

ORIGINAL ARTICLE

Unrelated donor or partially matched related donor peripheral stem cell transplant with CD34+ selection and CD3+ addback for pediatric patients with leukemias

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Unmodified peripheral stem cell transplants are associated with an increased risk of extensive chronic GVHD. T depletion may reduce this risk, but the risk of graft failure or relapse may increase. To decrease the risks of both extensive chronic GVHD and graft failure, we added back a defined dose of CD3+ cells to CD34+ selected PSCs. Twenty-four patients were evaluable for outcome analysis. Donors were unrelated (23) or related (1). Conditioning was thiotepa, cyclophosphamide, and total body irradiation. Cyclosporine was used post transplant. Following CD34+ selection, a total of 5×10^5 /kg CD3+ cells were infused. Donors were matched for 12 patients. The median CD34+ dose infused was 7.1×10^6 /kg. Engraftment occurred in all patients at a median of 14 days (10–19). Twelve patients are alive in remission 15–34 months (median, 25) post PSCT. GVHD occurred in 17 patients, but was >grade II in only 2. Chronic GVHD occurred in 61.5% of evaluable patients, but was limited to skin and perioral cavity. Two patients relapsed, and 10 patients died of non-relapse causes. This study demonstrates that PSCT with CD34+ selection and a defined dose of CD3+ results in prompt engraftment and may limit development of extensive chronic GVHD.

Bone Marrow Transplantation (2006) 37, 143–149.

doi:10.1038/sj.bmt.1705211; published online 7 November 2005

Keywords: leukemias; peripheral stem cells; CD34+ selection

hematopoietic progenitor cells that can be obtained following growth-factor mobilization and apheresis may result in faster neutrophil recovery, decreased transfusion requirements and faster immune reconstitution compared with bone marrow.^{2–6} However, PSCs contain at least one log more T cells than bone marrow, which theoretically could increase the risk of GVHD.⁷ Some studies have noted no increased risk of acute GVHD with matched sibling donor PSCT, despite the increase in T cells in PSCs.^{2,4,8} This may be as a result of the shift from Th1 to Th2-type lymphocytes with altered cytokine pattern as a result of G-CSF mobilization.^{9,10} Most studies have shown an increased risk of chronic GVHD, which is more likely to be refractory and extensive, resulting in increased late mortality.^{8,11–13} Although some studies reported a benefit from chronic GVHD with presumptive increased graft vs leukemia (GVL) effect for patients with high-risk leukemias, this benefit is nullified by higher mortality from GVHD in lower risk patients.¹⁴ A recent retrospective analysis compared pediatric patients who received PSCs or bone marrow from matched related donors, and demonstrated increased non-relapse mortality with PSCs, with more deaths from chronic GVHD.¹ Unrelated donor BMT is associated with an increased risk of both acute and chronic GVHD, and these concerns may be heightened with the increased T cell dose in peripheral blood. Despite the significant use of PSCs for matched sibling donor transplants, there have been few reports of PSC transplant for unrelated donors.

Elimination or reduction of CD3+ cells in PSCs may allow retention of the benefits of rapid engraftment kinetics, while reducing the risk of extensive chronic GVHD. Positive selection of CD34+ stem cells results in approximately 3–4 log reduction of CD3+ cells, with decreased risks of GVHD, but increased risks of risks of graft failure and relapse.^{15,16} An addition of defined dose of CD3+ cells at the time of infusion may alleviate the problem of graft rejection, yet allow for GVL and reduced chronic GVHD. To retain the advantages of PSCs, but reduce the risks of graft failure and GVHD, we developed a protocol to 'partially' T deplete PSCs by positive selection of CD34+ cells and an addback of CD3+ cells at the time of infusion.

Introduction

The use of peripheral stem cells (PSCs) for hematopoietic engraftment is becoming increasingly common, and these are now used in approximately 30% of pediatric transplants with matched related donors.¹ The larger numbers of

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Received 28 April 2005; revised 26 August 2005; accepted 29 August 2005; published online 7 November 2005

Patients and methods

Patients were eligible for this study if they had acute leukemias in first remission having failed primary induction therapy, or second remission, or chronic leukemias, and lacked an HLA matched sibling donor. Unrelated donor search was performed through the National Marrow Donor Program, if a related donor mismatched at one or two antigens was not available. Donors had to be willing and able to provide mobilized peripheral stem cells collected by apheresis. Donors underwent peripheral stem cell mobilization with G-CSF 10 µg/kg/day subcutaneously for five days. The Children's Hospital of Philadelphia Institutional Review Board approved the protocol, and parent or patient gave written consent.

