Outcome of Alloanergized Haploidentical Bone Marrow Transplantation after ex vivo Costimulatory Blockade: Results of Two Phase I Studies

Jeff K Davies¹,⁴, John G Gribben², Lisa L Brennan³, Dongin Yuk¹, Lee M Nadler¹,⁴ and Eva C Guinan³,⁵

¹Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA
²Department of Medical Oncology, St. Bartholomew's Hospital, London, United Kingdom
³Department of Pediatric Oncology, Dana-Farber Cancer Institute, Boston, MA
⁴Department of Medicine, Brigham and Women's Hospital
⁵Division of Hematology/Oncology, Children's Hospital, Boston, MA

Corresponding Author:
Eva C. Guinan M.D.
Associate Director, Center for Clinical and Translational Research
Associate Professor of Pediatrics
Harvard Medical School
Dana-Farber Cancer Institute
44 Binney Street
Boston, MA 02115
FAX 617-632-3770
Tel 617-632-4932
email: eva_guinan@dfci.harvard.edu

Scientific Section: Transplantation
Abstract

We report the outcome of 24 patients with high-risk hematological malignancies or bone marrow failure (BMF) who received haploidentical bone marrow transplantation (BMT) after \textit{ex vivo} induction of alloantigen-specific anergy in donor T cells by allostimulation in the presence of co-stimulatory blockade. 95% of evaluable patients engrafted and achieved full donor chimerism. Despite receiving a median T cell dose of 29 x 10^6/kg, only 5 of 21 evaluable patients developed Grade C (n=4) or D (n=1) acute graft-versus-host disease (GvHD), with only one attributable death. Twelve patients died from treatment-related mortality (TRM). Patients reconstituted T cell subsets and immunoglobulin levels rapidly with evidence of \textit{in vivo} expansion of pathogen-specific T cells in the early post-transplant period. Five patients reactivated CMV, only one requiring extended antiviral treatment. No deaths were attributable to CMV or other viral infections. Only one of 12 evaluable patients developed chronic GvHD. Eight patients survive disease-free with normal performance scores (median follow-up 7 years). Thus, despite significant early TRM, \textit{ex vivo} alloanergization can support administration of large numbers of haploidentical donor T cells, resulting in rapid immune reconstitution with very few viral infections. Surviving patients have excellent performance status and a low rate of chronic GVHD.
Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) offers a curative approach for many patients with hematological malignancies and certain non-malignant hematologic conditions. Although the majority of such patients will lack fully HLA-matched related donors, and many will also lack available HLA-matched unrelated donors, almost all will have available HLA-mismatched related donors. However, HLA-mismatched HSCT is associated with increased graft failure, GvHD and a higher risk of treatment failure. GvHD is predominantly mediated by alloreactive donor T cells which expand in vivo post-transplant. Non-selective T-cell depletion (nsTCD) of donor grafts is an efficient method for reducing alloreactivity and is very effective at preventing GvHD. While nsTCD has been combined with high CD34⁺ stem cell doses to achieve engraftment without severe GvHD in haploidentical HSCT, overall success of such strategies may be limited by delayed immune reconstitution, increased infectious complications, and higher relapse rates potentially associated with the loss of pathogen- and tumor-specific T cells.

Various experimental strategies to selectively deplete alloreactive T cells within the donor graft to prevent GvHD while preserving pathogen- and tumor-specific immunity have been developed with the aim of improving immune reconstitution after HLA-mismatched HSCT. Most of these approaches utilize a common mechanistic platform of ex vivo donor T cell stimulation by recipient alloantigens and subsequent removal or destruction of alloreactive T cells, identified by expression of activation markers, proliferation or metabolic activity.

An alternative to selective allodepletion is induction of allospecific anergy (hyporesponsiveness to subsequent restimulation with alloantigens) in donor T cells prior to HSCT. T cells require at least two signals to become activated: cognate antigen/MHC binding to the T cell receptor (Signal 1) and positive co-stimulatory signals from antigen presenting cells (APCs) (Signal 2). The predominant positive co-stimulatory signal to human CD4⁺ T cells is delivered via the CD28 receptor, constitutively expressed on the surface of 95% of human CD4⁺ T cells. This signal may be blocked by fusion proteins (such as CTLA4-Ig) or monoclonal antibodies (such as anti-B7.1 and –B7.2) that bind to the CD28 ligands B7.1 and B7.2 on antigen-
presenting cells (APCs). T cells stimulated with Signal 1 without Signal 2 enter a state of antigen-specific hyporesponsiveness. Thus recipient allospecific donor T cells can be rendered anergic by ex vivo stimulation with recipient alloantigens in the presence of co-stimulatory blockade (CSB).15

We previously reported early results of a clinical trial utilizing ex vivo induction of alloanergy in donor T cells within bone marrow (BM) grafts via CTLA4-Ig-mediated CSB.16 This technique permitted large doses of HLA-mismatched donor T cells to be infused at the time of BMT with reliable engraftment and establishment of rapid full donor chimerism without excess severe aGvHD. We now report the immune reconstitution, infection and acute and chronic GvHD characteristics and long-term follow-up of a much larger cohort of patients with high-risk hematological malignancies or BMF who received HLA-mismatched BMT after ex vivo CSB to induce alloanergy.
Methods

Patient and Donor Characteristics
Between March 1996 and March 2001, 24 patients entered Phase I studies of haploidentical BMT with \textit{ex vivo} CSB using CTLA4-Ig (n=19) or anti-B7-1 and -B7-2 antibodies (n=5) on Dana-Farber Cancer Institute IRB-approved protocols. Patients (or their guardians) gave informed consent in accordance with the Declaration of Helsinki. Follow up is reported through June 2008. Patients with high-risk hematological malignancies (subsequently also patients with congenital or acquired BMF other than Fanconi Anemia), were eligible as previously described.\textsuperscript{16} Patient and donor details are summarized in Table 1. The median age of adult patients was 26 years (range 19-50, n=7) while that of those aged <18 was 6.5 years (range 0.5-16, n=17). Of the 21 patients with hematologic malignancy, only 7 patients were in complete remission (CR; CR2=6, CR3=1) and 14 had progressive disease (PD; 5 having never attained remission) at time of BMT. UPNs 002 and 006 had failed prior autologous HSCT. All 3 patients with BMF were transfusion dependent having failed prior non-transplant therapy (if available). The haploidentical family donors consisted of parents (n=16), full siblings (n=5), half-siblings (n=2) and children (n=1). A number of different tissue-typing techniques were employed.

