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Safety and efficacy of plerixafor dose escalation for the mobilization of CD34⁺ hematopoietic progenitor cells in patients with sickle cell disease: interim results

Farid Boulad,^{1,2} Tsiporah Shore,³ Koen van Besien,³ Caterina Minniti,⁴ Mihaela Barbu-Stevanovic,⁵ Sylvie Wiener Fedus,⁶ Fabiana Perna,² June Greenberg,⁷ Danielle Guarneri,⁷ Vijay Nandi,⁵ Audrey Mauguen,⁸ Karina Yazdanbakhsh,⁵ Michel Sadelain² and Patricia A. Shi^{4,5}

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¹Department of Pediatrics, BMT Service, Memorial Sloan Kettering Cancer Center, New York; ²Center for Cell Engineering, Memorial Sloan Kettering Cancer Center, New York; ³Bone Marrow and Hematopoietic Stem Cell Transplant Program, Weill Cornell Medicine/ New York Presbyterian Hospital, New York; ⁴Sickle Cell Program, Division of Hematology, Albert Einstein College of Medicine, Bronx; ⁵Lindsley F. Kimball Research Institute, New York Blood Center, NY; ⁶Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York; ⁷Division of Hematology and Oncology, Weill Cornell Medicine /New York Presbyterian Hospital, NY and ⁸Department of Epidemiology and Biostatistics, Memorial Sloan Kettering Cancer Center, New York, NY, USA

ABSTRACT

Gene therapy for sickle cell disease is limited by the yield of hematopoietic progenitor cells that can be harvested for transduction or gene editing. We therefore performed a phase I dose-escalation study of the hematopoietic progenitor cell mobilizing agent plerixafor to evaluate the efficacy and safety of standard dosing on peripheral blood CD34⁺ cell mobilization. Of 15 patients enrolled to date, only one was chronically transfused and ten were on hydroxyurea. Of eight patients who achieved a CD34⁺ cell concentration >30 cells/ μ L, six were on hydroxyurea. There was no clear dose response to increasing plerixafor dosage. There was a low rate of serious adverse events; two patients developed vaso-occlusive crises, at the doses of 80 μ g/kg and 240 μ g/kg. Hydroxyurea may have contributed to the limited CD34⁺ mobilization by affecting baseline peripheral blood CD34 counts, which correlated strongly with peak peripheral blood CD34 counts. Plerixafor administration did not induce significant increases in the fraction of activated neutrophils, monocytes, or platelets. However, increased neutrophils positive for activated β 2 integrin and Mac-1 were associated with serious adverse events. In summary, plerixafor was well tolerated but did not achieve consistent CD34⁺ cell mobilization in this cohort of patients, most of whom were being actively treated with hydroxyurea and only one was chronically transfused. The study will continue with escalation of the dose of plerixafor and modification of hydroxyurea administration. *Clinicaltrials.gov identifier: NCT02193191.*

Correspondence:

pshi@nybc.org

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Introduction

Autologous gene therapy holds considerable promise for the treatment of patients with sickle cell disease (SCD).^{1,2} However, its successful application requires an adequate number of hematopoietic progenitor cells (HPC) for gene transfer or gene editing.³ Steady-state bone marrow has been the historical source of HPC for SCD gene therapy, but its harvest requires general anesthesia and has been complicated in current gene therapy trials by the need for repeated bone marrow harvests and a high rate of adverse events.⁴ Granulocyte colony-stimulating factor (G-CSF) is a standard method of mobilizing HPC but its use in SCD patients has been associated with vaso-occlusive complications and even death.⁵ The mechanism of action of G-CSF involves activation of neutrophils,⁶ and is also associated with endothelial cell, platelet, and coagulation system activation,⁷⁻¹¹ all of which may play a crucial role in sickle cell vaso-occlusion.¹²

In contrast to G-CSF, plerixafor is a bicyclam reversible small molecule inhibitor

Table 1. Patients' characteristics.

Dose level	Subject ID	Ethnicity	Age yrs	Gender	Genotype	Treatment regimen	Clinical complications (in addition to ACS)
80 µg/kg	1	African-American	33	M	SS	HU 16 mg/kg	Avascular necrosis, retinopathy
	2		21	F	SS	HU 26 mg/kg	≥ 3 vaso-occlusive crises per year
	3 (1*)	Hispanic	29	M	SS; thal(1/4)	No HU	≥ 3 vaso-occlusive crises per year
	4		32	M	SS; thal (1/4)	No HU	Deep venous thrombosis, priapism
	5		34	M	SS; thal (1/4)	HU 28 mg/kg	Leg ulcers, retinopathy
	6		25	F	SS; thal (1/4)	HU 16 mg/kg	Leg ulcers, retinal artery occlusion, retinopathy
160 µg/kg	7	African-American	25	F	SS	HU 25 mg/kg	Cerebral aneurysms (2-3 mm)
	8 (1*)		37	M	SS	HU 27 mg/kg	Avascular necrosis, retinopathy
	9		32	F	SS	Chronic transfusion – No HU	Avascular necrosis, iron overload, leg ulcers
240 µg/kg	10	African-American	46	M	SS; thal (1/4)	No HU	Leg ulcers, priapism
	12		38	M	SS	HU 17 mg/kg	Avascular necrosis, leg ulcers, priapism
	13		23	F	SS	HU 27 mg/kg	Avascular necrosis, retinopathy
	14		27	M	SS	HU 23 mg/kg	Avascular necrosis, priapism
	3 (2*)		31	M	SS- thal (1/4)	No HU	≥ 3 vaso-occlusive crises per year
	8 (2*)		38	M	SS	HU 27 mg/kg	Avascular necrosis, retinopathy

