

# A phase 1/2 study of chemosensitization with the CXCR4 antagonist plerixafor in relapsed or refractory acute myeloid leukemia

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**The interaction of acute myeloid leukemia (AML) blasts with the leukemic microenvironment is postulated to be an important mediator of resistance to chemotherapy and disease relapse. We hypothesized that inhibition of the CXCR4/CXCL12 axis by the small molecule inhibitor, plerixafor, would disrupt the interaction of leukemic blasts with the environment and increase the sensitivity of AML blasts to chemotherapy. In this phase 1/2 study, 52 patients with relapsed or refractory AML were treated with plerixafor in combi-**

**nation with mitoxantrone, etoposide, and cytarabine. In phase 1, plerixafor was escalated to a maximum of 0.24 mg/kg/d without any dose-limiting toxicities. In phase 2, 46 patients were treated with plerixafor 0.24 mg/kg/d in combination with chemotherapy with an overall complete remission and complete remission with incomplete blood count recovery rate (CR + CRi) of 46%. Correlative studies demonstrated a 2-fold mobilization in leukemic blasts into the peripheral circulation. No evidence of symptomatic hyper-**

**leukocytosis or delayed count recovery was observed with the addition of plerixafor. We conclude that the addition of plerixafor to cytotoxic chemotherapy is feasible in AML, and results in encouraging rates of remission with correlative studies demonstrating in vivo evidence of disruption of the CXCR4/CXCL12 axis. This study was registered at www.clinicaltrials.gov, no. NCT00512252. (*Blood*. 2012; 119(17):3917-3924)**

## Introduction

In acute myeloid leukemia (AML), the interaction of leukemic blasts with the BM microenvironment is postulated to be an important mediator of resistance to chemotherapy and disease relapse. Molecules that mediate adhesion to BM-stromal cells, such as VLA-4, LFA-1, CXCR4, and CD44, have been shown to provide antiapoptotic and antiproliferative effects to both normal CD34<sup>+</sup> stem cells and AML blasts.<sup>1-3</sup> The chemokine receptor, CXCR4, is expressed on both normal stem cells and AML blasts and serves as the principal regulator of stem cell homing and retention in the BM.<sup>4</sup> CXCR4 is a member of the 7 transmembrane G-coupled protein receptors. Engagement of its ligand, CXCL12, produced by marrow stroma results in receptor internalization and activation of multiple critical signal transduction pathways including PI3K/AKT, PKC $\zeta$ , and MAPK which are critical in cell proliferation and survival. Increased expression of CXCR4 has also been associated with an increased risk of relapse and poor outcome in AML.<sup>3,5-7</sup>

A bicyclam small molecule antagonist of CXCR4 binding to CXCL12, plerixafor, is currently approved for clinical use in combination with G-CSF as a stem cell mobilizing agent for patients with multiple myeloma or non-Hodgkin lymphoma undergoing autologous HSCT.<sup>8,9</sup> We hypothesized that disrupting the CXCL12/CXCR4 axis with plerixafor may augment the effects of chemotherapy. Previous work from our laboratory using a murine model of AML demonstrated that plerixafor can mobilize AML

blasts into the peripheral circulation. Furthermore, the addition of plerixafor sensitized leukemic blasts to the effects of cytotoxic chemotherapy and increased the overall survival (OS) of leukemic mice treated with the combination of plerixafor and chemotherapy compared with chemotherapy alone.<sup>10</sup> Based on these data supporting chemosensitization by plerixafor in AML, we conducted a phase 1/2 study of plerixafor in combination with mitoxantrone, etoposide, and cytarabine (MEC) for the treatment of patients with relapsed or refractory AML.

## Methods

### Trial design

This was an open-label, single-arm, phase 1/2 study conducted at Washington University School of Medicine. Eligible participants were between the ages of 18 and 70 years of age, diagnosed with AML according to WHO criteria with relapsed or refractory disease. Patients were required to have adequate organ function defined as creatinine  $\leq 1.5 \times$  institutional upper limit of normal and an aspartate aminotransferase, alanine aminotransferase, and total bilirubin  $\leq 2$  times the institutional upper limit of normal and left ventricular ejection fraction of  $> 40\%$  on multigated acquisition scan. In addition, a peripheral blood blast count  $\leq 20 \times 10^3/\text{mm}^3$  was required before starting treatment. Subjects with acute promyelocytic leukemia, active CNS leukemia, or who had been previously treated with the

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combination of mitoxantrone, etoposide, and cytarabine were excluded from the study.

All patients were required to provide written informed consent. The study protocol was approved by the Human Research Protection Office at Washington University.

### Study treatment

Plerixafor was administered by subcutaneous injection (SQ). The first dose of plerixafor was followed by a 24-hour observation period (day 0) to study the kinetics of mobilization of leukemia. On the subsequent days 1 to 5, plerixafor was administered 4 hours before chemotherapy which consisted of mitoxantrone 8 mg/m<sup>2</sup>/d intravenously over 30 minutes, etoposide 100 mg/m<sup>2</sup>/d intravenously over 60 minutes, and cytarabine 1000 mg/m<sup>2</sup>/d intravenously over 60 minutes. Colony-stimulating factors were prohibited during the first 14 days of treatment but were permitted afterward at the discretion of the treating physician.

