Ultra Low-Dose IL-2 for GVHD Prophylaxis after Allogeneic Hematopoietic Stem Cell Transplantation Mediates Expansion of Regulatory T Cells without Diminishing Antiviral and Antileukemic Activity

Alana A. Kennedy-Nasser1, Stephanie Ku1, Paul Castillo-Caro1, Yasmin Hazrat1, Meng-Fen Wu2, Hao Liu2, Jos Melenhorst3, A. John Barrett4, Sawa Ito4, Aaron Foster1, Barbara Savoldo1, Eric Yvon1, George Carrum1,3, Carlos A. Ramos1,3, Robert A. Krance1,3, Kathryn Leung1, Helen E. Heslop1,3, Malcolm K. Brenner1,3, and Catherine M. Bollard1,3

Abstract

Purpose: GVHD after allogeneic hematopoietic stem cell transplantation (alloSCT) has been associated with low numbers of circulating CD4+CD25+FoxP3+ regulatory T cells (Tregs). Because Tregs express high levels of the interleukin (IL)-2 receptor, they may selectively expand in vivo in response to doses of IL-2 insufficient to stimulate T effector T-cell populations, thereby preventing GVHD.

Experimental Design: We prospectively evaluated the effects of ultra low-dose (ULD) IL-2 injections on Treg recovery in pediatric patients after alloSCT and compared this recovery with Treg reconstitution post alloSCT in patients without IL-2. Sixteen recipients of related (n = 12) or unrelated (n = 4) donor grafts received ULD IL-2 post hematopoietic stem cell transplantation (HSCT; 100,000–200,000 IU/m2/C2 per week), starting < day 30 and continuing for 6 to 12 weeks.

Results: No grade 3/4 toxicities were associated with ULD IL-2. CD4+CD25+FoxP3+ Tregs increased from a mean of 4.8% (range, 0%–11.0%) pre IL-2 to 11.1% (range, 1.2%–31.1%) following therapy, with the greatest change occurring in the recipients of matched related donor (MRD) transplants. No IL-2 patients developed grade 2–4 acute GVHD (aGVHD), compared with 4 of 33 (12%) of the comparator group who did not receive IL-2. IL-2 recipients retained T cells reactive to viral and leukemia antigens, and in the MRD recipients, only 2 of 13 (15%) of the IL-2 patients developed viral infections versus 63% of the comparator group (P = 0.022).

Conclusions: Hence, ULD IL-2 is well tolerated, expands a Treg population in vivo, and may be associated with a lower incidence of viral infections and GVHD. Clin Cancer Res; 20(8); 2215–25. ©2014 AACR.

Introduction

In patients with leukemia, those who develop GVHD after allogeneic hematopoietic stem cell transplantation (H SCT) may have a lower incidence of leukemia relapse than patients not developing GVHD, a finding that suggests that residual leukemic cells present after the preparatory regimens can be eliminated by donor cellular mechanisms. Unfortunately, this graft-versus-leukemia (GvL) effect is often associated with GVHD, a leading cause of morbidity and mortality occurring in 30% to 60% of patients, with mortality reaching 50%. A hallmark of acute GVHD (aGVHD) is the activation and expansion of alloreactive T cells (1). Although alloreactive T cells present in a SCT graft are responsible for GVHD, removal of T cells entirely may compromise engraftment by increasing the risk of rejection by the residual recipient immune system, increasing infection risks, and reducing GvL (2). Therefore, a balance must be maintained between the benefits of donor T cells (GvL, infection control) and their risks (GVHD). Accumulating evidence suggests that lower levels of circulating regulatory T cells (Tregs) following allogeneic hematopoietic SCT (alloSCT) in humans are associated with a higher incidence of GVHD. A causal link for this association is suggested by murine models that show infusion of donor Tregs at the time of transplant, or shortly thereafter, prevents GVHD while maintaining the GvL effect. Several groups are, therefore, evaluating the adoptive
Allogeneic hematopoietic stem cell transplantation (alloSCT) is recognized as the treatment of choice for several hematologic malignancies. However, GVHD is one of the most adverse consequences of the procedure. GVHD occurs in approximately 30% to 70% of patients undergoing alloSCT, increasing morbidity and mortality, as well as the cost of care. Standard prophylactic therapies are often ineffective and lead to significant complications including organ damage and impaired immune recovery with resultant life-threatening infections and an increased risk of relapse. Thus, more effective and less-toxic therapies are urgently needed to prevent GVHD, while still preserving the virus specific and the graft versus tumor effect post SCT. We have directly translated a novel strategy to prevent GVHD by expanding donor-derived Tregs in vivo using ultra low-dose interleukin (IL)-2 in patients after alloSCT. Hence, this innovative approach has the potential to prevent GVHD without increasing morbidity and mortality for virus infections and relapse.

