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Phase I Study of the Safety and Pharmacokinetics of Plerixafor in Children Undergoing a Second Allogeneic Hematopoietic Stem Cell Transplantation for Relapsed or Refractory Leukemia

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A B S T R A C T

The safety, pharmacokinetics, and biological effect of plerixafor in children as part of a conditioning regimen for chemo-sensitization in allogeneic hematopoietic stem cell transplantation (HSCT) have not been studied. This is a phase I study of plerixafor designed to evaluate its tolerability at dose of .24 mg/kg given intravenously on day −4 (level 1); day −4 and day −3 (level 2); or day −4, day −3, and day −2 (level 3) in combination with fludarabine, thiotepa, melphalan, and rabbit antithymocytic globulin for a second allogeneic HSCT in children with refractory or relapsed leukemia. Immunophenotype analysis was performed on blood and bone marrow before and after plerixafor administration. Twelve patients were enrolled. Plerixafor at all 3 levels was well tolerated without dose-limiting toxicity. Transient gastrointestinal side effects of National Cancer Institute–grade 1 or 2 in severity were the most common adverse events. The area under the concentration-time curve increased proportionally to the dose level. Plerixafor clearance was higher in males and increased linearly with body weight and glomerular filtration rate. The clearance decreased and the elimination half-life increased significantly from dose level 1 to 3 ($P < .001$). Biologically, the proportion of $CXCR4^+$ blasts and lymphocytes both in the bone marrow and peripheral blood increased after plerixafor administration.

INTRODUCTION

The outcome in children with relapsed leukemia undergoing a second allogeneic hematopoietic stem cell transplantation (HSCT) is poor, with a 5-year overall survival ranging from 30% [1-3] to 48% [4]. Children may experience lower regimen-related toxicity from a second transplantation compared with adults [2,4]; however, recurrent disease remains a predominant cause of death. Thus, novel therapeutic strategies are needed to overcome leukemia resistance to improve the outcome after second HSCT. Interaction between leukemia cells and the bone marrow stromal environment is postulated to be an important mediator of this resistance [5,6]. The chemokine receptor CXCR4 is expressed in acute myeloid leukemia (AML) [7] and acute lymphoblastic leukemia [8], binds to CXCL12 expressed by the marrow stroma, and promotes survival of the leukemic cells. Increased expression of CXCR4 has been associated with an increased risk of relapse and poor outcome in acute lymphoblastic leukemia [9], and AML [10,11].

Plerixafor (Mozobil, Sanofi USA, Bridgewater, NJ) is a reversible inhibitor of the binding of CXCL12 to CXCR4. It is FDA approved for use in combination with granulocyte colony–stimulating factor to mobilize hematopoietic stem cells in patients with non-Hodgkin's lymphoma and multiple myeloma undergoing autologous transplantation [12,13]. In a murine model of AML, mice treated with chemotherapy plus plerixafor had lower tumor burdens and improved overall survival compared with mice treated with chemotherapy alone [14]. A phase I/II study of plerixafor in adults with relapsed or refractory AML showed that the drug was well tolerated and disrupted the CXCR4/CXCL12 axis [15].

The toxicity, pharmacokinetics, and biological effect of plerixafor when used in children as part of conditioning for an allogeneic HSCT is not known. We conducted a phase I trial to investigate the maximum tolerated dose of plerixafor, pharmacokinetics, and cell surface expression of CXCR4.

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METHODS

Study Population

The study was conducted at St. Jude Children's Research Hospital in Memphis, Tennessee, and was approved by the hospital's institutional review board. Consent was obtained from all parents and/or guardians, and assent was obtained from all children older than 7 years of age.

Eligibility criteria included age $\leq$ 21 years, a hematologic malignancy that had relapsed after prior allogeneic HSCT, and a scheduled bone marrow stem cell graft from a 7/8 or 8/8 HLA allele–matched related or unrelated donor. Patients needed adequate renal, hepatic, cardiac, and pulmonary function as determined by institutional guidelines. Exclusion criteria included active central nervous system malignancy, neuromuscular dysfunction, or ongoing treatment for acute or chronic graft-versus-host disease (GVHD). All patients had a performance score of 100.

