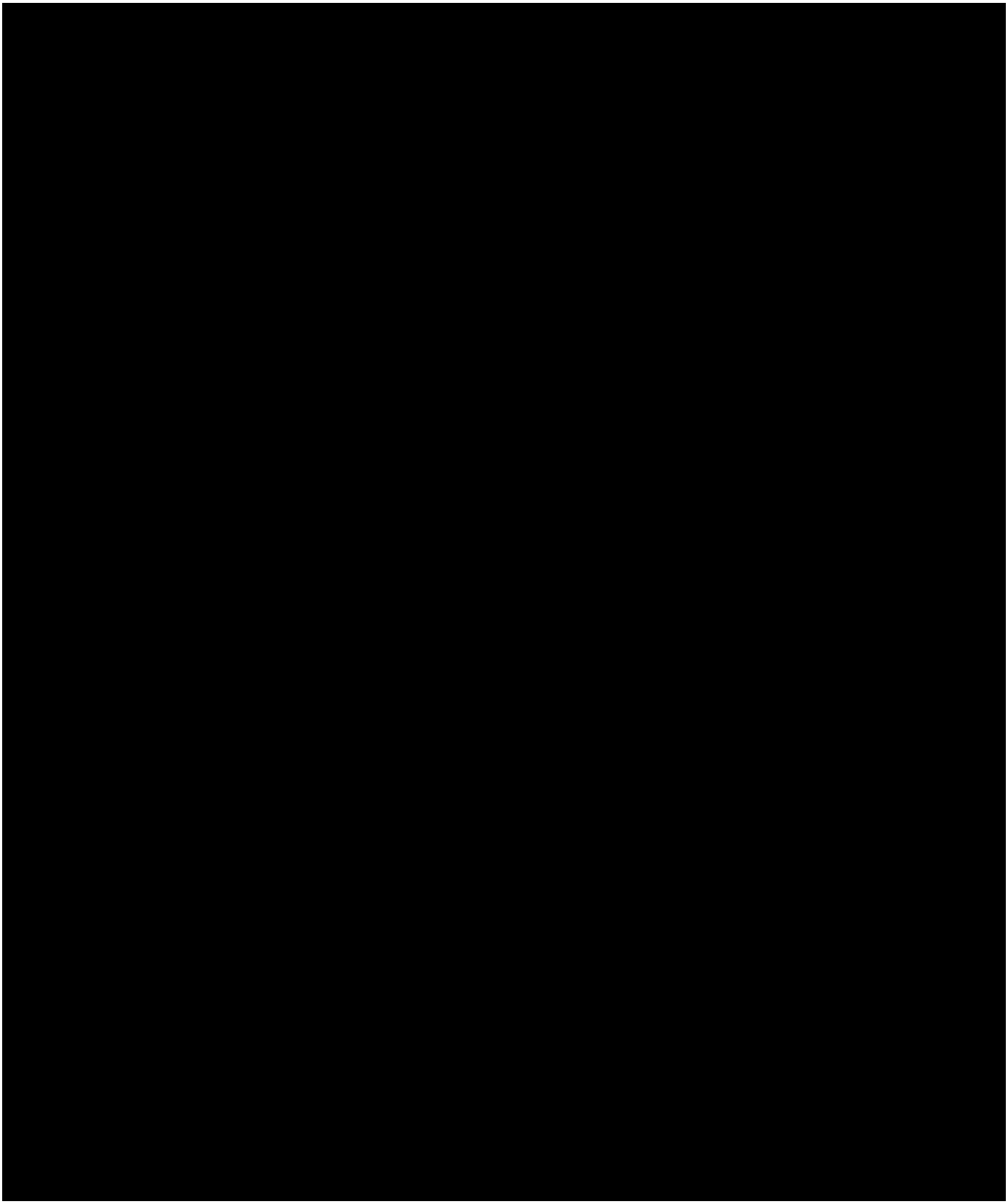
Opioids in particular are associated with dose-limiting side effects, such as confusion, drowsiness, nausea, respiratory depression and the potential for dependence and abuse *(Kelly 2008)*. The alarming level of opioid abuse in the United States (US) has led some to focus on multimodal pain management approaches that decrease or eliminate the use of opioids altogether *(Wardhan 2017)*. Key to this approach is the need to develop new, effective non-opioid analgesic therapies.

Human and animal genetic and pharmacology data support $Na_v1.7$ as a promising target for the development of non-opioid pain therapeutics. $Na_v1.7$ is preferentially expressed in peripheral somatic and visceral sensory neurons within the dorsal root ganglion, including nociceptors, olfactory sensory neurons, and sympathetic ganglion neurons *(Dib-Hajj 2013, Dib-Hajj 2017)*. Conditional, or global deletion of $Na_v1.7$ in mice led to reduction or loss of inflammatory pain responses induced by a range of stimuli *(Nassar 2004, Yeomans 2005, Minett 2012, Gingras 2014)*. Congenital insensitivity (indifference) to pain (CIP) in humans is linked to loss-of-function mutations in both alleles of SCN9A, the gene encoding $Na_v1.7$ *(Cox 2006)*. By contrast, dominantly inherited gain-of function missense mutations in SCN9A are found in patients with inherited erythromelalgia (IEM) and paroxysmal extreme pain disorder (PEPD), who report flushing and severe, episodic pain triggered by mild warmth or exercise (in IEM) or bowel movement (in PEPD) *(Yang 2004, Fertleman 2006)*.

2.2. Nonclinical Experience with ST-2427

ST-2427 is a highly selective, state-independent inhibitor of human voltage-gated Na^+ ion channel 1.7 ($Na_V1.7$) intended for the treatment of acute, postoperative pain.

[redacted]



[redacted]

[redacted]

[redacted]

2.3. Primary Objective

The primary objectives of the study are to evaluate the safety and tolerability of ST-2427 IV Infusion. [redacted]

2.4. Secondary Objectives

The secondary objective of the study is to measure the PK of ST-2427 IV infusion.

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design

Owing to its cationic nature, ST-2427 has low cellular permeability and is expected to have low bioavailability in humans if administered orally. As such, ST-2427 will be administered via IV in this trial. Furthermore, study drug will be administered slowly over a 1-hour infusion to mitigate/eliminate the number and severity of clinical observations which, in rats and monkeys, were generally observed at the time of highest drug concentration.

Subjects will be enrolled into the following sequential cohorts and randomized 4:2 to receive ST-2427 or placebo:

3.1.1. Single Ascending Dose Phase (SAD)

Cohort	Dose mg	Fold Increase from Previous Cohort	Predicted Peak Concentration ng/mL
1	5	—	200
2	10	2	375
3	15	1.5	565
4	22	1.5	820
5	33	1.5	1190

Subjects will be asked to participate in this study for a period of approximately 5 weeks: 4 weeks of Screening, 1 day of study treatment, 1 week of follow-up.