Translational Relevance

Allogeneic hematopoietic stem cell transplantation (alloSCT) is recognized as the treatment of choice for several hematologic malignancies. However, GVHD is one of the major adverse consequences of the procedure. GVHD occurs in approximately 30% to 70% of patients undergoing alloSCT, increasing morbidity and mortality, as well as the cost of care. Standard prophylactic therapies are often ineffective and lead to significant complications including organ damage and impaired immune recovery with resultant life-threatening infections and an increased risk of relapse. Thus, more effective and less-toxic therapies are urgently needed to prevent GVHD, while still preserving the virus specific and the graft versus tumor effect post SCT. We have directly translated a novel strategy to prevent GVHD by expanding donor-derived Tregs in vivo using ultra low-dose interleukin (IL)-2 in patients after alloSCT. Hence, this innovative approach has the potential to prevent GVHD without increasing morbidity and mortality for virus infections and relapse.

Materials and Methods

Patients

Subjects under 70 years of age who met standard criteria to receive an alloSCT from a matched related donor (MRD) or unrelated donor were eligible for this clinical trial. Individuals were eligible for treatment if their Karnofsky/Lanksy score was ≥50. Patients with severe intercurrent infection, severe organ dysfunction, or GVHD grade 2 were ineligible. All protocols were approved by the Baylor College of Medicine institutional review board (IRB). The study was also registered with Clinical trials.gov NCT00539695.

Transplant conditioning regimens

The IL-2–treated patients (n = 16) received standardized total body irradiation (TBI)-based conditioning regimens for patients undergoing transplant for malignant disease as previously published (8, 9). Patients receiving alternative donor grafts also received alemtuzumab. As additional GVHD prophylaxis, all patients received targeted doses of calcineurin inhibitor FK506 (tacrolimus) with mini-MTX (5 mg/m² on days +1, 3, 6, and 11) following our transplant standard operating procedures (SOP).

Administration of ULD IL-2

This was a phase II study to evaluate safety and efficacy of low-dose IL-2 in the prevention of severe (grade 3 or 4) aGVHD in alloSCT recipients. We used a fixed ULD of IL-2. Between day 7 and 30 (median, 28 days) post alloSCT, recombinant human IL-2 (Proleukin; Novartis) was started and continued for 12 weeks. Patients received 1 to 2 × 10⁵ IU/m²/dose subcutaneously three times per week (generally Monday/Wednesday/Friday) for the first 6 weeks. If this dose was tolerated, patients could continue to receive IL-2 at the same dose for an additional 6 weeks. Treg numbers were measured and GVHD assessed every week while on IL-2 (generally before the Wednesday dose), and monthly thereafter, for 1 year. Patients were evaluated monthly for 1 year for acute or chronic GVHD (cGVHD). If a patient developed greater than grade 2 GVHD while on IL-2, therapy was halted and patients were treated using standard institutional guidelines. Patients were routinely monitored for viral infections according to our institutional SOPs. All patients were regularly monitored for disease status according to our institutional SOP, including (i) morphologic analysis of bone marrow samples to assess "conventional" remission status and (ii) minimal residual disease analyses of marrow and peripheral blood samples using chromosomal markers. As an additional measure of disease recurrence, recipients were regularly monitored for (iii) the level of donor chimerism in myeloid and lymphoid cells in the blood and marrow, as per standard institutional protocols.

