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A Phase II, Multicenter, Single-Arm Study to Evaluate the Safety and Efficacy of Deferasirox after Hematopoietic Stem Cell Transplantation in Children with β-Thalassemia Major

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A B S T R A C T

We conducted a prospective, phase II, multicenter, single-arm study to evaluate the efficacy and safety of deferasirox in patients age >2 to <18 years with β-thalassemia major (TM) who underwent hematopoietic stem cell transplantation (HSCT) and had evidence of iron overload (serum ferritin >1000 μg/L; cardiac MRI T2* <20 ms, or liver iron concentration [LIC; by MRI R2] ≥5 mg/g). Patients received deferasirox at an initial dose of 10 mg/kg/day, with up-titration to a maximum of 20 mg/kg/day. The study continued for 52 weeks and included a total of 27 patients (mean age, 9.1 ± 3.8 years; 70.4% male). One patient (3.7%) was lost to follow-up. The majority of patients (n = 20; 74.1%) were able to achieve the intended dose of 20 mg/kg/day. No deaths occurred. A total of 134 adverse events (AEs) were reported in 25 patients (92.6%) during the study. The majority of patients had grade 1 or 2 AEs, with only 8 patients (29.6%) experiencing grade 3 AEs. Only 10 AEs occurring in 4 patients (14.8%) were suspected to be related to deferasirox (ALT/AST increase, n = 4; urinary tract infection, n = 1). The deferasirox dose had to be adjusted or interrupted for 6 AEs occurring in 4 patients (14.8%). A total of 6 serious AEs occurred in 3 patients (11.1%), none of which were suspected to be related to deferasirox. From baseline to week 52, there were decreases in median concentrations of alanine aminotransferase (ALT), from 30.0 to 17.0 IU/L, and aspartate aminotransferase (AST), from 35.5 to 26.0 IU/L. Median serum creatinine and cystatin C concentrations were similar at baseline and week 52. There was a continuous and significant decrease in median serum ferritin level from 1718.0 μg/L at baseline to 845.3 μg/L following 52 weeks of therapy ($P < .001$); 9 patients (33.3%) achieved a level of <500 μg/L. There was also a significant decrease in median LIC (from 8.6 to 4.1 mg/g; $P < .001$) and an increase in median cardiac T2* (from 26.0 to 28.0 ms; $P = .520$) from baseline to week 52. Our findings indicate that deferasirox treatment at doses up to 20 mg/kg/day reduces the iron burden in children with TM post-HSCT, with a manageable safety profile.

INTRODUCTION

Replacement of mutant hematopoietic cells through the use of hematopoietic stem cell transplantation (HSCT) is the sole available curative therapy for patients with transfusion-dependent β-thalassemia major (TM), and is now an established approach to correcting the defective erythropoiesis, particularly when matched sibling donors are available. HSCT is now widely applied, with substantial experience developed in Turkey [1]. Disease-free survival exceeds 80% with HLA-matched sibling donor transplants [2-6]. Moreover, improvements in management of graft-versus-host disease (GVHD) and better means of inducing graft tolerance have encouraged the use of unrelated donors and umbilical cord blood as the hematopoietic stem cell source for patients lacking a matched sibling donor. Clinical guidelines to inform the optimal approach for HSCT in TM are now available as well [6].

It is now established that uncontrolled iron overload secondary to regular transfusions is the primary source of disease morbidity and mortality in children and adults with TM, owing to detrimental effects on growth, development, and organ function, especially with regard to the heart, liver, and endocrine glands [7]. Although HSCT cures the genetic defect in globin synthesis in TM, patients continue to be at risk of the clinical consequences of iron overload acquired in their

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transfusion-dependent years [8]. Progressive liver fibrosis has been reported even years after HSCT in patients with evidence of iron overload [9]. Moreover, several mechanisms have been proposed linking iron to bone marrow toxicity and transplant failure [8]. Accordingly, adequate management of iron overload before HSCT and, more importantly, after HSCT in those with persisting clinically significant iron levels remain essential to ensure that the benefits of such a comprehensive procedure are not masked by the complications of iron overload [8].

