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Title: Peripheral blood stem cell collection for sickle cell disease (SCD) patients using plerixafor

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Subjects of study:	Number	Sex	Age range
Patients	15	Either	18 and above

Project involves ionizing radiation? No
Project uses "Durable Power of Attorney"? No
Off site project? No
Multi-institutional project? Yes

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Precis:

The constitution of blood relies upon hematopoietic stem cells (HSCs), which stay in the bone marrow and differentiate to all lineages of peripheral blood cells. HSC transplantation is the only curative option currently available for sickle cell disease (SCD) patients either via allogeneic HSC transplantation or HSC-targeted gene therapy. Granulocyte-colony stimulating factor (G-CSF)-mobilized HSCs are frequently utilized in the adult setting of HSC transplantation because of the faster hematologic recovery as compared to bone marrow. As an autologous HSC source for gene therapy, bone marrow harvest has been generally employed since G-CSF has been prohibitive in SCD patients due to granulocyte stimulation and the associated reports of vaso-occlusive crises, multi-organ failure, and death. However, when bone marrow harvest is used, the amounts of collected cells are limited and anesthesia is required. In order to obtain HSCs in large numbers without anesthesia, patients will undergo mobilization followed by large volume apheresis. Plerixafor is an alternative treatment for mobilization without direct stimulation to granulocytes, and it is theoretically applicable for SCD patients. The primary endpoint of this study is to obtain sufficient amounts of HSCs collected from the peripheral blood in SCD patients after plerixafor mobilization with an acceptable safety profile. The harvested products will be stored as backup for patients undergoing gene therapy as well as allogeneic HSC transplantation.

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1.0 Introduction and Background

Sickle cell disease (SCD) is the first disease to be described at the molecular level and has long been targeted for treatment at the molecular level. The disease is associated with significant morbidity and early mortality, with the most recent estimates of median lifespan only into the 40s. SCD affects one of every 600 African Americans in the United States alone, with around 100,000 Americans with the disease, but in Africa, an estimated 300,000 births per year are affected by SCD.[1] This orphan disease is historically underserved, and represents an area of significant health disparity.

β-globin is an essential component of hemoglobin, and a specific mutation (20A>T) of β-globin distinguishes SCD. The mutated β-globin leads to hemoglobin polymerization upon deoxygenation in areas of low oxygen tension. As a result, patients develop recurrent pain crisis, anemia, infections, acute chest syndrome, and neurologic events, as well as sudden death as the most serious consequences of this disease.[2] In addition, iron overload due to chronic transfusion increases morbidity and mortality in SCD patients. The medical costs of this disease are enormous, with estimates of $40,000 per patient per year (year 2000 figures) for chronic red cell transfusion and chelation alone, and rising costs with age at $231,000 per year in total costs for adults due to accumulating end organ damage have recently been described.[3]

Red cell transfusions are commonly used to treat patients with SCD. Red cells are infused as needed to treat symptoms of anemia, acute and severe complications such as acute chest syndromes and strokes, or before elective surgeries. Red cell transfusions can also be given at timed interval (also known as chronic transfusion therapy) to decrease the incidence of stroke in pediatric patients with abnormal trans-cranial Doppler velocities (internal carotid or middle cerebral artery velocity >200 cm/s).[4] While transfusions can prevent further neurologic events in patients at risk, iron overload is common, resulting in significant end-organ toxicity. Thus, iron chelation has become an important part in the care of patients with SCD.

Hydroxyurea, the one and only FDA approved drug for this disease, results in a significant reduction in the number of pain crisis per year and a decreased frequency of acute chest syndrome,[5] and has become the treatment of choice for many individuals with SCD. Unfortunately, neither red cell transfusions nor hydroxyurea is curative, nor do they appear to reverse established end-organ damage. Hydroxyurea has been suggested to improve survival in patients with SCD.[6] However, life expectancy remains significantly shortened compared to the national average with that of an affected male being 47 years versus the national average of 72.[7]

The only established cure for patients with SCD is allogeneic bone marrow transplantation, and the procedure was initially only applied in children.[8-10] In adults, the higher burden of accumulated end-organ damage would be expected to result in higher transplantation associated mortality and morbidity.[8,11,12] Recently, we established allogeneic hematopoietic stem cell (HSC) transplantation for adult SCD patients, the vast majority of whom meet disease severity entry criteria, by using reduced intensity conditioning.[13,14] This strategy can cure more than 90% of transplanted SCD patients; however, it requires an HLA-matched sibling donor (without SCD) that can be found for ~10% of patients, leaving 90% of patients with SCD without a curative option.

We are developing both haploidentical allogeneic and autologous HSC-targeted gene therapy for SCD patients who lack an HLA-matched sibling donor. Though we have seen success in around

half of the patients undergoing haploidentical allogeneic transplants, rejection rates remain high, and prolonged cytopenias have been observed in some SCD patients after rejection prompting us to require back up harvests for all patients on this experimental protocol. In addition, we are developing gene therapy approaches which utilize the patients' own HSC. With gene therapy, harvested HSC products are immunomagnetically purified for the CD34+ HSC population, and the purified HSCs are genetically modified to correct the β-globin gene in *ex vivo* culture by gene addition using lentiviral vectors. The genetically modified autologous HSCs are subsequently infused after conditioning. Our initial patients treated with a gene therapy approach have required multiple bone marrow harvests to proceed to transplantation on this protocol which also requires back up bone marrow harvesting.

Peripheral blood is the current preferred source for HSCs in both autologous and allogeneic transplant applications. Mobilization of HSCs into the circulation can be achieved by the administration of granulocyte-colony stimulating factor (G-CSF) and/or plerixafor, allowing the collection of bone marrow derived HSCs from the peripheral blood by apheresis.[15,16] The mobilized product can be further enriched for the primitive progenitor population by CD34+ cell selection. However, G-CSF leads to intolerable adverse effects in SCD patients including vaso-occlusive crisis, multi-organ failure, and even death, probably due to granulocyte stimulation associated with G-CSF.[17-21]

Plerixafor is an alternative treatment for HSC mobilization. Plerixafor is an inhibitor of the CXCR4 chemokine receptor and blocks binding of its cognate ligand, stromal cell-derived factor-1α (SDF-1α). The CXCR4 and SDF-1α play an important role in the homing of human HSCs to the marrow compartment, and the CXCR4 can help to anchor HSCs to the bone marrow matrix.[22,23] Treatment with plerixafor resulted in elevations in circulating HSCs, and human CD34+ cells mobilized by plerixafor were capable of engraftment with long-term repopulating capacity.[24] Plerixafor can theoretically be applied safely to SCD patients, since it mobilizes HSCs into the peripheral blood without direct stimulation of granulocytes.[22,25]

Therefore, we sought to evaluate the use of plerixafor to mobilize HSCs in SCD patients. Since our initial publication demonstrating the efficacy of allogeneic transplantation in adults with severe SCD, interest in curative approaches has increased substantially. We have screened more than 750 individuals with SCD at the NIH Clinical Center, and performed transplantation in over 75 SCD patients at our single center. We continue to see 3 new patient screens per week for consideration for protocol entry in our clinical trials testing allogeneic and autologous transplant strategies in this disorder. In this pilot trial, we seek to address one of the major limitations moving forward for autologous gene transfer/editing approaches – autologous HSC harvesting. The ability to safely and reliably collect HSCs in SCD could transform our approach to this devastating disease, meeting a significant unmet need.

2.0 Objectives

To obtain sufficient amounts of HSCs collected from the peripheral blood in SCD patients after plerixafor mobilization without serious adverse events.

2.1. Determine the safety profile associated with plerixafor administration in SCD. (Appendix)

2.2. Determine the number of CD34+ cells/kg of body weight achievable with plerixafor mobilization in SCD
2.3. Compare both the CD34+ cell/kg yield and side effects among SCD individuals

3.0 Scientific and Clinical Justification

Harvest of HSCs is required for both allogeneic HSC transplantation and autologous HSC-targeted gene therapy. Our efforts to develop curative therapies for SCD span this spectrum of modalities, but our efforts are encumbered by the relative contraindication of G-CSF usage in SCD, and thus the most utilized method for HSC collection. The availability of a safe, noninvasive means for HSC harvest from individuals with SCD would accelerate efforts at developing HSC based approaches, and mobilization with plerixafor could fill this void. Thus, the current work seeks to determine the safety of plerixafor mobilization in individuals with SCD undergoing experimental transplantation procedures with haploidentical HSC donors, gene-modified autologous HSCs, or other allogeneic transplant protocol where prolonged cytopenia is expected.