*Indicates first and second enrollments for indicated subject. ACS: acute chest syndrome.; HU: hydroxyurea.

of the chemokine receptor CXCR4 and prevents binding of its ligand CXCL12 or stromal cell derived factor-1 α to induce HPC mobilization.¹³ We hypothesized that plerixafor's mechanism of action would lead to less marked increases in white blood cell (WBC) counts and therefore less cell and coagulation system activation in SCD and supported this with data from a pre-clinical study involving a sickle cell mouse model.¹⁴ Nevertheless, the safety of plerixafor in SCD patients remains a matter of concern because of possible activation of WBC and neutrophils which could still lead to vaso-occlusive complications and the risk of early death in SCD.¹⁵⁻¹⁹ As CXCR4 is expressed on most WBC and is involved in the retention of these cells in bone marrow, a standard dose of plerixafor of 240 µg/kg increases all major WBC subsets (neutrophils, lymphocytes, monocytes) in normal donors about 3- to 4-fold.²⁰ Notably, however, in SCD patients who received G-CSF, not all patients who had highly elevated WBC counts experienced vaso-occlusive complications, and conversely, not all patients who experienced vaso-occlusive complications had highly elevated WBC counts,⁵ suggesting that WBC activation rather than WBC count *per se* may contribute to vaso-occlusion in SCD.

Another issue of concern is whether enough peripheral blood CD34⁺ cells can be mobilized in SCD patients with plerixafor. The mean and median peak CD34⁺ counts using plerixafor alone in normal donors are only ~25/µL.^{13,20} SCD patients might mobilize particularly well, in that SCD patients might have increased circulating HPC even at steady state, although more so during a crisis.^{21,22} Furthermore, SS and S β^0 patients tend to be autosplenectomized, and data from patients with thalassemia showed that splenectomized patients mobilized about twice as many peripheral blood CD34 cells with plerixafor alone as non-splenectomized patients.²³

Another consideration when using plerixafor is whether to withhold hydroxyurea, the recommended standard of care for most SCD patients. Hydroxyurea may inhibit mobilization and withholding hydroxyurea for 2 weeks leads to a degree of spontaneous mobilization that abets

drug-induced mobilization.^{23,24} However, Richard *et al.* showed that two of the three SCD patients whose hydroxyurea was withdrawn for 2 weeks developed painful crises following the withdrawal. Given these considerations, we designed a prospective phase I dose escalation study of both the safety and efficacy of plerixafor in patients with SCD in which the patients continued on their standard outpatient treatment used for disease control. We have completed the dosing cohorts through to the standard plerixafor dose of 240 µg/kg and report the interim results here.

Methods

Study design

This study is conducted under FDA IND 122657, registered in ClinicalTrials.gov as NCT02193191, and approved by the Institutional Review Boards of Memorial Sloan Kettering, Weill Cornell Medical College and the New York Blood Center. The study design is a 3 + 3 dose escalation study with six levels of escalation: doses of 80, 160, 240, 320, 400, and 480 µg/kg. There are two primary endpoints: (i) efficacy, defined by the achievement of a HPC mobilization level of 30 CD34⁺ cells/µL; and (ii) safety, defined by the occurrence of serious adverse events (≥ grade 3) that are at least possibly plerixafor-related (including vaso-occlusive events).

At any dose level, the occurrence of at least one grade 3 serious adverse event results in the addition of three more patients to the initial three-patient dosing cohort. The occurrence of two grade 3 serious adverse events at a particular dose-level signifies that the maximal tolerated dose has been exceeded and that the previous dose-level is the maximum tolerated dose. The trial will be stopped upon the occurrence of one grade 4 or 5 serious adverse event at least possibly related to plerixafor. Patients are followed for adverse events for 1 month after administration of the plerixafor. This design provides the following probabilities of escalation based on the true chance of a dose-limiting toxicity at a specific dose level:

