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## Diabetes Limits Stem Cell Mobilization Following G-CSF but Not Plerixafor



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Previous studies suggest that diabetes impairs hematopoietic stem cell (HSC) mobilization in response to granulocyte colony-stimulating factor (G-CSF). In this study, we tested whether the CXCR4 antagonist plerixafor, differently from G-CSF, is effective in mobilizing HSCs in patients with diabetes. In a prospective study, individuals with and without diabetes ( $n = 10/\text{group}$ ) were administered plerixafor to compare CD34<sup>+</sup> HSC mobilization; plerixafor was equally able to mobilize CD34<sup>+</sup> HSCs in the two groups, whereas in historical data, G-CSF was less effective in patients with diabetes. In a retrospective autologous transplantation study conducted on 706 patients, diabetes was associated with poorer mobilization in patients who received G-CSF with/without chemotherapy, whereas it was not in patients who received G-CSF plus plerixafor. Similarly in an allogeneic transplantation study ( $n = 335$ ), diabetes was associated with poorer mobilization in patients who received G-CSF. Patients with diabetes who received G-CSF without plerixafor had a lower probability of reaching  $>50/\mu\text{L}$  CD34<sup>+</sup> HSCs, independent from confounding variables. In conclusion, diabetes negatively impacted HSC mobilization after G-CSF with or without chemotherapy but had no effect on mobilization induced by G-CSF with plerixafor. This finding has major implications for the care of patients with diabetes undergoing stem cell mobilization and transplantation and for the vascular regenerative potential of bone marrow stem cells.

Diabetes leads to severe multiorgan complications that collectively reduce life expectancy (1). Recently, it has been demonstrated in humans and rodents with diabetes that hyperglycemia damages the bone marrow (BM)

microenvironment, causing microangiopathy, neuropathy, and remodeling of the hematopoietic stem cell (HSC) niche (2–4). As a result, mobilization of HSCs in response to granulocyte colony-stimulating factor (G-CSF) is impaired in diabetes (5–7). To describe this dysfunction, the expression “diabetic stem cell mobilopathy” has been coined (8). Diabetic stem cell mobilopathy may have important implications for the care of patients with diabetes undergoing HSC mobilization for autologous or allogeneic HSC transplantation. In addition, the BM is believed to harbor subsets of vascular progenitor cells, including endothelial progenitor cells (EPCs) (9). Based on a wealth of experimental studies, EPCs are believed to contribute to vascular repair and compensatory angiogenesis, thereby providing protection from cardiovascular disease (CVD) (10). Importantly, HSC and EPC mobilization alone or combined with cell therapy is being used to treat cardiac and peripheral ischemic diseases (9,11). EPCs are profoundly reduced and impaired in patients with diabetes (12), whereas restoration of EPC mobilization may protect from diabetic vascular disease (13).

The mechanism of G-CSF–induced HSC mobilization is indirect and still incompletely understood (14). The most downstream event is thought to be the reduction of intramedullary concentrations of the chemokine and HSC retention factor CXCL12 (SDF-1 $\alpha$ ), which is known to be a potent chemoattractant for HSCs and is a prime candidate for mediating HSC trafficking in and out of the BM through its receptor CXCR4 (15). The CXCR4 antagonist plerixafor (formerly AMD3100) is used for HSC mobilization in patients with lymphoma and multiple myeloma, in combination with G-CSF. Plerixafor induces rapid mobilization of HSCs in humans ( $\sim 4\text{--}9$  h) and in mice ( $\sim 1\text{--}3$  h)

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by competitive blockade of the CXCL12/CXCR4 axis in both the osteoblastic and vascular niches (14,16). The administration of plerixafor in addition to G-CSF has proved superior to G-CSF alone in inducing HSC mobilization (17). Furthermore, preclinical findings demonstrate that plerixafor is effective in mobilizing HSCs and EPCs in animal models of diabetes (3,18–20), although no data in humans are so far available. A comparison of the effects of plerixafor versus G-CSF in diabetes would provide novel insight into the mechanisms responsible for “stem cell mobilopathy” and also guide clinical practice. In this study, we aimed to confirm previous findings that suggest that diabetes impairs HSC mobilization in response to G-CSF and to test whether the direct CXCR4 antagonist plerixafor is effective in mobilizing HSCs in patients with diabetes. To this end, we report results from one small prospective clinical trial with a historical control comparison group and two large retrospective studies.

