Supplemental Online Content

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Supplement 1. Trial protocol

This supplemental material has been provided by the authors to give readers additional information about their work.

MST-188-01

EVALUATION OF PURIFIED POLOXAMER 188 IN VASO-OCCLUSIVE CRISIS OF SICKLE CELL DISEASE (EPIC):

A PHASE 3 RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTICENTER CLINICAL TRIAL OF MST-188 (PURIFIED POLOXAMER 188) INJECTION IN SUBJECTS WITH SICKLE CELL DISEASE EXPERIENCING VASO-OCCLUSIVE CRISIS

Sponsor	Mast Therapeutics, Inc. 12390 El Camino Real, Suite 150 San Diego, California 92130 USA	
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1. PROTOCOL AGREEMENT

Protocol Number:	MST-188-01
Protocol Title:	Evaluation of Purified Poloxamer 188 in Vaso-Occlusive Crisis of Sickle Cell Disease (EPIC): A Phase 3 Randomized, Double-Blind, Placebo-Controlled, Multicenter Clinical Trial of MST-188 (purified poloxamer 188) Injection in Subjects with Sickle Cell Disease Experiencing Vaso-Occlusive Crisis
Amendment Number:	Amendment 6 Dated: 20 November 2014

The undersigned agree that this protocol, dated 20 November 2014, shall apply to the conduct of the study identified above. By signing below, the Investigator agrees to conduct the study identified above in accordance with the terms set forth herein.

On behalf of the Sponsor:

Edwin Parsley, DO
Chief Medical Officer
Mast Therapeutics, Inc.

Date 20 NOV 2014
(DD-MMM-YYYY)

Institution
(please print) _____

Investigator Name
(please print) _____

Investigator Signature _____

Date (DD-MMM-YYYY) _____

2. SYNOPSIS

Name of Sponsor/Company: Mast Therapeutics, Inc. (Mast Therapeutics)

Name of Investigational Drug Product: MST-188 (purified poloxamer 188) Injection [MST-188; also known as ANX-188 (purified poloxamer 188) Injection or ANX-188]

Name of Investigational Drug Substance: purified poloxamer 188 (vepoloxamer)

Study Title:

Evaluation of Purified Poloxamer 188 in Vaso-Occlusive Crisis of Sickle Cell Disease (EPIC): A Phase 3, Randomized, Double-Blind, Placebo-Controlled Multicenter Clinical Trial of MST-188 (purified poloxamer 188) Injection in Subjects with Sickle Cell Disease Experiencing Vaso-Occlusive Crisis

(Protocol No. MST-188-01)

Phase of Development: 3

Lead Clinical Investigator: James Casella, MD

Objectives:

- The primary objective is to demonstrate the efficacy of MST-188 in reducing the duration of vaso-occlusive crisis (VOC) in subjects with sickle cell disease (SCD). The duration of VOC will be measured from the time of randomization to the time at which a subject receives the last dose of parenteral opioid analgesia for the treatment of VOC prior to hospital discharge.
- The secondary objectives are:
  - To compare the re-hospitalization rate (for VOC) between the treatment arms.
  - To compare the occurrence of acute chest syndrome (ACS) between the treatment arms.

Study Design:

This study is a Phase 3, randomized, double-blind, placebo-controlled, multi-center study in subjects with SCD hospitalized for acute pain typical of a VOC and who require treatment with parenteral opioid analgesia. Subjects will be randomized 1:1 to receive blinded MST-188 or blinded placebo as a continuous intravenous (IV) infusion. Blinded MST-188 or blinded placebo will be prepared in infusion bags or bottles for IV administration using a positive-pressure, volumetric infusion pump. Blinded MST-188 or blinded placebo will be administered as a 1-hour loading dose infusion immediately followed by a 48-hour continuous maintenance infusion. Two 24-hour infusion bags or bottles will be prepared to administer the blinded MST-188 or blinded placebo during the continuous maintenance infusion period. The maintenance infusion will be administered for at least 12 hours and up to 48 hours. The maximum total infusion duration will be 49 hours.

Randomization will be stratified by: age (≥4 to <16 years or ≥16 to ≤65 years), the use of hydroxyurea

(yes or no), and pain score as measured using the Wong-Baker FACES® Pain Rating Scale (0 to 10 scale; Appendix A) at the time of randomization (<8 or ≥8).

Subjects who meet all eligibility requirements will be centrally registered and randomly assigned 1:1 to MST-188 or placebo using an Interactive Web Response System (IWRS). The infusion of blinded MST-188 or blinded placebo should be initiated as soon as possible after randomization, preferably within 2 hours after randomization. The infusion of blinded study drug should be initiated within 24 hours of the subject's presentation to the center with VOC.

Subjects completing this study may be eligible to participate in an open-label extension study to evaluate the safety of repeat exposures of MST-188 in VOC. A separate protocol is being contemplated based on understandings gained during the course of this study, input from experts and approval of appropriate ethics committees and regulatory bodies. Selected sites will be invited to participate in the open-label extension study.

Sub-study: Pharmacodynamic Assessments

Pharmacodynamic (PD) laboratory biomarkers will be evaluated in a sub-study to this trial. Comparing treatment-related change in selected laboratory biomarkers may reveal information with regard to: 1) the pathophysiology and natural history of VOC, 2) treatment effect, and 3) MST-188 mechanism of action. Subjects at selected investigative centers may elect to participate in the PD sub-study. Participating sites will be selected based on expected sample transit time to the testing facility to ensure sample viability. The samples will not be used for genetic testing and will be stored for a maximum of 10 years or as allowed by local laws or institutional guidelines.

Number of Subjects: A maximum of 388.

Study Centers: Approximately 70 centers in the Americas, Europe, and the Middle East.

Study Population: Male and female subjects (ages ≥4 and ≤65 years) with SCD (HbSS, HbSC, HbSβ^+thal, or HbSβ^0thal) experiencing VOC who require hospitalization and treatment with parenteral opioid analgesia.

Eligibility Criteria:

Inclusion Criteria:

1. Written documentation of informed consent and assent as applicable.
   Note: Minors must provide assent to participate in this study at an age-appropriate level determined and approved by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) for the study center.
2. Subject is ≥4 and ≤65 years of age.
3. Subject has confirmed diagnosis of HbSS, HbSC, HbSβ^+thal, or HbSβ^0thal.
4. Subject is experiencing acute pain typical of VOC and requires treatment with parenteral opioid analgesia.
5. Subject has been in moderate to severe pain as a result of the current VOC for no more than 24 hours at the time of presentation to the study center and for at least 4 hours prior to randomization.

6. Subject is hospitalized or in the process of admission for VOC at time of randomization.
7. If the subject is taking hydroxyurea, the dose is expected to remain stable through discharge.
8. If sexually active, the subject agrees to use reliable contraception while participating in this study and for at least 30 days after discontinuation of blinded study drug infusion.
9. If the subject is female and of child-bearing potential, must have negative pregnancy test (urine or serum).

Exclusion Criteria:

1. Subject has suspected ACS, including either:

   a) baseline chest X-ray indicating a new pulmonary infiltrate or

   b) subject has acute respiratory symptoms consistent with ACS or with acute asthma attack.
2. Subject has platelet count <80,000/mm^3.
3. Subject has a known or suspected bleeding disorder.
4. Subject has inadequate liver function defined as ALT >3X the institution's upper limit of normal.
5. Subject has the following serum creatinine value:
   - Age ≥4-7 years: >0.8 mg/dL (>70.7 µmol/L)
   - Age ≥8-13 years: >0.9 mg/dL (>79.6 µmol/L)
   - Age ≥14 years: >1.0 mg/dL (>88.4 µmol/L)

   Subjects with a confirmed diagnosis of HbSC
   - Age ≥18 years: >1.2 mg/dL (>106.1 µmol/L)
6. Subject is pregnant or nursing.
7. Subject has had an episode of painful crisis requiring hospitalization within the preceding 14 days.
8. Subject has been transfused within the past 14 days.
9. Subject is already hospitalized for any condition other than the current VOC.
10. Subject uses opioid analgesia on a daily basis for any reason.
11. Subject is currently receiving another investigational drug or has received any investigational drug within 30 days prior to randomization.
12. Subject presents with complications related to SCD, such as: aplastic crisis, priapism, sepsis, stroke, hepatic or splenic sequestration, or any complication expected to require surgical intervention.
13. Subject has experienced >5 hospitalizations for VOC in the prior 6 months.
14. Investigator believes subject is suffering from chronic pain (e.g., necrotic tissue resulting from repeated prior VOCs) and not acute pain associated with an ongoing VOC.
15. Subject is otherwise not an appropriate study candidate, in the Investigator's judgment.
16. Subject has been previously enrolled in the present trial or any prior MST-188 clinical trial.

Study Treatment and Mode of Administration:

Investigational Product:

MST-188 is a clear, colorless, sterile, non-pyrogenic, aqueous solution for IV administration. MST-188 will be supplied in 100-mL glass vials containing 15 g of purified poloxamer 188 (150 mg/mL). The drug product contains a 0.01M citrate buffer and sodium chloride to adjust the total sodium content to be equivalent to that in 0.45% sodium chloride solution in water for injection; the resulting osmolarity of the solution is approximately 312 mOsm/L. MST-188 contains no bacteriostatic agents or preservatives.

Placebo Product:

Placebo product is 0.45% saline, which will be obtained from the pharmacy.

Dosing Plan:

Blinded study treatment (either placebo or MST-188) will be administered intravenously with a positive-pressure, volumetric pump. Both blinded study treatments will be administered on a volume matched (mL/kg) basis as a 1-hour loading dose infusion, followed by a continuous maintenance infusion for 48 hours. The maximum total infusion duration will be 49 hours.

The Principal Investigator or Sub-investigator may determine that a subject no longer requires parenteral opioid analgesia for the treatment of VOC (e.g., has received their last parenteral opioid dose) and is prepared for discharge from the hospital prior to the planned completion of the 49 hour infusion. The subject should have completed at least 12 hours of the maintenance infusion prior to hospital discharge.

If the subject will be discharged from the hospital prior to the completion of the 49-hour infusion of blinded study treatment, the study drug infusion should be discontinued immediately before hospital discharge.

The administered dose of MST-188 is based on subject weight (kg). Dose and administration are based on the following:

- A loading dose of 100 mg/kg will be infused for 1 hour.
- Immediately following the loading dose infusion, MST-188 will be administered as a continuous maintenance infusion at a dose of 30 mg/kg/hr for at least 12 hours and up to 48 hours.

The administered volume of placebo (0.45% saline) is based on subject weight (kg).

- Placebo will be infused for 1 hour.
- Immediately following the one-hour loading placebo infusion, placebo will be administered as a continuous maintenance infusion for at least 12 hours and up to 48 hours.

The total duration of the infusion will be a maximum of 49 hours.

Additional information about the administration of blinded MST-188 or blinded placebo during the Treatment Phase is outlined in the Pharmacy and Study Operations Manuals.

Analgesic Use and Concomitant Medications:

Analgesic drug choices will be limited to the following:

Note: Parenteral routes of administration include intravenous, subcutaneous, and intramuscular injections. Transdermal administration of opioids is not permitted.

Parenteral Opioids: Morphine, hydromorphone, nalbuphine, oxycodone, and tramadol, and approved parenteral formulations of permitted oral opioids.

Parenteral NSAIDS: Ketorolac

Oral Opioids: Codeine, hydrocodone, hydromorphone, morphine, oxycodone

Oral Non-Opioid Analgesics: Acetaminophen, aspirin, diclofenac sodium, ibuprofen, naproxen

Other Oral Analgesics: Tramadol

Long-acting variants of the oral analgesics noted above may be utilized.

The primary endpoint of the study is the time from randomization to last parenteral opioid dose for the treatment of VOC prior to hospital discharge. The endpoint is based on the treatment goal with parenteral opioids, which is to reduce pain to a level that the subject can manage at home with oral analgesics and be discharged for home as quickly as possible with as little analgesic intervention as possible. Diversity in pain management practices (including when and why parenteral opioids are discontinued) may confound interpretation of study results and must be minimized. Attention to the Pain Management Guidelines (Appendix B) is required.

Prohibited medications:

The use of systemic corticosteroids (oral, IV, or intramuscular injection) during screening and throughout hospitalization will not be permitted. Investigators should carefully consider the latter restriction when considering enrollment of those subjects who may pose risks of steroid withdrawal at the time of study entry or during their participation in the study; however, the use of inhaled corticosteroids will be permitted.

The use of L-glutamine will not be permitted during screening, hospitalization and throughout the 30-Day Post-Infusion visit.

Efficacy Assessments:

Primary:

- Time from randomization to the last dose of parenteral opioid analgesia for the treatment of VOC prior to hospital discharge.

Secondary:

- Re-hospitalization rate.
- Occurrence of ACS.

Safety Assessments:

Safety assessments include adverse events (AEs), clinical laboratory evaluations, physical examinations

and vital sign measurements.

The AE reporting period begins at randomization and continues through 30 days after the end of infusion. All AEs that occur in randomized subjects during the AE reporting period must be reported to the Sponsor, whether or not the event is considered related to study treatment. Any AE that occurs beyond the AE reporting period that the Investigator assesses as possibly related to study treatment also should be reported to the Sponsor. All subjects will be monitored for complications of SCD throughout the treatment and follow up periods.

An external independent Data Safety Monitoring Board (DSMB) will meet and review safety data regularly following randomization of the first subject in the study. The DSMB will have a separate charter and manual guiding its operations. The DSMB will make its recommendations to the Sponsor, who will implement the recommendations following any clarifying discussions that are needed.

Pharmacokinetic Assessments:

Blood samples will be collected from all subjects for pharmacokinetic (PK) assessments.

Plasma samples will be collected at baseline (within 1 hour prior to the start of the loading dose infusion), at the end of the 1-hour loading dose infusion (± 15 minutes), at the end of the continuous maintenance infusion (± 30 minutes), and 6 hours (± 2 hours) and 12 hours (± 2 hours) post end of the continuous maintenance infusion.

Sub-study – Pharmacodynamic Assessments:

Selected Investigative centers will participate in the Pharmacodynamic Assessments sub-study. Sites will be selected based on expected sample transit time to the testing facility to ensure sample viability. Subjects will provide consent to participate in the PD assessments.

Blood samples will be collected from subjects who consent to participate for the following assessments:

- Proteomics
- RNA assay
- High-sensitivity C-reactive protein (hs-CRP)
- Secretory phospholipase A(2) (sPLA2)
- D-dimer

Exploratory Urine Biomarkers:

Eligible investigative centers that have adequate access to and storage space in a -80^{o}C or colder freezer will collect urine biomarker specimens for exploratory analyses. If adequate access and storage conditions are available, random urine samples (approximately 10 mL) will be collected from all subjects before initiation of the blinded infusion (baseline), 24 hours (± 2 hours) after the initiation of the infusion, at the end of the continuous maintenance infusion (± 2 hours), immediately prior to hospital discharge, and at the 30-Day Post Infusion Follow-Up visit.

Urine samples will be collected for the following assessments:

- Kidney Injury Molecule-1 (KIM-1)

- N-acetyl-beta-D-glucosaminidase (NAG)
- Neutrophil gelatinase associated lipocalin (NGAL)
- Urine creatinine (Cr)
- Urine sodium (Na)

Urine biomarker samples must be stored at -80°C or colder and will be assayed at a central laboratory at periodic intervals throughout the trial. The above exploratory urine biomarkers will be used to provide additional insights regarding the subject's renal function prior to, during, and after study participation. These samples will not be used for genetic testing and will be stored for a maximum of 10 years or as allowed by local laws or institutional guidelines.

Statistical Methods:

A maximum of 388 subjects will be randomized in this study.

Efficacy Analyses:

Efficacy analyses will be performed on the intent-to-treat population, consisting of all randomized subjects, and on the per-protocol population.

Primary Endpoint

- The primary endpoint is the time in hours from randomization until the last dose of parenteral opioid analgesic for the treatment of VOC prior to hospital discharge.
- The mean times in the two treatment groups will be compared using an analysis of covariance (ANCOVA) model with effects for treatment group (MST-188 or placebo), age group (≥4 to <16 years or ≥16 to ≤65 years), use of hydroxyurea (yes or no), and pain score as measured using the Wong-Baker FACES® Pain Rating Scale (Appendix A) at the time of randomization (<8 or ≥8).

Secondary Endpoints

- Re-hospitalization for VOC: The percent of subjects who are re-hospitalized for VOC within 14 days after discharge from the hospital will be compared between the two treatment groups using Fisher's exact test.
- Occurrence of ACS: The percent of subjects who meet the protocol definition of ACS within 120 hours of randomization will be compared between the two treatment groups using a stratified Fisher's exact test.

Safety Analyses:

The safety and tolerability of MST-188 will be determined by AE, clinical laboratory evaluations, physical examinations, and vital sign measurements. Subjects who receive any quantity of blinded MST-188 or blinded placebo are considered evaluable for safety. Subjects will be grouped based on the actual study treatment received.

Treatment-emergent AEs from the start of the blinded infusion through 30 days after the end of the infusion will be summarized by Medical Dictionary for Regulatory Activities (MedDRA™) Version 14.1 (or higher) System Organ Class and Preferred Term. The incidences and percentages of subjects

experiencing each AE preferred term will be summarized by treatment group. Adverse events will also be summarized by National Cancer Institute Common Terminology Criteria for Adverse Events, Version 4.03 (NCI-CTCAE) grade and by causality (relationship to study treatment). Grade 3-4 AEs, serious adverse events (SAEs), and AEs leading to withdrawal or treatment discontinuation will also be summarized by preferred term.

Laboratory results will be classified according to NCI-CTCAE. Laboratory results not corresponding to a NCI-CTCAE term will not be graded. Incidences of laboratory abnormalities will be summarized by treatment group.

Vital signs and physical examination results will be summarized for each treatment group with descriptive statistics.

Pharmacokinetic Analyses:

Pharmacokinetic analyses will be performed as described in a separate PK sampling and analysis procedure protocol.

Pharmacodynamic Analyses - Sub-study:

All subjects who have PD data at baseline and at least one post-treatment value will be included in the PD analyses.

Pharmacodynamics will be summarized by descriptive statistics.

Exploratory Urine Biomarkers:

Subjects who have urine biomarker data for all time points will be included in the exploratory urine biomarker analyses.

Exploratory urine biomarker data will be summarized by descriptive statistics.

3. SCHEDULE OF EVENTS

	Pre-Screening	**Screening**	**Baseline (pre-treatment)**	**Treatment Phase (Infusion period)**	**Post-treatment Phase (Daily assessment until discharge)**	**15 Day Post-Infusion Contact**	**30-Day Post-Infusion Follow-up Visit[a]**	**30-Day Post-Hospitalization Contact[m]**
Informed Consent / Assent	X	X						
Medical History / Eligibility		X						
Physical Examination (Height only at screening)		X		X *(brief –end of infusion)*	X *(at time of discharge)*		X	
Body Weight[b]		X		X	X		X	
Vital Signs Including Oxygen Saturation (SpO_2)		X	X[c]	X[c]	X[d]		X	
Wong-Baker FACES® Pain Assessment (immediately post-consenting and just prior to randomization)		X						
Subject Analgesia Assessment and Consideration of Analgesia Dose Adjustment[e]			X	X	X			
Recording of Analgesic Requirements (parenteral and oral medication administration)		X	X	X	X			
Clinical Laboratory Testing[f]		X		X	X		X	
Urine or Serum Pregnancy Test[g]		X						
Chest X-Ray[h]		X		X *(as clinically indicated)*	X *(as clinically indicated)*			
Administration of Blinded MST-188 or Blinded Placebo[i]				X				
Pharmacokinetic Samples[j]			X	X	X			
Recording of Fluid Intake and Output[k]			X	X	X			
Concomitant Medication Monitoring[l]		X	X	X	X	X	X	X
Adverse Event Monitoring			X	X	X	X	X	

	Pre-Screening	Screening	Baseline (pre-treatment)	Treatment Phase (Infusion period)	Post-treatment Phase (Daily assessment until discharge)	15 Day Post-Infusion Contact	30-Day Post-Infusion Follow-up Visit[a]	30-Day Post-Hospitalization Contact[m]
Monitoring for re-hospitalization due to recurrent VOC						X	X	X
Telephone Contact[m]						X		X
Pharmacodynamic Sub-study Biomarker Samples (hs-CRP, sPLA2, D-dimer) & Proteomic and RNA Assay Samples[n]			X	X				
Exploratory Urine Biomarkers[o]			X	X	X *(at time of discharge)*		X	

[a] Thirty days (+ 2 days) after the completion of blinded infusion, the subject will return for a 30-Day Post-Infusion Follow-Up visit for safety evaluations.

