Brief Articles

Selectively T Cell-Depleted Allografts from HLA-Matched Sibling Donors Followed by Low-Dose Posttransplantation Immunosuppression to Improve Transplantation Outcome in Patients with Hematologic Malignancies

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We evaluated a photodepletion technique to selectively deplete host-reacting T cells from human leukocyte antigen (HLA)-matched sibling stem cell transplantations with the goal of reducing posttransplantation immunosuppression to improve antimalignancy effects postallografting. Donor lymphocytes were stimulated with irradiated expanded recipient T lymphocytes in an ex vivo mixed lymphocyte reaction. Alloactivated T cells preferentially retaining the photosensitizer 4,5-dibromorhodamine 123 (TH9402) were eliminated by exposure to visible light. Twenty-four patients with hematologic malignancies (16 high risk) conditioned with fludarabine, cyclophosphamide, and total-body irradiation received a CD34-selected stem cell allograft from an HLA-matched sibling along with 5 × 10^6/kg selectively depleted donor T cells. Low-dose cyclosporine was used for posttransplantation immunosuppression. Eleven patients survived at a median of 30 months. Probabilities (± SEM) for overall and disease-free survival are 39% ± 12% and 30% ± 12%, respectively, whereas grade III-IV acute graft-versus-host disease (aGVHD) was 13% ± 7%. Six patients relapsed, with a relapse probability of 27% ± 10%. These results suggest that selectively photodepleted allografts in matched sibling transplantations followed by low-dose immunosuppression may protect against severe aGVHD but is associated with delayed immune recovery.

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INTRODUCTION

A persisting limitation of allogeneic stem cell transplantation (SCT) is the close association between the beneficial graft-versus-leukemia (GVL) effect and life-threatening graft-versus-host disease (GVHD), both mediated by alloreacting donor T lymphocytes. Transplant outcomes can be improved by preventing GVHD by posttransplantation immunosuppression or T cell depletion of the allograft. However, both maneuvers restrict the full potential of the GVL effect to prevent relapse of the malignancy, which remains 1 of the major limitations to the improvement of SCT outcome [1]. One strategy to prevent GVHD while conserving the GVL effect is to selectively deplete the T lymphocytes that cause GVHD before SCT [2]. Preclinical experiments demonstrate that if donor lymphocytes alloactivated by lymphocytes from the patient are eliminated, myeloid leukemia-specific lymphocytes remain that may mediate GVL [3-5]. This

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approach requires the alloactivation of donor T cells by leukemia-free patient-derived antigen-presenting cells (APCs). Alloactivated donor lymphocytes can then be targeted for removal by antibodies to activation markers, dye-dilution, apoptosis induction, or photodepletion (PD) [2]. Selective depletion (SD) techniques have been evaluated in several clinical SCT trials [6-8]. In haploidentical transplantations, SD donor lymphocyte infusions contributed to posttransplantation immune reconstitution with reduced rates of infection and lowered nonrelapse mortality (NRM).

We previously used a CD25 immunotoxin to selectively deplete allografts to reduce posttransplantation immunosuppression and enhance GVL effects [7]. With low-dose cyclosporine as posttransplantation immunosuppression, there was a 12% probability of grade III–IV GVHD and relapse rates of 56% in 16 elderly patients with advanced hematologic malignancies. Acute GVHD was associated with low donor regulatory T cell numbers and poor depletion efficiency. To avoid potential disadvantages of the CD25 activation marker as a target for allodepletion, we chose to use a PD technique, previously validated in MHC-mismatched donor-recipient pairs in mice and humans [9,10]. In this approach, alloactivated donor T cells are eliminated after light exposure based on their preferential retention of the photosensitizer 4,5-dibromorhodamine 123 (TH9402; Kiadis Pharma, The Netherlands). We established a clinical-grade, good manufacturing practice-based SD approach that depleted alloreactive T cells from mixed lymphocyte reactions (MLRs) in mismatched and matched pairs without compromising third-party alloreactivity [11]. Here we present a clinical trial in 24 patients with hematologic malignancies receiving a myeloablative preparative regimen followed by a CD34-cell selected transplant together with 5 × 107/kg PD donor T cells on day 0 from an HLA identical sibling donor. Low-dose cyclosporine (CSA) was used as sole GVHD prophylaxis, but was eliminated in a stepwise fashion in successive cohorts. Results suggest that PD can decrease acute GVHD (aGVHD) while maintaining GVL.