Histocompatibility testing

Before transplantation, HLA typing on the patient and potential donors was performed by DNA-based methodology for A, B, C, DRB1 and DQB1 loci. HLA-C was performed at the low/intermediate level. HLA A, B, DRB1 and DQB1 were analyzed at the allele level by high-resolution typing. Loci A, B and DRB1 were first analyzed at the intermediate resolution level by a reverse SSOP (sequence specific oligonucleotide probes) methodology (RELI™ Dynal Inc.) and thereafter, depending on the typing, appropriate kits (Genovision Inc.) were selected for high-resolution typing. HLA-DQB1 typing was directly performed at the high-resolution level using 'One Lambda' SSP kits. These methodologies have been previously tested and validated.^{17,18}

Conditioning and GVHD prophylaxis

Conditioning included thiotepa 5 mg/kg days -6, -5, cyclophosphamide 60 mg/kg days -4, -3, and total body irradiation 1200 cGy/6 fractions days -2, -1, 0. Patients with CML in first chronic phase received anti-lymphocyte globulin 1.5 mg/kg days -2, -1, 0 for additional immunosuppression. Lung shielding after 800 cGy was performed for the last 14 patients, when concerns arose regarding engraftment syndrome and pulmonary toxicity.

GVHD prophylaxis included cyclosporine infusion beginning day 1 to achieve serum levels of 200–400 ng/dl. This was changed to oral tacrolimus when tolerated, as per institutional practice to improve compliance, with levels maintained between 4–12 ng/ml. Weaning commenced at day +100 in the absence of GVHD. Methylprednisolone 1 mg/kg in 2 divided doses was begun at day +8 for the last 14 patients to alleviate potential engraftment syndrome. This was rapidly weaned in the absence of GVHD once engraftment occurred.

Supportive care

All patients were in HEPA filtered private rooms, and given prophylactic acyclovir, trimethoprim/sulfamethoxazole, gut decontamination with oral amoxicillin and gentamicin, and fluconazole. Intravenous gamma globulin 400 mg/kg was given every other week for three months. CMV antigenemia was monitored weekly once engraftment occurred. No growth factors were used post SCT.

Peripheral stem cell processing and CD3+ adback

CD34+ selection was performed with Isolex 300i, which resulted in an average T cell depletion of 4.2 logs. The positive fraction had an average purity of 94%, and CD3+ cells obtained from the negative fraction were added to the positively selected product at the time of infusion to achieve the defined CD3+ dose of 5×10^5 /kg. The remaining portion of the negative fraction was cryopreserved in multiple aliquots for donor lymphocyte infusions, if needed.

Post transplantation evaluation

The day of engraftment was the first of 3 consecutive days on which the neutrophil count was ≥ 500 ul. Chimerism was documented by variable nucleotide tandem repeats (VNTRs). Patients with CML had quantitative bcr-abl monitoring by polymerase chain reaction every 3 months starting six months post BMT.

Patients who engrafted were evaluable for acute GVHD, and patients who survived more than 100 days were evaluable for chronic GVHD. Graft vs host disease was graded according to established criteria.¹⁹ Acute GVHD was treated with methylprednisolone or prednisone 1–2 mg/kg and tapered weekly according to response. For patients who did not respond, additional agents were used, including Daclizumab, and mycophenolate mofetil.

Statistical analysis

All statistical analyses were performed in STATA 8.0. (STATA Corp., College Park, TX). The cut-off date for analysis was 3/1/05. For all patients, an event was defined as relapse or non-relapse mortality, and the Kaplan–Meier model was used for survival analysis.

Results

Twenty-five patients were enrolled on this study from 2002–2004. One patient's cells were poorly viable on dye exclusion (Trypan blue) testing upon receipt, and this did not allow for manipulation. This patient was excluded from further analysis of engraftment and GVHD. She received haploidentical PSCs for engraftment.

Clinical characteristics of the 24 evaluable patients are shown in Table 1. All patients with acute leukemias were in remission. One patient with MDS had failed a prior syngeneic transplant, and another patient had secondary AML following therapy for ALL. One patient with ALL developed pancytopenia and histiocytosis for which he was referred for transplantation. Donors were matched for 12 patients (Table 2). Patients received a median of 7.1×10^6 /kg CD34+ cells (range, 1.3–12.4). Nine patients received doses greater than 8×10^6 /kg. Engraftment occurred in all patients, with median time to ANC > 500 of 14 days (10–19). Fifteen patients had platelet recovery, with platelets $> 20 \times 10^9$ /l at a median of 20 days (13–33), and platelets $> 50 \times 10^9$ /l at 21 days (15–98). The other nine patients died prior to platelet recovery. Patients received steroids for a median of 29 days (11–83).