### Statistical analysis

Response to treatment was assessed according to International Working Group standards for AML.<sup>11</sup> All patients who received at least one dose of plerixafor were considered evaluable for response and toxicity. A standard 3 + 3 design was used in the phase 1 portion starting with a plerixafor dose of 0.08 mg/kg and escalating by 0.08 mg/kg for each successive cohort up to a maximum of 0.24 mg/kg/d. The phase 2 dose was defined as the highest dose of plerixafor ≤ 0.24 mg/kg at which ≤ 1 of 6 patients experienced a dose-limiting toxicity (DLT).

For phase 2, the primary end point was the rate of complete remission plus complete remission with incomplete blood count recovery (CR + CRi). A bivariate design in 2 stages was used to allow an interim analysis of toxicity and remission rates.<sup>12</sup> A composite measure of unacceptable rates of toxicity combined the frequency of 30-day treatment-related mortality plus delayed hematopoietic recovery of > 42 days in the absence of leukemia. The maximum acceptable rate of regimen-related mortality plus delayed hematologic toxicity was < 25% with an expected rate of ≤ 10%. The minimum acceptable rate of CR + CRi was set at ≥ 30%, and the expected rate of CR + CRi with plerixafor was 50%. For phase 2, the probability of accepting a poor response ( $\alpha$  1) = 0.10, probability of accepting a toxic drug ( $\alpha$  2) = 0.15, and the probability of rejecting a good drug ( $\beta$ ) = 0.15 (ie, 85% power to accept a good nontoxic treatment). The completed phase 2 portion included a total of 46 patients, 20 in the first stage and 26 in the second stage.

### Flow cytometry

Immunophenotypic analyses were performed using a lyse/no-wash protocol. Samples were analyzed with a BD Biosciences FACScan flow cytometer modified with a second, 633-nm laser. The Ab combinations used included FITC-conjugated CD45 (clone HI30), peridinin chlorophyll protein-cyanine 5.5 (PerCP-Cy5.5) conjugated CD38 (clone HIT2), allophycocyanin-conjugated CD34 (clone 581) and PE-conjugated CD184 (clone 12G5 or 1D9), CD49d (clone 9F10), and CD123 (clone 32 703). All Abs were purchased from BD Biosciences except for CD123, which was obtained from R&D Systems. Blasts were identified using a CD45/SSC log-gating strategy. Appropriate isotype-matched negative controls were used in the panel of mAbs to assess background fluorescence intensity. Absolute cell counts were performed with SPHERO AccuCount fluorospheres (Spherotech Inc). To obtain quantitative analysis of fluorescence intensity, histograms were made and the relative mean fluorescence intensity (RMFI) were calculated by dividing the MFI of the analyzed marker by the MFI of respective isotype control using FlowJo (TreeSoft). The absolute blast (CD45<sup>dim</sup>/SSC<sup>low</sup>) cell counts were calculated from the number of fluorospheres according to the manufacturer's instructions: (number of events in test sample/number of bead events) × (flow count fluorospheres assayed concentration/volume of test sample). At least 2000 AccuCount fluorosphere events were collected per analysis.

### Transmigration assays

Transmigration assays were performed as previously described.<sup>13</sup> Briefly, peripheral blood samples from patients were collected at baseline and

6 hours after injection of plerixafor. PBMCs (10<sup>6</sup> cells) were added into the upper chamber of a 5- $\mu$ m pore-size transwell with CXCL12 (200 ng/mL) added to the bottom chamber. After 4 hours, input and migrated cells were collected and the number of blasts enumerated by flow cytometry.

### In vitro plerixafor treatment

Units of human umbilical cord blood were obtained from the St Louis Cord Blood Bank (Cardinal Glennon Children's Hospital, St Louis, MO). Immunomagnetic selection of CD34<sup>+</sup> hematopoietic stem and progenitor cells (HSPCs) from cord blood was performed using a CD34 cell isolation kit and AutoMACS device (Miltenyi Biotec). For coculture experiments, OP9 stromal cells (ATCC) were seeded into 96-well plates. After overnight incubation, CD34<sup>+</sup> HSPCs or PBMCs from healthy donors or AML patients were added to OP9 cultures or cultured alone (2-5 × 10<sup>4</sup> cells/well) for 16-18 hours in R-10 medium ± 1  $\mu$ M plerixafor. Cells were harvested using accutase (Sigma-Aldrich), washed, stained, and analyzed by flow cytometry. Discrimination of dead cells from viable cells was performed using 7-amino-actinomycin D (7-AAD; Sigma-Aldrich). Stromal cells were distinguishable from hematopoietic cells based on relative size and granularity characteristics.