Conditioning regimen included fludarabine 30 mg/m^2 on day −9 through day −5, thiotepa 5 mg/kg/dose for 2 doses on day −4, melphalan 70 mg/m^2 on day −3 and −2, and rabbit antithymocytic globulin (rATG) 3 mg/kg/day on days −3 through −1 after a test dose of 1 mg/kg on day −4. GVHD prophylaxis included tacrolimus starting day −2, sirolimus starting day 0, and methotrexate 5 mg/m^2 on days +1, +3, and +6. Patients at risk for cytomegalovirus or herpes simplex reactivation received prophylaxis with acyclovir, and all patients received prophylaxis with metronidazole, cotrimoxazole, and antifungals in accordance with institutional guidelines.

Plerixafor Dose Schedule

Plerixafor .24 mg/kg/day was given intravenously at 3 dose levels (1 dose, day −4; level 1), (2 doses, days −4 and −3; level 2), (3 doses, days −4, −3 and −2; level 3). Plerixafor was administered 5 hours before chemotherapy at each dose level.

Pharmacokinetic Testing

Blood samples for pharmacokinetic testing were obtained before plerixafor and 30 minutes and 1, 2, 6, 12, and 24 hours after infusion of each dose. Samples were spun and sera were cryopreserved, batched, and run at a later date. Samples prepared by a protein precipitation extraction procedure in sodium heparin plasma were analyzed by liquid chromatography/tandem mass spectrometry over the concentration range of 5 to 1000 ng/mL. The API 5000 (AB SCIEX, Framingham, MA) was operated in the Multiple Reaction Monitoring mode under optimized conditions for detection of plerixafor and AMD16617$^+$ ions formed by electrospray ionization. Calibration standards were placed at the beginning and end of each bio-analytical run.

Pharmacokinetic Analysis

The population pharmacokinetic and individual post hoc estimates were determined using nonlinear mixed effects modeling performed with Monolix (version 4.2.2, www.monolix.org). A 2-compartment pharmacokinetic model with first-order elimination was fit to the data. Parameters estimated included systemic clearance (L/hour or L/hour/kg), volume of distribution (L/kg), intercompartmental clearance (L/hour, or L/hour/kg), and volume of peripheral compartment (mL, or L/kg). The interindividual variability of the parameters was assumed to be log normally distributed. A proportional residual error model was used with assumed normal distribution of the residuals. Estimates of area under the concentration-time curve from 0 to 72 hours (AUC, ng $\times$ hour/mL), maximum concentration (ng/mL), and minimum concentration (ng/mL) were determined using the individual post hoc estimates.

Covariates, including demographics, glomerular filtration rate (GFR) as assessed by Tc99m renal clearance, serum creatinine, aspartate transaminase, alanine transaminase, bilirubin, and absolute neutrophil count were evaluated to determine their significance in explaining pharmacokinetic variability. These covariates were considered significant in a univariate analysis if their addition to the model reduced the objective function value at least 3.84 units ($P < .05$, based on the chi-square test for the difference in the 2-log likelihood between 2 hierarchical models that differ by 1 degree of freedom), and the covariate term was significantly different than 0 ($P < .05$, t-test).

