Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Biol Blood Marrow Transplant 24 (2018) 2259–2264

Biology of Blood and Marrow Transplantation

journal homepage: www.bbmt.org

Alternative Donors

Reduced-Intensity Conditioning and Dual T Lymphocyte Suppression with Antithymocyte Globulin and Post-Transplant Cyclophosphamide as Graft-versus-Host Disease Prophylaxis in Haploidentical Hematopoietic Stem Cell Transplants for Hematological Malignancies

Arjun Datt Law[†], Maria Queralt Salas[†], Wilson Lam, Fotios V. Michelis, Santhosh Thyagu, Dennis (Dong Hwan) Kim, Jeffrey Howard Lipton, Rajat Kumar, Hans Messner, Auro Viswabandya[*]

Allogeneic Blood and Marrow Transplantation Program, Division of Medical Oncology and Hematology, Princess Margaret Cancer Centre, Toronto, Canada

Article history:
Received 2 May 2018
Accepted 4 July 2018

Key Words:
Haploidentical hematopoietic stem cell transplantation
Post-transplant cyclophosphamide
Antithymocyte globulin
Dual T cell suppression

A B S T R A C T

Haploidentical hematopoietic stem cell transplantation (haploHSCT) with conditioning regimens using post-transplant cyclophosphamide (PTCy) for peripheral blood stem cell (PBSC) grafts is limited by comparably higher rates of acute and chronic graft-versus-host disease (GVHD). Antithymocyte globulin (ATG) may mitigate this risk. We evaluated haploHSCT after reduced-intensity conditioning (RIC) with ATG, PTCy, and cyclosporine to prevent rejection and GVHD. Fifty adults underwent haploHSCT from August 2016 to February 2018. RIC included fludarabine (30 mg/m^2/day on days −5 to −2), busulfan (3.2 mg/m^2/day on days −3 and −2), and total body irradiation (200 cGy) on day −1. Unmanipulated PBSCs were infused on day 0. GVHD prophylaxis included ATG (4.5 mg/kg over days −3 to −1), PTCy (50 mg/kg/day on days +3 and +4), and cyclosporine from day +5. Median age was 56 years (range, 22 to 70 years); 25 (73.5%) patients were in first complete remission (CR1), 5 (14.7%) were in second complete remission (CR2), and 8 (23.5%) had active disease. Median time to neutrophil engraftment was 16 days (range, 8 to 43 days). At day +100, the cumulative incidence of acute GVHD of any grade, and grades III to IV was 38.3% and 5.2%, respectively. Mild chronic GVHD was seen in 15.5%. Cytomegalovirus (CMV) reactivation occurred in 37 (74%) cases and CMV disease occurred in 4 (11.5%) cases. Epstein-Barr virus (EBV) reactivation occurred in 21 (61.8%) patients. The incidence of histologically confirmed post-transplantation lymphoproliferative disorder (PTLD) was 5.8%. Four patients received rituximab. There were no CMV, EBV, or PTLD-related deaths. Six-month and 1-year overall survival (OS), cumulative incidence of relapse (CIR), and nonrelapse mortality (NRM) were 73.9%, 10.2%, and 19.4%, respectively, and 48.1%, 16% and 38.2%, respectively. Infection was the most common cause of death (18%). Unmanipulated haploidentical PBSC transplantation following RIC with ATG, PTCy, and cyclosporine as a GVHD prevention strategy results in low rates of acute and chronic GVHD.

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative therapy for patients with high-risk or advanced hematological disorders [1]. An HLA-matched sibling donor is considered the first choice. Approximately 70% of patients do not have a suitably matched sibling donor (MSD) available for transplantation. Alternatives such as matched unrelated donors (MUDs) can be identified for only 50% to 60% of patients, with the donor search and procurement process requiring a median of 4 months [2]. For patients who do not have suitable MSDs or MUDs, alternative stem cell sources include unrelated single or double umbilical cord blood transplants, HLA-mismatched unrelated donors (MMUDs), and HLA haploidentical family members. Despite the expansion of MUD registries and cord blood banks, donor availability can often be uncertain particularly for under-represented ethnic minorities, which may be subject to prolonged and unproductive registry searches [3]. The use of haploidentical donors reduces this uncertainty to a large degree, as almost all patients have an immediately available related donor with whom they share a single HLA haplotype. This not only facilitates a reduced time to transplantation, but also provides a more reliable source of donor

Financial disclosure: See Acknowledgments on page 2264.

