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A Phase 1 Study of the CXCR4 Antagonist Plerixafor in Combination With High-Dose Cytarabine and Etoposide in Children With Relapsed or Refractory Acute Leukemias or Myelodysplastic Syndrome: A Pediatric Oncology Experimental Therapeutics Investigators' Consortium Study (POE 10-03)

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Abstract

Background—Plerixafor, a reversible CXCR4 antagonist, inhibits interactions between leukemic blasts and the bone marrow stromal microenvironment, and may enhance chemosensitivity. A phase 1 trial of plerixafor in combination with intensive chemotherapy in children and young adults with relapsed or refractory acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and myelodysplastic syndrome (MDS) was performed to determine a tolerable and biologically active dose.

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CONFLICT OF INTEREST STATEMENT

The authors have no relevant financial interests to disclose.

AUTHOR CONTRIBUTIONS

All authors provided substantial contributions to the work described in this paper, which consisted of at least one of the following: study conception/design, study investigations, data acquisition, data analysis/interpretation, writing the manuscript, and reviewing/ revising the manuscript. All authors provided final approval of the version to be published.

Procedure—Plerixafor was administered daily for 5 days at 4 dose levels (6, 9, 12, and 15 mg/m²/dose) followed four hours later by high-dose cytarabine (every 12 hours) and etoposide (daily).

Results—Nineteen patients (13 AML, 5 ALL, 1 MDS) were treated. The most common grade 3 or greater nonhematologic toxicities attributable to plerixafor were febrile neutropenia and hypokalemia. There were no dose limiting toxicities (DLTs). Plerixafor exposure increased with increasing dose levels and clearance was similar on days 1 and 5. Eighteen patients were evaluable for response. Two patients achieved complete remission (CR) and 1 patient achieved CR with incomplete hematologic recovery (CRi); all 3 had AML. No responses were seen in patients with ALL or MDS. Plerixafor mobilized leukemic blasts into the peripheral blood in 14 of 16 evaluable patients (median 3.4-fold increase), and degree of mobilization correlated with surface CXCR4 expression.

Conclusions—Plerixafor, in combination with high-dose cytarabine and etoposide, was well-tolerated in children and young adults with relapsed/refractory acute leukemias and MDS. While biologic responses were observed, clinical responses in this heavily-pretreated cohort were modest.

Keywords

pediatric; leukemia; CXCR4; plerixafor; tumor microenvironment

INTRODUCTION

Due to stepwise advances in the diagnosis and treatment of pediatric leukemia, current therapeutic regimens cure the majority of children diagnosed with ALL and AML. However, up to 20% of children with ALL and up to 40% of children with AML do not achieve long-term remissions.[1] In order to predict which patients are at the highest risk of relapse, much work has been focused on factors responsible for therapeutic resistance—such as cytogenetic abnormalities, gene rearrangements, and genetic mutations—that are intrinsic to the leukemia.[2] In parallel, researchers have demonstrated that leukemic blasts are protected by the bone marrow microenvironment.[3–5] Therefore, the interaction between leukemic blasts and nonhematopoietic, stromal cells in the bone marrow microenvironment may be an extrinsic mechanism of chemotherapy resistance.[6]

The cell surface receptor CXCR4 and the chemokine CXCL12 (stromal cell-derived factor-1, SDF-1) are crucial factors in microenvironment-mediated protection of leukemic blasts.[6] CXCR4 is present on the surface of hematopoietic stem cells (HSCs), several types of white blood cells, and lymphoid and myeloid leukemic blasts.[7,8] CXCL12 is produced by a variety of cells in the bone marrow microenvironment, including osteoblasts, endothelial cells, and mesenchymal stromal cells.[9] The CXCR4/CXCL12 axis mediates proliferation, quiescence, and survival of benign and malignant hematopoietic cells.[10–12] Activation of CXCR4 by CXCL12 also governs migration, homing, and retention of HSCs and leukemic blasts within the bone marrow.[13–18] Plerixafor (AMD3100, Mozobil, Genzyme, Cambridge, MA) is a first-in-class reversible small-molecule antagonist of CXCR4. Plerixafor is approved by the Food and Drug Administration for HSC mobilization

