Supplemental Online Content

Casella JF, Barton BA, Kanter J, et al. Effect of Poloxamer 188 vs Placebo on Painful Vaso-Occlusive Episodes in Children and Adults With Sickle Cell Disease. *JAMA.* Published online April 20, 2021. doi:10.1001/jama.2021.3414

Supplement 2. Statistical analysis plan

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

Statistical Analysis Plan

Mast Therapeutics, Inc.

Protocol MST-188-01

EVALUATION OF PURIFIED POLOXAMER 188 IN VASO-OCCLUSIVE CRISIS (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 Version: Amendment 6, 20 November 2014

Sponsor: **Mast Therapeutics, Inc.**
3611 Valley Centre Dr., Suite 500
San Diego, California 92130
USA

Prepared by: **Bruce A. Barton, Ph.D.**
Division of Biostatistics and Health Services Research
Department of Quantitative Health Sciences
University of Massachusetts Medical School
368 Plantation Street
Worcester, MA 01605

Version: **03**

Author: Bruce A. Barton 8/17/2016
Bruce A. Barton, Ph.D. DATE
Statistician, University of Massachusetts Medical School

Approver: Ed Parsley, D.O. 08/17/2016
Edwin Parsley, D.O. DATE
Chief Medical Officer and Sr. Vice President, Mast Therapeutics, Inc.

Approver: Mark Longer 08/17/2016
Mark A. Longer, Ph.D. DATE
Vice President, Regulatory Affairs and Quality, Mast Therapeutics, Inc.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS **4**
DEFINITIONS **6**
1. INTRODUCTION **7**
2. STUDY OBJECTIVES **7**
3. STUDY DESIGN AND PLAN **7**
4. DETERMINATION OF SAMPLE SIZE **8**
5. GENERAL ANALYSIS CONSIDERATIONS **9**
6. ANALYSIS POPULATIONS **9**
7. STUDY POPULATION **10**
7.1 Subject Disposition 10
7.2 Protocol Deviations 10
7.3 Demographic and Baseline Characteristics 12
7.4 Prior and Concomitant Medications 12
7.5 Treatment Compliance 13
8. EFFICACY ANALYSES **13**
8.1 Primary and Secondary Efficacy Endpoints 13
8.2 Pharmacodynamic Endpoints 14
8.3 Tissue Oxygenation Sub-study Endpoints 14
8.4 Pharmacokinetic Endpoints 15
8.5 Baseline Values 15
8.6 Adjustments for Covariates 15
8.7 Handling of Dropouts, Missing Data, or Withdrawal of Consent 16
8.8 Interim Analysis and Data Monitoring 17
8.9 Examination of Subgroups 17
8.10 Multiple Comparisons/Multiplicity 18
8.11 Multicenter Studies 18
9. METHODS OF EFFICACY ANALYSIS **19**
9.1 Primary Efficacy Endpoint Analyses 19
9.2 Sensitivity Analyses of the Primary Endpoint 19
9.3 Secondary Endpoint Analyses 21
9.3.1 Re-hospitalization for VOC 21
9.3.2 Occurrence of Acute Chest Syndrome 22
9.4 Pharmacodynamic Endpoint Analyses 22
9.5 Tissue Oxygenation Sub-Study Endpoint Analyses 23
9.6 Opioid Usage Analyses 24
9.7 Pharmacokinetic Analyses 26
10. SAFETY ANALYSES **26**
10.1 Extent of Exposure 26
10.2 Adverse Events 27
10.3 Clinical Laboratory Evaluation 28
10.3.1 Analyses of Laboratory Values with Regard to Efficacy and Safety 29

10.4 VITAL SIGNS 30
10.5 FLUID INTAKE AND OUTPUT 31
10.6 PHYSICAL EXAMINATION 31
11. OTHER EXPLORATORY ANALYSES 31
11.1 URINE BIOMARKERS 31
11.2 ADDITIONAL PARAMETERS 32
12. CHANGES TO PROTOCOL-SPECIFIED ANALYSES 33
13. REFERENCES 33
14. VERSION HISTORY 35

LIST OF ABBREVIATIONS

ACS	acute chest syndrome
AE	adverse event
ANCOVA	analysis of covariance
ANOVA	analysis of variance
ALT/SGPT	alanine aminotransferase
AST/SGOT	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC	area under the curve
BP	blood pressure
bpm	beats per minute
BUN	blood urea nitrogen
Cr	creatinine
CRF	case report form
CSR	clinical study report
CXR	chest X-ray
DSMB	Data and Safety Monitoring Board
EPIC	Evaluation of Purified Poloxamer 188 in Vaso-occlusive Crisis
FACES	Wong-Baker FACES™ Pain Rating Scale
g	gram
GGT	gamma-glutamyl transpeptidase
h	hour
HbSβ^0thal	Hemoglobin beta thalassemia 0 genotype of SCD
HbSβ^+thal	Hemoglobin beta thalassemia + genotype of SCD
HbSC	Hemoglobin SC genotype of SCD
HbSS	Hemoglobin SS genotype of SCD
hgb	Hemoglobin
HPF	high power field
hs-CRP	high sensitivity C-reactive protein
HU	hydroxyurea
IM	intramuscular
IV	intravenous
ITT	Intent-to-Treat
kg	kilogram
KIM	Kidney Injury Molecule-1
LDH	lactate dehydrogenase
LPO	last dose of parenteral opioid
MCHC	mean corpuscular hemoglobin concentration

LIST OF ABBREVIATIONS

MCV	mean corpuscular volume
MedDRA	Medical Dictionary for Regulatory Activities
MEU	morphine equivalent units
mg	milligram
min	minute
mL	milliliter
mm	millimeter
NA	not applicable
NAG	*N*-acetyl-beta-D-glucosaminidase
NGAL	neutrophil gelatinase associated lipocalin
NSAID	nonsteroidal anti-inflammatory drug
PD	pharmacodynamic
PDF	portable document format
PEG	percutaneous endoscopic gastrostomy
PNG	portable network graphics
PO	oral
PP	per-protocol
RBC	red blood cell
RDW	red cell distribution width
RNA	ribonucleic acid
SAE	serious adverse event
SAP	statistical analysis plan
SAS®	Statistical Analysis System (registered trademark)
SC	subcutaneous
SCD	Sickle cell disease
SD	standard deviation
SE	standard error
SOP	Standard Operating Procedure
sPLA2	secretory phospholipase A(2)
TD	transdermal
TEAE	treatment-emergent adverse event
TETRAE	treatment-emergent treatment-related adverse event
VOC	vaso-occlusive crisis
WBC	white blood cell
WHO	World Health Organization

DEFINITIONS

Intent-to-Treat Population	All randomized subjects. The analysis of efficacy outcomes with the ITT population will consider allocation of subjects to treatment groups as randomized.
Per-Protocol Population	All randomized subjects *except* those subjects whose duration of maintenance infusion of blinded study treatment (either placebo or MST-188) was less than 12 hours, those subjects who did not receive a parenteral dose of opioid analgesic after randomization, 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^{0}thal; or those subjects who were 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.
Safety Population	All randomized subjects who received any dose of blinded study treatment (either placebo or MST-188). 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.
Treatment-emergent AE	Adverse event with an onset date after the start of the study drug infusion or an adverse event starting prior to the study drug infusion and reported as possibly, probably or definitely related to treatment.
Treatment-emergent Treatment-related AE	Adverse event judged as possibly, probably, or definitely related to treatment.

1. INTRODUCTION

This document contains a technical and detailed elaboration of the principal features of the analysis described in the Mast Therapeutics, Inc. protocol MST-188-01, entitled "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." This plan includes detailed procedures for executing the statistical analysis of the primary and secondary variables and other data. Any deviations from this plan will be documented in the clinical study report (CSR).

2. STUDY 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 (using date and time as recorded by the randomization system) to the time at which a subject receives the last dose of parenteral opioid (LPO) analgesic for the treatment of VOC prior to hospital discharge (using date and time as recorded in medical records and on the EPIC case report forms [CRFs]).

The secondary objectives are:

- To compare the incidence of re-hospitalization for VOC within 14 days after discharge between the treatment arms. The information for date and cause of re-hospitalization will be taken from the medical records as recorded on the EPIC CRFs.
- To compare the incidence of protocol-defined acute chest syndrome (ACS) within 120 hours of randomization between the treatment arms. The information for date for diagnosis of ACS will be taken from the EPIC CRFs.

3. 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 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 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 infusion will be administered for at least 13 hours (1 hour loading and 12 hours maintenance) up to a maximum total infusion duration of 49 hours (1 hour loading and up to 48 hours maintenance).

