GlycoMimetics, Inc.

A PHASE 2 RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED STUDY OF THE EFFICACY, SAFETY, AND PHARMACOKINETICS OF GMI-1070, A PAN-SELECTIN INHIBITOR, IN SUBJECTS HOSPITALIZED FOR SICKLE CELL VASO-OCCLUSIVE CRISIS

PROTOCOL NUMBER:	GMI-1070-201
PROTOCOL VERSION AND DATE:	Version 5.0 dated 16 January 2012
INCORPORATING AMENDMENTS:	Amendments 1, 2, 3, and 4
PREVIOUS PROTOCOL VERSIONS:	Version 1.0 dated 4 November 2009 Version 2.0 dated 14 June 2010 incorporating Amendment 1 Version 3.0 dated 17 December 2010 incorporating Amendment 2 Version 4.0 dated 19 May 2011 incorporating Amendment 3
DRUG:	GMI-1070 Injection, 30 mg/mL
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For Contract Research Organization (CRO) and other contact information, see Study Reference Manual.

SPONSOR'S APPROVAL

PROTOCOL NUMBER: GMI-1070-201

PROTOCOL TITLE: A Phase 2 Randomized, Double-Blind, Placebo-Controlled Study of the Efficacy, Safety, and Pharmacokinetics of GMI-1070, a Pan-Selectin Inhibitor, in Subjects Hospitalized for Sickle Cell Vaso-Occlusive Crisis

PROTOCOL VERSION: Version 5.0 dated 16 January 2012

This protocol has been approved by GlycoMimetics, Inc. (GMI).

SPONSOR'S REPRESENTATIVE:

Vice President, Clinical Development
GlycoMimetics, Inc.

INVESTIGATOR'S AGREEMENT

I have read and reviewed this protocol (GMI-1070-201), and I agree to conduct this trial according to this protocol, to comply with its requirements subject to ethical and safety considerations, and to conduct the trial in accordance with all applicable regulations and International Conference on Harmonization Guidelines on Good Clinical Practice (ICH E6[1]). I further agree to comply with all other applicable federal, state, and local laws and regulations in connection with my conduct of this trial, including, without limitation, all applicable medical information privacy rule requirements.

I understand that the Sponsor may decide to suspend or terminate the study at any time for whatever reason; such a decision will be communicated to me in writing. Conversely, should I decide to withdraw from execution of the study I will communicate my intention immediately in writing to the Sponsor.

(Signature) **Date**

(Print name)

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ABBREVIATIONS AND DEFINITIONS

Abbreviation	Definition
AE	Adverse event
ABG	Arterial blood gases
ALT	Alanine transaminase (SGPT)
ANCOVA	Analysis of covariance
AST	Aspartate transaminase (SGOT)
AUC	Area under the curve
CHF	Congestive heart failure
CFR	Code of Federal Regulations
CL	Clearance
CL_r	Renal clearance
C_{max}	Maximum concentration
C_{min}	Minimum concentration
CNS	Central nervous system
Conc	Concentration
CRF	Case report form
CRO	Contract research organization
CRP	C-reactive protein
CSF	Cerebral spinal fluid
CVA	Costo-vertebral angle
DRAE	Disease related adverse event
DSMB	Data Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMI-1070	GMI-1070 Injection, 30 mg/mL
Hb	Hemoglobin
Hb F	Fetal hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
hsCRP	High sensitivity C-reactive protein
ICAM	Intracellular adhesion molecules
ICH	International Conference on Harmonization
ICU	Intensive Care Unit
IEC	Independent Ethics Committee
IRB	Institutional Review Board
ITT	Intent to treat
IV	Intravenous

Abbreviation	Definition
IVM	Intravital microscopy
IVRS	Interactive voice response system
LDH	Lactate dehydrogenase
NSAID	Nonsteroidal anti-inflammatory drug
pRBC	Packed Red Blood Cells
PBS	Phosphate buffered saline
PCA	Patient-controlled analgesia
PD	Pharmacodynamics
PFT	Pulmonary function test
PIPEDA	Personal Information Protection and Electronic Documents Act
PK	Pharmacokinetics
PMA	Platelet-monocyte aggregates
RBC	Red blood cells
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SCD	Sickle cell disease
SCD-Sβ^+-thal	Sickle cell disease Hb β^+-thalassemia
SCD-Sβ^0-thal	Sickle cell disease Hb β^0-thalassemia
SCD-SC	Sickle cell disease Hb C
SCD-SS	Sickle cell disease Hb S
SGOT	Aspartate transaminase (AST)
SGPT	Alanine transaminase (ALT)
$t_{½}$	Apparent terminal half-life
TEAE	Treatment-emergent adverse event
t_{max}	Time to maximum concentration
ULN	Upper limit of normal
US	United States
VAS	Visual analog scale
VCAM	Vascular cell adhesion molecules
VOC	Vaso-occlusive crisis
V_z	Apparent volume of distribution at the terminal phase
WBC	White blood cell
WHO	World Health Organization
WNL	Within normal limits

STUDY SYNOPSIS

Protocol Number: GMI-1070-201	**Study Drug:** Intravenous GMI-1070

Title of the Study:
A Phase 2 randomized, double-blind, placebo-controlled study of the efficacy, safety, and pharmacokinetics of GMI-1070, a pan-selectin inhibitor, in subjects hospitalized for sickle cell vaso-occlusive crisis

Clinical Phase: 2

Sites: Approximately 20 in the United States and Canada

Objectives:

Primary Objective:

- Evaluate the effect of multiple intravenous (IV) doses of GMI-1070 at two different dose levels on time to resolution of vaso-occlusive crisis (VOC) in subjects 12 to 60 years of age hospitalized for sickle cell VOC

Secondary Objectives:

- Evaluate the effect of multiple IV doses of GMI-1070 at two different dose levels on clinical activity in subjects 12 to 60 years of age hospitalized for sickle cell VOC
- Evaluate the safety of multiple IV doses of GMI-1070 at two different dose levels in subjects 12 to 60 years of age hospitalized for sickle cell VOC
- Evaluate the pharmacokinetics (PK) of multiple IV doses of GMI-1070 at two different dose levels in subjects 12 to 60 years of age hospitalized for sickle cell VOC
- Evaluate biomarkers of adhesion and inflammation at two different dose levels in subjects 12 to 60 years of age hospitalized for sickle cell VOC

Methodology:

Study Design

This is a randomized, double-blind, placebo-controlled trial of multiple IV doses of two different dose levels of GMI-1070 or placebo in subjects hospitalized for sickle cell VOC. The original protocol called for administration of GMI-1070 as a loading dose of 20 mg/kg followed by 10 mg/kg every 12 ± 2 hours. Based on the PK profile observed in an interim PK analysis of the first 10 subjects and as allowed in section 8.2.5, the dose level was modified mid-study (in protocol version 4.0) to administer GMI-1070 as a loading dose of 40 mg/kg followed by 20 mg/kg every 12 ± 2 hours.

Investigators are encouraged to identify subjects with VOC who are likely to be admitted, and approach them for consent and eligibility criteria early in the process of treatment for VOC. All subjects should be dosed with study drug within 24 hours of initial medical evaluation in the Emergency Department/clinic for VOC (defined as time of first medical evaluation excluding triage).

In addition, investigators are encouraged to identify subjects who may be appropriate for the study before they develop VOC. These subjects may be approached with educational

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information about the study. Such education will not replace the need to obtain informed consent (and assent where applicable) at the time of hospitalization for VOC.

The study will enroll until approximately 76 evaluable subjects (defined as those who have received at least 1 dose of study medication) have been enrolled. During Visit 1, eligible subjects will be randomized in a 1:1 ratio to either GMI-1070 or placebo using an interactive voice response system (IVRS). Study drug will be administered as a loading dose of 40 mg/kg followed by 20 mg/kg every 12 ± 2 hours until the subject meets criteria for resolution of VOC, or up to 7 days of treatment (maximum 15 doses including the loading dose), whichever comes first. If the subject has no improvement in VOC after 5 days (eg, no change in visual analog scale [VAS] and no weaning of IV pain meds), study drug should be discontinued.

Resolution of VOC is defined as the first of the following to occur:

- Sustained decrease in pain score of at least 1.5 cm out of 10 cm since baseline; AND transition to oral pain medications per hospital procedures (subject is on no IV pain medications),
- OR readiness for discharge as stated by the physician and subject,
- OR discharge to home.

For the purpose of this study, 'sustained' is defined as 2 consecutive pain scores at least 4 hours apart as reported by the subject on a VAS; 'baseline' is defined as the pain score just prior to the first dose of study drug as reported by the subject on a VAS.

Visits for follow-up examinations will be made 36 ± 12 hours post last dose (Visit 2) (ideally this will occur prior to discharge from the hospital), on day 7 ± 3 post last dose (Visit 3), and on day 28 ± 5 post last dose (Visit 4).

Pharmacokinetics Sampling

Plasma sampling for PK will be done during the study. A total of 9 samples will be drawn from each subject. Each sample will be 3 mL. Urine sampling for PK will also take place, in a single 12-hour urine collection.

Optional Site-specific Biomarkers

In a subset of subjects at sites capable of performing these tests, intravital microscopy (IVM) testing for microvascular blood flow and additional sampling for other biomarkers of adhesion and inflammation will be performed.

Number of Subjects:

The study plans to enroll until approximately 76 evaluable subjects (defined as those who have received at least 1 dose of study medication) have been enrolled.

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Diagnosis and Main Criteria for Admission:

Inclusion Criteria:

To be eligible for inclusion, each subject must fulfill each of the following criteria at screening, and must continue to fulfill these criteria prior to dosing:

1. 12 to 60 years of age
2. Confirmed diagnosis of sickle cell disease (HbSS or HbS-β^0thalassemia)
3. Diagnosis of VOC at the time of enrollment
4. Hospitalized or in process of admission at the time of enrollment
5. Able to receive the first dose of study drug within 24 hours of initial medical evaluation in the Emergency Department/clinic for VOC;
   - Subjects treated as an outpatient within the past 48 hours for the same VOC episode may be enrolled if dosing is also expected within 24 hours of their second (admitting) presentation.
6. Documented and observed written informed consent (and assent, where applicable)

Exclusion Criteria:

1. Infection, diagnosed or strongly suspected, as evidenced by one or more of the following:
   - Fever >39° C (102.2° F)
   - In the presence of fever ≥38.5° C (101.3° F), 1 of the following:
     - Positive findings (suspicious for infection) on diagnostic tests, such as cerebral spinal fluid [CSF] evaluation, radiographs, or bacterial culture of normally sterile sites
     - Exam findings leading to diagnosed or strongly suspected bone or joint infection
     - Determination by physician that bacterial or serious systemic viral infection is likely (eg, influenza, mononucleosis)
     - Subjects may be included with uncomplicated urinary tract infections (provided they do not have fever ≥38.5° C [101.3° F] or costo-vertebral angle [CVA] tenderness), and/or suspected minor viral syndromes (upper respiratory infection symptoms but no symptoms suggestive of bacterial infection other than uncomplicated otitis media or, uncomplicated streptococcal pharyngitis)
2. Acute chest syndrome, diagnosed or strongly suspected, as evidenced by a new infiltrate on chest radiograph, and 1 or more of the following:
   - Fever >39° C (102.2° F)
   - Hypoxia (confirmed by arterial blood gases [ABG] with paO_2 <70 mmHg)
   - Chest pain
   - Suspicious findings on exam (tachypnea, intercostal retractions, wheezing, and/or rales)
3. Sickle cell disease (SCD) pain atypical of VOC, including hepatic or splenic sequestration, cholecystitis, or pneumonia.
4. Acute stroke, acute priapism, severe avascular necrosis of the hip/shoulder when the presenting pain is only in the affected hip/shoulder

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5. Serum creatinine:
   - >1.2 mg/dL for subjects 16 to 60 years of age
   - >1.0 mg/dL for subjects 12 to 15 years of age
6. Alanine transaminase (ALT/SGPT) >2x upper limit of normal (ULN) (based on clinic laboratory normal range)
7. Hemoglobin <5 g/dL
8. Platelets <100,000/mm^3
9. Recent (within the past 30 days) major surgery, hospitalization for other than VOC, documented serious bacterial infection requiring antibiotic treatment, or significant bleeding
10. Hospitalization for uncomplicated VOC, or treated with parenteral pain medications in other medical settings such as the emergency department or day hospital for uncomplicated VOC, within past 14 days;
    - Subjects may be included if treated as an outpatient within the past 48 hours for the same VOC episode.
11. Recent (within the past 90 days) cerebrovascular accident, transient ischemic attack, or seizure
12. pRBC transfusions in the past 14 days
13. Systemic steroid therapy within 48 hours prior to enrollment or expectation that therapy may be used during the study (inhaled or topical steroids are allowed)
14. For those on chronic or long-acting opioids, a change in dose in the past 14 days OR pain requiring medical attention in the past 14 days (change in opioid medication for acute pain in the past 48 hours and directly related to this VOC admission is allowed)
15. Greater than 5 episodes of hospitalization for VOC in the past 6 months (180 days)
16. Medical or psychiatric condition that, in the opinion of the investigator, may pose a risk to the subject for participation or interfere with the conduct or results of the study
17. Currently receiving, or has received within the previous 4 weeks, any other investigational agent
18. Previous administration of GMI-1070
19. Expectation that the subject will not be able to be followed for the duration of the study
20. Pregnant or lactating female; or female of childbearing potential or male unable or unwilling to comply with birth control methods or abstinence during the course of the study
21. Active use of illicit drugs and/or alcohol dependence, as determined by the investigator

Study Drug, Dose, and Mode of Administration:

Study drug will be supplied in vials containing 8.3 mL of sterile solution for IV administration. Active vials will contain GMI-1070 at 30 mg per mL; placebo vials will contain 8.3 mL of sterile vehicle (phosphate-buffered saline pH 6.5 [PBS]).

Active drug will be labeled "GMI-1070 Injection, 30 mg/mL," and will be administered as a loading dose of 40 mg/kg followed by a dose of 20 mg/kg every 12 ± 2 hours for up to 7 days

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(maximum of 15 doses including the loading dose). Placebo will be labeled "PBS Placebo, 8.3 mL/vial," and will be administered as a loading volume equivalent to that of active drug, followed by a maintenance volume equivalent to that of active drug every 12 ± 2 hours for up to 7 days (maximum of 15 doses including the loading dose).	

Duration of Treatment:

- Planned duration of treatment period: up to 7 days (maximum 15 doses including the loading dose)
- Planned duration of enrollment and follow-up: up to 35 days including dosing and follow-up (28 days post last dose)

Criteria for Evaluation:

Endpoints for Primary Objective:

- The primary objective, resolution of VOC, will be defined as:
  - Sustained decrease in pain score of at least 1.5 cm out of 10 cm since baseline; AND transition to oral pain medications per hospital procedures (subject is on no IV pain medications),
  - OR readiness for discharge as stated by the physician and subject,
  - OR discharge to home.

Endpoints for Secondary Objectives:

Clinical Activity

- Pain management and medications
  - Amount and duration of IV analgesics
- Complications of disease
  - Rate of VOC complications
- Biomarkers of adhesion and inflammation
  - Microvascular blood flow using IVM
  - Markers of adhesion
  - Markers of inflammation
  - hsCRP
- Healthcare and resource utilization
  - Time to readiness for discharge (per subject and physician)
  - Rate of transfer to Intensive Care Unit (ICU)
  - Diagnosis leading to transfer to ICU
  - Duration of hospital length of stay
  - Oxygen use
  - IV antibiotic use
  - Number and type of transfusions

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Safety

- Adverse events (AEs) and local tolerability
- Dose-limiting toxicity
- Disease related adverse events (DRAEs)
- Physical examinations
- Vital signs
- Clinical laboratory testing: complete blood count with differential, platelets, reticulocyte count, alanine transaminase (ALT, SGPT), aspartate aminotransferase (AST, SGOT), bilirubin (fractionated) , blood urea nitrogen, creatinine, electrolytes with glucose, lactate dehydrogenase (LDH), and urinalysis

PK in Plasma and Urine

- Parameters as compared to data from previous human studies

Statistical Methods:

Data will be summarized to evaluate the difference between treatment groups.

Primary Objective

Efficacy:

- Time to resolution of VOC will be evaluated by testing the null hypothesis of no difference between treatment groups using the F-test from analysis of covariance model, controlling for sex, age and hemoglobin electrophoresis.

Secondary Objectives

Clinical Activity:

- Intensity of VOC, IV opioid and IV nonsteroidal anti-inflammatory drug use, complications of VOC, other health and resource utilization outcomes will be summarized to evaluate the difference between treatment groups using appropriate statistical methods.
- Descriptive statistics will be used to characterize changes from baseline in biomarker test results (IVM, other markers) in subjects for whom these data are available.

Safety:

- Incidence, severity, and relationship to study medication of all treatment-emergent adverse events (TEAEs), including DRAEs, will be tabulated by treatment group
- Descriptive statistics will be used to characterize changes from baseline in laboratory test results

Pharmacokinetics:

- PK parameters including, but not limited to maximum concentration (C_{max}), time to

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maximum concentration (t_{max}), and area under the curve (AUC) will be calculated

- Urinary excretion of GMI-1070 will be calculated

An independent Data Safety Monitoring Board (DSMB), consisting of hematologists/sickle cell experts, and other appropriate members, will be convened. An interim safety review will be performed by the DSMB and Sponsor Medical Monitor after treatment of the first 10 subjects under 18 years, and additionally after 25 and 50 subjects of all ages have been treated with the study drug. This review will include AEs and any other pertinent information. The DSMB and the Sponsor's Medical Monitor will review select data listings to ascertain the existence of any safety concerns for which the study should be terminated, as described in Section 6.3.6.4 Dose Limiting Toxicities and Study Stopping Rules. The Sponsor Medical Monitor will remain blinded.