in adults with non-Hodgkin lymphoma and multiple myeloma, in combination with granulocyte-colony stimulating factor (G-CSF).[19,20] Interestingly, in preclinical models of ALL[11,21–23] and AML[24–26] plerixafor disrupts leukemia-stroma interactions, mobilizes leukemia cells from the bone marrow, and enhances sensitivity to anti-leukemic therapies. Initial trials of plerixafor as a chemosensitizing agent in adults with relapsed/refractory[27] and newly-diagnosed AML[28] demonstrated both safety and efficacy. This strategy has not been tested in the pediatric population. We report the results of a Pediatric Oncology Experimental Therapeutics Investigators' Consortium (POETIC) phase 1 trial of plerixafor in combination with chemotherapy in children and young adults with relapsed/refractory acute leukemias and MDS (NCT01319864).

METHODS

Patient Eligibility

Eligible patients had relapsed (first or greater relapse) or refractory AML, ALL, secondary AML/MDS, or acute leukemia of ambiguous lineage and were between the ages of 3 and 30 years at the time of study entry. Patients with AML/MDS or leukemia with ambiguous lineage were required to have >5% blasts in the bone marrow, while patients with ALL were required to have >25% blasts in the bone marrow. Prior to entering this study, patients must have fully recovered from the acute toxic effects of all prior chemotherapy, immunotherapy, or radiotherapy. At the time of study entry, eligible patients could not have received myelosuppressive therapy or long-acting myeloid growth factor within 14 days, or biologic therapy or short-acting myeloid growth factor within 7 days. At least 90 days must have elapsed since hematopoietic stem cell transplant (HSCT) and patients must not have had evidence of active graft-versus-host disease (GVHD) requiring immunosuppressive medications. Other eligibility criteria included Karnofsky/Lansky scores >50%, serum creatinine the upper limit of normal (ULN) for age or calculated creatinine clearance/radioisotope glomerular filtration rate (GFR) 70mL/min/1.73m², total bilirubin <1.5× ULN for age or normal conjugated bilirubin for age, alanine transaminase (ALT) <5× ULN unless due to leukemic involvement, and shortening fraction 27% by echocardiogram or ejection fraction 50% by gated radionuclide study.

Exclusion criteria included absolute blast count >50,000/μL despite cytoreduction with hydroxyurea or leukapheresis, presence of leukemic blasts in the central nervous system (CNS2 or CNS3), uncontrolled active systemic infection, and/or a second cancer other than secondary AML. Pretreatment with intrathecal chemotherapy was allowed to achieve CNS1 status.

The protocol was approved by the institutional review boards/institutional ethics committees of participating institutions. Written informed consent was obtained from all patients or their legal guardians and age-appropriate assent was obtained according to the guidelines of each participating institution.

Drug Administration and Study Design

The primary objective of this phase 1 trial was to determine the safety and tolerability of plerixafor in combination with reinduction chemotherapy in pediatric and young adult patients with relapsed or refractory acute leukemias and MDS. Secondary objectives were to 1) estimate the response rate, 2) determine the pharmacokinetics of plerixafor in this treatment regimen and patient population, 3) quantify mobilization of leukemic blasts into the peripheral blood, 4) measure the quantitative expression of CXCR4 on leukemic blasts at baseline, and 5) evaluate changes in quantitative expression of CXCR4 on residual leukemic blasts after cycle 1. This phase 1 dose-escalation study utilized the rolling six trial design, [29] which allowed between 2 and 6 patients to be enrolled concurrently onto a given dose level.