In patients transplanted on our haploidentical transplantation protocol to date, 5 of 21 patients with SCD did not recover their platelet counts to >50k/uL by 100 days' post-transplant. Because of the risk of intracerebral hemorrhage in patients with SCD and severe thrombocytopenia, backup collection of autologous HSCs by bone marrow harvesting is now routinely performed in SCD patients undergoing haploidentical transplantation. Additionally, gene therapy applications targeting autologous HSCs require bone marrow harvesting in SCD subjects, and the experimental nature of this work requires back up harvesting. The need for back up HSCs on these existing protocols demands that patients undergo a bone marrow harvest procedure with the associated risks. This protocol will therefore be offered to patients with SCD enrolling on to our haploidentical transplantation and gene therapy studies as an alternative to bone marrow harvest for collection of autologous HSCs.

The risks of plerixafor administration in SCD patients are not known. However, G-CSF administration was reported to induce vaso-occlusive crisis during mobilization in several SCD patients, likely due to granulocyte expansion associated with G-CSF.[17,19-21,26] Tragically, one SCD patient developed multi-organ failure and death after G-CSF mobilization despite close monitoring during the 5 day mobilization.[19] The risks of plerixafor administration, in contrast, are expected to acceptable. Plerixafor has a different mechanism of action and acts by blocking CXCR4 to directly interfere with binding between HSCs and bone marrow niche, while G-CSF markedly increases bone marrow cellularity with granulocyte expansion to permit egress of HSCs to peripheral blood.[27] The different mechanism of plerixafor results in less bone marrow expansion, less granulocyte stimulation, and lower peak of leukocyte counts than G-CSF.[17,22,25,27,28] Plerixafor effects are brief, with only one time injection at one day, while G-CSF requires injection for 5 days. Bone pain is minimal after a single dose of plerixafor, as compared to very common with 5-6 days of G-CSF.[24]

After single plerixafor administration (240 μg/kg) in patients with hematological malignancies, 67% of patients (16/24) achieved sufficient amounts of CD34+ cells (≥2.0x10e6 CD34+ cells/kg) without complication, while a second administration and apheresis was required to obtain a sufficient CD34+ cell yield in 21% of patients (5/24).[24] In addition, a higher dose (480 μg/kg) of plerixafor administration in healthy volunteers resulted in greater amounts of HSC mobilization without severe adverse events, as compared to the standard dose (240 μg/kg).[29]

As compared to these strategies, bone marrow harvest does not require drug administration for mobilization. However, bone marrow harvesting requires anesthesia (similar to surgery), which significantly increases risks of SCD-related complications.[30]

Following HSC mobilization, apheresis is required for HSC collection. The risks of apheresis are expected to be less than marrow harvest, since apheresis in SCD patients is established for red blood cell exchange. In contrast to simple transfusion, risks of apheresis also include hypovolemia or circulatory overload, citrate toxicity with hypocalcemia, infection, and hematoma or thrombosis at the site of catheter placement.[31-33] In our current open and active gene therapy protocol, 14-H-0155, the first 4 patients required at least 2 marrow harvests. When repeat HSC mobilization and collection is needed, the risks can be additive, and thus the risk to benefit ratio appears to favor apheresis.

Table 1. abbreviated list of adverse events with apheresis or marrow harvest

HSC collection by apheresis	HSC collection by bone marrow harvest
• Central venous catheter placement (all patients) • Toxicity from citrate containing anticoagulant; hypocalcemia (in 20% of patients) • 4-6 hours of apheresis (in all patients) • Hypovolemia or circulatory volume overload (in 20% of patients) • Hematoma from central venous catheter (<10%)	• General anesthesia, about 2 hours (in all patients) • Inpatient management of postoperative pain (in all) • Large intravascular volume shift, about 1-1.5 liters of marrow are aspirated, replaced by equal or greater volume of supportive IV fluid (in all) • Hematoma (in 20%) • Infection (10%)

4.0 Study Design

This is a single cohort phase I study to determine the safety of plerixafor mobilization and apheresis in SCD patients. With amendment C, this protocol becomes a multi-institute study with the inclusion of St. Jude Children's Research hospital as a participating site.

At Participating Site:
For St. Jude Children's Research Hospital, see Appendix II for site-specific protocol changes and activities.

5.0 Eligibility Assessment

SCD patients who are age 18 or greater and: (a) are to enroll on a clinical trial with curative intent in which a backup bone marrow harvest is required (e.g. allogeneic HSCT), or (b) are willing to donate hematopoietic stem cell collection for future gene therapy/gene editing study, are eligible for study entry. Patients who are not receiving long-term transfusion therapy will receive prophylactic red cell exchange prior to plerixafor administration to target the fraction of hemoglobin S <30% to reduce the incidence of vaso-occlusive crisis and other sickle related complications. Patients on red cell transfusions can either maintain on transfusions or undergo a modified exchange (e.g. mini-exchange) prior to plerixafor. Hydroxyurea treatment should be

stopped at least 2 weeks before mobilization, since the washout period is important for bone marrow recovery to reduce the myelosuppressive effects of hydroxyurea.[34,35]

Plerixafor may have adverse effects on a fetus *in utero.* Furthermore, it is not known if Plerixafor has transient adverse effects on the composition of sperm.

For this trial, male subjects will be considered of non-reproductive potential if they have azoospermia (whether due to having had a vasectomy or due to an underlying medical condition).

Female subjects will be considered of non-reproductive potential if they are either:

postmenopausal (defined as at least 12 months with no menses without an alternative medical cause; in women < 45 years of age a high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. In the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.);

OR

have had a hysterectomy and/or bilateral oophorectomy, bilateral salpingectomy or bilateral tubal ligation/occlusion, at least 6 weeks prior to screening;

OR

has a congenital or acquired condition that prevents childbearing.

Female and male subjects of reproductive potential must agree to avoid becoming pregnant or impregnating a partner, respectively, while receiving study drug by complying with one of the following contraception regimens if sexually active:

Single method (one of the following is acceptable):
• intrauterine device (IUD)
• vasectomy of a female subject's male partner
• contraceptive rod implanted into the skin
Combination method (requires use of two of the following):
• diaphragm with spermicide (cannot be used in conjunction with cervical cap/spermicide)
• cervical cap with spermicide (nulliparous women only)
• contraceptive sponge (nulliparous women only)
• male condom or female condom (cannot be used together)
• hormonal contraceptive: oral contraceptive pill (estrogen/progestin pill or progestin-only pill), contraceptive skin patch, vaginal contraceptive ring, or subcutaneous contraceptive injection

Subjects should be informed that taking the study medication may involve unknown risks to the fetus (unborn baby) if pregnancy were to occur during the study. All patients must agree to an effective method of contraception while on study treatment and for at least 3 months following Plerixafor treatment (including both female patients of child-bearing potential and male patients with partners of child-bearing potential). Effective birth control includes: a) birth control pills, depot progesterone, or an intrauterine device plus one barrier method, or b) two barrier methods.

Effective barrier methods are: male and female condoms, diaphragms, and spermicides (creams or gels that contain a chemical to kill sperm). For patients using a hormonal contraceptive method, information about any interaction of Plerixafor with hormonal contraceptives is not known.. If there is any question that a subject of childbearing potential will not reliably comply with the requirements for contraception, that subject should not be entered into the study.

All patients will be evaluated first with a medical history and physical examination. Baseline laboratory tests include:

- complete blood count and differential
- hemoglobin analysis
- blood chemistry studies (electrolytes and liver and renal function tests)
- ABO blood typing
- serological tests for syphilis (RPR)
- hepatitis B and C (HBsAg, Anti-HCV)
- HIV (anti-HIV 1/2)
- HTLV-I/II (anti-HTLV-I/II)
- abdominal ultrasound (baseline)
- other relevant tests that investigators consider important to establish a complete medical history will be performed
- All female patients of childbearing age will be required to undergo a serum or urine pregnancy test.