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

The Follow-up Phase will consist of post-hospitalization contacts for the monitoring of safety and re-hospitalization due to recurrent VOC. All safety/adverse events occurring within 30 days of discontinuation of blinded study drug infusion will be analyzed and presented in the form of frequency tables and listings. Adverse events occurring within 30 days after initial hospital discharge will be presented in the form of listings in the CSR.

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 (HU; yes or no), and pain score as measured using the Wong–Baker FACES® Pain Rating Scale at the time of randomization (< 8 or ≥ 8).

In previous amendments of the protocol, the allowable age group was ≥ 8 to < 18 years. Stratification was 8-11 and 12-18. Patients who were 8-15 years old in the early stratification will be included in the younger age strata for the main study when strata are included in analysis. Patients who were 16-17 years of age in the early stratification will be included in the older age strata for these analyses.

The study will be monitored by an external independent Data and Safety Monitoring Board (DSMB) which will review of 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.

Subjects completing the 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 will be prepared for the extension study.

4. DETERMINATION OF SAMPLE SIZE

The sample size was based on detecting a difference between treatment groups in the mean time from randomization to the LPO 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% for each group (yielding a standard deviation of 51.84 for the placebo group and 43.2 for the MST-188 group). 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 with unequal variances using PASS 13 sample size software (Hintze, J. (2014). PASS 13. NCSS, LLC. Kaysville, Utah, USA. www.ncss.com). To account for a dropout/missing primary outcome rate of 3% or less, the total maximum sample size will be 388 (194 per arm).

5. GENERAL ANALYSIS CONSIDERATIONS

The statistical analyses will be reported using summary tables, figures, and data listings which will be developed as appropriate to reflect the analyses described in this Statistical Analysis Plan (SAP). Unless otherwise noted, all statistical testing will be two-sided and will be performed at the 0.05 significance level. Continuous variables will be summarized with means, standard deviations, medians, minimums, and maximums. Selected summaries will also include geometric means. Categorical variables will be summarized by counts and by percentage of subjects in corresponding categories. All summary tables will be presented by treatment group. Baseline summaries will also include a total summary column.

Individual subject data obtained from the CRFs, pharmacodynamic data, and any derived data will be presented by subject in data listings. Listings will be sorted by treatment group and subject.

The analyses described in this plan are considered *a priori*, in that they have been defined prior to database lock and prior to breaking the blind. Any analyses performed subsequent to breaking the blind will be considered post-hoc and exploratory. Post-hoc analyses will be labeled as such on the output and identified in the CSR.

All analyses and tabulations will be performed using Statistical Analysis System® (SAS®) Version 9.3 or higher. Tables and listings will be generated in portable document format (PDF) directly from SAS®. Figures will be presented as portable network graphics (PNG) files in Microsoft® Word files. Upon completion, all SAS® programs will be validated by an independent programmer. Standard operating procedures (SOPs) related to SAS® programming, quality control, confidentiality and security, and analysis will be used throughout the execution of this SAP and are listed in Section 13.

6. ANALYSIS POPULATIONS

The following populations will be defined for this study:

- The ITT population will consist of all randomized subjects and will be used for the primary efficacy analyses. The analysis of efficacy outcomes with the ITT population will consider allocation of subjects to treatment groups as randomized.
- 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 per-protocol (PP) population will include all randomized subjects except those subjects whose duration of maintenance infusion of blinded study treatment (either placebo or MST-188) was less than 12 hours, those subjects who did not receive a parenteral dose of opioid analgesic after randomization, those subjects who did not have a diagnosis of HbSS, HbSC, HbSβ^+thal, or HbSβ^0thal, or those subjects who were not ≥ 4 and ≤ 65 years of age at randomization. The analysis of efficacy outcomes with the PP population will consider allocation of subjects to treatment groups as received.
- The pharmacodynamic (PD) population will consist of all randomized subjects who have at least one PD assessment.
- The urine biomarker population will consist of all randomized subjects who have at least one urine biomarker assessment.
- The tissue oxygenation sub-study population will consist of all randomized subjects who have at least one tissue oxygenation assessment.

Patients who withdraw consent will be included in any appropriate population, with data analyzed only to the point of withdrawal of consent to collect or utilize such data. Analytic strategies for these situations are included below.

7. STUDY POPULATION

7.1 Subject Disposition

Subject disposition information will be summarized for all subjects by treatment group. Summaries will include number of subjects screened, number of subjects randomized, number of subjects in each analysis population, primary reason for treatment discontinuation, number of subjects completing all protocol visits and/or contacts, number of subjects remaining on study through hospital discharge, and reason for early termination, including withdrawal of consent by patient (either withdrawal of consent for continued active treatment or for continued active treatment plus any further trial participation, such as safety follow-up or continued data collection).

A listing of subjects excluded from the PP population, including reasons for exclusion, will be provided. A listing of study medication by batch number will also be provided.

7.2 Protocol Deviations

Protocol deviations that could potentially affect the efficacy or safety conclusions of the study, i.e., major protocol deviations, will be identified prior to database lock and unblinding of treatment information.

A listing of all major protocol deviations will be provided and the number of subjects with major protocol deviations will be summarized by treatment group.

Major Protocol Deviations include but are not limited to the following:

Screening Procedures:

- Qualifying screening procedures were not performed including chest X-ray, laboratory testing for platelet count, creatinine, ALT, or pregnancy test, as applicable; and hospitalization for a condition other than VOC.

Inclusion/Exclusion Criteria:

- At time of randomization, age < 4 or > 65.
- Absence of diagnosis of HbSS, HbSβ^0thal, HbSβ^+thal or HbSC.
- Hospitalization for previous episode of VOC within 14 days of study hospital admission.
- Duration of moderate to severe pain of VOC is > 24 hours from the time of presentation to randomization or < 4 hours prior to the time of randomization.
- At time of randomization, acute chest syndrome exists.
- Subject received blood transfusion within 14 days prior to randomization.

Study Drug Administration:

- Duration of maintenance infusion of study drug < 12.0 hours, unless VOC was considered clinically resolved or the study drug was discontinued due to a documented adverse event.
- Stopping rules for administration of study drug not followed, i.e., study drug administration continued even though one of the following occurred:
  - creatinine increased 50% and an absolute increase ≥ 0.3 mg/dL (≥ 27 μmol/L)
  - elevation of SGPT/ALT > 12.0 times institutional upper limit of normal
  - elevation of SGPT/ALT > 8.0 times institutional upper limit of normal **and** direct bilirubin > 2.0 times the subject's baseline value **and** direct bilirubin > 2.0 times institutional upper limit of normal
  - platelet value < 50,000/mm^3

Prohibited Medications/Procedures:

- Use of systemic corticosteroids for VOC or L-glutamine during screening or treatment phase.
- Elective surgery (including the placement of a percutaneous endoscopic gastrostomy [PEG] feeding tube) or administration of other investigational treatments for SCD VOC.
- Transdermal administration of opioids, post randomization.

Efficacy:

- Time of LPO analgesic administration is missing.

7.3 Demographic and Baseline Characteristics

Demographic variables include age at randomization, sex, ethnicity, and race. Other baseline characteristics include height, weight, sickle cell genotype, HU use, FACES® pain score at presentation and randomization, duration of moderate to severe pain at presentation, medical history, prior hospitalizations, and clinic geographic region. Demographic and baseline characteristics will be summarized by treatment group for the Safety, ITT, and PP populations. By-patient listings of demographics, baseline characteristics, and medical history will be provided.

7.4 Prior and Concomitant Medications

Verbatim medication terms on CRFs will be mapped to Anatomical Therapeutic Chemical (ATC) class and Preferred Names using the World Health Organization (WHO) dictionary (WHO-DD).

Concomitant medications are defined as medications that were taken after randomization. This includes medications that were started prior to first dose of study drug and continued after first dose of study drug, as well as those medications that were started after the first dose of study drug. Any medications that were stopped prior to randomization are considered prior medications. If it is uncertain whether a medication is prior or concomitant, due to partial or missing start/end dates, the medication will be considered concomitant.

Concomitant medications excluding opioid analgesics will be summarized by treatment group by WHO ATC class and preferred name. These summaries will present the number and percentage of subjects using each medication. Subjects may have more than one medication per ATC class and preferred name. At each level of subject summarization, a subject is counted once if he/she reported one or more medications at that level. The summary table will be ordered by descending order of incidence of ATC class and preferred name within each ATC class, for all subjects combined.

Other concomitant measures (e.g., fluids, blood products, and other non-pharmaceutical therapies) will be summarized in the same way as for concomitant medications.