PK data from the first 10 subjects receiving a loading dose of 20 mg/kg followed by 10 mg/kg every 12 ± 2 hours have been analyzed; and resulted in amendment to the protocol with a change in dose level as allowed in protocol section 8.2.5.

PK data from the first 10 subjects receiving the new dose level, a loading dose of 40 mg/kg followed by 20 mg/kg every 12 ± 2 hours, will be similarly evaluated in a blinded, aggregate analysis in order to ensure that anticipated GMI-1070 plasma concentration levels are achieved with the new dose level. This information will be utilized to confirm or adjust dose, if necessary, for the remainder of the study. In addition, the first 10 subjects under 18 years of age will be similarly evaluated in a blinded aggregate analysis (regardless of dose level).

The sample size is also expected to provide initial safety and PK conclusions sufficient to contribute to the design of subsequent trials.

Table 1. Schedule of Assessments

Visit	Visit 1: During Hospitalization [1,2]				Post Last Dose		
		IV dosing Every 12±2 Hours up to Day 7			Visit 2	Visit 3	Visit 4
	Pre-dose Baseline	Loading Dose	At Least q4 Hours (while awake)	Daily Assessments	36±12 hrs	7±3 Days	28±5 Days (or early withdrawal)
Informed consent[3]	✔						
Inclusion/exclusion criteria	✔						
Randomization[4]	✔						
Demographics	✔						
Medical history	✔						
Physical examination	✔ [5]			✔ [6]	✔ [6]	✔ [6]	✔ [5]
Height, weight	✔ [7]					✔ [8]	
Vital signs[9]	✔			✔	✔	✔	✔
Clinical laboratories[10]	✔			✔	✔	✔	✔
Urinalysis	✔			✔ [11]	✔	✔	✔
Pregnancy test (serum or urine)	✔					✔	✔
VAS pain scale	✔		✔		✔	✔	✔
AEs	✔	✔	✔	✔	✔	✔	✔
Concomitant medication	✔	✔	✔	✔	✔	✔	✔

[1] All subjects should be dosed with study drug within 24 hours of presentation to the Emergency Department/clinic at the latest. Dosing will continue q12 hours until drug is discontinued, for a maximum of 15 doses including the loading dose. Assessments will continue until subject is discharged from the hospital or Day 28, whichever comes first.

[2] Subjects who remain hospitalized after Day 7 will undergo Visit 1 assessments on each additional day of hospitalization up to Day 28, with VAS scores daily instead of q4 hours on Days 13 to 28. Visits 2, 3, 4 should be completed at the assigned times whether the subject is discharged from the hospital or remains hospitalized.

[3] Informed consent (and assent, where applicable) must be obtained from all subjects.

[4] All eligibility criteria must be met prior to randomization.

[5] Full physical examination.

[6] Targeted physical examination.

[7] May be performed within 7 days prior to baseline.

[8] Weight only.

[9] Vital signs should be done prior to loading dose, and post-dose for all doses. Oxygen levels may be included as appropriate.

[10] Complete blood count with differential, platelets, reticulocyte count, high sensitivity C-reactive protein, ALT, AST, bilirubin (fractionated), blood urea nitrogen, creatinine, electrolytes with glucose, LDH. Hb electrophoresis sample to be collected at Pre-dose Baseline only; where possible may be performed on CBC sample. Results of this test are not required for study entry.

[11] Performed every other day.

1 BACKGROUND INFORMATION

This is the fourth trial for the clinical development of intravenous (IV) GMI-1070. The trial will be conducted in compliance with the protocol, the International Conference on Harmonization (ICH) Guideline on Good Clinical Practice (ICH E6[1]; GCP), and applicable local and federal regulatory requirements. This study drug is being developed for the treatment of VOC in SCD. The subject population will comprise hospitalized subjects 12 to 60 years of age who are experiencing VOC.

GMI-1070 is a pan-selectin antagonist, a compound found to inhibit selectin binding *in vitro* and to inhibit selectin-mediated effects *in vivo*. Selectin binding is a key early step in the inflammatory process leading to leukocyte adhesion and recruitment to inflamed tissue. Selectin binding has been shown to be involved in many disease processes that involve inflammation. There are no other known approved therapeutic agents in this class.

1.1 Sickle Cell Disease

SCD is one of the most prevalent genetic disorders in the United States (US), affecting over 80,000 people.[2] It is a chronic condition with substantial morbidity and mortality, and is responsible for more than 75,000 hospitalizations per year in the US with an average stay of 6.1 days.[3] Both children and adults are affected, and greater mortality is seen in those with more severe disease. The gene is found most commonly in people of African or Arab-Indian descent, and the disease is seen most commonly in these regions or in areas to which these populations have migrated, including North America and Europe.

SCD refers to a group of autosomal recessive inherited disorders of the β-globin gene. A single nucleotide substitution results in the presence of valine instead of glutamic acid in the β-globin chain. The resulting polymerization of hemoglobin S (HbS) when deoxygenated is the primary indispensable event in the molecular pathogenesis of SCD.[4] Individuals homozygous for HbS have sickle cell anemia (SCD-SS). Those who are compound (double) heterozygotes have 1 copy of Hb S and 1 copy of a hemoglobin that interacts with Hb S such as Hb C (SCD-SC), β^+-thalassemia (SCD-Sβ^+-thal), or β^0-thalassemia (SCD-Sβ^0-thal).[5]

1.2 Vascular Occlusion in the Pathogenesis of Sickle Cell Complications

SCD is associated with a number of serious and potentially disabling conditions that have similar symptoms but vary in severity by genotype. The most notable complication of SCD is VOC, an extremely painful and serious consequence of SCD presumably resulting from acute ischemic tissue injury. Individuals with the SCD-SS and SCD-Sβ^0-thal genotypes tend to experience similar disease severity, which is more severe than for other genotypes. SCD-SC and SCD-Sβ^+-thal are associated with a milder form of sickle cell disease.[4,5,6]

Over the course of a year, about 60% of patients with SCD-SS will have at least 1 severe VOC, which typically presents as episodes of pain and inflammation at 1 or more sites, of varying degrees of severity, and occurring at varying intervals throughout life. These pain crises, as VOC episodes are also known, are the clinical hallmark of SCD, and are responsible for the vast majority of hospitalizations (>90%), and result in significant morbidity, mortality, and interruption of daily functioning. The onset of pain is unpredictable, and has been described as periodic, self-limited episodes of excruciating musculoskeletal pain (although visceral, soft tissue, and other locations have been reported), occurring as early as 6 months of age.[7] In addition, readmission to the hospital for recurring or repeat episodes of VOC within a short time of discharge is common, with reports of up to 16% of patients readmitted within one week.[8] Other problems include ischemic and hemorrhagic stroke, acute chest syndrome, splenic or hepatic sequestration, dactylitis, osteonecrosis, priapism, leg ulcers, and nephropathy.[4,9,10,11] Most SCD-related deaths occur during acute VOC, and are due to acute chest syndrome, stroke, or multi-organ failure.[12] Patients can become symptomatic with pain, infection, or splenic sequestration as early as 6 months of age; thus, VOCs are an important cause of morbidity and mortality throughout life, resulting in disruption of the individual's education, psychosocial development, and employment as well as causing severe pain, hospitalization, and premature death.

The generally understood etiology of VOC involves dual mechanisms: a mechanical component, by which adhesive sickle red blood cells (RBCs) become caught in the post-capillary venules, and an associated inflammatory response in which white blood cells (WBC) adhere to the endothelium (Figure 1).

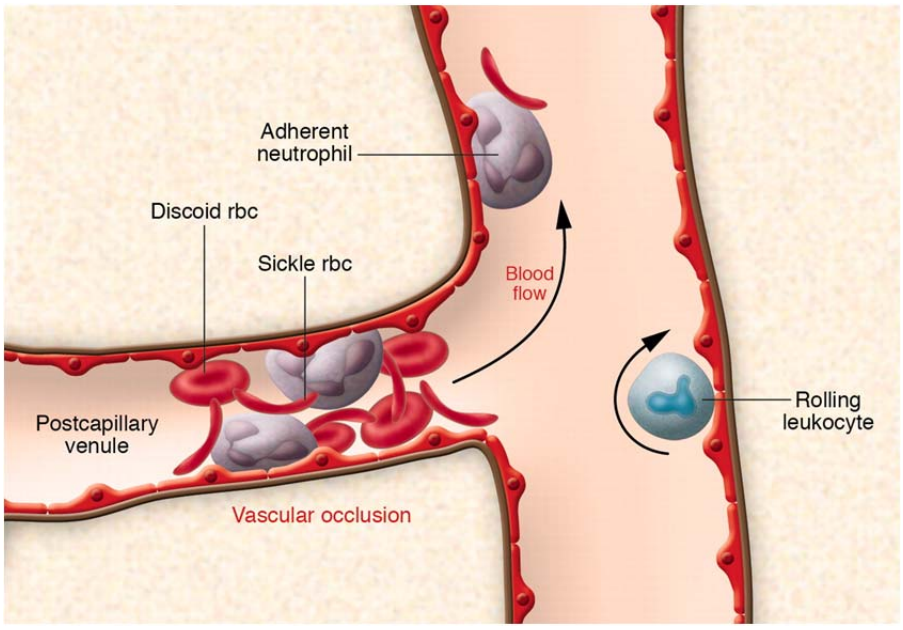


Figure 1: Sickled Red Blood Cells and Neutrophils Occlude Vasculature[5]

The underlying pathogenesis of RBC sickling is the deoxygenation of HbS, which results in polymerization of the hemoglobin, distortion of the RBC, and loss of deformability of the cell.[13] These "sickled" RBCs are a primary component of the occlusive process and cause vascular injury through interactions with endothelial cells.[9]

The other primary component is adhesion, since the stickiness of the cells causes cell aggregates of WBC-RBC, WBC-WBC, and WBC-platelet to form. This adhesion is selectin-mediated and contributes to the VOC process. Cell aggregates block the vasculature and create a trap for the sickled RBCs, resulting in obstruction of blood flow.[5,14]

Finally, vaso-occlusion resulting from sickled and adherent RBCs also causes slowing of blood flow in post-capillary venules, local tissue hypoxia, and further tissue inflammation, resulting in more deoxygenation and sickling of RBCs, and propagation of the occlusion. This is sometimes called secondary recruitment of sickled cells and occluded vessels.[4] Diapedesis (leukocyte migration into the tissues) and sickle-related microvascular occlusion also take place in postcapillary venules. Pain is the result of initial and ongoing occlusion and ischemia and is thought to be nociceptive.[i,15,11] It is particularly severe for patients with the SCD-SS genotype, who have been observed to have a higher mortality than other genotypes.[12]

Of note is that soluble E-selectin, vascular cell adhesion molecules-1 (VCAM-1), and intracellular adhesion molecules-1 (ICAM-1) levels are higher in sickle cell patients at baseline than in normal volunteers. Further evidence suggests that soluble E-selectin and ICAM-1 are increased during acute VOC when compared to baseline in patients with SCD.[16] In addition, increased soluble E-selectin levels have been shown to correlate with increased mortality in SCD patients.[17] Leukocytes adherent to the endothelial cell wall in postcapillary and collecting venules interact with sickled RBCs, leading to vaso-occlusion in sickle-cell mice. However, sickle cell mice lacking E- and P-selectin were protected from developing vaso-occlusion.[18] An understanding of the role that selectins play in cell aggregation and the pathophysiology of VOC is increasing.[4,19]

1.3 Treatment of Vaso-Occlusive Crisis

Nonpharmacologic management of pain includes cutaneous stimulation (transcutaneous electrical nerve stimulation), heat, cold, vibration, distraction, relaxation, massage, music, guided imagery, self-hypnosis, self-motivation, acupuncture, and biofeedback.[20]

[i] Nociceptive pain is initiated by nociceptors, the nerves that sense and respond to damage (fractures, burns, bumps, bruises, and inflammation) in a particular part of the body. This differs from neuropathic pain, which is the result of an injury or malfunction in the peripheral or central nervous system (CNS) and which sometimes also occurs in sickle cell disease.

Pharmacologic management of pain includes the use of 3 major classes of compounds: non-opioids (nonsteroidal anti-inflammatory drugs [NSAIDs], topical agents, tramadol, and corticosteroids), opioids, and adjuvants (antihistamines, antidepressants, benzodiazepines, and anticonvulsants).[20] Adjuvant treatments for pain are typically used for chronic pain. NSAIDs are the first line of therapy for acute VOC pain, and are typically prescribed on an outpatient basis. Opioids in repeated and increasing doses are usually the first line of treatment for emergency department or inpatient care required for acute VOC. Common drugs include codeine (oral, for mild pain); or for moderate to severe pain, IV morphine, or morphine-equivalent opioids such as oxymorphone, levorphanol, fentanyl, and methadone.[20] Opioid agonists may also be used in the management of sickle cell pain in adults. They decrease or modify the perception of pain at the level of the CNS.[20]

Studies of pain in the emergency room setting have shown that changes in pain that are clinically significant to the patient are 1.3-1.5 cm on a 10 cm visual analog scale (VAS).[21] Further, smaller studies of pain in the setting of sickle cell vaso-occlusive crisis lend support to this size of change being pertinent for sickle cell pain as well.[22]

Other supportive measures for VOC include hydration, supplemental oxygen, transfusion, and treatment of any concurrent infections or other inciting medical conditions. No treatment is available to interfere with the sickling or vaso-occlusive process.

Current long-term medical management of SCD includes use of hydroxyurea, which is used to increase Hb F (fetal hemoglobin) concentration and reduce the number of pain crises by preventing their development.[20]

1.4 Intravital Microscopy

Intravital microscopy (IVM) is a non-invasive imaging technique used for studying dynamic cell interactions and microvascular morphometry in living tissue. Variations of this technique have been used to measure microvascular blood flow and perfusion in both human and animal studies for multiple diseases, including diabetes, cardiovascular disease, and more recently SCD. Some sites with the capability to perform IVM on sickle cell patients may include this in the study, as described in this study protocol.

1.5 Biomarkers

Individuals with SCD display markers and the phenotype of a chronic mild inflammatory condition. Some markers which have been explored in the past include adhesion molecules (selectins, integrins, and activated integrins), cell-cell aggregates, C-reactive protein (CRP), and other markers of cell adhesion and tissue inflammation. Biomarker experiments in this study will be designed to determine whether GMI-1070 affects any such markers in the setting of VOC. Some biomarkers previously studied in SCD are noted below.

Kato, et al[17] measured levels of soluble endothelium-derived adhesion molecules in patients with SCD not in crisis (steady state). Soluble E-selectin (sE-selectin), sP-selectin, sVCAM-1, and ICAM-1 were measured and all were statistically significantly elevated except ICAM-1. All 4 adhesion molecules were elevated and showed a statistically significant relationship to each other with the exception of sICAM-1 to sP-selectin. The presence of sE-selectin in the blood was positively associated with hepatic dysfunction, reticulocyte count, triglyceride level, and linked independently to levels of CRP. Over a 4-year period, mortality was strongly and significantly (P=0.002) associated with levels of sE-selectin in the blood whereas no significant correlation was seen with levels of sP-selectin (P=0.36). These results suggest that early mortality in SCD patients is associated with endothelial activation.

Wun, et al[23] reported that monocytes are activated in SCD patients, which may be related to the increased occurrence of platelet adherence and aggregation. The mean percentage of platelet-monocyte aggregates (PMA) in Caucasian controls is 14%, whereas African-Americans is 25%, and in SCD patients is 45%.

Okpala, et al[24] published data showing high expression of αMβ2 integrins (MAC-1), CD18, and CD62L (L-selectin) on neutrophils of SCD patients. Lum, et al[25] showed that steady state SCD patients expressed elevated levels of MAC-1 and *activated* CD18 on neutrophil surfaces.

Zennadi, et al[26] demonstrated that RBC adhesion to peripheral blood mononuclear cells induced an E-selectin mediated adhesion to endothelial cells. Hidalgo, et al[27] published a paper showing that E-selectin is crucial in activating β2 integrins (MAC-1) at the leading edge of crawling neutrophils to bind and capture RBCs and platelets.

Belcher, et al[28] have reported a 12-fold increase in CRP levels in sickle cell serum compared to normal serum. As stated above, Kato, et al[17] showed a correlation (albeit independent) between sE-selectin and CRP levels in the blood of sickle cell patients.

1.6 Nonclinical Information

Nonclinical testing of GMI-1070 has been performed in mice, rats, rabbits, and cynomolgus monkeys. Pharmacodynamic studies have demonstrated that GMI-1070 administration can inhibit selectin binding and biological activity as it relates to induction of selectin-mediated inflammation. Single-dose IV pharmacokinetic (PK) studies in mice (20 mg/kg), rats (20 mg/kg), and monkeys (150 mg/kg), showed a similar half-life ($t_{½}$) for mice, rats, and monkeys (1.25 hours, 1.4 hours, and <3 hours, respectively).

The 14-day repeat-dose IV toxicology studies showed a no observed adverse effect level in mice of 150 mg/kg, and in cynomolgus monkeys of 75 mg/kg. Monkeys used in this pivotal toxicology study ranged in age from 21 to 35 months (mean 29 months) – the human age equivalent of 11-12 years.