True probability of toxicity 0.10 0.20 0.30 0.40 0.50 0.60

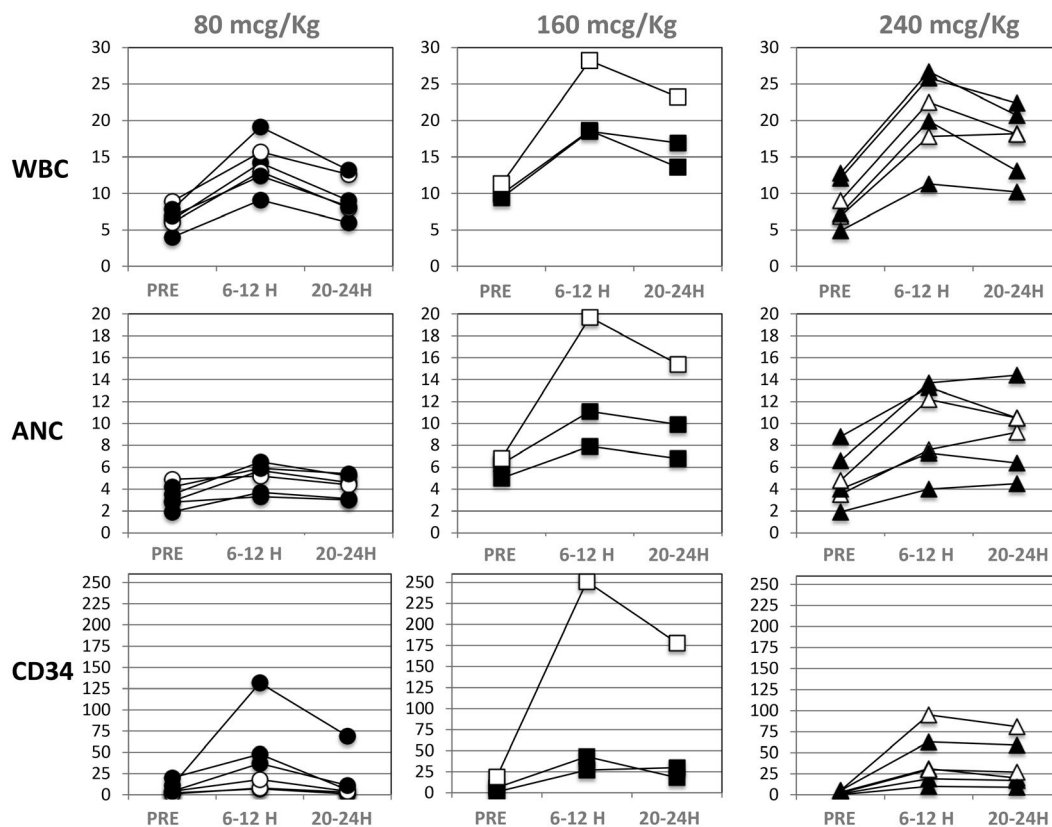


Figure 1. Peripheral blood white blood cell, absolute neutrophil, and CD34 cell counts. There is a trend of increasing white blood cell count (WBC, $P=0.05$) and absolute neutrophil count (ANC, $P=0.03$), but not CD34 concentration ($P=0.65$) with increasing dose of plerixafor. The graphs show the peripheral blood WBC, ANC, and CD34 concentrations of 15 patients with SCD treated with 80 (circles), 160 (squares) and 240 (triangles) $\mu\text{g}/\text{kg}$ of plerixafor prior to administration of plerixafor (PRE), 6-12 h and 20-24 h after plerixafor. Patients on hydroxyurea are represented by filled circles, squares and triangles, patients off hydroxyurea are represented by open circles, squares and triangles.

Probability of escalation: 0.91 0.71 0.49 0.31 0.17 0.08

For the efficacy endpoint, the dose escalation will continue to 480 $\mu\text{g}/\text{kg}$ unless all patients at a preceding dose level achieve a peripheral blood CD34⁺ concentration of at least 30 cells/ μL . In the present dose-escalation phase, no leukapheresis is performed. If and when the efficacy endpoint is safely reached, the study will proceed to a leukapheresis phase (including preclinical transduction and editing) in three patients.

Eligible subjects are adults with SS or S β^0 disease, normal renal and liver function, hemoglobin concentration ≥ 6 g/dL, WBC count $\geq 3,000/\mu\text{L}$, absolute neutrophil count (ANC) $\geq 1,500/\mu\text{L}$, and platelet count of $\geq 150,000/\mu\text{L}$. Eligible subjects are admitted to the Clinical Research Center at Weill Cornell Medical College. A single subcutaneous injection of plerixafor (Sanofi-Genzyme) is administered in the evening between 8-9 pm. The protocol calls for peripheral blood sampling at three time points (baseline, 0-2 h prior to plerixafor; peak between 6-12 h after the plerixafor dose; at the presumed return to baseline between 20-24 h after the dose): for reasons of feasibility and patient comfort issues, the peak sample was consistently drawn at a mean of 12 ± 1 h after plerixafor administration and the return to baseline sample at a mean of 20 ± 0.29 h after the dose. Since patients have pre-existing anemia, for reasons of safety no more than a total of 105 mL of blood is drawn at all three time points combined.

Peripheral blood CD34 testing

Flow cytometric evaluation of the collected peripheral blood is performed using a FACS Canto flow cytometer (Becton

Dickinson Biosciences, San Jose, CA, USA) and FACS Diva software (BD Biosciences). Samples are stained and analyzed within 2-12 h of collection using a modification of the International Society of Hematology and Graft Engineering (ISHAGE) method (see *Online Supplementary Methods*).

CD34⁺CD38⁻ enumeration

Mononuclear cells are isolated from 2 mL peripheral blood by Ficoll-Hypaque Plus density centrifugation. CD34⁺ cells are purified by positive selection (MidiMACS™ LS Columns, Miltenyi) and stained with CD34 (BD PharMingen) and CD38 (Invitrogen).