Conditioning Regimen, \textit{ex vivo} Manipulation of BM and GvHD Prophylaxis
Prior to conditioning, patient peripheral blood mononuclear cells (PBMC) obtained by leukapheresis were cryopreserved for subsequent use as APCs during \textit{ex vivo} coculture. Patients received 1400cGy total body irradiation (175cGy BID D-6-D-3) and cyclophosphamide 1800 mg/m\textsuperscript{2} on D-2 and D-1. The first 8 patients also received cytosine arabinoside 3g/m\textsuperscript{2} q12h for 6 doses. All patients also received methylprednisolone q12h for 4 doses ending no later than 2 hours prior to BMT. The method of \textit{ex vivo} alloanergy induction with CSB with CTLA4-Ig (Repligen, Waltham, MA, USA) was as previously described.\textsuperscript{16} Briefly, donor BM was harvested on D-2 and co-cultured \textit{ex vivo} with irradiated recipient PBMC for 36-40 hours in the presence of CTLA4-Ig, washed and re-infused on D0. For the last 5 patients 10µg/ml humanized murine IgG2 monoclonal anti-B7.1 and -B7.2 antibodies (Wyeth, Madison, NJ, USA), replaced CTLA4-Ig in \textit{ex vivo} co-cultures. All patients received cyclosporine and short-course methotrexate, and 23 received folinic acid after some or all methotrexate doses.
Measurement of Residual Alloprecursor Frequency

Residual alloprecursor frequencies in alloanergized BM were determined in some patients by helper T lymphocyte precursor frequency assay (hTLPf), as previously described.\textsuperscript{16}

Supportive Care

Patients received oral, non-absorbable antibiotics from admission until neutrophil engraftment, fluconazole for fungal prophylaxis and \textit{Pneumocystis Carinii} prophylaxis with trimethoprim-sulfamethoxazole or pentamidine during conditioning and after D+30 or discharge. Patients received acyclovir prophylaxis at 100 mg/m\textsuperscript{2} q12h if they were seropositive for HSV or at 250 mg/m\textsuperscript{2} q8h if the donor or recipient was CMV-seropositive. Intravenous immunoglobulin (IVIg) was given weekly (400-500 mg/kg) until trough levels were self-sustaining at IgG >500 mg/dL. Patients were monitored for CMV reactivation at weekly intervals by detection of CMV antigenemia. Patients who became CMV antigenemic received intravenous ganciclovir therapy.

Chimerism and Engraftment

Hematopoietic chimerism was determined on unfractionated blood or BM samples by fluorescence in situ hybridization in recipients with sex-mismatched donors or by polymerase-chain-reaction amplification of sequence specific primers or oligonucleotide probes for HLA class I and class II donor and recipient antigens.

Definitions of acute and chronic GvHD and Performance Status

Patients were evaluable for aGvHD, if they successfully engrafted (graded with the International Bone Marrow Transplant Registry Severity Index) and cGvHD if they reached D+100 without relapse (graded with consensus criteria).\textsuperscript{17,18} Wherever possible, histological confirmation of clinical diagnoses of aGvHD was sought. Performance Status of surviving patients was assessed using Karnofsky or Lansky scoring, appropriate to age.\textsuperscript{19,20}

Immune Reconstitution

Patients surviving beyond D+100 without relapse were eligible for immune reconstitution analysis. T, B and NK cell subsets were quantified by at 1, 2, 3, 4, 6, 9 and 12 months after BMT by flow cytometric analysis of PBMCs using FITC/PE/PC5-labeled antibodies
against CD3, CD4, CD8, CD19, CD56, CD45RA/RO and CD62L antibodies (Becton Dickinson, San Jose, CA, USA). Serum Immunoglobulin levels were determined by standard rate nephelometry (Dade Behring, Deerfield IL, USA). CMV (HLA-A2 NLVPMVATV) multimer analysis was performed on patients with HLA A*0201+ and A*0206+ donors as this multimer binds both HLA-A*0201 and HLA-A*0206-restricted CMV-specific T cells.21 PBMC were co-stained with CD8-PC5, CD3-FITC and isotype-PE control antibody or PE-conjugated multimers (Proimmune, Oxford, England), previously titrated for optimal staining. PBMC from HLA A2- healthy donors served as additional negative controls. EBV (HLA-A2 GLCTLVAML) multimers were also used. A minimum of 100 multimer-positive events was acquired. The percentage of multimer-positive cells in the CD3+/CD8+ lymphocyte gate was expressed as a proportion of the CD8+ cells (with the negative control value subtracted).

**Definitions and statistical aspects**

Data were analyzed using the Statistical Package for the Social Sciences (SPSS) (v. 10.0) software (SPSS Inc., Chicago, IL, USA). Associations between categorical variables was assessed using the χ-squared test. Overall survival was estimated using the method of Kaplan and Meier.22 Transplant-related mortality (TRM), and relapse were calculated by the cumulative-incidence method using NCSS software (with relapse and TRM as the competing risks respectively).23 To summarize the overall kinetics of immune reconstitution, the area under the curve (AUC) for each lymphocyte subset was calculated using the trapezoidal rule. To analyze categorical factors affecting immune reconstitution, mean AUC at 4, 6 and 12 months was compared between groups using a 2 sample t test (Graphpad Prism v4). P values less than 0.05 were considered significant.
Results

Engraftment and Chimerism

Cell doses infused are detailed in Table 1. Two patients failed to engraft with one dose of \textit{ex vivo} manipulated BM. UPN019 failed to engraft after receiving \textit{ex vivo} manipulated BM from an initial BM harvest of only $0.7 \times 10^6$ CD34$^+$ cells/kg. An additional dose of \textit{ex vivo} alloanergized BM was administered on D+40 from a second BM harvest from the same donor ($0.6 \times 10^6$ CD34$^+$ cells/kg) but no engraftment occurred. This patient subsequently underwent nsTCD CD34-selected peripheral blood HSCT at another center, with the other parent as donor, engrafted without aGVHD but developed fatal disseminated zoster infection. UPN004 received $2.3 \times 10^6$ CD34$^+$ cells/kg but had not engrafted by D+30. An additional dose of \textit{ex vivo} alloanergized BM ($0.6 \times 10^6$ CD34$^+$ cells/kg) was administered from a second BM harvest from the same donor on D+35 without reconditioning and engraftment occurred at D+54. Two patients died (D+8 and D+22) too early to evaluate for engraftment. Of the remaining 20 patients, neutrophil engraftment occurred in all at a median D+21 (range D+13-29) and 11 (55\%) achieved an unsupported platelet count > 20,000/µL at a median D+46 (range D+19-204) with the remaining patients dying before platelet recovery. All patients who engrafted achieved 100\% donor chimerism at the first point of testing.