[*] Correspondence and reprint requests : Auro Viswabandya, MD, DM, FRCP (Edin), Allogeneic Blood and Marrow Transplantation Program, Allogeneic Blood and Marrow Transplantation Program, Division of Medical Oncology and Hematology, Princess Margaret Cancer Centre, Department of Medicine, University of Toronto, Rm-6-323, OPG Building, 700 University Avenue, Toronto, Ontario - M5G 1 × 6, Canada.

E-mail address: auro.viswabandya@uhn.ca (A. Viswabandya).

[†] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbmt.2018.07.008
1083-8791/

stem cells or lymphocytes in the event further interventions are required.

There remains considerable variation between centers in conditioning regimens, graft sources and choice of graft-versus-host disease (GVHD) prophylaxis to achieve lower nonrelapse mortality (NRM) and optimum long-term outcomes in haploidentical HSCT (haploHSCT). The combination of a nonmyeloablative conditioning regimen with antithymocyte globulin (ATG) and post-transplant high-dose cyclophosphamide (PTCy) has only been described in the setting of haploHSCT for sickle cell disease [4]. In our institution, we established a reduced-intensity conditioning (RIC) regimen consisting of fludarabine (Flu), reduced-dose busulfan (Bu), and total body irradiation (TBI) (total dose 200 cGy) combined with ATG and followed by PTCy and cyclosporine for the prevention of graft rejection and GVHD for haploHSCT recipients. We previously reported the use of this protocol in MUD transplants wherein lower rates of severe acute GVHD were demonstrated compared with non–ATG-based regimens [5]. In the present study, we report the use of this protocol in 50 consecutive patients who underwent haploHSCT at our center.

METHODS

Patient Selection

From August 2016 to February 2018, 50 adult patients with hematological malignancies underwent haploHSCT at the Princess Margaret Cancer Centre, Toronto, Canada. All patients were ≥18 years old with no upper age limit, had a Karnofsky Performance Score (KPS) >60%, and did not have active/untreated infection or human immunodeficiency virus seropositivity. Criteria for pretransplant organ function included left ventricular ejection fraction ≥45% without significant preexisting cardiac disease, pulmonary function testing demonstrating forced expiratory volume >50% predicted and diffusing capacity of carbon monoxide >50, normal/stable kidney function on biochemistry, and liver functions tests showing total bilirubin <2.5 times normal with transaminases <3 times the upper limit of normal. Informed consent was obtained from all patients. The use of this protocol was approved by the institutional board of ethics. All patients with positive donor specific antibodies were excluded.

HLA Typing and Donor Selection

High-resolution molecular typing for HLA class I (A, B, C) and class II (DR, DQ) was performed for recipients and donors when patients were referred to the Allogeneic Blood and Marrow Transplantation Program. Haploidentical related donors were selected in all cases where MSD or 9/10 MUD could not be identified. Peripheral blood stem cells (PBSC) were collected after granulocyte colony-stimulating factor (G-CSF) mobilization. A minimum dose of 6×10^8 $CD34^+$ cells/kg recipient body weight was requested in all cases. $CD3^+$ cell enumeration is not routinely performed in our institute, as unmanipulated stem cells are used in all cases. All donors provided informed consent.

Conditioning Regimen and Post-Transplant Immunosuppression

Patients received conditioning therapy with fludarabine 30 mg/m^2/day i.v. on days -5 to −2, busulfan 3.2 mg/m^2/day i.v. days −3 and −2, and 200 cGy of TBI on day −1. Rabbit ATG (thymoglobulin; Genzyme-Sanofi, Lyon, France) was administered in doses of .5 mg/kg on day −3, 2 mg/kg on day −2, and 2 mg/kg on day −1 (total 4.5 mg/kg).