[b] Daily body weight will be charted during treatment and post-treatment phase.

[c] Vital signs (blood pressure, pulse, respiration rate, temperature, and pulse oximetry [SpO2]) will be charted just prior to the start of the infusion, at 1 hour (± 15 minutes), 4 hours (± 15 minutes), every 8 hours (± 30 minutes) during the infusion of blinded MST-188 or blinded placebo and again at the end of the infusion (± 15 minutes).

[d] Vital signs will be charted every 8 hours (± 30 minutes) during the post treatment period until hospital discharge.

[e] The adequacy of the analgesia will be assessed and a consideration of dose adjustment of analgesia will be made by the treating physician or the nurse every 4 hours (± 1 hour) while the subject is awake beginning at randomization until discharge.

[f] Clinical Laboratory Tests include: Hematology (complete blood count [CBC] with differential platelet count, and reticulocyte count), Chemistry (Comprehensive Metabolic Panel plus indirect bilirubin, gamma-glutamyl transferase (GGT), lactate dehydrogenase [LDH]) and Standard Urinalysis with microscopic examination. Refer to Section 11.1 for additional details.

[g] If the subject is female and of child-bearing potential.

[h] Chest X-ray (posterior-anterior and lateral views) will be done at screening. Chest X-ray (posterior-anterior and lateral views) will be repeated during the Treatment or Post-treatment phases if signs or symptoms of pulmonary involvement are apparent.

[i] Study drug will be administered intravenously over 49 hours. There will be a 1-hour loading dose infusion followed by a 48-hour continuous infusion. The 48-hour continuous infusion will be administered as two 24-hour infusions. Refer to Section 10 for additional details.

[j] Pharmacokinetic (PK) samples will be collected at: baseline (within 1 hour prior to the start of the loading dose infusion), the end of the loading dose infusion (± 15 min), the end of the continuous maintenance infusion (± 30 min), 6 hours and 12 hours after end of the continuous maintenance infusion (± 2 hours). Refer to Section 11.1 for additional details.

[k] Fluid intake and output will be charted daily from randomization until discharge.

[l] All medications taken within 30 days prior to the subject's presentation to the site should be recorded.

[m] All subjects will be contacted (phone contact is permitted) 15 days (+ 2 days) after end of continuous maintenance infusion and 30 days (± 2 days) after discharge from the hospital for evaluation of re-hospitalization due to recurrence of VOC. If the 30-Day Post-Hospitalization contact occurs within 2 days of the 30-Day Post-Infusion Follow-Up visit, all subject information for both visits may be collected during the 30-Day Post Infusion Follow-Up visit.

[n] Sub-study Pharmacodynamic Assessments - Participating Centers Only: Pharmacodynamic (PD) blood samples will be collected from consenting study subjects at baseline and 48 hours (± 2 hours) after the initiation of the infusion. If the continuous maintenance infusion is less than the maximal 48 hours or is prematurely discontinued, collection of the laboratory biomarkers will occur at the time of the termination of the infusion (± 2 hours). Refer to Section 11.2 for additional details.

[o] Exploratory urine biomarker assessments: Participating Centers Only: urine samples (10 mL - random sample) will be collected from all subjects at baseline (pre-dose), 24 hours after the initiation of the infusion (± 2 hours), at the end of the maintenance infusion (± 2 hours), just prior to hospital discharge, and again, at the 30-Day Post Infusion Follow-Up visit. Participating centers must have -80°C or colder storage capabilities.

4. TABLE OF CONTENTS

LIST OF TABLES

5. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation or Specialist Term	Definition
ACS	Acute chest syndrome
AE(s)	Adverse event(s)
ALT	Alanine aminotransferase (see SGPT)
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AST	Aspartate aminotransferase (see SGOT)
AUC_{∞}	Area under the time-versus-concentration curve extrapolated from time zero to infinity
BTC	By-the-clock
BUN	Blood urea nitrogen
CDC	Centers for Disease Control and Prevention
CL_p	Plasma clearance
C_{max}	Maximum plasma concentration
Cr	Creatinine
CRA	Clinical Research Associate
D5W	5% dextrose in water
DEHP	Di-(2-ethylhexyl)-phthalate
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
eCRF	Electronic case report form
ED	Emergency Department
EPIC	Evaluation of Purified Poloxamer 188 in Vaso-Occlusive Crisis of Sickle Cell Disease, Study MST-188-01
FDA	United States Food and Drug Administration
GCP	Good Clinical Practice
GGT	Gamma-glutamyl transferase
$HbS\beta^0thal$	Hemoglobin beta thalassemia 0 genotype of SCD
$HbS\beta^+thal$	Hemoglobin beta thalassemia + genotype of SCD
HbSC	Hemoglobin SC genotype of SCD
HbSS	Hemoglobin SS genotype of SCD
HIPPA	Health Insurance Portability and Accountability Act of 1996

Abbreviation or Specialist Term	Definition
HPF	High power field
hs-CRP	High-sensitivity C-reactive protein
ICF	Informed Consent Form
ICH	International Conference on Harmonization
ICMJE	International Committee of Medical Journal Editors
ICS	Inhaled corticosteroids
IEC	Independent Ethics Committee
I/O	Intake and output
IRB	Institutional Review Board
ITT	Intent-to-treat
IV	Intravenous
IWRS	Interactive Web Response System
KIM-1	Kidney Injury Molecule-1
LDH	Lactate dehydrogenase
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MST-188	MST-188 (purified poloxamer 188) Injection [MST-188; also known as ANX-188 (purified poloxamer 188) Injection or ANX-188]
Na	Sodium
NAG	N-Acetyl-beta-D-glucosaminidase
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Events
NGAL	Neutrophil gelatinase associated lipocalin
PA	Posterior-anterior
PCA	Patient-Controlled Analgesia
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic
PO	Oral
PVC	Polyvinylcholoride

Abbreviation or Specialist Term	Definition
QT	Measure between Q wave and T wave in the heart's electrical cycle
QTc	QT Corrected (corrected QT interval)
RBC	Red blood cell
RDC	Remote Data Capture
RDW	Red cell distribution width
rt-PA	Recombinant tissue plasminogen activator
SAE(s)	Serious adverse event(s)
SCD	Sickle cell disease
SD	Standard deviation
SGOT	Serum glutamic-oxaloacetic transaminase (see AST)
SGPT	Serum glutamic-pyruvic transaminase (see ALT)
sPLA2	Secretory phospholipase A(2)
SpO_2	Oxygen saturation
StO_2	Tissue oxygen saturation
$t_{1/2}$	Half-life
T_{max}	Time to reach C_{max}
TOMT	Trioctyl trimellitate
U-CRA	Unblinded Clinical Research Associate
VOC	Vaso-occlusive crisis
V_{ss}	Volume of distribution at steady state
WBC	White blood cell

6. INTRODUCTION

MST-188 (purified poloxamer 188) Injection [MST-188; also known as ANX-188 (purified poloxamer 188) Injection or ANX-188] is formulated as a clear, colorless, sterile, non-pyrogenic, aqueous solution for intravenous administration. MST-188 has hemorheologic, antithrombotic, anti-inflammatory and cytoprotective properties. The active component of MST-188, purified poloxamer 188, is a linear block copolymer (mean molecular weight of 8,500 Daltons) comprised of a central core of hydrophobic polyoxypropylene flanked by chains (blocks) of hydrophilic polyoxyethylene.

MST-188 improves microvascular blood flow and reduces ischemia by lowering viscosity (particularly under low shear rate conditions) and adhesive, frictional forces. The mechanism of action for this effect is not fully understood. The MST-188 drug substance provides a hydrated, poorly compressible barrier that appears to block hydrophobic adhesive interactions (e.g., cell-cell, cell-protein, and protein-protein) in the bloodstream. As a result, there is a reduction in whole blood viscosity, red blood cell (RBC) aggregation, and adhesion to the vascular endothelium.[1] The mechanism of action for this effect is not fully understood.

6.1. Sickle Cell Disease

Sickle cell disease (SCD) is classified as an orphan disease. The Centers for Disease Control and Prevention (CDC) estimates that SCD affects 90,000 to 100,000 people in the United States.[2] Worldwide, affected individuals are predominantly of African, South and Central American, Caribbean, Indian, Mediterranean, and Middle Eastern heritage.[2] In the United States, SCD is most common in African Americans, occurring in 1 of every 500 births, and to a lesser extent in Hispanic Americans (1 of 36,000 births).[2] Severity of the disease varies greatly depending on the type of hemoglobin mutation inherited and other genetic factors.

Sickle cell disease is an autosomal recessive hemoglobinopathy characterized by “sickle-shaped” RBCs that result from mutant sickle hemoglobin.[3] Sickle hemoglobin, when not carrying oxygen, forms polymers that cause RBCs to deform into a sickle shape that can adhere to the vascular endothelium and interfere with microvascular blood flow and cause ischemia.[4] Though the clinical syndrome of SCD results from a complex pathophysiology, clinically significant sickling of RBCs predominately occurs during periods of hypoxia.[3] The syndrome is characterized by chronic anemia and frequent ischemic vaso-occlusion, which in turn can give rise to chronic organ damage. Clinical manifestations may include acute pain, recurrent severe painful crises, acute chest syndrome (ACS), pulmonary hypertension, bone or joint necrosis, priapism, and/or renal failure.[3] Additional manifestations in children with SCD include splenic sequestration, anemia, infection (e.g., pneumonia, pneumococcal meningitis, viral influenza, hepatitis), and hand-foot syndrome.[5]

The most common clinical complication that results in hospitalization of SCD patients is vaso-occlusive crisis (VOC), a severe and debilitating pain episode that is primarily responsible for more than 75% of hospital admissions.[6] Among patients with sickle cell anemia, an average rate of acute pain episodes of 80 per 100 patient-years was reported in the United States.[7]

Acute chest syndrome is a clinical complication of SCD that results in prolonged hospitalizations averaging 10.5 days.[8] Among SCD patients with the HbSS genotype, the reported incidence of patients with ACS was 12.83 per 100 patient-years.[9] Among patients with the S-β^0 thalassemia genotype, the reported incidence was 9.42 per 100 patient-years and among patients with SC genotype, the reported incidence was 5.16 per 100 patient-years.[9] Patients with ACS typically experience fever, cough, chest pain, chills, and difficulty breathing. Diagnosis is confirmed by the presence of a new pulmonary infiltrate evident on a chest X-ray.[10] Severe cases mimic that of acute respiratory distress syndrome and often involve multiple lung segments.[11] Adult SCD patients who have experienced at least one ACS event have a higher mortality than patients who do not have a history of ACS.[9]

6.1.1. Development Rationale

The life of patients with SCD is characterized by chronic pain punctuated by acute episodes of vaso-occlusion that result in severe pain and often begin in early childhood (4 months of age). Vaso-occlusion also deprives tissue of oxygen, resulting in end-organ damage. The cumulative effect of vaso-occlusion, and resulting ischemia and infarction, is significant. In 2006, based on data reported to the CDC, the mean age at death was 39 years; the percentage of individuals alive at 45 years was 35%.[2]

Although SCD has been recognized for more than a century, therapeutic options are extremely limited. Hydroxyurea is the only United States Food and Drug Administration (FDA)-approved treatment option for SCD. Administered chronically, hydroxyurea can be effective in decreasing the number of painful vaso-occlusive crises, but has not been shown to be effective in shortening the duration or decreasing the severity of an ongoing VOC. Thus, despite substantial research and understanding of the pathophysiology of SCD, there are no approved therapies to treat an ongoing VOC. Clinical care today is primarily supportive, consisting mostly of analgesia, and does not address the underlying cause of pain or the long-term cumulative effects of vaso-occlusion on end organ infarcts. New treatment modalities are needed to address this significant unmet need.

MST-188 has been investigated in seven clinical studies, including four studies in patients with SCD and three studies in healthy volunteers. In these prior clinical studies, including a Phase 3 multicenter, randomized, double-blind, placebo-controlled study in patients with SCD experiencing VOC, MST-188 was generally well tolerated. In the Phase 3 study, MST-188 was associated with a shorter duration of VOC and a higher proportion of subjects achieving crisis resolution by a pre-determined time point. Although the decrease in duration of VOC in the intent-to-treat (ITT) population did not reach statistical significance (p=0.072), Mast Therapeutics believes that features of the study design and the study not enrolling the originally-planned number of patients affected the efficacy results. In *post hoc* analyses, MST-188 demonstrated statistically significant efficacy results compared to placebo in subjects <16 years of age and those on concomitant hydroxyurea.

MST-188 can reduce RBC aggregation, cellular adhesion to vascular endothelium, and whole blood viscosity by inhibiting hydrophobic adhesive interactions. When infused during VOC, it can facilitate restoration of compromised microvascular blood flow.[12] As improvement in microcirculation occurs rapidly after infusion, use of MST-188 at the onset of VOC can be expected not only to shorten the duration of VOC and the associated pain, but also to limit the

cumulative tissue damage and end-organ dysfunction and failure commonly seen in patients with SCD.

Given the nonclinical and clinical findings from earlier work on MST-188 and the significant unmet medical need for treatment of VOC and other serious complications associated with SCD, such as ACS, continuing investigation of the therapeutic potential of MST-188 for patients with SCD in an effort to make a new treatment available for a desperate patient population is warranted.

6.1.2. MST-188 Mechanism of Action and Role in the Treatment of Sickle Cell Disease

MST-188 is a nonionic block copolymer surfactant that is administered intravenously shortly after the onset of vaso-occlusion. MST-188 can reduce erythrocyte aggregation, cellular adhesion to vascular endothelium and whole blood viscosity by inhibiting hydrophobic adhesive interactions. When infused during vaso-occlusive events, it can facilitate restoration of impaired microvascular blood flow.[12] Improved blood flow leads to improved tissue oxygenation. This in turn should reduce the burden of infarcted tissue.

As improvement in microcirculation occurs rapidly after infusion,[12] administering MST-188 at the onset of the VOC can be expected to shorten the duration of the crisis and the associated pain, as well as limit cumulative tissue damage and end-organ dysfunction and failure. As no therapeutic agents for an on-going VOC episode have completed the registration process leading to marketing, the development pathway and approval process for such drugs necessarily are unique and untested.

6.2. Human Studies

MST-188 has been administered to 94 healthy volunteers in three clinical studies and 211 subjects with SCD in four clinical studies (169 subjects in VOC and 42 subjects with ACS). The majority of SCD subjects were female (125/211, 59%) and Black (207/211, 98%). The median age was 19 years (range of 22 months to 53 years). The most common adverse events (AEs) were fever, bilirubinemia direct, pruritus, vomiting, nausea, constipation headache, tachycardia, pain, weight loss, bilirubinemia, anemia, abdominal pain, sickle cell crisis, thrombocytopenia, jaundice, rhinitis, and dyspnea. The most common Grade 3 and 4 AEs were bilirubinemia (direct and total), thrombocytopenia, increased serum glutamic-pyruvic transaminase (SGPT), and increased serum glutamic-oxaloacetic transaminase (SGOT). The hepatic-related events were transient and resolved by the Day-35 follow-up visit, except in subjects whose liver function tests had been elevated at baseline. Serious AEs were reported in 75 subjects and included ACS, infections, sepsis, pneumonia, sickle cell crisis, and bilirubinemia direct. No severe hypersensitivity reactions such as angioedema or anaphylaxis were reported.

6.2.1. Phase 1 Healthy Volunteer Studies

Three healthy volunteer studies of MST-188 included 94 participants. In two studies, safety and pharmacokinetics (PK) of MST-188 were studied in 6 healthy volunteers who received an intravenous (IV) infusion of 100 mg/kg for 1 hour followed by 30 mg/kg/hr for 47 hours and 24 subjects who received a total of three IV infusions (100 mg/kg over 1 hour) of MST-188. MST-188 was well-tolerated.

In a third study, MST-188 was evaluated in a four-period, four-arm, crossover design, randomized, placebo and active-controlled study for the evaluation of the effect of therapeutic and supratherapeutic single-dose MST-188 on the QT/QTc intervals in healthy volunteers; 64 subjects were treated with MST-188 during this study. The study excluded a QT prolongation with MST-188 at both therapeutic and supratherapeutic doses. There were no adverse effects of MST-188 on electrocardiogram (ECG) morphology or outliers of concern using generally accepted criteria for QTc and change in QTc. No serious adverse events (SAEs) were reported during the study. Plasma concentration data and analyses of pharmacokinetic data are pending.

6.2.2. Human Studies in Sickle Cell Disease

6.2.2.1. Phase 1 Study of MST-188 in Subjects with Vaso-Occlusive Crisis

A Phase 1 multicenter safety study was conducted to evaluate two dose levels of MST-188 in 17 adult subjects with acute VOC of SCD. The dosing regimen of a 100 mg/kg loading dose for 1 hour followed by a continuous maintenance dose of 30 mg/kg/hr for 47 hours was selected for further development in Phase 2 and 3 studies in subjects with SCD in VOC based on the PK and tolerability characteristics observed in this dose-ranging study.

6.2.2.2. Subjects with Sickle Cell Disease Experiencing Acute Chest Syndrome

A Phase 1, dose-escalating, multicenter study was conducted to evaluate the safety and PK of MST-188 in SCD subjects less than 65 years old with ACS. Forty-two subjects were randomized to 1 of 5 dose groups. MST-188 was administered as a continuous IV infusion over 24 hours. Subjects received a loading dose of 200 mg/kg/hr over 1 hour followed by one of the following maintenance doses: 40 mg/kg/hr, 60 mg/kg/hr, 80 mg/kg/hr, 100 mg/kg/hr, or 120 mg/kg/hr given for 23 hours. The median age was 19 years (range of 22 months to 38 years). Twenty-seven subjects (27/42, 64%) were <19 years old.

Forty-one subjects (41/42, 98%) experienced at least one AE. The most common AEs (incidence of >20%) were fever, pain, tachycardia, constipation, vomiting, bilirubinemia, bilirubinemia-direct, weight loss, and rhinitis. Grade 3 and 4 AEs were reported in 24 subjects (24/42, 57%) and included bilirubinemia, bilirubinemia direct, dehydration, increased SGOT/SGPT, pneumonia, and pain.

6.2.2.3. Open Label, Multiple Exposure Study in Subjects with Vaso-Occlusive Crisis

An open-label, multicenter study was performed to evaluate the safety of repeat exposures of MST-188 to subjects with SCD in VOC. Twenty-eight subjects (10 of whom had previously participated in the Phase 3 study of MST-188 in subjects with VOC) were enrolled. MST-188 was administered as a treatment for up to six episodes of VOC occurring within a period of one year from enrollment, provided that at least 14 days passed between each exposure. MST-188 was administered as a continuous IV infusion over 48 hours. Subjects received a loading dose of 100 mg/kg for 1 hour followed by 30 mg/kg/hr for 47 hours (total dose of 1510 mg/kg over 48 hours). MST-188 was administered as a treatment for up to six episodes of VOC occurring within a period of one year from enrollment, provided that at least 14 days passed between each exposure.

There were a total of 59 subject exposures to MST-188, including 31 repeat exposures. The number of exposures per subject ranged from 1 to 6. Seventeen subjects received 2 or more exposures of MST-188 and one subject received a total of 6 exposures. The majority of subjects were female (19/28, 68%) and all were Black. The median age was 17 years (range of 7 to 39 years). Sixteen subjects (16/28, 57%) were <19 years old.

The most common AEs (incidence of >20%) were fever, pruritis, bilirubinemia-direct, constipation, nausea, vomiting, tachycardia, abdominal pain, headache, thrombocytopenia, increased SGPT, urine abnormality, jaundice, and dyspnea. Grade 3 and 4 AEs were reported in 17 subjects (17/28, 61%) and included bilirubinemia, bilirubinemia direct, increased SGOT/SGPT, and thrombocytopenia.

