Safe and efficient peripheral blood stem cell collection in patients with sickle cell disease using plerixafor

by Naoya Uchida, Alexis Leonard, David Stroncek, Sandhya R. Panch, Kamille West, Eoghan Molloy, Thomas E. Hughes, Sara Hauffe, Tiffani Taylor, Courtney Fitzhugh, Jane S. Hankins, Megan Wilson, Akshay Sharma, Shengdar Q. Tsai, Mitch J. Weiss, Matthew Hsieh, and John F. Tisdale

Haematologica 2020 [Epub ahead of print]

Citation: Naoya Uchida, Alexis Leonard, David Stroncek, Sandhya R. Panch, Kamille West, Eoghan Molloy, Thomas E. Hughes, Sara Hauffe, Tiffani Taylor, Courtney Fitzhugh, Jane S. Hankins, Megan Wilson, Akshay Sharma, Shengdar Q. Tsai, Mitch J. Weiss, Matthew Hsieh, and John F. Tisdale. Safe and efficient peripheral blood stem cell collection in patients with sickle cell disease using plerixafor. Haematologica. 2020; 105:xxx


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Title
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Authorship Contribution:
N.U. designed the research, wrote the clinical protocol, performed experiments, and wrote the paper; A.L. analyzed results, made the figures, and wrote the paper; D.S. designed the research; S.P. performed experiments; K.W. performed experiments; T.H. performed experiments; S.H. performed experiments; T.T. performed experiments; C.F. designed the research; J.H. performed experiments; M.W. performed experiments; A.S. performed experiments; S.T. performed experiments; M.W. designed the research; M.H. designed the research, wrote the clinical protocol, performed experiments, and wrote the paper; J.T. designed the research, wrote the clinical protocol, and wrote the paper.

Running Title:
Stem cell mobilization with plerixafor in SCD

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Word Count:
Main Text = 1,500
Trial Registration:  
clinicaltrials.gov identifier: NCT03226691

Acknowledgements  
This work was supported by the Intramural Research Program of NHLBI and NIDDK at NIH. This study was partially funded by The Doris Duke Foundation (SQT, AS, MJW) and NHLBI (P01 HL053749 to MJW and SQT). Plerixafor (Mozobil™) was provided by Sanofi, Bridgewater, NJ, USA. The authors thank Zana Blaku, Wynona Coles, Katherine Roskom, Donna Chauvet, Dr. Yan Zheng for their pharmacy, clinical, and apheresis support, Claire Drysdale for review of the manuscript, and Neal Jeffries for his statistical support.
Hematopoietic stem cell (HSC) gene therapy is potentially curative for sickle cell disease (SCD);\(^{(1)}\) however, options for HSC collection are limited in this population,\(^{(2-4)}\) and investigation of the collection, efficiency, and safety of peripheral blood (PB) mobilization with plerixafor from start to finish is needed. Here we describe consistent, safe, and sufficient PB HSC collection and processing after plerixafor mobilization from the greatest number of participants reported to date and the first two-institutional study. Our data suggest plerixafor mobilized HSCs in SCD are enriched for long-term engrafting HSCs, which is not true of HSCs from SCD bone marrow (BM),\(^{(5)}\) supporting a paradigm shift in the optimal HSC source for patients with SCD.

This open-label phase I study was sponsored by NHLBI at NIH and was conducted at the NIH Clinical Center and St. Jude Children’s Research Hospital (SJCRH) (NCT03226691). All patients provided written informed consent for a protocol approved by each institution’s Institutional Review Board. Hydroxyurea (HU) was stopped at least 2 weeks prior to mobilization, and all participants received red blood cell exchange the day prior to mobilization and collection to target <30% sickle hemoglobin (HbS).