## RESEARCH DESIGN AND METHODS

### Prospective Study

#### Patients

The study was approved by the Ethical Committee of the University Hospital of Padova (2996P) and was performed in accordance with the Declaration of Helsinki. The trial was registered on clinicaltrials.gov (NCT02056210). The study was conceived as an off-label test of plerixafor administration alone in individuals without hematological diseases and not undergoing HSC transplantation or donation, with the sole purpose of comparing the extent of HSC mobilization between patients with and without diabetes. The primary end point was the fold change of CD34<sup>+</sup> cells per microliter before and after HSC mobilization. Secondary outcomes included stem/progenitor cell phenotypes and HSC function by colony-forming units count (CFU-C). Patients with diabetes aged 18–65 years were recruited among those referred to the diabetes outpatient clinic of the University Hospital of Padova. Both type 1 and type 2 diabetes patients were eligible. Individuals without diabetes aged 18–65 years were recruited from those referred to the same outpatient clinic for screening of other metabolic diseases. All provided written informed consent. Exclusion criteria were pregnancy or lactation; recent (within 2 months from study entry) surgery, trauma, or acute diseases; immune diseases (except from type 1 diabetes and autoimmune thyroiditis); chronic infectious diseases; hematologic malignancies either past or present; solid tumor known or strongly suspected; leukocytosis, leukopenia, or thrombocytopenia; solid organ transplant or immunosuppression; alteration of hepatic function (transaminases >2 upper limit of normality); severe chronic diabetic micro- or macroangiopathy; HbA<sub>1c</sub> >11%; deficit in renal function (estimated glomerular filtration rate <50 mL/min/1.73 m<sup>2</sup>); significant abnormalities of the peripheral lymphocyte immunophenotype; known hypersensitivity to plerixafor or its excipients; and

refusal or inability to provide informed consent. Women with childbearing potential could participate in the study if on oral contraception, and a negative pregnancy test was required before study entry. Women were also asked to continue oral contraception for 3 months after plerixafor administration. All medications for the treatment of diabetes and for other medical conditions were allowed during the study.

For all subjects, we recorded the following data: age, sex, BMI, blood pressure, smoking of one or more cigarettes per day, comorbidities, and current medications. For patients with diabetes, we recorded the prevalence of chronic complications, as reported in their electronic charts.

#### Treatment Protocol

After confirmation of eligibility, participants were subjected to a baseline blood sampling from the antecubital vein. Immediately after, plerixafor (Mozobil; Sanofi) was injected subcutaneously at the standard dose of 0.24 mg/kg body weight. Six hours after injection, the patients returned for a second blood sampling.

#### Flow Cytometry

Quantification of peripheral blood (PB) stem and progenitor cells was performed using multicolor flow cytometry, as described previously (6). In brief, after red blood cell lysis, 150  $\mu$ L of PB was stained with 10  $\mu$ L of fluorescein isothiocyanate-conjugated anti-human CD34 monoclonal antibody (mAb) (Becton Dickinson), 10  $\mu$ L of phycoerythrin-conjugated anti-human KDR mAb (R&D Systems), 10  $\mu$ L of allophycocyanin-conjugated anti-CD133 mAb (Miltenyi Biotec), and 10  $\mu$ L of PerCP-Cy5-conjugated anti-CD45 mAb (BD). The frequency of PB cells positive for the above reagents was determined by a two-dimensional side scatter-fluorescence dot plot analysis, after appropriate gating. We gated CD34<sup>+</sup> or CD133<sup>+</sup> PB cells in the mononuclear cell fraction and then examined the resulting population for the dual expression of KDR. At the intersection of the CD34 and CD133 gates, we identified CD34<sup>+</sup>CD133<sup>+</sup> cells, which were examined for KDR expression. For FACS analysis,  $5 \times 10^5$  cells were acquired and scored using a FACSCalibur (BD). Data were processed using the FACSDiva software (BD). The same trained operator, who was blind to the clinical status of the patients, performed the tests throughout the study. Absolute progenitor cell counts per unit of blood were derived by multiplying fractional data per white blood cell count.

#### CFU-C

To test the functionality of mobilized CD34<sup>+</sup> HSCs from participants with and without diabetes mobilized with plerixafor, generation of HSC colonies from purified CD34<sup>+</sup> cells was performed as follows. CD34<sup>+</sup> cells were purified from PB samples drawn immediately prior to and 6 h post plerixafor administration using the Diamond CD34 Isolation Kit (Miltenyi Biotec), which is specifically designed to yield Lin<sup>-</sup>CD34<sup>+</sup> HSCs from human PB. Then, HSC colonies were cultured from 500 purified CD34<sup>+</sup> cells

plated on methylcellulose using the MethoCult colony-forming cell assay (Stem Cells Inc.). After 2 weeks in methylcellulose, colonies were visualized under an inverted optical microscope, counted, and scored.

### **Historical Controls**

In a previous trial performed at our site (NCT01102699), 38 individuals with and without diabetes received a single 5  $\mu\text{g}/\text{kg}$  body weight dose of subcutaneous G-CSF to test rapid (24 h) HSC mobilization response (6). Details on this study are provided elsewhere (6). For the current study, we reanalyzed data from  $n = 10$  subjects with and  $n = 10$  without diabetes extracted as the best matching sample for comparison with subjects enrolled and treated in the current trial. Both studies used identical inclusion/exclusion criteria. Extraction and matching were performed by an ad hoc-generated bootstrapping procedure. Data on PB CD34<sup>+</sup> HSC mobilization was blinded at the time of patient selection.

### **Statistical Analysis**

Normality of the variables was checked using the Kolmogorov-Smirnov and Shapiro-Wilk tests. Variables with markedly skewed distributions were log transformed. Data are expressed as mean  $\pm$  SE if normal, or as percentage where appropriate. The paired Student *t* test was used for within-group comparisons, whereas comparisons of continuous variables between the groups of subjects with and without diabetes were performed using the unpaired two-tail Student *t* test, and the  $\chi^2$  test was used for the comparison of categorical variables. Linear correlations were checked using the Pearson *r* coefficient. Statistical significance was accepted at  $P < 0.05$ ; SPSS version 21 was used.

