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

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 HSC in SCD are enriched for long-term engrafting HSC, which is not true of HSC 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 the National Heart, Lung and Blood Institute at the National Institutes of Health (NIH) and was conducted at the NIH Clinical Center and St. Jude Children's Research Hospital (SJCRH) (*clinicaltrials.gov identifier: NCT03226691*). All participants provided written informed consent for a protocol approved by each institution's Institutional Review Board. Hydroxyurea (HU) was stopped at least two 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 μg/kg) of plerixafor (Mozobil™, Sanofi, Bridgewater, NJ, USA) 4 hours (h) before leukapheresis. Participants in the NIH cohort also received 325 mg aspirin. If the minimum target CD34$^+$ cell dose of 1.5x10^6 cells/kg (goal target 2.0x10^6 cells/kg) was not obtained, a second subcutaneous dose (240 μg/kg) of plerixafor was administered the next day followed by repeat collection. Blood samples were drawn before and 2 h after plerixafor administration, as well as at the start and end of apheresis. Participants were observed on an inpatient basis for at least 24 h after apheresis and received outpatient follow up 3-10 days after discharge. Other methods are described in *Online 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 years) and 47% were male (n=7). Mean hemoglobin (Hb) was 9.2 gm/dL (7.3-13.6 gm/dL), 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 days). 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 (1.0-41.0 cells/μL) (Figure 1A). Median CD34$^+$ cell counts varied substantially after plerixafor administration, averaging 38.5 cells/μL (3.0-152.0 cells/μL) at 2 h, 52.0 cells/μL (9.0-183.0 cells/μL) at 4 h/start of apheresis, and 21.0 cells/μL (2.0-129.0 cells/μL) at the completion of apheresis (Figure 1A and 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

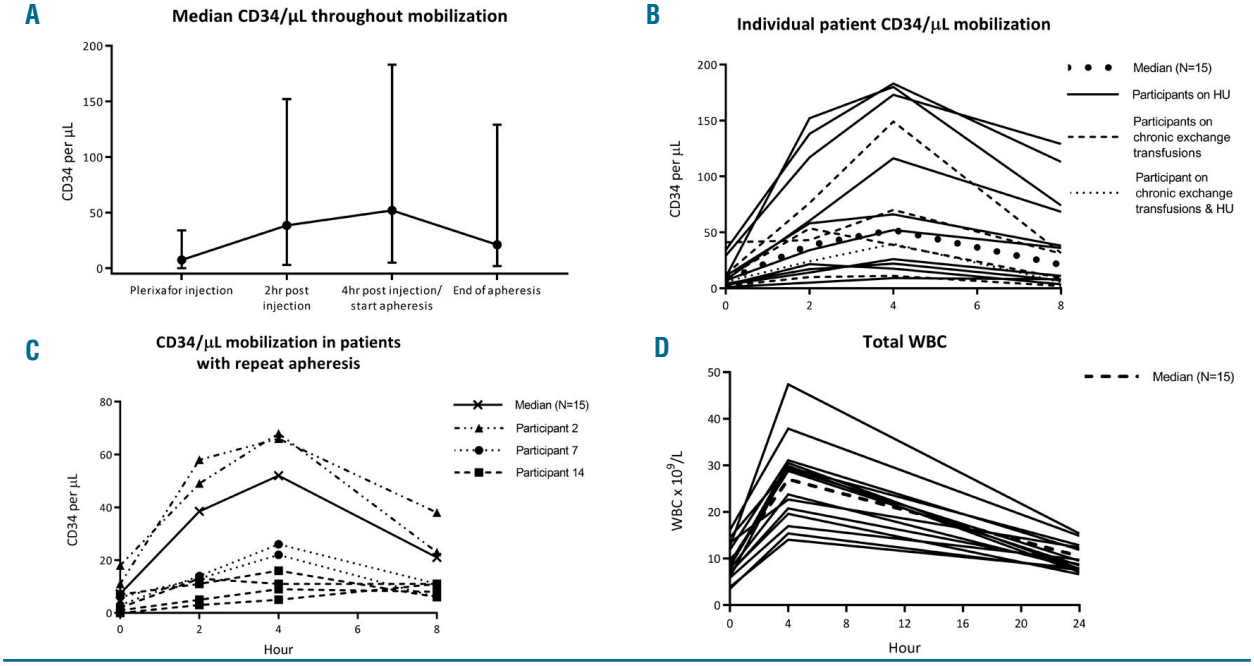

Figure 1. CD34 plerixafor mobilization and collection in participants with sickle cell disease. (A-D) CD34$^+$ cell count per μL was drawn before plerixafor administration (baseline), 2 hours (h) after plerixafor, 4 h after plerixafor (start of apheresis), and at the completion of apheresis (variable 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 hydroxyurea (HU) that was stopped at least 2 weeks prior to mobilization and collection; (C) three participants underwent more than one apheresis demonstrating consistent mobilization with each plerixafor mobilization and apheresis; (D) total white blood cell (WBC) count (x10^9/L) was obtained before and after the start of apheresis and returned to baseline within 24 h.