Participants received a single subcutaneous dose (240\(\mu\)g/kg) of plerixafor (Mozobil\textsuperscript{TM}, Sanofi, Bridgewater, NJ) 4 hours before leukapheresis. Participants in the NIH cohort also received 325mg aspirin. If the minimum target CD34\(^+\) cell dose of 1.5\(\times\)10\(^6\) cells/kg (goal target 2.0\(\times\)10\(^6\) cells/kg) was not obtained, a second subcutaneous dose (240\(\mu\)g/kg) of plerixafor was administered the next day followed by repeat collection. Blood samples were drawn before and 2 hours after plerixafor administration, as well as
at the start and end of apheresis. Participants were observed on an inpatient basis for at least 24 hours after apheresis and received outpatient followup 3-10 days after discharge. Other methods are described in Supplementary text 1.

Fifteen participants with SCD (HbSS n=13, HbSC n=1, HbSβ+ n=1) were enrolled at SJCRH (n=3) or NIH (n=12) between July 2017 and February 2019. Median age was 29 years (20-50) and 47% were male (n=7). Mean Hb was 9.2gm/dL (7.3-13.6), with an average %HbS pre- and post-exchange transfusion of 57.9% (18.1-87.1%) and 27.0% (15.1-37.7%), respectively. Most participants were on HU prior to study entry (n=11); remaining participants were maintained on regular exchange transfusions (n=5, one participant was on both HU and exchange transfusion). HU was stopped a median of 17 days prior to mobilization and collection (range 15-34). The most common SCD-related complications prior to study entry were iron overload (n=11), vaso-occlusive crisis (VOC) (n=10), and acute chest syndrome (n=7).

Median baseline CD34+ cell count was 7.3 cells/μL (range 1.0-41.0) (Figure 1A). Median CD34+ cell counts varied substantially after plerixafor administration, averaging 38.5 cells/μL (3.0-152.0) at 2 hours, 52.0 cells/μL (9.0-183.0) at 4 hours/start of apheresis, and 21.0 cells/μL (2.0-129.0) at the completion of apheresis (Figures 1A-B).

Two participants did not achieve the minimum CD34+ target and underwent a second procedure the following day (Figure 1C). One of these participants achieved the target after a second procedure. The other participant underwent a repeat cycle after a 30 day
wait period, requiring 2 additional apheresis and yielding a total collection of 1.9x10^6 CD34+ cells/kg. A third participant underwent repeat collection on day 2 despite meeting the initial target after one apheresis (total day 1 = 2.9x10^6 CD34+ cells/kg) in order to store additional backup per allogeneic protocol.

The total white blood cell (WBC) count increased by an average of 3.2-fold over baseline values (1.7-5.0) to an average peak WBC count of 26.5x10^3/μL (14.1-47.4). All WBC counts returned to baseline within 1-2 days (Figure 1D). Median WBC, CD34+, CD19+, and CD3+ cells/kg in the final apheresis product after one (n=12) or two (n=3) collection procedures are shown in Figure 2A-C.

Mean whole blood flow rate during apheresis was 61.1ml/min (40-75). The average number of liters and total blood volumes processed during one apheresis was 17.8 L (10.6-30.1) and 4.5 (3-7), respectively (Figure 3A). Mean CD34+ collection efficiency was 32.2% (14.8-59.4%). Mean hematocrit in the collected product was 4.5% (2.7-7.5%).

Spearman's correlation test was used to assess the relationship between baseline and pre-apheresis CD34/μL, total CD34+ cells/kg collected, and total blood volume processed. (Figures 3B-F). There was a strong positive correlation between baseline CD34/μL and pre-aphereis CD34/μL \( (r_s=0.8426, \ p=0.001) \) (Figure 3B) and therefore a positive correlation between total CD34+ cells/kg collected and either baseline CD34/μL \( (r_s=0.7776, \ p=0.001) \) (Figure 3C) or pre-apheresis CD34/μL \( (r_s=0.8122, \)
p=0.001) (Figure 3D). Participants with the lowest pre-apheresis CD34 cell count generally underwent higher blood volume processing ($r_s=-0.1443$, $p=0.59$) (Figure 3E) in an effort to achieve target yields. In general, higher blood volume processing did not correlate to higher total CD34+ cells/kg yields ($r_s=-0.2104$, $p=0.43$) (Figure 3F). Participants with the lowest pre-apheresis CD34+ cell count/μL demonstrated the lowest total CD34+ collection/kg regardless of processing volumes (Supplementary text 2).