Although phlebotomy is feasible in patients with TM post-HSCT, in view of the correction of underlying anemia, evidence in patients with hereditary hemochromatosis indicates that compliance with phlebotomy may vary owing to the inconvenience of frequent clinic visits and the discomfort associated with the procedure [10,11]. In addition, in a small subset of patients, coexisting mild anemia through a heterozygous sibling donor, vascular access, or other contraindications to phlebotomy can limit treatment options. Thus, the use of iron chelation therapy to treat iron overload in patients with TM post-HSCT is a possible alternative approach, although the risk/benefit of drug therapy compared with phlebotomy has not been established in the setting of randomized phase III trials.

The use of the subcutaneous iron chelator deferoxamine has shown efficacy in iron reduction in the post-HSCT setting, although concerns about compliance with parenteral therapy in ex-thalassemic patients remain. The potential neutropenia associated with the use of the oral chelator deferiprone also raises concerns regarding its suitability in a post-HSCT setting [8]. Deferasirox is a once-daily oral iron chelator with established efficacy and safety in patients with secondary iron overload due to transfusions and in patients with non-transfusion-dependent thalassemia [7,12-14]. Data on the use of deferasirox in the post-HSCT setting in patients with TM are limited to a few case series and small clinical trials [15-17], which have shown favorable results regarding the drug's safety profile and ability to manage iron overload in this patient population.

In this multicenter phase II study, our aim was to evaluate the safety and efficacy of deferasirox in a cohort of children with TM who had undergone successful HSCT and continue to show evidence of iron overload, to provide further evidence on its role in managing patients in this setting.

MATERIALS AND METHODS

Patient Eligibility

The study included male and female patients age >2 to <18 years with a confirmed diagnosis of TM. Patients had to have undergone HSCT within a minimum of 6 months and a maximum of 2 years prior to screening, with a washout period of at least 3 months following immunosuppressive or any other nephrotoxic therapy. They also had to have clinically significant iron overload at screening, defined by a serum ferritin level of >1000 μg/L, a cardiac magnetic resonance imaging (MRI) T2* of <20 ms, or a liver iron concentration (LIC) by MRI R2 of ≥5 mg Fe/g dry weight (dw). Exclusion criteria were any contraindication for treatment with deferasirox according to the local prescribing information; history of hypersensitivity to the study drug or excipients; transfusion dependence following HSCT; receipt of phlebotomy for treatment of iron overload after HSCT; receipt of any other iron chelation therapy, including experimental drugs, after HSCT; severe complications of HSCT (eg, acute or chronic GVHD); clinical symptoms of cardiac dysfunction (eg, dyspnea, chest pain, exercise intolerance, edema, ascites, arrhythmia, hypertension); severe concomitant illnesses (eg, cancer or AIDS); significant medical condition or systemic disease interfering with the ability to take part in this study; presence of a surgical or medical condition that might significantly alter the absorption, distribution, metabolism, or excretion of the study drug; active inflammatory diseases that may interfere with the accurate measurement of serum ferritin; significant proteinuria as indicated by a urine protein-to-urine creatinine ratio of >.5 mg/mg in a non-first-void urine sample at screening visits 1 or 2; calculated creatinine clearance ≤60 mL/minute on 2 measurements during screening visits 1 and 2; serum creatinine level exceeding the upper limit of normal (ULN) on 2 measurements during screening visits 1 and 2; alanine aminotransferase (ALT) >3 times the ULN at screening visits 1 or 2; clinical evidence of active hepatitis B virus (HBV; positive hepatitis B surface antigen with negative hepatitis B surface antibody) or HCV (positive HCV antibody and detectable HCV RNA with ALT exceeding the ULN); history of positive HIV serology (determined by enzyme-linked immunosorbent assay); history of clinically relevant ocular and/or auditory toxicity related to iron chelation therapy; known diagnosis of cirrhosis (confirmed by biopsy if available); pregnancy or breastfeeding; illicit drug use and/or alcohol use; history of noncompliance; unwillingness or inability to comply with the study procedures or undergo study assessments, including MRI; concomitant therapy with hydroxyurea, erythropoietin, or butyrates; and involvement in a clinical trial with another compound.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and in compliance with Good Clinical Practice, including the archiving of essential documents. The study protocol was reviewed by an Independent Ethics Committee or Institutional Review Board at each participating center. Informed consent was obtained from each patient or legal guardian in writing before participation. The investigator, or a person designated by the investigator, informed the patient or legal guardian of all pertinent aspects of the trial, including the written information and the approval/favorable opinion by the Independent Ethics Committee/Institutional Review Board.