The starting dose of plerixafor was 6 mg/m²/dose (Dose Level 1, DL1), which was approximately 80% of the dose tested in the phase 2 adult AML trial.[27] Dose levels for subsequent groups of patients included 9 mg/m²/dose (DL2), 12 mg/m²/dose (DL3), and 15 mg/m²/dose (DL4). Dose escalation was based on a prior study of the safety and mobilization of CD34+ cells using escalating doses of plerixafor, which demonstrated a trend toward higher peak CD34+ counts at doses up to 0.48 mg/kg (roughly equivalent to 15 mg/m²) in healthy adult volunteers.[30] In case of DLT, the study allowed for one dose de-escalation level (DL-1, 3 mg/m²/dose). On day 1, patients received standard age-based doses of intrathecal methotrexate (ALL patients) or cytarabine (AML, MDS, and leukemia with ambiguous lineage patients). On days 1 through 5, plerixafor was administered intravenously (IV) once daily over 30 minutes followed 4 hours later by high-dose cytarabine (1 gram/m² IV every 12 hours) over 1 hour and etoposide (150 mg/m² IV daily) over 1 hour. The timing of plerixafor prior to chemotherapy administration was the same as that studied in the phase 2 adult AML chemosensitization trial, and was based on preclinical and clinical data suggesting that this timing would maximize the interruption of leukemia-stroma interactions. [27]

Toxicity was graded during cycle 1 using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), version 4.0. DLT was defined as any grade 3 non-hematologic toxicity that persisted for >48 hours without resolution to grade 2, and was possibly, probably, or definitely attributable to plerixafor, excluding alopecia; anorexia; grade 3 vomiting or diarrhea that resolved to grade 2 within 48 hours; grade 3 nausea that resolved to grade 2 within 7 days; asymptomatic grade 3 elevation in amylase, lipase, or total bilirubin that returned to grade 2 within 7 days; grade 3 elevation in hepatic transaminases or alkaline phosphatase that returned to grade 2 within 14 days; grade 3 fever with neutropenia; and grade 3 infection. Failure to recover a peripheral absolute neutrophil count >500/μL or a non-transfusion-dependent platelet count >20,000/μL by day 56, without evidence of residual leukemia, was also considered a DLT. Attribution of toxicities to the study drug were determined by treating physicians. Patients evaluable for DLT were defined as those who: 1) experienced DLT after receiving at least one dose of plerixafor; or 2) did not experience DLT and received 80% of planned doses of plerixafor.

Response was assessed via bone marrow aspiration performed at the end of cycle 1 (upon count recovery but no later than day 28). Patients who achieved a response of stable disease or better were eligible to receive a second cycle of therapy, as long as all grade 3 toxicities had resolved to baseline or < grade 2. DLT was assessed in the first cycle only. Patients evaluable for response were defined as those who: 1) met the definition of progressive disease after receiving at least one dose of plerixafor; or 2) did not meet the definition of progressive disease, received ≥ 80% of planned doses of plerixafor, and had the required response evaluation performed at the end of the first course of therapy.

Pharmacokinetic Studies

Peripheral blood (2 mL) for pharmacokinetic studies was collected at hour 0 (immediately prior to the plerixafor infusion), within 5 minutes prior to the end of the plerixafor infusion, and hours 1, 2, 4, 8, and 24 on days 1 and 5 of cycle 1. Blood for pharmacokinetic studies was collected from a site separate from the line used for plerixafor infusion. Plasma was then separated by centrifugation at $3,000 \times g$ for 10 minutes, divided into 2 equal aliquots, stored at -80°C , and analyzed in batches. Plerixafor plasma concentrations were measured using a validated LC-MS-MS analytical method.

Plasma pharmacokinetic parameters were estimated by non-compartmental analysis using Phoenix WinNonlin 7.0 (Certara). Maximum plasma concentration (C_{max}), area under the concentration-time curve from time 0 to 24 hours ($\text{AUC}_{0-24\text{h}}$), terminal half-life ($t_{1/2}$) and systemic clearance were estimated from the observed concentration-time data.

Correlative Biology Studies

To measure CXCR4 expression, bone marrow aspirates were obtained at study entry (baseline) and post cycle 1. Peripheral blood mononuclear cells were isolated via Ficoll density centrifugation as previously described.[22] Cells were then prepared for flow cytometry analysis as previously described.[23] Briefly, cells were stained with antibodies against human CD45, CD34, CD19, CD33, CXCR4 (12G5, eBioscience, San Diego, CA), and isotype controls, and read on a FACSCalibur (all from BD Biosciences, San Diego, CA, except as noted). Analysis to identify leukemic blasts was performed using FlowJo software for flow cytometry (FlowJo, LLC, Ashland, OR) and surface CXCR4 expression in the blast population was quantified as mean fluorescence intensity (MFI) relative to isotype control.