Sentinel dosing will be used in Cohort 1 wherein the first 2 subjects (one ST-2427, one placebo) will be randomly chosen to receive study drug and be followed for up to 2 days before any other subject is dosed in that cohort. Dosing of the remaining 4 subjects (3 ST-2427, 1 placebo) in the cohort will commence after safety review by the Principal Investigator and Sponsor. Study assessments will be performed at the days/times defined in Table 4. Subjects enrolled in the SAD will return to the clinic for additional assessments and sample collections at approximately 8 days (± 2 days) after the start of infusion.

3.1.2. General

Eligible subjects will be scheduled to return to the clinic and be admitted for a confinement period of 3 days in the SAD. The confinement period will begin the day before the start of study

drug administration to confirm eligibility for the study and for the completion of baseline assessments.

Subjects will return to clinic on Day 8 ± 2 days for the Follow-Up visit.

Safety and tolerability of each regimen will be assessed by adverse event (AE) monitoring, clinical laboratory tests, vital signs, body weight, electrocardiograms (ECGs), and physical examinations. Enrollment into Cohorts 2 through 5 will commence after all subjects in the prior cohort have completed the Follow-Up visit, and safety and PK data from all prior cohorts have been reviewed by a Data Monitoring Committee (DMC) consisting of the Principal Investigator, a medical representative from the Sponsor, and a PK expert, and no clinical concerns are identified.

3.2. Number of Subjects

Approximately 30 subjects will be enrolled into this study, 6 subjects in each of the 5 cohorts.

3.3. Treatment Assignment

Subjects who meet all criteria for enrollment will be randomly assigned to 1 of 2 treatment groups, ST-2427 or placebo, following a 4:2 allocation. A randomization schedule will be produced by an unblinded statistician using a validated computer program. Once baseline data (Day –1) has been reviewed and subjects are determined to be eligible for the study, they will be assigned a unique randomization number. Investigators and subjects will remain blinded to the study drug assignment and only certain designated staff (such as pharmacy personnel) will be unblinded to study treatment.

Sealed code break envelopes will be provided by an unblinded statistician prior to the start of the study. During the study, the blind is to be broken by an Investigator only when subject safety is at risk and unblinding must occur to determine effective medical intervention for the subject. All unscheduled unblinding events must be reported to the medical monitor and SiteOne Therapeutics, Inc. as soon as possible.

3.4. Dose Adjustment Criteria

The dose levels have been selected to reach defined peak concentrations as predicted from PK parameters in animal studies. If it is found in the initial study cohort that the achieved peak concentrations at a group level deviate from the predictions, doses will be adjusted accordingly

in subsequent cohorts to achieve the defined peak concentrations. All subjects will receive the dose defined for the cohort to which they were randomized.

3.4.1. Safety Criteria for Adjustment or Stopping Doses

The following stopping criteria will be used to inform decisions to continue drug infusion in any given subject:

3.4.2. Pharmacokinetic Criteria for Adjustment or Stopping Doses

If it is found in the initial study cohort that the achieved peak concentrations at a group level deviate from the predictions, doses will be adjusted accordingly in subsequent cohorts to achieve the defined peak concentrations.

3.5. Criteria for Study Termination

The study will run to completion unless the Sponsor decides to terminate it earlier, e.g., following a recommendation from the DMC based on review of all available PK and safety data.

Table 4: Schedule of Assessments in Part A (Single Ascending Dose)

	Screening	CONFINEMENT																			Follow-Up/EOS/ET
Time (days)	-28 to -2	-1	1																2	3	8± 2
Time[a] (hours)			Pre-dose	0	0.25	0.5	0.75	1	1.15	1.3	1.5	2	2.5	3	4	6	8	10	12	24	48	
Informed consent	x																				
Medical/medication history	x	x																			
Physical Examination	x	x																		x[b]	x
Weight, height, BMI	x	x																			
Serum Pregnancy Test	x	x																			
[redacted]																					
Local Irritation at the infusion site					x														x		
[redacted]																					
HIV, hepatitis B, hepatitis C testing	x																				
COVID-19 testing[f]		x																			
Follicle stimulating hormone[g]	x																				
Review inclusion/exclusion	x	x																			
Randomization		x																			
Admission to clinic		x																			
Confinement[h]		x																			
Discharge from clinic																				x	
Adverse events	x																				
Concomitant Medications	x	x																			
Study drug administration[i]				x																	
LABORATORY MEASURES																					
Urine drug and alcohol tests	x	x																			
Clinical chemistry	x	x																	x	x	x
Hematology	x	x																	x	x	x
Coagulation tests	x																				
Urinalysis	x	x																	x		x
Blood PK sample[j]			x		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	

	Screening	CONFINEMENT																		Follow-Up/EOS/ET
Time (days)	-28 to -2	-1	1															2	3	8± 2
Time[a] (hours)			Pre-dose	0	0.25	0.5	0.75	1	1.15	1.3	1.5	2	2.5	3	4	6	8	10	12	24	48	
Urine PK sample[k]			x	x											x		x		x	x	x	

a Time is defined relative to the start of the first infusion of study drug. For example, an assessment scheduled to occur at time = 2 hour must be performed 2 hours after the start of study drug infusion, i.e., 1 hour after the end of the infusion.

b A symptom-directed physical exam will be conducted prior to discharge.

c [redacted]

Orthostatic tests will be performed pre-dose (within 60 minutes prior to study drug administration), and at 0.5, 1, 1.5, 2, and 2.5 hours (± 10 minutes) after start of infusion. For these tests, subjects should be standing for 3 minutes with measurements of heart rate and blood pressure (SBP, DBP) at 1, 2, and 3 minutes after standing

d [redacted]

e [redacted]

f COVID-19 test will be performed after Screening is complete and subject is confirmed, but prior to check-in to the unit

g A blood sample will be collected from women and tested for follicle stimulating hormone to confirm eligibility for this study unless documented evidence of surgical sterilization is available and reviewed by qualified study personnel.

h Subjects will be confined at the clinic from the day before study drug infusion through at least 48 hours after the start of study drug infusion. This is predicted to exceed the time necessary for the concentration of ST-2427 to decrease to <5% of the peak concentration. During the study, the period of confinement may be extended if the drug elimination is found to be slower than predicted in the first cohort(s). Subjects will be discharged only if clinically significant AEs, if any, are resolved.

i Study drug is to be administered intravenously as a 1–hour infusion. A ±10-minute window is allowed for the duration of infusion, and a ±15-minute window is allowed for the start of infusion relative to the nominal time.

j Whole blood samples collected for PK analysis should be collected at the specified time. When blood sample collections coincide with other assessments, assessments should be conducted in the following order: [redacted] Whole blood samples for PK will be collected at the following time points:

- Day 1: pre-dose, and 0.25, 0.5, 0.75, 1, 1.15, 1.3, 1.5, 2, 2.5, 3, 4, 6, 8, 10, 12, 24, 48
- Predose PK samples should be collected within approximately 60 minutes prior to dosing

k A pre-dose urine sample will be collected at any time prior to dosing on Day 1 for analysis of pre-dose ST-2427 concentrations in the urine. Subjects are to be instructed to empty their bladders prior to dosing on Day 1. All urine will be collected for interval analysis of PK from the start of the first infusion until at least 48 hours later. Urine will be pooled 0 hr – 4 hr, 4 hr – 8 hr, 8 hr – 12 hr, 12 hr – 24 hr, and 24 hr – 48 hr collection windows and the collection time and volume for each collection recorded. Pooled urine samples will be maintained refrigerated and processed at the end of each pooled collection.