Toxicity

Twelve patients died of TRM. Two died of fungal infection, 2 from identified bacterial septicemia and one from a syndrome consistent with bacterial sepsis (organism unidentified). Four of these infection-related deaths occurred in patients with PD at BMT, whereas only one occurred in patients in CR. Five patients died from non-infectious toxicity; 2 from multi-organ failure (one of whom also had histological evidence of cerebral toxoplasmosis post-mortem), one from diffuse alveolar hemorrhage and one from hepatic veno-occlusive disease. One patient, for whom aGvHD was considered the primary cause of death, died with radiological evidence of fungal sinus infection while receiving immunosuppressive therapy. One patient was found dead at home, cause undetermined. Median time of TRM was D+53: three-quarters of toxic deaths occurred before D+100 and all before D+200 (Table 2). Cumulative incidence of TRM was 50\%. Recipient age (above median) was the only factor significantly associated with overall
TRM. Median duration of hospitalization for the 13 patients who survived to discharge was 70 days (range 31-100).

**GvHD**

Eight of 21 evaluable patients (38%) had clinical findings consistent with aGVHD, clinically graded B (n=3), C (n=4) and D (n=1) diagnosed at a median time of D+38 (range 20-64). In addition, UPN017 developed diarrhea in the day prior to death from VOD, and post-mortem GI biopsies were consistent with aGvHD. Eight patients received treatment with systemic corticosteroids with only 2 requiring additional treatment. All patients responded with improvement of symptoms attributable to aGvHD. UPN 018 developed presumed sinus fungal infection and died while on immunosuppressive treatment for aGvHD. No other death was attributable to aGvHD (Table 3). There was no significant difference in median infused cell doses of CD34+, CD3+, CD4+ or CD8+ cells in assessable patients who developed aGvHD and those who did not. Nine patients had alloreactive cell frequencies estimated by hTLPf assay before and after *ex vivo* manipulation of BM, 7 of whom were assessable for aGvHD. There was no significant difference in median hTLPf (or dose) in those who developed aGvHD and those who did not. Only one of 12 evaluable patients (8%) developed *de novo* chronic GvHD (with GI tract and skin involvement), on D+145 after discontinuing aGvHD prophylaxis on D+122, resolving after receiving slowly-tapering immunosuppression until D+355. The cumulative incidence of cGvHD (at 8 years) was only 8%.

**Viral reactivation and infection**

Of 11 at-risk patients (donor and/or recipient seropositive for CMV), 5 patients experienced a total of 7 episodes of CMV reactivation (45%, Table 2). UPNs 004, 021 and 024 reactivated on single occasions at D+64, D+32 and D+44 respectively. All cleared CMV antigen with 3 days of anti-viral treatment. UPN 018 reactivated on D+37 and again on D+103, clearing CMV antigen rapidly after treatment with ganciclovir on both occasions. UPN 009, who had a history of CMV-associated acute hepatic necrosis requiring intensive care immediately prior to BMT reactivated on D+32 and cleared CMV antigen after 14 days of treatment with Foscarnet, reactivating again on D+150, clearing CMV antigen by D+154 after further Foscarnet treatment. No CMV disease and no other clinically significant viral infections or post-transplant lymphoproliferative disorder (PTLD) occurred.
Immune Reconstitution

Absolute lymphocyte count (ALC) recovery, determined in all 21 patients surviving beyond D+30, occurred rapidly (Figure 1A). The median ALC at D+30 ($\text{ALC}_{\text{D+30}}$) was $0.42 \times 10^9$/L. Although no pre- or post-BMT factors were statistically significantly associated with the $\text{ALC}_{\text{D+30}}$ there was a trend towards a higher $\text{ALC}_{\text{D+30}}$ in surviving patients compared to those that died of any cause ($p=0.10$, paired t test).

Eight of 9 evaluable patients surviving without relapse beyond D+100 were assessed for T cell subset reconstitution. CD4$^+$ T cell reconstitution (Figure 1B) was rapid with a median CD4$^+$ count of 90/µL at 1 month and almost 500/µL at 3 months. 3/8 patients achieved CD4$^+$ T cell counts above 200/µL by 2 months, 5/8 had CD4$^+$ counts above 200 /µL by 4 months and all by 9 months. CD8$^+$ T cell reconstitution was also rapid, with 5/8 patients achieving CD8$^+$ counts greater than 200/µL at 2 months, 7/8 by 4 months and all by 12 months (Figure 1C). B cell reconstitution was less rapid, although the median CD19 count was greater than 200/µL by 4 months (Figure 1D). We saw an early peak in CD56$^+$ NK cell immune reconstitution at 3 months (Figure 1E). Reconstituting CD4$^+$ T cells were predominantly memory cells and emergence of naïve CD4$^+$ T cells was not seen until 6 months (Figure 1F). In 2 patients in which CD8 memory cell subsets were measured by dual-color CD45RA and CD62L expression, the majority of reconstituting memory CD8 cells had a CD45RA$^-$ CD62L$^-$ effector memory phenotype (data not shown). Endogenous immunoglobulin production recovered rapidly (Figure 1G) and IVIg replacement was discontinued at a median of 4 months post-BMT.

To evaluate factors affecting the kinetics of T cell reconstitution, AUC analysis was performed (Table 4). There was significantly greater CD4$^+$ T cell AUC at 4 months in patients aged below (compared to those aged above) the median, and there was also a trend towards greater CD4$^+$ T cell AUC in younger patients at 12 months and CD8$^+$ T cell AUC at 4 months. Neither malignant versus non-malignant diagnosis nor administered CD34$^+$, CD4$^+$ and CD8$^+$ cell doses had any effect on CD4$^+$ or CD8$^+$ T cell AUC at 4, 6 or 12 months. Similarly, there was no significant difference in CD4$^+$ or CD8$^+$ T cell AUC at 4, 6 or 12 months between patients who developed aGvHD and those who did not. However, although no significant difference was seen in CD4$^+$ T cell AUC at 3, 6 or 12 months, there was a trend towards greater CD8$^+$ T cell AUC at 4 months and 6 months.
and significantly greater CD8+ T cell AUC at 12 months in those that reactivated CMV compared to those who did not.