5.1 Inclusion criteria:

5.1.1. SCD patients who are 18 or older, and (a) planned to enroll in an active allogeneic HSCT study where back-up autologous HSCs are needed; OR (b) are eligible for an allogeneic HSCT study [i.e. have the same disease severity as group (a), but no active allogeneic HSCT study is available], and are willing to donate autologous HSCs for a future gene therapy, gene editing, or allogeneic HSCT study.

5.1.2. Adequate renal function: serum/plasma creatinine <1.5 mg/dL.

5.1.3. Adequate liver function: direct bilirubin and ALT <5 times the upper limit of normal range.

5.1.4. Blood counts: WBC >3,000/mm3, granulocytes >1,000/mm3, hemoglobin>7.0g/dL, platelets>150,000/mm3.

5.1.5. Female patients of childbearing age should have a negative serum pregnancy test within one week of beginning plerixafor administration, have had a hysterectomy, post-menopausal, or absence of a menses for over a year.

5.1.6. Meets NIH Department of Transfusion Medicine (DTM) eligibility criteria for blood component donation for *in vitro* research use (negative serologic tests for syphilis, hepatitis B and C, HIV, and HTLV-1).

5.1.7. Ability to give informed consent to participate in the protocol.

5.1.8 Female and male individuals of reproductive potential must agree to one of the contraceptive regimens stated above if sexually active

5.2 Exclusion criteria:

5.2.1. Pregnancy. Female patients of childbearing age should have a negative serum pregnancy test within one week of beginning plerixafor administration, except those that have had a hysterectomy, post-menopausal, or an absence of a menses for over a year.
5.2.2. Active viral, bacterial, fungal, or parasitic infection.
5.2.3. History of cancer, excluding squamous carcinoma of the skin and cervical carcinoma in situ.
5.2.4. Active and painful splenomegaly or splenomegaly (size greater than upper limit of normal) determined by ultrasound.
5.2.5. Allergy to plerixafor.

At Participating Site:
For St. Jude Children's Research Hospital, see Appendix II for site-specific protocol changes and activities.

6.0 Treatment Plan

At NIH

6.1 Prophylactic red blood cell exchange or simple red cell transfusions will be given to patients prior to plerixafor administration targeting HbS <30% to reduce the incidence of vaso-occlusive crisis and other events that may be associated with high hemoglobin S levels. Hydroxyurea treatment should be stopped at least 2 weeks before mobilization, since the washout period is important for bone marrow recovery after myelosuppressive effects of hydroxyurea.[34,35]

6.2 Patients undergoing HSC mobilization will be admitted on a telemetry medical unit. They will then receive a single-dose subcutaneous administration of plerixafor (Mozobil®) at 240 μg/kg. (If the CD34+ cell yields are not sufficient for a minimal target dose in each protocol, a second plerixafor administration can be performed at the same dose for mobilization and apheresis the following day.)

6.3 Patients will be monitored continuously by telemetry. Leukapheresis will start approximately 4-12 hours after plerixafor is given. Mononuclear cells will be collected using an automated cell separator or apheresis device. Whole blood is withdrawn from one venipuncture site at a rate of 60-80 mL/min and then conveyed to the separator. If IV access is inadequate, central lines will be inserted prior to apheresis. Leukapheresis will be performed by the Apheresis Unit under supervision of the medical staff of the Department of Transfusion Medicine at the NIH Clinical Center with periodic EKG monitoring, or in a nursing unit where continuous cardiac monitoring (telemetry) can start with plerixafor administration and through the apheresis procedure.

6.4 After successful blood withdrawal, blood is separated into cells and plasma by centrifugation. The light density mononuclear cells are collected into a component bag, and the remaining cells and plasma are re-infused into the patient via a second venipuncture site. Acid Citrate Dextrose formula A (ACD-A) is used as the anticoagulant at a whole blood to anticoagulant ratio of 13:1. Prophylactic intravenous calcium infusions are used in all procedures. Maximal extracorporeal blood volume during the procedure ranges from

300-400 mL. Sufficient volumes of blood will be processed to obtain target CD34+ cells of 2.0x10e6 CD34+ cells/kg (minimum of 1.5x10e6 cells/kg).

6.5 The harvested peripheral blood apheresis products will be cryopreserved using a controlled rate freezer. All excess cells beyond 2.0x10e6 CD34+ cells/kg will be available for immediate CD34+ cell selection and freezing for research testing cell culture, lentiviral transduction, gene editing, and xenotransplantation.

6.6 After successful mobilization and stem cell collection, patients will remain in the hospital for 1-3 days to monitor for side effects. The total inpatient duration is about 1 week. 3-10 days after discharge from hospital, patients will be evaluated in outpatient clinic. Laboratory and other testing will be obtained as clinically indicated.

6.7 Additional blood samples may be drawn before plerixafor administration, 2 hours after plerixafor, when starting apheresis, and at the end of apheresis to study CD3, CD34, and total nucleated cell counts. The total amount of blood to be drawn including the screening labs will adhere to the policy for adult patients.

6.8 Patients will complete this study 10 days after the last dose of plerixafor, and be taken off this protocol.

At Participating Site:
For St. Jude Children's Research Hospital, see Appendix II for site-specific protocol changes and activities.

7.0 Sample Collection, Storage, and Biospecimen and Data Management Plan

At NIH

7.1 Data management

The PI will be responsible for overseeing entry of data into a password-protected electronic system that complies with NIH security standards and NHLBI DIR policy, and for ensuring data accuracy, consistency and timeliness of entry. The PI, associate investigators, research nurses, and/or a contracted data manager will assist with the data management efforts. All human subject personally identifiable information (PII) eligibility and consent verification will be recorded in conformity with DIR policy. Primary and final analyzed data will have unique codes so that research data can be attributed to an individual human subject participant.

7.2 Intended use

Blood samples and data collected and stored for research purposes will be used to study alternative stem cell selection strategies and culture techniques. Research samples will be stored indefinitely in NIH freezers. Patient clinical data will be protected and tracked using standard operating procedures in the medical record department. The IRB will be notified if research samples are unintentionally destroyed or lost.

7.3 End of study procedures

Data will be stored in a password-protected database in conformity with NHLBI DIR policy or in a publicly accessible research repository until they are no longer of scientific value. Data may be destroyed only when permitted by the clinical director and approved by the IRB.

7.4 Loss or destruction of data

Should the research team become aware that a major breech in our plan to protect research subject confidentiality and trial data has occurred, the clinical director and IRB will be notified.

7.5 Data sharing and future use of data

Research data may be shared with qualified non-collaborator recipients following publication of the primary research results after removal of PII and IRB approval. Future research use of data not defined in the research protocol may occur only after IRB review and approval or an exemption from the NIH Office of Human Subjects Research Protections (OHSRP). Refusal of a research subject participant to permit future use of data--other than required in the protocol or by the FDA--will be honored. Limitations in data sharing and future use of data due to contractual obligations (e.g., CRADAs) or intellectual property proceedings (such as patent filings) will be honored.

7.6 Future use of biospecimens

After analyzing the biospecimens for primary research purposes as described in the protocol, remaining samples suitable for future research must be stored in manner that conforms with DIR policy (such as BSI) or in a publicly accessible research biospecimen repository following IRB or OHSRP approval. Biospecimens may be destroyed only when permitted by the clinical director and approved by the IRB.
Any future research use of biospecimens not defined in the protocol in which NHLBI investigators are engaged in research (e.g., they are undertaking research activities and hold the key that identifies research subjects) requires IRB review and approval. Coded biospecimens (NHLBI investigators hold the key that identifies research subjects) to be shared outside of NIH for future research use requires IRB review and approval (for research collaborations) or submission of a determination to OHSRP (for non-collaborative research), and an executed transfer agreement. Unlinked biospecimens (no key to identify research subjects exists) to be shared outside of NIH for future research use requires submission of a determination to OHSRP and an executed transfer agreement. There are a few types of biospecimens that do not require IRB or OHSRP approval for future research use outside of NIH, such as specimens from deceased individuals (refer to OHSRP SOP5, Appendix 1 for complete list); an executed transfer agreement is required in these special cases. Refusal of a research subject participant to allow for future use of identifiable biospecimens--other than required in the protocol or for appropriate regulatory purposes --will be honored.