Study Design and Assessments

This prospective, phase II, multicenter, single-arm study was conducted across 7 treatment centers in Turkey. All included patients received oral deferasirox dispersible tablets at a starting dose of 10 mg/kg/day, with dosage escalation permitted up to a maximum of 20 mg/kg/day. Dosage escalation by 5 mg/kg/day was allowed every 3 months to reach the maximum dose unless the patient was experiencing adverse events (AEs). Dosage adjustments or interruptions according to weight, iron parameters, AEs, and renal and hepatic laboratory measures followed the local prescribing information. Treatment was planned for 52 weeks (end of study) or until the patient's serum ferritin level dropped below 500 μg/L. Patients were withdrawn from the study in the event of death, pregnancy, use of prohibited concomitant medications (ie, chronic systemic corticosteroids [prednisone equivalent of >10 mg/day for >2 weeks] or any investigational drug), withdrawal of consent, loss to follow-up, study drug discontinuation or interruption for more than 6 months because of AEs or abnormal tests, or administrative reasons. A steering committee supervised the study and regularly reviewed safety data. After completion of the study, patients were treated at the physician's discretion according to the local standard of care.

To determine eligibility, patients were evaluated at 2 screening visits and were included in the study if they met the prespecified inclusion and exclusion criteria. Each participating center included a minimum of 3 patients during a 12-month recruitment period. Included patients were followed from baseline through regular visits every 28 days, during which the following were assessed: vital signs, physical examination and body weight, concomitant diseases and medications, AEs (including severity and relationship to study drug), and laboratory evaluations (complete blood count [CBC], blood urea nitrogen [BUN], serum creatinine, cystatin C, proteinuria, serum ferritin, ALT, aspartate aminotransferase [AST], gamma-glutamyl transferase [GGT], and total and direct bilirubin). In addition, vital signs, BUN, serum creatinine, cystatin C, proteinuria, and AEs were assessed each week during the first 4 weeks of the study, and CBC, BUN, serum creatinine, cystatin C, proteinuria, serum ferritin, ALT, AST, GGT, and total and direct bilirubin were assessed every 2 weeks in the first 8 weeks of the study. AEs, BUN, serum creatinine, proteinuria, ALT, and AST were also assessed for a minimum of 5 days after 14 days of dosage escalation. Hepatitis serology (hepatitis A, B, and C viruses), electrocardiography, echocardiography, liver MRI (R2), and cardiac MRI (T2*) were assessed at baseline and week 52. All patients were followed for AEs for 28 days after the last dose of study drug.

Statistical Analysis

The primary objective of the study was to determine the safety of deferasirox in the treatment of iron overload after HSCT in children with TM over a 52-week (12-month) period based on the incidence, type, relationship to study drug, and severity of AEs, including renal, hepatic, biochemical, and hematologic parameters. Secondary objectives included evaluation of the change in serum ferritin level from baseline to week 52, change in other indices of iron overload (cardiac iron and LIC by MRI) from baseline to week 52, and percentage of patients achieving a serum ferritin level <500 μg/L at weeks 28 and 52. Analyses for efficacy (changes in iron overload parameters) were done for all patients who had started the study and had received at least 1 dose (full analysis set). Analyses for safety were

performed in all patients included in the study (safety set). Descriptive statistics were summarized as percentage, mean ± SD, or median (range) as appropriate. The Friedman test and Wilcoxon test were used to test for changes in iron indices over time. The Wilcoxon test was performed to test the significance of pairwise differences using Bonferroni correction to adjust for multiple comparisons. All *P* values were 2-sided, with the level of significance set at <.05.