A 7-day repeat-dose IV toxicology study in juvenile rats showed a no observed adverse effect level of 150 mg/kg/day, and a 14-day repeat-dose IV toxicology study in juvenile rats of 35 mg/kg/day.

In the presence of human, rat, mouse, and monkey hepatocytes, GMI-1070 was metabolically stable.

Additional information may be found in the Investigator's Brochure.

1.7 Clinical Information

A summary of clinical information from 2 clinical studies (GMI-1070-101 and GMI-1070-102), and preliminary data from a third study (GMI-1070-103), is provided in the following sections. Additional information may be found in the Investigator's Brochure.

1.7.1 Safety

Study GMI-1070-101 evaluated single doses of IV GMI-1070 at 2, 5, 10, 20, and 40 mg/kg. Study GMI-1070-102 evaluated multiple doses of 5, 10, and 20 mg/kg given every 8 hours for 4 days, and a loading dose of 40 mg/kg followed by multiple doses of 20 mg/kg given every 8 hours for 2 days. These studies evaluated a total of 72 healthy adult subjects (54 active, 18 placebo).

A third study, GMI-1070-103, recently completed enrollment. This was an open-label study of IV GMI-1070 in adults with stable SCD. Fifteen subjects have received a 20 mg/kg dose followed by a 10 mg/kg dose of IV GMI-1070.

GMI-1070 has demonstrated excellent safety and tolerability in these studies as demonstrated by:

- No clinically significant electrocardiogram or physical exam findings
- With 1 exception, all adverse events (AEs) in subjects receiving GMI-1070 were Grades 1 or 2. Severe symptomatic anemia (grade 4) that occurred in 1 subject with stable SCD, was considered to be remotely related to the study medication, and was resolved with therapy.
- No serious AEs (SAEs) occurred

In both healthy adult studies, mild local irritation at the site of IV administration was observed to occur more frequently in higher dose groups with multiple doses, in both the GMI-1070 and placebo treatment groups. Two subjects in the 40 mg/kg dose group of the phase 1 studies developed a rash. One of these was in Study GMI-1070-101 and received a single dose of GMI 1070 (40 mg/kg), and 1 subject was in Study GMI-1070-102 and received 3 doses of GMI-1070 (a single 40 mg/kg dose, and two 20 mg/kg doses). None of these observations prevented dose escalation or progression to the next study.

Local irritation (Grade 1) was reported in one subject in Study GMI-1070-103.

Headache was reported in 21 subjects (19 active, 2 placebo) across all three studies to date. One subject in Study GMI-1070-102 who received placebo reported a severe (Grade 3) headache; all others (including all reported in subjects receiving GMI-1070) were Grade 1 or 2. Table 2 summarizes all treatment-emergent adverse events (TEAE) across all three IV GMI-1070 studies.

Table 2. TEAE in Previous Studies with GMI-1070

Study	Treatment	No. (%) of Subjects	No. of Adverse Events	Severity Grade by Number of TEAE Episodes			
				Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	(Grade 4)
GMI-1070-101	2 mg/kg (n=6)	1 (17%)	8	7	1	0	0
	5 mg/kg (n=6)	4 (67%)	12	10	2	0	0
	10 mg/kg (n=6)	2 (33%)	3	3	0	0	0
	20 mg/kg (n=6)	3 (50%)	6	6	0	0	0
	40 mg/kg (n=6)	2 (33%)	4	4	0	0	0
	Placebo (n=10)	3 (30%)	11	10	1	0	0
GMI-1070-102	5 mg/kg (n=6)	5 (83%)	12	12	0	0	0
	10 mg/kg (n=6)	4 (67%)	8	8	0	0	0
	20 mg/kg (n=6)	6 (100%)	17	15	2	0	0
	Loading dose (n=6)	6 (100%)	16	14	2	0	0
	Placebo (n=8)	7 (88%)	37	31	5	1	0
GMI-1070-103	20 mg/kg load 10 mg/kg dose (n=15)	9 (60%)	16	5	3	0	1

In study GMI-1070-103, sixteen AEs have been reported in 9 subjects, and all AEs were mild with the exceptions noted. Reported AEs included hip pain (1), headache (4 subjects reporting 5 events, 1 moderate), mild and moderate pain crisis 13 and 23 days respectively after dosing (2), moderate emesis (1), severe symptomatic anemia requiring transfusion (1), moderate leukocytosis (1), sore throat (1), cough (1), itching (1), a moderate urinary tract infection (1), and moderate hypokalemia (1). Moderate headache (1) and leukocytosis (1) were the only AEs noted as Probably Related to study drug by the Investigator. Finally, only one AE of moderate headache was noted by the Investigator as Possibly Related to study drug. No serious AEs were reported.

Also in study GMI-1070-103, clinical laboratory testing indicated small mean increases in overall WBC and neutrophil counts 24 hours after dosing, which returned to baseline at 7 days; this finding was not noted in the healthy volunteer studies. In all but one subject, these changes did not constitute AEs and the clinical significance of these changes is unknown. In one subject, moderate leukocytosis was reported as an

AE, associated with elevated neutrophil count and hsCRP – all of which returned to baseline at 7 days, with no other symptoms reported or noted in the subject. All other hematology (including RBC indices, reticulocyte and platelet counts), chemistry (including electrolytes, glucose, BUN, creatinine, and ALT), and urinalysis test results remained unchanged from baseline in all study subjects. No notable trends in vital signs were observed during the study. The majority of subjects had no changes in physical examination findings, and those findings noted were not considered clinically significant.

The elevated peripheral WBC and neutrophil counts may reflect the prevention or interruption of adhesion of neutrophils to the endothelial cells on the blood vessel wall, which would be predicted by the selectin-inhibition mechanism of the drug.

It was concluded by the investigators and the Medical Monitor that for studies GMI-1070-101, GMI-1070-102, and GMI-1070-103, all doses observed demonstrated minimal safety concerns for continuing clinical trials at the already tested dose levels, up to and including multiple doses of 20 mg/kg TID (60 mg/kg/day) for 4 days and alternatively a loading dose of 40 mg/kg followed by 20 mg/kg TID (60-80 mg/kg/day) for 2 days.

1.7.2 Pharmacokinetics

After a single dose of GMI-1070 in healthy volunteers, mean values for C_{max}, $AUC_{(0-t)}$, and $AUC_{(inf)}$ increased in a dose-proportional manner, providing evidence of linear PK. The median values for T_{max} ranged from 0.341 to 0.417 hours and were not dependent on dose. There were no dose related trends in either CL or V_z and the mean elimination half-life ($t_{½}$) ranged from 6.67 hours to 7.41 hours and was independent of dose. (Table 3)

Table 3. GMI-1070 Pharmacokinetics Post Single Dose in Healthy Adults

Parameter[1]	Cohort				
	2 mg/kg N=6	**5 mg/kg N=6**	**10 mg/kg N=6**	**20 mg/kg N=6**	**40 mg/kg N=6**
C_{max} (µg/mL)	19.1±2.39	53.6±4.60	137±20.5	256±43.7	454±53.2
T_{max} (h)	0.417	0.355	0.383	0.417	0.341
$AUC_{(0-t)}$ (hr*µg/mL)	117±19.5	288±37.9	794±104	1312±141	2563±423
$AUC_{(inf)}$ (hr*µg/mL)	120±17.7	291±38.9	802±109	1321±148	2582±431
kel (h^{-1})	0.0941±0.0083	0.1028±0.0151	0.0976±0.0124	0.1058±0.0154	0.1007±0.0083
$t_{1/2}$ (h)	7.41±0.64	6.85±0.90	7.19±0.81	6.67±0.96	6.93±0.58
CL (mL/h/kg)	0.284±0.042	0.292±0.043	0.211±0.029	0.255±0.028	0.264±0.041
V_z (L/kg)	0.181±0.022	0.171±0.017	0.130±0.006	0.145±0.011	0.157±0.022
Ue (mg)	140±13.3	332±28.0	709±71.5	1412±179	2755±524
Fe (% dose)	90.2±9.54	93.9±10.5	102±8.08	98.0±6.31	99.1±11.7
CL_r (mL/min	20.5±4.17	19.5±3.20	15.1±2.65	18.2±3.43	18.2±4.16

[1]Arithmetic mean ± standard deviation (N) except for T_{max} for which the median (N) is reported.

Analysis of the plasma concentration and urinary excretion data for GMI-1070 in healthy adults after IV administration of 5, 10, or 20 mg/kg every 8 hours for 4 days or 40 mg/kg x 1 + 20 mg/kg every 8 hours for 2 days indicates linear PK over that range of doses (Figure 2). The loading dose achieved steady state immediately. The elimination $t_{1/2}$ was ~8 hours, a minimum of 89% of the dose was recovered in the urine, and CL_r was less than filtration clearance, consistent with tubular reabsorption.

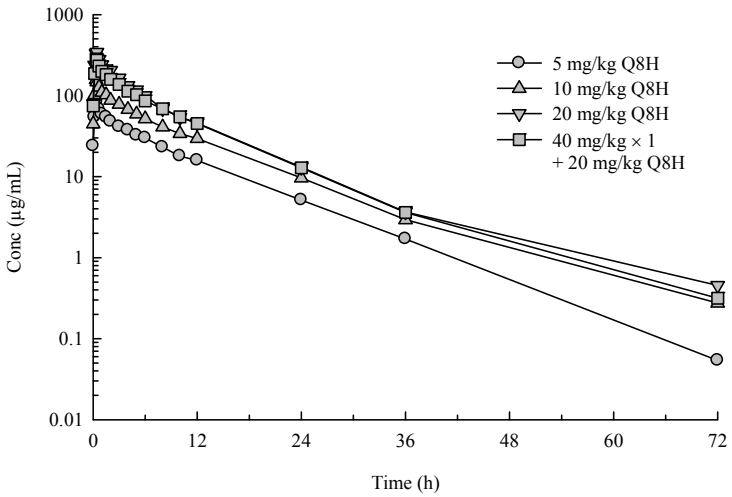


Figure 2: Mean plasma concentrations of GMI-1070 after the final dose during intravenous administration of 5, 10, or 20 mg/kg every 8 hours or 40 mg/kg × 1 + 20 mg/kg every 8 hours to healthy adults under fasted conditions - semi-logarithmic axes

Data from 14 subjects in the GMI-1070-103 study indicate that the pharmacokinetics of GMI-1070 in stable SCD subjects not in VOC are well characterized by a two-compartment model; estimates for clearance, volume of distribution, $t_{½}$, and CL_r were consistent with those in healthy volunteers. As seen in the mean plasma concentrations (Figure 3), there was excellent agreement between the observed plasma GMI-1070 concentrations and those predicted by the pharmacokinetic model for all patients. (Table 4)

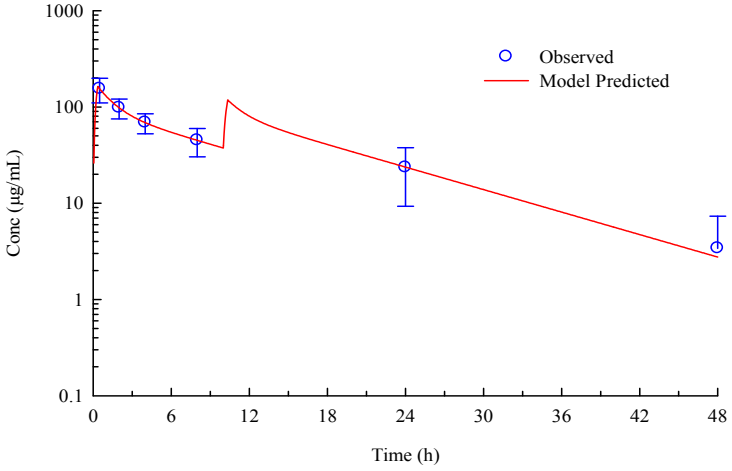


Figure 3: Observed (mean ± standard deviation) and model predicted plasma concentrations of GMI-1070 after intravenous infusion of 20 mg/kg (loading dose) and 10 mg/kg (maintenance dose) to patients with SCD — semi-logarithmic axes

Table 4. Pharmacokinetic parameters of GMI-1070-103

Parameter	GMI-1070 20 mg/kg + 10 mg/kg[1] Arithmetic mean (SD) N = 14
Primary[2]	
Total plasma clearance (CL; mL/h/kg)	19.6 (6.42)
Volume of the central compartment (V1; mL/kg)	120 (30.7)
Intercompartmental clearance (CLD2; mL/h/kg)	31.6 (17.2)
Volume of the peripheral compartment (V2; mL/kg)	64.2 (19.6)
Secondary[2]	
Volume of distribution at steady state (V_{ss}; mL/kg)	184 (40.1)
Maximum observed drug concentration (C_{max}; µg/mL)	167 (48.4)
Area under the plasma concentration-time curve to infinity ($AUC_{[inf]}$; h x µg/mL)	1129 (367)
Apparent first-order distribution rate constant (α; h^{-1})	0.8610 (0.3985)
Apparent first-order terminal elimination rate constant (β; h^{-1})	0.0969 (0.0249)
Elimination half-life ($t_{1/2}$; h)	7.70 (2.38)
Tertiary[3]	
Renal clearance for the collection period (CL_r; mL/min)	18.0 (7.94)

SD = standard deviation.
1 The first dose was a loading dose and the second dose was administered 10 ± 1 hours later.
2 Primary parameters were those used to fit the model and secondary parameters were estimated from the primary parameters.
3 Tertiary parameters were estimated from the primary parameters plus the urinary excretion of GMI-1070.

1.7.3 Dose Selection

The initial dose used in this study, a 20 mg/kg loading dose followed by a 10 mg/kg dose every 12 hours for up to 7 days, was selected based on non-clinical pharmacokinetic/ pharmacodynamic (PK/PD) studies in a SCD mouse model as well as experience with human dosing in healthy volunteer studies, GMI-1070-101 and GMI-1070-102, and human dosing in stable SCD subjects not in VOC, study GMI-1070-103. The safety profile of GMI-1070 was excellent, including up to a 40 mg/kg loading dose followed by a 20 mg/kg dose every 8 hours, or a total of 60 mg/kg per day for 4.3 days. The PK profile from these clinical studies supports using a 20 mg/kg loading dose to achieve steady state immediately, and continuing dosing at 10 mg/kg every 12 hours to maintain plasma levels above a minimum concentration (C_{min}) of 16-20 µg/mL associated with effect in the SCD mouse model.

As per study protocol section 8.2.5, the PK data from the first 10 subjects in this study were evaluated in an interim analysis to confirm the dose. The analysis was performed using a population PK model developed from rich datasets of the previous PK studies. In the population PK model there was no difference between healthy

subjects and those with SCD not in VOC. Because SCD subjects in VOC are in a different physiologic state and treatment paradigm than those not in VOC (most notably they may have higher cardiac output and are heavily hydrated), and clearance of GMI-1070 is through the kidney, it was not known if PK parameters would differ. This interim PK analysis was planned to confirm or adjust dose, if necessary, for the remainder of the study.

This interim PK analysis indicated that SCD subjects in VOC appear to have a higher rate of clearance of GMI-1070 as compared to the healthy volunteer and subjects with SCD not in VOC (as seen in studies GMI-1070-101, GMI-1070-102 and GMI-1070-103, respectively). None of the covariates examined (age, gender, weight, height, organ function or hemoglobin) appeared to explain this difference between the predicted and the observed levels. Half the subjects included in the interim PK analysis were between the ages of 14-25 years. The data, incorporated into the model, demonstrated a mean C_{min} of 11 µg/mL, with the individual patient range between 4 – 24 µg/mL. This is below the 16-20 µg/mL minimum both desired based on non-clinical efficacy studies and expected based on previous human studies. The average time above 20 µg/mL actually achieved is 7.2 hours (60.2%) of the dosing interval. In contrast, the expected time above C_{min} at the outset of the study was 90-100% of the dosing interval. Furthermore, the average time above 15 µg/mL, which represents the lower end of the C_{min} target, is only 8.6 hours of a 12 hour dosing interval (71.9% of the dosing interval).

Non-clinical and biomarker data from the GMI-1070-103 study suggest that the levels of GMI-1070 achieved with the loading dose of 20 mg/kg followed by 10 mg/kg dose every 12 hours would likely still be within the range necessary to show a biological effect. Nevertheless, given the excellent safety profile seen to date and the desire to evaluate clinical effect at the original target plasma levels in the context of this Phase 2 study, the Sponsor has elected to adjust the dose to reach these original target levels. This is considered appropriate for all age groups in the study, as age was not identified as a covariate for PK behavior. As allowed in protocol section 8.2.5, the dose level is being adjusted to achieve the desired plasma concentration of GMI-1070.

PK modeling using the established population PK model and the new data suggests that a loading dose of 40 mg/kg followed by 20 mg/kg every 12 hours will result in a mean C_{min} of 21.4 µg/mL. In addition, this will result in an average time above 20 µg/mL of 10.3 hours of a 12 hour dosing interval (85.8% of the dosing interval).

Previous studies have evaluated doses up to and including this dose level (40 mg/kg followed by 20 mg/kg every 12 hours), and in fact up to a greater total daily dose (60-80 mg/kg/day in the GMI-1070-102 study) with minimal safety concerns. In addition, comparison of PK data across the 3 previous studies indicates that the slower clearance rate in earlier studies resulted in higher total exposure than that anticipated in SCD subjects in VOC (Table 5). Higher total exposure combined with minimal safety concerns seen in previous studies suggests that the increase in dose in SCD subjects with VOC is acceptable from a safety risk perspective.