Monitoring patients who did not receive IL-2

To determine the pattern of recovery of Tregs and the rates of GVHD in recipients of alloSCTs who did not receive IL-2, we studied a contemporaneous group of recipients on our IRB-approved immune monitoring protocols, receiving the same conditioning regimens (and the same GVHD prophylaxis and supportive care elements), as the IL-2 study patients but who were either not approached or declined participation in the IL-2 study. Kinetics of Treg recovery and other immune reconstitution studies were determined at time points post alloSCT comparable with IL-2 recipients. An intention-to-treat analysis was performed, and only patients who would have otherwise been eligible for the IL-2 study by day 28 were included in the data collection, which was the day when the majority of the study patients

Published OnlineFirst February 26, 2014; DOI: 10.1158/1078-0432.CCR-13-3205
started IL-2. Blood was drawn monthly after SCT for 6 months, then every 3 months until 12 months. Peripheral blood mononuclear cells (PBMC) were frozen and the samples were batched. These patients were also evaluated for GVHD monthly for 3 months and then every 3 months until 12 months, similar to patients who received IL-2.

**Immunization of both the related donor and recipient before transplantation to produce high and sustained levels of antibody protection**

We immunized a subset of donor and recipient pairs with tetanus toxoid 5-week pretransplant because such dual immunization consistently produces high level and sustained tetanus toxoid antibody responses when such antigen-primed donor B cells are transferred into an antigen-containing environment. Related donors received tetanus toxoid 1 to 10 days before stem cell harvest, while selected transplant recipients were given the toxoid 7 to 10 days before SCT. Recipients received a tetanus toxoid booster 3 months post alloSCT. The impact of IL-2 on humoral immune responses was evaluated by measuring tetanus antibody immunoglobulin G (IgG) on serum samples obtained pre- and post-IL-2 infusion using a quantitative ELISA. A patient with a postvaccination to prevaccination ratio of less than 1.5 was considered a nonresponder, a ratio of 1.5 to less than 3.0, a limited responder, and a ratio of 3.0 or greater, a normal responder.

**Evaluating immune reconstitution of T cells after alloSCT**

Flow cytometric analyses were used to quantify T cells (CD3, CD4, and CD8) and T-cell subsets, including naïve and memory T cells, and natural killer (NK) cells. Specifically, we evaluated the percentage of CD4 and CD8 T cells, NK cells (CD56+CD3-), naïve (CD45RA+CCR7+), central-memory (CD45RO-CCR7+), effector-memory (CD45RO+CCR7-), T cells, and terminal-differentiated effector (TEMRA; CD45RO-CCR7-) T cells.

**Evaluation of Tregs post SCT**

PBMCs were isolated from patients using Ficoll–Hypaque density-gradient centrifugation and frozen in liquid nitrogen, enabling batching of tests to minimize interassay variation. To determine Treg phenotype, we used the intracellular FoxP3 Treg kit (eBioscience) and CD4 and CD25 fluorescent antibodies (BD Biosciences). All analyses used a FACSCalibur flow cytometer (BD Biosciences) and CellQuest Pro software. Absolute lymphocyte counts were measured to determine the absolute frequency of Tregs.

**Evaluation of Treg function**

Treg-induced suppression was demonstrated by effects on thymidine uptake in mixed lymphocyte reactions. PBMCs were separated into CD4+CD25- and CD4+CD25+ fractions using fluorescent cell sorting or magnetic bead separation. The CD4+CD25- fraction (responder cells) was plated into a 96-well plate coated with anti-CD3 (OKT3). The CD4+CD25+ Treg fractions were added at a 1:1 ratio and incubated at 37°C. On day 3, each well was pulsed with 1 μCi of 3H-thymidine. After 18 hours of additional incubation, plates were harvested and processed for counting.

