A 2-step approach to myeloablative haploidentical stem cell transplantation: a phase 1/2 trial performed with optimized T-cell dosing.

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Gross, Dolores; Carabasi, Matthew; Filicko-O'Hara, Joanne; Kasner, Margaret; Wagner, John L; Colombe, Beth; Cornett Farley, Patricia; O'Hara, William; Flomenberg, Phyllis; Werner-Wasik, Maria; Brunner, Janet; Mookerjee, Bijoyesh; Hyslop, Terry; Weiss, Mark; and Flomenberg, Neal, "A 2-step approach to myeloablative haploidentical stem cell transplantation: a phase 1/2 trial performed with optimized T-cell dosing." (2011). *Department of Medical Oncology Faculty Papers*. Paper 10.  
[https://jdc.jefferson.edu/medoncfp/10](https://jdc.jefferson.edu/medoncfp/10)
As submitted to:

Blood

And later published as:

A TWO-STEP APPROACH TO MYELOABLATIVE HAPLOIDENTICAL STEM CELL TRANSPLANTATION: A PHASE I/II TRIAL PERFORMED WITH OPTIMIZED T CELL DOSING
doi: 10.1182/blood-2011-07-365338

October 27, 2011

vol. 118 no. 17

pp.4732-4739

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GROSSO et al.              T CELL OPTIMIZATION IN HAPLO-HSCT

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Category: Clinical Trials and Observations
Abstract

Studies of haploidentical hematopoietic stem cell transplantation (HSCT) have identified threshold doses of T cells below which severe graft-versus-host (GVHD) is usually absent. However, little is known regarding optimal T cell dosing as it relates to engraftment, immune reconstitution, and relapse. To begin to address this question, we developed a 2 step myeloablative approach to haploidentical HSCT in which 27 patients conditioned with total body irradiation (TBI) were given a fixed dose of donor T cells (HSCT step 1), followed by cyclophosphamide (CY) for T cell tolerization. A CD34 selected HSC product (HSCT step 2) was infused after CY. A dose of 2 x10^8/kg T cells resulted in consistent engraftment, immune reconstitution, and acceptable rates of GVHD. Cumulative incidences of grade III-IV GVHD, non-relapse mortality and relapse-related mortality were 7.4%, 22.2%, and 29.6% respectively. With a follow-up of 28-56 months, 3 year probability of overall survival for the whole cohort is 48% and 75% in patients without disease at HSCT. In the context of CY tolerization, a high, fixed dose of haploidentical T cells was associated with encouraging outcomes especially in good risk patients, and can serve as the basis for further exploration and optimization of this 2 step approach.

Presented in part in abstract form at the 51st annual meeting of the American Society of Hematology, New Orleans, La.

ClinicalTrials.gov Identifier NCT00429143
Introduction

Until recently, HLA haploidentical HSCT has often been associated with disappointing clinical outcomes limiting the widespread application of this approach. Higher rates of infection and relapse, two consequences of the T cell depletion required to prevent severe GVHD in recipients of HLA mismatched grafts, adversely impact long term survival particularly in patients transplanted late in their disease course. Because only a subset of appropriate transplant candidates has an HLA identical sibling or unrelated donor, the development of safer, more efficacious transplant approaches using haploidentical donors would provide potential transplant options for patients who lack well-matched donors.

Based on murine models, clinical approaches to haploidentical transplantation initially relied on aggressive T cell depletion techniques. Ex-vivo T cell depletion with soy bean agglutinin and E rosetting or the use of monoclonal antibodies such as T10B9 resulted in marrow products containing T cell doses in the range of $10^4$ to $10^5$ T cells/kg of recipient body weight. This degree of T cell depletion was associated with the attenuation of severe GVHD and provided consistent engraftment, particularly when anti-thymocyte globulin (ATG) was also administered.\(^1\,^2\) The correlate of a high degree of T cell depletion to avoid GVHD is delayed post-transplant immune recovery,\(^3\,^9\) mortality from infection and relapse,\(^1\,^10\,^16\) and higher rates of graft rejection as compared to T cell-containing regimens.\(^17\) Ruggeri and colleagues\(^18\) demonstrated that the higher relapse rates associated with T cell depletion can be moderated in part by maximizing natural killer (NK) cell alloreactivity. However, infectious mortality still remains an obstacle to long term survival even with this approach.