**PATIENTS AND METHODS**

Patients

Patients with hematologic malignancies, including T-lymphocytic malignancies, between the ages of 17 and 75 years, and with an HLA-identical sibling donor were eligible for this study. Between July 2007 and October 2009, 24 patients underwent a PD SCT from an HLA-identical sibling on the National Heart, Lung and Blood Institute institutional review board approved protocol (07-H-0136; clinical trials registration number NCT00467961, FDA IND #13305), and donors provided written informed consent before enrolling in the transplantation protocol.

**Generating Alloactivated Donor Lymphocytes**

The techniques used to produce the PD product were previously reported [11]. Briefly, recipient stimulator lymphocytes were obtained by CD3-selection (Miltenyi Biotech, Bergisch Gladbach, Germany) from a single leukapheresis product and cultured in X-VIVO 20 (Cambrex Bio Science, Walkersville, MD) supplemented with 2.0% autologous heat-inactivated plasma and 100 IU/mL interleukin-2 (Proleukin; Chiron, Everyville, CA) in gas-permeable polylefin tissue culture bags (Lifecell PL732; Baxter Cellular Therapies, Deerfield, IL) precoated with OKT3 antibody (murumonab-CD3, Orthoclone OKT3; Ortho Biotech, Horsham, PA). Cells were incubated at 37°C, in 7% CO2, and 90% humidity, adding medium to maintain the cell concentration between 0.5 × 10⁶ and 1.5 × 10⁶ cells/mL. Stimulator cells were harvested after 10 to 12 days, concentrated, washed, and cryopreserved in 5% DMSO. SCT donors underwent 8- to 12-liter leukapheresis to collect 8 to 12 × 10⁹ mononuclear cells. Stimulator cells were thawed, irradiated to 25 Gy, resuspended in X-VIVO 20 (Cambrex Bio Science) supplemented with 2.0% autologous heat-inactivated plasma at a concentration of 5 × 10⁶ cells/mL, and combined with responder cells at a ratio of 1:1. Responders and stimulators were cocultured for 72 hours at 37°C in 7% CO2. Cocultures were performed in gas-permeable polylefin tissue culture bags (Lifecell PL732).

**PD**

Cocultures were washed and resuspended at a concentration of 5 × 10⁶ cells/mL and incubated at 37°C with 7.5 μM of 4,5-dibromorhodamine 123 (TH9402; Kiadis Pharma, Amsterdam, The Netherlands). After 40 minutes of incubation, cells were centrifuged and resuspended in TH9402-free medium for 90 minutes to accelerate dye efflux, and then exposed to 5 Joule/cm² light (514 nm wavelength; Theralux device, Model 514, Kiadis Pharma). Cells were cryopreserved in 5% DMSO and 6% pentastarch, and maintained in liquid nitrogen (−200°C to −150°C), thawed, and transfused after product release criteria (≥70% viability with CD3+ ≥50% of viable cells, endotoxin <5 EU/mL, mycoplasma negative, and all microbiology cultures without growth) were satisfied.

**Transplant Procedure**

Patients were conditioned with 125 mg/m² of fludarabine (Flu; days −8 to −4) and 120 mg/kg of cyclophosphamide (Cy; days −3 to −2), and age-adapted fractioned totalbody irradiation (TBI) in 8 fractions days −7 to −4 (total 12 Gy for patients ≤55 years.
and 4 Gy for patients >55 years). After leukapheresis to make the PD product, donors were mobilized with granulocyte-colony stimulating factor (G-CSF) for 6 days before apheresis. The stem cell collection was depleted of T lymphocytes by CD34+ positive selection (Miltenyi CliniMacs System, Miltenyi Biotec Inc., Auburn, CA). On the day of transplantation, all patients received a fixed dose of 5 × 10^6/kg viable PD CD3+ cells, together with a CD34-selected 3-4 log T cell-depleted stem cell product (3.1-9.5 × 10^6 CD34 cells/kg) from their HLA-matched sibling.

**Study Design**

The trial was designed to safely evaluate the efficacy of PD to prevent severe aGVHD. To mitigate any GVHD, all patients received low-dose CSA for GVHD prophylaxis to achieve a plasma level of 100-200 ng/mL, starting day -4 before transplantation. To evaluate if CSA was needed to prevent severe GVHD after PD, we used a sequential 3-cohort CSA deescalation design, with grade III-IV aGVHD as the primary endpoint: In the absence of more than 3 cases of grade III-IV aGVHD per cohort, CSA could be tapered by day +90 (cohort 1), day +45 (cohort 2), and day +3 (cohort 3). The cohort size of 17 patients was based on an expected rate of acute grade III/IV GVHD of 12% to 41% from prior transplantation experience with low-dose CSA [7] and provided a power of >85% (α = 0.05).