### **Retrospective Studies**

A retrospective analysis of HSC mobilization was approved by the local institutional review board (HRPO 201301067) and conducted by the Bone Marrow Transplant Unit at the Washington University School of Medicine (WUSM).

### **Autologous HSC Transplantation Substudy**

Our collaborators at WUSM performed a retrospective chart review of 803 consecutive adult (age 18 years or older) patients who underwent autologous PB HSC collection from January 2008 through December 2011 at their institution. Patients were considered eligible for analysis if they had a diagnosis of multiple myeloma (MM), non-Hodgkin's lymphoma (NHL), or Hodgkin's lymphoma (HL) and had not undergone previous HSC collection or transplantation. Diabetes was defined based on self-reported or clinical-reported history, as well as fasting plasma glucose and/or HbA<sub>1c</sub> levels reported in hospital and ambulatory charts. Patients were divided based on the mobilization regimen: G-CSF with/without chemotherapy versus G-CSF with plerixafor.

### **Allogeneic SC Transplantation Substudy**

Our collaborators at WUSM performed a retrospective chart review of 488 consecutive adult patients who underwent apheresis for allogeneic PB HSC donation for a family member from 2006 through 2013 at their institution. Donors who received any mobilization regimen other than G-CSF alone or had undergone a previous PB stem cell donation were excluded. Diabetes was defined based on self-reported or clinical-reported history, as well as fasting plasma glucose and/or HbA<sub>1c</sub> levels reported in hospital and ambulatory charts.

### **Statistical Analysis**

Data are expressed as median (interquartile range) or as percentage where appropriate. Comparisons of continuous variables between the groups of subjects with diabetes and without diabetes were performed using the Mann-Whitney *U* test, and the  $\chi^2$  test was used for the comparison of categorical variables. After univariate analysis, multivariate logistic regression analysis was used to assess the independent association between diabetes and achievement of a CD34<sup>+</sup> HSC level  $>50/\mu\text{L}$  (dependent categorical variable), adjusting for potential confounders. Statistical significance was accepted at  $P < 0.05$ , and SPSS version 21 was used.

## **RESULTS**

### **Prospective Plerixafor Mobilization Trial**

Clinical characteristics of patients with and without diabetes in this prospective trial are summarized in Table 1. Each group had identical age and sex but differed for end-organ complications and medications. However, patients with diabetes were relatively well controlled and had a low prevalence of comorbidities and complications. Figure 1, Supplementary Fig. 1, and Table 2 summarize the major findings.

Plerixafor induced a significant mobilization of CD34<sup>+</sup> HSC in subjects with and without diabetes, and there were no differences in their fold changes compared with baseline (Fig. 1). Plerixafor also induced significant increases in PB CD133<sup>+</sup> and CD34<sup>+</sup>CD133<sup>+</sup> HSC phenotypes, and CD34<sup>+</sup>KDR<sup>+</sup> and CD34<sup>+</sup>CD133<sup>+</sup>KDR<sup>+</sup> EPC phenotypes in both groups with no differences in the respective fold changes (Supplementary Fig. 1). Baseline CD34<sup>+</sup>KDR<sup>+</sup> EPC levels were lower in subjects with diabetes as compared with those without diabetes ( $42.4 \pm 10.0$  vs.  $92.3 \pm 20.3/\text{mL}$ ;  $P = 0.044$ ). Plerixafor significantly increased CD133<sup>+</sup>KDR<sup>+</sup> EPCs only in patients with diabetes, but the fold change was not significantly higher than in control subjects. No differential mobilization response was detected between patients with type 1 versus type 2 diabetes, and no correlation was found between HbA<sub>1c</sub> and mobilization of any stem/progenitor cell phenotype. A trend for an inverse correlation between fold change in CD34<sup>+</sup> HSCs and diabetes duration was detected ( $r = -0.61$ ;  $P = 0.061$ ;  $n = 10$ ). Although statins have been shown to stimulate EPCs (21), no association was found between statin use and stem/progenitor cell mobilization.

**Table 1—Characteristics of patients in the prospective plerixafor mobilization trial and in the historical G-CSF trial data**