## Results

### Patient characteristics

Fifty-two patients with relapsed or refractory AML were treated on this protocol. Baseline characteristics of the patients are shown in

**Table 1. Patient characteristics (N = 52)**

Characteristic	N
<b>Median age, y (range)</b>	52 (18-70)
≥ 60 (%)	8 (15)
<b>Sex (%)</b>	
Male	22 (42)
Female	30 (58)
<b>ECOG performance status (%)</b>	
0	29 (55.8)
1	12 (23.1)
2	3 (5.8)
Unknown	8 (15.4)
<b>AML source (%)</b>	
De novo AML	43 (82.7)
Therapy related	4 (7.7)
Prior MDS/MPD	6 (11.5)
<b>Cytogenetic risk category (%)</b>	
Favorable	7 (13.5)
Intermediate	29 (55.8)
Poor	16 (30.8)
<b>Peripheral blood (range)</b>	
WBC, mean in K/mcL	7.7 ± 9.8 (3-40.5)
PB blast, mean %	19 ± 23.5 (9-89)
<b>Prior transplantation (%)</b>	
Autologous	3 (5.8)
Allogeneic	6 (11.5)
<b>Treatment indication (%)</b>	
Primary refractory to 1 induction	8 (15.4)
Primary refractory to 2+ inductions	3 (5.8)
First relapse, first salvage	37 (71.2)
CR1 < 6 mo	12
CR1 < 12 mo	26
First relapse, ≤ second salvage	3 (5)
Second relapse	1 (1.9)

ECOG indicates Eastern Cooperative Oncology Group; AML, acute myeloid leukemia; MDS, myelodysplastic syndrome; MPD, myeloproliferative disease; WBC, white blood cell count; PB, peripheral blood; and CR, complete remission.

**Figure 1. Phase 1 mobilization kinetics.** Measurement of (A) total leukocytes, and (B) AML blasts in the peripheral blood after administration of a single dose of plerixafor 0.08 mg/kg (n = 3), 0.16 mg/kg (n = 3), or 0.24 mg/kg (n = 6).

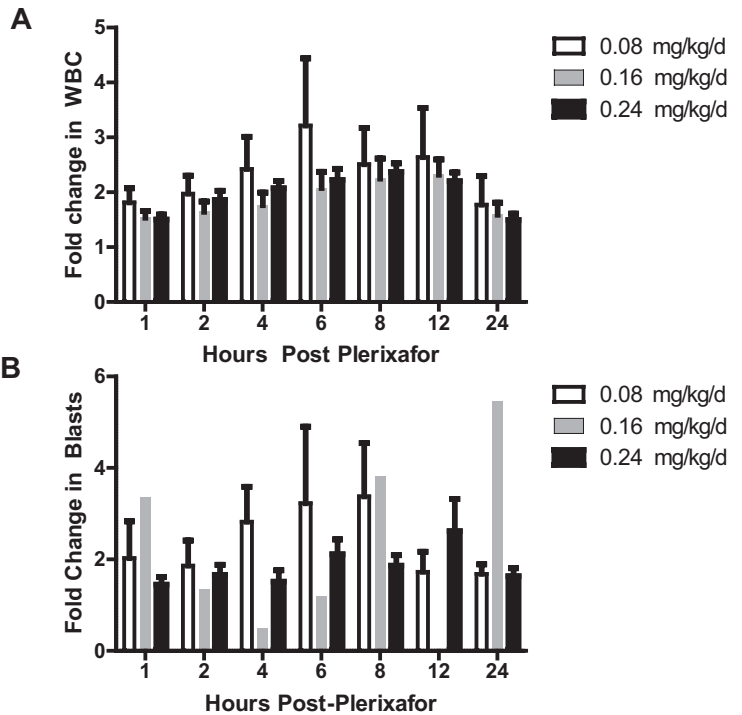


Table 1. The patient population had a median age of 52 years (18-70 years). The majority of patients were in first relapse receiving their first salvage regimen, and two-thirds of those had an initial CR1 duration of < 12 months. Eight patients had previously undergone a HSCT and 9 had secondary AML either arising from therapy or a previous myelodysplastic syndrome or myeloproliferative disease.

**Phase 1 dose escalation**

In phase 1, 3 subjects were treated at an initial plerixafor dose of 0.08 mg/kg/d. The plerixafor dose was escalated to a maximum of 0.24 mg/kg using a 3 + 3 design. The maximum dose of 0.24 mg/kg/d was the dose used in the phase 3 studies for stem cell mobilization, and limited safety data were available at higher doses.<sup>9,14</sup> At the 0.08 mg/kg/d and 0.16 mg/kg/d doses, 1 patient of 3 each achieved a complete remission. No dose-limiting toxicities were observed in any cohort and the 0.24 mg/kg/d dose of plerixafor was chosen for the phase 2 expansion.

To determine the kinetics of leukemic mobilization, we analyzed peripheral blood at serial time points after administration of plerixafor on day 0. Similar to normal HSC mobilization, peak

mobilization of both total leukocytes and AML blasts occurred ~ 6-12 hours after the administration of plerixafor (Figure 1). We were unable to detect a dose-response relationship between plerixafor and leukemic mobilization because of substantial interpatient variability in mobilization and the limited numbers of patients analyzed at the lower plerixafor doses in the phase 1.