Table 5. Comparison of AUC and C_{max} in the Phase 1 and Phase 2 Studies with GMI-1070 on Day 1 and at Steady State over a 24-hour Period

		Day 1 Mean (SD)		Steady State Mean (SD)	
Study Reference	**Regimen, mg/kg (Load/Maintenance)**	**C_{max}**	**$AUC_{(0-24)}$**	**C_{max}**	**$AUC_{(0-24)}$**
102	20 TID (no load)	237 (54.9)	2268 (184)†	349 (35.6)	4323 (381)†
102	40/20	445 (55.8)	2896 (203)†	292 (21.7)	3768 (282)†
103	20/10	167 (48.4)	1402*	116*	1108*
201	20/10	190 (55.2)*	1058 (449)*	105 (34.3)*	767 (347)*
New Dose 201	40/20	380 (110.4)*	2116 (897)*	210 (68.8)*	1534 (694)*

† $AUC_{(0-24)}$ calculated from $AUC_{(0-8)}$ data
* Modeled PK data

Given the excellent safety profile seen to date and that C_{min} is the PK parameter selected to set the dose, the dose will be adjusted to a loading dose of 40 mg/kg followed by 20 mg/kg every 12 ± 2 hours in order to achieve the desired plasma concentrations.

2 STUDY OBJECTIVES AND PURPOSE

2.1 Rationale for this Study

GMI-1070 is a potent, rationally designed glycomimetic inhibitor of E-, P-, and L-selectins *in vitro*. GMI-1070 has been shown to inhibit inflammation in several animal models of disease, including the VOC of SCD. This study drug is being developed for the treatment of VOC in SCD. In the present study, subjects 12 to 60 years of age who are hospitalized for VOC will be evaluated for the efficacy, safety, and PK of GMI-1070.

Amendment 1 expanded assessment of GMI-1070 from adults aged 16 to 45 to adolescents aged 12 to 15 years. As an inherited disease, sickle cell presents early in childhood and patients in all age groups, from infancy through adulthood, experience VOC. Adolescents generally have fewer co-morbidities and lower incidence of chronic pain and opioid narcotic use, such that GMI-1070 may have a greater treatment effect in younger patients experiencing VOC than adults. GMI-1070 has been well-tolerated in clinical trials to date; and based on the mechanism of action no differences in safety or efficacy would be expected in children as compared to adults. Thus, inclusion of adolescent subjects in clinical studies throughout clinical development is a critical component of the program.

Safety and PK data collected in the 2 prior studies of IV GMI-1070 in healthy adults and 1 study in subjects with stable SCD, have demonstrated an excellent safety profile and linear, predictable PK parameters at a range of doses for up to 4 days of treatment. In addition to the human safety data and based on results of GMI's nonclinical testing in juvenile monkeys and rats, GMI believes that assessment in adolescents aged 12 to 15 years would be safe and well-tolerated.

Amendment 2 revised eligibility criteria in response to further review of criteria in similar studies, with the consensus of the study investigators and DSMB Chair. These criteria included changing the upper age limit to 60, limits on previous transfusions and frequency of recent hospitalizations for VOC.

Amendment 3 modified the dose of GMI-1070 to a loading dose of 40 mg/kg followed by 20 mg/kg every 12 ± 2 hours. This was in response to the PK interim analysis of the first 10 subjects as per protocol section 8.2.5. The PK interim analysis indicated that SCD subjects in VOC appear to have a higher rate of clearance of GMI-1070 as compared to healthy volunteers and subjects with SCD not in VOC (as seen in studies GMI-1070-101, GMI-1070-102, and GMI-1070-103 respectively). The dose level was correspondingly increased to achieve the desired plasma concentration of GMI-1070.

Amendment 4 revised eligibility criteria in response to further review of criteria in similar studies, with the consensus of the study investigators and DSMB Chair. These criteria revisions included allowing subjects to be enrolled within 24 hours of presentation to the ED or after up to 2 days of outpatient parenteral treatment, and changing the limits on previous transfusions.

The present study seeks to build on these data by evaluating the safety and PK of two dose levels of GMI-1070 in subjects 12 to 60 years of age experiencing VOC. This study will also evaluate efficacy of GMI-1070 at two dose levels and gather data on biomarkers of adhesion and inflammation, and additional clinical activity with respect to pain and complications. Moreover, the dose adjustment will allow for the comparison of two dose levels in the same study, adding to the clinical pharmacology dataset for GMI-1070 in the treatment of VOC.

2.2 Study Objectives

2.2.1 Primary

- Evaluate the effect of multiple intravenous (IV) doses of GMI-1070 at two different dose levels on time to resolution of vaso-occlusive crisis (VOC) in subjects 12 to 60 years of age hospitalized for sickle cell VOC

2.2.2 Secondary

- Evaluate the effect of multiple IV doses of GMI-1070 at two different dose levels on clinical activity in subjects 12 to 60 years of age hospitalized for sickle cell VOC

- Evaluate the safety of multiple IV doses of GMI-1070 at two different dose levels in subjects 12 to 60 years of age hospitalized for sickle cell VOC
- Evaluate the pharmacokinetics (PK) of multiple IV doses of GMI-1070 at two different dose levels in subjects 12 to 60 years of age hospitalized for sickle cell VOC
- Evaluate biomarkers of adhesion and inflammation at two different dose levels in subjects 12 to 60 years of age hospitalized for sickle cell VOC

3 STUDY DESIGN

This is a randomized, double-blind, placebo-controlled trial of multiple IV doses of GMI-1070 or placebo in subjects hospitalized for sickle cell VOC. Investigators are encouraged to identify subjects with VOC who are likely to be admitted, and approach them for consent and eligibility criteria early in the process of treatment for VOC. All subjects should be dosed with study drug within 24 hours of first medical evaluation in the Emergency Department/clinic.

In addition, investigators are encouraged to identify those subjects who may be appropriate for the study before they develop VOC. These subjects may be approached with educational information about the study. Such education will not replace the need to obtain informed consent at the time of hospitalization for VOC.

The study will enroll until approximately 76 evaluable subjects (defined as those who have received at least 1 dose of study medication) have been enrolled. During Visit 1, eligible subjects will be randomized in a 1:1 ratio to either GMI-1070 or placebo using an IVRS. Study drug will be administered as a loading dose of 40 mg/kg followed by 20 mg/kg every 12 ± 2 hours until the subject meets criteria for improvement of pain, or up to 7 days of treatment (maximum 15 doses including the loading dose), whichever comes first. If the subject has no improvement in VOC after 5 days (eg, no change in VAS and no decrease in IV pain medication requirement), study drug should be discontinued.

Resolution of VOC is defined as the first of the following to occur:

- Sustained decrease in pain score of at least 1.5 cm out of 10 cm since baseline; AND transition to oral pain medications per hospital procedures (subject is on no IV pain medications),
- OR readiness for discharge as stated by the physician and subject,
- OR discharge to home.

For the purposes of this study, ‘sustained’ is defined as 2 consecutive pain scores at least 4 hours apart as reported by the subject on a VAS; ‘baseline’ is defined as the pain score just prior to the first dose of study drug as reported by the subject on the VAS.

Visits for follow-up examinations will be made 36 ± 12 hours post last dose (Visit 2) (ideally this will occur prior to discharge from the hospital), on day 7 ± 3 post last dose (Visit 3), and on day 28 ± 5 post last dose (Visit 4).

Pharmacokinetics Sampling

Plasma sampling for PK will be done during the study. A total of 9 samples will be drawn from each subject. Each sample will be 3 mL. Urine sampling for PK will also take place, in a single 12-hour urine collection.

Optional Site-specific Biomarkers

In a subset of subjects at sites capable of performing these tests, IVM testing for microvascular blood flow and additional sampling for other biomarkers of adhesion and inflammation will be performed.

4 SELECTION AND WITHDRAWAL OF SUBJECTS

4.1 Number of Subjects

The study plans to enroll until approximately 76 evaluable subjects (defined as those who have received at least 1 dose of study medication) have been enrolled.

4.2 Inclusion Criteria

To be eligible for inclusion, each subject must fulfill each of the following criteria at screening, and must continue to fulfill these criteria prior to dosing:

1. 12 to 60 years of age
2. Confirmed diagnosis of sickle cell disease (HbSS or HbS-β^0thalassemia)
3. Diagnosis of VOC at the time of enrollment
4. Hospitalized or in process of admission at the time of enrollment
5. Able to receive the first dose of study drug within 24 hours of initial medical evaluation in the Emergency Department/clinic for VOC;
   - Subjects treated as an outpatient within the past 48 hours for the same VOC episode may be enrolled if dosing is also expected within 24 hours of their second (admitting) presentation.
6. Documented and observed written informed consent (and assent, where applicable)

4.3 Exclusion Criteria

1. Infection, diagnosed or strongly suspected, as evidenced by 1 or more of the following:
   - Fever >39° C (102.2° F)
   - In the presence of fever ≥38.5° C (101.3° F), 1 of the following:

  - Positive findings (suspicious for infection) on diagnostic tests, such as CSF evaluation, radiographs, or bacterial culture of normally sterile sites
  - Exam findings leading to diagnosed or strongly suspected bone or joint infection
  - Determination by physician that bacterial or serious systemic viral infection is likely (eg, influenza, mononucleosis)
  - Subjects may be included with uncomplicated urinary tract infections (provided they do not have fever ≥38.5° C [101.3° F] or CVA tenderness), and/or suspected minor viral syndromes (upper respiratory infection symptoms but no symptoms suggestive of bacterial infection other than uncomplicated otitis media or, uncomplicated streptococcal pharyngitis)

2. Acute chest syndrome, diagnosed or strongly suspected, as evidenced by a new infiltrate on chest radiograph, and 1 or more of the following:
   - Fever >39° C (102.2° F)
   - Hypoxia (confirmed by arterial blood gases [ABG] with paO_2 <70 mmHg)
   - Chest pain
   - Suspicious findings on exam (tachypnea, intercostal retractions, wheezing, and/or rales)
3. SCD pain atypical of VOC, including hepatic or splenic sequestration, cholecystitis, or pneumonia.
4. Acute stroke, acute priapism, severe avascular necrosis of the hip/shoulder when the presenting pain is only in the affected hip/shoulder
5. Serum creatinine:
   - >1.2 mg/dL for subjects 16 to 60 years of age
   - >1.0 mg/dL for subjects 12 to 15 years of age
6. Alanine transaminase (ALT/SGPT) >2x ULN (based on clinic laboratory normal range)
7. Hemoglobin <5 g/dL
8. Platelets <100,000/mm^3
9. Recent (within the past 30 days) major surgery, hospitalization for other than VOC, documented serious bacterial infection requiring antibiotic treatment, or significant bleeding
10. Hospitalization for uncomplicated VOC, or treated with parenteral pain medications in other medical settings such as the emergency department or day hospital for uncomplicated VOC, within past 14 days;
    - Subjects may be included if treated as an outpatient within the past 48 hours for the same VOC episode
11. Recent (within the past 90 days) cerebrovascular accident, transient ischemic attack, or seizure
12. pRBC transfusions in the past 14 days
13. Systemic steroid therapy within 48 hours prior to enrollment or expectation that therapy may be used during the study (inhaled or topical steroids are allowed)
14. For those on chronic or long-acting opioids, a change in dose in the past 14 days OR pain requiring medical attention in the past 14 days (change in opioid medication for acute pain in the past 48 hours and directly related to this VOC admission is allowed)

15. Greater than 5 episodes of hospitalization for VOC in the past 6 months (180 days)
16. Medical or psychiatric condition that, in the opinion of the investigator, may pose a risk to the subject for participation or interfere with the conduct or results of the study
17. Currently receiving, or has received within the previous 4 weeks, any other investigational agent
18. Previous administration of GMI-1070
19. Expectation that the subject will not be able to be followed for the duration of the study
20. Pregnant or lactating female; or female of childbearing potential or male unable or unwilling to comply with birth control methods or abstinence during the course of the study
21. Active use of illicit drugs and/or alcohol dependence, as determined by the investigator

4.4 Withdrawal of Subjects

A subject is free to withdraw from the study at any time for any reason without prejudice to their future medical care by the physician or at the institution.

The Investigator or Sponsor may also withdraw the subject at any time. The primary reason for withdrawal must be recorded in the medical record and on the withdrawal case report form (CRF). If a subject is withdrawn for more than one reason, each reason should be documented in the medical record and the most medically significant reason should be noted on the CRF.

Reasons for withdrawal may include:

- If consent is withdrawn or the subject refuses to continue treatment and/or procedures/observations
- Occurrence of unmanageable AEs or if the subject requires concomitant medication disallowed under this protocol (refer to Section 5.2)
- If clinically significant and concerning changes in any clinical or laboratory measurements warrant withdrawal as determined by the Investigator (refer to Section 6.3.6.2 for a description of clinical laboratories in the context of adverse events)
- If the subject becomes pregnant
- For other reasons (eg, significant protocol violation, non-compliance)

The withdrawal of a subject from the study should be discussed where possible with the Medical Monitor before the subject stops study drug. Subjects withdrawn will not be replaced. Any comments (spontaneous or elicited) or complaints made by the subject and the reason for termination, date of stopping study drug, and the total amount of study drug administered must be recorded in the medical record and transcribed onto the CRF.

Subjects who prematurely stop administration of study drug but continue on the study will continue to be followed as per the procedures outlined in Table 1 up to Day 28.

All efforts will be made to obtain safety data, assigned PK samples, and other endpoint data from subjects, and these data will be included in the analysis.

Subjects who withdraw early from the study, will be encouraged to have all Day 28 evaluations, and any PK evaluations due at the time they withdraw, completed at the time of study withdrawal.

If a subject is unintentionally unblinded, study data collection should continue and the subject should continue to receive study drug.

If a subject is lost to follow-up after discharge from the hospital, at least three documented attempts to contact and see the subject must be made, one of which must include sending a certified letter to the subject's last known address requesting that they return to the study site for final safety evaluations.

The Sponsor may be contacted if clarification is required on a case-by-case basis.

4.5 Study Termination

The Sponsor reserves the right to terminate the study at any time. A description of study termination based on safety concerns is described in Section 6.3.6.4.

5 STUDY DRUG AND CONCOMITANT TREATMENTS

5.1 GMI-1070 and Placebo

5.1.1 Description

The official product name, as indicated in the Investigator's Brochure, is 'GMI-1070 Injection, 30 mg/mL,' and will be referred to as 'GMI-1070' in this protocol. For further details regarding the exact composition of the product, please refer to the Investigator's Brochure.

Study drug will be supplied in vials containing 8.3 mL of sterile solution for IV administration. Active vials will contain GMI-1070 at 30 mg per mL; placebo vials will contain 8.3 mL of sterile vehicle (phosphate-buffered saline pH 6.5 [PBS]).

5.1.2 Dosing Administration

After consent (and assent, where applicable) has been given and documented and the subject meets all inclusion/exclusion criteria, a written authorization (prescription/ order/instruction signed by the investigator, who should be a study physician listed on Form Food and Drug Administration [FDA] 1572), is required prior to providing any subject with study drug.

The pharmacist will not be blinded, but all study personnel in direct contact with the subject will be blinded. Active drug will be labeled "GMI-1070 Injection, 30 mg/mL," and will be administered as a loading dose of 40 mg/kg followed by 20 mg/kg every 12 ± 2 hours for up to 7 days (maximum of 15 doses including the loading dose). Placebo will be labeled "PBS Placebo, 8.3 mL/vial," and will be administered as a loading dose of volume equivalent to that of active drug, followed by a maintenance dose of volume equivalent to that of active drug every 12 ± 2 hours for up to 7 days (maximum of 15 doses including the loading dose).

Study drug administration will occur at a steady rate over a period of 20 minutes from the start of the IV infusion, and will be followed by a saline or heparin flush (saline preferred) to ensure clearance of the IV tubing of any remaining study drug. New tubing is to be used with each administration of study drug. Study drug should not be administered concurrently through the same IV line with any medications, or with IV solutions other than saline-based solutions.

The planned schedule for dosing every 12 ± 2 hours should remain as *planned,* irrespective of adjustments for timing based on the 2 hour window for dosing. Dosing schedule should not be adjusted for actual dosing times. For example:

Table 6. Sample Dosing Schedule

Dose	Planned Time	Actual Time
First dose (am)	9 am	9 am
Second dose (pm)	9 pm	10 pm
Third dose (am)	9 am	8:30 am
Fourth dose (pm)	9 pm	10:30 pm

5.1.3 Discontinuation of Study Drug

Subjects will be treated with study drug until they meet the following criteria for resolution of VOC:

- Sustained decrease in pain score of at least 1.5 cm out of 10 cm since baseline; AND transition to oral pain medications per hospital procedures (subject is on no IV pain meds)
- OR readiness for discharge as stated by the physician and subject
- OR discharge to home

For the purposes of this study, 'sustained' is defined as 2 consecutive pain scores at least 4 hours apart as reported by the subject on a VAS; 'baseline' is defined as the pain score just prior to the first dose of study drug as reported by the subject on the VAS.