Transmission of Data to Outside Investigators:
Coded samples and data may also be sent to our collaborators for analysis. No samples or data collected on this study will be sent outside NIH without prior IRB approval and fully executed material transfer agreement (MTA).

Collaborators include Madhusudan V. Peshwa, Ph.D., MaxCyte, Inc. and Dr. Bill Lundberg, CRISPR Therapeutics Director, will receive de-identified excess CD34+ cells to conduct preclinical studies aimed at genetically modifying hematopoietic stem cells as a treatment for sickle cell disease. These studies will be performed in vitro only and will not be returned to patients.

Collaborator include Prafulla Bhad, with Novartis Institute for Biomedical Research, will receive the bone marrow and peripheral blood sample from sickle cell patients to demonstrate comparability between the results obtained from processing of healthy volunteer bone marrow hematopoietic stem and progenitor cells obtained from healthy donor bone marrow derived HSPC and those from the sickle cell disease patient bone marrow. They will also get the available mobilized peripheral blood CD34+ samples from Dr. Tisdale for similar comparability studies.

Collaborator, Kenneth M. Huttner, with Bioverativ, may receive the bone marrow and mobilized peripheral blood sample from sickle cell patients for ZFN-editing testing. Bioverativ will be investigating gene expression characteristics of these populations. All data derived by Bioverativ from the NIH-sourced CD34+ stem cells that relates to stem cell editing will be shared with the NIH SCD research teams.

Collaborator, KaiHsin Chang, with Editas Medicine, may receive peripheral blood, leukocytes, and CD34+ cells from sickle cell patients. Editas is developing genome editing strategies to use CRISPR/Cas9 to introduce mutations in DNA that are associated with reactivation of fetal hemoglobin or to correct the mutation in the beta-globin gene that causes sickle cell disease.

Collaborator, Donald B. Kohn, M.D., with UCLA, may receive CD34 cells to perform gene editing to correct the sickle cell mutation and will assay in both culture and xenografted mice.

7.7 Loss or destruction of data/samples

Should we become aware that a major breech in our plan for tracking and storage of samples has occurred, the IRB will be notified.

7.8 Publication Policy

Given the research mandate of the NIH, subject data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection institutional approval such as prospective NIH IRB review and approval or an exemption from the NIH Office of Human Subjects Research Protections (OHSRP). Data will not be sent outside the NIH without IRB notification and an executed MTA or CTA.

7.9 Privacy and Confidentiality

All efforts, within reason, will be made to keep subjects' protected health information (PHI) and private identifiable information (PII) private. Using or sharing ("disclosure") such data must follow federal privacy rules. Under certain circumstances, the United States Office of Human Research Protections (OHRP), and the NIH Intramural Institutional Review Board (IRB), will be

able to inspect and copy confidential study-related records which identify participants by name. Therefore, absolute confidentiality cannot be guaranteed.

At Participating Site:
For St. Jude Children's Research Hospital, see Appendix II for site-specific protocol changes and activities.

8.0 Biostatistical Considerations

8.1 Sample size

This is a pilot study of safety of plerixafor mobilization in SCD. This study will accrue a maximum of 15 SCD patients at plerixafor 240 μg/kg-and safety will be determined by successful accrual without reaching stopping rules.

8.2 Stopping rules

Death and life-threatening adverse events: Drug dosing in further participants will be held pending IRB review, if any subject develops any grade IV or V (death) adverse events (Common Terminology Criteria for Adverse Events [CTCAE] version 4.0 or equivalent, see http://ctep.cancer.gov/reporting/ctc.html) considered possibly or definitely related to plerixafor within 10 days of administration of the drug.

Non-pain crisis related events: The allowance of a 20% rate for grade III AEs (attributable to plerixafor) is reasonable, given that 30-40% of participant's experience headaches or GI symptoms after plerixafor and a minority of these adverse events might lead to a grade III AE. Drug dosing in further participants will be held pending IRB review, if stopping rules are met in Table 2. Both pain crisis and acute chest syndrome are considered in a separate set of stopping rules below.

We examined the performance of the stopping rule against a range of true non-pain grade III (or higher) AE probabilities – between 10% and 50%. This range was chosen to span plausible AE rates based on clinical experience. The probability of concluding whether the regimen is unsafe (or safe) was determined by the percentage of 100,000 simulated trials in which the stopping rule boundary was reached (or not reached) for a given AE probability. Each simulated trial involved generating 15 dichotomous (yes/no) AE outcomes with the given AE probability and it was determined if the cumulative count of AEs reached the stopping boundary. The boundary was chosen after considering a range of different possible combinations of sample sizes and stopping boundaries. This boundary was selected because the performance balances a strong probability of stopping the trial when the true AE rate is too high (30% or more) and allowing the trial to continue when the AE rate is relatively low (less than 20%).

Table 2 – Stopping rule: Stop if 2 non-pain grade 3 or higher AEs observed in first 5 individuals, 4 AEs after evaluating 6-10 individuals, or 5 AEs after evaluating 11-15 individuals

True, Unknown AE probability	Probability Conclude Safe	Probability Conclude Unsafe
0.50	0.04	0.96
0.40	0.14	0.86

0.30	0.37	0.63
0.20	0.67	0.33
0.15	0.81	0.19
0.10	0.91	0.09

Pain crisis related events: Pain crisis is excluded from the criteria of grade III, while acute chest syndrome is included as grade III or IV. We anticipate up to a rate of 40% for pain crisis (attributable to plerixafor). This rate reflects that among the patients with SCD that have undergone allogeneic HSCT at our center, approximately 30-35% have chronic pain requiring chronic opioid usage and had frequent hospitalizations for pain at baseline. This prevalence is similar to the sickle cell center in Milwaukee, where 34 of 115 patients had 5-11 hospitalizations per year and 17 patients had ≥12.[36] Additionally, all the patients in our current gene therapy study (14-H-0155) had at least 4-5 days of hospitalization after marrow harvest, indicating that pain induced by the harvest procedure is substantial in all patients. The pain from plerixafor would be expected to be lower. Thus, we define pain crisis for this protocol to be (1) duration of hospitalization for the entire HSC collection (e.g. from plerixafor to discharge) to be more than 4 days due to pain that is considered to be sickle related crisis; or (2) hospitalization for pain crisis after discharge from the initial HSC collection and within 10 days of the final plerixafor dose; or (3) escalation in pain treatment (e.g. from oral to IV narcotics administration or from as needed doses of IV to PCA narcotics)..

Hence drug dosing in further participants will be held pending IRB review, if stopping rules are met in Table 3.

Table 3 – Stopping rule: Stop if 3 pain AEs (defined above) observed in first 5 individuals, 5 AEs after evaluting 6-10 individuals, or 6 AEs after evaluating 11-15 individuals.

True, Unknown AE probability	Probability Conclude Safe	Probability Conclude Unsafe
0.60	0.03	0.97
0.50	0.13	0.87
0.40	0.36	0.64
0.30	0.66	0.34
0.20	0.90	0.10

The boundaries for pain AEs were determined in a manner similar to the boundary for grade III AEs. Participants will be dosed sequentially, with no overlap during the 10-day monitoring period, (including hospital admission to clinic visit post-stem cell collection) to allow assessment of adverse events in each participant before proceeding to the next. If stopping rules are met at 240 ug/kg, dose reduction may be proposed at that time.

9.0 Data Safety and Monitoring Plan

The side effects of plerixafor mobilization and apheresis should be less than that of G-CSF, which are well described and vast experience with the procedure in both normal volunteers and family donors exists in the Clinical Center. In addition, we will evaluate for vaso-occlusive crisis for SCD

patients, for which vast experience also exists in the Clinical Center. Grade 3 and higher AEs and SAEs will be collected from the start of first dose of plerixafor administration to 10 days after the last dose of plerixafor. For subjects enrolled at the NIH, AEs and SAEs outside of this window of time will be tracked on the natural history protocol (04-H-0161), screening protocol (08-H-0156), or transplant protocol (03-H-0170, 14-H-0077, 17-H-0069).