Much of the data regarding the permissible content of T cells in the donor innocula was necessarily based on the correlation between T cell doses and the development of GVHD as opposed to immune reconstitution. Severe GVHD was infrequent to absent with T cell doses of
less than $1 \times 10^5$/kg in adults undergoing matched sibling HSCT \(^{19}\) and with less than $5 \times 10^4$/kg in children undergoing haploidentical HSCT.\(^{20}\) Huang et al.\(^{21}\) reported encouraging rates of disease-free survival but a cumulative incidence of chronic GVHD that exceeded 50% when a median T cell dose of $2.4 \times 10^9$/kg was administered with ATG to recipients of haploidentical HSCT utilizing combined blood and marrow products from recombinant human granulocyte colony-stimulating factor (G-CSF) primed donors.

The use of T cell add-back,\(^{22}\) selective lymphocyte depletion of donor grafts,\(^{23,24}\) and preemptive DLI\(^{25,26}\) are strategies used after T cell depleted haploidentical HSCT to preserve or restore the beneficial immune reconstituting effects of T cells. Another approach involves the use of post-transplant CY after non-myeloablative marrow grafts from haploidentical donors to preferentially delete activated lymphocytes as opposed to a non-selective depletion of all CD 3+ T cells.\(^{27-29}\) In one report,\(^{28}\) there were low infectious rates and little significant GVHD associated with the infusion of donor products containing a mean number of $4.2 \times 10^7$/kg T cells. Despite the use of these relatively high T cell-containing products, the rejection rate of 13% was higher, and the disease free survival rate was lower, than that reported by Huang et al.\(^{21}\) possibly due to the administration of comparatively less T cells, the use of a non-myeloablative conditioning regimen, or a combination of the two.

These trials demonstrate that outcomes after haploidentical HSCT can be influenced by the dosing, timing, and treatment of donor T cells. Potential barriers to further progress include the lack of consistency in T cell dosing from which to compare and optimize outcomes and methodologies to deliver consistent T cell doses at the time of HSCT. An ideal approach would maximize the number of “safe”, non-alloreactive T cells, avoiding the problems of GVHD while preserving the beneficial effects of T cells with regard to engraftment, infectious complications, and relapse. Moreover, while murine models of transplantation virtually always administer a
fixed dose of T cells to produce more consistent immunologic outcomes, human HSCT grafts contain a more highly variable number of passenger lymphocytes.

We therefore developed a 2 step myeloablative approach to haploidentical HSCT in which our primary goal was to provide a fixed, and ideally maximized, dose of T cells in the context of CY tolerization. Myeloablative rather than reduced intensity conditioning was used to provide more treatment intensity to high-risk patients. In the setting of myeloablative conditioning, we wished to maximize the number of T cells which could be safely administered and to use the higher number of stem cells which can be obtained from peripheral blood rather than marrow to avoid rejection. Because there was no data regarding T cell dosing with CY tolerization in a myeloablative setting, the trial was designed as a phase I/II study in which an optimal dose of T cells would be initially determined, based on the incidences of graft rejection and of GVHD. The goal of the phase II part of the study was to assess whether this optimized dose of cyclophosphamide-tolerized T cells would result in rapid immune constitution and low rates of severe infection, rejection, relapse, and significant GVHD, thus resulting in improved overall survival.

**Methods**

**The 2 step transplant regimen**

Patients received 12 Gy of TBI administered in 8 fractions over 4 days on days -9 to -6. After the last fraction of TBI, a DLI product was administered to deliver a specific dose of donor CD3+ T cells (see below) representing step one (the lymphoid portion) of the transplant. Days -5 and -4 were rest days. CY 60 mg/kg/day was given on days -3 and -2. Tacrolimus and Mycophenolate Mofetil were initiated on day-1 for GVHD prophylaxis. A CD 34 selected donor stem cell product was infused on day 0, representing step two (the stem cell portion) of the
transplant. Granulocyte-macrophage colony stimulating factor (GM-CSF) 250µg/m² was begun on day +1. No steroids were permissible until after the second dose of CY. In the absence of GVHD, Mycophenolate Mofetil was discontinued on day 28 after HSCT and a tacrolimus taper was initiated by day +60.