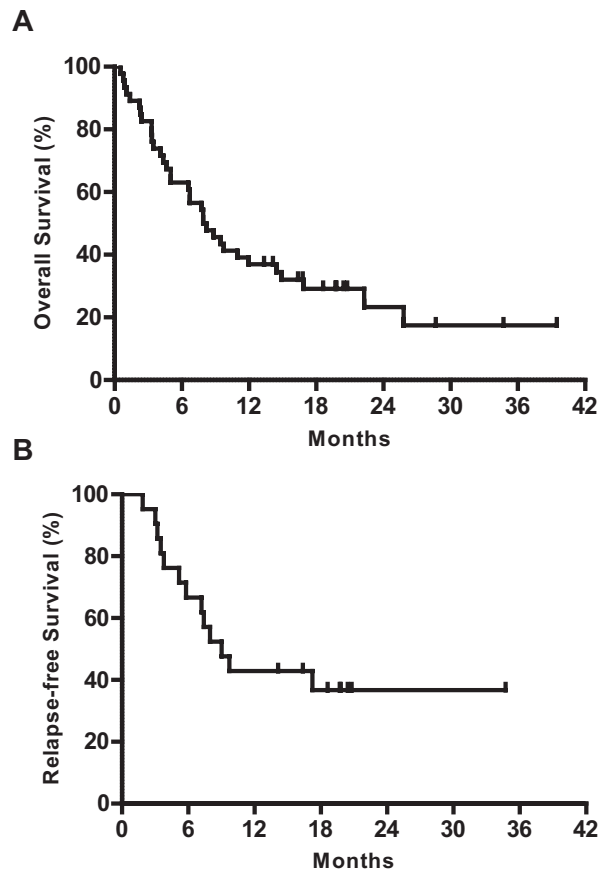
**Phase 2**

A total of 46 patients were treated with plerixafor 0.24 mg/kg/d. In one patient, a single dose of plerixafor was held because of grade 2 asymptomatic bradycardia which was judged to be possibly related to plerixafor. All other patients received the entire course of plerixafor and chemotherapy. Three deaths occurred during treatment (treatment related mortality, 6.5%). Two were because of sepsis and the third was because of an adverse transfusion reaction also occurring in the setting of febrile neutropenia. The most common nonhematologic serious adverse effects observed during the study included grade 3-4 fever (98%) and grade 3-5 infections (19%). A comprehensive table of treatment emergent adverse events regardless of attribution is provided in supplemental Table 1 (available on the *Blood* Web site; see the

**Table 2. Response evaluation 0.24 mg/kg/d cohort by treatment indication (N = 46)**

	N	Responses		Treatment failure			Response rate, %	
		CR	CRi	Persistent leukemia	Death during aplasia	Unknown	CR + CRi	CR only
<b>First relapse, first salvage, total</b>	32	15	3	12	1	0	56	47
CR1 < 6 mo	11	2	1	7	0	1	27	18
First salvage, CR1 6-12 mo	11	5	2	4	0	0	64	45
First salvage, CR1 > 12 mo	10	8	0	1	1	0	80	80
<b>Primary refractory, total</b>	10	2	0	6	2	0	20	20
1 induction	8	2	0	5	1	0	25	25
≥ 2 inductions	2	0	0	1	1	0	0	0
≥ Second relapse /salvage	4	1	0	3	0	0	25	25
<b>Total</b>	46	18	3	21	3	1	46	39

CRi indicates complete remission with incomplete blood count recovery.



**Figure 2. Survival.** Kaplan-Meier estimates of (A) OS and (B) RFS for the phase 2 (n = 46) cohort.

Supplemental Materials link at the top of the online article). For patients who achieved a CR or CRi (n = 21), neutrophil recovery (absolute neutrophil count > 1000/mm<sup>3</sup>) occurred at a median of 28 days after the start of chemotherapy (range 21-46 days). For those who achieved a CR (n = 18), platelet recovery (platelet > 50 000/mm<sup>3</sup>) occurred at a median of 28.5 days (16-42 days). In the phase 2, 29 patients (63%) subsequently received an allogeneic stem cell transplant including 16 (35%) who were in CR at the time of transplantation.

The overall response rate (CR + CRi) for phase 2 was 46% (95% CI, 30.9%-61.0%, Table 2). There was an increased probability of CR favoring patients with longer initial CR duration who were receiving their first salvage regimen ( $P = .025$ ). Although trends were identified for a decreased CR rate with increasing baseline white blood cell count and age, these covariates were not significantly associated with response.

For the phase 2 cohort, with a median follow up of 19.8 months, the median OS was 8.2 months with a relapse-free survival (RFS) of 9.0 months (Figure 2). One-year OS and RFS were 37% and 42.9%, respectively.