9.1 Safety Monitoring

At NIH

Principal Investigator: Accrual will be monitored by the principal investigator who will provide oversight, and will monitor accrual and safety data. The investigator and/or associate investigators will make study documents readily available for inspection by the local IRB, and the NHLBI Office of the Clinical Director staff for confirmation of the study data.

NIH Intramural IRB*:* Accrual and safety data will also be monitored and reviewed annually by the IRB. Prior to implementation of this study, the protocol and the proposed patient consent and assent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to 45 CFR 46. This committee will approve all amendments to the protocol or informed consent and conduct continuing annual review so long as the protocol is open to accrual or sample and/or data analysis continues.

NHLBI DSMB: The NHLBI Data and Safety Monitoring Board (DSMB) will review the protocol, progress report, accrual, efficacy and safety data at six- or twelve-month intervals as scheduled. All AEs and SAEs observed during the clinical trial and for which there is a relationship with the use of plerixafor, or the conduct of the study will be reported to the DSMB at the regularly scheduled DSMB meeting. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

9.2 Adverse events Definitions

Adverse Event (AE): Any untoward medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

Serious adverse events (SAEs): defined by federal regulations and include events which
- Are fatal or life threatening
- Result in significant or persistent disability
- Require or prolong inpatient hospitalization
- Result in a congenital anomaly or neoplasm
- Result from an overdose
- Are other conditions which in the judgment of the PI represent a significant hazard

Serious event: An event is serious if it meets the definition of a serious adverse event (above) or if it requires immediate corrective action by a PI and/or IRB to protect the safety, welfare or rights of subjects.

***Unanticipated Problem (UP)*:** Any incident, experience, or outcome that meets all of the following criteria:

1. **unexpected** in terms of nature, severity, or frequency in relation to
   a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
   b. the characteristics of the subject population being studied; and

2. **related or possibly related** to participation in the research; and

3. places subjects or others at a **greater risk of harm** (including physical, psychological, economic, or social harm) than was previously known or recognized.

***Unanticipated Problem that is not an Adverse Event*:** An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involves risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

Protocol Deviation (PD): Any change, divergence, or departure from the IRB approved research protocol.

Non-compliance: The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human research. Noncompliance may be further characterized as:

1. Serious non-compliance: Non-compliance that:
   a. Increases risks, or causes harm, to participants.
   b. Decreases potential benefits to participants.
   c. Compromises the integrity of the NIH HRPP.
   d. Invalidates the study data.

2. Continuing non-compliance: Non-compliance that is recurring. An example may be a pattern of non-compliance that suggests a likelihood that, absent an intervention, non-compliance will continue. Continuing noncompliance could also include a failure to respond to IRB requests to resolve previous allegations of non-compliance.

3. Minor (non-serious) non-compliance: Non-compliance that, is neither serious nor continuing.

9.3 Adverse event assessment

For both serious and non-serious adverse events, the investigator must determine both the intensity of the event and the relationship of the event to plerixafor administration. The investigator should record what action, if any, was taken to the planned administration of the investigational product due to the AE (i.e., discontinuation, modification, or interruption of the treatment).

Intensity will be assessed by the investigator using the NCI CTCAE, version 4.0. If the adverse event is not included in the CTCAE, then the investigator is to determine the intensity of the adverse event according to the following criteria:

Grade	**Definition**
Mild (Grade 1)	The AE is noticeable to the subject, but does not interfere with routine activity.
Moderate (Grade 2)	The AE interferes with routine activity, but responds to symptomatic therapy or rest.
Severe (Grade 3)	The AE significantly limits the subject's ability to perform routine activities despite symptomatic therapy.
Life-threatening (Grade 4)	The subject is at immediate risk of death.
Death (Grade 5)	Death

If the intensity (grade) changes within a day, the maximum intensity (grade) should be recorded.

Relationship will be determined by the investigator according to the criteria that follow. Relationship of adverse events to plerixafor will be determined after the start of plerixafor infusion:

Investigator Assessment	**Definition**	**Classification for Reporting Purposes**
Not Related	Exposure to the drug product did not occur, or the occurrence of the AE is not reasonably related in time, or the AE is considered unlikely to be related to the drug product.	Not Related
Unlikely Related	The study treatment and the AE were not closely related in time, and/or the AE could be explained more consistently by causes other than exposure to the drug product.	
Possibly Related	The study treatment and the AE were reasonably related in time, and the AE could be explained equally well by causes other than exposure to the drug product.	Related
Definitely Related	The study treatment and the AE were reasonably related in time, and the AE was more likely explained by exposure to the drug product than by other causes, or the drug product was the most likely cause of the AE.	

9.4 NIH Intramural-IRB and CD Reporting

Note: All IRB reportable events will be submitted by the coordinating center (NHLBI). Participating sites will submit those events to the NHLBI study coordinator, who will, in turn submit them to the IRB via an NIH Problem Report Form within the timeframes specified below.

9.4.1 Serious Events

Reports to the IRB and CD:
The PI must report serious UPs and serious PDs to the IRB and CD as soon as possible but not more than 7 days after the PI first learns of the event using the NIH Problem Report Form.

Reports to the IRB Chair and CD:
The PI must report all SAEs that do not meet the definition of UP to the IRB Chair and CD not more than 14 days after the PI first learns of the event using the NIH Problem Report Form.

9.4.2. Non-serious Events

Reports to the IRB and CD:
The PI must report all UPs that are not serious to the IRB and CD, and PDs that are not serious to the IRB, not more than 14 days after the PI first learns of the event using the NIH Problem Report Form.

Non-serious protocol deviations that result from normal subject scheduling variations or technical issues associated with sampling that does not impact the health of the subject or the interpretation of the study data will not be reported.

9.4.3 Deaths
The PI must report all deaths to the CD and IRB as soon as possible, but not more than 7 days after the PI first learns of the event.

We will not track subjects after stem cell donation. However, deaths and SAE will be reported through the primary transplant protocol.

9.4.4 Pregnancy
We will discuss the available options with patients who become pregnant during this study, and refer them for the appropriate consult or service.

9.5 Reports at the time of continuing IRB review

At continuing review, the PI will provide to the IRB a summary of:
• All UPs
• All PDs
• All AEs and SAEs grade 3 and higher will be collected from the start of first dose of plerixafor administration to 10 days after the last dose of plerixafor. (except for those granted a waiver of reporting)

Note: If, while preparing the continuing review, the PI identifies a greater frequency or level of severity of expected adverse events than was previously identified in the protocol or investigational brochure (IB), these should be reported separately as a UP. If such an observation occurs before the time of continuing IRB review, it should be reported to the IRB and CD as a UP in the time frames noted above, and summarized at the time of continuing review.

9.6 Reporting to Sanofi

9.6.1 Adverse Event Definition

An AE is any untoward medical occurrence associated with the use of the investigational product (active or placebo drug, biologic, or device) in a clinical investigation patient, which does not necessarily have a causal relationship with the investigational product. An AE can, therefore, be any unfavorable and unintended symptom, sign, disease or condition, or test abnormality whether or not considered related to the investigational product.

AEs may include, but are not limited to:

- Subjective or objective symptoms spontaneously offered by the patient and/or observed by the investigator or medical staff
- Clinically significant laboratory abnormalities
- A significant worsening of the patient's condition from study entry
- Disease signs and symptoms and/or laboratory abnormalities existing prior to the use of the study treatment that resolve but then recur after treatment
- Disease signs and symptoms and/or laboratory abnormalities existing prior to the use of the study treatment which increase in frequency, intensity, or a change in quality after treatment

9.6.2 Adverse Event Reporting

Adverse events will be graded according to CTCAE version 4(see section 9.3). Reference safety information to assess expectedness for Investigators will be IB. All adverse events except those clearly attributable to the underlying disease will be reported, including definitely, probably and possibly related. Both Serious and Non-Serious Adverse Events will be clearly noted in source documentation and listed on study specific Case Report Forms (CRFs). The Protocol Director (PD) or designee will assess each Adverse Event (AE) to determine whether it is unexpected according to the Informed Consent, Protocol Document, or Investigator's Brochure, and related to the investigation.