**Study design and endpoints**

The primary endpoint of the phase I part of the study was to determine the optimal (or maximum feasible) dose of CD3+ T cells that could be given with CY tolerization that would result in reliable engraftment without significant GVHD. After review of T cell numbers in allogeneic peripheral stem cell products at our institution, we hypothesized that 2 x 10⁸ CD3+ cells/kg would produce consistent engraftment and started the trial at that dose. The study design was such that the dose of T cells would be escalated if excessive graft failure was observed or decreased if excessive GVHD was observed. If excessive graft failure and GVHD were both observed at the same T cell dose, the study would close. The study was also designed to close if, after 4 dose adjustments (up or down) an appropriate T cell dose could not be identified. Once a dose was identified where 6 patients achieved successful engraftment without significant (grade III/IV) GVHD, the phase I part of this protocol would close, and subsequent patients would be treated at this dose in the phase II portion of the trial.

Since our goal was to develop a regimen that allowed haploidentical HSCT to be performed with low treatment related mortality (TRM) and since we anticipated a high relapse rate in the high risk patients likely to enter such a trial, the primary endpoint of the phase II part of the trial was demonstrate an overall survival of ≥ of 30% at 6 months. Secondary endpoints included the assessment of engraftment rates, immune reconstitution and infection, and incidence and severity of GVHD.
Recipient consent, eligibility, donor selection

Written informed consent was obtained for all of the patients in accordance with the Declaration of Helsinki. The study was approved by the Institutional Review Board of Thomas Jefferson University. Patients were eligible for inclusion if they had received front-line therapy for their disease, were without an available genotypically identical related donor, had an available related donor that was mismatched for ≥2 HLA antigens (HLA-A, B, C, DRB1) in the GVH direction, had adequate organ function as defined by a serum creatinine of ≤2.0 mg/dl or creatinine clearance of >40 ml/min, pulmonary diffusion capacity ≥45% (corrected for hemoglobin), and cardiac ejection fraction ≥45%, had a Karnofsky Performance Status ≥70%, were HIV negative, were not pregnant, and had no other active malignancies. Donors were selected to try to maximize anti-host alloreactivity based on factors such as a higher degree of HLA mismatch or the presence of KIR mismatches.

Collection of cells, graft characteristics and processing

Donors underwent apheresis on days -7 and -6 to collect the DLI product. The desired dose of CD 3+ cells was infused without manipulation after the last fraction of TBI on D-6. After collection of the DLI product, donors received subcutaneous injections of G-CSF, 5 µg/kg BID on days -5 through -1, and underwent apheresis for hematopoietic stem cells (HSC) on days -2 and -1. The HSC product underwent CD 34 selection using the Isolex 300i magnetic cell selection system (Baxter) followed by treatment with muromonab-CD3 (OKT-3 Ortho-Biotech) to decrease residual T cell amounts after selection. The product was washed after OKT-3 incubation prior to infusion to ensure that any infused OKT-3 was cell bound and that free OKT-3 was not administered. Processing and infusion of the HSC product occurred on day 0.
Definitions
White cell engraftment was defined as an absolute neutrophil count (ANC) of \( \geq 0.5 \times 10^9/L \) for at least 3 consecutive days post-transplant. Platelet engraftment was defined as a platelet count of \( \geq 20,000/\mu l \) without transfusion for the 7 preceding days. Toxicities were graded using National Cancer Institute (NCI) criteria (Common Toxicity Criteria). Acute GVHD was scored based on the Glucksberg system. Grades III-IV GVHD was termed “severe” GVHD. Chronic GVHD was based on the National Institutes of Health Consensus Criteria.