RESULTS

Patient Characteristics

Out of 39 screened patients, 27 patients (19 [70.4%] males) were included in the study. The mean patient age was 9.1 ± 3.8 years (median, 9.0 years; range, 3.0 to 16.0 years). The age distribution was >2 to ≤6 years, n = 8 (29.6%); >6 to <12 years, n = 12 (44.4%); and ≥12 to <18 years, n = 7 (25.9%). The mean body mass index was 17.0 ± 2.4 kg/m^2 (range, 13.7 to 21.9 kg/m^2). The median age at TM diagnosis was 7.4 months (range, 2.3 to 98.1 months), and the median interval from diagnosis to HSCT was 75.5 months (range, 10.1 to 179.2 months). Twelve patients were excluded, for abnormal laboratory results (n = 10), inability to perform study assessments (n = 1), or administrative reasons (n = 1).

Safety

Overall, 26 patients completed the 52-week study, with only 1 patient (3.7%) dropping owing to loss to follow-up. The majority of patients (n = 20; 74.1%) were able to achieve the intended deferasirox dose of 20 mg/kg/day.

There were no deaths reported during the study. A total of 134 AEs were reported in 25 patients (92.6%) (Table 1). None of the AEs was unexpected, considering the known safety profile of deferasirox. The most commonly reported AEs, occurring in >10% of patients, were anemia (n = 7), ALT increase (n = 7), cough (n = 7), pyrexia (n = 7), AST increase (n = 6), pharyngitis (n = 6), influenza (n = 5), diarrhea (n = 3), and vomiting (n = 3). Anemia and ALT and AST increases occurred within the first month of therapy (median time to occurrence, 28, 28, and 30 days, respectively). The majority of patients had grade 1 or 2 AEs, and only 8 patients (29.6%) had grade 3 AEs (ALT increase, n = 3; AST increase, n = 2; platelet count decrease, n = 1; herpes zoster infection, n = 1; pneumonia, n = 1). Of the 134 AEs reported, only 10 AEs occurring in 4 patients (14.8%) were suspected to be related to deferasirox (ALT or AST increase, n = 4; urinary tract infection, n = 1). The deferasirox dose had to be adjusted or interrupted for 6 AEs occurring in 4 (14.8%) patients (ALT, AST, or hepatic enzyme increase, n = 4; diarrhea, n = 1; vomiting, n = 1), and all AEs were resolved after dosage adjustment or interruption.

A total of 6 serious AEs occurred in 3 patients (11.1%) during the study, none suspected to be related to deferasirox. A 12-year-old male patient experienced an influenza-like illness and also underwent a surgical procedure, both of which necessitated hospitalization. The 2 events occurred within the first 2 months of the study, while the patient was receiving 10 mg/kg/day of deferasirox. A 5-year-old male patient had an hepatitis B virus infection with ALT and AST increases, which necessitated a deferasirox dosage adjustment. A 3-year-old male patient experienced a decreased neutrophil count, which required no subsequent action.

A total of 57 notable AEs reflecting prespecified laboratory changes were reported in the 27 patients (proteinuria, n = 9; ALT increase >3 times the ULN [if baseline was below the ULN] or >3 times baseline [if baseline was at or above the ULN], n = 9; absolute neutrophil count decrease, n = 6; AST increase >5 times the ULN, n = 3; platelet count <100 × 10^9/L, n = 2; serum creatinine increase >33% from baseline [mean of screening visits 1 and 2] and above the ULN at 2 consecutive visits at least 5 days apart, n = 1).