**Evaluating antiviral and antileukemia activity**

Of note, 20 to 40 mL of blood was taken at monthly intervals post alloSCT to measure the frequencies of virus-specific T cells. T-cell frequencies were measured in ELISPOT assays as previously described (10), using a cocktail of peptides (pepmixes) specific for viral antigens: CMV-pp65 and CMV-IE1, ad-hexon and ad-penton, and the Epstein-Barr virus (EBV) antigens BZLF1, EBNA1, EBNA3A, EBNA3B, EBNA3C, LMP1, and LMP2. In patients with myeloid and lymphoid malignancies, we measured the precursor frequency of cytotoxic T lymphocytes specific for the leukaemia/lymphoma–associated antigens MAGE A4, survivin, PRAME, and WT-1 using ELISPOT assays using pepmix pulsed PBMC (11, 12).

**Statistical considerations**

We used the Bryant and Day two-stage design incorporating toxicity and efficacy considerations to calculate sample size (13). We considered the treatment not effective if Treg expansion occurred in less than 50% of subjects. A treatment-related serious adverse event (SAE) was defined as any grade 3 or 4 toxicity considered related to IL-2 as indicated by the criteria listed in the National Cancer Institute (NCI) Common Toxicity Criteria version 3.0 (http://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev3.pdf). Percentages of Treg expansion, IL-2–related SAE events, and aGVHD were summarized descriptively. Summary statistics, including mean and medians, were calculated for the subsets of T cells. Immune reconstitution of Tregs in alloSCT patients was compared with patients who received the same conditioning but did not receive IL-2. The change in Treg and general immune reconstitution pre and 1-month post transplant was compared between the patients who received IL-2 and those who did not. We analyzed the difference in changes by a two-sample t test. aGVHD and viral infection rates were compared between groups using the Fisher exact test. T cell–specific reactivity to at least one leukemia-associated antigen was compared between groups using the Wilcoxon rank-sum test. Survival data were analyzed by the Kaplan–Meier method and the comparisons between groups were performed using the log-rank test. Overall survival was calculated from the time of transplant to death from any cause or censored at last follow-up. Relapse-free survival was calculated from the time of transplant to the date of relapse, death, or last follow-up, whichever occurred first.

**Results**

**Patient characteristics and GVHD**

We treated 16 patients with ULD IL-2 for 6 to 12 weeks after HSCT [12 MRD and 4 matched unrelated donor (MUD) SCT; Table 1]. Follow-up ranged from 5 to 40 months. Median age was 13 years and all patients had underlying
hematologic malignancies (acute lymphoblastic leukemia (ALL; n = 14), acute myelogenous leukemia (AML; n = 1), and non-Hodgkin lymphoma (NHL; n = 1). Adverse effects after IL-2 administration were all grade 1 only, and included muscle aches, arthralgias, fatigue, nausea, and decreased appetite. However, although there were no significant adverse events, 7 patients elected not to complete the full 12 weeks of therapy because of aversion to the injections given three times a week, a potential limitation of a pediatric-only study. To evaluate the tempo of immune reconstitution of Tregs in alloSCT patients who did not receive IL-2, we collected immune reconstitution and clinical data at monthly intervals after transplant from 16 recipients of related donor grafts (Supplementary Table S1) and 18 recipients of unrelated donor grafts (Supplementary Table S2). The median age of the MRD cohort was 8.5 years with all but 2 patients (myelofibrosis and lymphoma) receiving their transplant for ALL. In the MUD cohort, the median age was 9 years with all but 2 patients (CML and severe chronic active EBV infection syndrome [SCAEBV]) receiving their transplant for ALL. These patients received the same transplant conditioning regimens to the IL-2 study patients and only patients who would have been eligible to receive IL-2 by day 28 after HSCT were included in the analysis.