Post-transplant supportive care
Patients were monitored weekly with a quantitative CMV PCR assay performed on blood samples. If the test became positive, patients were pre-emptively treated with foscarnet or valganciclovir. Patients were given intravenous gamma globulin therapy every 3 to 4 weeks until IgG levels returned to the normal range.

Statistical analysis
The phase I portion of the trial was based on a continual reassessment method and the final T cell dose was determined based on the observed clinical outcomes as described above. Six month survival was estimated using the Kaplan-Meier method (SPSS software, version 12). Cumulative incidence of grades II-IV GVHD, grades III-IV GVHD, engraftment, and relapse were all calculated with death as a competing risk using R v 2.11.1.

Results
Patients
A total of 27 patients, median age of 52 years (range 19-67) with high risk hematologic malignancies were treated between the years of 2006 and 2009. Patient, donor, and disease
characteristics are listed in Table 1. Patients and donors were mismatched for 2 (n = 2), 3 (n=11), or 4 (n=13) HLA-A, B, C, or DR antigens in the GVHD direction. A single patient with zero mismatches in the GVH direction and 4 mismatches in the host-versus-graft (HVG) direction because of HLA homozygosity was treated on the trial.

**T cell dose (transplant step 1) and subsequent in vivo alloreaction**

The initial T cell dose in this trial was $2 \times 10^8$ CD $3^+$ cells/kg. This dose resulted in consistent engraftment and acceptable rates of severe GVHD. Consequently, no dose escalation or de-escalation was performed and all study patients received this dose except for one patient whose donor product contained only $1.7 \times 10^8$ T cells despite two days of apheresis. This patient engrafted and did not develop significant GVHD.

Unexpectedly, patients developed fever (median temperature 103.8°F) within 24 hours of the DLI. Virtually all patients developed diarrhea coincident with or shortly after onset of the fever. Approximately 25% of patients developed rash in the same time frame. Two of the patients who developed a rash and diarrhea in this time frame underwent biopsies which showed histopathologic evidence of GVHD. Fever and diarrhea were resistant to antipyretics and other supportive measures. However, in all cases, these symptoms abated after the second dose of CY. A typical fever curve is shown in Figure 1.

**CD34 dose (transplant step 2) and engraftment**

CD34 and residual CD3 content of the second step of the graft is summarized in Table 2. Two patients died before engraftment could be evaluated. One patient with a flare of Crohn’s disease the week prior to HSCT (GVH direction mismatches = 0) developed hypotension and adult respiratory distress syndrome shortly after transplant. He died on day +9 from a presumed bowel event related to his Crohn’s disease. A second patient died of respiratory
syncytial virus pneumonitis (GVH direction mismatches = 3) on day +1. These two patients were evaluated for toxicity only.

Twenty-three of the remaining 25 patients had full donor engraftment. Neutrophil recovery occurred at a median of 12 days (range = 9-15) and platelet recovery occurred at a median of 20.5 days (range = 15-46). Cumulative incidence of engraftment for neutrophils and platelets was 85.2% and 74.1% respectively (Figure 2). Two multiparous females with multiple HLA antibodies rejected grafts from their daughters. The first patient had an HLA class I anti-donor antibody and was successfully engrafted using a reduced intensity conditioning regimen and an alternate haploidentical donor. The second patient demonstrated an HLA class II anti-donor antibody. This patient, who had no suitable alternate donor, was given 4 doses of Rituximab and apheresis followed by a reduced intensity conditioning regimen and was successfully engrafted using the original donor. Both patients who rejected their transplants had markedly different temperature curves as compared to those who successfully engrafted, with an earlier appearance of the initial temperature rise but a far milder fever later in the course. Their fever curves were more typical during their second transplants. The fever curves of these patients are shown in Figure 1.