6.2.2.4. Phase 3 Study of MST-188 in Subjects with Vaso-Occlusive Crisis

A Phase 3 multicenter, randomized, double-blind, placebo-controlled study enrolled 255 subjects with SCD experiencing VOC. Among the 249 subjects who received study treatment (MST-188 or placebo), the majority were female (147/249, 60%) and Black (244/249, 98%). Four were Hispanic (4/249, 2%). The median age was 19 years (range of 9 to 53 years). One-hundred and twenty-two subjects (122/249, 49%) were <19 years old. Seventy-three subjects (73/249, 29%) were ≤15 years old. The majority of subjects had the sickle cell anemia (HbSS) genotype (180/249, 71%). Thirty-five subjects (35/249, 14%) had the sickle hemoglobin C (HbSC) genotype.

MST-188 was administered as a continuous IV infusion over 48 hours. Subjects were administered MST-188 at 100 mg/kg for 1 hour followed by 30 mg/kg/hr for 47 hours (total dose of 1510 mg/kg over 48 hours, N=126) or placebo (N=123). Six subjects did not receive study treatment.

MST-188 was well tolerated. No difference in the overall incidence of AE or SAEs was observed between the MST-188 and placebo groups. Adverse events with an increased incidence (>5% increase over placebo) in the MST-188 group compared with the placebo group included bilirubinemia direct (68/126, 54% vs. 46/123, 37%), bilirubinemia (27/126, 21% vs. 16/123, 13%), increased SGPT (15/126, 12% vs. 2/123, 2%), thrombocytopenia (31/126, 25% vs. 20/123, 16%), nausea (51/126, 41% vs. 42/123, 34%), vomiting (45/126, 36% vs. 35/123, 28%), weight loss (35/126, 28% vs. 18/123, 15%), and urticaria (8/126, 6% vs. 0%).

Grade 3 and 4 AEs were reported in 94 subjects in the MST-188 group (94/126, 75%) and 76 subjects in the placebo group (76/123, 62%). Many of these events were attributed to underlying disease. In the MST-188 group, the most common (>5%) Grade 3 or 4 AEs included headache, pain, transient thrombocytopenia, bilirubinemia, bilirubinemia direct, anemia, sickle cell crisis, and increased SGOT/SGPT. In the placebo group, the most common (>5%) Grade 3 or 4 AEs included transient thrombocytopenia, bilirubinemia, bilirubinemia direct, ACS, and pneumonia.

Serious adverse events were reported for 23% (29/126) and 22% (27/123) of the subjects in the MST-188 and placebo groups, respectively. Serious adverse events reported in the MST-188 group included ACS, pneumonia, bilirubinemia, increased SGOT, sepsis, and bone necrosis. Serious adverse events reported in the placebo group included sickle cell crisis, ACS, pneumonia, and anemia. One subject in the MST-188 group died due to a cardiopulmonary

arrest. The cardiopulmonary arrest was considered secondary to fat embolism based on autopsy. The underlying cause was judged by the Investigator to be due to SCD and not due to treatment with MST-188. Six subjects who received MST-188 discontinued treatment due to AEs that included fever, bilirubinemia/bilirubinemia-direct, supraventricular tachycardia, pruritus, anemia, embolus, thrombocytopenia, ACS, hypoxia, and dyspepsia.

In the urogenital body system, the AEs in the MST-188 group included dysuria (3/126, 2.4%), urinary tract infection (5/126, 3.9%), urinary incontinence (2/126, 1.6%), urine abnormality (3/126, 2.4%), and urinary retention (2/126, 1.6%). Acute renal failure was reported in one subject ≥19 years in the placebo group. Grade 3 or 4 increased creatinine was reported in 0.8% of the MST-188 group (1/126) and 0.8% in the placebo group (1/123). In subjects ≤19 years old there were no reports of acute renal failure and no Grade 3 or 4 renal toxicities were reported. Slightly more Grade 1 and 2 renal toxicities (as defined by increase creatinine) were reported in subjects <19 years old compared with subjects ≥19 years old.

The overall incidence of Grade 3 or 4 laboratory values (SGOT and bilirubin [total and direct], specifically) was higher in the MST-188 group compared with the placebo group. The increased incidence was observed both in subjects ≥19 years old and subjects <19 years old upon analysis by age. These events were transient and reversible by Day 35 of follow up, except when liver function tests had been elevated at baseline.

A higher incidence of decreased platelet counts was observed in subjects in the MST-188 group compared with the placebo group. Analysis by age demonstrated that this increased incidence of Grade 3 or 4 thrombocytopenia was observed in subjects ≥19 years old only (15/68, 22% vs. 5/59, 9% in the MST-188 and placebo groups, respectively). The increased incidence of Grade 3 or 4 thrombocytopenia was not observed in subjects <19 years old (15/58, 26% vs. 15/64, 23% in the MST-188 and placebo group, respectively).

6.2.3. Pharmacokinetics of MST-188

The largest study with PK data is the Phase 3 multicenter, randomized, double-blind, placebo-controlled study performed in subjects with SCD in VOC. MST-188 was administered as a continuous IV infusion over 48 hours. Subjects were administered a loading dose of 100 mg/kg for 1 hour followed by 30 mg/kg/hr for 47 hours (total dose of 1510 mg/kg over 48 hours. Blood samples were collected from 81 subjects who had received MST-188. Pharmacokinetic data is presented in Table 1.

Table 1: Summary of Pharmacokinetic Parameters in Subjects with Sickle Cell Disease in Vaso-occlusive Crisis (Study C97-1248)

Parameter	All Subjects	Subjects <16 years	Subjects ≥16 years	Hydroxyurea	No Hydroxyurea
	Mean (SD)	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**	**Mean (SD)**
Number of Subjects	81	28	53	20	61
C_{max} (mg/mL)	0.42 (0.42)	0.35 (0.20)	0.45 (0.50)	0.39 (0.47)	0.43 (0.41)
T_{max} (hr)	30.6 (11.2)	27.6 (9.04)	32.2 (11.9)	33.09 (13.22)	29.77 (10.45)
AUC_{∞} (mg·hr/mL)	13.7 (10.5)	11.3 (4.90)	14.9 (12.3)	10.95 (5.49)	14.59 (11.60)
Number of Subjects	77	27	50	19	58
$t_{1/2}$ (hr)	4.77 (1.94)	5.35 (3.12)	4.70 (1.84)	4.85 (1.71)	4.96 (2.56)
CL_p (mL/hr)	7744 (3817)	6599 (3020)	8443 (4030)	9986 (4373)	7115 (3286)
V_{ss} (L)	269 (31.4)	223 (123)	299 (172)	367 (216)	243 (122)

SD = Standard deviation; C_{max} = Maximum plasma concentration; T_{max} = Time to reach C_{max}; AUC_{∞} = Area under the time-versus-concentration curve extrapolated from time zero to infinity; $t_{1/2}$ = Half-life; CL_p = Plasma clearance; V_{ss} = Volume of distribution at steady state.

Analysis by analysis of variance (ANOVA) of the PK parameters for age group, sex, and hydroxyurea use identified that subjects receiving concurrent hydroxyurea cleared poloxamer 188 more rapidly from plasma than the subjects who were not concurrently receiving hydroxyurea (9986 mL/hr vs. 7115 mL/hr, respectively, p=0.019). This difference was also observed in analyses that were normalized for body weight (171 mL/hr/kg vs. 133 mL/hr/kg, p=0.019). The subjects receiving concurrent hydroxyurea also had larger volume of distribution at steady state (V_{ss}) than those not receiving hydroxyurea (V_{ss}=367 L vs. 243 L, respectively) which was also observed in analyses that were normalized for body weight (V_{ss}=6.25 L/kg vs. 4.53 L/kg, p=0.008, respectively). There were no significant differences in demographic characteristics between those receiving concurrent hydroxyurea and those not receiving hydroxyurea.

There were no differences among the subject subgroups regarding maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}), area under the time-versus-concentration curve extrapolated from time zero to infinity (AUC_{∞}), or half-life ($t_{1/2}$). For all subjects, the mean $t_{1/2}$ was 4.77 ± 1.94 hours. There were no differences between the male and female subjects in the total group or in any of the subgroups. In analyses normalized for body weight, differences in PK parameters were not observed between subjects <16 and >16 years old.

6.2.4. MST-188 Safety Summary

Across the dose ranges studied, MST-188 was generally well tolerated. The majority of AEs reported were mild or moderate intensity. The tolerability of MST-188 did not change

significantly with increasing exposure (increasing dose and/or duration). The safety profile was similar in subjects ≥19 years old and subjects <19 years old.

Among the 211 subjects with SCD who received MST-188, the most common AEs (incidence of >10%) were fever (174/211, 83%), bilirubinemia-direct (93/211, 44%), pruritus (82/211, 39%), vomiting (73/211, 35%), nausea (71/211, 34%), constipation (65/211, 31%), headache (57/211, 27%), tachycardia (52/211, 25%), pain (50/211, 24%), weight loss (48/211, 23%), bilirubinemia (946/211, 22%), anemia (44/211, 21%), abdominal pain (38/211, 18%), sickle cell crisis (38/211, 18%), thrombocytopenia (37/211, 18%), jaundice (27/211, 13%), rhinitis (27/211, 13%), and dyspnea (23/211, 11%).

The most common Grade 3 and 4 AEs were elevated liver function tests and thrombocytopenia: bilirubinemia-direct (92/211, 44%), bilirubinemia (46/211, 22%), thrombocytopenia (35/211, 17%), increased SGPT (21/211, 10%), and increased SGOT (10/211, 5%). The hepatic-related events were transient and resolved by the Day 35 follow-up visit, except in subjects whose liver function tests had been elevated at baseline. One Grade 3/4 event of embolus and one Grade 3/4 event of phlebitis occurred in the MST-188 treated subjects. Serious AEs were reported in 75 subjects (75/211, 36%). Serious AEs included ACS, infections, sepsis, pneumonia, sickle cell crisis, and bilirubinemia-direct. No severe hypersensitivity reactions such as angioedema or anaphylaxis were reported.

Deaths were reported for two subjects ≥19 years old and one subject <19 years old. One subject (29 years old) died from cardiopulmonary arrest secondary to a fat embolism, one subject (32 years old) died from sepsis and acute respiratory distress syndrome, and one subject (14 years old) developed line sepsis and died in the hospital. The cause was unknown. All three of these deaths were considered by the study Investigators to be unrelated or unlikely to be related to treatment with MST-188.

Refer to the current Investigator's Brochure for further details about MST-188. The Investigator's Brochure may be updated during the course of this study.

6.3. Investigational Product

MST-188 (purified poloxamer 188) Injection [MST-188; also known as ANX-188 (purified poloxamer 188) Injection or ANX-188] is a clear, colorless, sterile, non-pyrogenic, aqueous solution for IV administration. MST-188 will be supplied in 100-mL glass vials containing 15 g of purified poloxamer 188 (150 mg/mL). The drug product contains a 0.01M citrate buffer and sodium chloride to adjust the total sodium content to be equivalent to that in 0.45% sodium chloride solution in water for injection; the resulting osmolarity of the solution is approximately 312 mOsm/L. MST-188 contains no bacteriostatic agents or preservatives.

7. TRIAL OBJECTIVES AND PURPOSE

7.1. Primary Objective

The primary objective is to demonstrate the efficacy of MST-188 in reducing the duration of vaso-occlusive crisis (VOC) in subjects with sickle cell disease (SCD). The duration of VOC will be measured from the time of randomization to the time at which a subject receives the last dose of parenteral opioid analgesia for the treatment of VOC prior to hospital discharge.

7.2. Secondary Objectives

The secondary objectives are:

- To compare the re-hospitalization rate (for VOC) between the treatment arms.
- To compare the occurrence of acute chest syndrome between the treatment arms.

Refer to protocol Sections 11.1 and 14.8.2 for the definition of acute chest syndrome (ACS) occurring after subject enrollment.

8. SUBJECT SELECTION

8.1. Study Population

Male and female subjects (ages ≥4 and ≤65 years) with sickle cell disease (HbSS, HbSC, HbSβ^+thal, or HbSβ^0thal) experiencing VOC who require hospitalization and treatment with parenteral opioid analgesia.

8.2. Subject Inclusion Criteria

Each subject must meet the following criteria to be randomized in this study:

1. Written documentation of informed consent and assent as applicable.
   Note: Minors must provide assent to participate in this study at an age-appropriate level determined and approved by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) for the study center.
2. Subject is ≥4 and ≤65 years of age.
3. Subject has confirmed diagnosis of HbSS, HbSC, HbSβ^+thal, or HbSβ^0thal.
4. Subject is experiencing acute pain typical of VOC and requires treatment with parenteral opioid analgesia.
5. Subject has been in moderate to severe pain as a result of the current VOC for no more than 24 hours at the time of presentation to the study center and for at least 4 hours prior to randomization.
6. Subject is hospitalized or in the process of admission for VOC at time of randomization.
7. If the subject is taking hydroxyurea, the dose is expected to remain stable through discharge.
8. If sexually active, the subject agrees to use reliable contraception while participating in this study and for at least 30 days after discontinuation of blinded study drug infusion.
9. If the subject is female and of child-bearing potential, must have negative pregnancy test (urine or serum).

8.3. Subject Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from participation in the study:

1. Subject has suspected ACS, including either:
   a) baseline chest X-ray indicating a new pulmonary infiltrate or
   b) subject has acute respiratory symptoms consistent with ACS or with acute asthma attack.
2. Subject has platelet count <80,000/mm^3.
3. Subject has a known or suspected bleeding disorder.
4. Subject has inadequate liver function defined as alanine aminotransferase (ALT) >3X the institution's upper limit of normal.

5. Subject has the following serum creatinine value:
   - Age ≥4-7 years: >0.8 mg/dL (>70.7 µmol/L)
   - Age ≥8-13 years: >0.9 mg/dL (>79.6 µmol/L)
   - Age ≥14 years: >1.0 mg/dL (>88.4 µmol/L)

   Subjects with a confirmed diagnosis of HbSC
   - Age ≥18 years: >1.2 mg/dL (>106.1 µmol/L)
6. Subject is pregnant or nursing.
7. Subject has had an episode of painful crisis requiring hospitalization within the preceding 14 days.
8. Subject has been transfused within the past 14 days.
9. Subject is already hospitalized for any condition other than the current VOC.
10. Subject uses opioid analgesia on a daily basis for any reason.
11. Subject is currently receiving another investigational drug or has received any investigational drug within 30 days prior to randomization.
12. Subject presents with complications related to SCD, such as: aplastic crisis, priapism, sepsis, stroke, hepatic or splenic sequestration, or any complication expected to require surgical intervention.
13. Subject has experienced >5 hospitalizations for VOC in the prior 6 months.
14. Investigator believes subject is suffering from chronic pain (e.g., necrotic tissue resulting from repeated prior VOCs) and not acute pain associated with an ongoing VOC.
15. Subject is otherwise not an appropriate study candidate, in the Investigator's judgment.
16. Subject has been previously enrolled in the present trial or any prior MST-188 clinical trial.

8.4. Subject Enrollment

All subjects meeting the eligibility requirements will be considered for study enrollment regardless of race, religion, or gender. Subjects will be recruited from a population of subjects with SCD experiencing VOC. Each subject's medical record and screening assessments will be reviewed by the Investigator to determine his/her eligibility for the study.

Exceptions to eligibility criteria will not be granted by the Investigator or the Sponsor.

Subjects will be made aware of the protocol, its specific aims and objectives, and the potential risks and benefits the subject may incur. Each subject or subject's legally authorized or acceptable representative, in accordance with local regulations, must read, agree to, and sign a current IRB or approving IEC-approved Informed Consent Form (ICF) and, where applicable, assent form, before any study-related procedure is performed.

Eligibility screening and other pretreatment evaluations will be performed as described in Section 12.2 and Section 3 - Schedule of Events.

Designated personnel at the study center will register the subject using an Interactive Web Response System (IWRS) to confirm eligibility. Entry of the subject's screening eligibility (i.e., screening procedure information) into the IWRS initiates the registration of the subject in the study. Subjects who do not meet the eligibility criteria for inclusion to the trial will be designated as a screening failure. The reason the screened subject was not eligible to be included in the trial will be captured in the IWRS.

The subject is considered enrolled in the study if the subject is registered, all eligibility criteria are met, and the subject is randomized to a study treatment arm. Specific instructions for the registration and central IWRS randomization procedures are provided to the study center in a separate procedure manual.

8.5. Method of Assigning Subjects to Treatment Groups

Subjects will be centrally registered and randomly assigned 1:1 to MST-188 (Treatment Group A) or placebo (Treatment Group B). Enrolled subjects will be stratified at randomization by: age (≥4 to <16 years or ≥16 to ≤65 years), the use of hydroxyurea (yes or no), and pain score as measured using the Wong-Baker FACES® Pain Rating Scale at the time of randomization (<8 or ≥8).

Upon completion of randomization, the first dose of blinded MST-188 or blinded placebo should be administered, preferably within 2 hours.

8.6. Blinding

This is a double-blind study. All study personnel (Principal Investigator [PI]), research nurses, non-pharmacy monitors (Blinded-Clinical Research Associate [CRA], etc.), participating subjects, the Sponsor, and its designee(s) will remain blinded to treatment arm. Exceptions will include the pharmacists responsible for preparing blinded MST-188 or blinded placebo for IV administration, the designated unblinded pharmacy monitors (Unblinded-CRA [U-CRA]), and the randomization coordinators.

After randomization, the pharmacist will receive the subject number and treatment arm assignment. The designated pharmacists will prepare blinded MST-188 or blinded placebo in infusion bags or bottles.

After preparation of each infusion bag or bottle, the pharmacist will dispense the blinded MST-188 or blinded placebo by covering the bag or bottle with opaque sheaths and a foil cover and will cover the drip chamber of the IV set with foil to maintain the blind. The sheaths and foil covers should remain in place throughout the infusion. Each prepared infusion bag or bottle as well as the opaque sheaths will be labeled, "Do Not Shake". Infusion bags or bottles and opaque sheaths for the prepared study treatment infusion will be labeled according to pharmacy standards for investigational drug trials (refer to Section 10 for additional details). Additional information is provided in the pharmacy manual with instructions on drug storage, preparation, labeling, administration and disposal.

NOTE: Subject treatment assignments must not be unblinded during the study. There is no specific antidote or therapy for MST-188 toxicity. Therefore, the treatment of any AE or toxicity should be the same regardless of whether the subject is receiving MST-188 or placebo. If the PI

requires information about the blinded therapy the PI must contact the designated Medical Monitor.

9. INVESTIGATIONAL PLAN

9.1. Overall Study Design and Plan

This study is a Phase 3, randomized, double-blind, placebo-controlled, multi-center study in subjects with SCD hospitalized for acute pain typical of a VOC and who require treatment with parenteral opioid analgesia. Subjects will be randomized 1:1 to receive blinded MST-188 or blinded placebo as a continuous IV infusion. Blinded MST-188 or blinded placebo will be prepared in infusion bags or bottles for IV administration using a positive-pressure, volumetric infusion pump. Blinded MST-188 or blinded placebo will be administered as a one-hour loading dose immediately followed by a 48 hour continuous maintenance infusion. Two 24-hour infusion bags or bottles will be prepared to administer the blinded MST-188 or blinded placebo during the continuous maintenance infusion period. The maintenance infusion will be administered for at least 12 hours and up to 48 hours. The maximum total infusion duration will be 49 hours.

The treatment period for administration of blinded MST-188 or blinded placebo will not exceed a duration of 53 hours, which allows for a maximum of 4 hours (cumulative time) of infusion interruption.

A maximum of 388 subjects will be randomized.

Randomization will be stratified by: age (≥4 to <16 years or ≥16 to ≤65 years), the use of hydroxyurea (yes or no), and pain score as measured using the Wong-Baker FACES® Pain Rating Scale at the time of randomization (<8 or ≥8).

The study will be monitored by an external independent Data Safety Monitoring Board (DSMB) which will review the accumulated data following a regular schedule at least annually. The DSMB will have a separate charter guiding its operations. The DSMB will make its recommendations to the Sponsor which will implement the recommendations following any clarifying discussions that are needed.

The PK profile of MST-188 will be determined in all participating subjects.