Table 1 Clinical features of evaluable patients

M:F	13:11
Age (median), years	3–22 (10)
Unrelated: Related	23:1
ALL (CR2)	6
AML/MDS	10/3 (RA-1, RAEBT-2)
CML	3 (CP1–2, CP2–1)
JMML	2
CMV R/D	
Neg/Neg	11
Neg/Pos	8
Pos/Neg	4
Pos/Pos	1

CR2-complete second remission; CMV R/D- cytomegalovirus recipient/donor.

Table 2 HLA matching of evaluable patients

HLA match	No. patients
10/10 match	12
A mismatch	5
B and C mismatches	4
DQB1 mismatch	3

Event free survival for all patients enrolled was 50%, with 12 patients surviving in remission 14–36 months post SCT (median, 25) (Figure 1). All survivors have Karnofsky or Lansky activity scores of 100. CML patients were bcr-abl negative by PCR less than six months from PSCT, without GVHD. There were two relapses in AML patients at 3 and 18 months. The patient who relapsed at 18 months achieved a second remission with chemotherapy, and was 95% donor by VNTRs before a second non-myeloablative transplant using cryopreserved residual peripheral stem cells.

Non-relapse mortality (NRM) was 41.7%, with deaths occurring 32–85 days (median, 50) after SCT. Infection and pulmonary toxicity were responsible for NRM, and included CMV (2), idiopathic interstitial pneumonitis (IIP) (6), HHV6 (2), Pseudomonas (1). Both patients who died from CMV pneumonitis were seropositive with seronegative donors; one developed pneumonitis at day +20, with negative antigenemia and the second had persistent antigenemia, despite therapy with ganciclovir and foscarnet. CMV was noted on biopsy samples of both patients. Patients with HHV6 had positive PCR from blood, with one patient PCR positive in both CSF and pleural fluid. These patients had other symptoms consistent with HHV6, including encephalitis, skin rash and pneumonitis. Patients with IIP did not have any organisms identified by pathology, PCR or culture from bronchoalveolar lavage or thorascopic biopsy. Viral studies on these patients included RSV, parainfluenza, HHV6, adenovirus, and CMV. These patients developed capillary leak and oxygen requirement at the onset of engraftment. Three patients may have had risk factors that predisposed them to IIP; one had streptococcus viridans sepsis one week prior to development of IIP, one patient had prolonged mechanical ventilation during reinduction therapy, and one patient had

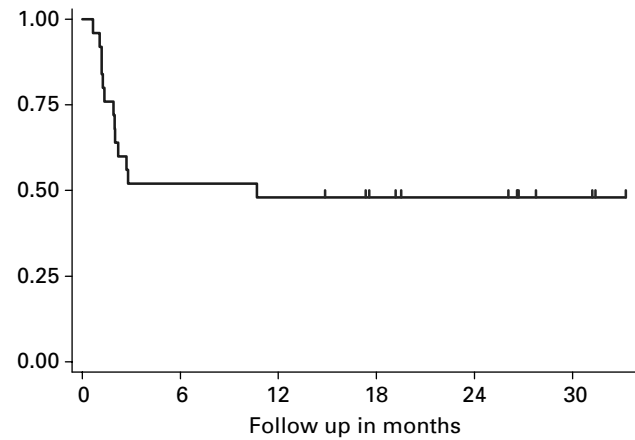


Figure 1 EFS of 25 patients enrolled on study.

Table 3 Immune function post SCT (median, day +356, range 241–431)

Normal	No. analyzed	Median	Range
IgG (583–1783 mg/dl)	10	571	453–1290
IgM (70–212 mg/dl)	10	50	11–109
IgA (70–498 mg/dl)	10	67	29–592
CD3+ (700–4200) ^a	5	425	122–1212
CD3/CD4+ (300–2000) ^a	10	148	15–885
CD3/CD8+ (300–1800) ^a	10	379	7–965
CD20+ (0–479) ^a	6	374	37–1075
CD56+ (90–900) ^a	10	156	31–610
CD4+/CD45RA+ (40.7–1121) ^a	8	264	2–721
CD4+/CD45RO+ (152.9–582) ^a	8	243	4–419

^aCells/ul.

a prior myeloablative transplant. Only one of these patients had lung shielding during TBI.

Acute GVHD developed in 17 patients, but was grade III–IV in only two (8.3%). All patients had skin involvement, with gut involvement in one. In the patient with grade IV GVHD it was concomitant with HHV6 disease. Chronic GVHD developed in eight of 13 (61.5%) evaluable patients, but was limited to skin involvement only in seven, and skin and perioral involvement in one. No patient developed extensive chronic GVHD. Two patients received additional therapy, including Daclizumab and mycophenolate mofetil. There has been resolution in seven patients; one remains on weaning therapy with complete resolution of skin involvement.