The more rapid expansion of CD8+ T cells in the 3 evaluated patients who reactivated CMV (UPNs 009, 021 and 024) compared to the 5 who did not is shown in Figure 2A. Serial frequencies of CMV-specific pentamer+ CD8+ T cells were determined in UPN 021 and 024, who both received BMT from HLA A2+ donors, to determine if the CD8+ T cell expansion included significant populations of CMV-specific T cells (shown for UPN 024 in relation to a single episode of CMV antigenemia in Figure 2B). Absolute levels of CMV-specific pentamer+ CD8+ T cells were detectable at levels above 10 cells/µL as early as D+50 and these levels were sustained (Figure 2C). A similar in vivo expansion of CMV-specific CD8+ T cells occurred in UPN 021, who in contrast to UPN024, received alloanergized BMT from an HLA A2+ CMV-seronegative donor. CMV-specific pentamer+ CD8+ T cells were not detectable in the donor peripheral blood pre-BMT or in the donor BM after alloanergization prior to infusion, but became detectable following a single episode of CMV antigenemia at D+37. A sustained expansion of CMV-specific pentamer+ CD8+ T cells was seen and no further episodes of CMV antigenemia occurred (Figure 2D).

A rapid and sustained expansion of EBV-specific pentamer+ CD8+ T cells was also observed in all 4 evaluated patients with HLA A2+ EBV-seropositive donors (Figure 2E).

Outcome
Actuarial event-free survival (EFS) was 33% at 10 years for the whole patient cohort. EFS was significantly higher in patients aged <18 years than in those aged >18 years. The difference in EFS in pediatric and adult patients was due to increased early TRM in adult patients (Figure 3 Panels A and B). No other factors statistically significantly affected EFS. Three of the 4 patients with hematological malignancies who relapsed/progressed did so before D+100. Of the 7 patients transplanted in second (or higher) CR, one relapsed at 48 months, 4 remained disease-free until the time of death from TRM (median D+48, range D+21-176) and 2 survive in CR at D+3909 and D+2647 (UPN 007 and 0024, respectively). Fourteen patients had PD at the time of BMT; 3 progressed at D+50, +60, and +90 (UPNs 016, 011 and 019 respectively); 8 died in clinical remission/without evidence of disease progression at a median of D+37 (range 8-
159) and 3 survive in CR at D+3708, +3520 and +2966 (UPNs 009, UPN 012 and UPN020 respectively). Three of 4 patients who relapsed/progressed had PD at time of BMT. All 3 patients transplanted for BMF are free of disease at a median of D+3394 (range 2890-3694). The cumulative incidence of relapse/progression (competing risk death in CR/without progression) was 17% (Figure 3 Panel C). Eight patients survive with a median follow-up of 7.2 years (Table 2). All are free of disease, have normal peripheral blood counts and immunoglobulin levels, and have demonstrated humoral responses to post-BMT vaccinations. None are on immunosuppressive or anti-infectious medications and all have normal performance scores. Actuarial overall survival at 10 years was 33% (Figure 3 Panel D).
Discussion

In these two Phase 1 clinical studies, myeloablative haploidentical BMT containing large numbers of alloanergized donor T cells resulted in acceptable engraftment and less severe aGVHD than historical reports using unmanipulated haploidentical BM. Moreover, alloanergized BMT was associated with rapid T cell reconstitution, in vivo expansion of pathogen-specific CD8+ T cells and early recovery of immunoglobulin production. While substantial early TRM was observed in these very high risk patients, no TRM was related to viral infection, and long-term disease-free survival uncomplicated by cGVHD was seen in one third of patients.

Alloanergization did not appear to impair immune reconstitution. The median ALC D+30 in our study (0.42 x 10^9/L), evaluated for all patients who survived to engraftment, was similar to that reported after conventional unmanipulated BMT from HLA-matched sibling donors (0.49 x 10^9/L). Both ALC and CD4+ T cell counts rose rapidly by 3 months. The presence of CD4+ cells with a predominantly memory phenotype during the initial period post-BMT, when rapid recovery of T cell counts occurred, was consistent with peripheral expansion of mature donor T cells contained in the BM graft. In contrast naïve CD4+ T cells were not detectable in the periphery until 6 months post-BMT. Although the favorable prognostic value of early ALC recovery reported after adult and pediatric matched sibling HSCT has not been established after haploidentical BMT, and cannot be inferred from our limited data, the observed recovery of ALC suggests donor lymphocytes maintained their capacity to expand in vivo after alloanergization. Indeed, rapid reconstitution of T cell subsets occurred in patients evaluable for immune reconstitution (all of whom were < 18 years of age). The median time to achieve a CD4+ T cell count of 300/µL was 3 months, faster than the 8 months observed after haploidentical CD34-selected BMT in children reported by Eyrich et al. and comparable to the 4 months observed after CD34-selected HSCT followed by infusion of 10^5 selectively allodepleted donor T cells/kg reported by Amrolia et al.

In contrast to a recent study of immune reconstitution after haploidentical HSCT, our AUC analysis demonstrated that CD4+ and CD8+ T cell recovery was not adversely affected by the presence of aGVHD, probably reflecting the modest dose and duration of corticosteroid treatment in most patients who developed aGVHD. Conversely, the
absence of marked acceleration of T cell recovery in patients with aGVHD suggests that alloantigen-driven expansion of alloenergized donor T cells in vivo did not occur, (or if it did, more rapid expansion was not associated with clinically apparent aGvHD). However, the marked acceleration of CD8+ reconstitution seen in patients with CMV reactivation may have been driven at least in part by CMV-antigens. Indeed, the expansion of CMV-specific CD8+ T cells seen in patients with HLA A2+ donors (both CMV-seropositive and -seronegative) demonstrates that alloenergized donor T cells retained their capacity to mount both effective memory and primary CMV responses in vivo after episodes of CMV reactivation. In both cases, absolute numbers of CMV-specific CD8+ T cells above the level shown to be protective against CMV (10/µl) after HSCT30 were rapidly attained and sustained. No repeat CMV reactivation occurred in these patients. Although we did not prospectively monitor patients for EBV reactivation, we saw no cases of PTLD despite transplantation of donor BM replete with B cells from 22 EBV seropositive donors. EBV-related PTLD remains a significant problem after nsTCD haploidentical HSCT, especially after transplantation of grafts containing significant numbers of donor B cells.4,31 A protective effect against EBV+ PTLD may have been conferred by expansion of donor EBV-specific CD8+ T cells, as directly demonstrated in patients with HLA A2+ EBV+ donors, consistent with previously reported efficacy of donor lymphocyte infusions in treating EBV+ PTLD.32