Pharmacodynamic laboratory biomarkers will be evaluated in a sub-study to this trial. Comparing treatment related change in selected laboratory biomarkers may reveal information with regard to: 1) the pathophysiology and natural history of VOC, 2) treatment effect, and 3) MST-188 mechanism of action. Selected investigative centers will participate in the pharmacodynamic (PD) sub-study. Sites will be selected based on expected sample transit time to the testing facility to ensure sample viability. The samples will not be used for genetic testing and will be stored for a maximum of 10 years or as allowed by local laws or institutional guidelines. Subject participation is optional.

Eligible investigative centers that have adequate access to and storage space in a -80^{o}C or colder freezer will collect urine biomarker specimens for exploratory analyses.

Subjects completing this study may be eligible to participate in an open-label extension study to evaluate the safety of repeat exposures of MST-188 in VOC. A separate protocol is being

contemplated based on understandings gained during the course of this study, input from experts and approval of appropriate ethics committees and regulatory bodies.

9.2. Treatment Duration

This study will consist of a 1) Screening / Baseline Phase, 2) Treatment Phase, 3) Post-treatment Phase, and 4) Follow-up Phase. The Screening/Baseline Phase will be conducted on an out-patient basis or on hospital admission with acute VOC and will include obtaining informed consent and assent, as applicable, a physical examination and medical history, and screening assessments. If the subject is successfully randomized, baseline assessments will be completed.

The Treatment Phase will start at randomization and will continue until the infusion of blinded MST-188 or blinded placebo is completed. Blinded MST-188 or blinded placebo will be administered as a one-hour loading dose infusion immediately followed by a 48-hour continuous maintenance infusion. The administration period of blinded MST-188 or blinded placebo during the Treatment Phase will not exceed a duration of 53 hours, which allows for up to 4 hours (cumulative time) of infusion interruption. If the blinded MST-188 or blinded placebo infusion is prematurely discontinued (<49 hours total duration of administration), the Treatment Phase will end at the termination of the infusion.

The Principal or Sub-investigator may determine that a subject no longer requires parenteral opioid analgesia for the treatment of VOC (e.g., has received their last parenteral opioid dose) and is prepared for discharge from the hospital prior to the planned completion of the 49-hour infusion. The subject should have completed at least 12 hours of the maintenance infusion prior to hospital discharge.

If the subject will be discharged from the hospital prior to the completion of the 49-hour infusion of blinded study treatment, the discontinuation of the study drug infusion should be just prior to hospital discharge.

The Post-treatment Phase will start when blinded MST-188 or blinded placebo infusion is discontinued and ends at the time of hospital discharge.

The Follow-up Phase will consist of post-hospitalization contacts for the monitoring of re-hospitalization due to recurrent VOC and safety evaluations completed 30 (+ 2) days after discontinuation of blinded MST-188 or blinded placebo.

The planned duration of the enrollment (i.e., first subject randomized to last subject randomized) is 24 months. The minimum follow-up duration is 30 days from last dose of blinded MST-188 or blinded placebo treatment. The planned duration of the entire study is approximately 26 months.

Subjects can participate in this study only once.

Treatment with blinded MST-188 or blinded placebo may be discontinued as outlined in Section 12.11.

10. STUDY TREATMENTS

10.1. Treatments and Administration

MST-188 (purified poloxamer 188) Injection [MST-188; also known as ANX-188 (purified poloxamer 188) Injection or ANX-188] is intended for IV administration only. Vial labels of MST-188 will indicate the product, lot number and concentration.

MST-188 will be provided to the pharmacy at each study center in single use glass vials.

MST-188 will be diluted with sodium chloride for injection (0.45%). MST-188 should not be diluted or admixed with other drugs. The dilution or mixing will be done by the pharmacy at the study centers.

Diluted MST-188 may be stored for up to 48 hours in refrigerated conditions (2°C - 8°C) prior to administration.

The duration of administration of prepared infusion bags of blinded MST-188 or blinded placebo during the Treatment Phase is outlined in the Pharmacy and Study Operations Manuals. The dose will be prepared by the pharmacist under aseptic conditions. Drug handling and manipulation instructions will be provided in the Pharmacy Manual and must be followed. Infusion bags or bottles with blinded labeling and diluted MST-188 or placebo (0.45% saline) will be provided for each subject.

After preparation of each infusion bag or bottle, the pharmacist will dispense the blinded MST-188 or blinded placebo by covering the bag or bottle with opaque sheaths and a foil cover and will cover the drip chamber of the IV set with foil to maintain the blind. The sheaths and foil covers should remain in place throughout the infusion. Each prepared infusion bag or bottle as well as the opaque sheaths will be labeled, "Do Not Shake."

Transfer of the prepared infusion bags or bottles from the pharmacy to the bedside via a pneumatic tube delivery system is prohibited.

Blinded study treatment (either placebo or MST-188) will be administered intravenously with a positive-pressure, volumetric pump. Study treatments, blinded MST-188 and blinded placebo, will be administered on a volume matched (mL/kg) basis as 1 hour loading dose infusion, followed by a continuous maintenance infusion for at least 12 hours and up to 48 hours. The infusion may be extended up to 53 hours to account for interruptions, which may not exceed 4 hours in aggregate.

MST-188 may be administered from glass bottles, trioctyl trimellitate (TOMT) plasticized or di-(2-ethylhexyl)-phthalate (DEHP) plasticized polyvinylcholoride (PVC) IV, or polyolefin infusion bags with standard TOMT or DEHP PVC or polyolefin IV infusion sets. Compatibility has been established for each of these materials.

Note: MST-188 is known to be physically incompatible with Alteplase (rt-PA; recombinant tissue plasminogen activator), amphotericin B colloidal suspension, amphotericin B cholesteryl sulfate complex, methylprednisolone sodium succinate, ofloxacin, levofloxacin, and minocycline hydrochloride and these drugs should not be infused in the same line as the blinded MST-188 or blinded placebo.

A list of drugs that have been shown to be physically compatible with MST-188 in a simulated Y-site infusion testing is provided in Appendix C and the Pharmacy Manual.

10.2. Dose Plan and Duration of Treatment

Subjects will be randomized to receive MST-188 (Treatment Arm A) or Placebo (0.45% saline from the pharmacy; Treatment Arm B). Both MST-188 and Placebo Treatment Arms will be administered on a volume matched (mL/kg) basis as a 1 hour loading dose infusion followed by a continuous maintenance infusion for at least 12 hours and up to 48 hours. The subject's dose of MST-188 will be calculated based on body weight (kg) and MST-188 will be diluted with 0.45% sodium chloride for injection prior to administration. The total infusion volume of blinded MST-188 or blinded placebo will be calculated based on the body weight (kg) of the subject.

Treatment Arm A:

The administered dose of MST-188 is based on subject weight (kg). Dose and administration are based on the following:

- A loading dose of 100 mg/kg will be infused for 1 hour.
- Immediately following the loading dose, MST-188 will be administered as a continuous maintenance infusion at a dose of 30 mg/kg/hr for at least 12 hours and up to 48 hours.

Treatment Arm B:

The administered volume of placebo (0.45% saline) is based on subject weight (kg).

- Placebo will be infused for 1 hour.
- Immediately following the placebo loading dose infusion, placebo will be administered as a continuous maintenance infusion for at least 12 hours and up to 48 hours.

The total infusion volume of placebo will be calculated based on the body weight (kg) of the subject. Placebo will be prepared and dispensed as matched infusion volume by body weight to preserve the blind.

Refer to the Pharmacy Manual for instructions for the preparation and administration of blinded MST-188 and blinded placebo.

Prior to the start of the infusion of blinded MST-188 or blinded placebo, an IV catheter will be inserted.

The infusion of blinded MST-188 or blinded placebo should be initiated as soon as possible after randomization, preferably within 2 hours. The infusion of blinded MST-188 or blinded placebo should be initiated within 24 hours of the subject's presentation to the center with VOC.

The entire contents of each infusion bag or bottle should be administered over the prescribed interval unless the infusion is discontinued per the discontinuation criteria.

The infusion should not be interrupted for longer than 4 hours in total. The total duration of the administration period of blinded MST-188 or blinded placebo during the Treatment Phase will not exceed 53 hours, which allows for up to 4 hours (cumulative time) of infusion interruption. If a single interruption exceeds 4 hours or the cumulative time for all study interruptions exceeds

4 hours, blinded MST-188 or blinded placebo infusion is to be permanently discontinued. All interruptions of the infusion must be documented as to the date, time, cause and duration.

If a subject's infusion is permanently discontinued for any reason prior to the expected 49-hour duration of infusion, the volume of fluid remaining in the bag or bottle and the infusion number should be recorded at the time blinded MST-188 or blinded placebo infusion is stopped.

Any fluid remaining in an infusion bag or bottle at the end of an infusion period will be recorded in the source records and the electronic case report form (eCRF). Partially full bags or bottles will be returned to the pharmacy for accountability and disposal or disposed of according to the policy of the institution.

The dates and times of initiation and termination of each stage of the infusion will be recorded in the source records and the eCRF. Additional information about the administration of blinded MST-188 or blinded placebo during the Treatment Phase is outlined in the Pharmacy and Study Operations Manuals.

10.3. Treatment Compliance

Trained medical personnel will administer blinded MST-188 or blinded placebo as specified in Section 10.1. Treatment compliance will be monitored by drug accountability records and treatment administration data as recorded in the patient's medical record and eCRF.

10.4. Packaging and Labeling

MST-188 (purified poloxamer 188) Injection [MST-188; also known as ANX-188 (purified poloxamer 188) Injection or ANX-188] is formulated as a clear, colorless, sterile, non-pyrogenic, aqueous solution for IV administration after dilution with 0.45% saline.

MST-188 is packaged in 100-mL vials containing 150 mg/mL (15 g/vial).

The drug product contains a 0.01M citrate buffer to control pH (citric acid, USP and trisodium citrate dihydrate, USP). The resulting osmolarity of the solution is approximately 312 mOsm/L. It contains no bacteriostatic agents or preservatives.

10.5. Storage and Proper Handling

MST-188 must be stored between 2°C and 8°C (36°F and 46°F).

10.6. Drug Accountability

Drug Accountability Records must be maintained at each study center where investigational products are stored and prepared. The PI will designate a pharmacist who will be responsible for tracking all MST-188 vials requested and delivered to the study center and its blinded administration to individual subjects.

It is the responsibility of the PI-designated pharmacist to ensure that a current record of investigational product disposition is continuously maintained. Drug accountability records must contain documentation of drug storage conditions, dose calculation and preparation, and disposition of the drug vials. Dose preparation and vial disposal records will include a signature by the pharmacist. Pharmacy records or logs must comply with applicable regulations and

guidelines. Mast Therapeutics or a designee will provide forms to facilitate inventory control if the staff at the investigational center does not have an established system that meets Mast Therapeutics' requirements.

Empty or partially used investigational vials should not be returned to Mast Therapeutics or a designee. Opened/entered vials of MST-188 can be disposed of at the investigational center as pharmacy waste following drug accountability reconciliation performed by the U-CRA. It is the PI's responsibility to arrange for the disposal of all used, partially used, empty, or unused vials provided that procedures for proper disposal have been established according to applicable federal, state, local, and institutional guidelines and procedures, and provided that appropriate records of disposal are kept. Refer to the Pharmacy Manual for instructions

10.7. Return of Investigational Product

Upon completion or termination of the study, all unopened investigational product must be returned to a Mast Therapeutics designee or, upon authorization by Mast Therapeutics or their designee, unused investigational product will be destroyed at the center. All investigational products returned to Mast Therapeutics' designee must be accompanied by the appropriate documentation and be clearly identified by protocol number and study center number on the outermost shipping container. Returned supplies should be in the original containers. The return of unopened investigational products will be arranged by the responsible study monitor or U-CRA.

11. STUDY PROCEDURES

11.1. Procedures Applying to all Subjects

Informed Consent and Assent

Prior to enrolling an individual in the study, the Investigator shall obtain the informed consent and, as applicable, assent of such individual or his or her legally authorized representative in accordance with Section 16.3. If the study subject is a minor, written informed consent must be obtained from the legal guardian or parent prior to the initiation of study-related screening procedures. Assent must be obtained from the study subjects in an age appropriate fashion. The study center's IRB/IEC must determine the age-specific requirement for assent according to local regulations. The informed consents and assents must be IRB/IEC approved prior to the initiation of the study. The subject must meet all inclusion and exclusion criteria as described in Section 8 of this protocol. Written permission will be given by each subject and/or legally acceptable representative prior to undergoing any protocol-specific evaluations and prior to receiving treatment.

Demographics

Demographics include gender, date of birth, race, and ethnicity.

Medical History

Medical history includes past and current medical conditions and treatments, chronically administered medications, including supplements, current medications and those taken within 30 days prior to presentation at the center, past and/or present hydroxyurea use, and genotype (HbSS, HbSC, HbSβ^+thal, or HbSβ^0thal).

Physical Examination:

Physical examination includes evaluation by body system, height (at screening only), and weight. A brief physical examination includes an evaluation by body system of previous abnormal and new findings since the last examination.

Vital Signs

Vital signs, including temperature, pulse rate, respiration rate, oxygen saturation (SpO_2 - pulse oximetry), and blood pressure, will be charted just prior to the start of the infusion, at 1 hour (± 15 minutes), 4 hours (± 15 minutes), every 8 hours (± 30 minutes) during the infusion of blinded MST-188 or blinded placebo and again at the end of the infusion. During the post-treatment period vital signs will be charted every 8 hours (± 30 minutes) until discharge.

Wong-Baker FACES® Pain Rating Scale

The Wong-Baker FACES® Pain Rating Scale is recommended for use for individuals age 3 and older.[13,14,15,16,17,18] A series of facial expression drawings with text descriptions displayed as a 0 to 10 scale will be used by study subjects to self-assess pain intensity (Appendix A). The

Wong-Baker FACES® assessment will be collected at the Screening visit immediately post-consenting and just prior to randomization (within 30 minutes).

Adequacy of Analgesia Assessment

Subjects will be assessed by the treating physician or the nurse for the adequacy of their analgesia, including consideration for an analgesia dose adjustment every 4 hours (± 1 hour), while the subject is awake, beginning at randomization until discharge. The assessment of analgesia adequacy and consideration of an analgesia dose adjustment will be performed and documented in the source records. Documentation of the completion of this assessment will be captured in the eCRF.

Clinical Laboratory Tests

Clinical laboratory tests are performed once daily during hospitalization. During the treatment period, laboratory collections will occur approximately 24 hours and 48 hours after initiation of the blinded MST-188 or blinded placebo infusion (or at end of infusion if the infusion is prematurely discontinued).

The clinical laboratory tests include the following:

1. Hematology profile:

   Hemogram: white blood cell (WBC), RBC, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW).

   Differential: bands, neutrophils, lymphocytes, monocytes, eosinophils, and basophils.

   Numerical platelet count.

   Reticulocyte count.

2. Chemistry profile: includes sodium, potassium, bicarbonate/CO_2, chloride, blood urea nitrogen (BUN), creatinine, glucose, total protein, albumin, uric acid, aspartate aminotransferase (AST)/SGOT, ALT/SGPT, alkaline phosphatase, total bilirubin, direct bilirubin, lactate dehydrogenase (LDH), calcium, gamma-glutamyl transpeptidase (GGT).
3. Urinalysis: routine dipstick measurements (specific gravity, pH, bilirubin, protein, blood) and microscopic analysis (WBC/high power field [HPF], RBC/HPF, Bacteria, Casts, Crystals) are required.
4. Serum or urine pregnancy test: in females of child-bearing potential (screening only).

Chest X-Ray

A baseline chest X-ray (posterior-anterior [PA] and lateral) will be obtained prior to study enrollment. Any additional chest X-rays (PA and lateral are preferred) will be obtained as clinically indicated during the treatment and post-treatment periods. Acute chest syndrome occurring after subject enrollment will be defined as described by the National Acute Chest Syndrome Study Group,[8] namely, the finding of a new pulmonary infiltrate involving at least one complete lung segment that is consistent with the presence of alveolar consolidation, but

excluding atelectasis. In addition, the patient must have at least one of the following: chest pain, a temperature of more than 38.5°C, tachypnea, wheezing, or cough.

Pain Management Guideline

A pain management guideline is outlined in Appendix B.

Pharmacokinetic Assessments

The PK profile of MST-188 will be determined in all subjects. Plasma samples will be collected for pharmacokinetic assessments for all subjects. Three milliliters of blood will be collected at each time point.

Sample collection times are listed in Table 2**.**

Table 2: Pharmacokinetic Blood Sampling Time Points

Nominal time points
0 - Baseline (within 1 hour prior to the start of the loading dose infusion)
End of one hour loading dose infusion (± 15 minutes)
End of the continuous maintenance infusion (± 30 minutes)[a]
6 hours after end of the continuous maintenance infusion (± 2 hours)
12 hours after end of the continuous maintenance infusion (± 2 hours)

[a] If the infusion is terminated prior to 48 hours, the end of infusion pharmacokinetic (PK) sample will be collected at the actual time of the discontinuation of the infusion. The timing of subsequent PK blood sample collections will proceed from the actual time of the discontinuation of the infusion. If the subject is discharged from the hospital within 12 hours after the completion of the infusion, a final PK sample will be collected at the time of discharge. The actual PK collection time points will be recorded on the eCRF.

Actual times for start and end of the infusion as well as actual times of all plasma sample collections will be recorded in the source documents and the eCRFs. Detailed instructions on sample collection, preparation, storage, and shipping will be provided in a separate procedure manual.

11.2. Sub-study - Pharmacodynamic Assessments

11.2.1. Laboratory Biomarkers

Several blood-based biomarkers have been used to reflect the individual organ and systemic dysfunction in SCD including measures of inflammation, coagulation, oxidative stress, apoptosis, hemolysis, and endothelial function. Evaluation of the underlying pathophysiological processes relevant to vaso-occlusive crisis (inflammation and coagulopathy) will be done using biomarkers that have been shown to correlate to risk for vaso-occlusive events in SCD.

Selected investigative centers will participate in the PD sub-study and collect the PD blood samples from subjects who provide informed consent for participation in the PD sub-study.

Blood will be collected for analysis of high-sensitivity C-reactive protein (hs-CRP), secretory phospholipase A(2) (sPLA2), and D-dimer. In addition, blood samples will be collected for global proteomic and gene expression analysis. Comparing treatment related change in these biomarkers may reveal important information with regard to: 1) the pathophysiology and natural history of vaso-occlusive crisis, 2) treatment effect, and 3) MST-188 mechanism of action.

Blood samples will be collected for assessment of the following laboratory biomarkers from all study subjects at baseline (prior to the start of the infusion) and again 48 hours (± 2 hours) after the initiation of the infusion. If the continuous maintenance infusion is less than the maximal 48 hours or is prematurely discontinued, collection of the laboratory biomarkers will occur at the time of the termination of the infusion (± 2 hours). The actual dates and times of the blood collections will be recorded in the source and eCRF.

- Proteomics
- RNA assay
- High-sensitivity C-reactive protein (hs-CRP)
- Secretory phospholipase A(2) (sPLA2)
- D-dimer

Detailed instructions on sample collection, preparation, storage, and shipping will be provided in a separate procedure manual.

11.3. Urine Biomarkers

Eligible investigative centers that have adequate access to and storage space in a -80^{o}C or colder freezer will collect urine biomarker specimens for exploratory analyses. If adequate access and storage conditions are available, random urine samples (approximately 10 mL) will be collected from all subjects before initiation of the blinded infusion (baseline), 24 hours (± 2 hours) after the initiation of the infusion, at the end of the continuous maintenance infusion (± 2 hours), immediately prior to hospital discharge, and at the 30-Day Post Infusion Follow-Up visit.

Urine samples will be collected for the following assessments:

- Kidney Injury Molecule-1 (KIM-1)
- N-acetyl-beta-D-glucosaminidase (NAG)
- Neutrophil gelatinase associated lipocalin (NGAL)
- Urine creatinine (Cr)
- Urine sodium (Na)

Urine biomarker samples must be stored at -80°C or colder and will be assayed at a central laboratory at periodic intervals throughout the trial. The above exploratory urine biomarkers will be used to provide additional insights regarding the subject's renal function prior to, during, and after study participation. These samples will not be used for genetic testing and will be stored for a maximum of 10 years or as allowed by local laws or institutional guidelines

11.4. Sub-study – Tissue Oxygenation

11.4.1. A Study of the Effects of MST-188 on Tissue Oxygen Saturation in Sickle Cell Subjects Experiencing a Vaso-Occlusive Crisis

A sub-study to evaluate the effects of MST-188 on microvascular blood flow in subjects with SCD experiencing a VOC, as indirectly measured by non-invasive measurements of tissue

oxygen saturation (StO_2), will be conducted at selected investigational sites in the United States. The sub-study is included in this protocol as Appendix D.