Research cell and coagulation activation studies

Peripheral blood samples drawn at baseline and after 12 h are stained within 1 h of collection for activation markers relevant to sickle vaso-occlusion^{12,25-28} and assessed by flow cytometry (BD FACSCanto™). For CD16b⁺ (1D3, Beckman Coulter) neutrophils: activated $\beta 2$ integrin (clone 24, abcam), activated Mac-1 (CBRM1/5, eBioscience), E-selectin-Fc chimera (724-ES, R&D Systems), L-selectin (DREG-56, eBioscience), Mac-1/CD11b (ICRF44, BD Pharmingen), and LFA-1/CD11a (HI111, BD Pharmingen). For CD14⁺ (M5E2, BD Pharmingen) monocytes: tissue factor (HTF-1, BD Pharmingen). For CD41⁺ (HIP2, BD Pharmingen) platelets: CD16b (1D3, Beckman Coulter) and CD14 (M5E2, BD Pharmingen). The percentages of positive cells and median fluorescent intensity (MFI) are assessed for each

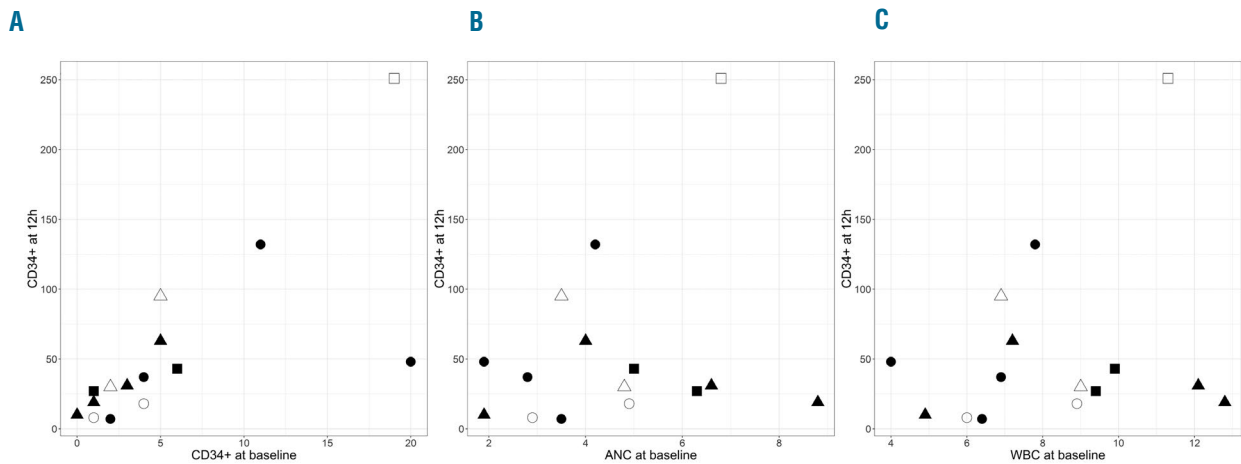


Figure 2. Correlation between post-plerixafor CD34⁺ cell counts and baseline cell counts. The CD34⁺ level at 12 h correlated positively with the baseline level of CD34⁺ ($P=0.0006$) but not baseline levels of absolute neutrophil count (ANC, $P=0.66$) or white blood cells (WBC, $P=0.49$). The graphs show the association between the value of peripheral blood CD34 concentration at 12 h after plerixafor and the baseline values of (A) CD34, (B) ANC and (C) WBC in 15 patients with SCD treated with 80 (circles), 160 (squares) and 240 (triangles) $\mu\text{g}/\text{kg}$ of plerixafor. Patients on hydroxyurea are represented by filled circles, squares and triangles, patients off hydroxyurea are represented by open circles, squares and triangles.

patient, with calculation of the absolute number of positive cells by multiplying the percentage of positive cells by the relevant number of cells obtained from a concurrent complete blood count. For plasma studies, whole blood is centrifuged at 2500 rpm for 15 min at 4°C and plasma frozen at $\leq -80^\circ\text{C}$ until batch testing. The following coagulation system activation markers relevant to sickle vaso-occlusion are tested:^{26,29} prothrombin fragment 1.2 (Enzygnost F1+2, Dade-Behring) and factor VIII (STA[®]-ImmunoDef VIII, Stago).

Statistical analysis

Absolute concentrations as well as the fold increases (ratio of peak at 12 h to baseline absolute concentrations) of standard clinical as well as research parameters were analyzed. For patient 8(2), to avoid computing correlations on an infinite value, we replaced the baseline value of 0 by 0.2 in the analyses using CD34⁺ ratio ($10/0.2 = 50$). When applying non-parametric statistics, the chosen value does not affect the results, as long as it is close to 0. The presence of a trend in increases of CD34⁺, ANC, and WBC counts (both at 12 h and fold increases) with increasing dose was tested using the non-parametric Cuzick test for trend. Correlations between baseline values, and values at 12 h or fold increases were estimated using the Kendall tau. A difference in the distribution of 12 h and fold increases according to the administration of hydroxyurea was investigated using a Wilcoxon test.

For analyses of cell activation and coagulation, Wilcoxon signed rank paired testing was performed on the combined dose cohorts for differences between 12 h and baseline values, whereas the presence of significant fold differences between dose levels was examined with the Kruskal-Wallis test. Correlations between values were estimated using the Kendall tau. Two-tailed P values <0.05 were considered statistically significant.