Concomitant opioid and nonsteroidal anti-inflammatory drug (NSAID)/non-opioid analgesics will be summarized based on the pre-defined categories, by route (parenteral, oral, or other) from the CRF, including the following:

- Parenteral opioids: Morphine, hydromorphone, nalbuphine, oxycodone, and tramadol, and approved parenteral formulations of permitted oral opioids.
- Disallowed opioids (e.g., Fentanyl) will be summarized separately.
- Parenteral NSAIDS, e.g., Ketorolac.
- Oral opioids, e.g., Codeine, hydrocodone, hydromorphone, morphine, oxycodone.
- Oral non-opioid analgesics: Acetaminophen, aspirin, diclofenac sodium, ibuprofen, naproxen, other NSAIDS.
- Other oral analgesics, e.g., Tramadol.

7.5 Treatment Compliance

Patients randomized to active therapy will receive MST-188 as a loading dose of 100 mg/kg for 1 hour followed by a continuous maintenance infusion dose of 30 mg/kg/hour for at least 12 hours and up to 48 hours. Patients randomized to the placebo arm will receive a 0.45% saline solution delivered at a volume and duration identical to that of active drug to preserve the blind.

A patient will be considered compliant if the patient receives at least 12 hours of maintenance infusion of blinded study treatment (either placebo or MST-188). Compliance will be summarized by treatment group and time interval for the Safety, ITT, and PP populations.

A by-patient listing of treatment compliance will be provided.

8. EFFICACY ANALYSES

The primary efficacy analysis, as well as the analyses of the secondary outcomes, will be based on the ITT population, whereas the same analyses based on the PP population will be run as supportive analyses. If a subject did not receive any parenteral dose of opioid analgesic after randomization, they will be assigned a value of 1 hour for separate reporting during analysis. A separate tabulation by treatment group of those who do not receive any parenteral dose of opioid analgesic after randomization will be included. These patients will be included with this value in the ITT analysis. If a subject did not receive at least 12 hours of maintenance drug infusion, they will be included in the ITT analyses, but not in the PP population.

8.1 Primary and Secondary Efficacy Endpoints

The primary endpoint is defined as the time in hours from randomization until the last administration of parenteral opioid analgesic (LPO) for the treatment of VOC prior to hospital discharge. The null (H_0) and alternative hypotheses (H_1) of the primary efficacy endpoint analysis will be as follows:

- H_0: No difference in the time from randomization to last parenteral opioid analgesic administration between the MST-188-assigned patients and the placebo-assigned patients.
- H_1: There is a difference in the time from randomization to last parenteral opioid analgesic administration between the MST-188-assigned patients and the placebo-assigned patients.

The secondary endpoints are:

- Re-hospitalization for a recurrence of VOC within 14 days of initial discharge from the hospital (yes or no). The null (H_0) and alternative hypotheses (H_1) of this secondary efficacy endpoint analysis will be as follows:
    - H_0: There is no difference in the proportion of patients who are re-hospitalized for a recurrence of VOC within 14 days of initial discharge from the hospital between the MST-188-assigned patients and the placebo-assigned patients.

- H_1: There is a difference in the proportion of patients who are re-hospitalized for a recurrence of VOC within 14 days of initial discharge from the hospital between the MST-188-assigned patients and the placebo-assigned patients.

- Occurrence of ACS within 120 hours of randomization, defined as described by the National Acute Chest Syndrome Study Group, 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 (yes or no). The null (H_0) and alternative hypotheses (H_1) of the secondary efficacy endpoint analysis will be as follows:
  - H_0: There is no difference in the proportion of patients with an occurrence of ACS within 120 hours of randomization between the MST-188-assigned patients and the placebo-assigned patients.
  - H_1: There is a difference in the proportion of patients with an occurrence of ACS within 120 hours of randomization between.

8.2 Pharmacodynamic Endpoints

Blood samples will be collected for assessment of laboratory biomarkers from subjects at selected investigative centers 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).

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

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

8.3 Tissue Oxygenation Sub-study Endpoints

The tissue oxygenation sub-study will be conducted at approximately 10 selected study sites in the United States. Approximately 30 eligible male and female sickle cell subjects are planned for the sub-study.

A noninvasive measurement of tissue oxygen saturation (StO_2) in the cerebral tissue of all sub-study subjects and skeletal muscle of study subjects who meet the age and body mass requirements of the measuring device will be used as a measure of microvascular perfusion. Peripheral oxygen saturation (SpO_2) of hemoglobin (hgb) will also be measured using conventional pulse oximetry.

The Fore-Sight® MC-2000 (Casmed Medical Systems, Inc., Branford, CT, USA) was utilized for the EPIC study to record StO_2 measurements. One minute (or up to three minutes, depending on stability of measurements at a particular time point) of data will be utilized for each measurement time point.

StO_2 and SpO_2 measurements will be collected at the following time points:

- Baseline (pre-treatment).
- 1 hour (± 30 minutes) after the initiation of the infusion of blinded study treatment (either placebo or MST-188).
- 8 hours (± 30 minutes) after the initiation of the infusion of blinded study treatment.
- 24 hours (± 30 minutes) after the initiation of the infusion of blinded study treatment.
- 48 hours (± 30 minutes) after the initiation of the infusion of blinded study treatment, or at the conclusion of the infusion 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 the infusion of blinded study treatment, the subject will return for a 30-Day Post-Infusion Follow-Up visit).

8.4 Pharmacokinetic Endpoints

Plasma samples will be collected for pharmacokinetic assessments at the following nominal time points:

- Baseline (prior to the start of the study drug infusion)
- End of 1 hour loading dose infusion (± 15 minutes)
- End of the continuous maintenance infusion (± 30 minutes)
- 6 hours after end of the continuous maintenance infusion (± 2 hours)
- 12 hours after end of the continuous maintenance infusion (± 2 hours)

Pharmacokinetic analyses will be described in a separate population pharmacokinetic data analysis plan.

8.5 Baseline Values

Unless otherwise specified, the baseline value for a study endpoint is the last non-missing value recorded prior to the start of study drug infusion.

8.6 Adjustments for Covariates

The primary efficacy analysis will include adjustments for the following covariates/stratification factors: age group (< 16 years or ≥ 16 years), use of HU (yes or no), and pain score as measured using the Wong–Baker FACES® Pain Rating Scale at the time of presentation and at the time of randomization (< 8 or ≥ 8). Other covariates of interest include: region (US or non-US), gender, and hemoglobin genotype (HbSS/other).

8.7 Handling of Dropouts, Missing Data, or Withdrawal of Consent

It is anticipated that there will be little or no missing or incomplete data for the primary endpoint since all data are collected during the patient's hospitalization. Thus, the primary analysis will be based on the observed time to LPO analgesic for the treatment of VOC for each subject. If a subject did not receive any parenteral dose of opioid analgesic after randomization, they will be assigned a value of 1 hour. A separate tabulation of these patients by treatment group will be presented. Because the natural log (ln) of time to LPO analgesic will be used in analysis of the primary outcome, these patients will be included in the ITT analysis with a time of log(1) = 0. The PP analysis will provide a sensitivity analysis of whether these patients (with a log time of 0) have an impact on the results. Patients who were randomized but did not receive at least 12 hours of study medication will be handled as indicated above under Study Populations.

In the event that some subjects have censored data, i.e. the LPO for the treatment of VOC recorded may not be the true last administration of an opioid, a sensitivity analysis will be performed using regression methods for right censored data (e.g., Cox regression models). For this analysis, subjects with incomplete follow-up will be censored at the time of their last observed parenteral opioid analgesic for the treatment of VOC.

Additional sensitivity analyses using multiple imputation techniques will also be used to replace both missing outcome data and any missing covariate data (as described in Section 8.6). SAS® PROC MI will be utilized to generate five sets of fully imputed data, after which SAS® PROC MIANALYZE will be used to conduct the same analysis as above for the main analysis of the primary outcome, stratified by imputation data set, with the results of the stratified analysis combined (through PROC MIANALYZE) to produce a single set of results. Two types of multiple imputations will be used: (1) a standard multiple imputation with missing data for subjects modeled from the observed data in that subject's treatment group; and (2) an "imputation under the null" with missing data for all subjects modeled from the observed data in the placebo group. If the results differ between the two methods, additional analyses will be conducted to better explicate the reasons, beyond treatment affect.

Prior to performing the multiple imputation, the determination will be made as to whether the missingness is: (1) completely at random (MCAR), (2) at random (MAR), or (3) not at random (i.e., informative missingness – MNAR). For the primary outcome, analyses will be generated using a logistic regression model (SAS® PROC LOGISTIC) with missingness (yes/no) as the outcome and baseline covariates and treatment group as predictors. If none of these factors are significantly related to missingness, the conclusion will be that the data are at least MAR. For the purpose of multiple imputation of the missing data, data have to be at least MAR for the imputation to be unbiased.