12. STUDY ACTIVITIES

A series of clinical tests and procedures will be performed at specified intervals throughout the study (Section 3 - Schedule of Events).

12.1. Pre-Screening Period

Potential subjects may be consented during a clinic or office visit provided that such informed consent and assent is in accordance with Section 16.3.

a. Signed Informed Consent Form and assent, if applicable (re-consenting will be performed at time of presentation).

b. Assent must be obtained in subjects per country/state/institutional requirements. Note: Minors must provide assent to participate in this study at an age-appropriate level determined and approved by the IRB/IEC for the study center.

12.2. Screening/Presentation

Screening evaluations are used to determine each subject's study eligibility and must be completed prior to randomization. Prior to conducting any other screening procedures with respect to a subject, the Investigator will either confirm that informed consent and assent, as applicable, was obtained for such subject during a pre-screening visit and/or obtain the informed consent and assent, as applicable, of such subject in accordance with Section 16.3.

Eligible subjects must be experiencing acute pain typical of VOC requiring treatment with parenteral opioids. The subject must have been in moderate to severe pain as a result of the current VOC for no more than 24 hours at the time of presentation to the study center and for at least 4 hours prior to randomization. Efforts should be made to secure subject assessment for study entry as soon as possible.

The following assessments will be completed prior to randomization:

a. Informed Consent: re-consenting and assenting, as applicable, if the screening consenting/assenting was obtained during a clinic or office visit

b. Eligibility status

c. Wong-Baker FACES® pain assessment. This assessment will be collected at the time of consenting or confirmation of consent, and again within 30 minutes prior to randomization

d. Medical history

e. Physical examination

f. Vital signs (blood pressure, pulse rate, temperature, respiration rate, SpO_2)

g. Height and weight

h. Clinical laboratory tests

i. Pregnancy test (urine or serum) if subject is female and is not pre-pubertal or surgically sterilized

j. Chest X-ray: PA and lateral views

k. Recording of parenteral and oral analgesics administered from the time of site presentation.

l. Recording of concomitant medication (include 30 days prior to presentation)

Results of all screening evaluations must be reviewed by the PI or his/her designee to ensure that all eligibility criteria have been satisfied prior to subject randomization. If all eligibility criteria are met, the subject will be randomized. If a subject is consented but is not randomized, the reason for ineligibility must be recorded in IWRS.

12.3. Baseline

The following assessments will be completed directly following randomization and prior to starting the infusion of blinded study drug:

a. Pharmacokinetic sample collection – pre-dose sample collected within 1 hour prior to the start of the loading dose infusion.

b. Recording of parenteral and oral analgesia administration beginning at the time of randomization.

c. Vital signs (just prior to the initiation of the infusion).

d. Adequacy of analgesia assessment and consideration of analgesia dose adjustment every 4 hours (± 1 hour) while the subject is awake beginning at the time of randomization.

e. Initiation of recording of daily fluid intake and output.

f. Adverse event monitoring.

g. Concomitant medication monitoring.

h. Pharmacodynamic Sub-study Assessments (if applicable): Laboratory biomarker sample collection (hs-CRP, sPLA2, D-Dimer, Proteomic, and RNA assay samples) – pre-dose collection.

i. Urine biomarker assessments (if applicable) – pre-dose collection.

12.4. Treatment Phase

Subjects fulfilling all of the eligibility criteria will be randomized and the blinded MST-188 or blinded placebo infusion should be initiated as soon as possible after randomization, preferably within 2 hours. The infusion of blinded MST-188 or blinded placebo should be initiated within 24 hours of the subject's presentation to the center with VOC.

The duration of the infusion will continue for 49 hours, until the full dose of blinded MST-188 or blinded placebo is administered or a criterion for discontinuation of the infusion is met.

The following evaluations will be completed during the treatment period:

a. Administration of blinded MST-188 or blinded placebo.

b. Vital signs (blood pressure, pulse, respiration rate, temperature, SpO_2) at 1 hour (± 15 min) and at 4 hours (± 15 min) after start of blinded infusion, then every 8 hours (± 30 min) during the remainder of the infusion, and at the end of the infusion (± 15 min).

c. Adequacy of analgesia assessment and consideration of analgesia dose adjustment every 4 hours (± 1 hour) while the subject is awake beginning at the time of randomization.

d. Daily body weight.

e. Clinical laboratory tests once daily at approximately 24 and 48 hours after initiation of the blinded MST-188 or blinded placebo infusion (or at end of infusion if the infusion is prematurely discontinued).

f. Brief physical at the discontinuation of the infusion.

g. Recording of daily fluid intake and output.

h. Chest X-ray (PA and lateral views) is to be repeated if clinical signs or symptoms of pulmonary involvement are apparent.

i. Pharmacokinetic sample collection at end of the one hour loading dose infusion (± 15 minutes) and again at the end of the continuous maintenance infusion (± 30 minutes). If the infusion is terminated prior to 48 hours, the end of infusion PK sample will be collected at the actual time of the discontinuation of the infusion.

j. Recording of parenteral and oral analgesic administration.

k. Adverse event monitoring.

l. Concomitant medication monitoring.

m. Pharmacodynamic Sub-study Assessments (if applicable): Laboratory biomarker sample collection at hour 48 (± 2 hours) after the start of the loading dose infusion. If the continuous maintenance infusion is less than the maximal 48 hours or is prematurely discontinued, collection of the laboratory biomarkers will occur at the time of the termination of the infusion (± 2 hours).

n. Urine biomarker assessments (if applicable): Collection of urine biomarker sample 24 hours after the initiation of the infusion (± 2 hours) and at end of the continuous maintenance infusion (± 2 hours).

12.5. Post-treatment Phase

The Post-treatment Phase will start when the infusion of blinded MST-188 or blinded placebo is discontinued and for the duration of hospitalization until discharge.

Assessments will be completed daily until discharge unless otherwise noted:

a. Recording of parenteral and oral analgesic administration change until discharge.

b. Vital signs (blood pressure, pulse rate, temperature, respiration rate, SpO_2), every 8 hours (± 30 minutes).

c. Adequacy of analgesia assessment and consideration of analgesia dose adjustment every 4 hours (± 1 hour) while the subject is awake beginning at the time of randomization.

d. Daily body weight.

e. Clinical laboratory tests.

f. Physical examination prior to discharge.

g. Recording of daily fluid intake and output until discharge.

h. Chest X-ray (PA and lateral is preferred) is to be repeated if clinical signs or symptoms of pulmonary involvement are apparent.

i. Adverse event monitoring.

j. Concomitant medication monitoring.

k. Pharmacokinetic sample collection 6 hours (± 2 hours) and again 12 hours (± 2 hours) after the discontinuation of the continuous maintenance infusion. If the infusion is terminated prior to 48 hours the timing of subsequent PK blood sample collections will proceed from the actual time of the discontinuation of the infusion. If the subject is discharged from the hospital within 12 hours after the completion of the infusion, a final PK sample will be collected at the time of discharge.

l. Urine biomarker assessments (if applicable): Collection of urine biomarker sample prior to hospital discharge.

m. The date and time of the decision to discharge the subject (discharge orders) will be captured on the eCRF.

12.6. 15-Day Post-Infusion Contact

The following assessments will be completed 15 days (+ 2 days) following the discontinuation of blinded MST-188 or blinded placebo. This assessment may be completed via telephone contact with the subject and does not require an office visit.

a. Adverse event monitoring

b. Monitoring for re-hospitalization due to recurrent VOC

c. Concomitant medication monitoring

12.7. 30-Day Post-Infusion Follow-up Visit

The following assessments will be completed 30 to 32 days following the discontinuation of blinded MST-188 or blinded placebo:

a. Physical examination

b. Vital signs (blood pressure, pulse rate, temperature, respiration rate, SpO_2)

c. Body weight

d. Clinical laboratory tests

e. Adverse event monitoring

f. Monitoring for re-hospitalization due to recurrent VOC

g. Concomitant medication including analgesic medication

h. Urine biomarker assessments (if applicable) – 30-day Post-Infusion sample

12.8. 30-Day Post-Hospitalization Contact

Subjects should be queried regarding re-hospitalization for VOC 30 days (± 2 days) following discharge. This assessment may be completed via telephone contact with the subject and does not require an office visit.

If this date of this contact falls within 2 days of the 30-Day Post-Infusion Follow-up visit, the visits may be combined.

12.9. Treatment Modifications and Management of Toxicity

Blinded MST-188 or blinded placebo will be administered as a one-hour loading dose infusion immediately followed by a 48-hour continuous maintenance infusion. The maintenance infusion will be administered for at least 12 hours and up to 48 hours. The maximum total infusion duration will be 49 hours. The administration period of blinded MST-188 or blinded placebo during the Treatment Phase will not exceed a duration of 53 hours, which allows for up to 4 hours (cumulative time) of infusion interruption.

Renal, hepatic, and hematologic function will be closely monitored. Blinded MST-188 or blinded placebo will be discontinued if the subject experiences:

- creatinine increased 50% and an absolute increase ≥0.3 mg/dL (≥27 µmol/L)
- elevation of SGPT/ALT >12.0 X institutional upper limit of normal
- elevation of SGPT/ALT >8.0 X institutional upper limit of normal
  and direct bilirubin >2.0 X the subject's baseline value
  and direct bilirubin >2.0 X institutional upper limit of normal
- platelet value <50,000/mm^3

12.10. Concomitant Medications and Therapy

All medications administered to subjects from the time of presentation to the center through the 30-Day Post-Infusion Follow-up visit will be recorded on the eCRF. Concomitant medications, taken by the subject within the 30 days prior to presentation, will be recorded on the eCRF.

No elective surgery including the placement of a PEG feeding tube, other investigational treatments, or additional sickle cell therapies are allowed during the treatment phase of this study.

The use of blood transfusions is permitted at the discretion of the treating physician.

12.10.1. Analgesic Therapy

The primary endpoint of the study is the duration of VOC as measured by the time from randomization to the last parenteral opioid dose for the treatment of VOC prior to hospital discharge. The endpoint is based on the treatment goal with parenteral opioids, which is to reduce pain to a level that the subject can manage at home with oral analgesics and be discharged for home as quickly as possible with as little analgesic intervention as possible. Diversity in pain management practices (including when and why parenteral opioids are discontinued) may confound interpretation of study results and must be minimized. Attention to the Pain Management Guidelines (Appendix B) is required.

12.10.2. Analgesic Classes and Routes of Administration

The names, dates, times, doses, amount administered, and routes of administration of all analgesics will be documented in the eCRF from 30 days prior to presentation to the site through the 30-Day Post Infusion Follow-Up visit.

The reason for change of dose or route of administration of analgesic medications should be captured.

Analgesic drug choices will be limited to the following after randomization:

Parenteral Opioids: Morphine, hydromorphone, nalbuphine, oxycodone, and tramadol, and approved parenteral formulations of permitted oral opioids.

Parenteral NSAIDS: Ketorolac

Oral Opioids: Codeine, hydrocodone, hydromorphone, morphine, oxycodone

Oral Non-Opioid Analgesics: Acetaminophen, aspirin, diclofenac sodium, ibuprofen, naproxen

Other Oral Analgesics: Tramadol

Long-acting variants of the oral analgesics noted above may be utilized.

12.10.3. Hydroxyurea

Concurrent therapy with oral hydroxyurea is allowed. The dose of hydroxyurea that the subject is receiving at presentation is expected to be maintained from randomization until discharge, unless contraindicated by the subject's clinical status.

Investigators will be allowed to reduce the hydroxyurea dose or stop hydroxyurea completely as required by the clinical situation, but they may not increase the dose during the study period. All changes in dose, amount, and date of such changes will be documented.

12.10.4. Prohibited Medications

The use of systemic corticosteroids (oral, IV, or intramuscular injection) during screening and throughout hospitalization will not be permitted. The Investigator should carefully consider the latter restriction when considering enrollment of those subjects who may pose risks of steroid withdrawal at the time of study entry or during their participation in the study. However, the use of inhaled corticosteroids (ICS) will be permitted.

The use of L-glutamine will not be permitted from screening through the 30-Day Post-Infusion Visit.

12.10.5. Fluids, General Care

Hydration, IV plus PO (oral) fluids, should be used judiciously. Increased fluids may be needed if the subject is dehydrated and/or if the subject is experiencing fluid loss (e.g., persistent fever). To prevent RBC dehydration and potential weight loss due to osmotic diuresis, careful monitoring of fluid intake and output (I/O) should be done from baseline until discharge. Subjects will be weighed daily. The investigator should adjust fluids/hydration accordingly if the subject's body weight decreases by more than 2% from baseline weight. There is no restriction on the type of IV fluids administered during the study period, but unless contraindicated, 5% dextrose in water (D5W)/0.45% saline is recommended.

12.11. Discontinuation of Study Drug

Blinded MST-188 or blinded placebo **will** be discontinued for the following reasons:

- If subject experiences:
  - creatinine increased 50% and an absolute increase ≥0.3 mg/dL (≥27 μmol/L)
  - elevation of SGPT/ALT >12.0 X institutional upper limit of normal
  - elevation of SGPT/ALT >8.0 X institutional upper limit of normal **and** direct bilirubin >2.0 X the subject's baseline value **and** direct bilirubin >2.0 X institutional upper limit of normal
  - platelet value <50,000/mm^3
- Discontinuation of the study by the Sponsor
- Subject withdraws consent. Every effort must be made to collect adverse events that may have occurred leading to the subject's withdrawal of consent.

A subject may be discontinued from study **treatment** for any of the following reasons:

- Adverse event (including intercurrent illness or inability to tolerate continued treatment due to unmanageable toxicity or serious adverse event)
- Protocol violations indicating the subject is unable to comply with the protocol
- The Treatment Phase exceeds a duration of 53 hours
- Investigator's determination that withdrawal from treatment is appropriate (with documentation of reason)
- Subject is ready for hospital discharge and has received his/her last dose of parenteral opioid

12.12. Criteria for Withdrawal of Study Subject from the Study or Study Assessments

A subject may be withdrawn from the **study or study assessments** for any of the following reasons:

- Subject refusal/withdrawal of consent
- Subjects may withdraw their consent to participate in the study at any time without prejudice. However, every effort must be made to contact the Investigator providing care to determine whether adverse events occurred. This is to maximize the safety data obtained from the trial and therefore maximize the safety of future patients treated with MST-188.
- Death
- Discontinuation of the study by the Sponsor
- Investigator's determination that withdrawal from the study is appropriate (with documentation of reason)

The reason for withdrawal will be recorded on the relevant eCRF. If possible, all subjects who withdraw from the study treatment prematurely should return for the 30-day Post Infusion Follow-up visit and safety assessments. Regardless of the reason for withdrawal, effort will be made by the study center to follow safety events from the time of withdrawal through resolution or until the event stabilizes.

12.13. Subject Replacement

Enrolled subjects who discontinue the study early for any reason will not be replaced and are not permitted to re-enter the study.

13. SAFETY ASSESSMENTS

13.1. Safety Parameters

Safety will be evaluated by tabulations of AEs and will be presented with descriptive statistics at baseline and follow up visits for each treatment group. Adverse events, SAEs, vital signs and clinical laboratory test results will be tabulated.

Safety will be assessed throughout the study in all subjects. Subject safety will be assessed by monitoring adverse events, clinical laboratory tests, vital signs and physical examinations. Commonly reported AEs in SCD are provided in Appendix E.

Subjects will be monitored from randomization through 30 days after the end of the continuous maintenance infusion for occurrence of AEs, as well as for changes in clinical status, vital sign measurements, and laboratory data. All subjects will be monitored for complications of sickle cell disease throughout the treatment and follow up periods. Subjects will also be monitored after the hospital discharge for re-hospitalization due to recurrent VOC.

13.2. Adverse Event Definition

An adverse event is any untoward medical occurrence associated with the use of the drug in a subject or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can be any unfavorable and unintended sign (including a laboratory finding), symptom or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

At each evaluation subjects should be interviewed in a non-directed manner to elicit potential adverse events from the subject. The occurrence of an AE will be based on changes in the subject's physical examination, laboratory results, and/or signs and symptoms.

All AEs (except Grade 1 and 2 laboratory abnormalities that do not require an intervention), regardless of causal relationship, are to be recorded in the eCRF and source documentation. The Investigator must determine the intensity of any AE according to the National Cancer Institute-Common Terminology Criteria for Adverse Events (NCI-CTCAE) Version 4.03 (see http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf) and their causal relationship.

All AEs will be monitored until either resolution of the event or determination by the Investigator that the event has become stable or irreversible. If an AE remains unresolved at the conclusion of the study, the Investigator and Mast Therapeutics Medical Monitor will make a clinical assessment as to whether continued follow-up of the AE is warranted.

13.3. Reporting Period for Adverse Events

Adverse events occurring from the time of presentation to the site but before randomization should not be recorded as AEs. Instead, study center personnel should record the occurrence and nature of these experiences on the medical history eCRF page. From randomization and

throughout the 30-Day Post Infusion Visit, any change in the condition, occurrence or nature of these experiences should then be recorded as AEs.

13.4. Evaluating Adverse Events

All observed or volunteered AEs, regardless of treatment group assignment or suspected causal relationship, will be reported in the eCRF. The Investigator will determine the seriousness, causality, and severity of each AE based on the following definitions:

13.4.1. Serious Adverse Events

An AE or suspected AE is considered serious if, in the view of the either the Investigator or Sponsor, it results in any of the following outcome

1. Death;
2. A life-threatening adverse event;
3. Inpatient hospitalization or prolongation of existing hospitalization, excluding hospitalization for VOC required for inclusion into the study;
4. A persistent or significant disability/incapacity;
5. Congenital anomaly/birth defect; or
6. Other events that, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above.

Appropriate diagnostic tests should be performed and therapeutic measures, if indicated, should be instituted. Appropriate consultation and follow-up evaluations should be carried out until the event has returned to baseline or is otherwise explained by the Investigator.

Follow-up data concerning the SAE (e.g., diagnostic test reports, physician's summaries, etc.) must be submitted to the Sponsor, as they become available, until resolution of the SAE.

13.4.2. Unexpected Adverse Event

An AE is considered unexpected if it is not described in the Investigator's Brochure or if it is of greater frequency and/or severity than mentioned in the Investigator's Brochure. Unexpected, as used in this definition, refers to an adverse drug event that has not been previously observed (e.g., included in the Investigator's Brochure) rather than from the perspective of such event not being anticipated from the pharmacological properties of the pharmaceutical product.

13.4.3. Relationship

The Investigator will assess the relationship of the event (not-related, unlikely-related, possibly related, probably related, or definitely related) to blinded investigational agent(s) using a 5-point scale. The relationship of an adverse event to blinded investigational agent(s) should be assessed using the following definitions:

Not Related: Evidence exists that the adverse event definitely has an etiology other than the investigational agent, such as a pre-existing condition or underlying disease, a concurrent illness, or concomitant medication, and does not meet any of the criteria above.

Unlikely: The causality of the AE is supported by enough evidence to reasonably suggest that the event could more likely be attributed to something other than the investigational product such as the underlying disease or an intercurrent illness or injury.

Possibly Related: A temporal relationship exists between the event's onset and blinded investigational agent administration. Although the event may appear unlikely to be related to the blinded investigational agent, a relationship cannot be ruled out with certainty, and/or the event cannot be readily explained by the subject's clinical state or concomitant treatment.

Probably Related: A temporal relationship exists between the event's onset and blinded investigational agent administration, and the event appears with some degree of certainty to be related to investigational agent treatment, based on the investigational agent's known therapeutic and pharmacologic actions. The event cannot be readily explained by the subject's clinical state or concomitant treatment. If the investigational agent is discontinued or the dose is reduced the event abates or resolves.

Definitely Related: Strong evidence exists that the blinded investigational agent caused the adverse event. There is a temporal relationship between the event's onset and blinded investigational agent administration, and strong therapeutic and pharmacologic evidence that the blinded investigational agent caused the event. The subject's clinical state and concomitant treatment have been ruled out as causes. If the blinded investigational agent is discontinued or the dose is reduced the event abates or resolves, but it reappears upon rechallenge.