To measure peripheral blood mobilization of leukemic blasts, paired aliquots of whole blood (4 mL) were collected from each patient immediately before the first dose of plerixafor (hour 0) and at hour 4. After red blood cell lysis, cells were counted using a hemacytometer and Trypan Blue exclusion. Flow cytometry was then performed as described above to identify leukemic blasts. Absolute peripheral blood leukemic blast counts were calculated by multiplying the percentage of leukemic blasts by the total cell count.

Student's t-tests were used to calculate p values. Alpha was set to 0.05. Correlation between CXCR4 expression and leukemic blast mobilization was assessed by Pearson's correlation coefficient.

RESULTS

Patient Characteristics

Twenty patients were enrolled between July 2011 and June 2013. One patient consented to the trial but did not initiate protocol therapy. One patient received <80% of planned plerixafor doses and was removed from protocol therapy for reasons other than DLT or progressive disease, and thus was not evaluable for DLT or response. This patient had pre-existing abdominal pain that worsened significantly after the first day of protocol therapy. Specifically, after receiving the first day of protocol therapy consisting of plerixafor (12 mg/m²), etoposide, and cytarabine, there was radiographic evidence of neutropenic colitis and treatment was discontinued. Given antecedent prolonged leukemia with cytopenias and the timing of clinical findings, the investigator determined this was not related to plerixafor but not in the patient's best interest to continue intensive protocol therapy. Therefore, 18 patients were evaluable for DLT and response; the characteristics of these 18 patients are summarized in Table 1. All patients with ALL had B-lineage ALL. Of note, one-third of patients (n=6) were in second or greater relapse. Specifically, the median number of prior treatment regimens was 2.5 (range 2–5) for patients with ALL and 2 (range 1–5) for patients with AML. Nearly half (n=8) of the evaluable patients had previously undergone HSCT.

Toxicity

Nineteen patients (including the inevaluable patient mentioned above) received at least one dose of study drug. Patients were treated at all 4 planned dose levels of plerixafor (3 patients at DL1, 5 at DL2, 5 at DL3, and 6 at DL4). Plerixafor dose de-escalation was not necessary. There were no DLTs or delays in count recovery attributable to plerixafor. In general, toxicities were consistent with intensive relapsed leukemia regimens. The most common grade 1 and 2 toxicities attributed to plerixafor occurring in >20% of patients included nausea (n=12); anorexia (n=11); vomiting (n=9); diarrhea and hypocalcemia (n=8 each); headache and hypoalbuminemia (n=7 each); abdominal pain (n=6); increased ALT (n=5); and constipation, fatigue, hyperglycemia, hypertriglyceridemia, and hypokalemia (n=4 each). The most common grade 3 or greater nonhematologic toxicities attributed to plerixafor that occurred in >10% of patients included febrile neutropenia (n=4), hypokalemia (n=3), anorexia, hyperglycemia, hypotension, mucositis, typhlitis, and vomiting (n=2 each) (Table 2).

Response

As shown in Table 3, 2 patients achieved CR (<5% bone marrow blasts and no evidence of circulating blasts or extramedullary disease, ANC >1000/μL, and platelet count >100,000/μL) and 1 patient achieved CRi (CR without recovery of ANC or platelet count to aforementioned levels). Notably, all 3 of these responses were in patients with AML. The 2 patients who achieved CR were treated at DL1 and DL3, while the 1 patient who achieved CRi was treated at DL4. No patients achieved partial remission (PR, no evidence of circulating blasts, decrease of at least 50% of blasts in the bone marrow with 5% and 25% blasts with recovery of peripheral counts). There were no responses in patients with ALL or MDS. Stable disease was reported in 44% of patients and 39% of patients had progressive disease.