Immune function studies, including immunoglobulins and lymphocyte subsets, were performed at 241–384 days post SCT and were available for a subset of surviving patients. These data are summarized in Table 3. No infections requiring intravenous antibiotics were observed after day 100.

Discussion

Most series have shown that unmodified PSCs for matched sibling donor transplants result in faster engraftment, but

an increased risk of extensive chronic GVHD, which may negatively impact upon survival. With unrelated PSCs, the risk of both acute and chronic GVHD might be expected to increase, as unrelated donor bone marrow transplantation is associated with higher risks of this complication. There have been few reports of unrelated donor PSCT. One study in patients with CML noted decreased transplant related mortality and improved overall survival in recipients of unrelated PSCs compared with bone marrow.²⁰ There were no differences in either acute or chronic GVHD between the two groups. Other studies in adult patients with leukemias initially showed no differences in outcome or acute or chronic GVHD between bone marrow and PSC unrelated donor transplants.^{21,22} However, a recently published follow-up confirmed the increased risk of extensive chronic GVHD in PSC recipients, and no survival advantage compared with bone marrow recipients.²³ In another non-randomized study of patients with acute leukemias, lower survival was noted in ALL patients with PSCs compared with bone marrow.²⁴ Unlike other studies, the incidence of acute GVHD was higher in ALL patients who received PSCs.

T cell depletion may decrease the risk of GVHD but is associated with a higher risk of graft failure and relapse. These major problems may be dependent upon type and degree of T cell depletion, as T cell depletion of bone marrow with narrow specificity antibodies may not have these disadvantages.²⁵ Our previous study with partially T cell depleted bone marrow for unrelated and partially matched related donors demonstrated a graft rejection rate of < 5%, and relapse rate that was comparable to T replete grafts.²⁶ The optimal number and type of T cells in a PSC graft to ensure engraftment and maintain potential graft vs leukemia effect is unknown. In addition, other factors, including the dose of CD34+ cells/kg and HLA disparity, must be included as factors in engineering the ideal PSC graft.^{27,28} T cell depletion of PSCs may be accomplished by positive selection methods, such as CD34+ selection, which has the advantage of eliminating B cells that may cause EBV-associated lymphoproliferative disease. The largest experience using CD34+ selected PSCs comes from studies of haploidentical related donors.²⁹⁻³¹ However, the extensive T cell depletion by this method may increase the risk of graft failure due to depletion of cells that may facilitate engraftment. A recent study compared three groups that received unrelated donor products, including bone marrow, unselected PSCs, and CD34+ selected PSCs. A higher rate of graft rejection occurred with the CD34+ selected PSCs; engraftment occurred in 95% of patients who received unselected PSCs, in contrast to 78% engraftment in recipients of CD34+ selected PSCs ($P < 0.001$).²¹ Bornhauser *et al.* used CD34+ selected PSCs for unrelated donors, with an infusion of 1×10^5 /kg CD3+ cells at the time of initial infusion or delayed until day +21. Graft failure occurred in 16% of patients¹⁵ and an increased rate of relapse and infectious complications was also noted. In a series of unrelated donor PSCT in 31 children with leukemias, Lang *et al.* used CD34+ selected PSCs³¹ without post transplant immunosuppression. Five patients did not engraft initially, but subsequently engrafted with additional immunosuppression. Non-

engraftment was associated with a CD34+ dose of $< 10 \times 10^6$ /kg. Although engraftment was achieved in all patients, the prolonged pancytopenia obviously increased infectious risks. Although 'mega' doses of CD34+ cells, at least 10×10^6 /kg, are associated with engraftment in CD34+ selected PSCs,^{30,31} patients on our study engrafted with cell doses that were much lower.