To determine whether patients with IL-2 developed severe aGVHD, all patients were followed for signs and symptoms of GVHD in both the IL-2 and control cohorts. No episodes of grade 2 to 4 aGVHD occurred in any recipient who received ULD IL-2, with only 3 of 16 (19%) patients developing grade 1 aGVHD: 2 recipients of MRD grafts (2 of 12; 17%) and 1 of a MUD graft (1 of 4; 25%). In contrast, of the 16 recipients of MRD grafts who did not receive IL-2, 6 (38%) developed grade 1 GVHD and 2 (13%) developed grade 2 to 4 GVHD. Furthermore, of the 18 recipients of MUD grafts who did not receive IL-2, 5 patients (28%) developed GVHD with 3 patients developing grade 1, 1 patient grade 2, and 1 patient grade 4 (Fig. 1A and B; Table 1 and Supplementary Tables S1 and S2). Although the study was not powered to evaluate the effects of ULD IL-2 on cGVHD, only 1 patient (MRD recipient) who received ULD IL-2 developed with cGVHD (skin). In contrast, of the patients who did not receive IL-2, 4 MRD recipients developed cGVHD (skin, hepatic, skin and oral, and skin and liver) and 2 MUD recipients developed cGVHD (both skin).

**Administration of ULD IL-2 rapidly increased Tregs after alloSCT**

Increases in the frequency of CD4⁺CD25⁺FoxP3⁺ Tregs from baseline were seen in all recipients who received ULD IL-2 after starting IL-2 (Fig. 2A). Flow cytometric analysis showed that their percentage of CD4⁺CD25⁺FoxP3⁺ Tregs increased from a mean of 4.8% (range, 0.0%–11.0%) pre-IL-2 to 11.1% (range, 1.2%–31.1%) following 6 weeks of IL-2 therapy (Fig. 2A). Tregs in the peripheral blood of the IL-2 recipients increased 1 week after starting IL-2 (to 10.5%) compared with recipients of MRD grafts who did not receive IL-2 (mean, 5.8%; P = 0.031; Fig. 2B and C). In contrast, in vivo expansion of Tregs was absent in the unrelated graft recipients given ULD IL-2, whose preparative regimen had included serotherapy with the T CD52 antibody alemtuzumab. All these patients had minimal detectable Treg numbers post-IL-2 (absolute Treg numbers ranged from 0–1 × 10³ cells/µL). However, even in these patients, there was a trend to higher Treg in the IL-2 patients compared with the control group at 3 months post SCT (2 months post IL-2; Fig. 2D and E). Combining the data for both groups, the Treg/Tcon ratio

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<th>Table 1. Recipients of ULD IL-2</th>
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Abbreviation: CR, complete remission.
ratio increased to a median of 1.3-times the baseline value at 3 months (range, 0–29).

**Administration of ULD IL-2 rapidly restored functional Tregs after alloSCT**

The Tregs obtained from the recipients of MRD grafts receiving IL-2 suppressed alloreactive responses in vitro because ³H-thymidine incorporation by activated T cells (CD4⁺ CD25⁺) stimulated with allogeneic OKT3 blasts was markedly suppressed by the addition of patient Tregs (CD4⁺ CD25hi Tregs, Fig. 2F and G). To determine whether the Tregs produced after IL-2 administration were of donor origin, PBMCs were analyzed for donor chimerism by FISH for sex chromosomes in sex-mismatched transplants and short tandem repeats measurement in same sex pairs. All patients were 100% donor during ULD IL-2 administration, suggesting that these expanded cells were of donor origin (data not shown).