target after a second procedure. The other participant underwent a repeat cycle after a 30-day wait period, requiring two additional apheresis and yielding a final 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 3.2-fold over baseline values (1.7-5.0 -fold) to an average peak WBC count of 26.5x10^9/L (14.1-47.4x10^9/L). 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 61.1 mL/minute (40-75 mL/minute). The average number of liters (L) and total blood volumes (TBV) processed during one apheresis was 17.8 L (10.6-30.1 L) and 4.5 TBV (3-7 TBV), 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 TBV processed. (Figure 3B-F). There was a strong positive correlation between baseline CD34/μL and pre-aphereis CD34/μL (rs=0.8426, *P*=0.001) (Figure 3B) and therefore a positive correlation between total CD34$^+$ cells/kg collected and either baseline CD34/μL (rs=0.7776, *P*=0.001) (Figure 3C) or pre-apheresis CD34/μL (rs=0.8122, *P*=0.001)

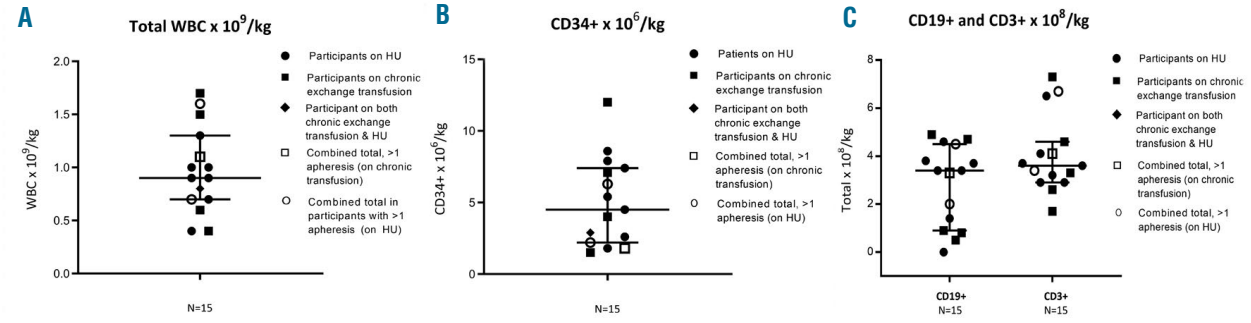

Figure 2. Final product characterization. (A-C) Total white blood cell (WBC) count, CD34$^+$, CD19$^+$, and CD3$^+$ cells/kg were assessed in the final product after one (n=12) or two (n=3) apheresis procedures. One subject who did not meet the target CD34$^+$ cells/kg dose after two apheresis procedures underwent repeat plerixafor mobilization and apheresis collection after a 30-day wait period, with total WBC, CD34$^+$, CD19$^+$, and CD3$^+$ counts combined from all four apheresis procedures for this participant. Median values with 95% confidence interval are shown. HU: hydroxyurea.