If at least one predictor is found in either logistic model that is related to missingness, the data may be missing not at random. Further analyses will be conducted to understand the "informativeness" of the missing data so that appropriate adjustments can be made. Because every case of MNAR is different, there is no one standard approach to handling the problem. All analyses and decisions made related to this investigation will be documented in the CSR.

The main analysis of the primary outcome will be compared to the sensitivity analyses with regard to treatment effect (i.e., the regression coefficient, s.e., and t-statistic/p-value from SAS® PROC GLM) to determine consistency of effect under the different approaches. If the differences do not change the interpretation of the outcome in terms of significance, then no further analyses will be done. If the different approaches do not give a consistent interpretation of the outcome, then additional analyses will be conducted to determine which patient(s) contributed to that change through either the censoring or the imputation. This will be done by generating the PRESS (predicted sum of squares) statistic for each patient for which either censoring or imputation was done. If a patient shows a larger PRESS statistic than others, investigation into why that patient is different will be undertaken. The results of this investigation will be documented in the CSR for this study. This same approach will be taken for the two secondary outcomes when a patient is missing complete information for those outcomes.

For outcomes that are outliers (i.e., a primary outcome that is more than 3 standard deviations higher than the mean outcome and has been validated as real by independent medical review by three blinded members of the steering committee (i.e., not a recording error)), two approaches will be taken for including those values in the main analysis of the primary outcome. First, a winsorization of the outlier value to the value that is the 3 standard deviation value will be used in the analysis to determine the change in the treatment effect. Second, outliers will be weighted according to their distance beyond the 3 standard deviation point so that: 3-3.9 standard deviations = 0.75 weight, 4-4.9 standard deviations = 0.50 weight, and 5.0 and beyond standard deviations = 0.25 weight. The results from these two analyses will be compared to the main analysis of the primary outcome for consistency and handled as detailed above for the missing data sensitivity analyses. The problem of outliers does not exist for the secondary outcomes, since those are yes/no outcomes.

8.8 Interim Analysis and Data Monitoring

An independent DSMB will meet and review safety data at least annually. There are no interim efficacy analyses planned for this study. Thus, there is no adjustment to the critical p-value for the primary efficacy outcome due to alpha spending as required by interim monitoring. The DSMB will have a separate charter guiding its operations.

8.9 Examination of Subgroups

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

- Age group (< 16 years or ≥ 16 years) as determined by age at randomization
- Gender (male or female)
- Use of HU (yes or no), the subjects considered to be on HU for this subgroup (HU=Yes) are those who were on HU at least 14 days prior to randomization and remained on HU until at least the day before Randomization
- 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 (≤ 12 hours or > 12 hours) using the date and time of moderate to severe VOC onset and the date and time of presentation
- Region/country (U.S., non-U.S.) as determined by EPIC study site
- HbSS versus other hemoglobin genotypes
- Age < 16 with HU usage at a U.S. EPIC study site (MST-188/Placebo)
- Age < 16 with HU usage at a non-U.S. EPIC study site

All data used to determine the subgroups will come from source verified screening data rather than data from the randomization system.

The analytic approach for these subgroups will be as follows. In the analysis of covariance (ANCOVA) model used for the main analysis of the primary outcome, these factors will be added along with terms for interaction with treatment. If the specific factor by interaction regression coefficient is significantly different from 0 at $p \leq 0.05$, then a descriptive analysis of the treatment outcome within the different components of the subgroup will be conducted and presented.

The treatment effect of the primary efficacy endpoint within subgroups will be displayed as the difference in the geometric means with the 95% confidence interval as well as the p-value from the ANCOVA model run within the subgroup. As a visual approach to presenting all of this information, a forest plot will be constructed to present the treatment effects within subgroups with 95% confidence intervals and symbol size varying by size of the subgroup (Alosh et al., 2015).

A similar approach will be taken for examination of safety within subgroups. For overall incidence of TEAEs by organ class and preferred term, as well as other major areas of safety concern, such as hepatotoxicity or renal toxicity, the difference in proportion of patients with TEAEs will be displayed in tabular form with the 95% confidence interval as well as in a forest plot to help visualize all of the information.

8.10 Multiple Comparisons/Multiplicity

The sequential testing approach discussed in Section 9.3 will be performed to control for the overall false positive rate at 0.05.

8.11 Multicenter Studies

Approximately 70 centers are expected to participate in the study, resulting in an average of fewer than 10 subjects per center. Summaries by country and by center within a country are planned. Region will be included in the main analyses of the primary and secondary efficacy outcomes.

9. METHODS OF EFFICACY ANALYSIS

9.1 Primary Efficacy Endpoint Analyses

The values for the primary endpoint, hours from randomization to last administration of any parenteral opioid analgesic for VOC, in the two treatment groups will be compared using an ANCOVA model with effects for the pre-specified stratification groups: (1) treatment group (MST-188 or placebo); (2) age group (< 16 years or ≥ 16 years); (3) use of HU (yes or no); and (4) pain score as measured using the Wong–Baker FACES® Pain Rating Scale at the time of randomization (< 8 or ≥ 8). The duration of VOC is the outcome for this model, with duration calculated per the algorithm below. Because data collection for the primary efficacy outcome will be in the hospital over a few days of time, we anticipate minimal missing data for this endpoint. For the patients who did not receive any parenteral opioid analgesics (and, thus, have not endpoint), a value of one hour will be imputed as their time. Because the primary endpoint is response time data, we will use a natural log transformation, which will be applied to the individual times prior to conducting this analysis. (Wagenmakers and Brown, 2007) For patients who did not receive any parenteral opioid analgesics, the imputed duration of 1.0 becomes a duration of 0.0 on the log scale. The least squares means and the least squares mean difference will be calculated on the log scale and transformed back to the original scale using the exponential function to obtain the geometric means and the geometric mean ratio. The reported p-value will be the p-value from the two-sided t-test of the least squares means for the two treatment groups from the above model.

Algorithm: The duration of VOC will be calculated as follows:

$$\text{Duration} = (\text{date:time}_{ld} - \text{date:time}_{r})/3600$$

Where date:time_{ld} is the date and time (in SAS® date:time format) of the LPO analgesic and date:time_{r} is the date and time (in SAS® date:time format) of randomization.

The SAS® date:time value is generated from SAS® Function Datetime, which returns the number of seconds from January 1, 1960 to the specified date:time. To convert the difference in seconds to the difference in hours, the difference is divided by 60 × 60 = 3600 to give a number of hours with a decimal representation of the number of minutes. As an example, a randomization date:time of September 1, 2015 at 8:15 AM and a date:time of LPO analgesic of September 7, 2015 at 10:25 AM yields a difference of 526200 seconds, or a duration of 146.17 hours.

9.2 Sensitivity Analyses of the Primary Endpoint

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 (or an alternative to guard against the inefficiency of the van Elteren test if the treatment effect varies across strata), stratified by age group (< 16 years or ≥ 16 years), use of HU (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. If the results of this sensitivity analysis are not consistent with those from the main analysis of the primary outcome, the

following assumptions for the ANCOVA model will be investigated: (1) linear relationship between predictors and outcome; (2) normal distribution of data/residuals; (3) no multicollinearity; (4) independent observations (no auto-correlation); and (5) homoscedasticity (i.e., constant variance of the residuals across the range of predictions). In this study, all observations are independent and log transformation for the outcome will create a normally distributed outcome variable. The other assumptions will be tested graphically for deviations from these assumptions. Any fixes for these will depend on the type and severity of the deviation, but will follow established procedures in the statistical literature and will be documented in the CSR.

Additional sensitivity analyses will include: (1) including/excluding patients with major protocol deviations in the main analysis; (2) including/excluding patients with extreme values for the primary endpoint (i.e., outliers); and (3) including/excluding patients with censored primary outcome data. As with all of the other sensitivity analyses, the goal is to determine if any of these analyses changes the interpretation of the main study results related to treatment effect. If so, then additional analyses will be conducted to determine the underlying cause(s) of that change and to document the results of these additional analyses as potential explanatory factors in the final interpretation of the study results. These analyses will be documented in the CSR.

As noted in Section 8.7, it is anticipated that there will be little or no missing or incomplete data for the primary endpoint. In the event that some subjects have censored data, a sensitivity analysis will be performed using regression methods for right censored data. 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. The SAS® PHREG procedure (i.e., Cox regression analysis) will be used for the analysis which will include the same covariates as in the main analysis ANCOVA model. Model parameters will be estimated using maximum likelihood with the regression coefficient, hazard ratio, and p-value for the treatment group term in the model reported as the predictor of interest in this analysis.