**Supportive Care**

Patients received transfusion support, standard catheter care, prophylaxis of viral infection with valacyclovir, fungal infection with fluconazole, and pneumocystis infection with sulfamethoxazole/trimethoprim in accordance with the Guidelines for Management in Allogeneic Hematopoietic Stem Cells Transplant Recipients published by the CDC [12].

**Definitions**

Acute leukemia in first complete remission (CR1), myelodysplastic syndrome (MDS) with Intermediate-I World Health Organization grading, and patients with chronic myelogenous leukemia (CML) in the chronic phase were categorized as standard risk for transplant. More advanced disease (second complete remission [CR2] or greater), primary refractory or relapsed disease, and secondary acute myelogenous leukemia (AML) were categorized as high risk for transplant. GVHD was categorized by the Glucksberg grading system [13,14]. Overall survival was calculated from the interval between the date of transplantation and death, or last follow-up visit. Relapsed disease for acute leukemia and MDS was defined by morphologic or cytogenetic evidence, in peripheral blood or bone marrow. For CML, relapsed disease was defined by hematologic, cytogenetic, or molecular evidence of recurrence. NRM was defined as the time from transplantation until death from infectious cause, graft failure, GVHD, or any other cause unrelated to disease. Engraftment was defined as absolute neutrophil count >500/μL for 3 consecutive days, and unsupported platelet count of ≥20,000 or 50,000/μL for 3 consecutive days or detection of donor DNA by PCR-short tandem repeat.

**Statistical Analysis**

Survival was measured to the last contact date or death. The probability of transplant outcomes was estimated according to the Kaplan-Meier method, and graphs were generated with the use of Prism 4.00 for Windows software (GraphPad Software, San Diego, CA). P values of .05 or less were considered significant. Date of analysis was December 1, 2010.

**RESULTS**

**Patients and Outcome**

Patient characteristics and outcome are shown in Table 1. The median age at SCT for the 14 males and 10 females was 43.5 years (range: 23-68). Indication for SCT included AML (n = 9), MDS (n = 7), acute lymphoblastic leukemia (ALL) (n = 4), non-Hodgkin lymphoma (n = 2), and CML (n = 2), with 16 of the patients considered high risk and 8 patients considered standard risk for relapse at the time of SCT. All 24 patients received a PD SCT with a median of 6.3 × 10^6/kg CD34+ stem cells (range: 3.1-9.5 × 10^6/kg) containing a median of 1.1 × 10^6/kg unmanipulated residual CD3+ cells (range: 0.7-10^5/kg). Median PD product viability was 90% for all products at time of cryopreservation (range: 70%-96%), and all PD products were infused at a predetermined dose of 5 × 10^6 viable CD3- cells/kg based on cryopreservation viability and quantification. Infusions of all cell products were well tolerated with no immediate serious adverse events.

**Engraftment**

Engraftment was rapid for all patients, with >95% donor myeloid chimerism occurring by day 14, and >95% donor T cell chimerism occurring for the majority of patients by day 30 (median 98%; range: 30%-100%). Neutrophil engraftment occurred by day 14, and on day 30 a median absolute lymphocyte count of 761/microL for 3 consecutive days, and unsup-ported platelet count of ≥20,000 or 50,000/μL for 3 consecutive days or detection of donor DNA by PCR-short tandem repeat.
Eight of the 24 patients developed aGVHD greater than grade I before day 100 after SCT (5 grade II, 3 grade III-IV). One patient developed aGVHD 108 days after SCT, after receiving an unmanipulated donor lymphocyte infusion to treat decreasing donor T cell chimerism. One patient had a recurrence of aGVHD on day 320 after SCT when she presented with grade IV skin GVHD occurring after an unmanipulated donor lymphocyte infusion given in her home country to treat a misdiagnosed recurrence of ALL (expansion of CD33^+^TdT^+^precursors noted in bone marrow specimen following medication-induced marrow aplasia). In the first cohort of 17 patients, CSA was tapered and stopped between days 90-110 in 12 patients. CSA was continued in 5 patients who developed grade II GVHD. No patient in this cohort developed severe aGVHD (grade III-IV). Because the criteria for continuing to the second cohort were met, 7 patients were entered into the second cohort where CSA was scheduled for taper on day 45. In 3 patients, CSA was successfully tapered without occurrence of severe GVHD, but 3 developed grade III-IV acute GVHD while on CSA treatment: 1 with grade III gastrointestinal symptoms on day 36, 1 with grade III liver and grade IV skin manifestations on day 27 following an episode of sulframethoxazole-induced toxic epidermal necrolysis, and 1 with grade III gastrointestinal and grade IV liver disease on day 64 (but still receiving CSA for earlier development of skin GVHD). Probabilities (±SEM) of aGVHD were 38% ± 10% for grades II-IV and 13% ± 7% for grades III-IV (Figure 1A). One patient experienced a recurrence of aGVHD of the gastrointestinal tract occurring after day 100, and received therapy with second-line aGVHD therapy. The 3 patients who developed grade III-IV GVHD died of infectious complications.