13.4.4. Severity of Adverse Events

Severity or intensity of adverse events will be graded using the NCI-CTCAE, Version 4.03, and reported as indicated on the eCRF. If an adverse event occurs which is not listed in NCI-CTCAE, the scale in Table 3 will be used.

Table 3: General Guideline for Clinical Descriptions of Severity for Adverse Events

Grade	Severity	Description
1	Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated. No limitation of usual activities.
2	Moderate	Minimal, local or noninvasive intervention indicated. Some limitations of usual activities
3	Severe	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated. Inability to carry out usual activities.
4	Life-threatening	Urgent intervention indicated; immediate hazard to life.
5	Fatal	Patient death

13.4.5. Serious Adverse Event Reporting

13.4.5.1. Timeframe for Reporting

Any SAE experienced by the subject after randomization, or within 30 days of the discontinuation of blinded MST-188 or blinded placebo, regardless of relationship assessment, must be reported (within 24 hours of the Investigator becoming aware of the event) by telephone, email, or facsimile (fax) transmission to the Sponsor or designee as listed below. Notification by fax is preferred. The fax/telephone numbers and email address listed below may be used during both business and non-business hours. During non-business hours a recorded message will provide the caller with the contact information for the on-call monitor.

All SAEs require that, in addition to telephone notification, a Serious Adverse Event Report Form be completed and forwarded either via facsimile or as a PDF via email to Theradex® at the fax number or email listed below within 24 hours of becoming aware of the event.

The Investigator will be able to contact the Theradex® Medical Safety Desk at all times:

Theradex® Safety Desk
Telephone: (609) 799-7580
Fax: (609) 799-1567
Email: SafetyDeskUS@Theradex.com

13.4.5.2. Follow-Up of Adverse Events

Any SAE or AE assessed as possibly, probably, or definitely related that led to treatment discontinuation (including clinically significant abnormal laboratory values that meet these criteria) and is ongoing 30 days after last dose of study treatment must be followed until either resolution of the event or determination by the Investigator that the event has become stable or irreversible. This follow-up guidance also applies to possibly, probably, or definitely related SAE that occur more than 30 days after last dose of study treatment. The status of all other continuing AEs will be documented as of 30 days after last dose of study treatment. Follow-up data concerning reported SAE must also be reported to the Sponsor or designee.

13.4.5.3. Regulatory Reporting

Reporting of SAEs by the Investigator to his or her IRB/IEC will be done in accordance with the standard operating procedures and policies of the IRB/IEC. Adequate documentation must be maintained showing that the IRB/IEC was properly notified.

Appropriate diagnostic tests should be performed and therapeutic measures, if indicated, should be instituted. Appropriate consultation and follow-up evaluations should be carried out until the event has returned to baseline or is otherwise explained by the Investigator.

Follow-up data concerning the SAE (e.g., diagnostic test reports, physician's summaries, etc.) also must be submitted to the Sponsor or their designee, as they become available, until resolution of the SAE.

13.5. Other Safety Considerations

13.5.1. Laboratory Data

All laboratory data obtained during the course of the study should be reviewed by the Investigator. Any abnormal value that leads to a change in subject management (e.g., dose delay or discontinuation, requirement for additional medication or monitoring) or is considered to be of clinical significance by the Investigator should be reported as an AE or SAE as appropriate, unless this value is consistent with the subject's present disease state or is consistent with values obtained prior to entry into the study.

13.5.2. Pregnancy

Women of child-bearing potential and fertile men with partners of child-bearing potential must use an effective method of contraception to avoid pregnancy throughout the study and for up to 30 days after the discontinuation of study treatment in such a manner that the risk of pregnancy is minimized. Females of child-bearing potential include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea ≥12 consecutive months; or women on hormone replacement therapy with documented serum follicle-stimulating hormone level ≥35 mIU/mL). Even women who are using oral, implanted, or injectable contraceptive hormones or mechanical products, such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy, are practicing abstinence, or whose partner is sterile (e.g., vasectomy), should be considered to be of child-bearing potential.

All women of child-bearing potential must have a negative serum or urine pregnancy test at screening. If the pregnancy test is positive, the subject must not receive study therapy and must not be enrolled in the study.

Prior to study enrollment, women of child-bearing potential and fertile men must be advised of the importance of avoiding pregnancy during trial participation and through 30-day follow-up period and the potential risk factors for an unintentional pregnancy. The subject or the subject's acceptable/authorized representative must sign an informed consent form documenting the subject's receipt of this information.

In addition, all women of child-bearing potential or fertile men with partners of childbearing potential should be instructed to contact the Investigator immediately if they suspect they or their partner might be pregnant (e.g., missed or late menstrual period) at any time during study participation.

If any subject or subject's partner becomes pregnant while on study or during the 30-day follow-up period, the individual must be followed up until birth or termination of pregnancy. Women who are determined to be pregnant while on-study or during the 30-day follow up period will be discontinued from the study and the follow-up procedures must be performed on the subject unless contraindicated by pregnancy. The pregnancy is to be immediately reported to Mast Therapeutics or their designee. The anticipated date of birth or termination of the pregnancy should be provided at the time of the initial report. The outcome of the pregnancy should be reported to Mast Therapeutics or their designee as soon as it is known. If the

pregnancy ends for any reason before the anticipated date initially reported the Investigator should notify Mast Therapeutics or their designee the following working day.

If the outcome of the pregnancy meets any criteria for classification as a SAE (including stillbirth, neonatal death, spontaneous abortion, or congenital anomaly - including that in an aborted fetus) the Investigator must follow the procedures outlined in Section 13.4.5 regarding the reporting of SAEs. Any neonatal death occurring ≤30 days after birth will be reported as a SAE.

Other appropriate pregnancy follow-up procedures should be considered if indicated.
In addition, the Investigator must report to Mast Therapeutics or their designee follow-up information regarding the course of the pregnancy, including perinatal and neonatal outcome. Infants should be followed for a minimum of 8 weeks.

13.5.3. Medication Errors

Any blinded MST-188 or blinded placebo dosing error that results in an AE, even if it does not meet the definition of serious, requires reporting within 24 hours to the Theradex® Medical Safety Desk.

13.6. Safety Monitoring

Safety monitoring of reported Adverse Events and laboratory findings will be conducted by the Medical Monitor on an ongoing basis. To minimize the possibility of exposing study subjects to unusual risk, the safety information from this study will be reviewed by an external independent DSMB on an ongoing basis. The DSMB will meet and review safety data at least annually following randomization of the first subject in the study and, based on its review, will make a recommendation to the study Sponsor as to whether or not it is safe to continue the study according to the protocol.

14. PLANNED STATISTICAL METHODS

14.1. General Considerations

The analysis of all safety and efficacy data will be performed by Mast Therapeutics or its designee. The analyses of safety outcomes will be based on the safety population. The primary efficacy analysis will be based on the ITT population. Additional analyses will be performed based on the per-protocol population.

Categorical data will be summarized as frequency and its corresponding percentage. Continuous data will be summarized using frequency (n), mean and standard deviation, median, minimum, and maximum.

14.2. Determination of Sample Size

The sample size was based on detecting a difference between treatment groups in the mean time from randomization to the last dose of parenteral opioid analgesic for the treatment of VOC. The calculations used a mean of 96 hours for the placebo group, a mean of 80 hours for the MST-188 group, a two-sided alpha of 0.05, and a coefficient of variation of 54%. Using these assumptions, a sample size of 188 per arm (376 total) provides 90% power to detect a difference between treatment groups using a two sample t-test. In order to account for dropout rate of 3% or less, the total maximum sample size will be 388 (194 per arm).

14.3. Analysis Populations

The following populations will be defined for this study:

- The **safety population** will include all randomized subjects who received any dose of MST-188 or placebo. The analysis of safety outcomes with the safety population will consider allocation of subjects according to the treatment received, regardless of which treatment they were randomized to receive.
- The **intent-to-treat population** will consist of all randomized subjects and will be used for the efficacy analyses. The analysis of efficacy outcomes with the ITT population will consider allocation of subjects to treatment groups as randomized.
- The **per-protocol population** will consist of all randomized subjects except those subjects whose duration of study drug infusion was less than 12 hours, those subjects who did not receive a parenteral dose of opioid analgesic after randomization, and those subjects who failed to satisfy either or both of the following major entry criteria: subject did not have a diagnosis of HbSS, HbSC, HbSβ^+thal, or HbSβ^0thal; or subject was not ≥4 and ≤65 years of age. The analysis of efficacy outcomes with the per-protocol population will consider allocation of subjects according to the treatment received.

14.4. Demographics and Baseline Characteristics

Descriptive statistics will be provided to summarize demographics and baseline characteristics parameters.

14.5. Analyses of Safety Outcomes

The safety and tolerability of MST-188 are determined by adverse events, clinical laboratory evaluations, physical examinations, and vital sign measurements. Subjects who receive any quantity of blinded MST-188 or blinded placebo are considered evaluable for safety.

14.5.1. Adverse Events

Treatment-emergent AEs will be summarized by Medical Dictionary for Regulatory Activities (MedDRA™) Version 14.1 (or higher) by system Organ Class and preferred term. The incidences and percentages of subjects experiencing each AE preferred term will be summarized by treatment group. Adverse events will also be summarized by NCI-CTCAE grade and by causality (relationship to study treatment). Serious adverse events and AEs leading to withdrawal, dose modification, or treatment discontinuation will also be summarized by preferred term.

14.5.2. Laboratory Results

Laboratory results will be classified according to NCI-CTCAE, Version 4.03. Laboratory results not corresponding to an NCI-CTCAE term will not be graded. Incidences of laboratory abnormalities will be summarized by treatment group. Summary statistics for each laboratory parameter, along with the change from baseline, will be tabulated by time point.

14.5.3. Other Safety Outcomes

Summary statistics for each of the vital sign parameters, along with the change from baseline, will be tabulated by time point.

14.5.4. Interim Safety Analyses

An independent DSMB will meet and review safety data at least annually. The Board will have a separate charter and operations manual guiding its operations. The DSMB will make its recommendations to the Sponsor who will implement the recommendations following any clarifying discussions that are needed.

14.6. Primary Objective Analyses

The primary endpoint is defined as the time in hours from randomization until the last administration of any parenteral opioid analgesic for the treatment of VOC prior to hospital discharge. The mean times in the two treatment groups will be compared using an analysis of covariance (ANCOVA) model with effects for treatment group (MST-188 or placebo), age group ($\geq$4 to <16 years or $\geq$16 to $\leq$65 years), use of hydroxyurea (yes or no), and pain score as measured using the Wong-Baker FACES® Pain Rating Scale at the time of randomization (<8 or $\geq$8), on the log transformed data.

Following a blinded data review, an assessment will be made regarding the frequency and magnitude of outliers, and the frequency of censored data. Based on this review, the statistical plan may be modified to change the primary analysis to one of the sensitivity analyses described in the next section.

14.7. Sensitivity Analyses

In order to assess the sensitivity of the ANCOVA results to any potential deviations from the underlying ANCOVA assumptions, a nonparametric analysis of the primary endpoint using the van Elteren test, stratified by age group (≥4 to <16 years or ≥16 to ≤65 years), use of hydroxyurea (yes or no), and pain score as measured using the Wong-Baker FACES® Pain Rating Scale at the time of randomization (<8 or ≥8), will also be provided. However, the ANCOVA analysis using the log transformed data will be the primary analysis.

It is anticipated that there will be very little or no missing or incomplete data for the primary endpoint. Thus the primary analysis will be based on the observed time to last parenteral opioid analgesic for the treatment of VOC for each subject. In the event that some subjects have censored data, in the sense that the last recorded parenteral opioid may not be the true last administration of an opioid (for example, if a subject withdraws from the study while still in the hospital), then a sensitivity analysis will be performed using regression methods for right censored data. For this analysis, patients with incomplete follow-up will be censored at the time of their last observed parenteral opioid analgesic for the treatment of VOC.

A Kaplan-Meier analysis of the primary endpoint will also be provided. The Kaplan-Meier estimates will be tabulated and graphed by treatment group. The log rank test, stratified by age group (≥4 to <16 years or ≥16 to ≤65 years), use of hydroxyurea (yes or no), and pain score as measured using the Wong-Baker FACES® Pain Rating Scale at the time of randomization (<8 or ≥8), will be used to compare the treatment groups. For this analysis, subjects with incomplete follow-up during hospitalization will be censored at the time of their last observed parenteral opioid analgesic for the treatment of VOC.

Additional sensitivity analyses using multiple imputations will also be considered.

14.8. Secondary Objectives Analyses

In order to control the overall false positive rate at 0.05, a sequential testing method will be used for the secondary endpoints. The primary analysis must be significant at the 0.05 level in order to declare significance for any secondary endpoint. If the primary analysis is significant at the 0.05 level, then the secondary endpoints will be analyzed in the following order:

1. Re-hospitalization for VOC
2. Occurrence of ACS

The first secondary endpoint will be tested at the 0.05 level, and if significant ($p < 0.05$), then the second secondary endpoint will be tested at the 0.05 level. If the analysis of the first secondary endpoint is not significant, then neither secondary endpoint will be declared significant.

14.8.1. Re-hospitalization for Vaso-occlusive Crisis

The number and percent of subjects who are re-hospitalized for a recurrence of VOC within 14 days of initial discharge from the hospital will be tabulated, and Fisher's exact test will be used to compare the two treatment groups.

14.8.2. Occurrence of Acute Chest Syndrome

The number and percent of subjects who develop ACS within 120 hours of randomization will be tabulated, and Fisher's exact test, will be used to compare the two treatment groups. Acute chest syndrome will be defined as described by the National Acute Chest Syndrome Study Group,[14] namely, the finding of a new pulmonary infiltrate involving at least one complete lung segment that is consistent with the presence of alveolar consolidation, but excluding atelectasis. In addition, the patient must have at least one of the following: chest pain, a temperature of more than 38.5°C, tachypnea, wheezing, or cough.

14.9. Subgroup Analyses

Analyses of the primary endpoint for the following subgroups will be provided:

- Age group (≥4 to <16 years or ≥16 to ≤65 years)
- Gender (male or female)
- Use of hydroxyurea (yes or no)
- Pain score as measured using the Wong-Baker FACES® Pain Rating Scale at the time of randomization (<8 or ≥8)
- Duration of moderate to severe pain at presentation (≥4 to 12 hours or >12 hours to ≤24 hours)

Additional subgroups may be specified prior to breaking the blind.

14.10. Pharmacodynamic Endpoints

Each of the pharmacodynamic endpoints (hs-CRP, sPLA2, D-dimer, proteomics, and RNA assay) will be summarized by time point using descriptive statistics.

14.11. Pharmacokinetic Endpoints

MST-188 plasma concentration data from this study will be used in combination with plasma concentration data from previous clinical studies to develop an appropriate population pharmacokinetic model for MST-188 which will aid in the justification of dose selection for this patient population. In addition, the pharmacokinetic information will be examined along with individual specific covariate factors (e.g., demographics, disease state, renal function, etc.) that are predictive of unexplained random variability. The specifics regarding the development of a population pharmacokinetic model for MST-188, the analysis of data, including assessment of covariate factors, will be described in a detailed population pharmacokinetic data analysis plan.

14.12. Exploratory Urine Biomarkers

Each of the exploratory urine biomarker analytes, KIM-1, NAG, NGAL, urine creatinine, and urine sodium, will be summarized by time point using descriptive statistics.

15. QUALITY CONTROL AND QUALITY ASSURANCE

An independent audit at the study center may take place at any time during or after the trial. This audit can be carried out by the Mast Therapeutics Quality Assurance department, Mast Therapeutics designee, or by a regulatory authority.

15.1. Quality Control

Quality control includes operational techniques and activities undertaken within the quality assurance system to verify that the requirements for quality of study activities have been fulfilled. Quality control should be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

15.2. Quality Assurance

Quality assurance includes all planned, systematic actions ensuring that the trial is performed, and study data are generated, documented and reported, in compliance with International Conference on Harmonization (ICH) Guideline for Good Clinical Practice as published in the Federal Register May 9, 1997.

15.3. Inspections

An inspection is a regulatory authority's official review of documents, facilities, records and any other resources deemed related to the clinical trial, at study centers, at the Sponsor's and/or contract research organization's facilities, or at any other establishment as appropriate.

15.4. Audits

An audit is a systematic, independent review of study-related activities and documents, to determine whether validated activities were conducted, and data were recorded, analyzed and accurately reported, according to the protocol, designated standard operating procedures, Good Clinical Practice (GCP) and applicable regulations.

16. ETHICS

Before initiating the study, the Investigator must obtain written, dated approval or favorable opinion, from the appropriate IRB or IEC, of the final study protocol and any amendments, the written Informed Consent Form and assent forms as applicable, and any updates, subject recruitment material such as advertisements, and any other written information for subjects. The written letter of IRB/IEC approval should refer to the final protocol number and date. Information on IRB/IEC membership, including members' names and functions, should be provided to the Sponsor. During the study, the Investigator should provide the IRB/IEC with all further documents requiring IRB/IEC review.

16.1. Ethics Review

16.1.1. Institutional Review Board /Independent Ethics Committee

The IRB or IEC is an independent body consisting of medical, scientific and non-scientific members. It is responsible for ensuring that study subjects' rights, safety and well-being are protected in accordance with the Declaration of Helsinki. IRB/IEC activities include review, approval and further review of the study protocol and amendments, and of the materials and methods used to obtain and document subjects' informed consent. It carries out these responsibilities by reviewing, and providing a favorable opinion of, the study protocol, the Investigator's suitability, the study facilities, and the materials and methods used to obtain and document subjects' informed consent.

The IRB's/IEC's legal status, composition, function, operations and relevant regulatory requirements may differ among countries, but should allow the IRB/IEC to act in accordance with GCP.

16.1.2. Ethics Committee Approval

The appropriate IRB/IEC must review and approve the protocol, the informed consent documents, and any and all other written materials or information to be provided to subjects or prospective subjects, including, without limitation, advertisements and subject recruitment materials. In addition, the IRB/IEC should conduct continuing review of the study periodically, but at least once per year. The Institution and the Investigator will provide Mast Therapeutics with documentation that the IRB/IEC has approved the study before the study may begin. The written letter of IRB/IEC approval or favorable opinion should refer to the final protocol number and date.

In addition, the Investigator must provide the following documentation:

- The IRB/IEC annual re-approval of the protocol.
- The IRB/IEC approval of any revisions to the informed consent or assent documents or amendments to the protocol.

Details of the IRB/IEC composition including names of their members, their qualifications and what function they perform on the committee (e.g., chairman, specialist, lay-member) will be

made available to the Sponsor to conform to regulations governing the conduct of clinical trials. If available, the constitution of the IRB/IEC must also be supplied.

16.2. Modification of Protocol

The Investigator should not implement any deviation from, or changes of, the protocol without agreement by Mast Therapeutics and prior review and documented approval/favorable opinion from the IRB/IEC of an amendment. The only exceptions are where necessary to eliminate an immediate hazard(s) to trial subjects, or when the change(s) involves only logistical or administrative aspects of the trial (e.g. change of telephone number[s]).

As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted:

a. to the IRB/IEC for review and approval/favorable opinion,

b. to the Sponsor for agreement and, if required,

c. to the regulatory authority(ies).

Protocol amendments must be written, signed and dated by Mast Therapeutics and the PI. Mast Therapeutics or its designee will ensure that the Investigators submit the necessary protocol amendments to the appropriate IRBs/IECs.

All approved protocol amendments must be clearly documented using standard procedures as defined by Mast Therapeutics, and must be signed and dated by Mast Therapeutics and the Investigator.

16.3. Written Informed Consent

As used in this protocol, the term “informed consent” includes all consent and/or assent given by subjects or their legally authorized/acceptable representatives, as applicable, and the term “minor” means a person who has not attained the legal age for consent to treatments or procedures involved in clinical investigations, under the applicable law of the jurisdiction in which the study will be conducted.

All subjects are to give informed consent, or, as applicable, assent in accordance within the origins of the Declaration of Helsinki, United States Code 21 CFR part 50 and that are consistent with ICH guidelines on GCP and regulatory requirements as applicable. Written informed consent with appropriate signatures and dates on the informed consent document must be obtained from each subject prior to the subject undergoing any study-specific procedures.