9.6.3 Serious Adverse Events (SAEs) Definition

A SAE is any adverse event that results in any of the following outcomes:

Death

- A life-threatening experience
- Requires inpatient hospitalization or prolongs existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Important medical events that may jeopardize the patient and may require medical or surgical intervention to prevent 1 of the outcomes listed above

Hospitalizations that occur under the following circumstances are not considered to be SAEs:

- were planned before entry into the clinical study;
- are for elective treatment of a condition unrelated to the studied indication or its treatment;
- occur on an emergency or outpatient basis and do not result in admission (unless fulfilling the criteria above), are part of the normal treatment or monitoring of the studied indication and not associated with any deterioration in condition.

9.6.4 Serious Adverse Events (SAEs) Reporting

All Serious Adverse Events (SAEs) will be tracked for 30 days or until resolution, or until the end of study (10 days after last dose of the study treatment).

SAEs, CTCAE Grade 3 and above, and all subsequent follow-up reports will be reported to the Safety Monitoring Committee (DSMC) using the study specific CRF regardless of the event's relatedness to the investigation. Following review by the DSMC, events meeting the IRB definition of 'Unanticipated Problem' will be reported to the IRB using eProtocol within 10 working days of DSMC review, or within 5 working days for deaths or life-threatening experiences.

SAEs, within 24 hours (US) or one business day (EU) of first knowledge of such serious adverse event, will need to notify Sanofi via fax, attention Sanofi Pharmacovigilance (PV), 908-203-7783 (US) or via email at: USPVmailbox@sanofi-aventis.com. Additionally, the Investigator will transmit to Sanofi PV an information copy of any such report sent to the governing regulatory authority, prior to or at the time of authority filing. The Investigator will make available to Sanofi promptly such records as may be necessary and pertinent to investigate any such expedited adverse event, if specifically requested by Sanofi.

Furthermore, the Investigator will inform Sanofi of the following:

- Any events that result in protocol amendments for safety reasons, as well as any safety related regulatory action such as a clinical hold of the Research;
- Any pregnancies occurring in patients who are exposed to the Product in connection with the Research.
- In addition, the Investigator will notify Sanofi within 24 hours (US) or one business day (EU) of first knowledge of any Product complaints (communication of dissatisfaction that alleges deficiencies related to the identity, quality, durability, effectiveness, safety, labeling, purity, stability, and appearance) by fax to 508-661-8771 (US) or Sanofi Customer Services Europe, +31 (0)35 699 1222.
- The Investigator will also inform Sanofi within 1 business day of becoming aware of any actions from any authority that may affect the performance of the Research

Safety reporting rules are to be complied with, according to current PV specifications (QGSD-007589). Sponsor is to provide Sanofi with: results relevant to final diagnosis of any SAE; routine transmission of any overdose with plerixafor; periodic reports; study report must contain section with safety review and conclusion –to be reviewed by Sanofi before finalization.

9.6.5 Pregnancy Reporting

The Investigator will inform Sanofi PV within 24 hours of the Investigator's first knowledge of pregnancy in a female patient or the female partner of a male patient at any time after the first dose of Plerixafor. Pregnant female patient(s) must not receive additional study treatment. The pregnancy will be followed until the outcome is known (i.e., delivery, elective termination, spontaneous abortion). The Investigator will obtain follow-up information no later than two months after the gestational period to obtain maternal, fetal, and neonatal outcome and any other relevant information. If the pregnancy results in the birth of a child, additional follow-up information may be requested. The Investigator must complete as much information as possible on the relevant Pregnancy Notification Forms (PNF) A and B, and fax the forms to the Sanofi Genzyme PV.

10.0 Human Subjects Protections

10.1 Rationale for Subject Selection

Subjects will be recruited through the Clinical Center Volunteer Office and through our clinic referrals. Accrual will be based solely upon protocol entry criteria and no selection will be based upon gender, race, or age (in those 18 years or greater in age).

10.2 Participation of children
Children are excluded from protocol entry as the study poses more than minimal risk without the prospect of direct clinical benefit.

10.3 Risks in relation to benefits
As of February 7, 2019, this study is now closed to new subject accrual and continues in data analysis only and the level of risk is now minimal.

For adult research participants level of risk is greater than minimal risk with no prospect of direct benefit but likely to yield generalizable knowledge about the subject's disorder or condition (45 CFR 46.406). Patients may withdraw from study at any time.

10.4 Rationale for the Exclusion of Pregnant Women

The participation in the study may lead to a risk to the fetus. For those reasons, we are excluding pregnant women. During screening, any prospective subject who reports being pregnant will be excluded from participating. In addition, we will measure serum or urine beta-HCG in women for childbearing age. Any subjects found to be pregnant will be excluded from this study.

10.5 Informed Consent Processes and Procedures
At NIH
Each participant will receive an oral and written explanation of the goals, procedures, and risks of this study. The Principal Investigator and those Associate Investigators who are listed on the cover page of the protocol with an asterisk next to their name may obtain informed consent from research participants. Consent will be obtained at the NIH Clinical Center. The original, signed informed consent document will be placed in the medical record, and the subject will receive a signed copy of the informed consent document.

At participating site:
After the study has been fully explained, written informed consent will be obtained from the subject or his/her legal representative prior to study participation. The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

10.6 Conflict of Interest

This is a covered protocol. The Principal Investigator assured that each associate investigator listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. No initial or subsequent members of the research team reported a potential conflict of interest. The National Institutes of Health reviews NIH staff researchers at least yearly for conflicts of interest. The following link contains details on this process http://ethics.od.nih.gov/forms/Protocol-Review-Guide.pdf. You may ask your research team for additional information or a copy of the Protocol Review Guide. This protocol may have investigators who are not NIH employees. Non-NIH Investigators are expected to adhere to the principles of the Protocol Review Guide but are not required to report their personal financial holdings to the NIH.

10.7 Risk and discomforts

10.7.1. Plerixafor:

Plerixafor is an inhibitor of the CXCR4 chemokine receptor and blocks binding of its cognate ligand, stromal cell-derived factor-1α (SDF-1α). The CXCR4 and SDF-1α are recognized to play an important role in the homing of human HSCs to the marrow compartment, and the CXCR4 can help to anchor HSCs to the marrow matrix, including SDF-1α. Treatment with plerixafor resulted in leukocytosis and elevations in circulating HSCs, and the plerixafor-mobilized CD34+ cells were capable of engraftment with long-term repopulating capacity. The plerixafor blocks CXCR4 to directly interfere binding between HSCs and bone marrow niche, while G-CSF markedly increases bone marrow cellularity with granulocyte expansion to permit egress of HSCs to peripheral blood.[27] The different mechanism of plerixafor results in less bone marrow expansion, less granulocyte stimulation, and lower peak of leukocyte counts than G-CSF.[17, 22, 25, 27, 28]

Common (≥10%) side effects:
[Plerixafor and G-CSF] The most common adverse reactions reported were diarrhea (37%), nausea (34%), fatigue (27%), injection site reactions (34%), headache (22%), arthralgia (13%), dizziness (11%), and vomiting (10%).

34% of patients had mild to moderate injection site reactions at the site of subcutaneous administration of plerixafor. These included erythema, hematoma, hemorrhage, induration, inflammation, irritation, pain, paresthesia, pruritus, rash, swelling, and urticarial.

[Plerixafor only] Adverse events were similar, including lightheadedness (44%); nausea, bloating, or flatulence (36%); injection site discomfort or warm sensation (28%); perioral paresthesia, loose stools, or diaphoresis (20%); and headache (16%).24 A recent study for a single administration of plerixafor revealed mild-moderate facial paresthesia (26%) and mild arrhythmia including sinus tachycardia (56%), premature ventricular contraction (21%), and sinus bradycardia (7%)[37].

Less common (1-10%) side effects:
Other adverse reactions that occurred in < 5% of patients but were reported as related to plerixafor during HSC mobilization and apheresis included abdominal pain, hyperhidrosis, abdominal distention, dry mouth, erythema, stomach discomfort, malaise, hypoesthesia oral, constipation, dyspepsia, and musculoskeletal pain.