TRM

There were three deaths due to multi-organ failure. These included the patient with a flare of Crohn’s disease described above. The other 2 deaths occurred in the patients who experienced primary graft failure. Although these patients were subsequently successfully engrafted, both died of multi-organ failure presumably due to the combined toxicities of the conditioning regimens used for two transplants in rapid succession.
Infection and immune reconstitution

Three of 27 patients (11%) died of infection (bacterial sepsis with subsequent brain abscess-1, progression of pre-existing fungal pneumonia-1, RSV pneumonia-1) during the transplant admission.

Sixteen patients alive and without evidence of recurrent disease at 6 months post HSCT were analyzed with regard to their immune reconstitution. Their median CD3+/CD4+ cell count at day +28 was 33.6 cells/µl (range 11.5-171.8), rising to a median of 104.6 cells/µl (range 10-403.27) at day +100. The median CD3+/CD8+ cell count at day +28 was 28.7 cells/µl (range 3.83-160.09), rising to 151.3 cells/µl (range 2.31-2379.8) at day 100. Lower CD3+/CD4+ and CD3+/CD8+ counts were associated with the use of corticosteroids for GVHD. The CD3+/CD4+ and CD3+/CD8+ counts for these patients are shown in Figure 3. Patients treated earlier in the trial who developed GVHD were treated more aggressively and appeared to have slower CD4 count recovery. There was no consistent pattern to CD4/CD8 ratios, although several patients developed very elevated CD3+/CD8+ counts at the time of infections.

No patient died of complications related to CMV reactivation, although the majority of patients who were CMV seropositive developed evidence of reactivation (15 of 18) whether their donor was CMV seropositive or not. There were no cases of CMV tissue infection.

GVHD

None of the 25 evaluable patients died from GVHD. Only 2 of 25 (8%) patients developed severe acute GVHD. One patient developed steroid responsive grade III intestinal GVHD and one patient, having previously developed stage III skin GVHD which resolved with steroids and photopheresis, later developed grade IV liver GVHD which responded to treatment with OKT-3. Fourteen others developed grade II skin GVHD, with the majority of these patients (11) having
stage III skin disease only without evidence of GVHD in the liver or GI tract. Their skin GVHD responded to steroids (n=8) or steroids plus photopheresis (n=3). Three patients had grade I GVHD responding primarily to topical steroids. Four of 25 evaluable patients (16%) developed chronic GVHD, score 1. Cumulative incidences of grades II-IV and grades III-IV GVHD were 59.2% and 7.4%, respectively (Figure 4).

Cumulative incidence of non-relapse mortality (NRM) at the time of the most recent follow-up was 22.2% (Figure 5).

**Relapse and overall survival**

Relapsed disease was the primary cause of death in patients treated on this trial. Eight of 25 patients (32%) experienced a relapse of their malignancies 49-327 days post HSCT. Cumulative incidence of death due to relapse (RRM) was 29.6% (Figure 5). Six of 13 patients with active malignancy at the time of HSCT subsequently relapsed, while only two of 12 patients who underwent transplant while in remission relapsed afterwards. All patients who relapsed ultimately died from their malignancy, and all patients who are alive are at least 28 months post HSCT without evidence of their disease.

Seventeen of 27 patients (63%) were alive 6 months after their transplant, satisfying the primary endpoint of the trial. Kaplan-Meier estimate of overall survival was 54% at 1 year and 48% at 3 years (median follow up 40 months, range 28-56 months). Patients without disease at the time of HSCT fared better with a projected OS of 75% at 3 years. Survival curves are shown in Figure 6. All surviving patients remain in CR and have been followed for a minimum of 24 months post-transplant.
Discussion

Our goals in developing this 2 step regimen were: to develop a myeloablative regimen for haploidentical HSCT that could be administered to patients with refractory or relapsed hematological malignancies initially, but would subsequently be appropriate for high risk patients earlier in their disease course who lacked other donor options; to avoid exposure of newly transplanted HSCs to high dose CY; to utilize peripheral blood rather than marrow as the stem cell source; and to fix and maximize the number of CD3 cells patients would receive in an effort to produce consistent outcomes following transplantation. Of these, we considered the standardization of the CD3 dose to be the most essential. Every animal transplant model carefully controls and fixes CD3 content, yet this is rarely done in clinical transplantation with the exception of trials of T cell depletion where the focus is often on a T cell threshold rather than a dose. Essentially we believed that controlling the T cell dose was equally important to the outcome of transplant as prescribing specific doses of radiation and chemotherapy.