Characteristic	Prospective plerixafor trial		Historical G-CSF trial	
	Without diabetes	With diabetes	Without diabetes	With diabetes
Number	10	10	10	10
Type 1/type 2	—	5/5	—	5/5
Age, years	44.0 ± 4.8	44.0 ± 3.3	40.3 ± 4.6	49.1 ± 4.3
Males/females	8/2	8/2	8/2	8/2
BMI, kg/m <sup>2</sup>	24.2 ± 1.5	26.8 ± 1.5	26.0 ± 1.6	27.5 ± 1.6
HbA <sub>1c</sub> , % (mmol/mol)	5.5 ± 0.1 (37 ± 0.7)	7.7 ± 0.3* (61 ± 2.3)	5.5 ± 0.2 (37 ± 1.3)	7.8 ± 0.5* (62 ± 4.0)
Diabetes duration, years	—	17.3 ± 3.3	—	13.7 ± 3.9
Hypertension, <i>n</i> (%)	1 (10)	3 (30)	2 (20)	6 (60)
Smoking habit, <i>n</i> (%)	1 (10)	1 (10)	1 (10)	1 (10)
Diabetic retinopathy, <i>n</i> (%)	0 (0)	2 (20)	0 (0)	2 (20)
Microalbuminuria, <i>n</i> (%)	0 (0)	0 (0)	0 (0)	0 (0)
Diabetic neuropathy, <i>n</i> (%)	0 (0)	1 (10)	0 (0)	2 (20)
Atherosclerotic CVD, <i>n</i> (%)	0 (0)	2 (20)	0 (0)	3 (0)
<b>Medications</b>				
Insulin, <i>n</i> (%)	—	6 (60)	—	6 (60)
Metformin, <i>n</i> (%)	—	3 (30)	—	5 (50)
Sulphonylureas, <i>n</i> (%)	—	1 (10)	—	1 (10)
Thiazolidinediones, <i>n</i> (%)	—	2 (20)	—	2 (20)
DPP-4 inhibitors, <i>n</i> (%)	—	1 (10)	—	2 (0)
ACE inhibitors, <i>n</i> (%)	0 (0)	2 (20)	1 (10)	5 (50)
Other antihypertensive, <i>n</i> (%)	0 (0)	1 (10)	0 (0)	1 (10)
Statins, <i>n</i> (%)	0 (0)	6 (60)*	0 (0)	4 (40)
Aspirin, <i>n</i> (%)	0 (0)	1 (10)	0 (0)	0 (0)

\**P* < 0.05 in subjects with diabetes vs. those without diabetes.

Plerixafor equally increased the clonogenic capacity of PB CD34<sup>+</sup> HSCs in subjects with and without diabetes, with no significant differences in the fold change between the two groups. The increased clonogenesis was mostly attributable to a significant increase in colony-forming units–granulocyte macrophage, whereas burst-forming unit–erythroid were overall unaffected (Supplementary Fig. 1).

Plerixafor significantly increased neutrophil, lymphocyte, monocyte, and eosinophil counts in both groups. Although neutrophil count was significantly higher in patients with diabetes post plerixafor, fold changes of all mature cell types did not differ significantly between groups (Table 2).

The drug was well tolerated and there were no severe adverse events (AEs). There were four minor AEs in four patients (20%): three cases of diarrhea (two subjects with diabetes and one without) and one case of abdominal pain (in a subject with diabetes). All these minor AEs occurred ~2–3 h after Mozobil injection and had already resolved at 6 h, without any specific intervention. At follow-up, no other AE was recorded.

#### Comparison with G-CSF–Mobilized Historical Cases

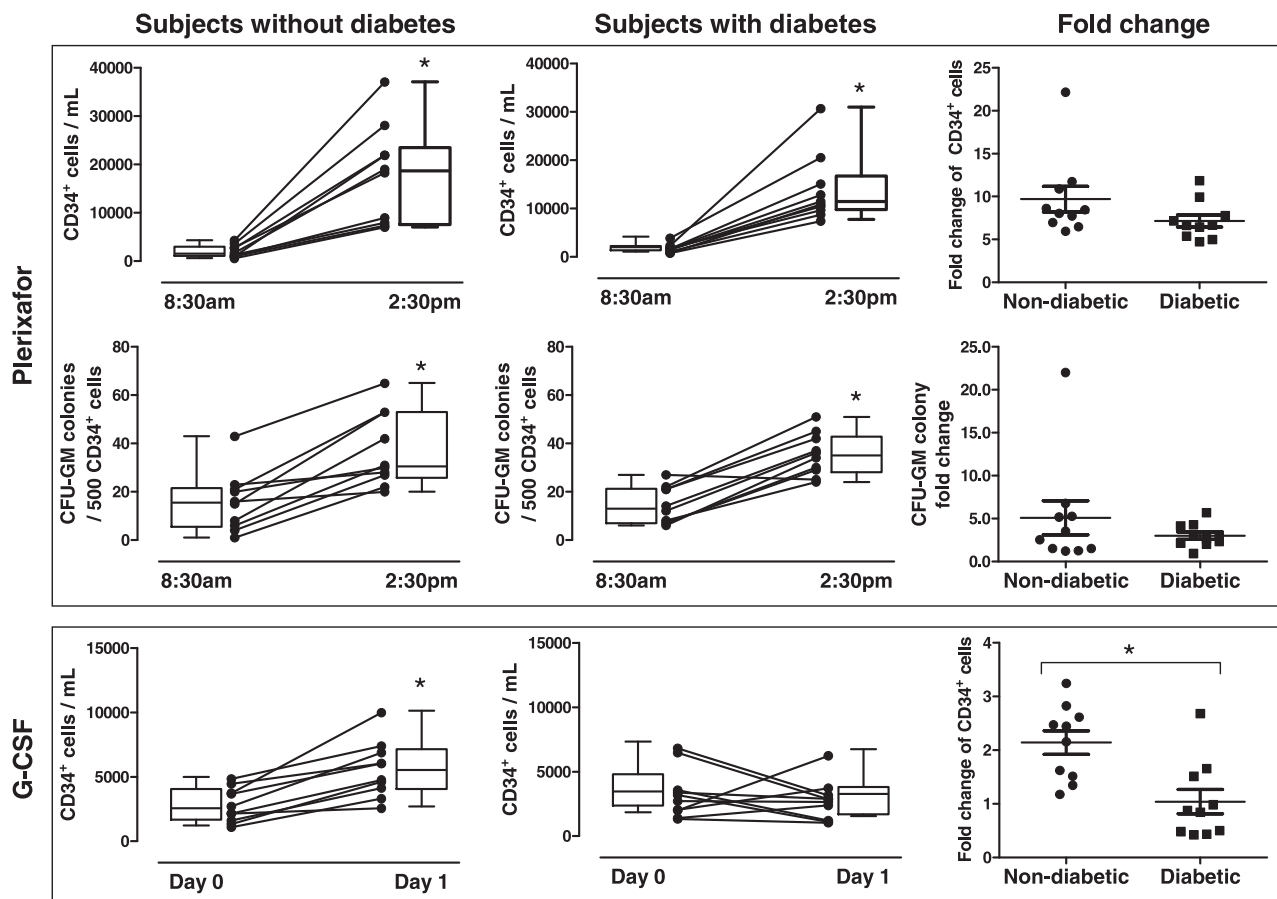
Twenty subjects (*n* = 10 with and *n* = 10 without diabetes) were extracted. As shown in Table 1, no statistically significant or clinically meaningful differences were present