Study drug should be discontinued if any of the following medical changes take place, and study evaluations should continue to be performed as outlined in Table 1:

- Subject is transferred to ICU for any medical reason

- Acute chest syndrome is diagnosed
- Acute stroke, or other acute CNS changes
- Fever >39° C (102.2° F); fever with negative chest radiograph does not require discontinuation of study drug unless determined to be medically appropriate by the investigator
- Elevated WBC >35,000/mm3
- No improvement in VOC (as determined by the investigator, based on VAS and opioid or pain medication requirements and the judgment of the investigator) after 5 days of study drug
- Unexpected rise in creatinine, consistent with acute renal failure
- Pregnancy

Note: If it has been determined that study medication should be stopped no weaning of study medication will take place.

5.1.4 Allocation of Subjects to Treatment

Subjects will be randomized in a 1:1 ratio to receive either GMI-1070 or placebo. Randomization blocks will be stratified by site using an IVRS. Instructions on use of the IVRS will be included in the Study Reference Manual.

Once IRB/IEC approval for Amendment 3 (Protocol Version 4.0) is received, all new subjects should be enrolled at the new dose level of GMI-1070 or placebo.

5.1.5 Blinding

GMI-1070 will be supplied as open-label vials and a third-party design for blinding will be employed. The subject, the Sponsor, the CRO and all other study personnel in direct contact with study subjects will be blinded as to treatment allocation. The pharmacist (or designee) will be unblinded, and will prepare and label all study drug/placebo in a blinded manner to maintain the blind for all other personnel. Study drug/placebo syringes will be covered with opaque tape or similarly effective blinding method, IV bags will be covered, and colored tubing sleeves will be supplied for IV tubing.

Unblinding by study site personnel for AEs may only be performed in emergencies where knowledge of the subject's treatment assignment is essential for further management of the subject's medical care. Unblinding a subject's treatment assignment by the investigator or study site personnel under any other circumstances will be considered a protocol violation.

The investigator should make every effort to contact the CRO's Medical Monitor prior to unblinding a subject's treatment assignment. Emergency unblinding for AEs will be performed by the CRO Medical Monitor. Alternatively, if required, emergency unblinding for AEs may be done through the pharmacist responsible for preparation of

the drug. This may be performed only if the subject's well-being requires immediate knowledge of the subject's treatment assignment.

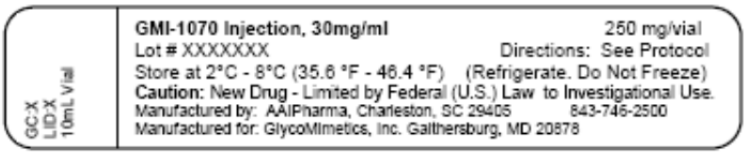
In the event of any unblinding, emergency or accidental, the CRO's Medical Monitor must be notified in writing within 1 working day, and the unblinding must be documented in the medical record at the investigative site as well as on the CRF. The date, time, and reason for breaking the blind must be recorded in the medical record.

5.1.6 Labeling, Packaging, Storage, and Handling

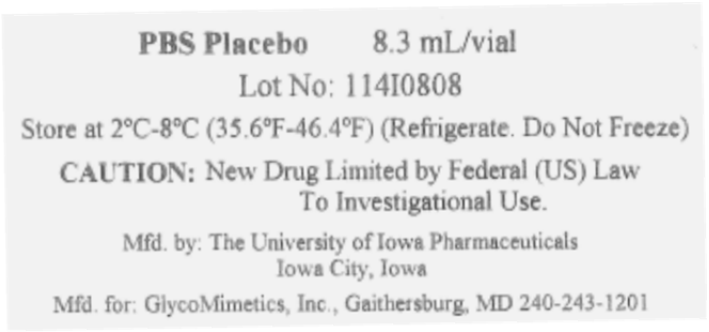
The study drug will be shipped to a designee at the study site and must be stored in a pharmacy or locked and secured in a storage facility with temperature control, accessible only to those individuals authorized by the investigator or designee.

The Sponsor will supply sufficient quantities of IV GMI-1070 and placebo to allow completion of this study. Study drug will be supplied to the pharmacy in vials containing 8.3 mL of sterile solution for IV administration. Active vials will contain GMI-1070 at 30 mg per mL.

All labeling will meet applicable regulatory requirements. Sample labels are below:



Placebo will be provided as vials containing 8.3 mL of sterile vehicle (phosphate-buffered saline [PBS], with a pH of 6.5).



Study drug must be refrigerated at a controlled temperature between 2° and 8° C. The investigator or designee is responsible for ensuring that the temperature is monitored throughout the total duration of the trial and that records are maintained; the temperature should be monitored continuously. Excursion from the established range will require site investigation as to cause and remediation. GMI will determine the ultimate impact of any excursions and will provide supportive documentation.

5.1.7 Compliance and Drug Accountability

Study drug must be used only as directed in the protocol. Study drug inventory and accountability forms will be maintained by the pharmacist or designee, and examined and reconciled by the unblinded monitor.

Study drug may not be relabeled. Study drug will not be destroyed or returned without explicit written instructions from the Sponsor. The Investigator must not dispense the study drug from, nor store it at, any site other than the study sites agreed upon with the Sponsor and approved by the IRB/IEC. Based on entries in the site drug accountability forms, it must be possible to reconcile drug delivered with that used and returned. All study drug must be accounted for and all discrepancies investigated and documented appropriately.

5.2 Concomitant Treatments

Treatments intended for other indications should be administered as usual. All concomitant treatments taken during the study (within 48 hours of randomization to the last post-dose follow-up assessment) should be recorded in the source document and CRF. All concomitant treatments should be confirmed and reviewed by the investigator or his/her designee prior to each dose for the duration of the study. Subjects taking systemic steroid therapy within 48 hours prior to enrollment, or those at baseline who have an expectation that therapy may be used during the study, will be excluded from enrollment. The use of inhaled or topical steroids, hydroxyurea, and NSAIDs will be allowed.

For the purposes of inclusion/exclusion, the term “illicit drugs” includes, but is not limited to, cocaine, heroin, ecstasy, and phencyclidine (commonly known as ‘PCP’). The use of marijuana is not excluded for the purposes of this study. Investigators are encouraged not to enroll subjects for whom addiction to prescribed opioids may interfere with interpretation of study endpoints.

Females of childbearing potential must be abstinent or agree to use one medically reliable method of contraception throughout the study period and for 30 days after the last dose of study drug. Acceptable methods include intrauterine devices, hormonal contraceptives (oral, depot, patch, or injectable), double barrier methods such as condoms or diaphragms with spermicidal gel or foam, or a surgically sterilized partner (vasectomy for 6 months minimum). If hormonal contraceptives are used, they should be taken according to the package insert. Women of child-bearing potential who are not currently sexually active must agree to use an acceptable method of contraception, as defined above, if they decide to become sexually active within 30 days after the last dose of study drug.

Sexually active male subjects should ensure that an acceptable method of contraception is used throughout the study period and for 30 days after the last dose of investigational product. Acceptable contraceptives include double barrier methods

such as condoms with spermicidal gel or surgical sterilization (eg, vasectomy for 6 month minimum).

5.2.1 Pain Management

Pain management should be approached according to the institution's usual procedures, with the following suggestions for standardized management:

- Recommended IV pain medications include morphine (preferred), hydromorphone, ketorolac, or fentanyl, used in accordance with standard clinical practice
- Initial treatment should aim to achieve pain control; pain control is defined as pain that does not require further escalation of pain medication OR subject reports pain is controlled
- Ongoing treatment should aim to maintain pain control; consider weaning IV opioids once pain is controlled (≤7/10)
- Discharge criteria related to pain may include improved pain score; on oral hydration; and on oral pain medications for at least 6 hours
- Patient-controlled analgesia (PCA) use (basal and bolus dosing) is encouraged for all subjects, per hospital procedure
- Subjects will be adequately hydrated per routine procedure and as medically indicated

Discharge instructions for pain management should include a minimum of 48 hours continuous treatment with oral opioid or similar pain medications. Subjects on chronic opioids may require different instructions, such as an opioid weaning regimen.

5.2.2 Transfusion

In all cases, the medical judgment of the responsible physician will determine the course of treatment.

The following suggestions are made for standardized management of transfusions in study subjects:

- Uncomplicated pain episodes generally do not require transfusion for treatment
- Conditions which may require transfusion as determined by the physician include: acute neurologic event, severe pneumonia or severe acute chest syndrome, acute arterial hypoxia (PaO2 <70 mmHg), severe anemia with cardiac decompensation, and/or symptomatic anemia (such as subjective shortness of breath or postural dizziness), worsening of anemia with an inadequate reticulocyte response, splenic or hepatic sequestration

6 STUDY PROCEDURES AND EVALUATIONS

6.1 Study Procedures

Refer to Table 1, for a list of procedures and times for assessments, and to Table 8 and Table 9 for a list of PK and biomarker assessments.

6.1.1 Pre-study Identification of Potential Study Subjects

Investigators are encouraged to identify subjects with VOC who are likely to be admitted, and approach them for consent and eligibility criteria early in the process of VOC care. Subjects with a history of frequent admissions for painful VOC in the past 12 months, and identified during routine clinical care as potentially eligible for study participation, may be approached with educational information about the study. Such education will not replace the need to obtain informed consent (and assent, where applicable) in accordance with applicable regulations and ICH E6[1] prior to administration of any study related procedures.

Where possible and as appropriate, subjects who are eligible for study participation should have admission procedures expedited to allow for early study enrollment and prompt management of VOC per study procedures.

All subjects should be dosed with the first dose of study drug within 24 hours of initial medical evaluation in the Emergency Department/clinic. Any initial dose of study drug administered more than 24 hours after initial medical evaluation will be considered a protocol deviation.

6.1.2 Pre-dose Baseline

Subjects will sign the informed consent form (and assent, where applicable), and eligibility will be determined. Eligible subjects will be randomized to a treatment group.

Sampling for biomarkers and IVM will be initiated at selected investigational sites.

Results of all eligibility assessments, particularly clinical laboratories and pregnancy tests, and radiographs where applicable, should be reviewed by the investigator or qualified designee before randomization and dosing with study drug is initiated. Eligible subjects will be randomized in a 1:1 ratio to treatment (either GMI-1070 or placebo) using an IVRS.

Note – A sample will be drawn for hemoglobin electrophoresis at baseline, but the result is not required prior to randomization or first dose.

6.1.3 Visit 1

Visit 1 is considered to be the period of hospitalization, during which subjects will be dosed and samples will be obtained for PK assessments and, where applicable, biomarkers and IVM.

All subjects should be dosed with study drug within 24 hours of initial medical evaluation in the Emergency Department/clinic at the latest. Dosing will continue q12 hours until study drug is discontinued. The weight measured at Baseline will be used to determine the appropriate dose administered throughout the study. Study drug will be administered as described in Section 5.1.2.

Results of all assessments, particularly AEs and VAS scores, should be reviewed by the investigator or qualified designee on a daily basis after dosing with study drug is initiated. At a minimum, this review will be documented by the reviewers dated initials.

The calendar date and 24-hour clock time of the beginning and end of each infusion will be recorded in the medical record and on the CRF.

PK sampling will commence as described in Section 6.4. Biomarker and IVM sampling will commence at selected sites. AE and concomitant medication information will be collected during this period.

Subjects who remain hospitalized after Day 7 will undergo the following assessments for each additional day of hospitalization up to Day 28:

- Days 8 to 12: Same assessments expected for Days 1 to 7 (Visit 1).
- Days 13 to 28: Same assessments expected for Days 1 to 7 (Visit 1) with VAS scores collected daily instead of q4 hours.
- Visits 2, 3, 4 should be completed at the assigned times whether the subject is discharged from the hospital or remains hospitalized.

6.1.4 Visit 2

Visit 2 will occur 36 ± 12 hours after the last dose of study medication, during which time the subject may or may not still be hospitalized.

6.1.5 Visit 3

Visit 3 will occur 7 ± 3 days after the last dose of study medication.

6.1.6 Visit 4

Visit 4 will occur 28 ± 5 days post last dose and is the last follow-up for the study.

6.2 Efficacy Evaluation

Resolution of VOC will be assessed via a 10 cm VAS scale (to be provided with the CRF), IV opioid and IV NSAID use, readiness for hospital discharge, and time of actual hospital discharge.

VAS data will be recorded a minimum of every 4 hours (while awake) throughout the inpatient study period. Subject pain cards will be provided for use at each pain assessment. The subject will be asked a general question, such as, "how is your pain now?" and the subject will record the answer on a continuous pain scale. The card will be placed in the source record.

6.3 Safety Evaluations

6.3.1 Physical Examination

A full physical examination includes a review of the following body systems:

- general appearance
- skin
- head, ears, eyes, nose, throat
- spine/neck/thyroid
- respiratory
- cardiovascular
- abdomen (including liver and kidneys)
- nervous system
- musculoskeletal

A targeted physical examination includes body systems selected at the discretion of the investigator. At a minimum, this will include respiratory, cardiovascular, and abdominal examinations. Additional examination components may be added at the discretion of the investigator.

Any abnormalities or changes in intensity noted during the review of body systems should be documented and reported in the medical record and on the appropriate CRF page. If a new clinically significant finding occurs at the time of or subsequent to dosing, an AE form must be completed. In addition, resolution of any abnormal, clinically significant findings during the study will be noted in the medical record and the CRF.

6.3.2 Vital Signs

Complete vital signs are considered to be body temperature, respirations, blood pressure, and pulse (taken after 5 minutes seated or supine). Oxygen levels may be included as appropriate.

During Visit 1 vital signs should be recorded pre- and post-dose. Otherwise, when the time of vital signs measurement coincides with a blood draw, the vital signs will be taken before the scheduled blood draw.

Abnormal vital signs are not to be considered AEs unless thought to be clinically significant by the investigator or treating physician. The sponsor will routinely evaluate vital signs to investigate possible emerging trends.

6.3.3 Clinical Laboratory Evaluations

All laboratory assays will be performed locally according to the laboratory's normal procedures. Reference ranges will be supplied by the local laboratory. Laboratory assays may be repeated once to rule out laboratory error, before determining if results are normal or abnormal. It is recognized that in SCD, laboratory values that are out of the reference range may in fact be baseline for that individual. When test results are outside the reference range, the investigator (or his/her sub-investigator) will indicate on the medical record/laboratory report and on the CRF whether the result is considered to be clinically significant or not. Out of range results not considered to be clinically significant by the investigator (or his/her qualified sub-investigator) will not be considered abnormal for the purposes of this study. Abnormal laboratory values (out of range and clinically significant) which are unexpected or not explained by the subject's clinical condition and which are a worsening from the subject's baseline should be repeated until confirmed, explained, or resolved, and should be reported as AEs. The sponsor will routinely evaluate clinical laboratory findings to investigate possible emerging trends. If urinalysis is positive for blood, protein, or leukocyte esterase, a microscopic examination for RBC, WBC, bacteria, and casts will be performed.

Data to be collected are noted below. Collection for clinical laboratory evaluations will occur once daily and time of collection must be recorded in the CRF.

Table 7. Clinical Laboratory Evaluations

Hematology	
Hemoglobin	Platelet count
Hematocrit	WBC count with differential
RBC count	High sensitivity C-reactive protein (hsCRP)
Reticulocyte count	Hemoglobin Electrophoresis (at Baseline only)
Serum Chemistry	
Blood urea nitrogen	Alanine transaminase (ALT/SGPT)
Creatinine	Aspartate aminotransferase (AST/SGOT)
Electrolytes with glucose	Fractionated bilirubin
	Lactate dehydrogenase (LDH)
Urinalysis	
pH	Bilirubin
Specific gravity	Blood
Protein	Nitrite
Glucose	Urobilinogen
Ketones	Leukocyte esterase

6.3.4 Pregnancy Test

A pregnancy test (urine or serum at the discretion of the investigator) is required for women of childbearing potential. Females who are surgically sterilized for at least 6 months OR more than 2 years postmenopausal can be excluded from this requirement.

Any pregnancies occurring during the study will be handled as described in Section 6.3.6.5.

6.3.5 Adverse Events and Serious Adverse Events

AEs and SAEs will be reviewed on an ongoing basis by the Sponsor Medical Monitor to identify safety concerns. Relevant safety issues will be communicated to participating investigator(s) and regulatory agencies as appropriate.

6.3.5.1 Definition of Adverse Events

An AE is any untoward medical occurrence in a subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. Adverse events may be reported by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test, or other means.

AEs include (International Conference on Harmonization E2A[29]):

- Any new undesirable medical experience or an unfavorable and unintended change of an existing condition that occurs during or after treatment, whether or not considered related to study drug
- Abnormal laboratory findings considered by the investigator to be clinically significant, ie, those that are unusual for the populations being studied or individual subject

The subjects will be instructed to inform the investigator or clinical staff of any AEs and intercurrent illnesses experienced during the trial. The inquiry will be posed in a non-specific manner using open-ended questions so as not to bias the response (eg, "How are you today?" or "How have you been since your last visit?"). Any subject who has an AE will be evaluated by the investigator and will be treated.

6.3.5.2 Grading of Adverse Events

Each sign or symptom reported will be graded in accordance with the World Health Organization (WHO) Common Toxicity Criteria (Appendix 1). The date of AE onset, time of onset, duration, severity, relationship, outcome, and use of concomitant treatment will be recorded for each event.

Except as noted below, the following severity classifications will be used if the AE does not have a toxicity grade listed in Appendix 1.