The initially tested T cell dose of 2 x 10^6/kg was associated with prompt engraftment, little significant GVHD, and prompt immunological recovery and thus met our phase I criteria for an optimal dose of T cells within the context of these immunologic outcome measures. In contrast to our own experience with T depleted haploidentical HSCT, deaths from infection at this T cell dose were very low despite a high rate of CMV reactivation. In many patients, the reactivation was accompanied by a rise in CD3+/CD4+ and CD3+/CD8+ T cells, although the circulating T cells were not tested for CMV specificity. In our prior experience with global T cell depletion, patients remained severely T lymphopenic throughout the course of viral infections. With CY tolerization, CMV reactivation was typically rapidly controlled once immune suppression was tapered. Twenty-one of twenty-seven patients (78%) survived until discharge and all subsequent deaths were related solely to relapsed disease and not TRM.
We did not anticipate the two rejections in patients with donor specific antibodies (DSA) especially in the context of a large T cell dose and the successful engraftment of a patient treated earlier in the trial with very broadly reactive anti-HLA antibodies, but who was without DSA. It is possible that the atypical early fevers in these two patients were a reflection of an antibody mediated lysis of the donor lymphocytes shortly after infusion which allowed residual host humoral and cellular immunity to reject the graft. Rejection resulting from DSA was not well described at the time this trial was launched and a retrospective review of the literature revealed only one trial that described this phenomenon.\textsuperscript{32} Few additional reports have been published since this time and the cumulative experience, including our own, suggests that the presence of DSA is a significant risk factor for rejection of haploidentical grafts.\textsuperscript{33,34} In the absence of DSA, over 60 patients treated with this 2 step approach either within this particular trial or subsequent to it have engrafted, demonstrating that without anti-donor antibodies, engraftment is consistent.

The incidence of GVHD was higher in this trial versus that reported in other haploidentical trials where CY tolerization was used as GVHD prophylaxis.\textsuperscript{28} This likely reflects the 5 fold higher T cell dose that was administered, the aggressive taper of immune suppression, efforts to select the donor predicted to be most alloreactive amongst the available family members and the more intensive conditioning regimen. Nonetheless, GVHD was primarily limited to skin and easily controlled with steroids or steroids and early photopheresis. Photopheresis is thought to ameliorate GVHD in part by increasing CD4+CD25+/FoxP3+ T regulatory cells (Tregs).\textsuperscript{35,36} The role of Tregs in controlling haploidentical GVHD after using this 2 step HSCT approach is an area of current inquiry.
Chronic GVHD was infrequent and was not severe. This positive outcome likely contributed to the absence of late non-relapse mortality as most patients did not require chronic immune suppression.

The febrile reaction, rash and diarrhea that resulted from the T cell infusion are reminiscent of the “haplo immunostorm” (HIS) described by Colvin and colleagues when they infused similar doses of T cells following 2 Gy of radiation without other cytoreduction. In that setting, HIS was thought to be mediated by a cytokine storm and not associated with GVHD or engraftment syndrome. In our 2 step approach, the reaction resolved completely after two doses of CY and morphological analysis of skin and gut biopsies were consistent with GVHD. In the Colvin et al. study, this phenomenon was observed when the CD3 dose reached $1 \times 10^8$/kg and was most pronounced at $2 \times 10^8$/kg, the dose utilized in this trial. Similar febrile reactions were not reported by O’Donnell and colleagues, probably because their median T cell dose was 5 fold lower than in our trial.

We believe that during this in vivo alloreaction, donor lymphocytes contribute to the immunologic elimination of residual malignancy. This is supported by the Colvin group’s observation that patients receiving haploidentical cellular therapy for resistant malignancy had clinical responses at the doses of T cells associated with HIS despite the minimal conditioning administered.