Immunophenotype Analysis

Blood samples and bone marrow aspirate for immunophenotype analysis were obtained before plerixafor administration on day −5 and after its administration on day −4 (before thiotepa administration). The cell content was phenotyped by flow cytometry using BD FACSCanto II flow cytometer (BD Biosciences, San Jose, CA), BD FACSDiva 6.0 software (BD Biosciences, San Jose, CA), and a red cell lysis/multicolor antibody protocol. The following monoclonal antibodies against cell surface or intracellular markers were used: Anti-CD45 APC-H7 (Clone 2D1), Anti-CD33 PE-Cy7 (Clone P67.6), Anti-sCD3 V450 (Clone UCHT1), Anti-CD7 FITC (Clone M-T701), Anti-CD5 PE-Cy7 (Clone L17F12), Anti-CD19 APC (Clone SJ25C1), Anti-CD33 APC (Clone P67.6), Anti-HLADR APC-H7 (Clone L243), Anti-CD184 (CXCR4) PE (Clone ID9), Anti-IgG 2a PE (Clone X-39; all from BD Biosciences, San Jose, CA); Anti-CD34 PerCP (Clone 581), Anti-cCD3 PerCP (Clone SK7; all from Biolegend, San Diego, CA); Anti-CD38 FITC (Clone T16; Beckman Coulter, Pasadena, CA) and Anti-CD133 APC (Clone AC133, Miltenyi Biotec, Cambridge, MA). Patient-specific combinations of 6 or 8 antibodies and Boolean gating scheme were used to identify the blasts for each patient and determine their CXCR4 expression. Matched isotype control was used to determine the upper limit of fluorescent background.

Toxicity

Dose-limiting toxicity (DLT) was defined as any grade IV organ toxicity not due to conditioning or underlying malignancy, attributable to plerixafor from the first dose on day −4 through day +7 after HSCT. Adverse events and toxicities due to plerixafor were assessed using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events, version 4.0.

Routine Evaluation

GVHD was assessed in accordance with published criteria [16]. Daily physical examination and blood testing, including complete blood count and serum chemistries, were obtained. The day of engraftment was defined as the first of 3 measurements on consecutive days of achieving an absolute neutrophil count > 500 cells/μL. *Primary graft failure* was defined as an absolute neutrophil count never meeting or exceeding 500 cells/μL for 3 measurements on consecutive days by day +30 after transplantation.

Statistical Design

The maximum tolerated dose was determined using a conventional phase I study design with cohorts of 3 to 6 patients each. The *maximum tolerated dose* was defined as the dose level immediately below the level at which 2 or more patients out of a cohort of 3 to 6 patients experienced a DLT. If no patient experienced a DLT at dose level 1 and 2, then a total of 6 patients were treated at level 3.

Patients were enrolled in the study between August 2010 and December 2012. All patients received the doses of plerixafor as scheduled. No patient was lost to follow up. The characteristics of patients are summarized using frequencies for categorical variables and mean, median, and range for continuous variables. SAS version 9.2 (SAS Institute, Cary, NC) was used for statistical analysis.

RESULTS

A total of 12 patients were enrolled in the study. Patient characteristics are outlined in Table 1. Of the 12 patients, 8 were in complete remission (CR) and 4 were in morphological relapse at the time of the second transplantation. One of them had blasts in the peripheral blood. Five patients received total body irradiation–based conditioning for the first transplantation. The median interval between the first and the second transplantation was 11 (range, 3 to 24) months. For the second transplantation, 10 donors were matched unrelated and 2 were matched siblings. Two patients did not engraft before they died, both on day +14, because of infection. For the remaining 10 patients, the mean time to neutrophil and platelet engraftment was 22 (range, 13 to 27) days and 34 (range, 17 to 64) days, respectively. The mean time to neutrophil and platelet engraftment for 10 historical controls at our center with the same conditioning regimen without plerixafor, during the same period was 19 (range, 14 to 23) days and 22 (range, 14 to 42) days, respectively. Criteria for primary graft failure was not met in this phase I trial. There were no effects of plerixafor administration on chimerism, which was full in both lymphoid and myeloid compartments after transplantation. The mean CD34$^+$ cell dose was 7.6 $\times$ 10^6 (range, 2.2 to 25.6 $\times$ 10^6) cells/kg. Grade II to IV GVHD was seen in 3 patients and no patient had chronic GVHD.

With a mean duration of follow-up of 332 (range, 14 to 754) days, 4 of the 12 patients (33%) are surviving at the time of this report, 2 (17%) without progression of disease. One of these patients was on dose level 2 and the other on dose level 3. All 4 patients were in CR before transplantation. Four patients died of progression of underlying disease on day +754 (dose level 1), day +295 (dose level 1), day +94 (dose level 2),