Results

Patients' characteristics

Fifteen subjects have been recruited to date for the study at the first three dose levels of 80, 160 and 240 $\mu\text{g}/\text{kg}$. Fourteen patients were enrolled from Montefiore

Medical Center (New York, USA) and one patient from The Mount Sinai Hospital (New York, USA) (Table 1). Two patients enrolled at dose levels 1 and 2 were subsequently re-enrolled in the study at a higher plerixafor dose (dose level 3). All subjects had a past history of moderate to severe acute chest syndrome, defined by requiring treatment with simple or exchange transfusion. Importantly, for safety and feasibility, patients were continued on their standard outpatient treatment being used to control their disease. Ten of 15 patients were on hydroxyurea, with a median HbF level of 12.4% (*Online Supplementary Table S1*) and median baseline ANC of 4100/ μL (*Online Supplementary Table S2*). Only one of the 15 subjects was receiving chronic transfusion therapy, with a HbF of 1.2% and HbA of 54%; this patient was also on deferasirox for the treatment of transfusion-related iron overload. HbA was absent in all other patients. In the non-transfused patients, HbF levels correlated strongly with hemoglobin concentration, hematocrit, and reticulocyte counts. Of nine patients for whom splenic imaging was available, seven had splenic atrophy (*Online Supplementary Table S1*).

Efficacy of CD34⁺ mobilization

Absolute WBC counts, neutrophil counts and CD34⁺ cell concentrations increased from baseline in all patients (Figure 1). Absolute monocyte and lymphocyte counts also increased from baseline (*Online Supplementary Table S2*). Our target goal of mobilizing at least 30 CD34⁺ cells/ μL was, however, reached in only 50% of patients given the plerixafor dose of 80 $\mu\text{g}/\text{kg}$, 33% of patients given 160 $\mu\text{g}/\text{kg}$, and 33% of patients given 240 $\mu\text{g}/\text{kg}$. Peak ANC ($P=0.03$) and WBC count ($P=0.05$), but not CD34⁺ cell count ($P=0.65$), increased with increasing dose level. As previously reported in healthy donors,^{13,30,31} there was a strong correlation of peak CD34⁺ count with baseline CD34⁺ concentration (Kendall tau=0.68, $P=0.0006$) but no correlation was observed with baseline ANC (Kendall tau=0.09, $P=0.66$) or baseline WBC count (Kendall tau=0.13, $P=0.49$) (Figure 2). There was also no correlation, as previously reported,¹³ with baseline platelet

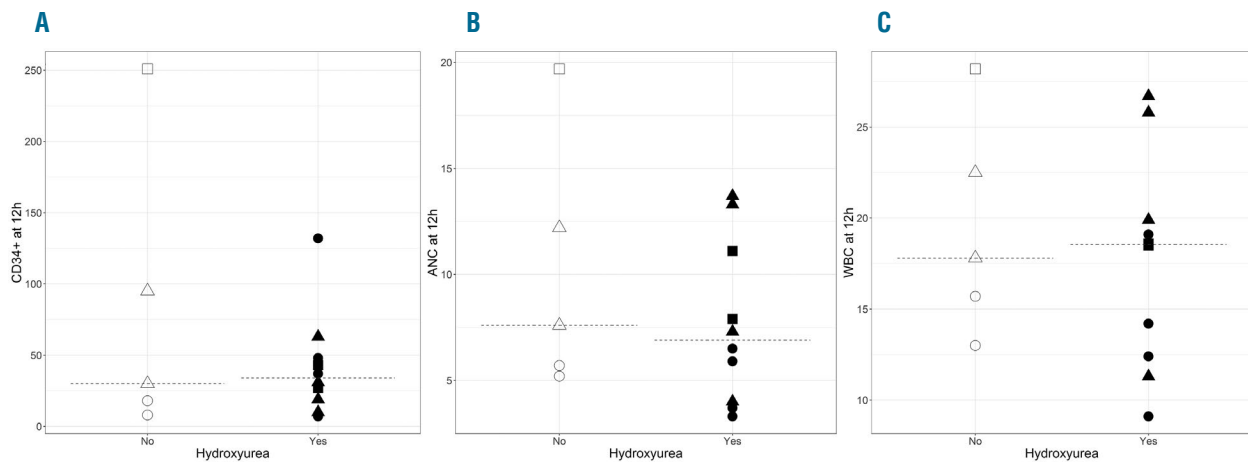


Figure 3. Association of hydroxyurea treatment with post-plerixafor cell counts. The use or not of hydroxyurea was not associated with different levels of CD34⁺ ($P=0.66$), absolute neutrophil count (ANC, $P=0.66$), or white blood cell count (WBC, $P=0.57$) 12 h after plerixafor. Peripheral blood (A) CD34⁺ concentrations, (B) ANC, and (C) WBC at 12 h after plerixafor in 15 patients with SCD treated with 80 (circles), 160 (squares) and 240 (triangles) µg/kg of plerixafor. Patients on hydroxyurea are represented by filled circles, squares and triangles, patients off hydroxyurea are represented by open circles, squares and triangles.

count (Kendall tau=0.30, $P=0.13$) or donor age (Kendall tau=0.14, $P=0.49$).

There was a significant increase in the median CD34⁺ fold increase with dose ($P=0.01$) (Online Supplementary Figure S1). A trend was observed for ANC ratio, although not statistically significant (1.6-, 1.8-, and 2.1-fold increases, $P=0.08$). No trend was seen for WBC ratio ($P=0.13$). With the caveat of statistical adjustment for patient 8(2)'s baseline CD34⁺ count of 0/µL, there was no correlation between CD34⁺ fold increase and baseline CD34⁺ (Kendall tau= -0.32, $P=0.11$), baseline ANC (Kendall tau=0.19, $P=0.32$), or baseline WBC (Kendall tau=0.22, $P=0.25$) (Online Supplementary Figure S2).