One of the rationales for the two step approach described here was that donor lymphocytes would be exposed to CY while hematopoietic stem and progenitor cells would not. While stem cells may be protected from mutagenic effects of CY due to their expression of aldehyde dehydrogenase, levels of this enzyme drop as cells diverge from the stem cell phenotype. Some current models of leukemogenesis posit that mutations occur in a post stem precursor
cell which, as a consequence, reacquires stem properties. Whether secondary MDS or AML will emerge as problems in regimens where HSCs are exposed to CY remains to be seen. The two step approach eliminates that issue, however big or small it may be. It does require 4 days of apheresis for most donors (2 for DLI and 2 for HSC), but this has been well tolerated to date.

The 52% and 48% one and three year overall survival rates observed in patients treated on this two-step regimen met the protocol criteria for an effective regimen. Based on the current literature, the results appear comparable to outcomes in matched sibling and unrelated donor HSCT in similar high risk groups of patients. All six patients treated for lymphoid diseases, four patients with high-risk ALL and two patients with chemotherapy-resistant non-Hodgkin lymphoma, are disease-free 28 to 52 months post-transplant. Whether these favorable outcomes were due to the use of TBI in the conditioning regimen or to the large number of haploidentical T cells administered with this approach is unknown. If the latter is true, it may alter the spectrum of diseases in which strong graft-versus-tumor (GVT) effects are observed after haploidentical transplantation.

Administration of the graft in two steps avoided the infusion of donor T cells that were skewed towards a TH2 phenotype since T cells were collected prior to administering G-CSF to the donors. Recognizing the high risk nature of the patients treated, we also administered GM-CSF rather than G-CSF after transplant in a further attempt to avoid G-CSF-induced polarization to a TH2 phenotype. Despite this strategy, relapsed disease in patients with AML or MDS was the primary cause of mortality. Interestingly, six of the eight (75%) recipients that relapsed, including the only two patients who relapsed following transplantation while in remission, were mothers receiving grafts from their children. Recent data supports a mechanism of long-lasting regulatory T cell mediated tolerance of maternal cells by offspring. While child to mother transplants have been associated with superior outcomes in other transplant settings, in our
limited population they were associated with higher rates of relapse. It is possible that the outcomes associated with this donor-recipient combination are dependent on the particular disease state and transplant regimen which together will determine whether an increase or decrease in alloreactivity is likely to be beneficial or detrimental.

Building on the platform of a large and consistent T cell dose, additional strategies can be explored with the potential to improve relapse rates in patients with advanced malignancies. Mayumi and Good demonstrated in murine models that the administration of CY 1-3 days after antigenic stimulation is the optimal time to establish allogeneic tolerance. \(^4^1\) The optimal timing in man has not been directly studied. It may be worthwhile to assess whether the interval between DLI and CY can be safely increased modestly in future trials as a means of allowing further immunologically mediated cytoreduction by the DLI prior to abrogation by CY. Other drugs preferentially cytotoxic to activated cells such as Melphalan \(^4^2\,^4^3\) could potentially be introduced in place of CY using this 2 step approach without concerns about cytotoxicity toward hematopoietic stem cells. This platform also allows the use of two haploidentical donors to further increase the GVT effect and eliminate the potential for leukemic escape due to uniparental disomy.\(^4^4\)

The results from this trial have been encouraging amongst patients undergoing transplant in remission with a follow up of in excess of two years for all patients. The low regimen related mortality and high overall survival suggests that this approach can be extended to better risk patients earlier in their disease course and should be further studied in larger trials.
Acknowledgements

We are grateful to all of the clinicians at the Jefferson Kimmel Cancer Center for their excellent care of our transplant patients.

Authorship

D.G. and N.F. designed and performed the research, analyzed and interpreted the results and drafted the manuscript. T.H. and D.G. performed statistical analyses. J.B., M.C., J.F.O, P.F. M.K., W.O., J.L.W, and M.W.W. performed the research and contributed to the writing of the paper. P.C.F., and B.C. performed and analyzed the research, and M.W. analyzed the research and contributed to the drafting of the manuscript.