Pharmacokinetics

Plerixafor pharmacokinetic parameters on days 1 and 5 are summarized in Table 4. C_{max} and AUC_{0-24h} values increased with increasing dose levels. Day 1 mean $\pm$ standard deviation (SD) plerixafor AUC_{0-24h} values at 12 mg/m² (DL3) and 15 mg/m² (DL4) were $5,074 \pm 380$ and $5,375 \pm 1852$ ng*h/mL, respectively. The day 1 half-life of plerixafor was similar at all 4 dose levels (4.33 ± 2.37 hours). Drug clearance was similar between days 1 and 5 ($p=0.197$) with values at all dose levels of 2.39 ± 1.42 L/h/m² and 1.95 ± 0.53 L/h/m², respectively.

Correlative Biology Studies

Adequate bone marrow aspirates for baseline correlative biology studies were obtained for 16 evaluable patients at study entry. Average surface expression of CXCR4 at baseline was variable (range of MFI 17.45 - 413.48). Consistent with previous reports,[8] surface CXCR4 expression was higher in patients with ALL compared to those with AML or AML/MDS (average MFI 167.5 in ALL vs. 51.94 in AML, $p=0.025$, Figure 1A).

Paired peripheral blood samples collected before and after dose 1 of plerixafor were obtained for 16 evaluable patients. In 14 of 16 patients, plerixafor mobilized leukemic blasts into the peripheral blood (Figure 1B), with a median 3.4-fold increase in circulating leukemic blasts ($p=0.006$). Further, baseline surface CXCR4 expression correlated positively with the degree of leukemic blast mobilization ($r=0.56$, $p=0.023$, Figure 1C). Compared to hour 0, surface CXCR4 expression in peripheral leukemic blasts was significantly higher at hour 4 (average MFI 46.4 at hour 0 vs. 62.53 at hour 4, $p=0.004$, Figure 1D).

Analysis of paired bone marrow aspirates from study entry and end of cycle 1 was able to be performed for 14 evaluable patients. Surface CXCR4 expression in leukemic blasts obtained from the bone marrow was not significantly different between baseline and the end of cycle 1 (average MFI 89.2 vs. 67.15 respectively, $p=0.258$, Figure 1E). Because only 3 patients had objective responses, we were unable to correlate response with CXCR4 expression, changes in CXCR4 expression, or mobilization of leukemic blasts into the peripheral blood (Supplemental Table 1). However, in patients with AML/MDS, responders had a greater degree of leukemic blast mobilization (median 3.3-fold increase) than non-responders (median 1.7-fold increase), but this difference was not statistically significantly different ($p=0.472$).

DISCUSSION

Plerixafor in combination with an intense chemotherapeutic regimen was well-tolerated in children and young adults with relapsed/refractory acute leukemias and MDS. Observed adverse events were largely consistent with toxicity profiles from conventional chemotherapy regimens for pediatric acute leukemias. Because CXCR4 is expressed on the surface of HSCs and white blood cells, an important finding was that the addition of plerixafor to high-dose cytarabine and etoposide did not lead to delayed count recovery in the patients studied. In initial adult AML chemosensitization trials, the addition of plerixafor to chemotherapy also did not result in delayed count recovery.[27,28] Compared to PK assessments in adult volunteers receiving intravenous plerixafor,[31] PK analysis in this

study revealed a similar half-life, but decreased clearance of plerixafor. Drug clearance did not differ between days 1 and 5, implying that there was no accumulation of plerixafor over the period studied.

As reported in other trials of plerixafor in children and adults with acute leukemias, [27,28,32] we observed that plerixafor mobilized leukemic blasts into the peripheral blood. Despite heterogeneous expression of surface CXCR4, mobilization occurred in the vast majority of patients in this trial. In addition, higher CXCR4 expression correlated with higher blast mobilization. Thus, it is possible that surface CXCR4 expression may predict responsiveness to plerixafor. For example, *in vitro* studies in ALL and AML cell lines found that chemotherapy-induced increases in surface CXCR4 expression were positively associated with the degree of chemosensitization by plerixafor.[23,26] In this trial, we hoped to explore this finding further, but, due to low patient numbers and limited responses, we could not correlate changes in surface CXCR4 expression with response in a statistically meaningful way.