The median ALT level decreased from 30.0 IU/L (range, 9.5 to 116.5 IU/L) at baseline to 17.0 IU/L (range, 9.0 to 205.0 IU/L) at week 52. The median AST level decreased from 35.5 IU/L (range, 17.0 to 66.5 IU/L) at baseline to 26.0 IU/L (range, 18.0 to 78.0 IU/L) at week 52. The median serum creatinine level was similar at baseline (.4 mg/dL; range, .2 to .7 mg/dL) and week 52 (.4 mg/dL; range, .2 to 1.0 mg/dL). The median cystatin C level was also similar at baseline (.8 mg/mL; range, .5 to 1.0 mg/mL) and week 52 (.7 mg/mL; range, .6 to 1.0 mg/mL).

Efficacy

There was a significant decrease in median serum ferritin level, from a baseline of 1718.0 μg/L (range, 873.7 to 2919.0 μg/L; 24 [88.9%] patients had a level ≥1000 μg/L) to 845.3 μg/L (range, 146.2 to 2740.0 μg/L) following 52 weeks of therapy (*P* < .001; Figure 1). The number of patients with a serum ferritin level <1000 μg/L increased from 3 (11.1%) at baseline to 16 (59.3%) at week 52. Two patients (7.7%) achieved a serum ferritin level <500 μg/L after 28 weeks, and 9 patients (33.3%) did so after 52 weeks of therapy. There was also a significant decrease in median LIC from a baseline of 8.6 mg Fe/g dw (range, 2.8 to ≥43.0 mg Fe/g dw; 23 [85.2%] patients had a value of ≥5 mg Fe/g dw) to 4.1 mg Fe/g dw (range, .9 to 12.5 mg Fe/g dw) following 52 weeks of therapy (*P* < .001). The number of patients with a favorable LIC value of <5 mg Fe/g dw increased from 4 of 25 (14.8%) evaluable patients at baseline to 16 of 25 (64.0%) evaluable patients at week 52. Cardiac T2* increased from a median of 26.0 ms (range, 4.5 to 41.0 ms; 2 [7.4%] patients had a value <20 ms) at baseline to 28.0 ms (range, 18.5 to 44.0 ms) at week 52, although the change did not reach statistical significance (*P* = .520). The number of patients with favorable cardiac T2* values of ≥20 ms increased from 25 (92.6%) at baseline to 26 (95.8%) at week 52.

Table 1
Adverse Events*

AE	Number of Patients	Not Suspected to be Drug-Related (n)	Suspected to be Drug-Related (n)
SAEs	3	ALT increase (1) AST increase (1) Neutrophil count decrease (1) Influenza-like illness (1) Surgical and medical procedures (1) Hepatitis B virus infection (1)	
AEs leading to dose adjustment/temporary interruption	6	Diarrhea (1) Vomiting (1)	ALT increase (2) AST increase (1) Hepatic enzyme increase (1)

* Some patients experienced more than one AE.