- Mild: The AE is of little concern to the subject and/or of no clinical significance. The event is not expected to have any effect on the subject's health or well-being.
- Moderate: The subject has enough discomfort to cause interference with or change in usual activities. The event is of some concern to the subject's health or well-being. The event may require medical intervention.
- Severe: The subject is incapacitated and unable to work or participate in many or all usual activities. The event is of definite concern to the subject and/or poses substantial risk to the subject's health or well-being. The event is likely to require medical intervention and/or close follow up.

The investigator (a study physician listed on Form FDA 1572) will review each event and assess its relationship to drug treatment (unrelated, possibly related, or probably related). The investigator should decide whether, in his or her medical judgment, there is a reasonable possibility that the event may have been caused by the investigational product.

The relationship of each AE to study drug will be assessed using the following guidance:

- Unrelated: The event can be readily explained by other factors such as the subject's underlying medical condition, concomitant therapy, or accident and no obvious temporal relationship exists between the investigational product and the event.

- Possibly related: There may be some temporal relationship between the event and the administration of the investigational product but there remains some ambiguity as to the cause.
- Probably related: The temporal relationship between the event and the administration of the investigational product is compelling, and/or follows a known or suspected response pattern to that product, and the event cannot be explained by the subject's medical condition, other therapies, or accident.

The outcome may be classified as follows:

- Resolved: The subject has fully recovered from the event with no residual effects observable.
- Stabilized: Effects of the event are constant. The likelihood of these effects changing (improving or worsening) is low.
- Ongoing: Effects of the event are still present and changing. The event is not considered stabilized or resolved.
- Death Due to Event[ii]: The event was the primary cause of death (may or may not be the immediate cause of death).
- Death Due to Other Event: The subject died but the event reported was not the primary cause of death.

6.3.6 Recording and Period of Observation

All AEs and SAEs occurring from time of first study procedure (informed consent, and assent, where applicable) through day 28 ± 5 post last dose will be collected and recorded in the source document. All AEs and SAEs occurring from first dose of study drug through day 28 ± 5 post last dose will be collected and recorded on the CRF, whether or not they are considered to be related to study medication. Whenever possible, recognized medical terms should be used. If the investigator is confident of a unified diagnosis, all related signs, symptoms, and abnormal laboratory test results are to be grouped together as a single AE on the CRF (eg, cough and rhinitis are to be reported as "upper respiratory tract infection"). However, signs and symptoms considered unrelated to a syndrome or disease are to be reported as individual events.

Clinically significant worsening of pre-treatment untoward events, after initiation of study drug, must be reported as new AEs.

Adverse events occurring secondary to other events are to be identified by the primary cause. Death is an outcome of an event. The event that resulted in death should be recorded on the AE page of the CRF.

For hospitalizations for surgical or diagnostic procedures, the illness leading to the procedure is to be recorded as an SAE. The procedure is to be documented in the narrative as "action taken" in response to the illness.

[ii] Death is an outcome of an event and not an event per se (sudden death or death of unexplainable causes can be reported but follow-up will be required until cause of death is determined).

Following discharge from the hospital, if the AE is still not resolved, the subject will be referred to the subject's own physician, and followed up until the findings return to normal or acceptable levels, as judged by the investigator. Where appropriate, medical tests and examinations will be performed to document resolution of event(s).

All AEs will be followed to resolution (the subject's health has returned to his/her baseline status or all variables have returned to normal), an outcome is reached, stabilization (the investigator does not expect any further worsening of the event), or the event is otherwise explained, regardless of whether or not the subject is still participating in the study. Where appropriate, medical tests and/or examinations will be performed to document resolution of events.

6.3.6.1 Disease Related Adverse Events (DRAEs)

It is recognized that VOC and pain are frequent and expected events in SCD, and will be present at baseline in all subjects enrolled in this study. Fluctuations in pain are the expected and usual course of VOC and will not be reported as AEs. Pain and fluctuations in pain will be collected as part of the primary study objective, and evaluated extensively in the efficacy analysis. Changes in pain resulting from causes other than VOC should be reported as AEs. The use of opioids for VOC episodes is usually required and considered routine. Subjects being admitted to the hospital for VOC will be expected to receive IV opioid or IV pain medications for treatment of the VOC. It is also recognized that subjects being discharged from the hospital for VOC will go home with prescribed opioid and other pain medications.

After discharge from the hospital following a VOC episode, subjects often have recurrent or repeat episodes of VOC. For the purposes of this study, <u>after</u> discharge from the hospital for the original event, VOC (new or recurring) shall be defined as an episode requiring a visit to a medical facility resulting in medical treatment for pain. When determining grade of an AE for new or recurring VOC, the investigator shall use the classification noted below.

- Mild: the pain can be managed at home (includes home oral opioid pain medication)
- Moderate: the pain requires medical intervention (includes clinic, day hospital, or emergency department care)
- Severe: the pain requires inpatient treatment

Patients with SCD, as a result of this condition, commonly have abnormal laboratory values and clinical findings. It is assumed that abnormalities in physical exam or laboratory findings consistent with the subject's underlying diagnosis of SCD or VOC will be present prior to dosing and therefore will not be treatment-emergent AEs (TEAEs).

In general, symptoms of SCD should not be classed as AEs if they are within the normal day-to-day fluctuation expected for the disease. However, significant

worsening of the symptoms should be recorded as an AE. Therefore, DRAEs that are also TEAEs will be reported as AEs per study procedures.

6.3.6.2 Vital Signs and Clinical Laboratory Evaluations

Abnormal vital signs are not to be considered AEs unless thought to be clinically significant by the investigator or treating physician.

Abnormal laboratory values which are out of range and clinically significant and which are a worsening from the subject's baseline should be reported as AEs. When evaluating such changes, the extent of deviation from the reference range or a subject's baseline value, the duration until return to the reference range/baseline, either while continuing treatment or after the end of treatment with the investigational product, and the range of variation of the respective parameter within its reference range, must be taken into consideration.

The investigator should decide, based on the above criteria and the clinical condition of a subject, whether a change in a vital sign or laboratory parameter is clinically significant and therefore represents an AE.

The Sponsor Medical Monitor will routinely evaluate clinical laboratory findings and vital signs to investigate possible emerging trends.

6.3.6.3 Serious Adverse Event

An SAE is any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Results in permanently disabling or incapacitating condition
- Requires inpatient hospitalization
- Prolongs inpatient hospitalization
- Is a congenital anomaly/birth defect
- Is an important medical event that may not result in death, be life-threatening, or require hospitalization but which, based upon appropriate medical judgment, may jeopardize that subject and may require medical or surgical intervention

All SAEs will be reported by the investigational staff to the contract research organization (CRO) SAE Hotline within 1 working day of their knowledge of the event and followed by a written report within 2 working days, whether or not the SAEs are deemed drug-related.

If an SAE occurs, the CRO SAE Hotline is to be contacted immediately (refer to contact information in the Study Reference Manual).

The initial report of an SAE should include the following minimum information: an identifiable subject; the study drug; an identifiable reporting source; and an event or

outcome that can be identified as serious. The investigator or designee must also inform the Institutional Review Board (IRB)/Independent Ethics Committee (IEC), in compliance with GCP and local IRB/IEC reporting guidelines, of an SAE, whether or not considered study-related. The initial written report to the CRO and to the IRB/IEC must be as complete as possible.

Questions regarding safety issues and any SAEs may be directed to the SAE Hotline or the CRO Medical Monitor (refer to contact information in the Study Reference Manual).

If deemed appropriate for medical treatment decision-making purposes, the randomization assignment for individual subjects may be unblinded (refer to Section 5.1.5 for details of unblinding).

The Sponsor will be responsible for reporting SAEs to regulatory agencies (FDA, Health Canada, or other national or foreign regulatory authorities) as required.

6.3.6.4 Dose Limiting Toxicities and Study Stopping Rules

Dose-limiting toxicity will be defined as treatment-emergent SAEs deemed by the Sponsor Medical Monitor and Data Safety Monitoring Board (DSMB) to be related to study drug per this protocol, and to reach a level of significance for the study that requires investigation before proceeding with further enrollment or dosing in the study.

The Sponsor Medical Monitor will perform ongoing review of all SAEs in a timely fashion, and will perform frequent reviews of all AEs and lab data in the study. Any concerns for safety identified by the Medical Monitor will be communicated to the DSMB for review.

An interim safety review will be performed by the DSMB and Sponsor Medical Monitor after treatment of the first 10 subjects under age 18 years, and additionally after 25 and 50 subjects of all ages have been treated with the study drug. This review will include AEs and any other pertinent information. There will be 2 levels of review. First, the Sponsor Medical Monitor and DSMB will review safety data in a blinded fashion, to identify any possible concerns. Second, the DSMB will hold a closed meeting to review safety data by treatment group. The DSMB Chair will have the option to request a fully unblinded review by the DSMB of any data deemed appropriate. The Medical Monitor will not attend an unblinded data review meeting. If any safety concerns arise, the DSMB will make a recommendation to the Sponsor Medical Monitor on how to proceed.

Enrollment in the study will be temporarily halted for further review of safety data if the following are seen and deemed to be possibly or probably related to study drug. This further assessment of safety data will include review by the DSMB, which may ask for blinded or unblinded data at the discretion of the Chair of the DSMB. The

Sponsor Medical Monitor and Coordinating Investigator may also take part in this review, but not in a manner that would unblind either of these individuals. Events that would trigger halting enrollment and a more in-depth safety review include:

- >30% of subjects with recurrent VOC requiring hospitalization within 7 days of discharge for the current study
- >30% of subjects with documented systemic bacterial infections in normally sterile sites (excluding uncomplicated urinary tract infection and otitis media)
- >5% of subjects with death from SCD-related cause or other medical cause during the study or within 28 days of last dose of study drug

In addition, the study may be halted for further review:

- If, for any reason involving safety, the investigator deems continuing the study inappropriate, and halts the study at that site
- If, for any reason, the Sponsor deems continuing the study inappropriate

If the study is halted temporarily or terminated, a written statement fully documenting the reasons for study halt or termination will be provided to the IRB/IEC and appropriate regulatory authorities.

6.3.6.5 Pregnancy

Pregnancy occurring in a female subject during the study period will be documented in the subject's source documents and reported on a Pregnancy Report form to the SAE hotline and to the IRB/IEC by the investigational staff within 1 working day of their knowledge of the event. Pregnancy itself is not an AE; it should be reported for tracking purposes. The investigator or designee will discontinue the pregnant subject from the treatment aspects of the study (PK testing, biomarker testing, and IV GMI-1070 administration), continue to follow her per protocol for safety outcomes only, and advise her to seek prenatal care and counseling from her primary care provider. Data on fetal outcome and breast-feeding will be collected for regulatory reporting and drug safety evaluation.

If the pregnancy is due to a subject's failure to comply with the contraceptive requirements of this protocol, this should also be documented as a protocol deviation.

Complications of pregnancy or congenital abnormalities in the newborn child will be reported appropriately as AEs.

The site clinical staff will request the pregnant subject to notify the site of the outcome of the pregnancy (ie, birth, loss, or termination). To help ensure this, the site clinical staff will follow-up with the subject until the end of pregnancy, with the subject's consent. This request and the subject's response will be documented in the subject's source document.

6.4 Pharmacokinetics Sampling

Plasma PK samples will be obtained prior to first dose and at 14 different timepoints following the start of the first dose for a total of 15. These timepoints have been selected so as to describe the time course of optimal plasma levels of GMI-1070, samples being obtained more frequently when plasma concentrations are changing rapidly and less frequently when they are changing more slowly. Although 15 different sample timepoints are identified, it is not practical to obtain that many samples from each individual in this target population, as a result of limited venous access and preexisting anemia. Thus, a "sparse sampling" approach, coupled with population PK methods, has been adopted to decrease the number of samples obtained from each subject. Each subject will be sampled 9 times from a pre-determined subset of the complete collection of timepoints. Subjects will be assigned to 1 of 2 sampling groups (Table 8 and Table 9). This approach – sparse sampling coupled with population PK analysis methods – is widely accepted as appropriate for determining PK parameters in clinical studies.

Beginning at the start of the second or third dose, urine will be collected over 12 hours for each subject. A sample of the urine collected will be tested to determine the concentration of GMI-1070. The urine concentration and volume will be used to determine the amount excreted over the collection period.

Samples will be shipped according to the Sponsor's instructions to the bioanalytical laboratory (refer to the Study Reference Manual for contact information). Specific details for sample handling, storage, and shipping may be found in the Study Reference Manual. Once PK testing is complete on these samples, they will be used for biomarker analysis. When all study-related testing is complete, the samples will be destroyed.

Pharmacokinetics Sampling

Total plasma sampling for PK will be performed at the following times during the period of hospitalization. For each set of doses, study staff should choose 1 of the doses for sampling based on timing of dose and staff availability:

Visit 1

Pre Loading Dose:

- Pre dose of study drug

Post Dose 2 or 3:

- 30 ± 10 minutes, and 1 ± 0.25, 2 ± 0.25, 4 ± 1, 8 ± 2, and 12 ± 2 hours post initiation of dosing
- Urine samples for PK will be collected for 12 hours starting immediately after administration of dose 2 or 3

Immediately Pre Dose 4 or 5:

- Pre dose of study drug

Immediately Pre Dose 6 or 7:

- Pre dose of study drug

Post Dose 6 or 7 (if still receiving study drug):

- 30 ± 10 minutes, and 1 ± 0.25, 2 ± 0.25, 4 ± 1, and 8 ± 2 hours post initiation of dosing

Visit 2

Post Last Dose:

- 36 ± 12 hours post initiation of last dose

Subjects will be divided into 2 groups (Group A or B) based on randomization for a sparse PK sampling plan. The group assignment will determine the sampling time for each subject. Each subject will have 9 samples of 3 mL blood drawn per sample; and each subject will have urine collected. Biomarker testing will be done on the plasma remaining from sparse PK samples.

Table 8. Group A Schedule of Sparse Pharmacokinetics, Biomarkers, and Intravital Microscopy Sampling Assessments

	Visit 1															Visit 2
			Post Dose 2 or 3								Post Dose 6 or 7 (if still receiving study drug)					Post Last Dose
			Min	Hour							Min	Hour				
ALL SITES	Pre-Loading Dose	Loading Dose	30± 10	1± 0.25	2± 0.25	4± 1	8± 2	12 ± 2[1]	Immed. Pre Dose 4 or 5	Immed. Pre Dose 6 or 7	30± 10	1± 0.25	2± 0.25	4± 1	8± 2	36± 12
PK, Group A	✔		✔		✔		✔		✔	✔		✔		✔		✔
Urinalysis			✔[2]													
SELECTED SITES																
IVM and Additional Biomarkers[3]	✔		✔			✔	✔		✔	✔						✔

Table 9. Group B Schedule of Sparse Pharmacokinetics, Biomarkers, and Intravital Microscopy Sampling Assessments

	Visit 1															Visit 2
			Post Dose 2 or 3								Post Dose 6 or 7 (if still receiving study drug)					Post Last Dose
			Min	Hour							Min	Hour				
ALL SITES	Pre-Loading Dose	Loading Dose	30± 10	1± 0.25	2± 0.25	4± 1	8± 2	12 ± 2[1]	Immed. Pre Dose 4 or 5	Immed. Pre Dose 6 or 7	30± 10	1± 0.25	2± 0.25	4± 1	8± 2	36± 12
PK, Group B	✔			✔		✔		✔	✔		✔		✔		✔	✔
Urinalysis			✔[2]													
SELECTED SITES																
IVM and Additional Biomarkers3	✔		✔			✔	✔		✔	✔						✔

[1] Must be drawn before next dose.

[2] Collected for 12 hours on the day of Dose 2 or 3, starting at beginning of dose.

[3] Sites capable of doing IVM and additional biomarker tests will do so.

6.5 Clinical Activity Evaluations

6.5.1 Pain Management and Medications

Pain will also be assessed via the amount and duration of IV opioid, and IV NSAID use, and the time to transition to oral pain medications.

6.5.2 Complications of Disease

Complications of VOC will be assessed by recording the occurrence of acute chest syndrome, transfusions, stroke, death, etc, during the study.

6.5.3 Biomarkers of Adhesion and Inflammation

Markers of adhesion and inflammation will be tested on plasma samples taken for PK sampling, using the left-over sample once PK testing is complete. Biomarker tests may include soluble selectins, integrins, cytokines, and other related markers. Once PK and biomarker testing for this study is complete, samples will be destroyed.

High sensitivity CRP (hsCRP), which can also be considered a biomarker, will be tested as part of the clinical lab sampling done at the investigative site, as noted under "clinical laboratories" on Table 1.

6.5.4 Optional Site-Specific Biomarkers

In a subset of subjects at sites capable of performing these tests, IVM testing for microvascular blood flow, and blood sampling for other biomarkers of adhesion and inflammation, may be performed at the times specified in Table 8 and Table 9 as feasible. Where applicable, this will be described in a site-specific addendum to the Study Reference Manual. This testing will not proceed until the local IRB/IEC has approved an informed consent form detailing the specific procedures.

For this subset of subjects, IVM and additional biomarker sampling may be performed at the following times, as feasible:

Visit 1

Pre Loading Dose:

- Pre-dose

Post Dose 2 or 3:

- 30 ± 10 minutes post initiation of dose 2 or 3
- 4 ± 1, and 8 ± 2 hours, post initiation of dose 2 or 3

Pre Doses 4 or 5:

- Immediately prior to dosing

Pre Doses 6 or 7:

- Immediately prior to dosing

Visit 2

Post Last Dose:

- 36 ± 12 hours post last dose

Sampling may continue for up to 2 days post last dose, as feasible. Additional sampling times may be considered on a per-site basis. For sites participating in this portion of the study, all IVM and additional biomarker sampling to be considered on a per-site basis will be described in detail in the Study Reference Manual for that site.