Surface CXCR4 expression, as measured by the 12G5 antibody, increased after plerixafor treatment (Figure 1D). The 12G5 CXCR4 antibody is specific for the site at which both CXCL12 and plerixafor bind. Previous work suggested that continuous exposure to plerixafor *in vitro* for 72 hours leads to increased surface expression of CXCR4.[23] In adults with AML, plerixafor also increased surface CXCR4 expression.[27,33] Higher levels of surface CXCR4 expression in residual leukemic blasts may lead to untoward consequences, including increased responsiveness to CXCL12, resulting in decreased sensitivity to chemotherapy.[23] Collectively, these findings may help optimize future trial design of plerixafor or other CXCR4 antagonists. For example, additional preclinical evaluation of plerixafor may identify dosing regimens that disrupt leukemia-microenvironment interactions without increasing surface CXCR4 expression. Additional CXCR4-targeted agents in clinical development include BL-8040 (formerly BKT-140),[34] LY2510924,[35] POL6326,[36] and ulocuplumab[37] Both BL-8040 and ulocuplumab have demonstrated safety and efficacy when combined with chemotherapy in adults with relapsed/refractory AML.[34,37]

We observed 3 complete responses (2 CR, 1 CRi) in this heavily-pretreated population of children with relapsed/refractory ALL, AML and MDS. However, the design of this study does not allow us to conclude that plerixafor was responsible for the CRs.

We were intrigued that all observed responses were in patients with AML. However, with the sample size of only 12, the point estimate of 25% for CR/CRi in AML patients has a 95% confidence interval ranging from 0.5% to 49.5%. Similarly, the lack of response seen in patients with ALL and MDS is difficult to interpret given the sample sizes of 5 and 1 patients, respectively. While the overall response rate on this study was modest, the study was not designed or powered to definitively address an efficacy question. Whether further study of this regimen is warranted will depend upon the relative enthusiasm for this approach compared to other novel approaches available for this patient population. With a CR+CRi rate of 25% (range 0.5% to 49.5%) in pediatric patients with heavily pretreated

relapsed AML, and no evidence of enhanced toxicity, this regimen is not an unreasonable candidate for further study.

It is also important to recognize that clinical trials of CXCR4 chemosensitization in acute leukemias, including this one, have focused on AML and B-lineage ALL. Recently, the potential importance of CXCR4 and CXCL12 in T cell ALL (T-ALL) was demonstrated in two elegant studies.[38,39] Silencing of CXCR4 by small hairpin RNA decreased leukemia engraftment and leukemia-initiating capacity in transplant models of murine and human T-ALL.[39] Further, administration of the CXCR4 inhibitor AMD3465 as a single agent significantly decreased leukemic burden in murine T-ALL and xenografts of primary T-ALL samples.[38] Therefore, CXCR4 inhibition may be a potential therapeutic approach in T-ALL. Because our trial did not enroll any T-ALL patients, further studies will be required to address this question.

It is possible that response rates could be improved by optimizing the treatment backbone with additional chemotherapeutic agents or targeted therapies, or by adding G-CSF, which has been shown to decrease CXCL12 production by stromal cells.[40] Remarkably, plerixafor and G-CSF have been shown to augment the effectiveness of targeted therapies in high-risk leukemias in preclinical models[22] and in clinical trials.[33] For example, plerixafor and G-CSF greatly enhanced the efficacy of the FLT3 tyrosine kinase inhibitor lestaurtinib in a xenograft model of infant *MLL*-rearranged ALL.[22] Also, a phase 1 trial of plerixafor, G-CSF, and sorafenib in adults with relapsed/refractory FLT3-ITD+ AML reported a CR+CRi rate of 31% in this very difficult-to-treat population, as well as a 41-fold increase in circulating blasts after mobilization.[33] Therefore, while the study reported here was associated with modest responses, targeting leukemia-stroma interactions through CXCR4 inhibition may yet prove to be an effective strategy in the treatment of high-risk leukemias.