Rare (<1%) side effects:
Mild to moderate allergic reactions were observed in less than 1% of patients within approximately 30 min after plerixafor administration. Symptoms generally responded to treatments (e.g., antihistamines, corticosteroids, hydration or supplemental oxygen) or resolved spontaneously. Severe side effects including anaphylaxis have been reported in patients receiving plerixafor.

Vasovagal reactions, orthostatic hypotension, and/or syncope can occur following subcutaneous injections (<1%). The majority of these events occurred within 1 hour of plerixafor administration. Because of the potential for these reactions, appropriate precautions should be taken.

Other side effects may include thrombocytopenia, splenic enlargement (potential for rupture), fever, bone pain, anorexia, insomnia, muscle pain, and infusion related reaction.

SCD specific side effects:
Recent reports document the provocation of vaso-occlusive crisis by G-CSF administration in individuals with SCD.[19, 20, 26] The risks of plerixafor in SCD are not known, but are expected be less than G-CSF, since it mobilizes HSCs without direct stimulation to granulocytes.

10.7.2. Risks related to apheresis:

Adverse reactions related to apheresis include lightheadedness, nausea, and pre-syncope due to vasovagal reactions and cutaneous paresthesia due to ACD-A, a calcium chelating anticoagulant. Vasovagal reactions are managed by postural manipulation and volume administration. Paresthesia are treated by slowing the rate of the anticoagulant infusion, increasing the rate of calcium replacement, or temporarily interrupting the procedure. Since patients with SCD would have a red cell exchange previously, the risk of apheresis for HSC collection are expected to be similar.

10.7.3 Other potential risks:

Anemia may occur from repeated blood sampling and leukapheresis. This can be prevented by initial screening and red blood cell exchange. Pain and bruising can result from needle sticks. Thrombocytopenia (platelet count <150,000/uL) can be expected in 10-20% of subject's post-apheresis. When observed, thrombocytopenia is usually moderate, self-limited, and clinically insignificant. Although the implications are not clear, splenic enlargement occurs in individuals undergoing standard mobilization for stem cell collection.[38,39] This enlargement is transient, resolving in 3-4 days.

11.0 Investigator Administrative Requirements

11.1Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonization (ICH) E6 (Guideline for Good Clinical Practice), the ethical principles that have their origin in the Declaration of Helsinki, Title 21 of the Code of Federal Regulations, Parts 50 (Protection of

Human Subjects), and 56 (Institutional Review Boards), and other appropriate regulatory requirement(s). The Investigator will be thoroughly familiar with plerixafor as described in the protocol. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Regulatory files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

11.2 IRB Submissions

The site IRB/IEC and other appropriate institutional regulatory bodies will review all appropriate study documentation in order to safeguard the rights, safety, and well-being of the subjects. The protocol, informed consent, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC and other appropriate institutional regulatory bodies by the Investigator.

11.3 Subject Information and Informed Consent

After the study has been fully explained, written informed consent will be obtained from the subject or his/her legal representative prior to study participation. The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

11.4 Subject Confidentiality

In order to maintain subject privacy, all CRFs, accountability records, study reports, and communications will identify the subject by initials and the assigned subject number. The Investigator will grant monitor(s) and auditor(s) from the NIH Principal Investigator or his designee and regulatory authority(ies) access to the subject's original medical records for verification of data gathered on the CRFs and to audit the data collection process. The subject's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

11.5 Protocol Compliance

The Investigator will conduct the study in compliance with the protocol provided by the NIH Principal Investigator, and given approval/favorable opinion by the IRB/IEC and other appropriate institutional regulatory bodies. Modifications to the protocol should not be made without agreement of both the Investigator and the NIH Principal Investigator. Changes to the protocol will require written IRB/IEC and other appropriate institutional regulatory body approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to subjects. The IRB/IEC may provide, if applicable regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval /favorable opinion of the IRB/IEC and other appropriate institutional regulatory bodies. The NIH Principal Investigator will submit all protocol modifications to the regulatory authority(ies) in accordance with the governing regulations.

When immediate deviation from the protocol is required to eliminate an immediate hazard(s) to subjects, the Investigator will contact the NIH Principal Investigator, if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be fully documented in the CRF and source documentation.

11.6 Direct Access to Source Data

Monitoring and auditing procedures developed by the NIH Principal Investigator will be followed, in order to comply with GCP guidelines.

Regulatory authorities, the IRB/IEC and other appropriate institutional regulatory bodies, and/or the NIH Principal Investigator may request access to all source documents, CRFs, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

11.7 Case Report Form Completion

CRFs will be completed for each study subject. It is the Site Principal Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the subject's CRF. Source documentation supporting the CRF data should indicate the subject's participation in the study and should document the dates and details of study procedures, AEs, and subject status. The Investigator will have access to an NHLBI-approved database to enter CRF data electronically.

12.0 Compensation

Participants will not be compensated for participating in this study.

13.0 Reimbursement for Travel

Reimbursement for travel will be in accordance with NHLBI travel policy reimbursement for food and lodging will be consistent with NIH and NHLBI guidelines.

14.0 Pharmaceuticals

- Plerixafor (Sanofi), Mozobil®

Plerixafor is available from the Clinical Center pharmacy in single-use clear glass vials containing 24 mg plerixafor in 1.2 mL vials (20 mg/mL). It is formulated as a sterile, preservative-free, clear, colorless to pale yellow, and isotonic solution for subcutaneous injection. The quantitative composition (per vial) is:

Plerixafor	24 mg
Sodium chloride	5.9 mg
Water for injection (qs ad) to	1.2 mL

(adjusted to a pH of 6.0 to 7.5 with hydrochloric acid and with sodium hydroxide)

Storage: The intact vials of plerixafor should be stored at 25°C (77°F); excursions permitted to 15-30°C (59-86°F).

Stability: The expiration date is indicated on the label. Do not use it after the expiration date.

Off-Label Use of Drugs

Plerixafor will be used beyond the indications specified in the Prescriber Information. These Off-Label uses will comply with prevailing community standards.

The use of these drugs for this protocol meets the requirements for exemption from the Investigational New Drug regulations, 21 CFR 312, specifically:

1. The investigational drug is lawfully marketed in the United States
2. The investigation is not intended to be reported to the FDA as a well-controlled study in support of a new indication for use of the drug product
3. The investigation is not intended to support a significant change in advertising to an existing lawfully marketed prescription drug product
4. The investigation does not involve a route of administration or dosage level or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.
5. The investigation will be conducted in compliance with the requirements for institutional review set forth in FDA regulations 21 CFR 56, and requirements for informed consent as set forth in FDA regulations 21 CFR 50
6. The investigation will be conducted in compliance with FDA regulations 21 CFR 312.7: Promotion and charging for investigational drugs.

15.0 References

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Appendix I

Individuals with sickle cell disease, after plerixafor mobilization and apheresis for stem cell collection

Date(s) of plerixafor injections:

Date(s) of apheresis:

Narcotics offered: yes or no Narcotics taken: yes or no, if yes, state for what kind of pain

CTCAE (common toxicity criteria for adverse events v 4.0, http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf)

	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Fever (pg 56)	38.0-39.0C 100.4-102.2 F	>39.0-40.0 C 102.3-104 F	>40 (>104F) for 24 hrs or less	>40C (>104F) for >24 hrs	Death
Fatigue (pg 56)	Relieved by rest	Fatigue not relieved by rest; limiting instrumental ADL	Fatigue not relieved by rest, limiting self-care ADL		
Rash, papulo-pustular, not acne (pg 79)	<10% body surface area	10-30%	>30%	>30%, extensive superinfection, need IV antibiotics	Death
Rash, pustular (pg 82)		localized	Need IV antibiotics or antimicrobials		
Pruritus (pg 184)	Mild or localized (topical treatment)	Intense, widespread, skin changes from scratching	Intense, widespread, skin changes from scratching, limiting self-care; oral steroids or immune-suppressive therapy		
Rash, acneiform (pg 184)	<10% body surface area	10-30%	>30%, limiting self-care, local superinfection, oral antibiotics	>30%, extensive superinfection, need IV antibiotics	Death