between the historical cases and those from the prospective trial. In the historical cases, low-dose G-CSF induced a twofold increase in CD34<sup>+</sup> HSCs after 24 h in individuals without diabetes, whereas no mobilization was seen in patients with diabetes. No differential mobilization response was detected between patients with type 1 versus type 2 diabetes, and no correlation was found between HbA<sub>1c</sub>, disease duration, or statin use and stem/progenitor cell mobilization.

#### Retrospective Autologous Transplantation Study

A total of 706 patients met the eligibility criteria for analysis. The median age was 57.8 years (range 18.4–74.6) and 58% (411) were male. Thirteen percent (*n* = 94) had been diagnosed with diabetes prior to mobilization. Fifty-three percent (*n* = 373) had MM, 38% (*n* = 266) had NHL, and 10% (*n* = 67) had HL. Sixty-three percent (*n* = 444) of patients received a mobilization regimen of G-CSF and plerixafor, 21% (*n* = 145) G-CSF alone, and 17% (*n* = 117) G-CSF and chemotherapy. The median peak PB CD34<sup>+</sup> HSC count (on day 1 of collection) after mobilization was 50/μL (range 0–1,678/μL).

Diabetes was not associated with poorer mobilization in patients who received a mobilization regimen containing G-CSF and plerixafor (diabetes median peak CD34/μL; 70/μL vs. nondiabetes median peak CD34/μL; 60/μL). However, diabetes was associated with poorer mobilization



**Figure 1**—Summary results of the prospective plerixafor trial and comparison with historical G-CSF data. For subjects with and without diabetes, changes in the levels of CD34<sup>+</sup> HSCs, as well as HSC colonies after plerixafor are reported as box and whisker plot, individual patient lines, and fold changes. \**P* < 0.05 vs. baseline or vs. nondiabetic (for fold-change comparison). CFU-GM, colony-forming units-granulocyte macrophage.

in patients who received a mobilization regimen containing G-CSF alone or with chemotherapy (diabetes median peak CD34/ $\mu$ L; 18/ $\mu$ L vs. nondiabetes median peak CD34/ $\mu$ L; 32/ $\mu$ L without; *P* = 0.018) (Fig. 2).

Patients who received a mobilization regimen containing G-CSF alone or with chemotherapy who had diabetes were also less likely to achieve a peak PB CD34<sup>+</sup> HSC count >50/ $\mu$ L than patients without diabetes (17% compared with 37%; *P* = 0.022). Subset analysis of patients with MM (*n* = 373) and patients with NHL and HL (*n* = 333) found similar results (data not shown). Ninety-four percent of patients who achieved a peak PB CD34<sup>+</sup> count >50/ $\mu$ L were able to meet the collection goal of  $5.0 \times 10^6$  CD34<sup>+</sup> HSCs in one apheresis procedure, whereas only 6% of patients who did not achieve a PB CD34<sup>+</sup> HSC count >50/ $\mu$ L did. A multivariate logistic regression model was created with the following independent variables: age, sex, BMI, diagnosis (HL, NHL, or MM), prior radiation, and diabetes diagnosis (Table 3). In patients who received G-CSF  $\pm$  chemotherapy, a diagnosis of diabetes was independently associated with lower probability of achieving a peak PB CD34<sup>+</sup> cell count >50/ $\mu$ L (odds ratio [OR] 0.30 [95% CI 0.11–0.79]; *P* = 0.015). When the

model was further adjusted for plasma glucose, diabetes remained significantly associated with poorer mobilization (OR 0.19 [0.06–0.62]; *P* = 0.006). Adjustment for HbA<sub>1c</sub> could not be performed, as it was not available for all patients. Fifty patients (19.1%) who received G-CSF with or without chemotherapy were on statins. Statin use was not associated with HSC mobilization in the univariate analysis, and when the multivariable model was further adjusted for statin use, results did not change.