A Kaplan-Meier analysis of the primary endpoint will also be provided using SAS® PROC LIFETEST. The Kaplan-Meier estimates will be tabulated and graphed by treatment group. The log rank test, stratified by age group (< 16 years or ≥ 16 years), use of HU (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.

The results from these analyses will be compared for consistency in interpretation of the treatment effect. If there are substantial differences in the treatment effect, additional investigation into differential censoring patterns between the two treatment groups will be conducted to determine if the frequency and time-relatedness of the censoring differs between the two treatment groups and the factors related to the censoring. The results of these analyses will be documented and included in the CSR.

9.3 Secondary Endpoint Analyses

To control the overall false positive rate at 0.05, a sequential testing approach will be used for the secondary endpoints. The primary efficacy endpoint analysis must be significant at the 0.05 level in order to declare significance for any secondary efficacy endpoint. If the primary endpoint efficacy analysis is significant at the 0.05 level, then the secondary efficacy 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 (i.e., $p \leq 0.05$), 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 for the purpose of claims.

9.3.1 Re-hospitalization for VOC

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 by treatment group. The denominator for the percentages will be the number of subjects with at least one follow-up visit/contact at least 14 days after the initial hospital discharge. In situations where subjects were re-hospitalized at the point of their 14 day follow-up, the data from their re-hospitalization will be used to qualify the subject as part of the denominator. Furthermore, if a subject does not have 14 days of follow-up data, but they were re-hospitalized for VOC prior to the 14 day mark, they will be counted as part of the denominator as their hospitalization is counted as part of the numerator.

Algorithm: To calculate this outcome, the same approach for date:time differences as for duration of VOC [Section 9.1] will be used.). If the difference between the date:time of original hospital discharge and 12:00 PM on the date of hospital readmission is less than 337.0 hours (14 days × 24 hours) and the primary cause of readmission is VOC, then the patient is counted as readmitted for the purposes of this outcome. The use of 12:00 PM on the date of hospital readmission is necessitated because the time of readmission was not captured on study CRFs.

SAS® PROC FREQ will be used to analyze the proportion of patients in each treatment group who are identified with this outcome. Depending on the number of events for this outcome, the likelihood ratio chi-square test or, if five events or less are observed, Fisher's exact test will be used to compare proportions between the two treatment groups. The difference in proportions between the treatment groups and a 95% confidence interval will also be provided. A logistic model (SAS® PROC LOGISTIC) will be constructed to analyze these data with predictive factors: (1) treatment group (MST-188 or placebo); (2) age group (< 16 years or ≥ 16 years); (3) use of hydroxyurea (yes or no); and (4) pain score as measured using the Wong–Baker FACES® Pain Rating Scale at the time of randomization (< 8 or ≥ 8). The reported statistic and p-value from this model will be that of the coefficient for treatment group. As a sensitivity analysis, we will conduct a time to event analysis with time from hospital discharge to re-hospitalization as the outcome. A Kaplan-Meier approach to generate survival curves for each

treatment group with a log-rank test for differences with special interest in the comparison of event rates at 14 days and at 30 days post discharge using SAS® PROC LIFETEST will be performed. In addition, a Cox regression analysis of the same time to event with predictors of interest as for the logistic model above, using SAS® using SAS® PROC PHREG will be conducted. If the number of events is sufficient, investigation of appropriate interactions with treatment to identify any subgroups that may have a significantly higher (or lower) time to re-hospitalization will be performed.

9.3.2 Occurrence of Acute Chest Syndrome

The number and percent of subjects with an occurrence of ACS will be tabulated by treatment group. ACS will be defined as described by the National Acute Chest Syndrome Study Group, 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.

Algorithm: To calculate this outcome, the same approach for date:time differences as above for duration of VOC above [Section 9.1] will be used. If the difference between the date:time of randomization and 12:00 PM (+12hrs) on the date of the ACS onset as reported by AE/SAE records is less than 121.0 hours, then the patient is counted as developing ACS for the purposes of this outcome. The window of hours was included because the time from the acquisition of the chest x-ray to the recording of the resulting diagnosis on the AE/SAE record was not instantaneous, with 12 hours thought to be a reasonable time frame for the delay in recording on the AE/SAE record. The choice of 12:00 PM was necessitated as the time of the diagnosis of the AE/SAE was not captured on the study CRFs.

ACS occurring within 120 hours of randomization will be tabulated by treatment group. The analysis will be the same as for re-hospitalization above for the analysis of proportions of patients in each treatment group with ACS at 120 hours post-randomization and at 30 days post-infusion both overall and adjusting for the same predictive factors as above. The survival analysis will also be the same as for the re-hospitalization outcome above (Section 9.3.1) with special interest in the comparison of event rates at 120 hours post-randomization and at 30 days post-infusions.

9.4 Pharmacodynamic Endpoint Analyses

Pharmacodynamic parameters will be summarized by time point and treatment group. The geometric mean and the geometric mean ratio (comparing 48 hours to Baseline) will be included in the summary statistics, due to the unknown nature of the distributions for each. The Wilcoxon rank sum test (SAS® PROC NPAR1WAY) will be used to compare the change from baseline values between the two treatment groups. The rank sum test will be performed two ways: using the change from baseline in the untransformed values and using the change from baseline in the log transformed values. We will also test for difference in response at 48 hours based on treatment and baseline values (categorized into quartiles) using Friedman's non-parametric two-way analysis of variance (ANOVA) by converting 48-hour values to ranks (SAS® PROC

RANK) and then using the ranks in a standard ANOVA (using SAS ®PROC GLM for flexibility in modeling).

9.5 Tissue Oxygenation Sub-Study Endpoint Analyses

As previously described (Section 8.3), StO_2 in the cerebral tissue and skeletal muscle is captured for 1 minute (or more) at each of the time points. The average value from each measurement site for each subject at each time point will be used for all analyses.

The relationship between SpO_2 values and StO_2 values will be assessed using scatterplots and Pearson/Spearman correlation coefficients (SAS® PROC CORR). All time points will be included separately in these summaries. In addition, Bland-Altman plots (both identity and agreement) will be generated to illustrate the agreement between SpO_2 and StO_2.

The StO_2 values (cerebral [right and left] and skeletal [forearm and calf]) and the SpO_2 values will be summarized by time point and treatment group. Longitudinal mixed effects models (SAS® PROC MIXED) will be fit to the data with all time points included in the model (baseline level as the reference for later values at 1, 8, 24, 48 hours, hospital discharge, and 30-day follow-up) to assess the change over time and the effect of covariates (including treatment) on that change. Patients without a true baseline (pre-study drug treatment) cannot be included in the descriptive change or area under the curve (AUC) analyses, but will be included in the longitudinal model at each time point where they have data.

The AUC for change from baseline in each tissue oxygenation parameter for each tested site will be calculated for each subject and each cumulative time period up to hospital discharge:

- Baseline to 1 hour
- Baseline to 8 hours
- Baseline to 24 hours
- Baseline to 48 hours/end of infusion
- Baseline to hospital discharge
- Baseline to Day 30

The AUC will be calculated using the trapezoidal rule, with baseline defined as time 0, and time since baseline used as the time axis. The calculation will use the technique from Pruessner (2003) - Equations 1 & 2 as the basis for the SAS program. The AUC values will be normalized by the length of the time interval, giving a weighted average change from baseline value for each time interval. The actual assessment date/times (as opposed to nominal times) will be used in the AUC calculations. For each AUC parameter the treatment arms will be compared using an ANCOVA model with effects for treatment group, age, and baseline oxygenation value.

The relationship between the primary endpoint (number of hours from randomization to LPO analgesic for the treatment of VOC) and the oxygenation parameters will be explored using scatterplots (SAS® PROC SGPLOT) and linear regression analyses (SAS® PROC GLM). Scatterplots of the primary endpoint versus StO_2 (cerebral and skeletal) and SpO_2 will be provided for each time point. Similar scatterplots will be provided based on the AUC parameters

for each time interval. Linear regression analysis will be performed with time to LPO analgesic as the dependent variable, and cerebral StO_2, skeletal StO_2, SpO_2, and age as the independent variables. Separate regressions will be carried out for each time point where the oxygenation parameters are assessed. Similar regression analyses will be provided based on the AUC parameters for each time interval. These regression analyses will be carried out separately for each treatment group and for both treatment groups combined in one model with treatment group as a predictor.