6.5.5 Healthcare and Resource Utilization

Healthcare and resource utilization will be assessed during hospitalization via the following:

- Time to readiness for discharge (per subject and physician)
- Rate of transfer to ICU
- Diagnosis leading to transfer to ICU
- Duration of hospital length of stay
- Oxygen use
- IV antibiotic use
- Number and type of transfusions

Readiness for hospital discharge will be determined by asking the question, "Is your pain now at the level where you can manage it at home with oral medications?" This question will be asked with pain score assessments once IV opioid doses are being weaned. The time at which the subject responds in the affirmative will be noted in the source record. The time at which the physician caring for the subject and/or the investigator agrees with a similar statement ("the subject's pain is at a level where it can be managed at home on oral medications") will also be noted in the record.

7 DATA COLLECTION AND DATA MONITORING

7.1 Data Management

Data from CRFs and other external data will be entered into a clinical database as specified in the CRO's data management plan. Quality control and data validation procedures will be applied to ensure the validity and accuracy of the clinical database.

Data from CRFs and other external data will be reviewed and checked for omissions, apparent errors, and values requiring further clarification using computerized and manual procedures. Data queries requiring clarification will be documented and returned to the investigational site for resolution. Only authorized personnel will make corrections to the clinical database, and all corrections will be documented in an audit trail.

7.2 Data Monitoring

After study initiation, the investigational site will be visited by the CRO on behalf of the Sponsor to review the CRFs and source documents for completeness, accuracy, protocol compliance, subject safety, and adherence to guidelines outlined in the Study Reference Manual. The CRO representative will highlight any discrepancies found between source documents and the completed CRFs and ensure that appropriate site personnel address the discrepancies. When a discrepancy results in corrected CRF data, the correction will be initialed and dated on the CRF. Uniform procedures will be discussed at the site initiation. In addition, the Sponsor may visit the site on occasion to review study documents and procedures.

8 STATISTICAL ANALYSIS

The statistical analyses described in this section will be performed as further outlined in the Statistical Analysis Plan (SAP), which will be finalized prior to completion of the clinical portion of the study and will be included in the Clinical Study Report for this protocol. The SAP will give a detailed description of the summaries and analyses (primary and secondary) that will be performed. Changes from analyses planned in the protocol will be documented in the SAP. If changes to principal features stated in the protocol are required, these will be documented in a protocol amendment.

For the purpose of analysis, the end of study is defined as the date of final database lock.

All variables will be summarized at specified time points by dose level. Categorical variables will be summarized using counts (n) and percents (%) and will be presented in the form n (%). Continuous variables (eg, height, weight) will be summarized using the number of observations, mean, standard deviation and/or standard error, and minimum and maximum values.

8.1 Analysis Populations

The following subject subsets will be defined for this study.

- Intent to Treat (ITT) population: the set of all randomized subjects who have received at least one dose of study treatment. Efficacy analyses will be

performed on subjects from this population. Subjects in the ITT population will be assigned to the treatment to which they were randomized, regardless of the treatment they actually received.

- Safety population: the set of subjects who received at least 1 dose of study drug and have at least 1 safety assessment. All safety analyses, including analyses of AEs, clinical laboratory results, vital signs, and physical examinations will be conducted on the Safety population. Subjects in the safety population will be assigned to the treatment they actually received, regardless of the treatment to which they were randomized.
- PK population: The set of subjects who receive at least 1 dose of study drug and who have sufficient post-dose blood samples taken to contribute to the sparse PK sampling analysis. Subjects in the PK population will be assigned to the treatment they actually received, regardless of the treatment to which they were randomized.

8.2 Statistical Methods

8.2.1 Subject Disposition

The disposition of subjects at all scheduled visits will be determined on the basis of evaluations made at each visit. The disposition will be summarized by treatment group.

8.2.2 Demographics and Baseline Characteristics

Demographics and subject characteristics obtained at the baseline evaluation, including age, genotype, race, ethnicity, weight, and hemoglobin electrophoresis results, will be summarized descriptively using the ITT population.

8.2.3 Efficacy Analysis

The efficacy analysis will be performed on the ITT population. Study site and subject age will be included in the model as permitted by sample size.

8.2.3.1 Primary Efficacy Analysis

The primary efficacy endpoint is the time to resolution of VOC from the time the subject receives his or her first dose. Resolution of VOC is defined as the first of the following to occur:

- Sustained decrease in pain score of at least 1.5 cm out of 10 cm since baseline; AND transition to oral pain medications per hospital procedures (subject is on no IV pain medications),
- OR readiness for discharge as stated by the physician and subject,
- OR discharge to home.

The null hypothesis of no difference in time to resolution of VOC between active and placebo treatment groups will be evaluated by using the F-test from an

analysis of covariance (ANCOVA) model, controlling for sex, age and Hemoglobin A level. No censoring is anticipated as the time to discharge will be known for all subjects. Inferential testing will be performed using a fixed-sequence testing procedure.

8.2.3.2 Secondary Efficacy Analysis

Comparisons of time to reduced pain score by at least 1.5 out of 10 cm from the baseline value and time to transition to oral pain medications between the active and placebo treatment groups will be presented by Kaplan-Meier plots stratified by treatment group. The survival distribution will be compared by log-rank test. If there is no censoring in these outcomes, the ANCOVA model similar to the analysis for time to resolution of VOC will be used instead.

The effects of study drug on time to readiness for discharge and time to discharge will also be summarized by treatment group and evaluated by ANCOVA model, controlling for sex and age. Time to readiness for discharge as noted by the subject alone, by the physician alone, and by both the subject and physician, will be considered.

The cumulative amount of IV analgesic use (opioid and NSAID) from admission to discharge will be standardized using morphine equivalents. This amount will be compared between active and placebo treatment groups by an ANCOVA model, controlling for age and sex. The hourly IV analgesic usage over time will be modeled longitudinally using a linear mixed model with subject as a random effect and treatment group, time, sex and age as fixed effects.

The intensity of pain as measured by VAS will be analyzed longitudinally using a linear mixed model with subject as a random effect and treatment group, time, sex, age, and baseline pain score as fixed effects.

The differences in percentage of subjects with complications of VOC including acute chest syndrome, transfusion, stroke and death between active and placebo treatment groups will be tested using Mantel-Haenszel Chi-Square test or Fisher's Exact test, depending on the number of subjects with events.

The mean hsCRP value and mean change from baseline value will be summarized at each time point. If the normal ranges are different among sites, the standardized values will be presented. The difference in values between treatment groups will be analyzed using a linear mixed model with subject as a random effect and treatment group, time, sex, age and baseline measurement as fixed effects.

The results from IV antibiotic usage will be presented in listings. ICU stay and transfusion data including frequency, timing related to VOC and diagnosis leading to transfer to ICU (leading to transfusion) will also be presented in listings.

8.2.4 Safety Analysis

Assessment of safety is a secondary objective of the study and will be performed on the safety population. Study site and subject age will be included in the analysis as permitted by sample size.

8.2.4.1 Adverse Events

All AEs, including SAEs, will be coded using the MedDRA dictionary. Incidence, severity, and relationship to study medication of AEs will be tabulated by treatment group. Comparison of AEs between treatment groups will be made using a Mantel-Haenszel chi-square test or a Fisher's Exact Test. Summaries will be provided for each of the following types of AEs:

- TEAEs
- Treatment-emergent SAEs
- Treatment-emergent SAEs that were reported as "possibly" or "probably" related to study drug
- TEAEs that were reported as "possibly" or "probably" related to study drug
- TEAEs by severity

TEAEs will be defined as those AEs that begin on or after the date/time of study drug administration. Drug-related AEs will include those AEs that are reported by the investigator as possibly or probably related to the study drug. With the exception of the TEAEs by severity summary, all summaries will present the number of AEs and the number and percent of subjects having an AE by system organ class and by specific AE preferred term. The TEAEs by severity summary will only report the number and percent of subjects. For all percentages, the subjects in the Safety population by treatment group will comprise the denominator.

In addition to these summary tables, subjects with treatment-emergent SAEs and subjects who discontinued prematurely due to a TEAE will be presented in separate subject listings. The listings will provide all of the information reported for that AE and will include the length of time from study drug administration to the occurrence of the AE. If any deaths occur in the study, a similar listing of all TEAEs for subjects who died will be provided.

8.2.4.2 Disease Related Adverse Events (DRAEs)

Disease related adverse events (DRAEs) are defined as AEs that occur during a subject's participation in the study, but which are expected for the condition (SCD, VOC), and may include:

- anemia (down to Hb $\geq$5)
- elevated WBC count (up to WBC $\leq$20,000/mm^3)
- unconjugated hyperbilirubinemia
- elevated markers of hemolysis (AST and LDH)

- proteinuria
- hematuria
- hypertension
- VOC-related swelling/erythema/warmth of an extremity
- nausea/vomiting/constipation
- low fever under 101.5° F
- dehydration
- pruritus
- increased somnolence
- fall in reticulocyte count

DRAEs will be reported and analyzed as AEs per study procedures. In addition to the analysis of all AEs per the section above, DRAEs will be analyzed by treatment group.

8.2.4.3 Vital Signs and Laboratory Parameters

For vital signs and continuous laboratory parameters, the observed value and the change in the value from baseline at each collection point during the hospitalization will be summarized. For categorical laboratory parameters that are collected in terms of abnormal or normal or can be classified into positive or negative results, the number and percent of subjects having values in each category will be presented at each scheduled collection time. For those categorical laboratory parameters that are reported in several descriptive categories (eg, urine color), the number and percent of subjects by each category will be presented. Graphs displaying population trends over time may be produced.

For both vital signs and laboratory parameters, clinically significant results will be listed with relevant subject information for assessment.

8.2.4.4 Physical Examinations

Significant changes in physical examination over the study visits will be listed by subject and summarized across the study population as appropriate.

8.2.5 Pharmacokinetic Analysis

Assessment of PK is a secondary study objective and will be performed on the PK population.

PK data from the first 10 subjects receiving study drug was evaluated in a blinded, aggregate population PK analysis using the methods described here. The purpose was to ensure that GMI-1070 plasma concentration levels in SCD patients in crisis are similar to SCD patients not in crisis (GMI-1070-103) and healthy volunteers (GMI-1070-101 and GMI-1070-102). This information resulted in amendment to the protocol with a change in dose level to achieve the originally targeted plasma concentration levels. In addition, the first 10 subjects under 18 years of age

(regardless of dose level) will be similarly evaluated in a blinded aggregate analysis.

An additional, similar analysis of PK data from the first 10 subjects receiving the new dose level, a loading dose of 40 mg/kg followed by 20 mg/kg every 12 ± 2 hours, will be performed to confirm that anticipated GMI-1070 plasma concentration levels are achieved with the new dose level.

All such analyses will maintain the blind for all those involved in the clinical execution of the study, and will be conducted as follows. A PK model was developed using the plasma concentration data from the 3 previous studies. Covariates (eg, body size, age, sex, etc.) will be incorporated if appropriate, and this model will be updated at each interim analysis. This model will then be used to predict the expected plasma concentration profile from the subjects in the present study, incorporating the actual dosing regimen and covariates. Graphics will be used to assess if the plasma concentration values obtained in the new study are systematically larger or smaller than those predicted based on previous studies. If the new population is biased upward or downward, this information will be utilized to confirm or adjust dose, if necessary, for the remainder of the study. Any required changes in dose of study drug will be addressed in an amendment to this study protocol.

At completion of the study, a formal population PK analysis will be performed, using the structural model developed in previous studies (adapting if appropriate based on the data from the new subjects). This model will accomplish the following goals: to determine if the PK parameters in these subjects differ from those in the 2 previous patient populations, to obtain a set of "typical" parameter values for GMI-1070 in sickle cell patients, to determine which, if any, covariates should be considered in dosing future patients, and to guide selection of the optimal dose magnitude and dose interval for future dosing.

PK parameters to be estimated will include:

CL	Total plasma clearance
CL_{D2}	Clearance from the peripheral (non-central compartment)
V_1	Volume of the central compartment
V_2	Volume of the peripheral (non-central compartment)
C_{min}	Minimum observed drug concentration
C_{max}	Maximum observed drug concentration
t_{max}	Time of the maximum drug concentration (obtained without interpolation)
α	Apparent distribution rate constant
β	Apparent terminal elimination rate constant
$t_{1/2}$	Apparent elimination half-life, calculated as $\ln(2)/\beta$

Urine

PK parameters will be computed from the individual urine concentrations using a noncompartmental approach. Appropriate validated PK software (eg, WinNonlin Professional) will be used.

U_e	The amount excreted in urine during the collection interval (C_{urine}), calculated as C_{urine} x urine volume.
CL_r	Renal clearance, calculated as $CL_r = Ue/AUC_{0-t}$. CL_r will be calculated for the collection interval.
% Dose	The percentage of drug recovered in urine, calculated as (Cum_{Ue})/Dose x 100.

8.2.6 Biomarker Analysis

Biomarker assessments are secondary study objectives and will be performed on the ITT population to the extent data is available. The mean microvascular blood flow measurement and biomarker measurements of adhesion, and inflammation will be summarized by treatment group and time point. If there are enough data, these outcomes will be analyzed using a linear mixed model similar to hsCRP analysis.

8.2.7 Study Drug Interim Analysis

An interim safety review will be performed by the DSMB and Sponsor Medical Monitor after treatment of the first 10 subjects under 18 years of age, and additionally after 25 and 50 subjects of all ages have been treated with the study drug. This review will include AEs and any other pertinent information. The DSMB and the Sponsor's Medical Monitor will review select data listings to ascertain the existence of any safety concerns for which the study should be terminated, as described in Section 6.3.6.4. The Sponsor's Medical Monitor will remain blinded.

In addition to the safety review, the DSMB and a sponsor representative (not otherwise involved in the study execution) will also review the efficacy (clinical activity) results in the first 50 subjects. Stopping criteria for the efficacy interim analysis are discussed in Section 8.3.2.

8.3 Statistical Considerations

8.3.1 Missing, Unused, or Spurious Data

Subjects who drop out of the study will not be replaced. All efficacy, safety, PK, and clinical activity data collected will be used to the extent possible. Analysis of improvement in pain is based on the assumption of a normal distribution and no censoring. If there are many subjects who withdraw from study prior to discharge, the survival analysis that takes censoring into account will be performed instead.

The additional clinical activity analyses using a longitudinal mixed model allow for missing data and do not require eliminating the subject for missing data.

8.3.2 Sample Size

Evaluable subjects are those who have received at least 1 dose of study medication. Subjects in the evaluable population will be used to determine when an adequate sample size has been achieved.

The study will enroll until a total of approximately 76 evaluable subjects (defined as those who have received at least 1 dose of study medication) have been enrolled. An interim analysis will be done after the first 50 subjects have completed the follow-up day 7 post last dose, to assess safety and efficacy. The efficacy test statistic for this interim analysis will be compared to a critical value corresponding to a 2-sided alpha of 0.005. To control study wise Type I error, an alpha spending approach will be utilized. The final analysis will then compare the test statistic to a critical value corresponding to 2-sided alpha of 0.045. Futility criteria will also be considered; these will be specified in detail in the statistical analysis plan.

A previous pilot study of treatments for pain in SCD showed the mean pain duration in placebo group is 103 hours with a standard deviation of 60 hours.[30] The original protocol sample sizing was done as follows. Assuming that the variances are the same in both groups and anticipating that the study drug will decrease pain duration by 40%, a total of at least 76 subjects (38 subjects each group) will be needed to detect a 40% decrease in VOC resolution and to achieve 83% power with a 2-sided alpha of 0.045.

With the addition of a second dose level, the power calculation for the study has been updated to compare the treatment difference between the placebo group and the pooled active group (ie, both dose levels combined). The sample sizing assumes that the placebo group has a mean pain duration of 103 hours with a standard deviation of 60 hours, and that both groups – placebo and pooled active groups – share a common standard deviation. Anticipating that the placebo and pooled active groups will each have about 38 subjects (ie, the pooled group will have 19 subjects from each of the dose levels) the study will have an 89% power to detect a 43.8% decrease in VOC duration with a 2-sided alpha of 0.045.

The sample size is also expected to provide initial safety and PK conclusions sufficient to contribute to the design of subsequent trials.

9 SPONSOR'S AND INVESTIGATOR'S RESPONSIBILITIES

This study will be conducted in accordance with current applicable regulations, ICH and local ethical and legal requirements.

9.1 Sponsor's Responsibilities

9.1.1 GCP Compliance

GlycoMimetics, and any third party to whom aspects of the study management or monitoring have been delegated, will undertake their roles for this study in compliance with all applicable regulations and ICH GCP Guideline E6.[1]

Visits to investigator sites will be conducted by representatives of GlycoMimetics or an appropriate designee to inspect study data, subjects' medical records, and CRFs in accordance with current GCP and the respective local and national government regulations and guidelines. Records and data may additionally be reviewed by auditors, interested commercial parties, or by regulatory authorities.

9.1.2 Regulatory Approval

GlycoMimetics will ensure that local regulatory authority requirements are met before the start of the study. GlycoMimetics or an appropriate designee will be responsible for the preparation, submission, and confirmation of receipt of any regulatory authority approvals required prior to release of investigational product for shipment to the study site.