Chronic GVHD (cGVHD)

Fourteen patients developed cGVHD: 12 limited and 2 extensive, with a probability (±SEM) of developing cGVHD of 65% ± 11%.

Relapse

Six high-risk patients (2 MDS, 1 ALL, 1 AML in second or subsequent remission, and 2 B cell malignancies transplanted with resistant disease) relapsed at a median of 187 days post-SCT (range: 147-349 days). Two patients with MDS (patients 13 and 19) and 1 patient with AML (patient 16) died of relapsed leukemic disease. The 2 patients with B cell malignancies (patient 3 with mantle cell lymphoma, patient 17 with diffuse large B cell lymphoma) responded...
completely to induction chemotherapy followed by an unmanipulated donor lymphocyte infusion, and currently survive in a complete remission (Table 1). One standard-risk patient with ALL (patient 24) relapsed on day 202, and was lost to follow-up after returning to his home country for palliative care 268 days after SCT. Patient 10 experienced a molecular relapse of CML after stopping imatinib therapy, 730 days after SCT. This patient is now in molecular remission on imatinib. The probability (±SEM) of hematologic relapse was 29 ± 13% for high-risk patients, and 27 ± 10% overall (Figure 1B).

Infectious Complications
Serious adverse events requiring hospitalization after SCT occurred frequently on this protocol and persisted beyond the early posttransplantation period. Patients experienced frequent viral reactivations, and recurrent bacterial, viral, and fungal infections. Twenty of 24 patients experienced reactivation of CMV with a median of 2 episodes (range: 1–3) requiring treatment in the first 100 days after SCT (including 2 initially CMV seronegative recipients receiving SCT from seropositive donors). Five patients experienced viral associated hemorrhagic cystitis (2 BK virus, 2 adenovirus, 1 both), and 1 patient died of respiratory syncytial virus pneumonitis (patient 1). Five patients experienced recurrent bacterial infections, 4 of whom died of bacterial pneumonia (patients 5, 9, 22, and 23). Four patients developed invasive fungal infections (1 zygomycosis, 3 aspergillus), 1 of whom died of extensive pulmonary zygomycosis (patient 15). Because of the unexpected duration and severity of these infectious complications, the trial was stopped after the 24th patient was enrolled.

NRM, Overall Survival, and Disease-Free Survival
Patients experienced a high rate of late NRM (Table 1 and Figure 1C), with a probability of 48% ± 15% in cohort I, 54% ± 22% in cohort II, and 53% ± 14% overall. Causes of death were: bacterial or fungal infection (5), respiratory syncytial virus (RSV) pneumonia (1), congestive heart failure (1), bronchiolitis obliterans syndrome (1), and sudden death with no identifiable etiology (1). Only 4 patients
had relapsed leukemia at the time of death. The probability of overall survival and DFS were 39% ± 12% and 30% ± 12%, respectively (Figures 1D and E).

DISCUSSION

An effective SD technique could provide a widely applicable technology to deliver an SCT with maximum GVL potential and minimal aGVHD, and improve outcome by reducing relapse, especially in high-risk patients. This study demonstrates the feasibility of using a PD technique to reduce T cells causing aGVHD from the graft before SCT. The primary objective of this protocol was to determine if depletion of alloreactive T cells before SCT by PD would avoid severe aGVHD within the first 3 months of transplantation and obviate the need for posttransplantation GVHD prophylaxis. In the first cohort, no patients developed grade III-IV aGVHD, indicating that the risk for severe aGVHD was low in the presence of low-dose CSA given during the first 90 days after SCT. However, determining whether CSA posttransplantation prophylaxis could be safely reduced to 45 days was not possible because of early termination of the trial. In the second cohort, CSA was successfully withdrawn on day 45 in only 3 out of 7 patients as a result of GVHD developing in 4 patients requiring continued CSA administration beyond day 45. Overall, 3 of the 24 patients enrolled in this study developed grade III-IV aGVHD before day 100, with a probability of 13% ± 7%. Thus, although the procedure was not completely effective, for most patients the infusion of 5 × 10^6/kg PD T cells successfully avoided severe aGVHD. Whether severe aGVHD was related to variables in the PD procedure or intrinsic variations, donor-recipient combination remains unanswered.