All participants except one successfully met the minimum target CD34+ cells/kg yield with two or fewer mobilization and apheresis procedures ($n=14$). Almost half the participants ($n=7$) had a CD34+ cells/kg yield $\geq 5.0 \times 10^6$ (5.4-12.0), which was achieved with only one apheresis in all but one participant.

Twelve final apheresis products contained a sufficient quantity of cells (median $6.3 \times 10^6$ CD34+ cells/kg, range 2.2-12.0) to allow for additional CD34+ selection, yielding an average CD34+ purity after selection of 94.7% (49.1-97.1%) (Figure 4A) and recovery of 46.8% (26-96%). Of note, the participant with 49.1% CD34+ purity required a second apheresis to meet the target, whereas all other participants achieved the CD34+ target after one apheresis. Notably, positively selected CD34+ cells demonstrated a CD34$^{\text{high}}$ phenotype, suggesting long-term engrafting ability. A median of 97% CD34+ cells were CD34$^{\text{high}}$ (73.6-99.4%) compared to 1.3% CD34$^{\text{low}}$ (0.09-24.4%) (Figure 4B). The gating strategy and comparison to previously published data on SCD BM vs. healthy, non-SCD BM is shown in Figure 4C-D, in which SCD BM is characterized by a minority of CD34$^{\text{high}}$ CD34+ cells.
Seven grade III AEs (two non-pain and five pain related) and one grade IV AE (non-pain – hemolysis) occurred, and each resolved with symptomatic treatment (Table 1). Eleven participants experienced pain (grade I-IV), with three patients accounting for the five pain related grade III-IV AEs. These three participants were hospitalized for 3, 5, and 7 days respectively, whereas the mean hospitalization for all 15 participants undergoing plerixafor mobilization and collection was 3.4 days (2-7). The level of HbS% did not correlated with the pain episodes. Participants who experienced VOC did not differ significantly from those who did not in their peak WBC count (Figure 5), absolute neutrophil count (Figure 5), or absolute monocyte count (data not shown). The participant with grade IV hemolysis experienced a delayed hemolytic transfusion reaction within one week of exchange transfusion and plerixafor mobilization.

Consistent with previous reports in a small number of patients,\textsuperscript{(8-11)} plerixafor mobilization in 15 participants with SCD resulted in safe and sufficient CD34+ cell collection. Plerixafor mobilization allowed high HSC yields sufficient for clinical gene therapy applications and for required back-up for allogeneic transplantation. Importantly, a median of 97% plerixafor mobilized HSCs demonstrated a CD34\textsuperscript{high} phenotype, suggesting collection of desirable long-term engrafting HSCs. This compares favorably to the minority CD34\textsuperscript{high} phenotype described for steady state SCD BM.\textsuperscript{(5)} Considering the risks of BM harvest in SCD patients and the poor quality and yield of HSCs obtained,\textsuperscript{(5)} our data indicate that plerixafor mobilization is a superior method for collecting HSCs from subjects with SCD.
In this study, participants with low baseline circulating CD34+ cells and low pre-
apheresis CD34+ cells had lower total CD34+ cells/kg regardless of volume of
processing (Figure 3B-E). Prolonged apheresis with larger processed blood volumes did
not equate to higher CD34+ recovery (Figure 3F) nor equivalent CD34+ recovery
among participants in the cohort despite different starting CD34+ baselines (Figure 3E).
Patients with low baseline CD34+ cells/μL potentially have lower collection efficiencies
and therefore would not benefit from higher blood volume processing. Participants who
required repeat mobilization, however, demonstrated a consistent CD34+ yield
suggesting that for maximal yield, repeat apheresis may be more beneficial than
prolonged collection. Additionally, in this particular patient population, less time on the
apheresis machine may reduce the risk of VOC.