#### Leukemia cell mobilization

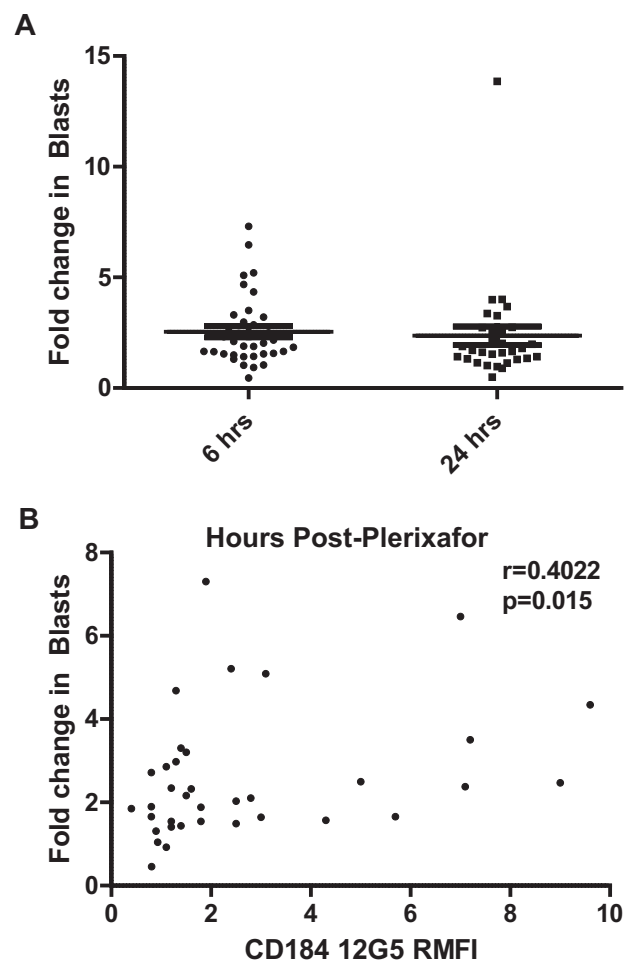
To determine the effect of plerixafor on leukemic cell mobilization, peripheral blood samples were collected at serial time points after administration of plerixafor on day 0. Peripheral blood blasts were identified both by morphology and by flow cytometry (CD45<sup>dim</sup>, SSC<sup>low</sup>). Similar to phase 1, mobilization of both total leukocytes and leukemic blasts was observed after treatment with plerixafor at 6 hours (median fold increase, 2.1) and remained elevated at

24 hours (median, 1.7) after administration (Figure 3A). Again, we observed substantial interpatient variability in the degree of mobilization. We then sought to determine whether the degree of mobilization correlated with CXCR4 expression on AML blasts as measured by flow cytometry. A modest correlation was seen between the expression of CXCR4 and the change in leukemic blasts in the peripheral blood between baseline and 6 hours after administration of plerixafor (Spearman  $R = 0.404$ ,  $P = .015$ , Figure 3B).

To determine whether leukemic blasts were mobilized preferentially over normal cells, we performed FISH from the peripheral blood in a subset of patients with informative cytogenetic abnormalities. No differences were observed between baseline and 6- and 24-hour time points, suggesting that both normal and leukemic cells were mobilized similarly in response to plerixafor (Table 3).

#### Expression of adhesion molecules

The expression of key adhesion molecules on AML blasts were measured in response to administration of plerixafor. We identified a unique pattern using 2 mAbs to CXCR4 (CD184), 12G5, and 1D9. The Ab, 12G5, recognizes an epitope involving the first and second extracellular domains of CXCR4<sup>15</sup> and inhibits plerixafor binding to CXCR4.<sup>16</sup> A different CXCR4 mAb, 1D9, binds to the N



**Figure 3. Phase 2 mobilization data.** (A) Change in AML blasts in the peripheral blood expressed as fold-change relative to baseline at 6 hours (n = 37) and 24 hours (n = 31) after administration of plerixafor 0.24 mg/kg. (B) Correlation between CXCR4 expression (CD184 12G5) in the peripheral blood at baseline and AML blast mobilization at 6 hours.



**Table 3. Peripheral blood FISH in plerixafor-treated patients**

Probe	Postplerixafor, no. of FISH <sup>+</sup> cells/total		
	0 h	6 h	24 h
MLL	128/200	106/200	136/200
MLL	142/200	130/200	120/200
CBFB	12/200	15/200	11/200
AML-ETO	151/200	150/200	162/200
AML-ETO	47/200	33/200	41/200
8	111/200	94/200	126/200
EGR1	92/200	146/200	152/200
EGR1	3/200	0/200	0/200
D20S108	192/200	199/200	196/200

terminus of CXCR4 and is not affected by plerixafor. We observed a decrease in 12G5 binding from pretreatment to 6 hours ( $P < .0001$ ) and an increase from 6 to 24 hours ( $P = .0001$ ) toward baseline indicating CXCR4 blockade by plerixafor in vivo. In contrast, we identified an increase in the binding of surface CD184-1D9 after plerixafor (Figure 4A-B) occurring between pretreatment and 6 hours ( $P < .0001$ ) and remaining elevated at 24 hours. We observed a similar increase in surface CXCR4 expression on human AML blast cells after mobilization with plerixafor in a NOD/SCID/IL2 $\gamma^{\text{null}}$  xenograft model of human AML (supplemental Figure 1). In contrast, treatment of leukemic NOD/SCID/IL2 $\gamma^{\text{null}}$  mice with G-CSF for 5 days induced CXCR4 receptor down-regulation on the human AML cells. For other adhesion molecules including CD49d (VLA-4), CD62L (L-selectin), and CD123 (IL3R $\alpha$ ), the relative MFI for Abs were unchanged after treatment with plerixafor (Figure 4B).