The recovery of B cell counts and independently sustained immunoglobulin levels demonstrated rapid functional B cell recovery in vivo. Unfortunately, a limitation of our immune reconstitution data is the absence of data similarly demonstrating functional pathogen-specific CD8+ responses. However, the low frequency of CMV reactivation and absence of both CMV disease and EBV+ PTLD strongly suggest that the expanded populations of CMV- and EBV-specific CD8+ cells we observed retained function in vivo. In common with other reports of graft manipulation techniques to selectively reduce alloreactivity, we also lack data demonstrating antigen-specific CD4+ responses. However, we have recently demonstrated that CMV-specific CD4+ T cells are retained after alloenergization in vitro. If such cells are also retained after alloenergized BMT, they might be capable of providing pathogen-specific memory responses needed to maintain CMV immunity in vivo post-BMT.33
The optimum dose of alloanergized T cells able to improve pathogen-specific immune reconstitution without significant GvHD remains to be determined, and may be significantly lower than the doses in this study. Although administration of alloanergized donor T cells was associated with aGVHD in 43% of patients, three-quarters of those with aGVHD responded to brief courses of corticosteroids. Steroid-refractory aGVHD only occurred in 2 patients (9%), despite a median T cell dose 2 logs greater than the dose (8-20 x 10⁴/kg) at which 30% of children undergoing nsTCD haploidentical HSCT were observed to develop steroid-refractory GvHD and 3 logs above a published threshold dose below which severe aGVHD was reliably prevented in the haploidentical setting.³⁴ ³⁵ AGvHD did not occur more often in those patients receiving CD3⁺, CD4⁺ or CD8⁺ T cell doses above median levels, and although alloreactive precursor frequency data was available on a relatively small proportion of patients, we also did not see an association between residual in vitro CD4⁺ alloresponses and aGVHD occurrence. A potential explanation for this observation might be that residual alloresponses after alloanergization are mediated in vivo by CD28⁻ donor T cells (which are predominantly CD8⁺), γδ T cells, or successfully alloanergized CD4⁺ donor T cells that had their anergic state temporarily reversed by high levels of cytokines in vivo.³⁶ ³⁷ We also observed an unusual distribution of organ involvement in patients with aGVHD, predominantly limited to the GI tract, suggesting that donor T cells with residual alloreactivity preferentially targeted the gut. Murine GI-specific alloresponses mediated by CD8⁺ cells are not abrogated by CD28-blockade; such cells may receive CD28-independent positive costimulatory signals via CD134.³⁸ ³⁹ Alternatively, GI tract aGVHD may have been disproportionately driven by non-allospecific mechanisms.

Our strategy resulted in a low cumulative incidence of cGvHD (8%) comparable to nsTCD haploidentical HSCT,⁴ ⁴⁰ and surviving patients were therefore free of cGvHD-associated morbidity and immunosuppression. In contrast, recent studies of T-replete G-CSF-stimulated haploidentical BMT have reported up to 70% cGvHD (and nearly 50% extensive cGvHD) in both adults and children.⁴¹

Recent series of nsTCD haploidentical HSCT have reported significant rates of late TRM in both children and adults, predominantly from delayed T cell immune reconstitution and viral infections, resulting in overall TRM of 40-60%.⁴ ⁴⁰ ⁴² ⁴³ In contrast we saw no significant morbidity or mortality related to viral infection and no TRM after 6 months
post-BMT. While the incidence of CMV reactivation in our study was lower than that in recent reports of nsTCD haploidentical HSCT, many such studies used detection techniques with greater sensitivity. However, the CMV reactivation observed was associated with high rates of fatal CMV disease in many such studies; up to 60% of TRM was related to CMV infection and up to 16% of all patients transplanted dying from human herpes virus-related infections, the vast majority from CMV. In our studies, there was no CMV-related TRM. Furthermore, we saw no mortality from adenovirus. In contrast, adenovirus-related deaths occurred in 10-18% of children with acute leukemia receiving nsTCD haploidentical HSCT (either by addition of alemtuzumab or CD34-selection of donor grafts).

We are encouraged that alloanergized BMT was associated with low incidences of steroid-refractory aGvHD, cGvHD and viral infection, and with rapid T cell reconstitution, a key area of therapeutic weakness in many other current strategies for haploidentical HSCT. The adoptive transfer of non-alloreactive donor T cell doses as low as $10^5$/kg has also been associated with improved viral-specific immune reconstitution after haploidentical CD34-selected HSCT, although relapse remained a significant problem, occurring in 7 of 16 patients. While early TRM and small patient numbers preclude any conclusions regarding long-term disease control, cumulative incidence of relapse/progression in our study was low at 17% with long follow-up.

The major limitation to our strategy, however, was significant early TRM, particularly in adult patients. While the engraftment rate was comparable to alternate strategies of haploidentical HSCT, the period of neutropenia was relatively long after alloanergized BMT (median 21 days). This may have contributed to infection-related TRM, all of which was caused by bacterial or fungal pathogens. Although the only factor significantly associated with risk of overall TRM was patient age, infection-related TRM occurred in 4 of 14 patients with hematological malignancy with PD, compared to only one of 10 patients in CR or with BMF, suggesting that patients with PD, most of whom had been heavily pre-treated, were at greater risk of infection-related TRM. Strategies employing high doses of CD34+ HSCs to achieve more rapid neutrophil recovery and reduced-intensity conditioning to minimize regimen-related toxicity have been successfully utilized in haploidentical HSCT and could reduce early TRM in the context of strategies utilizing alloanergization.
Encouraged by the rapid immune reconstitution after alloanergized BMT but mindful of the early TRM, we are undertaking a new study utilizing alloanergized donor T cells in haploidentical HSCT, modifying the strategy with megadose CD34+ HSCT and reduced intensity conditioning in older patients to minimize early TRM, followed by dose-escalating delayed infusion of alloanergized T donor cells to define the optimal dose of T cells able to improve immune reconstitution without causing severe GvHD.
Acknowledgements
The authors would like to thank the hospital staff who provided exceptional medical care and especially the patients and families who chose to participate in the studies.

These studies were supported by grants from the National Institutes of Health (P01 AI41584 and P50 HL54785) and the Frank J Hanna Jr Fund.

JKD is a recipient of a Career Development Award from the Leukemia and Lymphoma Society.