Several key factors may improve HSC collection and reduce complications in subjects
with SCD after plerixafor mobilization. These include discontinuation of HU,(8-13)
optimization of the apheresis collection interface by staff experienced in SCD,(10)
initiation of apheresis prior to 4 hours post-plerixafor, and customized determination of
blood volumes to be processed based on pre-apheresis CD34+ counts. Kinetics data in
subjects with SCD suggest that mobilization of CD34+ cells starts within 2 hours after
subcutaneous plerixafor administration,(14) peaking at 3-6 hours compared to 6-12 hours
in healthy donors.(10, 15) The chronically hyperproliferative marrow in SCD may partly
explain this early release of HSCs, supporting earlier apheresis initiation at 2 hours for
maximal CD34+ yield (Figure 1A).
Here we describe consistent, safe, and sufficient HSC mobilization, collection, and processing for patients with SCD from the greatest number of patients reported to date and the first two-institutional study. Plerixafor mobilized HSCs in SCD are enriched for an engrafting population, demonstrating their superior quality for transplantation applications.

**Funding:** This work was partially funded by The Doris Duke Foundation (SQT, AS, MJW) and NHLBI (P01 HL053749 to MJW and SQT).

**Author Disclosure Statement**

The authors have no relevant disclosures

**References**

### Table 1. Adverse Events

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DHTR: delayed hemolytic transfusion reaction; PTT: partial thromboplastin time; VOC: vaso-occlusive crisis. Data are n (%)
**Figure Description**

**Figure 1. CD34 Plerixafor Mobilization and Collection in Participants with Sickle Cell Disease.** A-D. CD34+ cell count per µL was drawn before plerixafor administration (baseline), 2 hours after plerixafor, 4 hours after plerixafor (start of apheresis), and at the completion of apheresis (variable for each participant); (A) median CD34+/µL with minimum and maximum values and (B) individual CD34+/µL values are shown. Five participants were on chronic exchange transfusion prior to study enrollment. All other participants (plus one on chronic exchange transfusion) were on HU that was stopped at least 2 weeks prior to mobilization and collection; (C) three participants underwent more than 1 apheresis demonstrating consistent mobilization with each plerixafor mobilization and apheresis; (D) total white blood cell (WBC) count (10³/µL) was obtained before and after the start of apheresis and returned to baseline within 24 hours.

**Figure 2. Final Product Characterization.** (A-C) Total WBC, CD34+, CD19+, and CD3+ cells/kg were assessed in the final product after one (n=12) or two (n=3) apheresis procedures. One subject who did not meet the target CD34+ cells/kg dose after two apheresis procedures underwent repeat plerixafor mobilization and apheresis collection after a 30 day period, with total WBC, CD34+, CD19+, and CD3+ counts combined from all four apheresis procedures for this participant. Median values with 95% confidence interval shown.
Figure 3. Apheresis Details. (A) Total blood volume processed on day 1 with median and 95% confidence interval shown; (B-F) spearman correlation was run to assess relationships; (B) baseline CD34+ cell counts correlated with pre-apheresis CD34+ count; (C-D) participants with a low baseline (C) or pre-apheresis (D) CD34+ cell count/µL had lower total CD34+ x10^6/kg yield; (E-F) prolonged and higher blood volume processing was utilized in participants with a low baseline CD34/µL but did not correlate with higher total CD34+ x10^6/kg yield.

Figure 4. CD34+ Product Quality. (A) Twelve final apheresis products underwent additional CD34+ selection based on a high total CD34+ cells/kg yield; (B) the majority of purified CD34+ hematopoietic stem and progenitor cells gathered by plerixafor peripheral blood mobilization are CD34^{high} (NIH cohort, n=9, available for analysis). Median values with 95% confidence interval shown. (C-D) CD34^{high} vs. CD34^{low} populations; SCD plerixafor mobilized HSC data is compared to our previously reported data in SCD vs. non-SCD BM;\(^5\) (C) gating strategy for determining CD34^{high} vs. CD34^{low} populations in CD34+ selected HSCs from healthy, non-sickle cell disease (SCD) bone marrow (BM) (historical data), SCD BM (historical data), and SCD plerixafor mobilized HSCs (current study); (D) plerixafor mobilized HSCs from subjects with SCD in this study are immunophenotypically distinct from previously obtained SCD BM HSCs, which demonstrate a predominantly CD34^{low} phenotype.\(^5\)