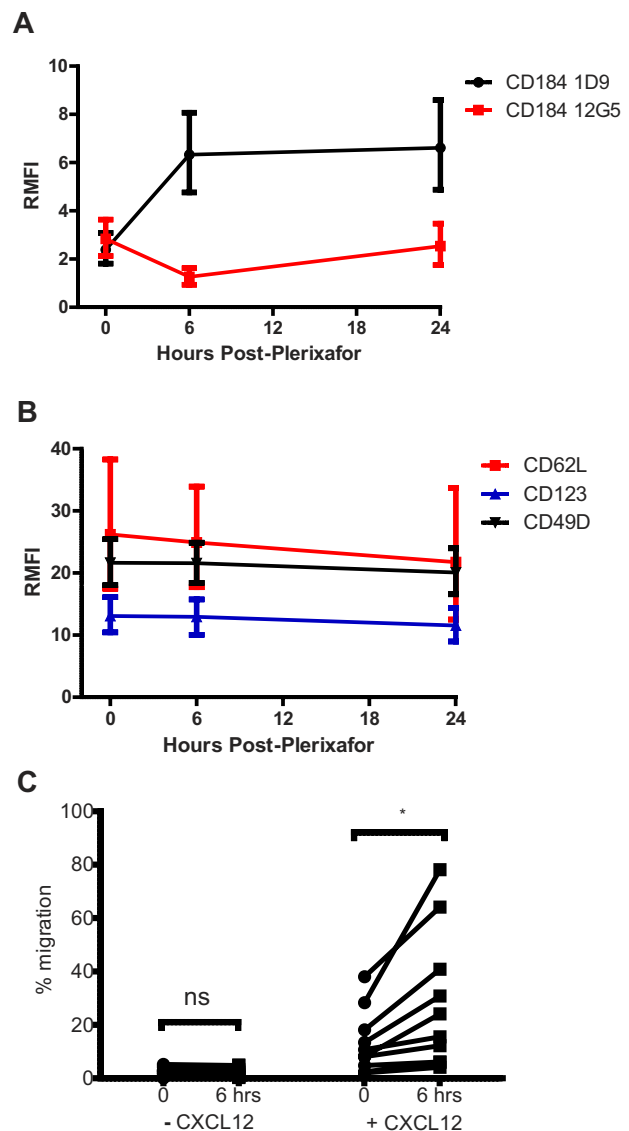
To determine whether differences in measured surface CXCR4 expression were associated with changes in chemotactic ability of the leukemic blasts, we performed transwell experiments using patient samples in a CXCL12 gradient. Compared with baseline, PBMCs from patients treated with plerixafor demonstrated increased chemotaxis in response to an SDF1 gradient (Figure 4C), suggesting that the increase in RFMI for the 1D9 is associated with increased CXCR4 receptor function.

To distinguish whether the increased expression of CXCR4 on circulating peripheral blood blasts was because of up-regulation of surface receptor rather than a mobilization of a high CXCR4-expressing population from the BM, we examined the expression of CXCR4 in response to plerixafor in vitro. Normal CD34<sup>+</sup> cells and lymphocytes, as well as AML blasts, demonstrated a decreased level of CXCR4 on the surface when cocultured in the presence of the OP9 stromal cell line (Figure 5). OP9 cells are known to express and secrete CXCL12<sup>17</sup> and support the growth of human hematopoietic progenitor cells. The down-regulation of CXCR4 on cells after coculture with the CXCL12-secreting OP9 cells was blocked by plerixafor. These findings are in agreement with previous studies by others showing inhibition of CXCL12-induced CXCR4 internalization by plerixafor.<sup>18,19</sup> Taken together, the data suggest that the levels of surface CXCR4 on circulating AML blast cells increase after in vivo plerixafor administration (Figure 4A) because of the plerixafor-mediated inhibition of CXCR4 internalization by CXCL12.