### Retrospective Allogeneic Transplantation Study

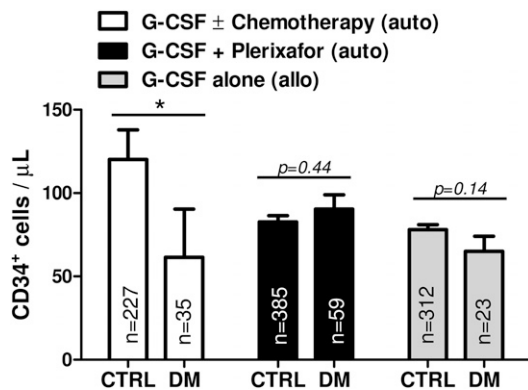
A total of 335 patients met the eligibility criteria for analysis. The median age was 53 years (range 18–76), and 50% (*n* = 167) were male. Only 7% (*n* = 23) had been diagnosed with diabetes prior to mobilization. The median peak PB CD34<sup>+</sup> cell count (on day 1 of collection) after mobilization was 70/ $\mu$ L (range 6–580/ $\mu$ L).

There was a trend of poorer mobilization for donors with diabetes (peak CD34/ $\mu$ L median 43/ $\mu$ L) compared with donors without (peak CD34/ $\mu$ L median 70/ $\mu$ L); however it was not statistically significant (*P* = 0.140) (Fig. 2). Donors with diabetes were less likely to achieve a peak PB CD34<sup>+</sup> count >50/ $\mu$ L (48% compared with

**Table 2—Effects of plerixafor on mature and stem cell populations in the prospective trial**

Variable	Subjects without diabetes			Subjects with diabetes			P value
	8:30 A.M.	2:30 P.M.	Fold change	8:30 A.M.	2:30 P.M.	Fold change	
<b>Mature cells</b>							
Neutrophils	3.3 ± 0.3	11.0 ± 0.7*	3.5 ± 0.4	3.5 ± 0.4	13.5 ± 1.0*#	4.0 ± 0.2	0.279
Lymphocytes	2.2 ± 0.3	7.3 ± 0.6*	3.4 ± 0.3	2.6 ± 0.3	6.7 ± 0.6*	2.8 ± 0.4	0.252
Monocytes	0.53 ± 0.03	2.36 ± 0.19*	4.4 ± 0.2	0.60 ± 0.06	2.09 ± 0.13*	3.7 ± 0.3	0.130
Eosinophils	0.19 ± 0.04	0.79 ± 0.11	4.7 ± 0.6	0.27 ± 0.07	0.88 ± 0.11*	4.7 ± 0.8	1.000
Basophils	0.03 ± 0.01	0.05 ± 0.01	2.2 ± 0.6	0.03 ± 0.01	0.07 ± 0.03	2.4 ± 1.3	0.889
<b>HSC</b>							
CD34 <sup>+</sup>	2,081 ± 401	17,798 ± 3,188*	9.7 ± 1.5	2,024 ± 282	14,077 ± 2,214*	7.2 ± 0.7	0.142
CD133 <sup>+</sup>	1,225 ± 218	12,093 ± 2,229*	10.8 ± 1.5	1,126 ± 143	8,417 ± 1,338*	8.0 ± 1.0	0.150
CD34 <sup>+</sup> CD133 <sup>+</sup>	967 ± 189	10,702 ± 2,241*	12.4 ± 2.0	760 ± 109	7,602 ± 1,180*	13.4 ± 3.9	0.820
<b>EPC</b>							
CD34 <sup>+</sup> KDR <sup>+</sup>	92.3 ± 20.3	227.4 ± 47.7*	2.9 ± 0.5	43.4 ± 10.0#	182.0 ± 43.4*	5.3 ± 1.6	0.156
CD133 <sup>+</sup> KDR <sup>+</sup>	41.4 ± 13.2	67.7 ± 14.8	5.7 ± 3.2	59.3 ± 10.4	185.4 ± 41.5*	4.9 ± 0.8	0.843
CD34 <sup>+</sup> CD133 <sup>+</sup> KDR <sup>+</sup>	17.3 ± 3.0	70.9 ± 20.1*	6.8 ± 1.8	10.7 ± 1.8	57.8 ± 16.5*	4.6 ± 0.9	0.290
<b>HSC colonies</b>							
Total	24.9 ± 6.3	52.8 ± 6.3*	3.2 ± 0.8	30.3 ± 4.6	56.3 ± 3.2*	2.5 ± 0.6	0.451
BFU-E	9.3 ± 2.9	15.7 ± 2.3	3.7 ± 1.4	16.1 ± 5.5	21.0 ± 2.7	5.3 ± 2.0	0.543
CFU-GM	15.7 ± 3.9	37.1 ± 4.8*	5.1 ± 2.0	14.5 ± 2.4	35.3 ± 2.8*	3.0 ± 0.4	0.323

Cell counts are expressed per milliliter. Colony number expressed per 500 CD34<sup>+</sup> cells. The P value refers to the comparison between fold changes. BFU-E, burst-forming units-erythroid; CFU-GM, colony-forming units-granulocyte macrophage. \*P < 0.05 in the comparison between values at 8:30 A.M. and values at 2:30 P.M. #P < 0.05 for subjects with diabetes vs. those without diabetes.