Hydroxyurea was not associated with differences in peak absolute CD34⁺ concentration ($P=0.95$), peak ANC ($P=0.59$) or peak WBC ($P=0.68$) concentrations (Figure 3); or with differences in the fold increases of CD34⁺ cell ($P=0.64$), ANC ($P=0.12$), or WBC ($P=0.36$) concentrations (Online Supplementary Figure S3).

In a subset of six patients, CD34⁺CD38⁻ cells were enumerated (Online Supplementary Table S3), showing a median 3-fold increase in CD34⁺CD38⁻ concentrations at 12 h.

Safety of plerixafor

There were no significant changes in hemoglobin concentration, hematocrit, or platelet counts with plerixafor treatment (*data not shown*, baseline values in Online Supplementary Table S1). Due to the occurrence of one serious adverse event at the 80 µg/kg dose and another one at the 240 µg/kg dose, an additional three patients were enrolled at each of these dose levels. The serious adverse events were both pain crises, possibly related to plerixafor (Online Supplementary Table S4), but also associated with other possibly contributory events. Patient 13 with a serious adverse event had the second highest peak ANC (and third highest peak WBC count) in the study, but high WBC count and ANC were not consistently associated with serious adverse events.

There were no significant differences between dose levels for any of the activation markers of vaso-occlusion tested. With the exception of tissue factor-positive (TF⁺) mono-

cytes at the 240 µg/kg dose, the median fold-changes in percentage of cells at every dose cohort were ≤ 1.1 , arguing against generalized plerixafor-mediated cell activation (Figure 4A). Median fold increases in absolute numbers of activated $\beta 2$ integrin-positive ($\alpha\beta 2^+$) neutrophils, activated Mac-1-positive (aMac-1⁺) neutrophils, and TF⁺ monocytes (160 µg/kg, 240 µg/kg) were close to 2 (Figure 4B). There were strong correlations between the fold increase in absolute numbers of neutrophils and fold increases in $\alpha\beta 2^+$ neutrophils (Kendall tau=0.85, $P<0.001$) and aMac-1⁺ neutrophils (Kendall tau=0.46, $P=0.02$). The absolute numbers of $\alpha\beta 2^+$ and aMac-1⁺ neutrophils were significantly increased (Online Supplementary Figure S4A,B), and the two patients with serious adverse events (gray arrows) had relatively high absolute numbers of $\alpha\beta 2^+$ and aMac-1⁺ neutrophils, albeit not the highest. There was also a significant increase in plasma prothrombin fragment 1.2 concentrations (Online Supplementary Figure S4C), but the two patients with serious adverse events had absolute concentrations and fold increases at 12 h that were lower than the median and mean for that measure. Both patients with serious adverse events had relatively high fold-increases in L-selectin^{neg} neutrophils and one had a large fold increase in TF⁺ monocytes (Figure 5), but their absolute numbers of L-selectin^{neg} neutrophils and TF⁺ monocytes were not particularly high (Online Supplementary Figure S4D,E). There were significant decreases for five parameters: percentage of $\alpha\beta 2^+$ neutrophils, MFI of $\alpha\beta 2$ neutrophils, percentage of TF⁺ monocytes, and percentage and absolute number of platelet-neutrophil aggregates (Online Supplementary Figure S5). There were no significant changes in the MFI of aMac-1⁺, Mac-1, LFA-1, or L-selectin on neutrophils (*data not shown*).

Discussion

Eight of 15 patients (53%) with SCD treated with plerixafor reached the peripheral blood CD34⁺ cell target count of at least 30 CD34⁺ cells/µL, including three of six patients treated at a dose of 240 µg/kg. This is in contrast