**No effect of ULD IL-2 on NK or CD8⁺ T cells’ immune reconstitution post SCT**

Because recovery of CD8⁺ T cells, CD56⁺/CD3⁻ NK cells, and CD56⁻/CD3⁺ NK-like T cells may play a critical role in engraftment and defense against relapse, we compared immune reconstitution in the treatment and control groups. In the recipients of MUD grafts, no significant differences in recovery of any cell subset was seen between treatment and control groups likely related to recent serotherapy use (data not shown). There was a significant increase of NK cells in the IL-2 treatment cohort at 1 week after starting therapy (Supplementary Fig. S1A) and a concomitant fall in CD8⁺ T cells (Supplementary Fig. S1B). These differences had disappeared by day 35. No significant differences were observed in absolute numbers or percentages of NK-like T cells (CD3⁻/56⁻) CD4⁺ CD3⁺ T cells, or CD19⁺ B cells (Supplementary Fig. S1C–S1E).

**Administration of ULD IL-2 can blunt humoral but not cellular immune responses**

To determine whether the rise in Tregs in patients who received ULD IL-2 was associated with blunted antibody-mediated antibacterial responses after alloSCT, we immunized related MRD and recipient pairs with tetanus toxoid 1 week pretransplant. As shown in Fig. 3A and B, we enrolled 3 patients in the treatment group and 4 controls. Patients who received IL-2 also received a 3-month tetanus booster. Figure 3B shows blunting of the tetanus antibody response in all 3 IL-2–treated patients; in contrast, 3 control patients responded well to the vaccine and the single nonresponder relapsed shortly after immunization.

**ULD IL-2 therapy does not impair virus-specific cell-mediated immunity and viral infection rates were low**

We next evaluated the cell-mediated immune recovery to viral antigens: adenovirus hexon and penton (Fig. 4A), BK virus VP1 and large T antigen (Fig. 4B), CMV IE1 and pp65 (Fig. 4C), and EBV antigens BZLF1, EBNA1, EBNA3A, EBNA3B, and EBNA3C (Fig. 4D). We detected specific reactivity against all viral antigens in all recipients of MRD grafts early after SCT, indicating equal recovery of virus-specific cellular immunity in the treatment and control groups (Fig. 4A–D). We next evaluated virus infection rates, defined as culture positive infection or PCR positivity in plasma or urine requiring treatment. In the MRD recipients, only 2 of 13 (15%) of the IL-2 patients developed viral infections (RSV and parainfluenza) as compared with 10 of 16 (63%) of the untreated patients (HHV6, VZV, BK virus, EBV, and CMV). Moreover, in the recipients of MUD grafts, 17 of 18 (95%) of patients without IL-2 developed viral infections versus 2 of 6 (33%) of the IL-2–treated group (P = 0.007), suggesting that IL-2 therapy does not increase (and may reduce) the risk of developing virus infections (Fig. 4E and F).

**Comparable GvL effects in treatment and control groups**

To detect tumor-specific T cells, the PBMCs were stained with HLA A2-restricted tetramers (Fig. 5A and B). T cell-specific reactivity to at least one leukemia-associated antigen (MAGE, PRAME, survivin, or WT-1) was detectable in IFN-γ ELISPOT assay in recipients of MRD grafts in both
treatment and control groups (Fig. 5C and D). No significant differences in IFN-γ spot-forming cells were observed between the treatment ($n = 9$) and control ($n = 6$) patients following stimulation with MAGE ($P = 0.862$), PRAME ($P = 0.563$), survivin ($P = 0.907$), or WT1 ($P = 0.862$), and there was no significant difference in overall survival (Fig. 5E) or...
relapse-free survival (Fig. 5F) between IL-2 treatment group and non-IL-2 group, which received either MRD or MUD SCT, respectively.

Discussion

The purpose of this study was to administer ULD IL-2 to patients post alloSCT to evaluate the effect on Treg immune reconstitution, with the ultimate goal of preventing moderate to severe (grades 2 to 4) aGVHD without increasing the risk for viral infection or relapse. Patients receiving ULD IL-2 showed a marked expansion of functionally suppressive Tregs in vivo compared with controls, without significant GVHD. We also noted a lower infection rate in the treatment group and comparable relapse rates. These results suggest that increasing Tregs using ULD IL-2 may be beneficial for outcome after HSCT.