**Figure 2**—Achieved CD34<sup>+</sup> HSC count in the retrospective studies. Patients undergoing HSC mobilization and collection for autologous transplantation (auto) were divided into those who received G-CSF with or without chemotherapy and those who received G-CSF with plerixafor. Subjects undergoing HSC mobilization and collection for allogeneic donation (allo) only received G-CSF. Then patients were divided based on a history of diabetes (DM) or not (CTRL). \**P* < 0.05 DM vs. CTRL.

70%; *P* = 0.038). Ninety-seven percent of donors who achieved a peak PB CD34<sup>+</sup> count >50/μL were able to meet the collection goal of 5.0 × 10<sup>6</sup> CD34<sup>+</sup> HSCs in one apheresis procedure (22), whereas only 22% of donors who did not achieve a peak PB CD34<sup>+</sup> cell count >50/μL did.

A multivariate logistic regression model was created with the following independent variables: age, sex, BMI, and diabetes (Table 3). A diagnosis of diabetes was independently associated with lower probability of achieving a peak PB CD34<sup>+</sup> cell count >50/μL (OR 0.23 [95% CI 0.09–0.61]; *P* = 0.003). When the model was further adjusted for plasma glucose, diabetes remained significantly associated with poorer mobilization (OR 0.23 [0.07–0.75]; *P* = 0.015). Adjustment for HbA<sub>1c</sub> could not be performed, as it was not available for all patients. Fifty-five patients (16.4%) were on statins. Statin use was not associated with HSC mobilization in the univariate analysis, and

when the multivariable model was further adjusted for statin use, results did not change.

**DISCUSSION**

In this study, we found that patients with diabetes can be mobilized with a plerixafor-containing regimen, whereas they poorly respond to mobilizing regimens based on G-CSF without plerixafor. This conclusion is supported by two independent lines of evidence: 1) a retrospective evaluation of >1,000 subjects with and without diabetes undergoing HSC mobilization and 2) a prospective trial mobilizing 10 patients with and 10 without diabetes with plerixafor alone, as compared with an equal historical cohort of patients who received low-dose G-CSF.

Previous studies have provided results suggesting that patients with diabetes do not adequately mobilize HSCs in response to G-CSF. In the translational study by Ferraro et al. (5), a small retrospective analysis of 62 patients with lymphoma or myeloma undergoing HSC mobilization with G-CSF with chemotherapy showed that diabetes was associated with a poor mobilizer phenotype (defined as <20 CD34<sup>+</sup> HSC/μL). In addition, the average glucose levels of poor mobilizers was significantly higher than those of good mobilizers (5). Soon after this preliminary report, we reported a study of HSC mobilization with a single dose of G-CSF (5 μg/kg) in participants with and without diabetes who were free from hematological disorders. Partial results from this study served as the historical cohort for the current trial. In that study, we found that whereas PB CD34<sup>+</sup> HSC doubled 24 h after low-dose G-CSF in control patients, the response was completely abolished in patients with diabetes (6). For ethical reasons, it was not possible to perform a full course of G-CSF stimulation (i.e., with 5–10 μg/kg for 5–10 days) for a research test, thereby limiting the clinical implications of such findings. For this reason, we performed a meta-analysis of clinical trials in which CD34<sup>+</sup> HSCs were mobilized with high G-CSF doses for the treatment of CVD in patients with and without diabetes. Upon

**Table 3**—Multivariable logistic regression analyses to determine the probability of mobilizing ≥50 PB CD34<sup>+</sup> HSC/μL with G-CSF ± chemotherapy in the retrospective studies

Variable	B coefficient ± SE	<i>P</i> value	OR (95% CI)
<b>Autologous</b>			
Age	0.006 ± 0.015	0.661	1.01 (0.98–1.03)
Sex, male	0.527 ± 0.285	0.065	1.69 (0.97–2.96)
BMI	4.3 × 10 <sup>-5</sup> ± 1.3 × 10 <sup>-4</sup>	0.733	1.00 (1.00–1.00)
Radiation	-1.132 ± 0.403	0.005	0.33 (0.15–0.71)
Diagnosis: HL	1.00 (reference)	0.022	1.00 (reference)
NHL vs. HL	0.360 ± 0.508	0.479	1.43 (0.53–3.88)
MM vs. HL	0.870 ± 0.318	0.006	2.39 (1.28–4.45)
Diabetes	-1.205 ± 0.497	0.015	0.30 (0.11–0.79)
<b>Allogeneic</b>			
Age	-0.036 ± 0.011	0.001	0.97 (0.94–0.98)
Sex, male	0.192 ± 0.257	0.455	1.21 (0.73–2.00)
BMI	0.098 ± 0.024	<0.001	1.10 (1.05–1.16)
Diabetes	-1.473 ± 0.501	0.003	0.23 (0.09–0.61)

meta-regression, a striking negative correlation was found between prevalence of diabetes in each trial and the levels of PB CD34<sup>+</sup> HSCs achieved after mobilization (7). Though it is impossible to generalize such findings to patients without CVD, this association strengthens the hypothesis that diabetes impairs the HSC-mobilizing activity of G-CSF.