Conflict-of-interest disclosure: Bijoyesh Mookerjee has stock options at the Incyte Corporation and at AstraZeneca. He is currently employed by the Incyte Corporation. The other authors declare no competing financial interests.

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References


Table 1. Patient Characteristics

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<td>Aplastic Anemia</td>
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</tr>
</tbody>
</table>

| Previous Transplant | 2 |
| Secondary Malignancy | 2 |
| Recipient/Donor Transplant Combinations | |
| Sibling to Sibling / Parent to Child / Child to Parent | 7 / 4 / 16 |

| CMV Serostatus Recipient (R) and Donor (D) | |
| R+/D+ | 12 |
| R+/D- | 6 |
| R-/D- | 9 |

<table>
<thead>
<tr>
<th># HLA Antigen Mismatches (GVH Direction) (A, B, Cw, DRB1)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Four</td>
<td>13</td>
</tr>
<tr>
<td>Three</td>
<td>11</td>
</tr>
<tr>
<td>Two</td>
<td>2</td>
</tr>
<tr>
<td>Zero†</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>KIR Mismatches‡‡</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>KIR ligand missing in recipient but present in donor</td>
<td></td>
</tr>
<tr>
<td>HLA-C Group 1</td>
<td>5</td>
</tr>
<tr>
<td>HLA-C Group 2</td>
<td>4</td>
</tr>
<tr>
<td>HLA-Bw4</td>
<td>1</td>
</tr>
<tr>
<td>HLA-C and HLA Bw4</td>
<td>2</td>
</tr>
<tr>
<td>No KIR Mismatch</td>
<td>15</td>
</tr>
</tbody>
</table>

* Based on cytogenetics, secondary disease, CNS/tissue involvement, or arising from MDS
† Patient had 4 mismatches in HVG direction only and was counted for toxicity only
‡‡ Missing self as defined by Ruggeri et al.18
Figure 1. Fever Curves after DLI Correlate with Clinical Events. A typical temperature curve from an engrafting patient is shown in green. Curves from the two patients with anti-donor antibodies who rejected their grafts are shown in red and blue. The boundary between the febrile and afebrile ranges (100.4 °F) is shown by the horizontal solid black line. The afebrile range is shaded gray. Engrafting patients generally developed within 24 hours after DLI. The fever spikes persisted despite the use of acetaminophen and other comfort measures until after the second dose of CY. In the setting of anti-donor antibodies, the patients developed fever within a few hours of the DLI, rapidly defervesced and remained afebrile thereafter.
### Table 2. Graft Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Step 1</th>
<th>Step 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3+/kg x10^8</td>
<td>2.0</td>
<td>3.6</td>
</tr>
<tr>
<td>Median (Range)</td>
<td>(1.7 in 1 patient, 2.0 in 26 patients)</td>
<td>(1.3-7.4)</td>
</tr>
<tr>
<td>CD34+/kg x10^6</td>
<td></td>
<td>0.51</td>
</tr>
<tr>
<td>Median (Range)</td>
<td></td>
<td>(0.13-6.9)</td>
</tr>
</tbody>
</table>
Figure 2. Neutrophil and Platelet Engraftment. Cumulative incidences of neutrophil and platelet engraftment were 85.2% and 74.1% respectively.
Figure 3. Immune Recovery after Haploidentical Transplant. CD3+/CD4+ cell counts (A) and CD3+/CD8+ cell counts (B) of 16 patients alive and disease-free at least 6 months post HSCT are illustrated.
Figure 4. Acute GVHD. Cumulative incidences of grades II-IV and III-IV GVHD were 59.2% and 7.4% respectively.
Figure 5. Relapse and Non-Relapse Related Mortality. Cumulative incidences of RRM and NRM were 29.6% and 22.2% respectively.
Figure 6. Probability of Overall Survival. Survival for all patients in the trial is shown as the solid black line (48% 3 year OS). Patients without marrow morphologic or radiographic evidence of disease at the time of transplant are shown with the dashed line (75% 3 year OS). Patients with marrow or radiographic evidence of disease are shown in the dotted line (27% 3 year OS).