We conclude that CXCR4 inhibition as a chemosensitization strategy is safe in children and young adults with relapsed/refractory acute leukemias and MDS. These findings support the consideration for the continued clinical development of bone marrow microenvironment-targeted agents in pediatric acute leukemias.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations key

ALL	Acute lymphoblastic leukemia
ALT	Alanine aminotransferase
AML	Acute myeloid leukemia
AUC	Area under the concentration-time curve
CNS	Central nervous system
CR	Complete remission
CRi	Complete remission with incomplete hematologic recovery
CTCAE	Common Terminology Criteria for Adverse Events
DL	Dose level
DLT	Dose-limiting toxicity
FLT3	Fms-like tyrosine kinase 3
FLT3-ITD	Fms-like tyrosine kinase 3-internal tandem duplication
G-CSF	Granulocyte-colony stimulating factor
GFR	Glomerular filtration rate
GVHD	Graft-versus-host disease
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplant
IV	Intravenous
MDS	Myelodysplastic syndrome
MFI	Mean fluorescence index
PK	Pharmacokinetic
POETIC	Pediatric Oncology Experimental Therapeutics Investigators' Consortium
PR	Partial remission
SD	Standard deviation
T-ALL	T cell ALL
ULN	Upper limit of normal

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Figure 1.

Summary of correlative biology studies. Data points represent individual patients and are color-coded by disease type (ALL in blue, AML in red, AML/MDS in black). **(A)** Surface expression of CXCR4 in leukemic blasts is higher in ALL than AML/MDS. Surface expression of CXCR4 was measured in bone marrow leukemic blasts at study entry (baseline). CXCR4 MFI in leukemic blast populations were normalized to isotype control. Average CXCR4 MFI was significantly higher in patients with ALL compared to patients with AML or AML/MDS (ALL 167.5 vs. AML 51.94, $p=0.025$). **(B)** Plerixafor mobilizes leukemic blasts into the peripheral blood. Blood was collected prior to dose 1 of plerixafor

(hour 0) and at hour 4. Mobilization of leukemic blasts by plerixafor was observed in 14 of 16 patients, with a median 3.4-fold increase in circulating leukemic blasts ($p=0.006$). **(C)** Mobilization of leukemic blasts by plerixafor is dependent on CXCR4 expression. Baseline surface CXCR4 expression and fold change in leukemic blast count after dose 1 of plerixafor were compared. The degree of leukemic blast mobilization correlated positively with baseline surface CXCR4 expression ($r=0.56$, $p=0.023$). **(D)** Treatment with plerixafor resulted in increased surface CXCR4 expression in circulating leukemic blasts. CXCR4 MFI of circulating leukemic blasts was measured prior to dose 1 of plerixafor (hour 0) and at hour 4. Plerixafor treatment induced a significant increase in surface CXCR4 expression ($p=0.004$). **(E)** Surface CXCR4 expression in bone marrow blasts did not differ significantly between baseline and the end of cycle 1. Surface expression of CXCR4 was measured in bone marrow leukemic blasts at study entry (baseline) and at the end of cycle 1.

Table 1

Characteristics of Patients Evaluable for Response (n=18).

	ALL	AML	AML/MDS
	5	12	1
Gender			
Male	3	2	0
Female	2	10	1
Race			
White	1	6	0
Black/African American	2	3	0
Asian	1	1	0
Other/Unknown	1	2	1
Age (Years)			
Median	14	13	20
Range	12 to 21	3 to 17	20
Molecular Abnormalities			
Complex (5 abnormalities)	3	3	0
FLT3/ITD	0	1	0
Hypodiploid	1	0	0
MLL-rearrangement	0	3	0
Monosomy 7	1	0	0
Pericentric inv(1)	0	0	1
Ring Chromosome 21	0	0	1
t(5;11)	0	1	0
t(6;9)	0	1	0
t(8;21)	0	1	0
t(9;22)	1	0	0
t(11;17)	0	2	0
t(14;20)	0	1	0
Trisomy 8	0	2	0
Prior Bone Marrow Transplant			
Yes	3	5	0
No	2	7	1
Prior Regimens (Number)			
Median	2.5	2	3
Range	2 to 5	1 to 5	3

Several patients had multiple molecular abnormalities. All patients with ALL had B-lineage ALL.