4. SELECTION AND WITHDRAWAL OF SUBJECTS

4.1. Subject Inclusion Criteria

Only subjects who meet all of the following criteria will be eligible for inclusion:

1. Healthy adult males and/or females (of non-childbearing potential), 18 to 55 years of age (inclusive) at the time of Screening.
2. Body mass index (BMI) within 18.0 to 35.0 kg/m^2, inclusive (minimum weight of at least 50.0 kg at Screening).
3. Medically healthy without clinically significant abnormalities at the screening visit, including physical examination and vital signs within the following ranges: heart rate 50 to 100 bpm, systolic blood pressure 100 to 149 mmHg; diastolic 70 to 94 mmHg.
4. The mean QTcF interval duration ≤450 msec for males and ≤470 msec for females measured from triplicate ECGs taken at least 1 minute apart with QT wave corrected for heart rate (HR) using Fredericia's method.
5. Hemoglobin/hematocrit, white blood cell (WBC) count, and platelet count equal to or greater than the lower limit of normal range of the reference laboratory (may be confirmed upon repeat testing without Sponsor approval).
6. Non-smokers (including tobacco, e-cigarettes, or marijuana), and no use of any tobacco product for at least 1 month prior to admission in the study.
7. Willing and able to provide written informed consent.
8. Willing and able to comply with all study assessments and adhere to the protocol schedule.
9. Have suitable venous access for blood sampling, as determined by an Investigator at screening.
10. If female, be of non-childbearing potential (e.g. post-menopausal as demonstrated by follicle stimulating hormone (>40 mIU/mL), amenorrhea for at least one year or surgically sterilized by tubal ligation, hysterectomy, bilateral oophorectomy and/or salpingectomy). Site personnel's review of the subject's medical records, medical examination, or medical history interview is acceptable evidence of female sterilization, verbal confirmation is adequate.

11. If male, willing not to donate sperm from the time of first study drug administration until 90 days after the final administration of study drug. If male and not intending to engage in sexual intercourse over the duration of the study, willing to agree to abstinence at screening. If male and engaging in sexual intercourse, willing to use a double barrier method of contraception (condom and spermicide). The latter criterion applies to all males (and/or female partners) including males who are surgically sterile and must be followed from the time of first study drug administration until 90 days after the final administration of study drug.

4.2. Subject Exclusion Criteria

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

1. History or presence of significant cardiovascular (including arrhythmia and ventricular tachyphylaxis), pulmonary, hepatic, renal, hematological, gastrointestinal, endocrine, immunologic, dermatologic, or neurological disease, including any acute illness or surgery within the past 3 months determined by an Investigator to be clinically relevant.
2. Creatinine, blood urea nitrogen (BUN), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) equal to 1.5 × upper limit of normal for the reference laboratory (may be confirmed upon repeat testing).

[redacted]

6. Positive urine drug/alcohol testing at Screening or Day –1.
7. Positive test results for HIV-1/HIV-2 Antibodies, Hepatitis B surface Antigen (HBsAg) or Hepatitis C Antibody (HCVAb).
8. Positive test results for COVID-19 (PCR or Antibodies).
9. History of substance abuse or alcohol abuse (defined as greater than 2 standard drinks per day) within the previous 2 years.

10. Use of any prescription medication or any over-the-counter medication, including herbal products and vitamins within 14 days or 5 half-lives (whichever is longer) prior to randomization.
11. Documented hypersensitivity reaction or anaphylaxis to any medication.
12. Blood donation (excluding plasma donation) of approximately 500 mL within 56 days prior to Screening, or receipt of a blood transfusion within 1 year of Screening.
13. Plasma donation within seven days prior to screening
14. Dosed in another investigational clinical trial within 30 days prior to Screening.
15. Any condition or prior therapy, e.g., seizures, or head trauma, that may lead to CNS effects during the study.
16. Documented or self-reported history of orthostatic hypotension or symptoms of hypotension such as dizziness, syncope, or blurred vision upon standing.
17. Any condition which is associated with increased brain permeability, e.g., cerebral ischemia, brain trauma, multiple sclerosis, brain tumors, brain infection.

4.3. Subject Withdrawal Criteria

There are no protocol-defined withdrawal criteria.

Subjects have the right to withdraw from the study at any time and for any reason without prejudice to their future medical care by the Investigator or at the institution. Subjects may also be discontinued from the study by the Investigator or the Sponsor for reasons such as safety, non-compliance, or ineligibility.

The Investigator will make a reasonable effort to determine the reason for withdrawal while respecting the subject's rights.

Reasons for removal from investigational product or observation might include:

- withdrawal of consent
- administrative decision by the Investigator or Sponsor
- hardship (e.g., move, family situation, change in job, etc.)
- pregnancy

- ineligibility
- major protocol deviation
- subject noncompliance
- AE
- death

Reasons for withdrawal will be reported in the corresponding CRF. Withdrawal of consent should only be indicated if no more specific reason can be ascertained.

For all subjects withdrawing from the study prior to the end of the study, a complete final evaluation including the assessments defined for the Follow-up visit should be made at the time of the subject's withdrawal.

5. TREATMENT OF SUBJECTS

5.1. Description of Study Drug

Table 5: Investigational Product

	Investigational Product	
Product Name:	ST-2427 for IV Infusion	Placebo (5% Dextrose Injection, USP)
Dosage Form:	Sterile Solution	Sterile Solution
Unit Dose (by Cohort)	250 mL total volume 1: 5 mg ST-2427 2: 10 mg ST-2427 3: 15 mg ST-2427 4: 22 mg ST-2427 5: 33 mg ST-2427	0 mg/mL
Route of Administration	Intravenous Infusion	Intravenous Infusion
Physical Description	Clear, colorless solution	Clear, colorless solution
Manufacturer	AMRI, Albany, NY Sterile solution will be prepared by Alta Sciences	Sourced by Alta Sciences

5.2. Concomitant Medications

No concomitant therapy is allowed between the start of confinement through the completion of the Follow-up visit. In addition, subjects are disqualified from participation in this study if they used any prescription medication or any over-the-counter medication, including herbal products and vitamins within 14 days or 5 half-lives (whichever is longer) prior to randomization.