Secondary analyses will include time to achieving a 25% increase in StO_2 (cerebral and skeletal) and SpO_2 using interval censored Cox regression models with SAS® PROC ICPHREG since StO_2 and SpO_2 are measured only as specific time points, so that continuous measures of these are not available.

9.6 Opioid Usage Analyses

The total opioid usage by any route will be calculated for each subject from the time of randomization to hospital discharge in 24-hour intervals. Each dose of opioid medication will be converted to morphine equivalents (in milligram) and these morphine equivalent doses will be summed for each subject per kilogram body weight. In the event the subject's principal investigator does not document the time for last VOC-related parenteral opioid dose for those subjects who continue to receive parenteral opioids for conditions *unrelated* to the VOC episode, three blinded members of the study Steering Committee will adjudicate when to set the time for the LPO analgesic for the treatment of VOC for that subject.

A comparison of opioid usage between treatment arms will be performed, after calculation of morphine equivalent units (MEU) per kg body weight. The calculation is as follows:

$$\text{MEU/kg calculation} = [\text{Dose in mg}\} \times [\text{MEU factor}] \div Wt\ in\ kg$$

The conversion factors utilized to calculated morphine equivalents will be based upon the following conversion factors:

Medication[1]	**Route**[2]	**MEU factor**
Buprenorphine	TD	0.1
Codeine	IM	0.1
Codeine (including Co-dafalgan and Solpadeine)	PO	0.05
Codeine	SC	0.1
Hydrocodone	PO	0.4
Hydromorphone	PO	2
Hydromorphone	IM	5
Hydromorphone	IV	5
Meperidine (Pethidine)	IM	0.1
Meperidine (Pethidine)	IV	0.1
Meperidine	PO	0.05
Morphine	IM	1

Morphine	IV	1
Morphine	SC	1
Morphine	PO	0.4
Nalbuphine	IV	1
Nalbuphine	IM	1
Nalbuphine	SC	1
Oxycodone	PO	0.63
Oxycodone	SC	1.5
Oxycodone	IV	1.5
Tramadol	PO	0.05
Tramadol	SC	0.1
Tramadol	IV	0.1
Tramadol	IM	0.1
Fentanyl	PO	0.05
Fentanyl	IV/IM	0.1
Fentanyl	SC	0.1
Fentanyl	TD	0.1
Fentanyl	IN	0.1
Butorphanol	IV	0.2
Methadone	PO	0.5
Oxymorphone	PO	1.0

1. Opioids recorded as combination products will be recorded and analyzed based upon the opioid component with the same MEU factor as a single-component opioid.
2. Abbreviations: IM, Intramuscular; IV, Intravenous; PO, Oral; SC, Subcutaneous; TD, Transdermal; IN, Intranasal

The following comparisons between treatment arms will be performed, adjusted for age (< 16, ≥ 16), sex, HU usage (yes/no), pain score at randomization (< 8, ≥ 8), region (US/non-US), and hemoglobin genotype (HbSS/other) in a mixed effects model, except as noted:

- MEU/kg calculated from time of randomization to first 12 hours, every 24 hours and from randomization to the earlier of time of decision to discharge or actual discharge. In situations where the decision to discharge is missing, the actual discharge will be used.
- MEU/kg calculated from start of loading dose to first 12 hours, every 24 hours and from start of loading dose to the earlier of time of decision to discharge or actual discharge. In situations where the decision to discharge is missing, the actual discharge will be used.
- MEU/kg for parenteral opioids only will be calculated from time of randomization to first 12 hours, every 24 hours, from randomization to LPO and from randomization to the earlier of time of decision to discharge or actual discharge. In situations where the decision to discharge is missing, the actual discharge will be used.
- MEU/kg for parenteral opioids only will be calculated from start of loading dose to first 12 hours, every 24 hours, from start of loading dose to LPO, and from start of loading dose to the earlier of time of decision to discharge or actual discharge. In situations where the decision to discharge is missing, the actual discharge will be used.

- MEU/kg for parenteral opioids administered via PCA vs. other will be calculated from randomization to LPO and from start of loading dose to LPO.
- Compare the total MEU/kg from randomization to actual hospital discharge.
- Compare the oral MEU/kg and between geographic regions and treatment arms.
- Compare the parenteral MEU/kg from randomization to actual hospital discharge.
- Total MEU/kg throughout hospitalization
- Compare the MEU/kg from start of study drug in 1st 24-hour period, and 24-hour periods through 48 hours.
- Compare total MEU/kg from start of loading dose to LPO.
- Subgroup comparisons of patients ages < 16 vs ≥ 16, by HU use versus none, by study region, and by hemoglobinopathy type will be performed for the above analyses as warranted (i.e., if sufficient sample sizes by different subgroups is found).
- Analyses of above comparisons by route of administration will be conducted as warranted (i.e., if sufficient sample sizes by different routes is found).

References:

Opioid Morphine Equivalent Conversion Factors retrieved from: https://www.cms.gov/Medicare/Prescription-Drug-Coverage/PrescriptionDrugCovContra/Downloads/Opioid-Morphine-EQ-Conversion-Factors-March-2015.pdf

Instructions for Morphine Equivalent Daily Dose (MEDD) retrieved from: http://palliative.org/NewPC/_pdfs/tools/INSTRUCTIONsMEDD.pdf

Total opioid usage in morphine equivalents will be summarized by treatment group and the Wilcoxon rank sum test will be used to compare the two treatment groups.

9.7 Pharmacokinetic Analyses

Pharmacokinetic analyses will be described in a separate population pharmacokinetic data analysis plan.

10. SAFETY ANALYSES

All safety analyses will be based on the Safety population.

10.1 Extent of Exposure

Summaries of exposure to study drug by treatment group will include duration of infusion (hours), volume infused (mL), weight adjusted amount of MST-188 infused (mg/kg), total amount of MST-188 infused (mg), and number of interruptions. Separate summaries will be provided for the loading dose infusion and the maintenance dose infusions. The duration of infusion will be calculated two ways, including and excluding any interruptions.

The number of infusion interruptions will be summarized using the number and percent of subjects in each of the following categories: 0, 1, > 1.

Subjects where duration of infusion or interruption cannot be calculated due to missing date and or time fields will be dropped from these analyses. When duration of interruption is given as a time range, the maximum time of the range will be used for calculations.

10.2 Adverse Events

All adverse event summaries will be restricted to treatment-emergent adverse events (TEAEs), which are defined as those adverse events (AEs) that occurred after the start of infusion and those existing AEs that worsened during the study or AEs which began prior to the start of infusion but are reported as possibly, probably or definitely related to treatment. If it cannot be determined whether the AE is treatment emergent due to a partial onset date or time, then it will be counted as treatment emergent. For an individual subject multiple AE records will be considered to be a single AE occurrence if the preferred terms are the same and the stop date of one AE is the same as the start date of the subsequent AE. Verbatim terms on case report forms will be mapped to preferred terms and system organ classes using the Medical Dictionary for Regulatory Activities (MedDRA; version 14.1 or higher).

The incidence of TEAEs will be summarized by treatment group along with the difference between treatment groups and 95% confidence interval around the difference. TEAEs will be analyzed with respect to incidence within each randomized treatment group as well as by severity, seriousness, potential relationship of the AEs to study medication. Incidence of TEAEs will be summarized by SOC and PT, and presented in descending order of incidence within each SOC, for all subjects combined. Subjects experiencing a fatal TEAE, a treatment-emergent SAE (hereafter referred to as SAE, except where noted), a TEAE leading to randomized study medication discontinuation, or a TEAE leading to study withdrawal will also be summarized by treatment group.

Summaries of the following types will include but not be limited to:

- Subject incidence of TEAEs and total number of unique TEAEs by MedDRA system organ class and preferred term.
- Subject incidence of treatment-emergent treatment-related adverse events (TETRAEs) and total number of unique TETRAEs by MedDRA system organ class and preferred term. Treatment related AEs are considered to be those reported as possibly, probably or definitely related to treatment. AEs with a missing relationship will be considered related for this summary.
- Subject incidence of TEAEs by MedDRA system organ class, preferred term, and highest severity. At each level of subject summarization a subject is classified according to the highest severity if the subject reported one or more events.
- Subject incidence of TETRAEs by MedDRA system organ class, preferred term, and highest severity. At each level of subject summarization a subject is classified according to the highest severity if the subject reported one or more events.