Perhaps helped by the rapid donor T cell engraftment, the GVL effects of the transplantation appeared to be well conserved after PD. Despite the fact that two-thirds of the patients were at a high risk for relapse, the probability of hematologic relapse was low (27% ± 10%). Of the 6 patients who relapsed, 2 had B cell lymphomas and were treated with chemotherapy followed by an unmanipulated donor lymphocyte infusion. Only 3 patients died of relapse as the primary cause.

Although day 100 overall mortality was only 4% and there was no severe acute GVHD in the first cohort, an unexpected outcome was the high rate of late infections that often accompanied chronic or persisting aGVHD, with a resultant late NRM (53%) and median survival of only 568 days. We also encountered unusual posttransplantation complications including 1 autoimmune hemolytic anemia, neutropenia related to T cell large granular lymphoproliferative disorder in 2 patients, and 3 cases of chronic renal failure, 2 requiring dialysis. Recurrent infectious complications also led to several prolonged hospitalizations and late readmissions more than 6 months after SCT. For these reasons, we elected to stop the study early.

The failure of allodepletion and development of severe aGVHD in 3 patients indicates either inability of recipient cells to activate donor cells or a failure of the PD process. In 3 large-scale preclinical experiments with PD, we demonstrated reduction in alloreactivity [10], but alloreacting cells generated between HLA-matched siblings are at the limit of detection by cytotoxic and helper T lymphocyte precursor (CTLp/HTLp) frequency assays, prohibiting the use of such assay results as release criteria [15]. Furthermore, it is generally acknowledged that no assay can reliably predict GVHD after matched related SCT. Our results are comparable to our earlier study using a CD25 immunotoxin for alloreduction where aGVHD and cGVHD occurred in 50% of patients [7]. However, these results contrast with encouraging data observed in HLA-mismatched transplants using the PD approach [9,16] or other techniques [6-8]. It is possible that selective T cell depletion techniques are less able to discriminate alloresponses from background noise in the weak allore sponses elicited by HLA-identical siblings, compared with the very powerful allore sponses elicited across HLA barriers. Alternatively, the T lymphocyte recipient APC used in our studies may have been less capable of eliciting stronger allore sponses than PBMC [6,16] or B cell [8] APC. Although T cell APC were chosen because of their reproducibility and reduced risk of depleting B cell or myeloid tissue-specific antigens (associated leukemic antigens) [3,5], they may have been inferior to other APCs for SD.

The high frequency of viral, bacterial, and fungal infections persisting beyond 3 months after SCT suggested that the PD process had affected graft T cell function. We therefore studied T cell subsets in the PD product and followed immune reconstitution in the PD recipients. We found a reduced frequency of central memory CD4^+ T cells in the PD product, and persisting CD4 deficiency and defective immunity to CMV 3 months after transplantation, which may explain the delayed infectious complications in our series [17].

CONCLUSION

In conclusion, this clinical trial shows that PD in the HLA-identical sibling SCT setting is effective in achieving rapid donor reconstitution and reducing incidence of severe aGVHD while maintaining a low relapse rate. However, using PD may preferentially deplete CD4^+ and memory T cells, resulting in a distortion of the immune repertoire and recurrent
infections. Modifications to the PD procedure are now planned to optimize donor T cell activation and eliminate nonselective depletion of T cells to better adapt the PD procedure for HLA-identical sibling SCT.

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AUTHORSHIP STATEMENT

S.M. designed research and wrote the article. Z.A.M. provided clinical care, analyzed data, and wrote the article. A.S. designed research and provided clinical care. V.F. supervised the preparation of clinical products. H.K. designed research and supervised the preparation of clinical products. D.F.S. designed research and supervised the preparation of clinical products. R.C. provided clinical care. S.F.L. designed research and supervised the preparation of clinical products. H.K. designed research and supervised the preparation of clinical products. K.R. designed research. A.J.B. provided clinical care. J.H. provided clinical care. B.S. designed research. K.R. designed research. A.S. designed research and provided clinical care, and wrote the article. S.M. received research funding from Kiadis Pharma, The Netherlands. The remaining authors declare no conflicts of interests.

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