T cell–replete PBSC grafts were infused on day 0. All patients received PTCy (cyclophosphamide 50 mg/kg/day i.v.) on days +3 and +4, with the first dose starting 72 hours after the start of allograft infusion. Four doses of mesna were administered on days +3 and +4 at a total daily dose of 80% of the cyclophosphamide dose. Cyclosporine was initiated at a dose of 2.5 mg/kg i.v. on day +5 and adjusted to achieve a therapeutic level of 200 to 400 μg/L (Figure 1). All patients received daily filgrastim 300 μg subcutaneously, starting on day +7 until neutrophils were $>1.5 \times 10^9$/L. Ursodeoxycholic acid 500 mg oral twice daily with meals (starting on the first day of conditioning) was used for prevention of sinusoidal obstruction syndrome (SOS) in all patients. Prophylactic antimicrobial therapy included posaconazole 300 mg daily, acyclovir 400 mg twice daily, ciprofloxacin 500 mg twice daily, and inhaled pentamidine 300 mg monthly. Postengraftment, cytomegalovirus (CMV) titer was monitored twice per week and Epstein-Barr virus (EBV) was monitored weekly. Cyclosporine tapering started around days +45 to 60 for all patients without GVHD. The schematic for the conditioning protocol and post-transplant immunosuppressive regimen is shown in Figure 1.

Engraftment and Chimerism Analysis

Neutrophil engraftment was defined as the first of 3 consecutive days with an absolute neutrophil count $\geq .5 \times 10^9$/L and platelet recovery was defined as a sustained and transfusion-independent platelet count $>20 \times 10^9$/L (first of 3 days) without transfusion times 7 days. Donor total chimerism was assessed in whole blood by PCR for variable number tandem repeat polymorphisms at days +30 and +60 in all patients. All patients underwent bone marrow examination at day +60 for marrow cellularity and disease monitoring.

Statistical Methods

Clinical features, post-transplant outcome, and side effects were collected through retrospective chart review. Last follow-up was updated in February 2018. The study was conducted in accordance with the Declaration of Helsinki, and was reviewed and approved by the Ethics Committee at the Princess Margaret Cancer Centre, Toronto, Canada.

Patient and disease characteristics were reported using descriptive statistics (count and percentage). The time to event was calculated from the date of transplant to the date of event or last date of patients known to be alive. The main outcome variables of interest included overall survival (OS), relapse-free survival (RFS), and nonrelapse mortality (NRM). The Kaplan-Meier method was used to estimate OS and RFS. The cumulative incidences of acute and chronic GVHD were calculated accounting for death and relapse as competing risks. The incidence rate of acute GVHD was estimated at day +100 and chronic GVHD at day +180. NRM was estimated using the cumulative incidence method accounting for relapse as a competing risk (Fine-and-Gray analysis). Survival rates were calculated 6 months and 1 year after haploHSCT. Analyses were performed using SPSS version 16.0 for Windows (SPSS Inc, Chicago, IL), and EZR [6].

RESULTS

Patient and Graft Characteristics

Table 1 shows baseline patient characteristics. Table 2 shows donor characteristics, graft parameters, and GVHD characteristics. Median follow-up for survivors was 168 days (range, 22 to 536 days), or 5 months (range, 0 to 17 months). Nearly 80% of donor-recipient pairs were mismatched at 5 HLA loci. Sixteen patients and donors were CMV mismatched.