Figure 5. Vaso-occlusive Adverse Events and White Blood Cell Counts.
Participants who experienced a vaso-occlusive (VOC) adverse event (AE) (n=11) did not differ significantly in their WBC count or ANC, compared to those who did not (n=4).
ANC data not available for SJCRH cohort. Median values with 95% confidence interval shown.
Figure 2

A. Total WBC $\times 10^9$/kg
- Participants on HU
- Participants on chronic exchange transfusion
- Participant on both chronic exchange transfusion & HU
- Combined total, >1 apheresis (on chronic transfusion)
- Combined total in participants with >1 apheresis (on HU)

B. CD34+ $\times 10^6$/kg
- Patients on HU
- Participants on chronic exchange transfusion
- Participant on both chronic exchange transfusion & HU
- Combined total, >1 apheresis (on chronic transfusion)
- Combined total, >1 apheresis (on HU)

C. CD19+ and CD3+ $\times 10^8$/kg
- Participants on HU
- Participants on chronic exchange transfusion
- Participant on both chronic exchange transfusion & HU
- Combined total, >1 apheresis (on chronic transfusion)
- Combined total, >1 apheresis (on HU)
Figure 4

A. CD34+ purity

B. CD34^{high} vs. CD34^{low} HSC populations in final apheresis product

C. Healthy, non-SCD BM
   SCD BM
   SCD plerixafor mobilized HSCs

D. CD34^{high} vs. CD34^{low} HSCs in SCD
   - SCD BM Derived HSCs
   - SCD Plerixafor Mobilized HSCs
Figure 5

VOC Adverse Event:
White Blood Cell and Absolute Neutrophil Counts

- □ Participants without VOC AE
- ● Participants with VOC AE
**Supplementary information**

**Supplementary text 1**

Leukapheresis was initiated approximately 4 hours after plerixafor administration using the Spectra Optia (continuous-flow mononuclear cell program) apheresis system (Terumo BCT Inc., Lakewood, CO). All cases used Acid Citrate Dextrose formula A for intra-procedural anticoagulation (initial whole blood to anticoagulant ratio of 12:1) and prophylactic IV calcium infusions. During apheresis, operators went slightly deeper into the buffy coat, targeting a hematocrit of 5-6%.

Adverse events (AEs) were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 4.0. All serious AEs were tracked for 30 days, until resolution, or until study completion (10 days after last dose of the study treatment), whichever was later. Separate stopping rules for pain and non-pain related AEs were designed to stop enrollment if data suggested the probability of a grade III AE attributable to plerixafor was >20%.

PB CD34+ cells/µL was measured by flow cytometry (BD FACSCanto II, BD Biosciences, San Jose, CA). Cell populations in the collection product were determined by flow cytometry using anti-human CD34 (clone 8G12), CD3 (SK7), and CD19 (SJ25C1) antibodies (BD Biosciences). CD34+ cells were selected using CliniMACS CD34 Reagent System (Miltenyi Biotec, Bergisch Gladbach, Germany).

**Supplementary text 2**

Of the two participants who required a second apheresis to achieve the minimum CD34+ cell target, baseline CD34+ count/µL was 1.0 and 3.0 CD34+/µL, respectively. Each underwent 15.0
and 15.1 L of processing (day 1) yielding 0.5x10^6 and 1.2x10^6 CD34+cell/kg, respectively, and 16.7 and 15.0 L of processing (day 2) to achieve similar collection yields (0.4x10^6 and 1.1x10^6 CD34+cell/kg, respectively), providing a combined total of 0.9x10^6 and 2.2x10^6 CD34+cells/kg, respectively. The participant with the highest blood volume processed (30.1 L) achieved 1.8x10^6 CD34+ cells/kg (baseline CD34+/µL=4.0), whereas the participant with the highest CD34+ cells/kg (12.0x10^6) achieved this target after processing 16.7 L (baseline CD34+/µL=12.0). The participant with the highest baseline CD34+ cells/µL (40.1) underwent 15.0 L of processing to achieve a final product of 4.0x10^6 CD34+ cells/kg. Overall this participant’s fold increase in CD34+ cell count/µL (1.7) was the lowest of the cohort (7.9, range 1.7-18.0).