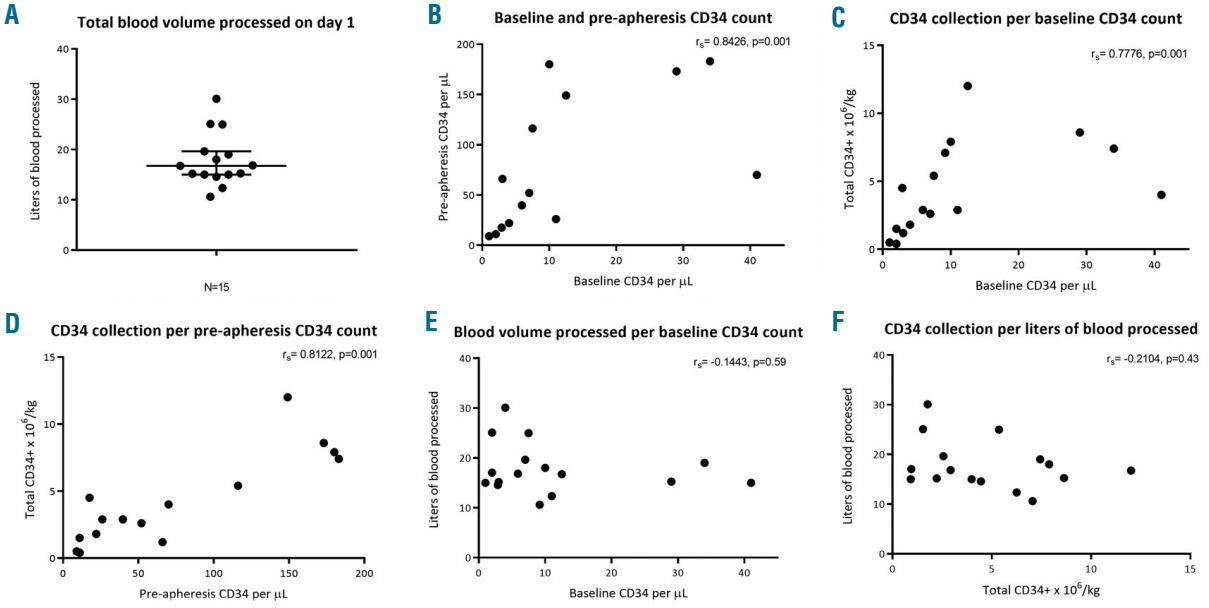

Figure 3. Apheresis details. (A) Total blood volume processed on day 1 with median and 95% confidence interval shown; (B-F) spearman correlation was run to assess relationships; (B) baseline CD34$^+$ cell counts correlated with pre-apheresis CD34$^+$ count; (C and D) participants with a low baseline (C) or pre-apheresis (D) CD34$^+$ cell count/μL had lower total CD34$^+$ x 10^6/kg yield; (E and F) prolonged and higher blood volume processing was utilized in participants with a low baseline CD34/μL but did not correlate with a higher total CD34$^+$ x 10^6/kg yield.

(Figure 3D). Participants with the lowest pre-apheresis CD34 cell count generally underwent higher blood volume processing (rs=-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 (rs=-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 (*Online 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 ≥5.0x10^6 (5.4-12.0x10^6), which was achieved with only one apheresis in all but one participant.

Twelve final apheresis products contained a sufficient quantity of cells (median 6.3x10^6 CD34$^+$ cells/kg, range 2.2-12.0 cells/kg) 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^{high} phenotype, suggesting long-term engrafting ability.[6-8] A median of 97% CD34$^+$ cells were CD34^{high} (73.6-99.4%) compared to 1.3% CD34^{low} (0.09-24.4%) (Figure 4B). The gating strategy and comparison to previously published data on SCD BM *versus*

Table 1. Adverse events.

	Grade I	Grade II	Grade III	Grade IV
Pain-related				
VOC	6 (40%)	5 (33%)	4 (27%)	
Non-pain-related				
DHTR				1 (7%)
Anemia			1 (7%)	
Headache	4 (27%)	1 (7%)	1 (7%)	
Myalgia	1 (7%)			
Insomnia	1 (7%)	1 (7%)		
Acute kidney injury	1 (7%)			
Decreased platelets	1 (7%)	1 (7%)		
Hypomagnesemia	5 (33%)	1 (7%)		
Hyponatremia	2 (13%)			
Hypokalemia	4 (27%)	2 (13%)		
Hypocalcemia	4 (27%)	1 (7%)		
Hypophosphatemia	1 (7%)			
Confusion	1 (7%)			
Hypotension	1 (7%)			
Fatigue	2 (13%)	2 (13%)		
Pruritis		1 (7%)		
Anorexia	1 (7%)			
Bloating		2 (13%)		
Fever	1 (7%)			
Injection site reaction	1 (7%)			
Flushing	1 (7%)			
Paresthesia	5 (33%)	1 (7%)		
Blurred vision		2 (13%)		
Nausea/mesis	2 (13%)	1 (7%)		
Tinnitus	1 (7%)			
Edema	2 (13%)			
Prolonged PTT	1 (7%)			
Hyperglycemia	1 (7%)			
Urinary retention		1 (7%)		
Hematuria	1 (7%)			
Increased bilirubin	1 (7%)			
Irregular heart rate	1 (7%)			
Dyspepsia	1 (7%)			

DHTR: delayed hemolytic transfusion reaction; PTT: partial thromboplastin time; VOC: vaso-occlusive crisis. Data presented as number (%).