Author Contributions
JKD performed immune reconstitution assays, collected data, performed statistical analysis and wrote the manuscript
JGG designed the clinical study, provided patient care and critically reviewed the manuscript
LLB provided patient care and collected data
DY performed immune reconstitution assays
LMN developed and provided important reagents and laboratory resources and critically reviewed the manuscript
ECG conceived and designed the clinical study, provided patient care and wrote the manuscript

Conflict of Interest Disclosure
The authors have no conflicts of interest to disclose
References

33. Davies JK, Yuk, D, Nadler, L.M., Guinan, E.C. T cells can be rendered hyporesponsive to alloantigen without loss of pathogen or tumor immunity. Transplantation. 2008;IN PRESS
### Table 1  Patient, Donor and Graft Characteristics

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age</th>
<th>Sex</th>
<th>Disease</th>
<th>Status†</th>
<th>Donor</th>
<th>HLA Mismatches*</th>
<th>Infused Cell Doses, x 10⁶/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>GvH  HvG CD34⁺ CD3⁺ CD4⁺ CD8⁺</td>
<td></td>
</tr>
<tr>
<td>001</td>
<td>4</td>
<td>F</td>
<td>B-ALL</td>
<td>CR2</td>
<td>Mother</td>
<td>4  4   6.5  55  41  18</td>
<td></td>
</tr>
<tr>
<td>002</td>
<td>26</td>
<td>M</td>
<td>DLBC</td>
<td>PD</td>
<td>Half-brother</td>
<td>1  2</td>
<td>2.0  17  13  07</td>
</tr>
<tr>
<td>003</td>
<td>6</td>
<td>M</td>
<td>T-ALL</td>
<td>CR2</td>
<td>Father</td>
<td>5  5   2.1  16  11  06</td>
<td></td>
</tr>
<tr>
<td>004</td>
<td>15</td>
<td>M</td>
<td>B-ALL</td>
<td>PD⁺</td>
<td>Sister</td>
<td>4  4</td>
<td>2.3  32  21  10</td>
</tr>
<tr>
<td>005</td>
<td>7</td>
<td>F</td>
<td>AML</td>
<td>PD⁺</td>
<td>Father</td>
<td>4  4</td>
<td>NE$  26  10  09</td>
</tr>
<tr>
<td>006</td>
<td>23</td>
<td>M</td>
<td>T-NHL</td>
<td>PD</td>
<td>Mother</td>
<td>4  4</td>
<td>1.3  12  04  07</td>
</tr>
<tr>
<td>007</td>
<td>7</td>
<td>F</td>
<td>B-ALL</td>
<td>CR2</td>
<td>Mother</td>
<td>3  3</td>
<td>1.3  12  09  02</td>
</tr>
<tr>
<td>008</td>
<td>20</td>
<td>F</td>
<td>T-ALL</td>
<td>CR3</td>
<td>Father</td>
<td>3  3</td>
<td>3.7  52  28  20</td>
</tr>
<tr>
<td>009</td>
<td>12</td>
<td>M</td>
<td>AML</td>
<td>PD⁺</td>
<td>Father</td>
<td>4  4</td>
<td>1.8  42  16  10</td>
</tr>
<tr>
<td>010</td>
<td>1.5</td>
<td>F</td>
<td>AMT</td>
<td>N/A</td>
<td>Sister</td>
<td>2  2</td>
<td>3.9  20  11  07</td>
</tr>
<tr>
<td>011</td>
<td>0.5</td>
<td>F</td>
<td>AML</td>
<td>PD⁺</td>
<td>Father</td>
<td>4  3</td>
<td>6.0  47  22  22</td>
</tr>
<tr>
<td>012</td>
<td>16</td>
<td>M</td>
<td>T-ALL</td>
<td>PD⁺</td>
<td>Father</td>
<td>4  4</td>
<td>NE$  31  16  10</td>
</tr>
<tr>
<td>013</td>
<td>4</td>
<td>F</td>
<td>SCN</td>
<td>N/A</td>
<td>Mother</td>
<td>4  4</td>
<td>6.3  66  27  37</td>
</tr>
<tr>
<td>014</td>
<td>19</td>
<td>M</td>
<td>B-ALL</td>
<td>PD</td>
<td>Half-brother</td>
<td>4  4</td>
<td>NE$  23  14  11</td>
</tr>
<tr>
<td>015</td>
<td>45</td>
<td>F</td>
<td>B-ALL</td>
<td>CR2</td>
<td>Brother</td>
<td>2  2</td>
<td>1.4  20  15  08</td>
</tr>
<tr>
<td>016</td>
<td>50</td>
<td>F</td>
<td>AML</td>
<td>PD</td>
<td>Son</td>
<td>3  2</td>
<td>3.5  48  32  23</td>
</tr>
<tr>
<td>017</td>
<td>6</td>
<td>F</td>
<td>AML</td>
<td>PD</td>
<td>Father</td>
<td>2  2</td>
<td>NE$  34  19  2.4</td>
</tr>
<tr>
<td>018</td>
<td>42</td>
<td>M</td>
<td>MDS</td>
<td>PD</td>
<td>Sister</td>
<td>2  2</td>
<td>1.9  15  08  09</td>
</tr>
<tr>
<td>019</td>
<td>16</td>
<td>F</td>
<td>AML</td>
<td>PD</td>
<td>Mother</td>
<td>4  3</td>
<td>1.1  07  04  02</td>
</tr>
<tr>
<td>020</td>
<td>12</td>
<td>F</td>
<td>B-ALL</td>
<td>PD</td>
<td>Brother</td>
<td>4  5</td>
<td>4.2  129 18  95</td>
</tr>
<tr>
<td>021</td>
<td>6</td>
<td>M</td>
<td>AMT</td>
<td>N/A</td>
<td>Father</td>
<td>2  3</td>
<td>3.1  68  35  31</td>
</tr>
<tr>
<td>022</td>
<td>6</td>
<td>F</td>
<td>B-ALL</td>
<td>PD</td>
<td>Father</td>
<td>4  4</td>
<td>12.3 11  12  31</td>
</tr>
<tr>
<td>023</td>
<td>4</td>
<td>M</td>
<td>B-ALL</td>
<td>CR2</td>
<td>Father</td>
<td>4  4</td>
<td>3.8  21  18  12</td>
</tr>
<tr>
<td>024</td>
<td>4</td>
<td>F</td>
<td>B-ALL</td>
<td>CR2</td>
<td>Father</td>
<td>4  4</td>
<td>9.8  43  24  13</td>
</tr>
</tbody>
</table>

* Median Cell Doses

---

| Number of mismatched between donor and recipient at A, B, Cw (where known), DR and DQ loci at serological typing level
† Disease Status Summary: 7/21 CR, 14/21 PD, 3 N/A (Bone Marrow Failure patients)
‡ Patients who had failed to attain remission with induction therapy
§ Peripheral blood leukaphereses of UPN 005, 012, 014 and 017 were contaminated with CD34⁺ leukemic blasts rendering infused CD34⁺ cell count determination non-evaluable
UPN indicates Unique Patient Number; HvG, Host-versus-Graft; GvH, Graft-versus-host; F, Female; M, Male; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; DLBC diffuse large B cell non-Hodgkin lymphoma; SCN, severe congenital neutropenia; MDS, myelodysplastic syndrome; AMT, amegakaryocytic thrombocytopenia; CR, complete remission; PD, progressive disease; N/A, not assessable; NE, Non-Evaluable; POS, positive; NEG, negative
### Table 2 Toxicity and Outcome