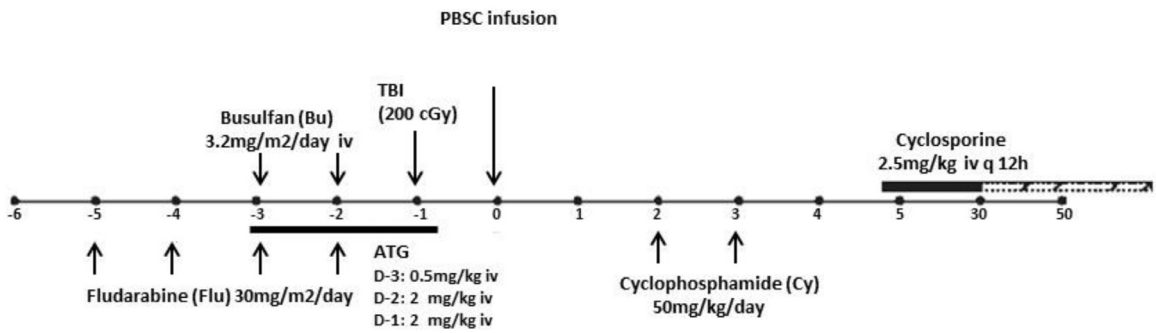

Figure 1. HaploHSCT conditioning and post-transplant immunosuppressive regimen. D indicates day.

While cryopreserved grafts were used in the majority, 10 patients received fresh product. The hematopoietic cell transplantation comorbidity index score was ≥3 in almost 40% cases.

Median time to neutrophil engraftment was 17 days (range, 8 to 43 days). Median time to platelet engraftment was 22 days (range, 7 to 217 days). Engraftment syndrome (characterized by fever, fluid retention/weight gain, hypotension, and hypoxemia in the absence of documented infection, and temporally correlated with count recovery) occurred in 3 (6%) patients, and all cases were successfully treated with steroids. Ninety-four percent of patients achieved donor chimerism >95% at day +30. Four (8%) patients failed to engraft by day +30. One patient developed secondary graft failure 3 months post-transplant. This included 1 patient with primary myelofibrosis, 1 with myelodysplastic syndrome, and 2 with acute myelogenous leukemia. Median of 9.94×10^6 $CD34^+$/kg recipient body weight (range, 8.2 to 11.7×10^6 $CD34^+$/kg recipient body weight) were infused. No donor-specific antibodies were documented. All patients had developed CMV reactivation, and 1 patient with secondary graft failure had biopsy-proven CMV colitis. EBV reactivation was documented in 2 cases, and 1 developed asymptomatic BK viruria.

Hospitalization, Organ Toxicity, and Infections

Median length of hospitalization was 32 days (range, 13 to 83 days). Post-transplant clinical course, organ toxicity, and infections are summarized in Supplementary Tables 1 and 2. Three (6%) cases developed low-grade cytokine release syndrome following stem cell infusion. This occurred on day 0 in all cases and resolved after cyclophosphamide administration in all cases by day +4. Mild mucositis was seen in all patients, while 9 (18%) patients developed grade 3 to 4 mucositis requiring short-term total parenteral nutrition. Mild-to-moderate SOS was documented in 7 (17.4%) patients, with grade 3 SOS in 1 case. All cases resolved with fluid restriction and diuretics. Ten patients developed features of fluid overload, and 3 patients developed cardiogenic pulmonary edema secondary to fluid retention and hypoalbuminemia during the first 30 days during the post-transplant course. All cases responded to aggressive diuresis and fluid optimization, and no patient needed intensive care support. No ischemic events occurred. Three patients developed pericardial complications (2 cases with pericarditis and 1 myopericarditis) during follow-up. All effusions were mild, resolved spontaneously, and did not show features of cardiac tamponade. Diagnostic or therapeutic pericardiocentesis was not performed. One patient demonstrated an appreciable decline in left ventricular ejection fraction on follow-up echocardiography at day+60 but remained asymptomatic. One patient with a history of anthracycline-associated cardiomyopathy with induction chemotherapy sustained a fatal ventricular arrhythmia while undergoing treatment for CMV reactivation and GVHD.

Transient elevation of renal parameters without oliguria was seen in 13 (26%) patients. Creatinine returned to baseline in all cases with conservative management.