5.3. Treatment Compliance

All study drug will be administered to all subjects by qualified site personnel during the confinement period. As such, treatment compliance is not relevant to this study. Missed doses will be assumed to be for reasons of subject safety, withdrawal, dosing error or other protocol deviation.

5.4. Randomization and Blinding

Subjects who meet all criteria for enrollment will be randomly assigned to 1 of 2 treatment groups, ST-2427 or placebo, following a 4:2 allocation. A randomization schedule will be produced by an unblinded statistician using a validated computer program. Once baseline data has been reviewed and subjects are determined to be eligible for the study, they will be assigned a unique randomization number. Investigators and subjects will remain blinded to the study drug assignment and only certain designated staff (such as pharmacy personnel) will be unblinded to study treatment.

Sealed code break envelopes will be provided by an unblinded statistician prior to the start of the study. During the study, the blind is to be broken by an investigator only when subject safety is at risk and unblinding must occur to determine effective medical intervention for the subject. Any unblinding events must be reported to the medical monitor and SiteOne Therapeutics, Inc. as soon as possible.

After all Follow-Up assessments are complete for subjects in a given cohort, safety and PK data will be reviewed by the DMC to determine whether to proceed to the next cohort, and the dose of ST-2427 to be administered in the next cohort.

In the event a pre-defined protocol stopping criterion is met, or a serious adverse event is observed for which knowledge of the randomization code would guide treatment decisions, the Investigator may be unblinded. In such cases, the Sponsor must be informed of the decision to unblind and provided all associated evidence within 24 hours.

Subjects who are randomized but withdraw prior to participation in the study will be replaced. Subjects that withdraw consent prior to dosing or discontinue due to adverse events or stopping criteria may be replaced.

6. STUDY DRUG MATERIALS AND MANAGEMENT

6.1. Study Drug

The study drug substance (ST-2427) will be manufactured by AMRI, Albany, New York in accordance with current good manufacturing practices (GMPs).

The placebo, Sterile 5% Dextrose Injection, USP (D5W) will be obtained commercially by the study site.

6.2. Study Drug Packaging and Labeling

Study drug substance (ST-2427) will be supplied to the pharmacy at the study site as non-sterile drug substance powder. Study drugs will be labeled according to the local regulatory requirements.

6.3. Study Drug Storage

ST-2427 will be stored frozen at –20°C, protected from light.

The D5W will be stored and handled according to the manufacturer label.

6.4. Study Drug Preparation

ST-2427 for IV infusion sterile solutions will be prepared by the clinical site pharmacy. The drug substance will be reconstituted in sterile 5% dextrose injection, USP. No other excipients will be added to the infusion solution. The site pharmacy will prepare the infusion solutions in a sterile compounding area and follow the requirements in USP <797> *Pharmaceutical Compounding – Sterile Preparations.*

Concentrations will be determined by the dose group and total volume of infusion. Refer to the *Study Pharmacy Manual* for detailed instructions.

6.5. Administration

Four subjects in each cohort will be randomized to receive ST-2427. ST-2427 will be administered via IV over 1 hour (± 10 minutes).

Two subjects in each cohort will be randomized to receive placebo. The placebo drug will be the matched volume of D5W administered via IV over 1 hour (± 10 minutes) to maintain the blind.

6.6. Study Drug Accountability

Study drug will be stored, inventoried, reconciled, and destroyed according to applicable regulations.

6.7. Study Drug Handling and Disposal

Any remaining study drug will be returned to SiteOne Therapeutics, Inc. or its designee for destruction or destroyed by the site (if the site has appropriate facilities and written procedures to dispose of clinical trial materials).

7. PHARMACOKINETIC ASSESSMENTS

Blood samples will be collected at protocol-defined time points for analysis of ST-2427 concentrations in whole blood using a validated analytical method. In addition, urine samples will be collected for analysis of ST-2427 concentrations in urine using a validated analytical method. A PK manual will be provided.

7.1. Blood Sample Collection

Blood will be collected from an accessible vein at the protocol-defined time points.

[redacted]

After each collection interval, primary samples will be shipped by overnight carrier to the address below. Duplicate samples will be stored at the clinical site for shipment as and when requested.

[redacted]
Alturas Technology Park
1324 Alturas Drive
Moscow, Idaho 83843
UNITED STATES OF AMERICA
Telephone No.: 208.883.3400
E-mail: [redacted]
cc: samplecustodian@alturasanalytics.com
cc: [redacted]

Before shipping, the recipient (lhvozda@alturasanalytics.com, lmalsack@alturasanalytics.com, and samplecustodian@alturasanalytics.com), Study Director, Study Coordinator, and Study Monitor will be notified by e-mail as to the date and method of sample shipments.

7.2. Urine Sample Collection

Urine samples will be collected at the protocol-defined intervals in the SAD.

During each protocol-defined urine collection interval, urine will be pooled in 0hr - 4hr, 4hr – 8hr, 8hr – 12hr, 12hr – 24hr and 24hr – 48hr collection windows and the collection time and volume for each collection recorded. Pooled urine samples will be maintained refrigerated and processed at the end of each 4-hour pooled collection. Approximately 200 µL of urine from each 4-hour pool will be added to a tube containing approximately 200 µL of ammonium acetate buffer (provided by Alturas Analytics as a 500 mM, pH 5.4 ammonium acetate buffer and stored at ambient conditions) and placed on wet ice or chilled cryoracks for up to 6 hours until stored in a freezer, set to maintain approximately –70°C. This will be repeated 2 more times to create 3 samples of urine/ammonium acetate mixture for every 4-hour pool. Any remaining urine will be discarded.

Samples will be collected, stored, and shipped as described above for the whole blood samples.

7.3. Sample Analysis

Whole blood and urine analysis for ST-2427 will be conducted by Alturas Analytics using validated HPLC-MSMS methods (TM20-573, urine; TM20-574, whole blood:buffer), and in accordance with the guidelines indicated in Alturas Analytics' SOPs.

Incurred sample reproducibility will be conducted in accordance with Test Site standard operating procedures on whole blood and urine samples. Results will be included in the analysis report.

When requested, ST-2427 concentrations in blood and urine will be transferred to the Sponsor-designated PK expert. These data will be analyzed using standard compartmental models (described in Section 9) for determination of PK parameters in time for the DMC meetings at the end of each cohort.