The Investigator will explain the nature, purpose and risks of the study and provide the subject and, as applicable, the legally authorized/acceptable representative with a copy of the study information sheet, if applicable. The subject and, as applicable, his or her legally authorized/acceptable representative will be given sufficient time to consider the study’s implications before deciding whether to participate.

For any subject who is a minor, the written informed consent must be obtained from such minor’s legally authorized/acceptable representative. In addition, when the IRB/IEC determines that assent is required, minors must provide assent to participate in the study at an

age-appropriate level as determined by the IRB/IEC. Each subject must meet all inclusion and exclusion criteria previously described in Section 8 of this protocol.

An authorization prepared and executed in compliance with the Health Insurance Portability and Accountability Act of 1996 (HIPAA) and any corresponding state requirements, to the extent such state requirements are not preempted under HIPAA, that permits the Investigator to use and disclose "protected health information" (as defined in the privacy rule issued under HIPAA, 45 C.F.R. Parts 160 and 164) to Mast Therapeutics and their respective representatives and agents, including, but not limited to, clinical trial service providers must be obtained.

It is the Investigator's responsibility to ensure that informed consent is obtained from each subject and, as applicable, legally authorized/acceptable representative and for obtaining the appropriate signatures and dates on the informed consent document prior to the performance of any protocol procedures and prior to the administration of blinded MST-188 or blinded placebo. The Investigator will retain each subject's original ICF and assent and will supply all enrolled subjects with a copy of their signed documents.

The ICF and assent form may need to be revised during the trial should important new information become available that may be relevant to the safety of the subject. In this instance the IRB/IEC must review and approve the revised protocol and ICF and assent; existing subjects must be informed of the changes, and signed consent obtained for the new changes.

Should there be any amendments to the protocol that would directly affect the subject's participation in the trial (e.g., a change in any procedure), the ICF and assent as applicable must be amended to incorporate this modification. Re-consenting and re-assenting, as applicable, will indicate subjects agree to continue participation in the trial.

17. DATA HANDLING AND RECORDKEEPING

17.1. Data Collection

Study subject data will be recorded using a Remote Data Capture (RDC) System. Clinical Research Coordinators, Data Managers, Research Nurses, or other appropriate personnel must be designated at each study center for entering subject data into RDC onsite. System use training will be provided for each individual designated to enter, review, or verify subject data before access to the RDC system is provided. Investigators will also be required to complete RDC system training to permit access to approve entered or corrected patient data. Access to the RDC system will be limited to trained personnel only.

Data entry for each subject enrolled and treated in the study must commence as soon as possible. Study subject data should be entered into the RDC system within ten (10) business days following completion of such Study Subject visit.

17.2. Inspection of Records

A representative or designee of Mast Therapeutics will visit the Investigator periodically for the purpose of monitoring the progress of this study in accordance with GCP guidelines and applicable regulations. Mast Therapeutics or Mast Therapeutics' designees may communicate with the Investigator by e-mail or telephone calls to discuss the progress of the study and review data quality. It is the responsibility of the Investigator to be present or available for consultation during scheduled monitoring visits. During these routine visits, all data pertaining to a subject's participation in this clinical investigation must be made available to the study monitor. Verification of clinical trial procedures and/or data, shall not violate the confidentiality of the subject, to the extent permitted by the applicable laws and regulations and that, by signing a written ICF, the subject or the subject's legally authorized/acceptable representative is authorizing such access.

In addition, Mast Therapeutics or Mast Therapeutics' designees, the FDA or another national regulatory authority may audit the study center in depth for study quality assurance. This audit may include review of all source documents, treatment records, original clinic case notes, etc. Patient confidentiality will be maintained at all times and consent for this review will be obtained before entry of the patient into the study by the patient's signature on the consent form.

17.3. Missing Data

To maintain reliability and confidence in the study endpoints, every effort should be made to minimize missing and incomplete data. Missing key variable data points can introduce substantial bias to the study endpoints. Timely data entry will assist in the detection of missing or inconsistent data and adherence to the study treatment and protocol, permitting actions to obtain the data or the institution of corrective measures. All missing data points will require an eCRF entry of the reason the data point is missing. Centers are encouraged to locate and supply the missing data points within 48 hours of notification by Mast Therapeutics or their designee.

Mast Therapeutics will monitor data collection and follow up assessments for missing data in an on-going manner throughout the study. Remote and on-site monitoring will permit real-time tracking and reporting of missing data points. Electronic data capture systems and centralized monitoring tools will identify data error rates and protocol deviations in real time and will help to identify potential risks for more intensive monitoring. Missing study data will be characterized by measurements such as rate, inconsistencies, outliers, prevalence of error types, systematic or significant errors of data items, ineligible participants, and delays in reporting or recording data.

Corrective and preventative actions may need to be undertaken by participating centers at Mast Therapeutics' request to improve the collection of data and minimize missing data points. Study centers that cannot consistently and reliably capture data associated with eligibility criteria, informed-consent documentation, critical study endpoints, protocol-required safety assessments, study drug accountability, and study-blind maintenance may be discontinued from participation by Mast Therapeutics.

17.4. Retention of Records

Study centers should retain essential documents until at least two years after the last approval of all outstanding marketing applications for MST-188 in an ICH region, or at least two years after formal discontinuation of the drug's clinical development. These documents should be retained longer, however, if required by applicable regulations or in an agreement with Mast Therapeutics. Mast Therapeutics is responsible for informing the Investigator when these documents no longer need to be retained.

17.5. Financial Disclosure

The Investigator shall in a timely manner provide all information to Mast Therapeutics necessary to comply with any disclosure requirements imposed on Mast Therapeutics, including, but not limited to, any information required to be disclosed in connection with any financial relationship between Mast Therapeutics and the Investigators and other healthcare professionals involved in the study, as well as any immediate family members thereof, pursuant to applicable law, including, but not limited to, 21 CFR Part 54 or foreign equivalents, or otherwise reasonably requested by Mast Therapeutics. Such disclosure shall be in a form and manner reasonably satisfactory to Mast Therapeutics. The required disclosure includes, but is not necessarily limited to, information related to financial interests that the Investigator or Sub-investigators hold in Mast Therapeutics or compensation received by the Investigator or Sub-investigators from Mast Therapeutics for activities other than conducting the study. The Investigator must promptly update this information if any relevant changes occur during the study and for one year following completion of the study. The Investigator also shall keep records regarding all payments made, and costs, expenditures and expenses incurred, in connection with the study, and, upon Mast Therapeutics' request, shall provide Mast Therapeutics with information regarding such costs, expenditures and expenses.

18. FINANCING AND INSURANCE

The compensation to be paid by Mast Therapeutics for performing the study will be documented in a separate clinical trial agreement to be signed by the study center and Mast Therapeutics before the study begins.

Subjects may be reimbursed for reasonable study-related travel expenses. Maximum amounts for such reimbursement will be agreed on in advance and documented in the clinical trial agreement between the Institution and Mast Therapeutics.

The clinical trial agreement between the study center and Mast Therapeutics will dictate the insurance coverage to be maintained by the Institution, the Investigator and Mast Therapeutics in connection with the study.

19. PUBLICATION POLICY

To the extent possible, the International Committee of Medical Journal Editors (ICMJE) guidelines (current as of the date of publication) will be followed. Mast Therapeutics recognizes the value of disseminating research results, and the Investigator shall be free to publish information about data and results obtained through the conduct of the study at the Institution, subject to the terms set forth below and in the clinical trial agreement between the Institution and Mast Therapeutics.

The Investigator may independently publish information about data and results obtained through the conduct of the study at the Institution only following the earlier of (i) the first publication by Mast Therapeutics of the overall results of the multi-center study, which shall take into consideration data generated from all clinical study centers participating in the multi-center study, and FDA approval of the New Drug Application for MST-188 (purified poloxamer 188) Injection [MST-188; also known as ANX-188 (purified poloxamer 188) Injection or ANX-188], or (ii) eighteen (18) months after final database lock for the multi-center study. Subject to the foregoing, where the Investigator desires to independently publish information about data and results obtained through the conduct of the study at the Institution, the Investigator shall, at least thirty (30) days prior to the proposed date for submission for publication or disclosure, furnish Mast Therapeutics with a written copy of the proposed publication or disclosure relating to the study, including, but not limited to, disclosures in papers or abstracts or at research seminars, lectures, professional meetings, or poster sessions. During such 30-day period, Mast Therapeutics shall have the right to review and comment upon such publication or disclosure and to require the removal of any confidential information (other than study results) therefrom, which removal shall be promptly accomplished by the Investigator. In addition, upon Mast Therapeutics' request, during the foregoing 30-day period, the Investigator shall delay submission for publication or disclosure for a period not to exceed sixty (60) days from the date of such request to permit Mast Therapeutics to file patent applications or to otherwise seek intellectual property protection related to information contained in such publication or disclosure. If Mast Therapeutics does not provide the Investigator with comments or suggested revisions, or with the request described in the preceding sentence, within thirty (30) days following Mast Therapeutics' receipt of such publication, then the Investigator may submit such publication without further obligation to Mast Therapeutics regarding its right to review.

The requirements set forth above should not be construed as a means of restricting publication, but rather are intended solely to ensure concurrence regarding data, evaluations, and conclusions, to provide an opportunity for Mast Therapeutics to share with the Investigator any new and/or unpublished information of which he or she may be unaware, and to allow the Sponsor to protect any confidential information or intellectual property contained in the proposed publication or disclosure.

Subject to applicable law, Mast Therapeutics and its designees shall have the right to review, use and disclose any and all data and other information developed or arising out of the study as Mast Therapeutics, in its sole discretion, deems appropriate, including, but not limited to, in submissions to the FDA and other regulatory authorities. The Investigator understands that, to

allow the use of information gained in this study, he or she is obligated to provide Mast Therapeutics or its designee with all test results and complete study data.

20. REFERENCES

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21. APPENDICES

Appendix A: Wong-Baker FACES® Pain Rating Scale

Appendix B: Pain Management Guideline

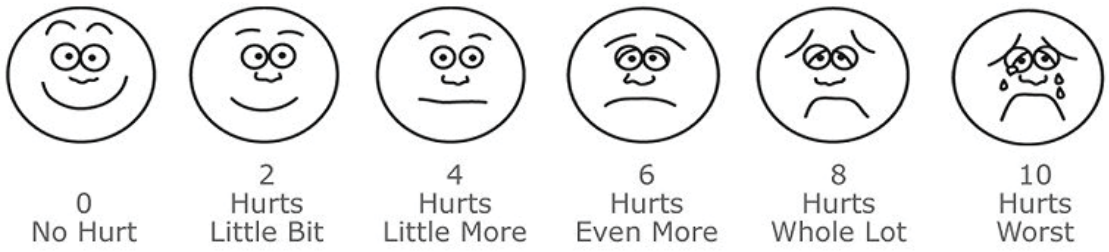
Appendix C: Drugs Demonstrated to be Physically Compatible with MST-188

Appendix D: Tissue Oxygenation Sub-study

Appendix E: Complications of Sickle Cell Disease

Appendix A: Wong-Baker FACES® Pain Rating Scale

Wong-Baker FACES® Pain Rating Scale



Explain to the person that each face is for a person who feels happy because he has no pain (hurt) or sad because he has some or a lot of pain. **Face 0** is very happy because he doesn't hurt at all. **Face 2** hurts just a little bit. **Face 4** hurts a little more. **Face 6** hurts even more. **Face 8** hurts a whole lot. **Face 10** hurts as much as you can imagine, although you don't have to be crying to feel this bad. Ask the person to choose the face that best describes how he is feeling.

Rating scale is recommended for persons age 3 years and older.

Appendix B: Pain Management Guideline

This study will utilize the time to last parenteral opioid dose prior to hospital discharge as the primary endpoint. This may be influenced by the methods of pain control used at each site. While approaches to pain management vary considerably from institution to institution, it is recognized that as uniform an approach to pain management as possible among clinical sites will help standardize determinations of the endpoint. All Investigators are strongly encouraged to adhere to these guidelines, unless alternative treatment is medically necessary.

These guidelines consist of three main parts:

1. Permitted Analgesics and Routes of Administration
2. Guidelines for Emergency Department or Other Acute-Care Setting
3. Guidelines for In-Patient Setting
    a. Patient-Controlled Analgesia (PCA)
    b. Time-Contingent/By-the-Clock Analgesia
    c. Factors in Discontinuing Parenteral Opioid Analgesics
    d. Supportive Care

1. Permitted Analgesics and Routes of Administration

Analgesic Classes and Routes of Administration

The names, dates, times, doses, amount administered, and routes of administration of all analgesics will be documented in the eCRF from 30 days prior to presentation to the site through the 30-Day Post Infusion Follow-Up visit.

Analgesic drug choices will be limited to the following:

Parenteral Opioids: Morphine, hydromorphone, nalbuphine, oxycodone, and tramadol, and approved parenteral formulations of permitted oral opioids.

Parenteral NSAIDS: Ketorolac

Oral Opioids: Codeine, hydrocodone, hydromorphone, morphine, oxycodone

Oral Non-Opioid Analgesics: Acetaminophen, aspirin, diclofenac sodium, ibuprofen, naproxen

Other Oral Analgesics: Tramadol

Long-acting variants of the oral analgesics noted above may be utilized.

2. Guidelines for Emergency Department or Other Acute-Care Setting

Optimally, methods of pain control utilized prior to a patient being admitted to this study will be as uniform as possible. Implementation of the following guidelines in the acute-care setting will increase consistency among clinical sites. Investigators are encouraged to discuss the following guidelines with personnel from the Emergency Department (ED) or other acute-care setting from which sickle cell disease patients typically are admitted to the institution. Investigators also

should request that they are notified when patients potentially eligible for this study present to the acute-care center.

1. Treatment in the acute care setting should be nurse-administered, time-contingent/by-the-clock (BTC) at set intervals.
2. Parenteral opioid therapy should be initiated at 0.10 – 0.15 mg/kg of morphine, or 0.015-0.02 mg/kg of hydromorphone or nalbuphine, given as an IV loading dose and every two hours thereafter (the BTC dose).
3. Reassess in 15-30 minutes: if pain relief is judged as inadequate by patient or physician, then administer a dose of ¼ to ½ of the BTC dose (the titration dose).
4. Repeat reassessment in 15-30 minutes and continue opioid titration, if necessary, until relief.
5. Once pain relief is judged adequate, based on number and timing of titration doses, adjust the BTC dose up or down (or continue) to maintain relief.
6. Administer the BTC dose every 2 hours.
7. Continue pain assessment at least every two hours.
8. If necessary due to poor relief between BTC doses, provide opioid demand doses (¼ of the BTC dose).
9. Patient may decline the BTC dose if pain relief is judged to be adequate at the time the dose is due. If the BTC dose is declined, the patient should be given the option to start oral analgesics.
10. If pain relief is judged to be adequate in the acute-care setting and patient and physician feel that (s)he no longer requires parenteral pain medication, the patient is no longer a candidate for this study.
11. If patient and physician believe the crisis is unlikely to resolve sufficiently with continued treatment in the acute-care setting to allow successful home analgesic therapy, patient remains eligible for this study and may be admitted as per institutional policy.

3. Guidelines for In-Patient Setting

Dosing of analgesics through PCA is recommended. However, at the discretion of the attending physician and patient, time-contingent/by-the-clock dosing may be utilized.

A. Patient-Controlled Analgesia

1. PCA may be started in the acute care setting or in the inpatient unit as per institutional protocol.
2. PCA should be a combination of a continuous “background” or basal infusion and a bolus “demand” or rescue dose that is delivered when the patient pushes the button. The goal is to control pain through the basal infusion to the extent that it limits demand doses to no more than two per hour. Basal dosing may be omitted for individual contraindication (for example, sleep apnea).

3. Initial dose rates for the basal infusion should be in the range of 0.02-0.06 mg/kg/hr for morphine, while demand doses should be in the range of 0.015-0.05 mg/kg with a six-minute to eight-minute lockout. Equi-analgesic doses of hydromorphone or nalbuphine may be used. Doses within these ranges should be determined in collaboration with the patient, considering what has worked in prior admissions, as well as (if available) the BTC dose used and the number of demand doses needed in the acute-care setting.
4. Pain should be assessed and dosing adjusted every 6-8 hours in the first 24 hours. After an acceptable dosing schedule has been obtained, pain assessments and dosing adjustments every 12 hours are appropriate based on number of demand doses, current pain intensity and degree of pain relief. Dosing adjustments also should be made as follows:
    - If patient administers more than 8 demand doses in 4 hours, then the basal infusion rate should be increased by 10%.
    - If patient goes 4 hours without administering any demand doses, then the basal rate should be reduced by 10%.
    - If patient indicates that pain has improved over the previous 12 hours, then the basal infusion rate should be reduced by 20% (physician has the discretion to reduce by a larger amount if patient agrees that a larger reduction is appropriate).
    - Basal infusions should not be increased in anticipation of sleep or at night.
5. When the basal rate has been reduced to half the starting dose and patient goes 4 hours without administering any demand dose, the basal infusion should be stopped and substituted for an equi-analgesic dose of long-acting oral opioid. Unless the Investigator determines otherwise, the basal infusion should be discontinued based on these criteria regardless of the time of day/night at which they occur (late evening/nighttime; while patient is sleeping; etc.).
6. Parenteral demand doses can be stopped when the pain has sufficiently resolved that the patient and physician feel that the pain can be effectively managed with oral analgesics alone. See “Factors in Discontinuing Parenteral Opioid Analgesics.”

B. Time-Contingent/By-the-Clock Analgesia

1. A nurse-administered, time-contingent/BTC dosing regimen will be continued after admission to the in-patient unit if PCA is not used. BTC doses, together with “demand” or rescue doses, should be used to manage pain. Care will be taken to make sure that doses are not omitted in the transition from the ED or other acute-care setting to the inpatient unit.
2. The initial BTC dose should be based on the efficacy of the BTC dose/schedule used in the ED or acute-care setting and the number of demand doses needed. The duration between BTC doses should be every 2 hours. The order should be written to

allow the patient to decline a dose if pain relief is judged to be adequate at the time the dose is due.

3. Demand doses are used to help make adjustments to the initial BTC dose and address breakthrough pain. During the early stages of treatment, demand doses are titrating doses to get pain under control. Later in treatment, demand dosing informs adjustments in BTC dosing.
4. Demand doses should be ordered q 1 hourly at ¼ of the BTC dose. Reassessment to ensure adequate pain relief should occur 15-30 minutes after administration of any demand dose.
5. After adequate pain control is maintained, BTC dosing should be spaced to q 3-4 hours.
6. Pain should be assessed and the BTC dose adjusted every 12 hours based on number of demand doses or refused doses, current pain intensity and degree of pain relief. Adjustments to the BTC dose also should be made as follows:
   - If demand doses are needed during two successive dosing intervals, then the BTC dose should be increased by 10%.
   - If patient declines any BTC dose, the BTC dose should be reduced by 10%.
   - If patient indicates that pain has improved over the previous 12 hours, then the BTC does should be reduced by 20% (physician has the discretion to reduce by a larger amount if patient agrees that a larger reduction is appropriate).
   - When pain improves, adjustments to the BTC dose should be reductions in dose, not interval.
7. Parenteral opioid analgesics can be stopped when the pain has sufficiently resolved that the patient and physician feel that the pain can be effectively managed with oral analgesics alone. See "Factors in Discontinuing Parenteral Opioid Analgesics."

C. Factors in Discontinuing Parenteral Opioid Analgesics

In assessing whether pain can be effectively managed with oral analgesics alone, the physician should take into account the following:

1. Patient vital signs are normal (body temperature, pulse rate, blood pressure, respiratory rate).
2. Patient can walk without assistance (for example, to the bathroom).
3. Patient is comfortable (relaxed) and willing to interact with hospital staff. Patient is not in distress (fetal position, curled up, avoiding movement out of a concern of triggering pain).
4. Patient is engaging in activities (going to playroom, participating in crafts).
5. Patient is interacting (talking) with hospital staff and not avoiding.

6. Patient has appetite.
7. Patient is sleeping for normal periods.
8. Patient has improvement in the number of sites of pain or in specific pain locations.
9. If taken, recent pain scores indicate the acute phase of the crisis is resolving.
10. Patient believes the acute phase of the crisis is resolving.