9.1.3 Protocol Management

All protocols and amendments will be prepared by GlycoMimetics. If it becomes necessary to issue a protocol amendment during the course of the study GlycoMimetics or an appropriate designee will notify the investigators and collect documented Investigator Agreements to the amendment.

Any change to this protocol significantly affecting the safety of subjects, the scope of the investigation, or the scientific quality of the study, requires a written protocol amendment that must be approved by the Sponsor, the investigator, and the IRB/IEC before implementation.

Examples of amendments requiring such approval are:

- An increase in drug dosage or duration of exposure of subjects
- A significant change in the study design (eg, addition or deletion of a group or period)
- An increase in the number of invasive procedures to which subjects are exposed
- Addition or deletion of a test procedure for safety monitoring

These requirements for approval should in no way prevent any immediate action from being taken by the investigator or Sponsor in the interest of preserving the safety of all subjects included in the study. If an immediate change to the protocol is felt to be necessary by the investigator and is implemented by him/her for safety

reasons, the Sponsor should be notified within 2 working days and the IRB/IEC should be informed within a reasonable timeframe.

Changes to the protocol affecting only administrative aspects of the study do not require formal protocol amendments or IRB/IEC approval; however, the IRB/IEC must be kept informed of such administrative changes. Examples of administrative changes not requiring formal protocol amendments and IRB/IEC approval include:

- Minor changes in wording (ie, typographical errors, deletion of double-entry words or sentences, adding words for clarification)
- Clarification/modification of procedure instructions
- Change/correction of addresses or phone numbers
- Addition of new clinical sites

9.1.4 Additional Study Sites

The study may be expanded to additional clinical sites, at the discretion of the Sponsor.

9.2 Investigator's Responsibilities

9.2.1 GCP Compliance

The investigator must undertake to perform the study in accordance with ICH GCP Guideline E6[1] and the applicable regulatory requirements.

It is the investigator's responsibility to ensure that adequate time and appropriate resources are available at the study site prior to commitment to participate in this study.

The investigator will maintain a list of appropriately qualified persons to whom the investigator has delegated significant trial-related tasks. An up-to-date copy of the *curriculum vitae* for the investigator and sub-investigator(s) will be provided to GlycoMimetics or an appropriate designee before starting the study.

If the subject has a primary physician the investigator should, with the subject's consent, inform them of the subject's participation in the trial.

9.2.2 Protocol Adherence

The investigator must adhere to the protocol as detailed in this document. The investigator will be responsible for enrolling only those subjects who have met protocol eligibility criteria.

It is the investigator's responsibility to communicate with his/her local IRB/IEC to ensure accurate and timely information is provided at all phases during the study. In particular the appropriate approvals must be in place prior to recruitment, notification of any SAEs during the study must take place and the IRB/IEC must be informed of study completion.

9.3 Documentation and Retention of Records

9.3.1 Case Report Forms

CRFs will be supplied by GlycoMimetics or an appropriate designee and should be handled in accordance with instructions from GlycoMimetics/designee.

The investigator is responsible for maintaining adequate and accurate medical records from which accurate information will be transcribed onto CRFs. CRFs should be filled out completely by the investigator or designee as stated in the site delegation list. All CRFs should be completed in a neat legible manner to ensure accurate interpretation of the data; a black ball-point pen is preferred to ensure the clarity of reproduced copies of all CRFs.

All requested information must be entered on CRFs. If an item is not available or is not applicable, this fact should be indicated without leaving a blank space. Incorrect entries should be crossed out with a single line.

The CRFs must be reviewed, signed, and dated by the investigator.

Once the study monitor has verified the contents of the completed CRF pages against the source data, the duplicate pages will be collected and forwarded to GlycoMimetics or an appropriate designee for data entry. Queries may be raised if the data are unclear or contradictory. The investigator or designee must address all queries.

9.3.2 Recording, Access and Retention of Source Data

Source data to be reviewed during this study will include, but is not limited to: subject's medical file, subject pain cards, original laboratory reports, histology, and pathology reports.

All key data must be recorded in the subject's medical records.

The investigator must permit authorized representatives of GlycoMimetics, the respective national, local or foreign regulatory authorities, the IRB/IEC, auditors, and interested commercial parties to inspect facilities and original records relevant to this study, regardless of media.

The monitor (auditors, IRB/IEC, or regulatory inspectors) may check the CRF entries against the original source documents (regardless of media). The consent form and documents in compliance with applicable privacy regulations (eg, Health Insurance Portability and Accountability Act authorization [HIPAA] and Personal Information Protection and Electronic Documents Act [PIPEDA]) will include a statement by which the subjects allow the monitor/auditor/inspector from GlycoMimetics or an appropriate designee, national or local regulatory authorities, or the IRB/IEC access to source data (eg, subject's medical file, appointment books, original laboratory reports, X-rays, etc.) which substantiate information

recorded in the CRFs. The informed consent form (and assent, where applicable) and applicable authorizations to disclose private information should be worded to ensure the above listed entities access to the essential documents for the entire retention period described below.

As described in the ICH GCP Guidelines, 'essential documents', including CRFs, source documents, consent forms, laboratory test results, and investigational product inventory records, should be retained by the investigator until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period however if required by the applicable regulatory requirements or by an agreement with GlycoMimetics.

These records must be made available at reasonable times for inspection and duplication, if required, by a properly authorized representative of the US FDA in accordance with the US Code of Federal Regulations (CFR) 21 CFR 312.68, or by Health Canada or other national or foreign regulatory authorities.

9.3.3 Investigational Product Accountability

All investigational product required for completion of this study will be provided by GlycoMimetics. The recipient will acknowledge receipt of the investigational product indicating shipment content and condition. Damaged supplies will be replaced. Accurate records of all investigational product dispensed, used, and returned, and if applicable, destroyed, will be maintained.

9.3.4 Audit/Inspection

To ensure compliance with relevant regulations, data generated by this study must be available for inspection upon request by representatives of national and foreign regulatory authorities, GlycoMimetics or an appropriate designee, interested commercial parties and the IRB/IEC for each study site.

9.4 Ethical Considerations

This study will be conducted in accordance with the US CFR 21, local, state, and national regulations and ICH guidelines.

9.4.1 Informed Consent

It is the responsibility of the investigator to obtain written informed consent (and assent, where applicable) from subjects. All consent and assent documentation must be in accordance with applicable regulations and ICH E6. [1] Each subject or the subject's legally authorized representative is requested to sign the subject informed consent form (as a certified translation, if applicable) after the subject has received and read the written subject information and received an explanation

of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences and the subject's rights and responsibilities.

A copy of the informed consent documentation (consent form and assent, where applicable) must be given to the subject or the subject's legally authorized representative. If applicable, it will be provided in a certified translation of the local language. Signed consent forms must remain in each subject's study file and must be available for verification by the study monitors at any time.

The investigator will provide the sponsor with a copy of the IRB/IEC approved consent (and assent where applicable) forms, and a copy of the IRB/IEC's written approval, prior to the start of the study.

The sponsor reserves the right to delay initiation of the study at a site where the informed consent (and assent, where applicable) forms do not meet the standards of applicable regulations and ICH E6.[1]

9.4.2 IRB/IEC Approval

It is the responsibility of the investigator to submit this protocol, the informed consent document, relevant supporting information, and all types of subject recruitment information to the IRB/IEC for review, and all must be approved prior to study initiation at that site. Prior to implementing changes in the study, GlycoMimetics and the IRB/IEC must also approve any revised informed consent documents and amendments to the protocol.

Investigational product supplies will not be released and the subject recruitment may not begin until written approval from the IRB/IEC to proceed with the study has been received by GlycoMimetics or an appropriate designee.

The investigator is responsible for keeping the IRB/IEC aware of the progress of the study and of any changes made to the protocol, but in any case at least once a year. The investigator must also keep the local IRB/IEC informed of any serious and significant adverse events.

9.5 Confidentiality

All US-based investigational sites and laboratories or entities providing support for this study, must, where applicable, comply with the HIPAA of 1996. An investigational site that is not a Covered Entity as defined by HIPAA must provide documentation of this fact to GlycoMimetics or an appropriate designee. Canadian sites must comply with applicable privacy regulations (eg, PIPEDA).

9.6 Publication Policy

The detailed obligations regarding the publication of any data, material results or other information generated or created in relation to the study shall be set out in the agreement between each investigator and the sponsor.

10 REFERENCES

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11 APPENDICES

APPENDIX 1 WHO COMMON TOXICITY CRITERIA

These criteria may be found at http://www.fda.gov/cder/cancer/toxicityframe.htm (accessed 24 March 2009).

Hematology					
Toxicity	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
WBC (x103/l)	4	3.0 - 3.9	2.0 - 2.9	1.0 - 1.9	< 1.0
Platelets (x103/l)	WNL	75.0 - normal	50.0 – 74.9	25.0 - 49.9	< 25.0
Hemoglobin (g/dL)	WNL	10.0 - normal	8.0 - 9.9	6.5 - 7.9	< 6.5
Granulocytes/ Bands (x103/l)	2	1.5 - 1.9	1.0 - 1.4	0.5 - 0.9	< 0.5
Lymphocytes ($x10^3$/l)	2	1.5 - 1.9	1.0 - 1.4	0.5 - 0.9	< 0.5
Hemorrhage	none	mild, no	gross, 1 - 2 units transfusion per episode	gross, 3 - 4 units transfusion per episode	massive, > 4 units transfusion per episode
Coagulation					
Toxicity	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Fibrinogen	WNL	0.99 - 0.75 x N	0.74 - 0.50 x N	0.49 - 0.25 x N	< 0.25 x N
Prothrombin time (Quick)	WNL	1.01 - 1.25 x N	1.26 - 1.50 x N	1.51 - 2.00 x N	> 2.00 x N
Partial thromboplastin time	WNL	1.01 - 1.66 x N	1.67 - 2.33 x N	2.34 - 3.00 x N	> 3.00 x N
Metabolic					
Toxicity	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Hyperglycemia (mg/dL)	< 116	116 - 160	161 - 250	251 - 500	> 500 or ketoacidosis
Hypoglycemia (mg/dL)	> 64	55 - 64	40 - 54	30 – 39	< 30
Amylase	WNL	< 1.5 x N	1.5 - 2.0 x N	2.1 - 5.0 N	> 5.0 x N
Hypercalcemia (mg/dL)	< 10.6	10.6 - 11.5	11.6 – 12.5	12.6 – 13.4	13.5
Hypocalcemia (mg/dL)	> 8.4	8.4 - 7.8	7.7 - 7.0	6.9 - 6.1	6
Hypomagnesemia (mg/dL)	> 1.4	1.4 - 1.2	1.1 - 0.9	0.8 - 0.6	0.5

Abbreviations: WNL=within normal limits.

WHO COMMON TOXICITY CRITERIA (cont)

Gastrointestinal

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Nausea	none	able to eat reasonable intake	intake significantly decreased but can eat	no significant intake	—
Vomiting	none	1 episode in 24 hours	2 - 5 episodes in 24 hours	6 - 10 episodes in 24 hours	> 10 episodes in 24 hours or requiring parenteral support
Diarrhea	none	increase of 2 - 3 stools/day over pre-Rx	increase of 4 - 6 stools/day, or nocturnal stools, or moderate cramping	increase of 7 - 9 stools/day, or incontinence, or severe cramping	increase of > 10 stools/day or grossly bloody diarrhea, or need for parenteral support
Stomatitis	none	painless ulcers, erythema, or mild soreness	painful erythema, edema, or ulcers but can eat solids	painful erythema, edema, or ulcers and cannot eat solids	requires parenteral or enteral support for alimentation

Liver

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Bilirubin (N = 17 µmol/L)	WNL	-----	< 1.5 x N	1.5 - 3.0 x N	> 3.0 x N
Transaminase (SGOT, SGPT)	WNL	2.5 x N	2.6 – 5.0 x N	5.1 - 20.0 x N	> 20.0 x N
Alk Phos or 5 nucleotidase	WNL	< 2.5 x N	2.6 – 5.0 x N	5.1 - 20.0 x N	> 20.0 x N
Liver- clinical	No change from baseline	-----	-----	Precoma	hepatic coma

Kidney, bladder

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Creatinine	WNL	< 1.5 x N	1.5 – 3.0 x N	3.1 - 6.0 x N	> 6.0 x N
Proteinuria	No change	1 (+) or < 0.3 g% or 3 g/L	2 - 3 (+) or 0.3 - 1.0 g% or 3 - 10 g/L	4 (+) or > 1.0 g% or > 10g/L	nephrotic syndrome
Hematuria	Negative	microscopic only	gross, no clots no Rx needed	gross and clots bladder irrigation	requires transfusion or cystectomy

WHO COMMON TOXICITY CRITERIA (cont)					
Weight gain/loss					
Toxicity	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Weight gain/ loss	< 5.0 %	5.0 – 9.9 %	10.0 – 19.9 %	20.00%	-----
Pulmonary					
Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Pulmonary	none or no change	asymptomatic, with abnormality in PFTs	dyspnea on significant exertion	dyspnea at normal level of activity	dyspnea at rest
Cardiac					
Toxicity	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Cardiac arrhythmias	none	asymptomatic, transient, requiring no therapy	recurrent or persistent, no therapy required	requires treatment	requires monitoring; or hypotension, or ventricular tachycardia or fibrillation
Cardiac function	none	asymptomatic, decline of resting ejection fraction by less than 20 % of baseline value	asymptomatic, decline of resting ejection fraction by more than 20% of baseline value	mild CHF, responsive to therapy	severe or refractory CHF
Cardiac ischemia	none	non-specific T- wave flattening	asymptomatic, ST and T wave changes suggesting ischemia	angina without evidence of infarction	acute myocardial infarction
Cardiac- pericardial	none	asymptomatic effusion, no intervention required	pericarditis (rub, chest pain, ECG changes)	symptomatic effusion; drainage required	tamponade; drainage urgently required
Hypertension	none or no change	asymptomatic, transient increase by greater than 20 mm Hg (D) or to > 150 / 100 if previously WNL. No treatment required.	recurrent or persistent increase by greater than 20 mm HG (D) or to > 150 / 100 if previously WNL. No treatment required.	requires therapy	hypertensive crisis
Hypotension	none or no change	changes requiring no therapy (including transient orthostatic hypotension)	requires fluid replacement or other therapy but not hospitalization	requires therapy and hospitalization; resolves within 48 hours of stopping the agent	requires therapy and hospitalization for > 48 hours after stopping the agent

Abbreviations: PFT=pulmonary function test, CHF=congestive heart failure,.

WHO COMMON TOXICITY CRITERIA (cont)

Neurologic					
Toxicity	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Neuro: sensory	none or no change	mild paresthesias; loss of deep tendon reflexes	mild or moderate objective sensory loss moderate paresthesias	severe objective sensory loss or paresthesias that interfere with function	-----
Neuro: motor	none or no change	Subjective weakness; no objective findings	mild objective weakness without significant impairment of function	objective weakness with impairment of function	paralysis
Neuro: cortical	none	mild somnolence or agitation	moderate somnolence or agitation	severe somnolence, (>50 % waking hours), agitation, confusion, disorientation, or hallucinations	coma, seizures, toxic psychosis
Neuro: cerebellar	none	Slight incoordination, dysdiadochokinesia	intention tremor, dysmetria, slurred speech, nystagmus	locomotor ataxia	cerebellar necrosis
Neuro: mood	no change	mild anxiety or depression	moderate anxiety or depression	severe anxiety or depression	suicidal ideation
Neuro: headache	none	mild	moderate or severe but transient	unrelenting and severe	-----
Neuro: constipation	none or no change	mild	moderate	severe	ileus >96 hours
Neuro: hearing	none or no change	Asymptomatic, hearing loss on audiometry only	Tinnitus	hearing loss interfering with function but correctable with hearing aid	deafness not correctable
Neuro: vision	none or no change	-----	-----	symptomatic subtotal loss of vision	blindness

Pain					
Toxicity	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Pain *	none	mild	moderate	severe	req. narcotics

WHO COMMON TOXICITY CRITERIA (cont)

Skin

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Skin	none or no change	scattered macular or papular eruption or erythema that is asymptomatic	scattered macular or papular eruption or erythema with pruritus or other associated symptoms	generalized symptomatic macular, papular or vesicular eruption	exfoliative dermatitis or ulcerating dermatitis

Alopecia

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Alopecia	no loss	mild hair loss	pronounced or total hair loss	-----	-----

Allergy

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Allergy	none	transient rash, drug fever < 38°C (100.4°F)	urticaria, drug fever 38°C (100.4°F), mild bronchospasm	serum sickness, bronchospasm requiring parenteral medication	anaphylaxis

Local

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Local	none	pain	pain and swelling with inflammation or phlebitis	ulceration	plastic surgery indicated

Fever of unknown origin

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Fever of unknown origin	none	37.1° - 38.0° C 98.7° - 100.4° F	38.1° - 40.0°C 100.5° - 104°F	> 40.0°C > 104.0°F for less than 24 hours	> 40.0°C (>104°F) for more than 24 hours or accompanied by hypotension

Infection

Toxicity	Grade 0	Grade 1	Grade 2	Grade 3	Grade 4
Infection	none	mild	moderate	severe	life-threatening

WHO COMMON TOXICITY CRITERIA (cont)	
Additional events	
Toxicity	
Asthenia	Analogous to Karnofsky index (WHO grading)
Chills	Analogous to fever
Peripheral edema	analogous to weight gain
Anorexia	analogous to weight loss

*See Sections 6.3.5.2 for grading of pain related to sickle cell disease.