<table>
<thead>
<tr>
<th>UPN</th>
<th>Age</th>
<th>Status</th>
<th>EBV</th>
<th>CMV</th>
<th>GvHD</th>
<th>Relapse</th>
<th>OS</th>
<th>Day</th>
<th>COD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>D/R</td>
<td>PTLD</td>
<td>D/R</td>
<td>Reactivation</td>
<td>Disease</td>
<td>Acute*</td>
<td>Chronic</td>
</tr>
<tr>
<td>001</td>
<td>4</td>
<td>CR2</td>
<td>+/+</td>
<td>No</td>
<td>+/+</td>
<td>No</td>
<td>No</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>002</td>
<td>26</td>
<td>PD</td>
<td>UNK/+</td>
<td>No</td>
<td>-/-</td>
<td>No</td>
<td>No</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>003</td>
<td>6</td>
<td>CR2</td>
<td>+/+</td>
<td>No</td>
<td>-/-</td>
<td>No</td>
<td>No</td>
<td>B</td>
<td>No</td>
</tr>
<tr>
<td>004</td>
<td>15</td>
<td>PD</td>
<td>+/+</td>
<td>No</td>
<td>+/+</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>005</td>
<td>7</td>
<td>PD</td>
<td>+/+</td>
<td>No</td>
<td>-/-</td>
<td>No</td>
<td>No</td>
<td>C</td>
<td>N/A</td>
</tr>
<tr>
<td>006</td>
<td>23</td>
<td>PD</td>
<td>+/+</td>
<td>No</td>
<td>-/-</td>
<td>No</td>
<td>No</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>007</td>
<td>7</td>
<td>CR2</td>
<td>+/+</td>
<td>No</td>
<td>-/+</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>008</td>
<td>20</td>
<td>CR3</td>
<td>+/+</td>
<td>No</td>
<td>-/-</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>009</td>
<td>12</td>
<td>PD</td>
<td>+/+</td>
<td>No</td>
<td>-/+</td>
<td>D+32,150**</td>
<td>No</td>
<td>No</td>
<td>Yes†</td>
</tr>
<tr>
<td>010</td>
<td>1.5</td>
<td>N/A</td>
<td>+/-</td>
<td>No</td>
<td>-/-</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>011</td>
<td>0.5</td>
<td>PD</td>
<td>+/+</td>
<td>No</td>
<td>-/-</td>
<td>No</td>
<td>No</td>
<td>B</td>
<td>N/A</td>
</tr>
<tr>
<td>012</td>
<td>16</td>
<td>PD</td>
<td>+/+</td>
<td>No</td>
<td>-/-</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>013</td>
<td>4</td>
<td>N/A</td>
<td>+/+</td>
<td>No</td>
<td>+/-</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>014</td>
<td>19</td>
<td>PD</td>
<td>+/+</td>
<td>No</td>
<td>+/-</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>015</td>
<td>45</td>
<td>CR2</td>
<td>+/+</td>
<td>No</td>
<td>-/-</td>
<td>No</td>
<td>No</td>
<td>C</td>
<td>No</td>
</tr>
<tr>
<td>016</td>
<td>50</td>
<td>PD</td>
<td>+/+</td>
<td>No</td>
<td>-/-</td>
<td>No</td>
<td>No</td>
<td>B</td>
<td>N/A</td>
</tr>
<tr>
<td>017</td>
<td>6</td>
<td>PD</td>
<td>+/+</td>
<td>No</td>
<td>+/-</td>
<td>No</td>
<td>No</td>
<td>B</td>
<td>N/A</td>
</tr>
<tr>
<td>018</td>
<td>42</td>
<td>PD</td>
<td>+/+</td>
<td>No</td>
<td>+/+</td>
<td>D+37,103</td>
<td>No</td>
<td>D</td>
<td>No</td>
</tr>
<tr>
<td>019</td>
<td>16</td>
<td>PD</td>
<td>+/-</td>
<td>No</td>
<td>-/-</td>
<td>No</td>
<td>No</td>
<td>N/A†</td>
<td>N/A</td>
</tr>
<tr>
<td>020</td>
<td>12</td>
<td>PD</td>
<td>+/+</td>
<td>No</td>
<td>-/-</td>
<td>No</td>
<td>No</td>
<td>C</td>
<td>No</td>
</tr>
<tr>
<td>021</td>
<td>6</td>
<td>N/A</td>
<td>+/+</td>
<td>No</td>
<td>-/+</td>
<td>D+32</td>
<td>No</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>022</td>
<td>6</td>
<td>PD</td>
<td>+/-</td>
<td>No</td>
<td>-/-</td>
<td>No</td>
<td>No</td>
<td>N/A</td>
<td>No</td>
</tr>
<tr>
<td>023</td>
<td>4</td>
<td>CR2</td>
<td>+/+</td>
<td>No</td>
<td>-/+</td>
<td>No</td>
<td>No</td>
<td>N/A</td>
<td>No</td>
</tr>
<tr>
<td>024</td>
<td>4</td>
<td>CR2</td>
<td>+/+</td>
<td>No</td>
<td>+/-</td>
<td>D+44</td>
<td>No</td>
<td>C</td>
<td>No</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9/21†</td>
<td>5/21‡</td>
</tr>
</tbody>
</table>
CMV Risk Summary: 13/24 low risk (Donor AND Recipient CMV seronegative) 11/24 high risk (Donor AND/OR recipient CMV seropositive)

* IBMTR Severity Index Criteria
† cGvHD resolving after 6 months immunosuppression
‡ Diagnosed on histological appearance of GI biopsies post mortem
§ UPN018 died of probable fungal infection (radiological evidence) whilst on immunosuppression for aGvHD
¶ UPN019 failed to engraft despite two infusions on alloanergized BMT
# IBMTR grades B-D
‡ IBMTR grades C-D
** UPN 009, who had a history of prior CMV disease, was given extended anti-CMV treatment.