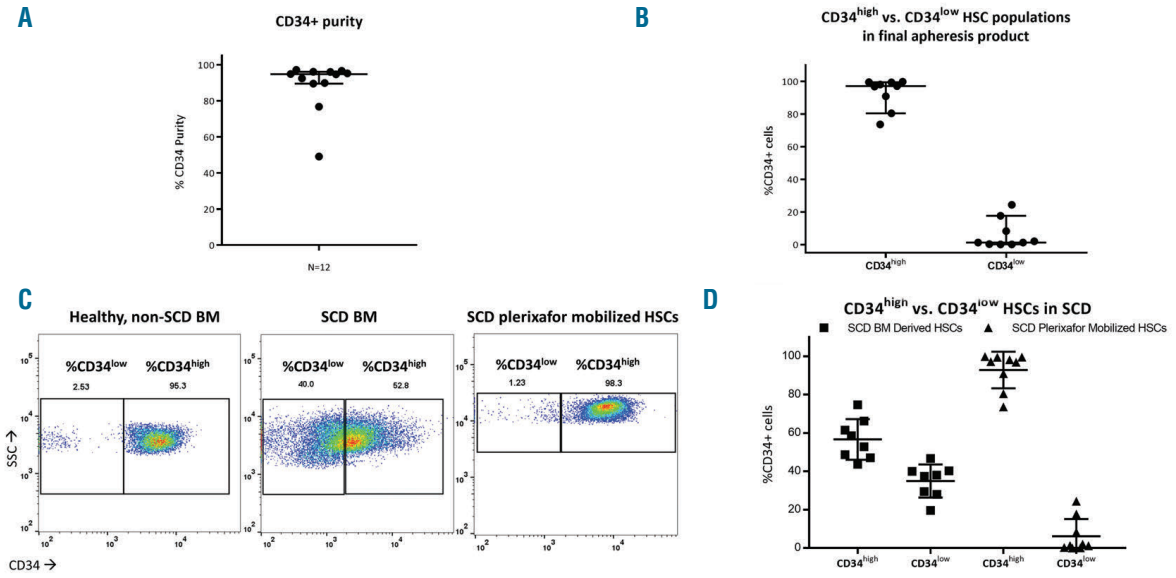

Figure 4. CD34⁺ product quality. (A) Twelve final apheresis products underwent additional CD34⁺ selection based on a high total CD34⁺ cells/kg yield; (B) the majority of purified CD34⁺ hematopoietic stem cell (HSC) and progenitor cells gathered by plerixafor peripheral blood mobilization are $CD34^{high}$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

healthy, non-SCD BM is shown in Figure 4C and D, in which SCD BM is characterized by a minority of $CD34^{high}$ CD34⁺ cells.[5]

Seven grade III adverse events (AE) (two non-pain-related 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 participants accounting for the five pain-related grade III-IV AE. 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 days). The level of HbS% did not correlate with the pain episodes. There was no significant difference in peak WBC count (Figure 5), absolute neutrophil count (Figure 5), or absolute monocyte count (*data not shown*) between participants who experienced VOC and those who did not. 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,[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 backup for allogeneic transplantation. Importantly, a median of 97% plerixafor mobilized HSC demonstrated a $CD34^{high}$ phenotype, suggesting collection of desirable long-term engrafting HSC. This compares favorably to the minority $CD34^{high}$ phenotype described for steady state SCD BM.[5] Considering the risks of BM harvest in SCD patients, and the poor quality and yield of HSC obtained,[5] our data indicate that plerixafor mobilization is a superior method for collecting HSC from subjects with SCD.

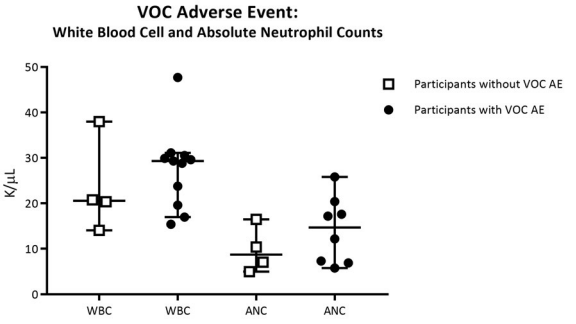

Figure 5. Vaso-occlusive adverse events and white blood cell (WBC) counts. There was no significant difference in WBC count or absolute neutrophil count (ANC) between participants who experienced a vaso-occlusive (VOC) adverse event (AE) (n=11) and those who did not (n=4). ANC data not available for the St. Jude Children's Research Hospital (SJCRH) cohort. Median values with 95% confidence interval are shown.

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 h 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 h after subcutaneous plerixafor administration,[14] peaking at 3-6 h compared to 6-12 h in healthy donors.[10,15] The chronically hyperproliferative marrow in SCD may partly explain this early release of HSC, supporting earlier apheresis initiation at 2 h 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 HSC in SCD are enriched for an engrafting population, demonstrating their superior quality for transplantation applications.

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