8. ASSESSMENT OF SAFETY

8.1. Safety Parameters

8.1.1. Demographic/Medical History

Demographics, including sex, age, race, ethnicity, and medical history including body weight, height, BMI, concomitant medications, and child-bearing potential (for women) will be recorded during Screening. These data will be used to evaluate eligibility for the trial. Demographics and medical history will be summarized as Baseline characteristics in the Clinical Study Report.

In addition, exploratory analyses of the effects of Baseline parameters (e.g., sex, body weight) on ST-2427 PK may be undertaken (refer to Section 9.2).

[redacted]

8.1.3. Weight, Height and Body Mass Index

Body weight and height will be measured and recorded for all subjects in the SAD during screening and upon admission for the confinement (on Day –1). BMI will be computed from body weight and height at all observation time points.

8.1.4. Physical Examination

The Investigator will perform a physical examination on all subjects at Screening, upon admission for the confinement, at the Follow-Up visit and for Symptom directed Physical Examination at discharge. Physical examinations will include assessments of possible medical signs or symptoms from clinical observation and subject interview per site SOPs. All findings will be recorded on case report forms.

8.1.5. Local Injection Site Irritation

Consistent with Food and Drug Administration (FDA) guidance, *Guidance for Industry Toxicity Graading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials, (2007)*, the assessment of local irritation at the injection site will be performed at the visits noted in Table 4 and Appendix D. The scale consists of 4 categories with each graded on a scale from 1 to 4 based on clinical abnormality. The scale is provided in Appendix D.

8.1.6. Electrocardiogram (ECG)

At Screening, ECGs will be recorded from all subjects in the SAD to evaluate and confirm subject eligibility for the trial.

Telemetry will be performed for detection of arrhythmias for 12 hours on Day 1 in SAD.

ECGs will be extracted at time points detailed in Schedule of Events.

8.1.7. Laboratory Assessments

Blood samples will be collected at pre-defined time points for evaluation of clinical laboratory tests (Appendix B), and the PK of ST-2427. The total blood volume to be collected from each subject in this study is approximately 65.2 mL during participation in the SAD (Appendix C). All hematology and blood chemistry analyses will be conducted by a central laboratory.

8.1.7.1. Hematology

At Screening, at the protocol-defined time points during the confinement periods for the SAD, and at the Follow-Up visit, blood samples will be collected for analysis of hematology. At each of these time points, approximately 4 mL of venous blood will be collected into potassium EDTA tubes.

8.1.7.2. Blood Chemistry

At Screening, at the protocol-defined time points during the confinement periods for the SAD, and at the Follow-Up visit, blood samples will be collected for analysis of clinical chemistry. At each of these time points, approximately 5 mL of venous blood will be collected in tubes without anticoagulant.

An additional approximately 2.7 mL venous blood sample will be collected into a sodium citrate tube for coagulation tests at Screening.

8.1.7.3. Urinalysis

Urinalyses will be conducted using spot urine sample collections at Screening, Check-In (Day -1), 24 hours, and at the Follow-up visit.

In addition, separate spot urine samples will be collected at Screening for analysis of drugs of abuse (Section 8.1.7.5).

8.1.7.4. Virus Serology

A single, approximately 8.5-mL blood sample will be collected at Screening for testing for human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg), or hepatitis C antibodies (HCV). All tests will be conducted by a central laboratory.

8.1.7.5. Drug Screen

At Screening and upon admission for the confinement, a urine alcohol test will be conducted, and a urine sample will be collected for testing for drugs of abuse. Urine drug tests will be conducted by a central laboratory and will screen for amphetamine, methamphetamine, benzodiazepines, barbiturates, cannabinoids, cocaine, cotinine, phencyclidine, methadone, and opioids.

8.1.7.6. Evaluation of Child-Bearing Potential

Women who enroll into this study must be of non-child-bearing potential, e.g., post-menopausal as demonstrated by follicle stimulating hormone, or surgically sterilized by tubal ligation, hysterectomy, bilateral oophorectomy and/or salpingectomy. During Screening, a blood sample will be collected from women and tested for follicle stimulating hormone to confirm eligibility for this

study unless documented evidence of surgical sterilization is available and reviewed by qualified study personnel.

8.2. Adverse and Serious Adverse Events

8.2.1. Definition of Adverse Events

8.2.1.1. Adverse Event (AE)

An AE is the development of an undesirable sign (including an abnormal laboratory finding), symptom, medical condition or the deterioration of a pre-existing medical condition following or during exposure to a pharmaceutical product, whether or not considered casually related to the product. In clinical studies, an AE can include an undesirable medical condition occurring at any time, including baseline or washout periods, even if no study treatment has been administered.

It is the responsibility of the investigator to document all AEs that occur during the study. All AEs that occur after any patient/subject has been enrolled, before treatment, during treatment, or at any time up to the Follow-Up visit, whether or not they are related to the study, must be recorded on the appropriate case report forms (CRFs).

8.2.1.2. Serious Adverse Event (SAE)

A SAE is an AE occurring during any study phase (i.e., Baseline, treatment, washout, or Follow-Up), and at any dose of the investigational product, comparator, or placebo, that fulfils one or more of the following:

- Results in death
- It is immediately life-threatening
- It requires in-patient hospitalization or prolongation of existing hospitalization
- It results in persistent or significant disability or incapacity
- Results in a congenital abnormality or birth defect
- It is an important medical event that may jeopardize the patient or may require medical intervention to prevent one of the outcomes listed above.

All SAEs that occur after any patient/subject has been enrolled, before treatment, during treatment, or at any time up to the Follow-Up visit, whether or not they are related to the study, must be recorded on the appropriate CRF.

Definition of Terms

Life-Threatening: An AE is life-threatening if it places the subject at immediate risk of death from the event as it occurred (i.e., it does not include an event that, had it occurred in a more severe form, might have caused death). For example, drug induce hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening, even though drug induced hepatitis can be fatal.

Hospitalization: An AE requiring hospitalization should be considered an SAE. Hospitalization scheduled for an elective procedure or treatment of a pre-existing condition that has not worsened during the participation in the trial (e.g., elective surgery for a pre-existing condition that has not worsened), or for a routine clinical procedure that is not the result of an AE is not considered as an AE, either serious or non-serious, according to the defined criteria.

In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency department for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting.

Disability/Incapacity: An AE is disabling or incapacitating if the event results in a substantial disruption of a subject's ability to conduct normal life functions.

8.2.1.3. Other Adverse Events

Other AEs will be identified by the Drug Safety Investigator and if applicable also by the Clinical Study Team Investigator during the evaluation of safety data for the Clinical Study Report. Significant AEs of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the patient/subject from the study, will be classified as other AEs. For other AEs, a narrative may be written and included in the Clinical Study Report.