Rash, maculo-papular (pg 185)	<10% body surface area	10-30%	>30%, limiting self-care		
Injection site reaction (pg 58)	Tender +/- warmth, redness, or itching	Pain, edema, phlebitis	Ulceration or necrosis, severe tissue damage, operative intervention indicated	Life-threatening, urgent intervention	Death
Nausea (pg 46)	Loss of appetite, no change in eating habits	Decrease oral intake, no weight loss	Tube feeding, TPN, or hospitalization		
Vomiting (pg 54)	1-2 episodes (separated by 5 min) in 24 hrs	2-5 episodes in 24 hrs	6 or more episodes in 24 hrs, tube feeding, TPN, or hospitalization	Life threatening, urgent intervention	Death
Anorexia (pg 114), similar to nausea	Loss of appetite, no change in eating habits	Decrease oral intake, no weight loss	Tube feeding, TPN, or hospitalization	Life threatening, urgent intervention	Death
Insomnia (pg 144)	Mild difficulty falling asleep, staying asleep or waking up early	Moderate difficulty	Severe difficulty		
Dizziness (pg 131)	Mild unsteadiness or sensation of movement	Moderate	Severe		
Presyncope (pg 137)		Present (near fainting)			
Syncope (pg 139)			Fainting, orthostatic collapse		
Abdominal pain (pg 28)	Mild	Moderate, limiting instrumental ADL	Severe, limiting self-care		
Bone pain, specify where, including back, hips, or other joints (pg 120)	Mild	Moderate, limiting instrumental ADL	Severe, limiting self-care		
Headache (pg 133)	Mild	Moderate, limiting instrumental ADL	Severe, limiting self-care		

Myalgia, muscle pain (pg 124)	Mild	Moderate, limiting instrumental ADL	Severe, limiting self-care		
IV site or infusion related reaction (pg 57)	Mild, infusion not interrupted, no intervention	Interrupted, but prompt response to treatment	Prolonged, recurrence of symptoms, or hospitalization	Life threatening	Death
Pharyngitis (pg 81)		Localized, local treatment	IV antimicrobials, radiologic or operative intervention	Life threatening	Death

Appendix II-As of January 18, 2019, this site is considered closed to accrual but open to follow-up
St. Jude Children's Research Hospital site-specific protocol changes and activities

The following changes and clarifications are requested for St. Jude Children's Research Hospital.

Section 3.0 Scientific and clinical justification
This research protocol (Plerixafor mobilization) will be offered to adult patients with SCD (ages 18 to 25) to assess feasibility and success of plerixafor mobilization and harvest of mobilized peripheral blood HSCs for future use in transplantation or gene therapy trials. At St. Jude, currently there are no open haploidentical hematopoietic stem cell transplant (HSCT) or gene therapy studies for children with SCD. However, for adults with SCD, a haploidentical bone marrow transplant research study is open and enrolling patients at UT/Methodist Hospital in Memphis. St. Jude participants may wish to enroll in a haploidentical HSCT or gene therapy trial following participation in the current study. Until then, their HSCs will be cryopreserved. All excess cells beyond 3.0x10e6 CD34+ cells/kg will be used for research and to develop various therapeutic approaches for sickle cell disease, including gene therapy, gene editing, drug discovery and development and other novel approaches to increase fetal hemoglobin as discussed in section 7.2.

Section 5.0 Eligibility assessment
Eligibility criteria will be as described in the NIH parent protocol except where noted. At St. Jude, SCD patients who are between the ages of 18 and 25 and are willing to donate hematopoietic stem cell collection for future gene therapy/gene editing study are eligible for study entry (criteria (b) of the parent protocol). Due to St. Jude being a pediatric hospital, participants will need to be no older than 25 to enroll at St. Jude. There are no clinical trials with curative intent open at St. Jude currently. In the future, if and when such trials become available, these subjects will be offered participation in those trials and the harvested HSCs will be used in those trials if the subjects so desire. Additionally, should any of these adult participants undergo haploidentical HSCT at UT/Methodist Hospital, their remaining unmanipulated HSCs will be made available for clinical purposes as needed, as back-up in case of graft failure or rejection, just like in the parent protocol at NIH/NHLBI.

Patients on chronic red cell transfusion therapy or chronic erythrocytapheresis therapy will be eligible.

Patients will need to have a working central line for access in order to be eligible for this protocol.

Section 5.1 Inclusion criteria
5.1.1 At St Jude, Inclusion criteria will be as described in NIH parent protocol 5.0 part (b).

Section 6.0 Treatment plan
6.1 Only one dose of Plerixafor will be administered and only one HSC apheresis will be performed at St. Jude irrespective of the CD34+ cell yield.
6.2 Leukapheresis will be performed in the Apheresis unit by the Apheresis team at St. Jude.
6.3 All excess cells beyond 3.0x10e6 CD34+ cells/kg will be used for sickle cell disease research at St Jude as described in 7.2 below.
6.4 Due to St. Jude Children's Research Hospital being a pediatric facility, if a participant experiences a prolonged complication as a result of participating in the protocol, he or she will be transferred to an adult hospital. An adult intensive care provider will be identified to serve as a liaison between St. Jude Children's Research Hospital and the adult hospital should a transfer need to take place. The intensive care unit at St. Jude Children's Research Hospital has agreed to accept adult participants from this protocol and will only

initiate a transfer to an adult hospital should the participant require a prolonged stay or develop multiple organ dysfunction.

Section 7.0 Sample Collection, Storage and Biospecimen and Data Management Plan
7.1 Data management
Data will be entered by the study coordinator in the Clinical Trial Management team within the Department of Hematology through electronic Case Report Forms provided by the sponsor.

St. Jude may share samples from the excess of 3.0x10e6 CD34+ cells/kg collected for research purposes in a de-identified manner.

7.2 Intended Use
At the time of collection, all excess cells beyond 3.0x10e6 CD34+ cells/kg will be allocated for research purposes and stored (coded and de-identified) in a research lab at St. Jude in the Department of Hematology. These HSCs will be used primarily for gene-editing studies to demonstrate comparability and efficacy of gene-editing in bone marrow-derived and peripheral blood-derived hematopoietic stem and progenitor cells obtained from those with SCD. The remaining cells will be stored at St. Jude in the Human Application Lab and will be available for clinical purposes if needed in the future. In the future, if a participant receives a curative therapy (stem cell transplant, gene editing, etc) and does not require these stored cells as backup, or expires, then the remaining stored clinical samples will be used for research.

7.8 Publication Policy:
Primary objectives are to determine the safety and efficacy of Plerixafor for mobilizing HSCs—data related to these objectives will be shared with Dr. Tisdale at the NHLBI. Data arising from laboratory studies of bone marrow and mobilized patient HSCs belongs to St. Jude investigators and will be shared with outside investigators at the discretion of St. Jude investigators.

7.9 Privacy and Confidentiality
Study codes will be used in place of an identifier, such as a medical record number. The list containing the study number and the medical record number will be maintained in a locked file and will be retained indefinitely. Samples will be handled and processed by Tissue Resources at St. Jude by trained personnel used to handling human specimens and with strict procedures for protecting participant confidentiality. De-identified samples will only be forwarded to the Hematology research lab.

The medical records of study participants may be reviewed by the NHLBI, St. Jude IRB, FDA, or clinical research monitors only.

Section 8.0 Biostatistical Consideration
8.1 St. Jude will enroll up to 4 participants.

Section 9.0 Data Safety and Monitoring Plan
9.1 Safety Monitoring
St. Jude PIs and study team are responsible for ensuring protocol compliance. 100% of eligibility checklists and consents will be reviewed for accuracy and completeness by the Eligibility Coordinators. Continuing reviews by the IRB and CT-SRC will occur at least annually

The NHLBI and the NHLBI Data Coordinating Center will be responsible for monitoring the St. Jude site.

9.4 NIH Intramural IRB and CD Reporting

St. Jude PI will report AEs to the NHLBI in time of submission of protocol to the NIH Intramural IRB for continuing review. St. Jude PI will report all SAEs to Dr. Tisdale within 72 hours of learning about the event.

Section 11.0 Compensation

Subjects at St Jude will receive $250 per inpatient stay day up to $750 and $50 per outpatient clinic visit to compensate them for wage losses due to time away from their jobs during study participation, for a max of $800 per participant.