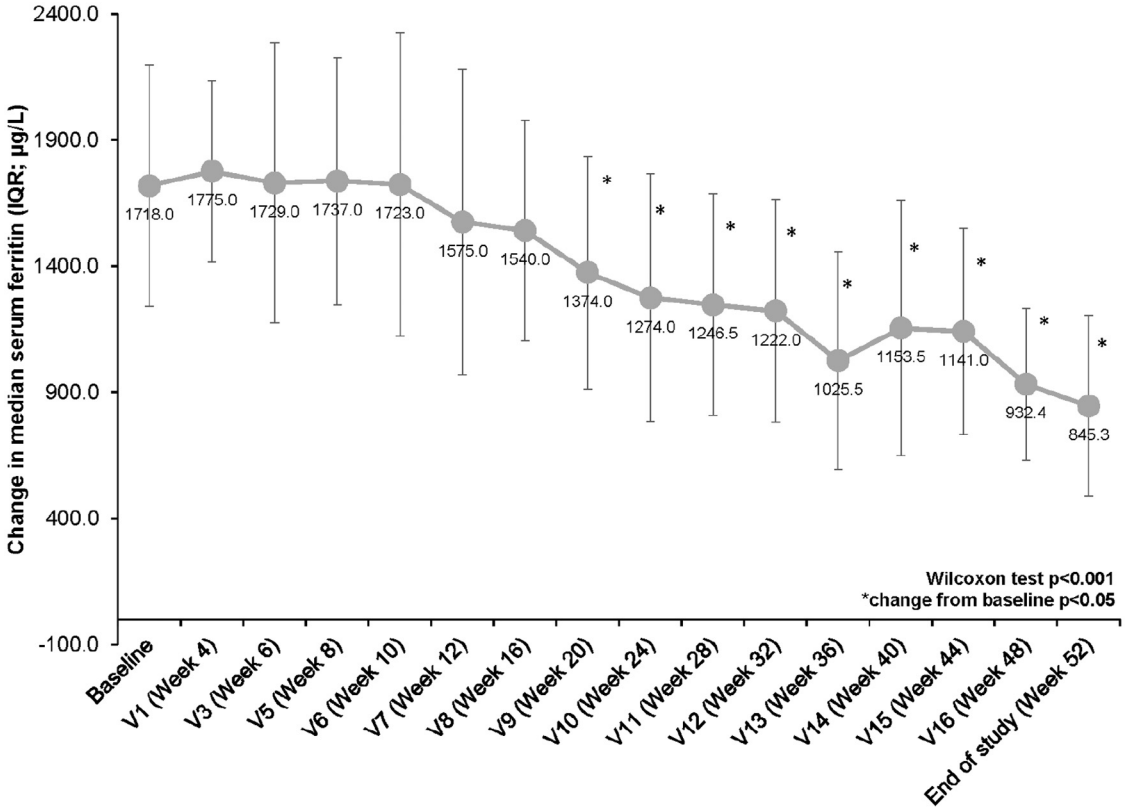

Figure 1. Change in median serum ferritin level during the study. V, visit.

DISCUSSION

Our data establish that deferasirox at doses up to 20 mg/kg/day is safe and effective in managing iron overload in children with TM who had undergone HSCT. The evidence indicating the efficacy and safety of deferasirox in preventing or reducing iron overload in cohorts of thalassemia patients is now well recognized. In transfusion-dependent patients with TM, deferasirox significantly reduces systemic, hepatic, and cardiac iron overload and helps patients transition from high-risk to low-risk thresholds in the respective iron indices, as evidenced from a large clinical trial program involving patients as young as 2 years [12,18-20]. It also helps preserve cardiac function, stabilizes or improves hepatic fibrosis, and can reverse endocrinopathies and osteoporosis [18,19,21-24]. High deferasirox doses of >30 mg/kg/day are often needed in patients with severe iron overload [25]. The safety profile of deferasirox has also been consistent across clinical trials, with AEs being managed clinically by close monitoring of renal and hepatic laboratory values, annual examinations for ocular and auditory toxicity, and dosage adjustments and/or interruptions for renal, hepatic, skin, and gastrointestinal AEs or laboratory abnormalities [12,18-20,25]. In patients age ≥10 years with non-transfusion-dependent thalassemia, deferasirox has also shown consistent efficacy and safety in reducing systemic and hepatic iron overload at dosages up to 20 mg/kg/day, with more recent evidence reporting efficacy and safety of dosage escalation up to 30 mg/kg/day in these patients with severe iron overload [26,27]. Our study adds to this body of evidence by extending efficacy and safety data to patients with TM who have undergone HSCT. This group remains a unique patient population considering its exposure to continuous transfusional iron during the transfusion-dependent phase before HSCT, then transitioning to a non-transfusion-dependent phase with persistent iron overload in the absence of ongoing iron intake.