Recombinant high-dose human IL-2 has been used therapeutically for many years, most often in adult cancers and HIV. To reduce toxicity while retaining beneficial immunomodulatory effects, more recent clinical trials conducted in patients with HIV or solid tumors (e.g., renal cell carcinoma or melanoma) have used IL-2 in doses 1 to 2 log lower than the original studies (14–16). Low-dose IL-2 (usually between $6 \times 10^5$ IU/m$^2$ to $9 \times 10^6$ IU/dose) has also been given following SCT to augment antitumor immunity. Low-dose regimens are safe and well tolerated (17–21), and although not measurably enhancing GvL, there was no evidence that the risk of GVHD was increased, and some evidence to suggest that the incidence and severity of this complication was, in fact, reduced (18).

The investigators also observed that patients receiving low-dose IL-2 also had expanded CD4$^+$ CD25$^+$ FoxP3$^+$ populations. This possible link between IL-2–augmented CD4$^+$ CD25$^+$ FoxP3$^+$ T cells and inhibition of GVHD is supported by a recent study showing that low-dose IL-2 infusions in adult patients with steroid refractory cGVHD increased circulating Tregs and reduced the severity of their cGVHD (22, 23). We, therefore, developed a study that exploited the high expression of the IL-2-CD25 receptor on the Treg population to prospectively administer ULD IL-2 (1 $\times 10^5$ IU/m$^2$) to pediatric patients after alloSCT and measure the effects on Treg and other immune reconstitution, and assess the effects of this regimen on GVHD, infection, and relapse.

In pediatric recipients of MRD transplants, the administration of ULD IL-2 was associated with a rapid (1–2 weeks) doubling in the number of circulating Tregs, indicating early amplification of this population. In contrast, IL-2–mediated effects on Treg numbers were both delayed and reduced in the recipients of MUD grafts, a patient group who had received the CD52 antibody alemtuzumab as part of their conditioning regimen. Alemtuzumab may persist at lymphodepleting levels beyond 4 weeks in SCT recipients after HSCT, which likely reduces the pool of preexisting IL-2 responsive Treg and depletes any newly emergent cells with this functional phenotype. Only by 3 months posttransplant did we observe an increase in Treg in the MUD ULD IL-2 group, and even then, the difference from controls was a trend without reaching conventional statistical significance. Nonetheless, in the MUD groups as in the MRD patients, the incidence of GVHD and of viral infections was lower in individuals receiving IL-2 than in untreated controls, so that even in the presence of alemtuzumab, sufficient Tregs may be present to suppress GVHD while preserving virus-specific cell-mediated immunity. These findings suggest that

Figure 3. In vivo Treg expansion may negatively affect humoral immunity. Donor–recipient pairs were immunized with tetanus toxoid 1-week pre-SCT and received a booster at 3 months post-SCT. Compared with patients who did not receive ULD IL-2 (A), the patients who received ULD IL-2 (B) showed blunting of tetanus antibody response postbooster.
shorter-acting immunosuppression, for example with horse ATG, might allow superior Treg recovery in this subset of ULD IL-2–treated patients.

Although this study was not designed to show a causal connection between the enhancement of Treg and the reduction of GVHD, growing evidence suggests a pivotal role for Tregs in the modulation of GVHD. Donors with higher natural frequencies of Tregs produce less GVHD in their recipients (25), while increasing the proportion of Tregs in the SCT reduces GVHD in mismatched SCT recipients (26). Moreover, Tregs infused post SCT can prevent GVHD in both murine models and in preliminary studies in man (27, 28). Hence, our findings add further weight to a central role of Tregs in the control of GVHD.