8.3. Relationship to Study Drug

An Investigator who is qualified in medicine must make the determination of relationship to the investigational product for each AE (Unrelated, Possibly Related, or Probably Related). The Investigator should decide whether, in his or her medical judgment, there is a reasonable possibility

that the event may have been caused by the investigational product. If no valid reason exists for suggesting a relationship, then the AE should be classified as "unrelated." If there is any valid reason, even if undetermined, for suspecting a possible cause-and-effect relationship between the investigational product and the occurrence of the AE, then the AE should be considered "related."

If the relationship between the AE/SAE and the investigational product is determined to be "possible" or "probable" the event will be considered related to the investigational product for the purposes of expedited regulatory reporting.

8.4. Recording Adverse Events

AEs spontaneously reported by the patient/subject and/or in response to an open question from the study personnel or revealed by observation will be recorded during the study at the investigational site. Clinically significant changes in laboratory values, blood pressure, and pulse need not be reported as AEs. However, abnormal values that constitute an SAE or lead to discontinuation of administration of study drug must be reported and recorded as an AE. Information about AEs will be collected from the Screening at enrollment until the end of the study. SAE information will be collected from the first administration of study drug until the Follow-up visit. The AE term should be reported in standard medical terminology when possible. For each AE, the investigator will evaluate and report the onset (date and time), resolution (date and time), intensity, causality, action taken, serious outcome (if applicable), and whether or not it caused the patient to discontinue the study.

Intensity will be assessed according to the following scale:

- Grade 1: Mild (awareness of sign or symptom, but easily tolerated)
- Grade 2: Moderate (discomfort sufficient to cause interference with normal activities)
- Grade 3: Severe (incapacitating, with inability to perform normal activities)
- Grade 4: Potentially Life Threatening (emergency room visit or hospitalization)

Should a pregnancy occur, it must be reported and recorded on the pregnancy reporting form. Pregnancy in itself is not regarded as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth, or congenital abnormality) must be followed up and documented even if the patient was discontinued from the study.

All reports of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as SAEs. Elective abortions without complications should not be handled as AEs.

8.5. Reporting Adverse Events

All SAEs (related and unrelated) will be recorded from the first administration of study drug until the Follow-Up visit. All SAEs discovered by the Investigator at any time should be reported. All SAEs must be reported to SiteOne Therapeutics, Inc. within one business day of the first awareness of the event. The Investigator must complete, sign and date the SAE pages, verify the accuracy of the information recorded on the SAE pages with the corresponding source documents, and send a copy by telephone or email to SiteOne Therapeutics, Inc.

Additional follow-up information, if required or available, should all be emailed to SiteOne Therapeutics, Inc. within one business day of receipt and this should be completed on a follow-up SAE form and placed with the original SAE information and kept with the appropriate section of the CRF and/or study file.

SiteOne Therapeutics, Inc. is responsible for notifying the relevant regulatory authorities of certain events. It is the Principal Investigator's responsibility to notify the IRB or IEC of all SAEs that occur at his or her site. Investigators will also be notified of all unexpected, serious, drug-related events (7/15 Day Safety Reports) that occur during the clinical trial. Each site is responsible for notifying its IRB or IEC of these additional SAEs.

9. STATISTICS

9.1. Statistical Methods

Treatment effects on safety and tolerability will be evaluated using descriptive statistics (mean, standard deviation, median, minimum, maximum) of the number and proportion of subject experiencing adverse events by system organ class, and preferred term.

Prior to dosing on Day 1, three separate measurements of supine blood pressure (BP; diastolic blood pressure [DBP], systolic blood pressure [SBP]) and heart rate (HR) will be recorded one minute apart. These values will be averaged and used to establish the baseline from which analyses of change from baseline will be computed.

For purposes of monitoring safety, AEs will be graded using the *Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers (FDA, 2007)* which is appropriate for healthy subjects.

Descriptive statistics will be used to evaluate the treatment effects on clinical laboratory assessments including clinical chemistry, hematology, and urinalysis.

9.2. Pharmacokinetic Analyses

PK modeling will be performed using compartmental methods. PK parameters including but not limited to C_{max}, T_{max}, Volume of distribution, Elimination half-life, and AUC after the ST-2427 infusion in the SAD.

Descriptive statistics will be used to summarize PK parameters, and ST-2427 concentrations in the urine by cohort.

In addition, exploratory analyses of the effects of some baseline characteristics (e.g., sex, body weight) on ST-2427 PK parameters may be undertaken.

9.3. Analysis of ECGs

ECGs will be collected during SAD as indicated in the Schedule of Events. Data from the ECGs will be used to determine cardiodynamic effects including concentration-QTc analysis. Details of this analysis will be given in a separate SAP and will include change-from-baseline ECG parameters (heart rate (HR), PR, QTcF and QRS), categorical outliers for HR, QTcF, PR and QRS and frequency of treatment emergent T- and U-wave abnormalities. For QTcF, the following categories will be used in the outlier analysis:

- Categorical analyses: number and proportion of subjects meeting the following corrected QT categories: >450 msec, >480 msec, and >500 msec,

9.4. Analysis Populations

The safety population will include all subjects who received at least one dose of study drug. Subjects will be analyzed as treated. The safety population will be used for all analyses of safety endpoints.

The PK population will include all subjects who received at least one dose of study drug, and who have sufficient data for evaluation of at least one PK parameter. Subjects will be analyzed as treated. Subjects randomized to placebo will not be included in the analysis.

9.5. Handling of Missing Data

Every effort will be made to query missing or spurious data prior to unblinding for DMC reviews and database lock.

Missing safety or PK data will not be imputed.

Deviations from the protocol will be recorded and assessed for potential to impact the trial outcomes.

9.6. Sample Size

Descriptive, non-inferential statistics are planned for the primary endpoint analyses. Based on clinical judgement, a sample size of 30 (20 randomized to ST-2427, and 10 randomized to placebo) is expected to be adequate to identify major treatment effects.

9.7. Data Monitoring Committee Reviews

A DMC consisting of the Principal Investigator, a medical representative from the Sponsor, and a PK expert, will be appointed for this study. Enrollment into Cohorts 2 through 5 will commence after all subjects in the prior cohort have completed the Follow-Up visit, and safety and PK data from all prior cohorts have been reviewed by the DMC, and no clinical concerns are identified. The DMC will additionally examine all available clinical and PK data to determine the dose of ST-2427 to be administered in the next cohort.

The study will run to completion unless the Sponsor decides to terminate it earlier, e.g., following a recommendation from the DMC following review of all available PK and safety data.

10. DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

10.1. Study Monitoring

Before an investigational site can enter a subject into the study, a representative of SiteOne Therapeutics, Inc. will:

- Determine the adequacy of the facilities
- Discuss with the Investigator(s) and other personnel their responsibilities with regard to protocol adherence, and the responsibilities of SiteOne Therapeutics, Inc. or its representatives. This will be documented in a Clinical Study Agreement between SiteOne Therapeutics, Inc. and the Investigator.