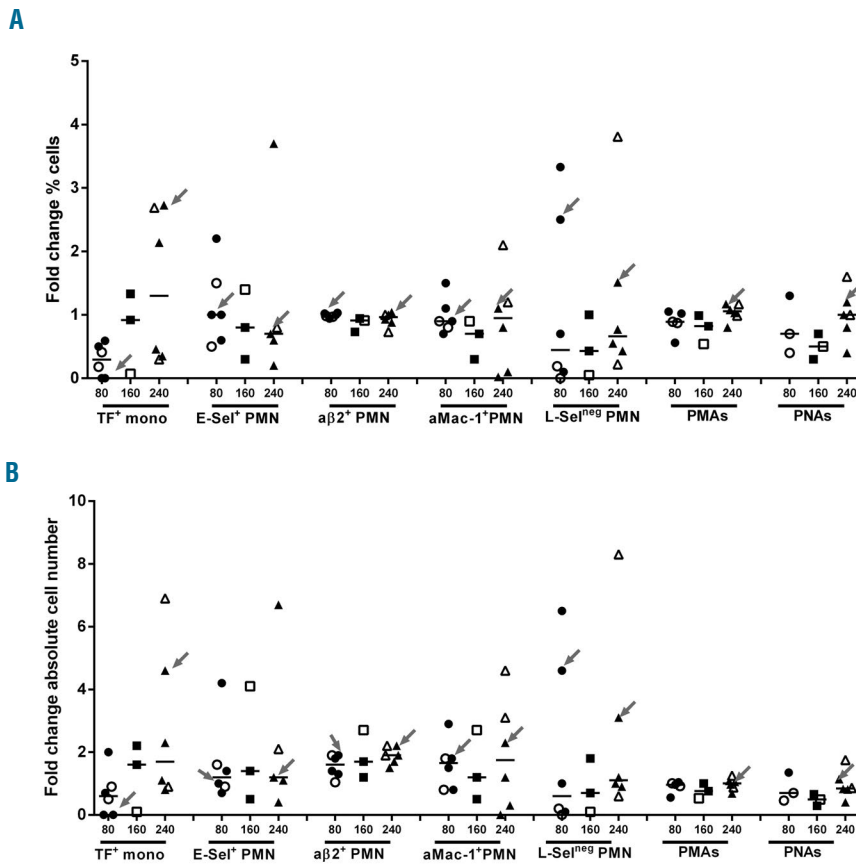


Figure 4. Fold changes in the percentage of cells expressing activation markers, according to plerixafor dose. (A) Other than % TF⁺ monocytes, median fold changes in % cells were ≤ 1.1 . (B) Median fold changes in absolute number of cells for TF⁺ mono, a β 2⁺ PMN, and aMac-1⁺ PMN were closer to 2 than 1. Fold changes are ratios of 12 h to baseline values. Horizontal lines represent medians. Filled shapes represent hydroxyurea-treated patients, open shapes represent patients not treated with hydroxyurea. Gray arrows point to the values for the two SAE patients who had serious adverse events. TF⁺ mono: tissue-factor-positive monocytes; E-Sele⁺ PMN: E-selectin-positive neutrophils; a β 2⁺ PMN: activated β 2 integrin-positive neutrophils, aMac-1⁺ PMN: activated Mac-1-positive neutrophils; L-Sele⁺ PMN: L-selectin-negative neutrophils; PMA: platelet-monocyte aggregates; PNA: platelet-neutrophil aggregates.

with the findings of a recent study by Tisdale *et al.*, in which mobilization was effective in seven of seven SCD subjects (100%) at a dose of 240 $\mu\text{g}/\text{kg}$.⁴ It should be noted that patients in the National Institutes of Health study were off hydroxyurea and had been transfused for at least 2 months to achieve a HbS <20-30%^{4,32} while in our study, ten of the 15 patients were on stable doses of hydroxyurea (for at least 1 year) and only one patient was on chronic transfusion. Although hydroxyurea, a ribonucleotide reductase inhibitor, causes myelosuppression and was recently found to reduce CD34 counts in peripheral blood and bone marrow,³³ there is no definitive evidence that hydroxyurea negatively affects numbers or quality of cell cycle-quiescent hematopoietic stem cells or immature bone marrow progenitors as opposed to more mature myeloid-erythroid progenitors.^{35,37} Indeed, in our study, although we did not achieve consistent efficiency in CD34 cell mobilization, no correlation was found between hydroxyurea use, and absolute or fold increases in CD34⁺ cells/ μL .

We observed wide inter-donor variability in CD34 mobilization with plerixafor, as previously reported in normal donors (CD34 peaks between 4-157/ μL)¹³ and in patients with SCD (CD34 peaks between 50-200/ μL).⁴ However, we also observed a strong correlation between baseline CD34⁺ and peak CD34⁺ concentrations, as previously reported with both G-CSF and plerixafor mobilization in healthy donors (Kendall tau=0.68, $P=0.0006$).^{15,30,31} Factors contributing to baseline CD34 count remain unclear, but our data and others' suggest that baseline CD34⁺ concentration may be affected by hydroxyurea-

related myelosuppression.^{24,33} Patient #8, a subject re-enrolled in the study, was particularly instructive regarding this hypothesis. This patient was clinically stable on hydroxyurea at a dose of 27 mg/kg and was enrolled twice at an interval of 13 months. At the time of his second treatment, however, he had a markedly lower baseline ANC (1900/ μL down from 6300/ μL) and platelet count (217,000/ μL down from 400,000/ μL), probably related to oscillatory non-toxic hematopoiesis seen in SCD with chronic and dose-intensive treatment with hydroxyurea (ANC oscillations between 2,000-6,000/ μL as determined from review of his clinical laboratory records).³⁸ This myelosuppression was associated with a baseline CD34 concentration of 0/ μL rather than 1/ μL , possibly contributing to the relatively low 12 h CD34⁺ concentration of 10/ μL at the 240 $\mu\text{g}/\text{kg}$ dose as compared to 27/ μL at the 160 $\mu\text{g}/\text{kg}$ dose. In brief, because hydroxyurea can decrease ANC and platelet count,³⁹ hydroxyurea-related myelosuppression may have contributed to the relatively poor CD34⁺ mobilization obtained in this cohort. However, avoiding hydroxyurea withdrawal might lower the risk of pain crises;²⁴ we, therefore, plan to explore timing plerixafor administration to the peak rather than nadir of hydroxyurea-related oscillatory hematopoiesis. Finally with regards to hydroxyurea therapy, data from the six patients in whom we enumerated CD34⁺CD38⁻ cells suggest that hydroxyurea may not adversely affect HSC, given that all patients except one (patient 10) were on hydroxyurea and a median 3-fold increase at 12 h was observed. Only 0.2-2.8% of CD34⁺ cells were CD38-negative, but this may be consistent with plerixafor's effect in

normal healthy donors, in that fewer HPC may be mobilized by plerixafor than by G-CSF, where up to 50% of G-CSF-mobilized CD34⁺ cells are CD38-negative.^{13,40}