- Subject incidence of TEAEs by MedDRA system organ class, preferred term, and relationship to study drug ("Not Related", considered not related, or unlikely related, versus "Related" which includes: Possibly, Probably, or Definitely related). An AE assessed as "Related" at any time during the subject's study participation will be assigned to the "Related" category. AEs with a missing relationship will be considered related for this summary.
- Subject incidence of serious TEAEs and total number of unique serious TEAEs by MedDRA system organ class and preferred term.
- Subject incidence of serious TETRAEs and total number of unique serious TETRAEs by MedDRA system organ class and preferred term.

All adverse events, including events that are not treatment emergent, will be included in a data listing. Separate listings will be provided for serious adverse events (SAEs), AEs leading to interruption of study drug, AEs leading to permanent discontinuation of study drug, and AEs leading to study withdrawal.

10.3 Clinical Laboratory Evaluation

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).

Laboratory results will be graded according to NCI-CTCAE, Version 4.03. Laboratory results not corresponding to an NCI-CTCAE term will not be graded.

Since some variation in the timing of the assessments is expected, a windowing procedure will be applied to assign values to time points. The number of hours from the start of infusion to each blood sample during the initial hospitalization will be computed (rounded to the nearest hour), and the samples will be assigned to study days based on the following windows:

Nominal Time Point Relative to Start of Infusion	Time Window (hours)
Day 2 (24 hours)	> 0 to 36
Day 3 (48 hours/End of Infusion)	> 36 to 60
Day 4 (72 hours)	> 60 to 84
Day 5 (96 hours)	> 84 to 108
Day 6 (120 hours)	> 108 to 132
Day 7 (144 hours)	> 132 to 156
Etc.	

If more than one value occurs in the same time window, the value closest to the nominal time point will be selected for analysis; for the 48 hour/End of Infusion time point, the value closest to the end of infusion will be selected. If there are two values that are equidistant from the nominal

time point, the latter value will be selected. Values after Day 7 of hospitalization will not be summarized, but all values will be included in the data listings.

Laboratory parameters will be summarized by treatment group using descriptive statistics at baseline and at each post-baseline time point (with windows for binning times as indicated in the table above). Changes from baseline will also be summarized. Baseline is defined as the last non-missing value prior to the start of study drug infusion. In addition to descriptive statistics, box-and-whisker plots will be generated for each parameter to illustrate the distribution of the parameter over time.

Shift tables (i.e., low-normal-high at baseline versus low-normal-high at follow-up in a 3-by-3 contingency table) will be provided for each hematology and chemistry parameter. Shift tables based on NCI-CTCAE grades will be provided for those parameters corresponding to an NCI-CTCAE term.

The clinical laboratory tests include the following:

Hematology profile:

- Hemogram: white blood cell (WBC), red blood cell (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.

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).

Urinalysis:

- Routine dipstick measurements (specific gravity, pH, bilirubin, protein, blood) and microscopic analysis (WBC/high power field [HPF], RBC/HPF, Bacteria, Casts, Crystals) were required.

10.3.1 Analyses of Laboratory Values with Regard to Efficacy and Safety

In addition to the above analyses of absolute and relative values of each laboratory measure, the following analyses will be undertaken to determine the relationship of laboratory values (both absolute and relative, i.e., change from baseline) with efficacy and safety outcomes within and between treatment groups. Results of these analyses will be reviewed in order to determine whether their interpretation and presentation will be included in the CSR.

- Association/no association of the occurrence of abnormal labs or longitudinal trends of laboratory values (either absolute or relative change) with occurrence and grade of AEs.

- Association/no association of the occurrence of abnormal labs or longitudinal trends of laboratory values (either absolute or relative change) with primary and secondary efficacy outcomes.
- Longitudinal change of urine biomarkers (either absolute or relative change) with occurrence and grade of AEs and with primary and secondary efficacy outcomes.
- Longitudinal change of pharmacodynamic quantitative measures (i.e., high-sensitivity C-reactive protein [hs-CRP], secretory phospholipase A(2) [sPLA2], d-dimer) with primary and secondary outcomes.
- Changes in proteomics and gene expression outcomes within and between treatment groups over time.
- Compare LDH (longitudinal changes and AUC over hospitalization) and alkaline phosphatase between the two treatment groups and correlate with primary and secondary efficacy outcomes.
- Analysis of the above questions by age (age < 16 years and ≥ 16 years) will be conducted as warranted (i.e., sufficient sample size with available information).

10.4 Vital Signs

Body weight is recorded daily during hospitalization and will be summarized using descriptive statistics at baseline, Day 1 to Day 7 of hospitalization, and at the 30-day post-infusion follow-up visit. Changes from baseline will also be summarized. Values recorded during hospitalization after Day 7 will not be summarized, but all values will be included in the data listings.

Temperature, pulse rate, respiration rate, oxygen saturation (SpO_2-pulse oximetry), and blood pressure, are assessed 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 are assessed every 8 hours (± 30 minutes) until discharge.

Vital signs (blood pressure, pulse rate and oximetry, respiratory rate, temperature, and body weight) will be summarized using descriptive statistics and frequency tabulations by treatment group. Changes from baseline for each of the vital signs variables will be summarized by treatment group.

Potentially clinically significant results will be determined using the following definitions:

Parameter	Absolute Criteria	Relative Criteria: Change Relative to Baseline
Systolic BP	< 80 mmHg	Decrease of ≥ 20 mmHg
Systolic BP	> 180 mmHg	Increase of ≥ 20 mmHg
Diastolic BP	< 40 mmHg	Decrease of ≥ 15 mmHg
Diastolic BP	> 105 mmHg	Increase of ≥ 15 mmHg
Pulse Rate	< 50 bpm	Decrease of ≥ 20 bpm
Pulse Rate	> 150 bpm	Increase of ≥ 20 bpm
Respiratory Rate	< 10 breaths/min	Decrease of ≥ 10 breaths/min
Respiratory Rate	> 36 breaths/min	Increase of ≥ 10 breaths/min
Temperature	> 39.5ºC	Increase ≥ 1°C
Body Weight	NA	Decrease ≥ 10%
Body Weight	NA	Increase ≥ 10%
Pulse Oximetry	< 88%	NA

Baseline is defined as the last non-missing value prior to the start of study drug infusion.

For each of the above definitions, the number and percent of subjects (stratified by age < 16 years of age and ≥ 16 years of age) having at least one potentially clinically significant result after the start of study drug infusion through end of blinded maintenance infusion will be tabulated by treatment group. Separate tabulations will be provided for the absolute criterion alone, the relative criterion (change relative to baseline) alone, and the combination of the two. For the combination, the two conditions must be met at the same assessment time.

10.5 Fluid Intake and Output

Fluid intake and output is monitored from baseline until discharge and recorded in 24-hour intervals. For each time interval, the fluid intake, fluid output, and fluid balance (intake – output) will be summarized by treatment group.

10.6 Physical Examination

Physical examination results will be presented in a data listing. No summaries are planned.

11. OTHER EXPLORATORY ANALYSES

11.1 Urine Biomarkers

Random urine samples (approximately 10 mL) will be collected from subjects at selected investigative centers 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. Results for assays of the urine samples will be judged to be analyzable if they are within established limits of quantification.

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)

Analyzable urine biomarkers will be summarized by time point and treatment group. The geometric mean and the geometric mean ratio (comparing each post-baseline time point to Baseline) will be included in the summary statistics. The Wilcoxon rank sum test will be used to compare the change from baseline values between the two treatment groups. The rank sum test will be performed two ways: using the change from baseline in the untransformed values and using the change from baseline in the log transformed values.

11.2 Additional Parameters

Results from analyses of each of the following exploratory parameters by treatment group (unless otherwise indicated) will be reviewed in order to determine whether their interpretation and presentation will be included in the CSR.

- Duration time in hours from start of loading dose until the administration of the LPO for the treatment of VOC prior to hospital discharge.
- Re-hospitalization for a recurrence of VOC within 72 hours of initial discharge from the hospital.
- Correlation of SpLA2 level with occurrence of ACS.
- Duration of hospitalization for those with occurrence of ACS compared to those without ACS.
- Analysis of the occurrence of ACS for those whose Screening/baseline CXR was assessed as “normal” or abnormal but limited to those with cardiac silhouette abnormalities.
- Compare the proportion of subjects receiving their LPO within 0-48 hours, 0-72 hours, 0-96 hours, 0-120 hours, 0-144 hours, and 0-168 hours, combined and by HU usage at baseline.
- Compare the proportion of subjects less than age 16 years receiving their LPO within 0-48 hours, 0-72 hours, 0-96 hours, 0-120 hours, 0-144 hours, and 0-168 hours.
- Compare the duration of hospitalization from the time of randomization to the earlier of time of decision to discharge or actual time of discharge.
- Compare duration of hospitalization from the time of randomization to the earlier of time of decision to discharge or actual time of discharge in children less than age 16.
- Compare the duration of hospitalization from time of study drug loading dose start to the earlier of time of decision to discharge, or actual time of discharge, and the actual time of discharge.