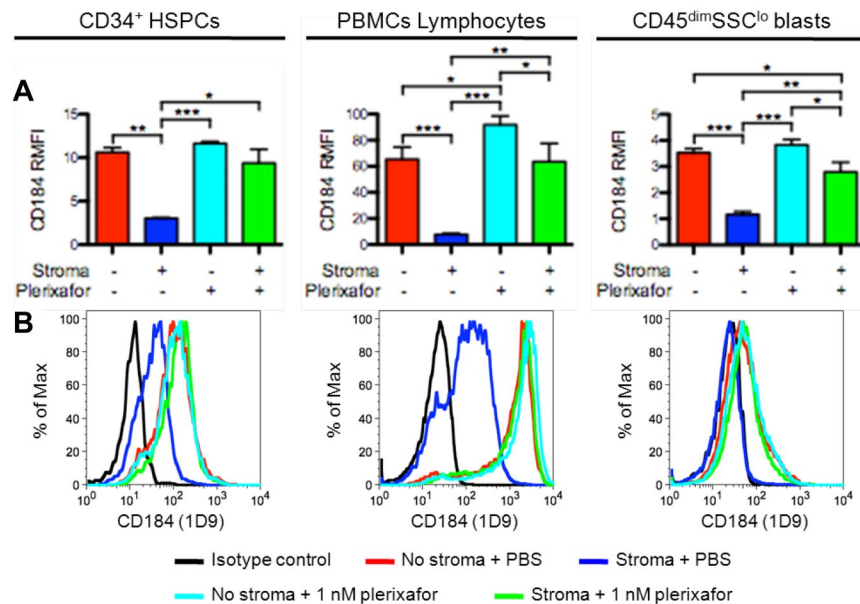
Finally, we attempted to correlate expression of various surface molecules to response to treatment. There were no detectable differences in the surface expression of CD123, CD184 (CXCR4), CD31, CD44, CD49D, CD58, CD62L, CD123, or CXCR7 in either peripheral blood or marrow by response to treatment (supplemental Table 2).

## Discussion

In this article, we demonstrate the safety and feasibility of combining plerixafor with chemotherapy in AML. Although plerixafor has an established safety profile when used in normal donors and patients with myeloma and lymphoma for stem cell mobilization, theoretical concerns exist in administering plerixafor to patients with acute leukemia. Because AML blasts express CXCR4, there was concern that mobilization of blasts by plerixafor may precipitate leukostasis. In fact, an early case report described the massive mobilization of AML cells after the administration of plerixafor and G-CSF.<sup>20</sup> Furthermore, normal HSCs mobilized with plerixafor may similarly be sensitized with chemotherapy that would result in marrow aplasia and delayed hematopoietic recovery after chemotherapy. However, neither symptomatic leukostasis



**Figure 4. Expression of adhesion molecules.** RFMI at baseline, 6 and 24 hours after administration of plerixafor 0.24 mg/kg for (A) 2 different mAbs against CD184 (CXCR4), 12G5, and 1D9 ( $n = 38$ ) and (B) CD62L (L-selectin,  $n = 24$ ), CD49d (VLA-4,  $n = 38$ ), and CD123 (IL3R $\alpha$ ,  $n = 38$ ). (C) Migration of PBMCs to CXCL12 (200 ng/mL) compared with no CXCL12 in transwell assay at baseline and 6 hours after administration of plerixafor ( $n = 10$ ).



**Figure 5. Surface expression of CXCR4 after in vitro culture with plerixafor.** Cord blood CD34<sup>+</sup> HSPCs or PBMCs from normal healthy donors or AML patients were incubated overnight at 37°C in the presence or absence of OP9 stromal cells and plerixafor (1 μM) as indicated. Cells were harvested with accutase, washed, stained with Abs to CD45 and CD184 (clone 1D9), and subjected to flow cytometry. Lymphocytes and AML blasts were discriminated by their CD45/SSC characteristics. (A) Relative changes in the amount of cell-surface-expressed CXCR4. RMFI ratios were calculated by dividing the MFI of CD184 by the MFI of a rat IgG2a isotype control. (B) Representative flow cytometric profiles. The flow cytometric histograms show overlays of CXCR4 surface expression on each cell type in the different culture conditions. The data (mean ± SD) are representative of 1 of 2 independent experiments, in which each condition was tested in duplicate or triplicate; \**P* < .05, \*\**P* < .01, \*\*\**P* < .001.

nor delayed hematopoietic recovery was observed in our study population.

We also demonstrate that plerixafor can mobilize leukemic blasts into the peripheral circulation. The degree of mobilization observed was relatively modest (~2.5-fold) and no evidence of hyperleukocytosis was observed. While the magnitude of mobilization was less than observed with plerixafor in normal donors, it is similar to what was observed in trials with plerixafor as a single agent for mobilization of autologous HSCs in patients with myeloma and non-Hodgkin lymphoma.<sup>21</sup> In addition, we did not observe any evidence of preferential mobilization of leukemic blasts over normal cells. The small sample size in the lower plerixafor dose cohorts (*n* = 3 in 0.08 and 0.16 mg/kg) precluded an analysis of a dose-response relationship between plerixafor and mobilization.

We observed that surface expression of CXCR4 increases in circulating AML blasts after administration of plerixafor both in vitro and in vivo. In addition to expressing surface CXCR4, hematopoietic cells are known to contain large intracellular stores of CXCR4. On binding of CXCL12, the receptor rapidly internalizes in a pertussis toxin-insensitive manner leading to decreased expression of surface CXCR4.<sup>22</sup> Of note is that recycling of CXCR4 to the surface from intracellular stores is both pertussis toxin-insensitive and inhibited by high osmotic states at 37°C. In addition, recycling of intracellular pools of CXCR4 to the surface may, in part, occur independent of receptor internalization. CXCR4 may accumulate on the surface when SDF-1-induced CXCR4 internalization is blocked with plerixafor. Functionally, these cells demonstrated increased chemotactic ability suggesting that this increased surface expression is associated with increased CXCR4 function. Although we cannot exclude the possibility that this phenomena may partly be due to the mobilization of a high CXCR4-expressing population from the marrow, the effect is more likely because of the plerixafor-mediated inhibition of CXCR4 internalization by CXCL12. By up-regulating CXCR4 after plerixafor-induced mobilization, leukemic blasts (as well as normal CD34 progenitors) may respond more robustly in response to diminishing SDF-1 gradients and enhance rehoming to the BM microenvironment.