Because we enrolled only one patient on chronic transfusion, we cannot assess any correlation between transfusion and CD34 mobilization, although notably this patient had the second highest baseline and highest peak CD34⁺ cell counts in our study. Other studies of plerixafor in SCD^{4,32} have initiated chronic transfusion based on the hypothesis that the inflammatory nature of SCD affects the bone marrow and transfusion assuages bone marrow inflammation and stress erythropoiesis. Although replicative and oxidative stress of HPC in bone marrow may occur,⁴¹⁻⁴⁴ there is limited evidence that HPC are damaged in SCD.³⁶ Five of our patients had HbF-associated increases in hemoglobin concentration and hematocrit to more than 10 g/dL and 30%, respectively (similar to values in chronically transfused patients) but HbF levels did not correlate with CD34 cell mobilization.

Based on our data, it is possible that continued dose escalation could result in greater efficacy of mobilization, since we observed a dose-related response in the median CD34⁺ cell fold increase ($P=0.01$), as also observed in healthy donors.⁴⁵ Patient 3, a repeat enrollment who had never been on hydroxyurea and was clinically stable, is instructive in that his 12 h peak CD34⁺ cell count following a plerixafor dose of 80 µg/kg was only 8/µL whereas at the dose of 240 µg/kg it was 40/µL, even though his baseline CD34⁺ cell concentrations (1/µL and then 2/µL) were similar, suggesting a dose-response to plerixafor. Notably, his two periods in the study were separated by 19 months, suggesting that, as with healthy donors,⁴⁶ intra-individual CD34⁺ cell counts in stable SCD patients not on hydroxyurea may remain stable over time. Based on these data and given the safety and continued dose response between 240 µg/kg and 480 µg/kg observed in healthy donors,²⁰ we plan to continue dose escalation in SCD patients through to the 480 µg/kg dose, barring significant adverse events. Adding the CXCR2 agonist, GROβ, might be useful.⁴⁷

Only two of 15 patients (13%) developed serious adverse events as compared to three of seven patients (43%) in the study of plerixafor mobilization in SCD by Tisdale *et al.*,⁴ although this must be qualified by the fact that the patients in the study by Tisdale *et al.* also underwent leukapheresis. Our low rate of serious adverse events could, however, also be due to chronic hydroxyurea therapy and the subsequent lower WBC and ANC peaks. As the fraction of activated neutrophils did not increase significantly with plerixafor, our low serious adverse event rate may be related to moderation of ANC elevations by hydroxyurea, reducing the absolute number of activated cells. Given the still uncertain risks of morbidity, as seen with G-CSF, the use of plerixafor in SCD requires further evaluation.

In summary, our present data suggest that, with regards the efficacy of CD34 mobilization, red blood cell transfu-

sion may be more effective than continuing standard of care. Whether red blood cell transfusion will remain more effective as we escalate the plerixafor dose (as safety allows) to 480 µg/kg, with protocol revisions for hydroxyurea-treated patients, is unknown. Finally, potential candidates for SCD gene therapy may not be able to receive regular red blood cell transfusions (e.g. if they have red cell alloimmunization or a history of hyperhemolysis) or may not be willing to do so (e.g. Jehovah Witnesses), even for the relatively short duration of 2-3 months.

This study has several limitations. Firstly, despite this study being the largest study to date of plerixafor administration in SCD patients, overall the number of patients involved remains small; thus comparisons, for example, between hydroxyurea-treated and non-hydroxyurea-treated patients, may not be representative of the actual populations. Secondly, we measured WBC, ANC and CD34 mobilization in this study only at ~12 and ~20 h after plerixafor administration. It is possible that an initial peak could have occurred at an earlier time (6-9 h) after plerixafor and could, therefore, have been missed. Nevertheless, CD34 cell concentrations remain at ~70% of peak levels at 12 h.^{20,45,48} Our current study will be amended to include the addition of earlier post-plerixafor assessments. Thirdly, we determined peripheral blood CD34 cell mobilization in the 15 patients treated with plerixafor, without performing apheresis. However, there is a well-described correlation between peripheral blood CD34 cell concentration and the ultimate CD34 cell dose obtained after apheresis. It is possible that technical adjustments may be required for this equation in the context of SCD. Finally, other than enumerating CD34⁺CD38⁻ cells, we did not further characterize CD34⁺ cells to study "stemness", for example by determining glycophorin A positivity and CD34 dimness.⁴ CD34⁺ or CD34⁺CD38⁻ enumeration is not specific for HSC⁴⁹ and it is, therefore, unclear whether patients had a true increase in HSC, as opposed to more mature lineage-committed CD34⁺ progenitors, which are either mobilized or present in bone marrow.⁴² We plan to characterize the CD34⁺ cells further as we move forward in the study, which is currently enrolling at the 320 µg/kg dose level. Despite mobilization of HSC possibly being less efficient with plerixafor than with G-CSF, plerixafor-mobilized HSC may have an engraftment advantage over G-CSF-mobilized HSC with regard to better retention of CXCR4, which facilitates homing.³²

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