During the study, a monitor will have regular contacts with the investigational site, for the following:

- Provide information and support to the Investigator(s)
- Confirm that facilities remain acceptable
- Confirm that the investigational team is adhering to the protocol, that data are being accurately recorded in the CRFs, and that investigational product accountability checks are being performed
- Perform source data verification. This includes a comparison of the data in the CRFs with the patient's medical records at the hospital or practice, and other records relevant to the study. This will require direct access to all original records for each patient (e.g., clinic charts).
- Record and report any protocol deviations not previously sent to SiteOne Therapeutics, Inc..
- Confirm AEs and SAEs have been properly documented on CRFs and confirm any SAEs have been forwarded to SiteOne Therapeutics, Inc. and those SAEs that met criteria for reporting have been forwarded to the IRB.

The monitor will be available between visits if the Investigator(s) or other staff needs information or advice.

10.2. Audits and Inspections

Authorized representatives of SiteOne Therapeutics, Inc., a regulatory authority, an Independent Ethics Committee (IEC), or an Institutional Review Board (IRB) may visit the site to perform audits or inspections, including source data verification. The purpose of a SiteOne Therapeutics, Inc. audit

or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, Good Clinical Practice (GCP) guidelines of the International Conference on Harmonization (ICH), and any applicable regulatory requirements. The Investigator should contact SiteOne Therapeutics, Inc. immediately if contacted by a regulatory agency about an inspection.

10.3. Institutional Review Board

The Principal Investigator must obtain IRB approval for the investigation. Initial IRB approval, and all materials approved by the IRB for this study including the patient consent form and recruitment materials must be maintained by the Investigator and made available for inspection.

11. QUALITY CONTROL AND QUALITY ASSURANCE

To ensure compliance with GCPs and all applicable regulatory requirements, SiteOne Therapeutics, Inc., or their representative may conduct a quality assurance audit.

12. ETHICS

12.1. Ethics Review

The final study protocol, including the final version of the Informed Consent Form, must be approved, or given a favorable opinion in writing by an IRB or IEC as appropriate. The Investigator must submit written approval to SiteOne Therapeutics, Inc. before he or she can enroll any patient/subject into the study.

The Principal Investigator is responsible for informing the IRB or IEC of any amendment to the protocol in accordance with local requirements. In addition, the IRB or IEC must approve all advertising used to recruit patients for the study. The protocol must be re-approved by the IRB or IEC upon receipt of amendments and annually, as local regulations require.

The Principal Investigator is also responsible for providing the IRB with reports of any reportable serious adverse drug reactions from any other study conducted with the investigational product. SiteOne Therapeutics, Inc. will provide this information to the Principal Investigator.

Progress reports and notifications of serious adverse drug reactions will be provided to the IRB or IEC according to local regulations and guidelines.

12.2. Ethical Conduct of the Study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki (refer to Appendix A) and are consistent with ICH/GCP, and applicable regulatory requirements.

12.3. Written Informed Consent

The Principal Investigator(s) at each center will ensure that the patient is given full and adequate oral and written information about the nature, purpose, possible risk, and benefit of the study. Subjects must also be notified that they are free to discontinue from the study at any time. The subject should be given the opportunity to ask questions and allowed time to consider the information provided.

The subject's signed and dated informed consent must be obtained before conducting any study procedures.

The Principal Investigator(s) must maintain the original, signed Informed Consent Form. A copy of the signed Informed Consent Form must be given to the subject.

[redacted]

14. PUBLICATION POLICY

The publication policy is detailed in the Clinical Trial Agreement.

The data generated in this clinical trial are the exclusive property of SiteOne Therapeutics, Inc. and are confidential. Written approval from SiteOne Therapeutics, Inc. is required prior to disclosing any information related to this clinical trial. Authorship on any publication of the results from this study will be at SiteOne Therapeutics, Inc. discretion based upon contributions to study enrollment, data analysis and interpretation of results as well as being consistent with the Uniform Requirements of the International Committee of Medical Journal Editors.

15. LIST OF REFERENCES

Alexandrou, A. J. et al.Subtype-selective small molecule inhibitors reveal a fundamental role for Nav1.7 in nociceptor electrogenesis, axonal conduction and presynaptic release. PLoS One 2016;11.

Apfelbaum JL, Chen C, Mehta SS, Gan TJ. Postoperative pain experience: results from a national survey suggest postoperative pain continues to be undermanaged. *Anesth Analg.* 2003 Aug;97(2):534-40.

Chou R, Gordon DB, de Leon-Casasola OA, Rosenberg JM, Bickler S, Brennan T, *et al.* Management of postoperative pain: a clinical practice guideline from the American Pain Society, the American Society of Regional Anesthesia and Pain Medicine, and the American Society of Anesthesiologists' Committee on Regional Anesthesia, Executive Committee, and Administrative Council. *J Pain*. 2016 Feb;17(2):131-57.

Cox JJ, Reimann F, Nicholas AK, Thornton G, Roberts E, Springell K, *et al.* An SCN9A channelopathy causes congenital inability to experience pain. *Nature.* 2006 Dec 14;444(7121):894-8.

Dib-Hajj SD, Yang Y, Black JA, Waxman SG. The Na(V)1.7 sodium channel: from molecule to man. *Nat Rev Neurosci.* 2013 Jan;14(1):49-62.

Dib-Hajj SD, Geha P, Waxman SG. Sodium channels in pain disorders: pathophysiology and prospects for treatment. *Pain.* 2017 Apr;158 Suppl 1: S97-S107.

Fertleman CR, Baker MD, Parker KA, Moffatt S, Elmslie FV, Abrahamsen B, *et al*. SCN9A mutations in paroxysmal extreme pain disorder: allelic variants underlie distinct channel defects and phenotypes. *Neuron.* 2006 Dec 7;52(5):767-74.

Gingras J, Smith S, Matson DJ, Johnson D, Nye K, Couture L, *et al*. Global Nav1.7 knockout mice recapitulate the phenotype of human congenital indifference to pain. *PLoS One.* 2014 Sep 4;9(9): e105895.

Jurcakova, D. et al.Voltage-gated sodium channels regulating action potential generation in itch-, nociceptive-, and low-threshold mechanosensitive cutaneous C-fibers. Mol. Pharmacol. 2018;94:1047–1056.

Katz J, Clairoux M, Redahan C, Kavanagh BP, Carroll S, Nierenberg H, *et al.* High dose alfentanil pre-empts pain after abdominal hysterectomy. *Pain*. 1996 Nov;68(1):109-18.