Table 2

Grade 2 and Higher Toxicities Possibly or Probably Related to Plerixafor (n=19 patients).

Toxicity type	Grade 2	Grade 3	Grade 4
Hematologic			
Anemia	3	7	
Lymphocyte count decreased		1	4
Neutrophil count decreased			8
White blood cell decreased		1	5
Platelet count decreased			11
Cardiovascular			
Ejection fraction decreased	1		
Hypotension		1	1
Constitutional			
Agitation	1		
Allergic reaction			1
Dizziness	1		
Fatigue	1		
Fever	3		
Dermatologic			
Erythroderma	1		
Flushing	1		
Localized edema			
Palmar-plantar erythrodysesthesia	1		
Rash, maculo-papular		1	
Skin ulceration		1	
Gastrointestinal			
Abdominal pain	3	1	
Anorexia	6	2	
Constipation	1		
Diarrhea	1	1	
Enterocolitis		1	
Gastrointestinal disorders, other	1	1	
Nausea	5		
Vomiting	3	2	
Mucositis, oral		2	
Infection			
Febrile neutropenia		4	
Infections and infestations, other	1	1	
Lung infection		1	
Sepsis			1
Skin infection		1	
Typhlitis		2	

Toxicity type	Grade 2	Grade 3	Grade 4
Laboratory abnormalities			
Alanine aminotransferase increased	1		
Aspartate aminotransferase increased	2	1	
Blood bilirubin increased	2	1	
GGT increased		1	
Hyperglycemia		2	
Hypertriglyceridemia	1		
Hypoalbuminemia	2		
Hypocalcemia	4		
Hypoglycemia	1		
Hypokalemia		2	1
Hypophosphatemia	2		
INR increased	1		
Neurologic			
Flank pain	1		
Headache	2		
Pain in extremity	1		
Syncope		1	
Oral pain	1		
Pain	1		
Renal			
Hypertension		1	
Respiratory			
Hypoxia		1	
Pulmonary edema		1	
Respiratory, thoracic, and mediastinal disorder, other	1	1	

Table 3

Responses to Protocol Therapy.

Total # Evaluable Patients	ALL	AML	AML/MDS
18	5	12	1
Response			
CR		2	
CRi		1	
PR			
SD	4	3	1
PD	1	6	

CR, complete remission; CRi, complete remission with incomplete hematologic recovery; PR, partial remission; SD, stable disease; PD, progressive disease.

Table 4

Plerixafor Pharmacokinetic Parameters.

Dose (mg/m ²)	Cmax (ng/mL)		AUC _{0-24h} (ng*h/mL)		t1/2 (h)		Clearance (L/h/m ²)	
	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5	Day 1	Day 5
6 (N = 3)	1,759 (1,309)	832 (96)	3,543 (1,765)	3,492 (1,051)	3.99 (0.23)	4.53 (0.60)	1.57 (0.81)	1.57 (0.93)
9 (N = 5)	1,380 (256)	1,661 (561)	4,718 (574)	5,130 (1,126)	4.16 (0.39)	4.51 (0.77)	1.90 (0.27)	1.78 (0.35)
12 (N = 4)	1,957 (355)	1,867 (447)	5,075 (380)	5,676 (1,474)	3.89 (0.17)	4.76 (0.34)	2.34 (0.18)	2.17 (0.62)
15 (N = 6)	1,933 (651)	2,358 (982)	5,375 (1,852)	6,976 (956)	4.95 (4.28)	4.78 (1.16)	3.24 (2.24)	2.14 (0.28)
All dose levels					4.33 (2.37)	4.66 (0.79)	2.39 (1.42)	1.95 (0.53)

Values are mean (standard deviation).