In the absence of ongoing transfusions, lower doses of deferasirox were used in our study compared with the mean doses commonly used in TM trials, but this was still associated with significant reductions in serum ferritin level. Of note, the proportion of patients with a serum ferritin level <1000 µg/L, which indicates improved survival and reduced morbidity in TM [28], increased by approximately 50% from baseline to the end of the study. Moreover, approximately one third of the patients achieved a serum ferritin level <500 µg/L, making them eligible for discontinuation of iron chelation. Similarly, LIC levels decreased significantly, and the proportion of patients with a favorable LIC value of <5 mg Fe/g dw increased by approximately 50%, with such values associated with reduced risk of morbidity, as established by studies of non-transfusion-dependent thalassemia [29]. Although the increase in cardiac T2* was not statistically significant, it should be noted that the majority of patients had normal values at baseline, with 1 of only 2 patients with a value <20 ms showing a shift to normal by the end of the study, thus alleviating the increased risk of cardiac complications at this threshold [30].

The safety profile observed in our study is consistent with the known AE profile of deferasirox, with the majority of AEs being grade 1 or 2 and none of the serious AEs related to the study drug. More importantly, there was no evidence of hepatotoxicity or nephrotoxicity, according to observed changes in hepatic and renal laboratory measures.

Initial evidence on the role of deferasirox post-HSCT in TM came from small case series. Unal et al. [16] reported 2

patients with TM who received deferasirox after HSCT in whom therapy was well tolerated and led to a decrease in hepatic and cardiac iron overload. In another study, our group reported our experience with 7 patients with TM who received deferasirox to manage iron overload because of poor compliance with phlebotomy and subcutaneous deferoxamine [17]. Serum ferritin levels were significantly decreased, and no adverse effects on AST, ALT, hemoglobin, or donor chimerism were reported. There was a significant rise in serum creatinine levels, but levels remained within normal limits for all patients [17]. More recently, Inati et al. [15] reported results from a prospective, randomized, 1-year clinical trial that compared the efficacy and safety of deferasirox (n = 12) versus phlebotomy (n = 14) for the treatment of iron overload in children with TM following HSCT. The evaluated patients had a similar age range (2 to 18 years; mean age, 12.4 years) as that in our study, and the applied deferasirox regimen also started with a dose of 10 mg/kg/day, with up-titration to a maximum of 20 mg/kg/day, with the maximum dose achieved in 5 patients (41.7%). Although the iron overload threshold for inclusion was lower than that in our study (LIC >3 mg Fe/g dw and serum ferritin >300 μg/L), the authors echoed our findings by showing that the patients receiving deferasirox had a significant decrease in serum ferritin levels (median decrease, 498 μg/L), LIC (mean decrease, 5.8 mg Fe/g dw), and non-transferrin-bound iron after 1 year of therapy. Nonetheless, the change in LIC with deferasirox was significantly greater than that observed with phlebotomy in patients with baseline serum ferritin ≥1000 μg/L (–8.1 versus –3.5 mg Fe/g dw) [15]. Similar to our study, the 1 patient who had cardiac siderosis in the deferasirox arm showed clinically relevant improvement. Adverse effects associated with deferasirox included skin rash, gastrointestinal symptoms, and increased liver enzyme levels, whereas difficulty with venous access and distress during the procedure were associated with phlebotomy [15].

In conclusion, deferasirox treatment at doses of up to 20 mg/kg/day was found to reduces iron burden in children with TM post-HSCT, with a manageable safety profile. These findings suggest a role for pharmacologic therapy to manage clinically significant iron overload in this patient population, especially when alternative measures, such as phlebotomy, are not feasible or convenient. Further research in larger patient samples with different admixtures that also examines the role of adherence in this patient population is warranted.