Kehlet H, Jensen TS, Woolf CJ. Persistent postsurgical pain: risk factors and prevention. *Lancet*. 2006 May 13;367(9522):1618-25.

Kelly JP, Cook SF, Kaufman DW, Anderson T, Rosenberg L, Mitchell AA. Prevalence and characteristics of opioid use in the US adult population. *Pain*. 2008;138(3):507-513.

Minett MS, Nassar MA, Clark AK, Passmore G, Dickenson AH, Wang F, *et al.* Distinct Nav1.7-dependent pain sensations require different sets of sensory and sympathetic neurons. *Nat Commun.* 2012 Apr 24; 3:791.

Nassar MA, Stirling LC, Forlani G, Baker MD, Matthews EA, Dickenson AH, *et al.* Nociceptor-specific gene deletion reveals a major role for Nav1.7 (PN1) in acute and inflammatory pain. *Proc Natl Acad Sci U S A*. 2004 Aug 24;101(34):12706-11.

Ramsay MA. Acute postoperative pain management. *Proc (Bayl Univ Med Cent).* 2000 Jul;13(3):244-7.

Sharrock NE, Urquhart BL, Ganz S, Williams-Russo PG. Epidural infusions of bupivacaine and fentanyl do not improve rehabilitation following one-stage bilateral total knee arthroplasty. *Ann Acad Med Singapore.* 1994 Nov;23(6 Suppl):3-9.

Wardhan R, Chelly J. Recent advances in acute pain management: understanding the mechanisms of acute pain, the prescription of opioids, and the role of multimodal pain therapy. *F1000Res.* 2017 Nov 29;6: 2065.

World Medical Association. Ethical Principles for Medical Research Involving Human Subjects. JAMA. 2013;310(20):2191-2194.

Yang Y, Wang Y, Li S, Xu Z, Li H, Ma L, *et al.* Mutations in SCN9A, encoding a sodium channel alpha subunit, in patients with primary erythromalgia. *J Med Genet.* 2004;41(3):171-174.

Yeomans DC, Levinson SR, Peters MC, Koszowski AG, Tzabazis AZ, Gilly WF, *et al.* Decrease in inflammatory hyperalgesia by herpes vector-mediated knockdown of Nav1.7 sodium channels in primary afferents. *Hum Gene Ther.* 2005 Feb;16(2):271-7.

Yeomans DC, Lu Y, Laurito CE, Peters MC, Vota-Vellis G, Wilson SP, *et al.* Recombinant herpes vector-mediated analgesia in a primate model of hyperalgesia. Mol Ther. 2006 Mar;13(3):589-97.

APPENDIX A:
WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Subjects (2013)
JAMA. 2013;310(20):2191-2194.

Preamble

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.

2. The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.

3. Consistent with the mandate of the WMA, the Declaration is addressed primarily to Investigators. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

General Principles

4. The Declaration of Geneva of the WMA binds the Investigator with the words, "*The health of my patient will be my first consideration,*" and the International Code of Medical Ethics declares that, "*A Investigator shall act in the patient's best interest when providing medical care.*"

5. It is the duty of the Investigator to promote and safeguard the health, well-being, and rights of patients, including those who are involved in medical research. The Investigator's knowledge and conscience are dedicated to the fulfilment of this duty.

6. Medical progress is based on research that ultimately must include studies involving human subjects.

7. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures, and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility. and quality.

8. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.

9. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.

10. It is the duty of Investigators who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the Investigator or other health care professionals and never with the research subjects, even though they have given consent.

11. Investigators must consider the ethical, legal, and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal, or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

12. Medical research should be conducted in a manner that minimizes possible harm to the environment.

13. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training, and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified Investigator or other health care professional.

14. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.

15. Investigators who combine medical research with medical care should involve their patients in research only to the extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the Investigator has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

16. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

APPENDIX B:
CLINICAL LABORATORY TESTS

Clinical Chemistry:

Serum Concentrations of:

- Sodium
- Potassium
- Chloride
- Total serum CO_2
- Blood urea nitrogen (BUN)
- Total Calcium
- Phosphorus
- Magnesium
- Glucose
- Creatinine
- Albumin
- Pre-albumin
- Total protein
- Alkaline phosphatase
- Alanine aminotransferase (ALT)
- Aspartate aminotransferase (AST)
- Lactate dehydrogenase (LDH)
- Total bilirubin
- Direct bilirubin
- Uric Acid
- Gamma-glutamyl transferase (GGT)
- Creatine phosphokinase (CPK)

Fasting Lipid Profile:

- Total cholesterol
- Triglycerides
- Low Density Lipoprotein (LDL)
- High Density Lipoprotein (HDL)
- Very Low Density Lipoprotein (VLDL)

Hematology:

- Hematocrit
- Hemoglobin
- Erythrocyte count (RBC)
- Leukocytes (WBC)

Absolute counts of:

- Neutrophils, segmented
- Neutrophils, juvenile (bands)
- Lymphocytes
- Monocytes
- Eosinophils
- Basophils
- Platelets

Urinalysis:

- Protein
- Specific gravity
- pH
- Glucose
- Ketones
  - Bilirubin
  - Urobilinogen
  - Blood
  - Nitrite
  - Leukocyte esterase
  - Urine creatinine
  - Urine uric acid

Other:

- Follicle stimulating hormone[a]
- Activated partial thromboplastin time (aPTT)
- Prothrombin time/International normalized ratio (PT/INR)
- HIV-1/HIV-2 Antibodies
- Hepatitis B surface Antigen (HBsAg)
- Hepatitis C Antibody (HCVAb)
- Urinary drug screen (UDS)

Abbreviations: RBC = red blood cells; WBC = white blood cells.

Note: All labs will be assayed by central laboratory unless otherwise noted.

a In women only for confirmation of non-child-bearing potential if documented evidence of surgical sterilization is not available

APPENDIX C:
BLOOD VOLUMES TO BE COLLECTED IN THIS STUDY

The tables below summarize the maximum volumes for all blood sampling during screening, standard laboratory, and monitoring tests during the study. Fewer samples may actually be collected, but this will not require a protocol amendment. Similarly, additional samples may be drawn if needed for safety assessments.

Maximum Blood Volume To be Collected

	Blood volume per sample (mL)	# Samples collected	Total Blood Volume (mL)	Total Blood Volume (pint)
Clinical Chemistry	5	4	25.5	0.053
Coagulation	2.7	1	2.7	0.006
Hematology	4	4	12	0.032
Pharmacokinetics	1	18	16	0.169
Virus serology	8.5	1	8.5	0.011
TOTAL	24.7	28	65.2	0.138

APPENDIX D:
TABLE FOR CLINICAL ABNORMALITIES ASSOCIATED WITH LOCAL REACTION TO INJECTABLE PRODUCT

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness *	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling **	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.