Abbreviations are explained in Table 1. In addition, D/R indicates Donor/Recipient serostatus; EBV, Epstein- Barr Virus; PTLD, Post-Transplant Lymphoproliferative Disorder; CMV, Cytomegalovirus; TRM, Treatment-related Mortality; GvHD, Graft-versus-Host Disease; OS, Overall survival; COD, cause of death; DAH, Diffuse Alveolar Hemorrhage; VOD, Veno-Occlusive Disease; MOF, Multi-Organ Failure
Table 3 Acute GvHD

<table>
<thead>
<tr>
<th>UPN</th>
<th>Day</th>
<th>Organ</th>
<th>Grade*</th>
<th>Treatment</th>
<th>Primary</th>
<th>Response</th>
<th>Secondary</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>003</td>
<td>D+64</td>
<td>GI</td>
<td>B</td>
<td>Corticosteroids</td>
<td>Complete</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>005</td>
<td>D+20</td>
<td>GI</td>
<td>C</td>
<td>Corticosteroids</td>
<td>Complete</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>011</td>
<td>D+49</td>
<td>GI</td>
<td>B</td>
<td>Corticosteroids</td>
<td>Complete</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>015</td>
<td>D+27</td>
<td>Skin, Liver</td>
<td>C</td>
<td>Corticosteroids</td>
<td>Partial</td>
<td>Dacluzimab, MMF</td>
<td>Complete</td>
<td></td>
</tr>
<tr>
<td>016</td>
<td>D+39</td>
<td>Skin</td>
<td>B</td>
<td>Corticosteroids</td>
<td>Complete</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>017</td>
<td>D+38</td>
<td>GI†</td>
<td>B</td>
<td>N/A</td>
<td>N/A</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>018</td>
<td>D+22</td>
<td>GI, Liver</td>
<td>D</td>
<td>Corticosteroids</td>
<td>Partial</td>
<td>Sirolimus, Tacrolimus</td>
<td>Died‡</td>
<td></td>
</tr>
<tr>
<td>020</td>
<td>D+25</td>
<td>GI</td>
<td>C</td>
<td>Corticosteroids</td>
<td>Complete</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>024</td>
<td>D+38</td>
<td>GI</td>
<td>C</td>
<td>Corticosteroids</td>
<td>Complete</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* IBMTR grading criteria
† diagnosed on histological appearance of GI biopsies post mortem
‡ UPN018 died of probable fungal infection (radiological evidence) whilst on immunosuppression for aGvHD

Abbreviations are explained in Table 1. In addition, GI indicates gastro-intestinal; MMF, Mycophenolate Mofetil
Table 4 Univariate analysis of factors influencing AUC for T cell subset immune reconstitution

<table>
<thead>
<tr>
<th>Factor</th>
<th>P value (two-tailed t test between mean AUCs)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CD4⁺ T cells</td>
</tr>
<tr>
<td></td>
<td>4m</td>
</tr>
<tr>
<td>Age (below median vs. above)</td>
<td>0.02*</td>
</tr>
<tr>
<td>Diagnosis (Malignancy vs. BMF)</td>
<td>0.76</td>
</tr>
<tr>
<td>CD34⁺ cell dose (above median vs. not above)</td>
<td>0.59</td>
</tr>
<tr>
<td>CD4⁺ or CD8⁺ T cell dose (above median vs. not above)</td>
<td>0.32</td>
</tr>
<tr>
<td>aGvHD (Grades B-D vs. less than Grade B) †</td>
<td>0.26</td>
</tr>
<tr>
<td>CMV (Reactivation vs. no reactivation) ††</td>
<td>0.43</td>
</tr>
</tbody>
</table>

*Statistically significant
†aGvHD (Grade B-D) n=3 vs. less than Grade B aGvHD (n=5)
‡CMV reactivation n=3 vs. no CMV reactivation n=5

Abbreviations are explained in Table 1. In addition, AUC indicates Area Under Curve.
Figure Legends

Figure 1  Immune Reconstitution after Alloanergized Haploidentical Bone Marrow Transplantation.

Panel A shows median Absolute Lymphocyte Count (ALC) Recovery (x10^9/L). Panel B shows CD4^+ T cell reconstitution, Panel C CD8^+ T cells, Panel D CD19^+ B cells and Panel E NK cells. Median absolute cell counts/µL are shown for panels B-E. Panel F shows the proportion of naive (CD45RA^+) and memory (CD45RO^+) cells in reconstituting CD4^+ T cells. Panel G shows median trough immunoglobulin levels for IgG, IgA and IgM (G/L).

Figure 2  Pathogen-specific Immune Reconstitution after Alloanergized Haploidentical Bone Marrow Transplantation.

Panel A shows CD8^+ T cell reconstitution in patients with and without CMV reactivation. Mean values for absolute CD8^+ cells/µL are shown. Panel B shows expansion of CMV-specific pentamer^+ CD8 cells in vivo in UPN 24 in relation to a single episode of CMV antigenemia. Panel C shows absolute levels of CMV-specific pentamer^+ CD8 cells in UPN 024 (grey dashed line indicates 10 cells/µL). Panel D shows a primary CMV-specific response in a CMV-seropositive patient (UPN 021) who received alloanergized BMT from an HLA A2^+ CMV-seronegative donor. CMV-specific pentamer^+ CD8 cells were not detectable in the donor pre-transplant or in the donor bone marrow after alloanergization prior to infusion, but became detectable following a single episode of CMV antigenemia at D+37. A sustained expansion of CMV-specific pentamer^+ CD8 cell was seen and no further episodes of CMV antigenemia occurred. Numbers in gated regions show HLA A2-restricted peptide (NLV) -pentamer^+ cells, expressed as percentage of CD3^+CD8^+ T cells. BM, Bone Marrow, PB, Peripheral Blood. ND = not detectable above negative control frequency in pentamer-stained HLA A2^+ donor cells. Panel E shows rapid and early expansion of EBV-specific pentamer^+ CD8 cells in 4 patients who received alloanergized BMT from HLA A2^+ EBV-seropositive donors.
Figure 3  Long-term Outcome after Alloanergized Haploidentical Bone Marrow Transplantation

Panel A shows actuarial event-free survival (EFS) in patients aged above and below 18 years. The difference in EFS in pediatric and adult patients was due to an increased incidence of early treatment-related mortality (TRM) in adult patients Panel B. P values for Panels 1 and 2 are for the Log-Rank Test. Cumulative incidence of relapse/progression (competing risk death without relapse/progression) was low at 17% for the whole patient cohort (Panel C) and actuarial Overall Survival at 10 years was 33% (Panel D).
Figure 1 Immune Reconstitution after Alloenergized Haploidentical Bone Marrow Transplantation
Figure 2 Pathogen-specific Immune Reconstitution after Alloanergized Haploidentical Bone Marrow Transplantation
Figure 3 Long-term Outcome after Alloanergized Haploidentical Bone Marrow Transplantation
Outcome of alloanergized haploidentical bone marrow transplantation after ex vivo costimulatory blockade: results of two phase I studies

Jeff K Davies, John G. Gribben, Lisa L Brennan, Dongin Yuk, Lee M Nadler and Eva C. Guinan