We aimed to replicate the results with partial T cell depletion of bone marrow by adding back a defined dose of T cells to PSCs. The addition of T cells has been shown to reduce the risk of graft failure, but the optimum number and type for addback is unknown. In a study by Urbano-Ispizua there was a correlation between T cell dose and engraftment in patients receiving matched sibling donor PSCs.³² In that study, the number of T cells below which there was an increased risk of graft failure was 2×10^5 /kg, as 14.8% of patients experienced graft failure when given less than this, compared with only 1% graft failure for those who received more. This study was complicated, however, by the variability of conditioning, as non-engraftment was higher in CML patients who received busulfan regimens.³² Another study using matched sibling donors used the Ceprate system for CD34+ selection that resulted in approximately 3 log T depletion. A median of 4.2×10^5 /kg CD3+ cells was infused, with a range from 1 to 20×10^5 /kg.³³ Both cyclosporine and methylprednisolone were used for GVHD prophylaxis, and all patients engrafted. GVHD developed in six of 20 patients, and was less than grade III, with chronic GVHD observed in only one of sixteen patients at risk with short follow up.³³ Based upon these studies, we decided to add back a slightly higher dose, 5×10^5 /kg CD3+, and use cyclosporine prophylaxis post transplant. All our patients engrafted, and despite HLA mismatches in almost half our patients, severe GVHD occurred in only two patients. However, chronic GVHD was observed in 66% of evaluable patients, which lends concern about the dose of CD3+ cells. Unlike studies in which no modification of PSCs was done, we were successful in limiting the extent of chronic GVHD as all patients have activity scores of 100, and extensive involvement was not observed.

There is tremendous diversity in the composition of a PSC graft in terms of CD34+ and CD3+ content. In particular, the dose of CD34+ cells in an unselected PSC graft may impact upon the development of chronic GVHD and outcome. One study found favorable effects in relapse free survival when CD34+ doses were greater than 3×10^6 /kg in a T depleted PSC graft.²⁷ However, this has not been substantiated in other studies, with increased chronic GVHD and decreased survival with high doses of CD34+. In one study a negative effect of doses greater than 3×10^6 /kg, with 75% of patients alive who received doses 1 to 3×10^6 /kg, in contrast to 52% for those who received larger doses.³⁴ Both Mohty and Zaucha *et al.* noted increased chronic GHVD with doses higher than 8×10^6 /kg.^{28,35} Our study was too limited to evaluate the effect of CD34+ dose in outcome and chronic GVHD. Unlike CD34+, the dose of CD3+ does not appear to correlate with either acute or chronic GVHD in an unmodified graft.^{28,36}

Engraftment syndrome has been described primarily in autologous PSCT, and it may be a significant cause of morbidity and mortality in children.³⁷ It is characterized by fever without infectious etiology, rash that can mimic GVHD, weight gain and pulmonary infiltrates.³⁸ The incidence of this syndrome in PSCT may be related to a higher cell dose and faster engraftment, as it usually occurs with the onset of engraftment.^{39,40} The release of proinflammatory cytokines, including IL-1 and TNF- α , have been implicated in its development.⁴¹ There are few reports of engraftment syndrome with allogeneic PSCT, but steroids may be used successfully.⁴² Treatment with steroids was initiated prophylactically when we became concerned about morbidity from engraftment syndrome, but the efficacy of this intervention was unclear in our study. Concerns regarding engraftment syndrome and pulmonary toxicity led to closure of the study.

Even at one year post SCT, our patients had a CD4+ / CD8+ <2, with elevated B and NK cells. Profound immunodeficiency and slow immune reconstitution, with an increased risk of infectious complications, develop after T depleted grafts, including CD34+ selected PSCs. Despite monitoring and prompt therapy with foscarnet or ganciclovir, CMV disease may develop with rapid onset of fatal pneumonitis.⁴³ We also experienced early onset of CMV pneumonitis in one patient, before antigenemia became positive, as well as CMV that did not respond to combined antiviral therapy. Prophylactic therapy in the highest risk group, CMV positive recipients with serology negative donors may help prevent this complication, but patients will remain at risk for the development of later onset disease.^{44,45} In addition, early onset of CMV drug resistance has been noted in other studies of T depleted recipients,⁴⁶ with the lack of an immune response allowing for high viral replication during therapy. Pediatric patients may be at particularly higher risk for this complication.⁴⁷ One patient with grade IV GVHD developed HHV6 disease concomitant with the onset of GVHD. HHV6 may initiate or exacerbate GVHD in T depleted grafts.^{31,48}

Partial T cell depletion as accomplished with CD34+ selection with an addback of CD3+ cells at the time of infusion for recipients of unrelated donor or partially matched related donor PSCT may allow for durable engraftment without loss of GVL. 'Megadoses' of CD34+ cells are not needed to accomplish engraftment, as in selection without the addition of CD3+ cells. Although chronic GVHD was observed, it was limited and not extensive as reported in series of unselected PSCT. However, engraftment syndrome and profound immunodeficiency resulting in viral infections remain a concern. Newer separation devices now allow removal of CD3+ cells, which is more consistent with our previous methods of T depletion, and we have begun to use this technology. As more information becomes available regarding subsets of T cells that mediate engraftment or GVHD, and technology for graft processing improves, we will be able to better 'engineer' the PSC product to take advantage of its unique properties.

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