The CR + CRi rate of 46% (CR = 39%) of the combination of plerixafor and MEC compares favorably with published studies of MEC alone. In a randomized phase 3 study of MEC with or without valspodar, the overall CR rate was 21% (*n* = 129) with no difference between the 2 arms.<sup>23</sup> Although the primary end point of a CR + CRi rate was achieved in this study, the results need to be interpreted with caution. In analyses of patients with relapsed or refractory leukemia, number, length of initial CR, and number of prior salvage chemotherapy regimens were associated with the probability of response.<sup>24</sup> Like all single-institution phase 2 studies, these results are highly influenced by patient selection and interpretation is limited by the relatively small patient numbers and short follow-up time. Furthermore, for relapsed AML, disease-free survival and OS is influenced by postremission therapy and the ability to successfully allograft these patients. Although we were able to successfully bridge the majority of patients to a potentially curative allogeneic stem cell transplant, the short OS of the entire cohort underscores the need for more effective salvage treatments for these patients.

In contrast to other studies, we did not observe an impact on the cell-surface expression of CXCR4 on either CR rates or OS.<sup>5,7,25</sup> Although there are several possible explanations, combining plerixafor with chemotherapy may abrogate the adverse prognostic feature of elevated CXCR4 expression. Alternatively, this may reflect differences in biology between a relapsed, refractory versus a de novo AML population.

This study represents our initial attempt to modulate the microenvironment to augment the sensitivity to chemotherapy in patients with AML. Disruption of the CXCR4/CXCL12 axis is hypothesized to act via 2 major pathways. First, physical detachment of AML cells by plerixafor can disrupt both contact-dependent and -independent mechanisms of chemotherapy resistance mediated by the stromal components. Second, plerixafor may disrupt CXCR4 signaling through its native ligand CXCL12 as well as activation of critical downstream signal transduction pathways including PI3K/AKT and MAPK prosurvival pathways.

Several factors have been found to regulate CXCR4 expression and function. CXCR4 has been shown to be up-regulated by HIF1α in the response to hypoxia.<sup>26</sup> CXCR4 inhibitors may also contribute

to the activity of FLT3 inhibitors.<sup>27</sup> Data from several laboratories have shown that G-CSF dramatically down-regulates the expression of SDF-1 in BM stroma and osteoblasts and CXCR4 expression on mobilized CD34 cells.<sup>28,29</sup> Recently, it has been described that plerixafor induces CXCL12 release from marrow stromal cells into the peripheral circulation and contributes to plerixafor-induced mobilization of hematopoietic progenitors.<sup>30</sup> Furthermore, our data in a NOD/SCID/IL2 $\gamma^{\text{null}}$  xenograft model of human AML suggest that G-CSF also down-regulates CXCR4 expression on human AML cells (supplemental Figure 1). As a follow-up to this study, we are currently exploring the combination of G-CSF and plerixafor to modulate the marrow microenvironment in patients with relapsed and refractory AML (NCT00906945). Plerixafor is known to act synergistically with G-CSF in stem cell mobilization. Preclinical and clinical studies have suggested that “priming” with GM-CSF or G-CSF concurrent with chemotherapy may result in superior outcomes for patients receiving standard induction therapy for AML.<sup>31,32</sup> Although it was presumed that G-CSF induces the proliferation of AML cells rendering them sensitive to cell-cycle agents such as Ara-C, an alternative explanation is that G-CSF also functions as a mobilizing agent, causing egress of AML cells from the BM to the peripheral blood.

The HSC niche is a complex interactive environment which includes cellular components such as osteoblasts, osteoclasts, vascular endothelial cells, stromal cells, extracellular components, and bone matrix. In addition to chemokine receptors such as CXCR4, these interactions between AML and the microenvironment are mediated by molecules including the  $\alpha$  integrins (VLA-4), selectins (L-selectin), and cell-surface glycoproteins (CD44). The expression and function of several of these molecules have been implicated in the response to chemotherapy although with varying results. For example, increased binding of soluble VCAM-1 but not surface expression of VLA4 was significantly associated with longer OS in patients with AML.<sup>6</sup> These molecules may provide alternative targets and candidates which may allow the simultane-

ous disruption of multiple adhesion pathways to modulate the effect of chemotherapy.

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## Authorship

Contribution: G.L.U., M.P.R., and J.F.D. designed and performed research, analyzed data, and wrote the manuscript; K.M.T. analyzed data; and I.H.M., K.M., L.M.H., S.K., C.N.A., A.F.C., K.E.S.-G., R.V., and P.W. performed research.

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