Neutropenic fever occurred in 42 (84%) of the patients with positive blood cultures in nineteen cases (38%). No proven invasive fungal infections were encountered during hospitalization for the transplant. Laboratory evidence of CMV reactivation by peripheral blood PCR was observed in 37 (74%) patients, with a median time to reactivation of 26 days (range, 13 to 61 days) post-transplant. CMV was also identified in bronchoalveolar lavage specimens in 3 cases and on intestinal biopsy in 1 case. All patients received intravenous ganciclovir or valgancyclovir until viremia clearance. Two patients required foscarnet therapy due to persistence of CMV viremia despite appropriate doses of ganciclovir. Twenty-seven (64%) patients developed EBV reactivation after transplantation with a median time of 56 days (range, 20 to 233 days). Four patients (8%) developed biopsy proven post-transplant lymphoproliferative disease (PTLD). PTLD was managed by tapering immunosuppression and weekly rituximab for a maximum of 4 doses.

Table 1
Clinical Characteristics of HaploHSCT Patients

	Overall (N = 50)
Age, yr	56 (22-73)
Sex	
Male	29 (58)
Female	21 (42)
Diagnosis	
AML	28 (56)
MDS	8 (16)
MPN	6 (12)
ALL	2 (4)
Lymphoma	5 (10)
BPDCN	1 (2)
Stage at transplant	
CR1	32 (76)
CR2	5 (10)
CR3	1 (2)
Active disease/partial response	12 (14)
HCT-CI	
<3	31 (62)
≥3	19 (38)
KPS	
≥80	43 (86)
<80	7 (14)

Values are median (range) or n (%).
AML indicates acute myelogenous leukemia; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; ALL, acute lymphoblastic leukemia; BPDCN, blastic plasmacytoid dendritic cell neoplasm; HCT-CI, hematopoietic cell transplant-comorbidity index; KPS, Karnofsky performance status.

Table 2
Donor Characteristics, Graft Parameters, and GVHD

Donor characteristics	Overall (N=50)
Donor relation, n (%)	
Sibling	12 (24)
Parent	9 (18)
Child	29 (58)
HLA mismatch, n (%)	
8/10	3 (6)
7/10	4 (8)
6/10	4 (8)
5/10	39 (78)
Donor age, yr, median (range)	37 (11-62)
Donor sex, n (%)	
Male	34 (68)
Female	16 (32)
CMV immune status (donor−recipient), n (%)	
Positive/positive	23 (46)
Negative/negative	6 (12)
Positive/negative	12 (24)
Negative/positive	9 (18)
Graft parameters	
Median $CD34^+$ cell dose/kg recipient body weight, $\times 10^6$/kg (range)	9.88 (3.73-28.6)
Median days to neutrophil engraftment (range)	17 (8-43)
Median days to platelet engraftment (range)	22 (7-217)
Cumulative incidence of GVHD, % (95% CI)	
Acute GVHD (any grade) at day +100	38.3 (23.8-52.7)
Acute GVHD grade II-IV at day +100	20.3 (9.8-33.5)
Acute GVHD grade III-IV at day +100	5.2 (.9-15.5)
Chronic GVHD (any grade) at 6 mo	15.5 (5.4-30.2)
Chronic GVHD (severe) at 6 mo	None

All patients responded with regression of affected nodal tissue (if any) and clearance of viremia. There were no EBV- or PTLD-associated deaths. Thirteen (26%) patients developed BK viruria, which was associated with hemorrhagic cystitis in 7 (14%) cases. Median time to develop BK viruria was 30 days (range, 5 to 55 days). All cases of hemorrhagic cystitis resolved with aggressive hydration and bladder irrigation. Other viral infections were diagnosed during the post-transplant period in 12 (24%) patients. Seven patients developed pulmonary viral infections: 3 caused by influenza A, 2 by respiratory syncytial virus, 1 by parainfluenza virus, and 1 by coronavirus. In addition, 1 patient developed oral cavity coxsackie virus infection and 4 patients had oral herpes simplex virus type 1 related mucositis. All cases successfully resolved with supportive measures and appropriate antivirals (acyclovir for herpes simplex virus and oseltamivir for influenza A). Proven or probable invasive fungal infection (positive galactomannan in bronchoalveolar lavage) was identified in 7 (14%) patients during the post-transplant course. One patient died from complications arising from fungal pneumonia.