D. Supportive Care

NSAIDS: Ketorolac may be used at 0.5 mg/kg (max 30 mg) IV, q 6 hours for 72 hours. Ibuprofen may be dosed at 10 mg/kg, PO, q 6 hours (400 mg, PO, 6 hour max) after discontinuation of ketorolac.

Stimulant medications: These are generally discouraged as are the initiation of anti-depressants and anti-convulsants during treatment of the acute pain event. Excessive sedation is best managed by opioid dose reduction or low dose naloxone infusion.

Side effect management: An oral laxative should be ordered on admission to reduce the possibility of opioid-induced constipation. Pruritis should be managed with oral agents to avoid excessive sedation. For a history of pruritis with opioids, the anti-histamine can be administered concomitantly with initiation of opioid therapy rather than waiting for pruritis to develop. Benadryl or hydroxyzine are first line agents. IV or oral ondansetron is encouraged for excessive nausea, as it is non-sedating, but metoclopramide or promethazine may also be considered.

These guidelines are based on the following publications and modified from guidelines written in part by Carleton Dampier:

1. Benjamin LJ, Dampier CD, Jacox AK, et al. (1999), Guideline for the Management of Acute and Chronic Pain in Sickle Cell Disease, APS Clinical Practice Guideline Series, No 1. Glenview, IL: American Pain Society.
2. Benjamin LJ, Dampier CD, Jacox AK, et al. (2001), Guideline for the Management of Acute Pain in Sickle Cell Disease-Quick Reference Guide for Emergency Department Physicians, APS Clinical Practice Guideline Series, No 1. Glenview, IL: American Pain Society.

Appendix C: Drugs Demonstrated to be Physically Compatible with MST-188

Drug Name		
Acetaminophen	Foscarnet	Pentazocine lactate
Acyclovir sodium	Furosemide	Phenylephrine hydrochloride
Amikacin sulfate	Ganciclovir sodium	Piperacillin sodium
Ampicillin sodium	Gentamicin sulfate	Piperacillin sodium-tazobactam sodium
Ampicillin sodium-sulbactam sodium	Granisetron hydrochloride	Potassium chloride
Aprepitant	Heparin sodium	Prochlorperazine edisylate
Atenolol	Hydrocortisone sodium phosphate	Promethazine hydrochloride
Aztreonam	Hydrocortisone sodium succinate	Propranolol hydrochloride
Brompheniramine maleate	Hydromorphone hydrochloride	Quinidine gluconate
Bumetanide	Hydroxyzine hydrochloride	Ranitidine hydrochloride
Buprenorphine hydrochloride	Imipenem-cilastatin	Streptokinase
Butorphanol tartate	Indomethacin sodium trihydrate	Ticarcillin disodium
Cefazolin and dextrose	Isoproterenol hydrochloride	Ticarcillin disodium-calvulanate potassium
Cefazolin sodium	Ketorolac tromethamine	Tobramycin sulfate
Cefepime HCl	Levorphanol tartate	Trimethoprim-sulfamethoxazole
Cefonicid sodium	Lidocaine hydrochloride	Vancomycin hydrochloride
Cefoperazone sodium	Lincomycin hydrochloride	Warfarin sodium
Cefotaxime sodium	Lorazepam	
Cefotetan sodium	Magnesium sulfate	
Cefoxitin sodium	Mannitol	
Ceftazidime (with sodium carbonate)	Meperidine hydrochloride	
Ceftriaxone sodium	Meropenem	
Chlorothiazide sodium	Metaraminol bitartate	
Chlorpheniramine hydrochloride	Methadone hydrochloride	
Cimetidine hydrochloride	Methocarbamol	
Ciprofloxacin	Metoclopramide hydrochloride	
Clindamycin phosphate	Metoprolol	
Codeine phosphate	Metoprolol tartate	
Dexamethasone sodium succinate	Metronidazole	
Dimenhydrinate	Mezlocillin sodium	
Diphenhydramine hydrochloride	Morphine sulfate	
Dobutamine hydrochloride	Moxifloxacin hydrochloride	
Dopamine hydrochloride	Nafcillin sodium	
Doxycycline hyclate	Nalbuphine hydrochloride	
Ephedrine hydrochloride	Naloxone	
Epinephrine hydrochloride	Netilmicin sulfate	
Erythromycin lactobionate	Nitroglycerin	
Ethacrynate sodium	Norepinephrine bitartate	
Famotidine	Ondansetron hydrochloride	
Fentanyl citrate	Orphenadrine citrate	
Fluconazole	Oxacillin sodium	
Folic acid	Oxymorphone hydrochloride	

Appendix D: Tissue Oxygenation Sub-study

A STUDY OF THE EFFECTS OF MST-188 ON TISSUE OXYGEN SATURATION IN SICKLE CELL SUBJECTS EXPERIENCING A VASO-OCCLUSIVE CRISIS

1. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
ANCOVA	Analysis of covariance
AUC	Area under the curve
CASMED	CAS Medical Systems, Inc.
EPIC	Evaluation of Purified Poloxamer 188 in Vaso-Occlusive Crisis of Sickle Cell Disease, Study MST-188-01
FDA	United States Food and Drug Administration
Hb	Hemoglobin
HbO_2	Oxygenated hemoglobin
$HbS\beta^0thal$	Hemoglobin beta thalassemia 0 genotype of SCD
$HbS\beta^+thal$	Hemoglobin beta thalassemia + genotype of SCD
HbSC	Hemoglobin SC genotype of SCD
HbSS	Hemoglobin SS genotype of SCD
MST-188	MST-188 (purified poloxamer 188) Injection [MST-188; also known as ANX-188 (purified poloxamer 188) Injection or ANX-188]
SCD	Sickle cell disease
StO_2	Tissue oxygen saturation
SpO_2	Peripheral oxygen saturation
VOC	Vaso-occlusive crisis

2. INTRODUCTION/BACKGROUND

MST-188 (purified poloxamer 188) Injection [MST-188; also known as ANX-188 (purified poloxamer 188) Injection or ANX-188] improves microvascular blood flow and reduces ischemia by lowering viscosity (particularly under low shear rate conditions) and adhesive, frictional forces. When infused in subjects with sickle cell disease (SCD) experiencing a vaso-occlusive crisis (VOC), MST-188 can facilitate restoration of compromised microvascular blood flow.[1] As improvement in microcirculation occurs rapidly after infusion, use of MST-188 at the onset of VOC can be expected to improve tissue oxygenation, shorten the duration of VOC and the associated pain, limit cumulative tissue damage, and limit end-organ dysfunction and failure commonly seen in patients with SCD. The ability of MST-188 to potentially reduce the duration of VOC in patients with SCD is currently being investigated in a multicenter, global clinical trial; MST-188-01, Evaluation of Purified Poloxamer 188 in Vaso-Occlusive Crisis of Sickle Cell Disease (EPIC).

This investigation is a sub-study of the EPIC study. The sub-study will investigate and quantify the effect of MST-188 on microvascular blood flow, indirectly measured by tissue oxygenation using a non-invasive method, and will investigate the relationship of tissue oxygenation to clinical outcomes such as the duration of VOC. A variety of methodologies to measure tissue oxygenation have previously been investigated in clinical studies.[2,3,4,5] The EPIC sub-study will use a noninvasive measurement of tissue oxygen saturation (StO_2) in the cerebral tissue and skeletal muscle of study subjects as a measure of microvascular perfusion. StO_2 will be determined using a United States Food and Drug Administration (FDA)-approved and CE-marked commercially-available instrument based on near-infrared spectroscopy that measures the absolute hemoglobin (Hb) saturation. This methodology has been previously evaluated in children with SCD for the measurement of cerebral tissue oxygen saturation.[6] Peripheral oxygen saturation (SpO_2) of Hb will also be measured using pulse oximetry to investigate the relationship between SpO_2 and StO_2.

Results from this sub-study will provide a greater understanding of the effect of MST-188 on microvascular blood flow, as indirectly measured by tissue oxygenation, and the relationship of tissue oxygenation to clinical outcomes assessed in the EPIC study.

3. SUB-STUDY DESIGN

This evaluation is a sub-study of the EPIC clinical trial. The sub-study will be conducted at approximately 10 selected study sites in the United States participating in the EPIC clinical trial. Approximately 30 eligible male and female sickle cell subjects, who are randomized to the EPIC clinical trial, will participate in the EPIC sub-study.

4. SUB-STUDY OBJECTIVE

The primary objective of the EPIC sub-study is to evaluate the effects of MST-188 on microvascular blood flow in subjects with SCD experiencing a VOC, as indirectly measured by non-invasive measurements of StO_2.

5. SUBJECT SELECTION

5.1. Study Population

Male and female subjects with SCD (HbSS, HbSC, HbSβ^+thal, or HbSβ^0thal) experiencing VOC who require hospitalization and treatment with parenteral opioid analgesia and are successfully randomized to study MST-188-01.

5.2. Subject Inclusion Criteria

1. Concurrently randomized in the MST-188-01 trial.
2. Be able to read, understand, and provide written informed consent and assent (as applicable) prior to any sub-study procedures.

5.3. Subject Exclusion Criteria

1. Injury or skin irritation of the forehead or extremities where the StO_2 device sensors will be placed.
2. Unwilling or unable to comply with the procedures of sub-study protocol.
3. Subject is otherwise not an appropriate study candidate, in the Investigator's judgment.

6. STUDY PROCEDURES

The CAS Medical Systems, Inc. (CASMED) FORE-SIGHT® Absolute Tissue Oximeter, MC-2000 Series will be used to noninvasively measure tissue oxygen saturation in the cerebral tissue and skeletal muscle. This instrument uses near-infrared spectroscopy to assess StO_2. The FORE-SIGHT® instrument consists of an optical transducer containing a laser light source (Class 1M laser output) and photodiode detectors, with a graphic display output screen.[7] The instrument will be used to measure StO_2 at the sub-study-designated time points. Bi-frontal sensors are applied to the subject's forehead and will provide separate readings for each cerebral hemisphere. The sensors will overlay the watershed zone between the anterior and middle cerebral arteries measuring the mix of arterial and venous blood.[7] Based on the manufacture's recommended use of FORE-SIGHT®, subjects ≥19 years of age or weighing >50 kilograms will not participate in the skeletal muscle StO_2 measurements. Sensors are applied to limb muscles of the forearm and leg calf and will provide separate readings for each extremity.

Operational use of FORE-SIGHT® will be in accordance with the manufacturer's User Manual provided with each instrument. Immediately prior to the placement of the sensors, the skin on the upper forehead and limb muscles, as applicable, of the subject will be cleaned with rubbing alcohol. Sensors should not be placed on high density hair areas. Two adhesive-backed sensors will be placed on the subject's forehead, one on the left side and one on the right side and avoiding the sinus cavities, to measure StO_2 of each cerebral hemisphere. After placement of the sensors, the operator will initiate collection of cerebral StO_2 data collection. The FORE-SIGHT® records a data point every 2 seconds, and one minute of data will be collected for each measurement time point.

If the subject is <19 years of age and weighs <50 kilograms, the cerebral sensors will be moved so that one sensor is located on one of the subject's forearms and one sensor is located on one of the subject's calf muscles. After placement of the sensors, the operator will initiate collection of skeletal StO_2 data for one minute.

Each subject's StO_2 measurements will be recorded on the FORE-SIGHT® USB memory drive after StO_2 measurements at each designated time point. SpO_2 measurements will be made using the pulse oximetry instrument routinely utilized in the clinic or hospital and will be collected at the conclusion of the StO_2 measurements.

StO_2 and SpO_2 measurements will be collected at the following times:

- Baseline (pre-treatment).
- 1 hour (± 30 minutes) after the initiation of the infusion of blinded study drug or placebo.
- 8 hours (± 30 minutes) after the initiation of the infusion of blinded study drug or placebo.
- 24 hours (± 30 minutes) after the initiation of the infusion of blinded study drug or placebo.
- 48 hours (± 30 minutes) after the initiation of the infusion of blinded study drug or placebo, or at the conclusion of the infusion of blinded study drug if the infusion is prematurely discontinued.
- Hospital discharge (defined as up to 60 minutes prior to discharge).
- 30-Day Post-Infusion Follow-up visit (Thirty days [+ 2 days] after the completion of blinded infusion, the subject will return for a 30-Day Post-Infusion Follow-Up visit).

7. MST-188-01 Sub-study Schedule of Events

	Screening	Baseline (Pre-Treatment)	Treatment Phase[1] (Hours)				Post-Treatment Phase	
			1	8	24	48[2]	Hospital Discharge[3]	30-Day Post-Infusion Follow-up Visit[4]
			After the Initiation of the Infusion of Blinded Study Drug					
Informed Consent/Assent	X							
Inclusion/ Exclusion	X							
StO_2 Measurement (cerebral tissue and skeletal muscle)		X	X	X	X	X	X	X
SpO_2 Measurement		X	X	X	X	X	X	X

Footnotes:

1. All measurement collection time points occurring during the treatment phase will be completed within ± 30 minutes of the designated times.
2. 48 hours (± 30 minutes) after the initiation of the infusion of blinded study drug or placebo, or at the conclusion of the infusion of blinded study drug if the infusion is prematurely discontinued
3. Hospital discharge is defined as up to 60 minutes prior to discharge.
4. Thirty days (+ 2 days) after the completion of blinded infusion, the subject will return for a 30-Day Post-Infusion Follow-Up visit.

Procedures and requirements for Safety Assessments, Quality Control and Quality Assurance, Ethics, Data Handling and Recordkeeping, Financing, and Insurance and Publication Policy are set forth in protocol MST-188-01 and apply in their entirety to this sub-study.

8. ANALYSIS PLAN

All analyses are exploratory. Descriptive statistics will be provided for the following endpoints and comparisons of MST-188 to placebo:

- Change in StO_2 from baseline at each of the following time points following initiation of blinded study drug infusion: 1 hour, 8 hours, 24 hours, 48 hours, hospital discharge, and 30-day post-infusion follow-up visit.
- Change in StO_2 from baseline as measured by area under the curve (AUC) for change in StO_2 from baseline over 1 hour, 8 hours, 24 hours, 48 hours, and hospital discharge.
- Change in SpO_2 from baseline at each of the following time points following initiation of blinded-study drug infusion: 1 hour, 8 hours, 24 hours, 48 hours, hospital discharge, and 30-day post-infusion follow-up visit.
- Change in SpO_2 from baseline as measured by AUC for change in SpO_2 from baseline over 1 hour, 8 hours, 24 hours, 48 hours, and hospital discharge.

For each endpoint, the treatment arms will be compared using an analysis of covariance (ANCOVA) model with effects for treatment group, age, and baseline StO_2 or SpO_2 as appropriate.

Relationships between StO_2 endpoints, SpO_2 endpoints, and the duration of VOC will be explored using scatterplots and regression analyses. For the regression analyses, the duration of VOC will be the dependent variable. The independent variables will include the StO_2 endpoint and age. These regression analyses will be carried out on the pooled treatment groups and separately within each treatment group.

Additional analyses may be specified prior to unblinding of the study.

9. FORE-SIGHT SYSTEM SPECIFICATIONS

The FORE-SIGHT® Absolute Tissue Oximeter is a noninvasive device that measures precise tissue oxygen saturation. The instrument is FDA approved and bears the CE mark, CE-0086. FORE-SIGHT® is intended for use as a skeletal muscle and cerebral tissue monitoring of absolute hemoglobin oxygen saturation of blood for individuals at risk for reduced blood flow or no-blood flow ischemic states.[7] FORE-SIGHT® operates on the principle that blood contains hemoglobin in two primary forms, oxygenated hemoglobin (HbO_2) and de-oxygenated Hb, which absorb near-infrared light in different, measurable ways. Cerebral tissue oxygen saturation (StO_2) levels are determined by the ratio of oxygenated Hb to total hemoglobin at the microvascular level (arterioles, venues, and capillaries) in the region of the cerebral tissue or skeletal muscle to which the sensor is applied: $\%StO_2$ = Oxygenated Hemoglobin/Total Hemoglobin = $(HbO_2/(HbO_2 + Hb)) \times 100\%$.[7]

The FORE-SIGHT® instrument incorporates CASMED's exclusive technology to project harmless near-infrared light (in multiple, precise wavelengths) into the tissue using disposable sensors. Laser light is projected in four precise wavelengths (<1nm) to capture information needed for an absolute indication of StO_2 levels. Four precise wavelengths are needed to

maximize the measurement accuracy of HbO_2 and de-oxygenated Hb in determining StO_2, to compensate for wavelength-dependent scattering losses and to account for interference from other background light absorbers (such as fluid, tissue, and skin pigmentation). Reflected-light is captured by detectors positioned on the sensor for optimal signal collection and removal of data from skin perfusion. After analyzing the reflected light, the FORE-SIGHT® device displays the tissue oxygen saturation level on the monitor as an absolute number and provides a graphical representation of historical values.

In comparison to pulse oximeters that monitor only arterial blood saturation from peripheral tissue and require pulsatile flow to operate, the FORE-SIGHT® device measures the balance of tissue oxygen supply to tissue oxygen demand. Measurement of StO_2 provides the clinician with an indication of the patient's tissue oxygen saturation status and can assist in uncovering low perfusion conditions and understanding the effectiveness of treatment approaches.

10. REFERENCES

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3. Raj A, Bertolone SJ, Mangold S, et al. Assessment of cerebral tissue oxygenation in patients with sickle cell disease: effect of transfusion therapy. *J Pediatr Hematol Oncol* 2004;26:279-83. [Abstract only].
4. Tavakkoli F, Nahavandi M,Wyche MQ, et al. Effects of hydroxyurea treatment on cerebral oxygenation in adult patients with sickle cell disease: An open-label pilot study. *Clin Ther* 2005;27:1083-8.
5. Raj AB, O'Brien LM, Bertolone SJ, et al. Cerebral oximetry improves detection of sickle cell patients at risk for nocturnal cerebral hypoxia. *Pediatr Pulmonol* 2006;41:1088-94. [Abstract only].
6. Quinn CT & Dowling MM. Cerebral tissue hemoglobin saturation in children with sickle cell disease. *Pediatr Blood Cancer* 2012;59:881-7.
7. FORE-SIGHT® Absolute Tissue Oximeter MC-2000 Series User Manual. CAS Medical Systems, Inc. Revision 19, May 2012.

Appendix E: Complications of Sickle Cell Disease

Pain - Acute (acute chest syndrome, cholecystitis, hand-foot syndrome [dactylitis], acute painful episode, priapism, right upper quadrant syndrome, splenic sequestration, hepatic sequestration) - Chronic (arthritis, arthropathy, aseptic [avascular] necrosis, leg ulcers, vertebral body collapse) - Mixed
Infection and Related Adverse Events - *S. pneumonia* - *H. influenza* - *N. meningitidis* - Viral influenza - Hepatitis - Fever - Sepsis - Acute chest syndrome - Bone pain (with fever)
Transient RC Aplasia - Anemia - Parvovirus B19 Infection
Stroke and CNS Disease - Transient Ischemic Attack - Brain infarction - Intracranial hemorrhage
Sickle Cell Eye Disease - Nonproliferative - Proliferative
Cardiovascular Manifestations (e.g., systolic murmurs, ventricular enlargement)
Pulmonary Complications - Acute chest syndrome - Systemic fat embolization syndrome - Reactive airway disease (asthma) - Pulmonary hypertension
Gall bladder and Liver - Cholelithiasis and biliary sludge - Acute and chronic cholecystitis - Viral and autoimmune hepatitis - Hemosiderosis/hemochromatosis - Hepatic vascular occlusion - Hepatic sequestration - Cholestasis (acute and chronic)
Splenic Sequestration
Renal Abnormalities - Hyposthenuria - Tubule dysfunction - Hematuria - Acute and chronic renal failure
Priapism
Bones and Joints - Bone marrow hyperplasia - Vaso-occlusive events (metaphyseal and diaphyseal infarcts, hand-foot syndrome, osteonecrosis) - Hematogenous infection (osteomyelitis, septic arthritis
Leg Ulcers

Source: National Institutes of Health: National Heart, Lung, and Blood Institute Division of Blood Disease and Resources (NHLBI). *The management of sickle cell disease*. 4th ed. Bethesda, MD: NHLBI; 2002.