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Recipient T Cell Exhaustion and Successful Adoptive Transfer of Haploidentical Natural Killer Cells

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Natural killer (NK) cells mediate surveillance for malignancy. In some chemotherapy refractory myeloid leukemia patients, adoptive transfer of NK cells from haploidentical donors can induce remission. We have previously shown that remission induction is linked to NK cell persistence at day +14, but the factors influencing NK cell persistence are unknown. To address this question, patient samples from a phase I trial of National Cancer Institute (NCI) IL-15 in whom either did or did not show NK cell expansion were compared with healthy donor control subjects. Before lymphodepleting chemotherapy, high absolute $CD3^+$ count was predictive of patients who failed to expand their haploidentical NK cell graft. Interestingly, both groups had elevated expression of inhibitory receptors and decreased cytokine production compared with control subjects, suggestive of T cell exhaustion among all patients before haploidentical NK cell infusion. At day +14, however, haploidentical NK cell expanders had persistence of recipient $CD8^+$ T cells with the most exhausted inhibitory phenotype (either PD-1^{high} or dual PD-1^+Tim-3^+) and elevated expression of T-bet and Eomes compared with NK cell nonexpanders and control subjects. This suggested that maintenance of an exhausted T cell state at day +14 permits haploidentical NK cell expansion and supports further efforts to selectively deplete recipient T cells or modulate their dysfunction.

INTRODUCTION

Natural killer (NK) cells are cytotoxic innate lymphoid cells that play a role in cancer surveillance [1]. Haploidentical (haplo-) NK cells can be safely infused after lymphodepleting chemotherapy, and successful in vivo expansion 14 days after infusion (day +14) is associated with remission induction in chemotherapy-refractory acute myeloid leukemia patients [2,3]. The factors that modulate haplo-NK cell expansion are unknown, and understanding the regulators could impact future adoptive haplo-NK cell therapeutics.

We hypothesized that recipient T cells dampen proliferation of adoptively transferred, MHC-mismatched haplo-NK cells and that patients with T cell dysfunction are more likely to have haplo-NK cell persistence. T cell exhaustion is an established state of T cell dysfunction occurring after chronic and continuous antigen stimulation and is well documented in human cancer [4,5]. It is characterized by progressive loss of effector functions, such as loss of cytokine production in response to stimulation; co-expression of multiple inhibitory receptors, including PD-1 and Tim-3; and the altered use of key transcription factors, Eomes and T-bet [4]. To test our hypothesis we used samples collected before and after haplo-NK cell adoptive therapy to investigate recipient T cell exhaustion in patients with or without successful in vivo haplo-NK cell expansion.

METHODS

Clinical Trial, NK Cell Expansion, and Patient Samples

This research was approved by the institutional review board, and all human participants gave written informed consent. NK cell persistence was prospectively determined at day +14 after adoptive transfer using short tandem repeat analysis performed on peripheral blood and correlated with NK cell absolute number [3]. Patients who had donor DNA and NK cells present at this time point had at least 75 donor NK cells/μL and were defined as "haplo-NK cell expanders" and those with no donor DNA and T cells present were defined as "haplo-NK cell nonexpanders." Using this definition we compared peripheral blood samples from 4 haplo-NK cell expanders, 6 haplo-NK cell nonexpanders, and 5 healthy donor control subjects. These patients were enrolled on University of Minnesota protocol (MT2010-10), which is a phase I dose escalation trial of National Cancer Institute (NCI) monomeric IL-15 administered i.v. after lymphodepleting chemotherapy (cyclophosphamide 50 mg/kg and fludarabine 25 mg/m^2) and NK cell infusion. Donor apheresis products were enriched for NK cells through T cell and B cell (CD3/CD19) depletion and then activated overnight in the presence of NCI IL-15 (10 mg/mL). On days +1 through +12 patients received daily i.v. infusions of NCI IL-15.

In Vitro Assays

Samples were thawed and incubated for 12 to 18 hours in RPMI media. For stimulation assays CD3/CD28 beads (Life Technologies, Carlsbad, CA) were added 1:1 to the media during this incubation. Antibodies used were as follows: L/D aqua (ThermoFisher, Carlsbad, CA), CD3-PreCP-Cy5.5, CD8-APC-C7,

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