Current immune-suppressive agents target both regulatory and effector T cells, thereby potentially affecting both alloreactive and antiviral/bacterial T-cell responses. The consequence is an increased risk of life-threatening infection that may offset the benefit from reduced GVHD. Murine models had suggested that increasing Tregs in recipients after alloSCT may substantially decrease the risk of GVHD without affecting responses to infectious agents (or tumors; ref. 29). In this study, we saw that patients treated with ULD IL-2 preserved their virus-specific immune responses measured in vitro, and had low rates of viral, fungal, and bacterial infections in vivo. Other studies, however, had shown that administration of IL-2 reduced the humoral immune response to vaccine antigens (30). Our studies confirmed this paradoxical activity, with preservation of antiviral activity in vitro and in vivo (judged by a low rate of viral infections) even in patients in whom the humoral immune response to tetanus toxoid antigen was diminished. This disparity suggests that Th2-associated antibody responses may be more susceptible to control by IL-2–induced human Treg than antiviral responses that consist predominantly of Th1 cytotoxic T-cell responses, but the mechanism for this differential effect remains poorly explained (31, 32). While Th1 cellular immune reconstitution is critical for protection against viruses, Th2-associated humoral immunity may be critical for protection against bacteria including pneumococcus, and it is possible that a larger series of patients would indeed show an increased risk for such infections, which may then require either antibiotic..
prophylaxis or administration of routine intravenous immunoglobulin.

Detection of WT-1 and PRAME-specific T cells in the peripheral blood of patients with lymphoid and myeloid malignancies after alloSCT strongly correlates with a low relapse rate after transplant, likely because these antigens are overexpressed by malignant target cells (33–35). Because IL-2 has a biphasic dose–response curve, in which low doses favor a Treg response and a high dose favors Th1 activation, there is a concern that the Treg induced by ULD IL-2 will increase Tregs that also suppress the antitumor response, leading to increased relapse rates. Our study shows that ULD IL-2 can increase Tregs without reducing tumor-specific immunity or increasing the relapse rate. This difference in effects on alloreactivity compared with the effects on antitumor (and antiviral) immunity may reflect the differences in the cell of origin of each response. The virus-specific populations are derived from memory T-cell populations, as are WT-1- and PRAME-specific T cells, which circulate in small numbers even in healthy individuals (36–38). In contrast, most alloreactive T cells able to stimulate GVHD may reside in the naïve population, which may have greater sensitivity to inhibition by Treg (39, 32).

Our findings highlight the potential of ULD IL-2 as an effective form of GVHD prophylaxis that may preserve immune responses to infectious agents and malignancy and the approach seems well suited to further study.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.
Authors' Contributions
Conception and design: A.A. Kennedy-Nasser, H.E. Heslop, M.K. Brenner, C.M. Bollard
Development of methodology: A.A. Kennedy-Nasser, S. Ito, B. Savoldo, C.M. Bollard
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.A. Kennedy-Nasser, S. Ku, Y. Hazzat, J. Melenhorst, A. Foster, E. Yvon, G. Carnum, C.A. Ramos, R.A. Krance, K. Leung, C.M. Bollard
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.A. Kennedy-Nasser, A.J. Barrett, K. Leung, C.M. Bollard
Study supervision: A.A. Kennedy-Nasser, R.A. Krance, K. Leung, C.M. Bollard
Sample processing, chart review and data analysis: P. Castillo-Caro

Acknowledgments
The authors thank A. Durrett for expert technical assistance and Munu Bilgi for research coordination.

Grant Support
This work was supported by NIH grant 1 R21 CA149967. This clinical trial was supported in part by the Clinical Research Center at Texas Children's Hospital, The Methodist Hospital and the Dan L. Duncan Institute for Clinical and Translational Research at Baylor College of Medicine. We also appreciate the support of shared resources by Dan L. Duncan Cancer Center support grant P30CA125123.

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Received December 5, 2013; revised January 8, 2014; accepted February 2, 2014; published OnlineFirst February 26, 2014.

References
7. Malek TR, Bayer AL. Tolerance, not immunity, crucially depends on IL-7.

18. Malek TR, Bayer AL. Tolerance, not immunity, crucially depends on IL-7.


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