- Compare duration of hospitalization from time of study drug loading dose start to the earlier of time of decision to discharge or actual time of discharge in children less than age 16.
- Compare duration of crisis as time from randomization to LPO in patients on HU versus without HU.
- Duration of time in hours from randomization to LPO for those on continuous concomitant naloxone drips compared to those without naloxone or with naloxone given on only an as needed (p.r.n.) basis.
- Duration time in hours from randomization to LPO for those with a Wong Baker FACES® score greater than or less than 8 at the time of presentation.
- Duration time in hours from start of loading dose to LPO for those with a Wong Baker FACES® score greater than or less than 8 at the time of presentation.
- Duration time in hours from randomization to LPO for subjects receiving the full 49 hours of test article infusion (1 hour loading dose and 48 hour maintenance infusion.
- Duration time in hours from start of loading dose to LPO for subjects receiving the full 49 hours of test article infusion (1 hour loading dose and 48 hour maintenance infusion).
- Duration of hospitalization from time of randomization to actual hospital discharge both overall and by region.
- Duration time in hours from randomization to time of decision to discharge both overall and by region.
- Duration of time in hours from randomization to LPO for subjects with reported time of onset of moderate to severe pain to start of loading dose less than or equal to 24 hours, greater than 24 hours but less than or equal to 48 hours, and those greater than 48 hours.
- Duration of time in hours from start of loading dose to LPO for subjects with reported time of onset of moderate to severe pain to start of loading dose less than or equal to 24 hours, greater than 24 hours but less than or equal to 48 hours, and those greater than 48 hours.
- Comparison of mean values of sPLA2 at each time point: baseline and 48 hours.
- Duration of ACS episode in hours as defined by reported date of onset and date of recovery from SAE reports.
- Proportion of subjects receiving transfusion of one or more units of packed red blood cells.
- Actual volumes of transfused blood products.

12. CHANGES TO PROTOCOL-SPECIFIED ANALYSES

No changes to the protocol specified analyses are planned.

13. REFERENCES

ICH Guideline E3, *Structure and Content of Clinical Study Reports* (July 1996, 1995)
ICH Guideline E9, *Statistical Principles for Clinical Trials* (September 1998)

QHS SOP SAS Coding Conventions, Revision 1, January, 2013
QHS SOP SAS Program Testing, Revision 0, January, 2013
QHS SOP SAS Program Risk Assessment, Revision 0, January, 2013
QHS SOP Data Management, Revision 0, June, 2013

Alosh M, Kritsch K, Huque M, et al. Statistical considerations on subgroup analysis in clinical trials. *Stat Biopharm Res*. 2015;7(4):286-303.

Duke SP, Jiang Q, Huang L, Banach M, Cherny M. Safety Graphics. In: Jiang Q, Xia HA, eds. *Quantitative Evaluation of Safety in Drug Development*. Boca Raton, FL: Chapman and Hall; 2015:196-222.

Pruessner JC, Kirschbaum C, Meinlschmid G, Hellhammer DH. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. *Psychoneuroendocrinology*. 2003;28(7):916-931.

Van Zandt T. How to fit a response time distribution. *Psychon Bull Rev*. 2000;7(3):424-465.

Wagenmakers E-J, Brown S. On the linear relation between the mean and the standard deviation of a response time distribution. *Psychol Rev*. 2007;114(3):830-841.

14. VERSION HISTORY

Version No.	Effective Date	Changes	Version Replaced
01	13 Nov 2015	Initial Version	New
02	13 Apr 2016	Major changes to provide more explicit information related to analyses and data handling: • Section 8.1 – statement of hypotheses to be tested for primary and secondary outcomes • Section 8.7 – full description of handling of missing data in analysis • Section 9.1 – full description of main analysis of primary outcome • Section 9.2 – full description of sensitivity analysis of primary outcome • Section 9.3 – full description of analyses of secondary outcomes, including the order of testing and the sensitivity analyses of these outcomes • Section 9.4 – full description of analysis of pharmacodynamic outcomes • Section 9.5 – full description of analysis of oxygenation sub-study data • Section 9.6 – full description of analysis of opioid data, including specific research questions of interest • Section 10.2 – full description of analysis of adverse events, including research questions of interest • Section 10.3 – full description of analysis of laboratory values, including research questions of interest • Section 10.4 – full description of analysis of vital signs, including updated table of clinically significant changes • Section 11.1 – full description of analysis of urine biomarkers • Section 11.2 – list of other research questions of interest • Section 13 – list of references, including relevant SOPs	01
03	17 Aug 2016	• List of Abbreviations – added “PDF” and deleted “RTF” • Definitions – revised definitions of “Treatment-emergent AE” and “Treatment-emergent Treatment-related AE” • Section 5 – revised to reflect that SAS output is in PDF format, rather than RTF format • Section 7.1 – revised language to clarify that study medication will be provided by batch number, rather than by subject and batch number	02

		• Section 7.2 – revised language to clarify protocol deviations due to study drug administration duration of maintenance infusion • Section 8.9 – ▪ added details regarding analyses of subgroups: – age group determined by age at randomization – use of HU (yes or no) clarified as subject who had been on hydroxyurea for at least 14 days prior to randomization – duration of moderate to severe pain at presentation clarified to describe that date and time of VOC onset and date and time of presentation will be used to determine duration – region/country clarified as determined by EPIC study site – added subgroup, "Age <16 with HU usage at non-U.S. EPIC study site" ▪ specified that all data used to determine subgroups will come from the SDV screening event data (rather than IWRS) • Section 9.1 – added language to clarify that effects are based on pre-specified stratification groups • Section 9.3.1 – ▪ revised language regarding the denominator for the percentages (subjects with at least 14 days of follow-up data or a re-hospitalization for VOC prior to the 14 day post-discharge time point) ▪ revised to reflect hospital readmission time set to 12pm on the date of the hospital readmission • Section 9.3.2 – revised to reflect the date of ACS onset set to 12pm (+12hrs) on the date of the reported ACS SAE/AE • Section 9.6 – ▪ revised to include additional MEU factors ▪ changed formatting for readability; changed table notes to footnotes ▪ added language to clarify data handling when date and time of decision to discharge is missing • Section 10.1 – added language on handling missing times for study drug infusion calculations • Section 10.2 – ▪ revised language regarding the definition of treatment-emergent AEs ▪ added language regarding the programming of continuation events ▪ revised language in the summary list to be consistent with new definition of treatment emergent; re-ordered summary list to be consistent with analytical presentation.	

Signature Certificate

Document Reference: 4Z8C4MJACJMUINDPFTCDLF

RightSignature
Easy Online Document Signing

Mark Longer
Party ID: PW97WYIDB42ZZPB8UNHP75
IP Address: 64.73.231.66
VERIFIED EMAIL: mlonger@mastthera.com

Electronic Signature:

Mark Longer

Multi-Factor
Digital Fingerprint Checksum
322425d84193878989793cdfc4b2e2ddcea6a78a

Ed Parsley
Party ID: 5CRUKGI7M44V4TV9SUGNTT
IP Address: 64.73.231.66
VERIFIED EMAIL: eparsley@mastthera.com

Electronic Signature:

Ed Parsley, D.O.

Multi-Factor
Digital Fingerprint Checksum
d7e29c22039cd187fb31ff0a792e538e85fecf3c

Timestamp	Audit
2016-08-17 16:31:08 -0700	All parties have signed document. Signed copies sent to: Wim DHaeze, Mark Longer, and Ed Parsley.
2016-08-17 16:31:08 -0700	Document signed by Mark Longer (mlonger@mastthera.com) with drawn signature. - 64.73.231.66
2016-08-17 16:31:08 -0700	Mark Longer verified the document passcode. - 64.73.231.66
2016-08-17 16:30:30 -0700	Document viewed by Mark Longer (mlonger@mastthera.com). - 64.73.231.66
2016-08-17 16:29:20 -0700	Document signed by Ed Parsley (eparsley@mastthera.com) with drawn signature. - 64.73.231.66
2016-08-17 16:29:19 -0700	Ed Parsley verified the document passcode. - 64.73.231.66
2016-08-17 16:28:36 -0700	Document viewed by Ed Parsley (eparsley@mastthera.com). - 64.73.231.66
2016-08-17 16:02:01 -0700	Document created by Wim DHaeze (wdhaeze@mastthera.com). - 64.73.231.66

This signature page provides a record of the online activity executing this contract.