Data reported in the current study, collected from a very large retrospective evaluation including >1,000 subjects undergoing HSC mobilization, clearly indicate that patients with diabetes mobilize less CD34<sup>+</sup> HSCs in response to G-CSF-based regimens without plerixafor, whereas they adequately respond to a plerixafor-containing regimen. In patients receiving G-CSF for HSC autologous transplantation or allogeneic donation, a history of diabetes was associated with a significantly lower probability of mobilizing  $\geq 50$  PB CD34<sup>+</sup> HSCs/ $\mu$ L. Achievement of at least 50 CD34<sup>+</sup> HSCs/ $\mu$ L allows a very high probability (94–97%) of apheresis success, which predicts engraftment and hematopoietic reconstitution (22,23). This effect of diabetes was not seen in patients undergoing HSC mobilization for autologous transplantation using G-CSF plus plerixafor for HSC mobilization. In parallel, the prospective trial indicates that plerixafor induces mobilization of CD34<sup>+</sup> and other stem/progenitor cells, including EPCs, in participants with diabetes as in those without diabetes. Interestingly, mobilized CD34<sup>+</sup> HSCs from both groups were equally functional as determined by clonogenic assays.

The reasons whereby diabetes impairs the mobilizing activity of G-CSF but not of plerixafor are being elucidated by preclinical studies. Whereas plerixafor directly disrupts the CXCL12/CXCR4 interaction, thereby allowing HSCs to egress the BM (16), the mobilizing effect of G-CSF is indirect and involves the sympathetic nervous system (24), the CXCL12 cleaving enzyme CD26/DPP-4 (25,26), as well as cells that regulate CXCL12 production (stromal cells, osteoblasts, and macrophages) (14,27). In animal models of diabetes, the BM undergoes extensive remodeling and functional changes (10), including sympathetic denervation (3), imbalances in cellular populations (4), and maladaptive regulation of CD26/DPP-4 (28), all of which can contribute to blunt response to G-CSF. In addition, experimental and clinical data also suggest that diabetes compromises ischemia-induced stem/progenitor cell mobilization (3,29,30). Yet, based on the normal response to plerixafor, we can speculate that the mechanisms responsible for this defect converge on the very downstream CXCL12/CXCR4 axis, whereas ready availability of functional HSCs does not seem to be affected.

Besides the hematological setting, HSC mobilization has been extensively studied and has moved to clinical application for the treatment of CVD (9). This is based on the assumption, derived from a large amount of preclinical data, that the BM harbors vascular progenitor cells, such as EPCs, that contribute to vascular repair and angiogenesis (31). Importantly, diabetes profoundly affects EPC number, function, and mobilization in response to

ischemia and G-CSF (12). It should be noted that although a threshold for successful HSC mobilization in hematopoietic disorders has been established, the extent to what mobilization could result in improvement of vascular outcome remains unclear. However, effective EPC mobilization in patients with diabetes treated with plerixafor suggests that modulation of the CXCL12/CXCR4 axis represents a major target to restore endogenous BM cell-mediated vascular repair, providing a means to treat CVD in diabetes. Finally, our data provide valuable information also because stem cell transplantation is being tested for the restoration of self-tolerance as part of intervention trials to preserve  $\beta$ -cell function in type 1 diabetes (32).

This study has limitations inherent to the retrospective protocols: only a previous diagnosis of diabetes was considered, thus leading to a potential underestimation of the true prevalence of the disease, and no data were available on HbA<sub>1c</sub>, disease duration, and antidiabetic medications. In addition, the comparison between the prospective plerixafor study and the historical G-CSF study cannot be generalized, as the sample sizes were small and G-CSF was administered at a subclinical dose. A larger parallel study directly comparing the mobilizing effects of full-dose G-CSF and plerixafor in patients with diabetes would strengthen our conclusions and provide correlations between mobilization and clinical covariates, such as HbA<sub>1c</sub>, diabetes duration, complications, and medications.

Current mobilization regimens are associated with a substantial failure rate irrespective of underlying disease (33). Diabetic stem cell mobilopathy may have important implications for the care of patients with diabetes undergoing HSC mobilization for autologous or allogeneic HSC transplantation. Although the addition of a CXCR4 inhibitor such as plerixafor for the mobilization of optimal stem cell numbers from both autologous transplant patients and from allogeneic donors may not be fully justified based on the results of this study and others, a better understanding of clinical features predictive of the poor mobilization, especially in patients with diabetes, may lead to improved tailored mobilization regimens in the future.

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**Author Contributions.** G.P.F. and M.F. designed the study; collected, analyzed, and interpreted data; and wrote the manuscript. R.C., M.D., N.P., L.M., and M.A. collected, analyzed, and interpreted data and reviewed the manuscript.



S.P. designed the study; collected, analyzed, and interpreted data; and edited the manuscript. J.D. and K.S.-G. designed the study, analyzed and interpreted data, and wrote the manuscript. A.A. designed the study, analyzed and interpreted data, and reviewed the manuscript. All authors provided final approval of the version to be published. G.P.F. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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