Graft-versus-Host Disease

Rates of acute and chronic GVHD were 44% and 10%, respectively. Acute GVHD was graded according to the Keystone criteria and chronic GVHD was graded in accordance with the National Institutes of Health Chronic Graft-versus-Host Disease Consensus [7,8]. Acute GVHD of any grade was identified in 20 (44%) patients; however, grade III to IV acute GVHD occurred in only 2 cases (4%). The cumulative incidence of acute GVHD of any grade, grade II to IV, and grade III to IV at day +100 was 38.3%, 20.3%, and 5.2%, respectively (Table 2). Skin (36%) was the most commonly affected site, followed by liver (14%) and gut (10%). First-line therapy of clinically significant acute GVHD consisted of topical or systemic steroids based on site and severity. The cumulative incidence of chronic GVHD at 3 months was 15.5%. No systemic immunosuppression was required for patients with chronic GVHD. No patients developed moderate or severe chronic GVHD (Table 3).

OS, RFS, CIR, and NRM

Nineteen (38%) patients died during follow-up. Table 4 lists the causes of death among transplanted patients. Five (10%) patients relapsed during the follow-up period with a median time to relapse of 4 months (range, 1 to 9 months). Six-month OS, RFS, CIR, and NRM were 73.9%, 57%, 10.2%, and 19.4%, respectively. No significant differences were found in OS (P = .411) and progression-free survival (P = .563) between patients in CR1 versus CR2 versus active disease. One-year OS, RFS, CIR, and NRM were 48.1%, 35.7%, 16%, and 38.2%, respectively (Table 2). Causes of death were relapse in 4 (8%) cases, graft failure in 2 (4%) patients, infection in 9 (18%) patients, multiorgan failure in 2 (4%) cases, acute GVHD in 1 case, and cardiac arrhythmia in 1 case (Figures 2–4).

Table 3
OS, CIR, and NRM

Median follow-up, d/mo, median (range)	168 (22-536)/5 (0-17)
% Survival (95% CI)	
OS	
6 mo	73.9 (55.4-85.7)
1 yr	48.1 (26.2-67.1)
RFS	
6 mo	57 (41-73)
1 yr	35.7 (15.7-55.7)
CIR	
6 mo	10.2 (3.1-22.3)
1 yr	16 (4.9-32.8)
NRM	
6 mo	19.4 (8.2-34.1)
1 yr	38.2 (18.9-57.4)

DISCUSSION

HaploHSCT was initially described in the late 1950s. This was historically associated with high rates of graft rejection, severe acute GVHD, and worse outcomes when compared with MSD transplants [9]. An important milestone in haploHSCT was the use of T cell–replete grafts in combination with post-transplantation high-dose cyclophosphamide [10]. In the setting of haploHSCT, PTCy decreases the risk of GVHD and graft rejection by targeting and inducing cell apoptosis of alloreactive T cells rapidly proliferating early after transplant, sparing regulatory T cells and preserving nondividing hematopoietic stem and progenitor cells [10–13]. PTCy may be associated with high rates of severe acute GVHD if used alone, with 4 of 5 patients receiving single-agent PTCy succumbing to fatal, steroid-refractory GVHD in 1 single-center report [14]. Therefore, post-transplant immunosuppression commonly includes additional agents such as calcineurin inhibitors, sirolimus, and/or mycophenolate mofetil [13,14]. HaploHSCT using unmanipulated T cell–replete PBSC grafts with PTCy, calcineurin inhibitors, and mycophenolate mofetil as GVHD prophylaxis have been associated with increased rates of acute and chronic GVHD and have shown commensurate outcomes with acceptable NRM when compared with other donor sources [12,15,16]. ATG has also been extensively studied as a method to prevent graft rejection, acute GVHD, and chronic GVHD in MSD and MUD transplants [17–19]. However, its effectiveness in modulating severe acute and chronic GVHD is offset by a higher rate of infectious complications, including CMV and EBV reactivation [20–22].

PTCy induces selective destruction of proliferating alloantigen–stimulated T cells. This effect is invaluable for haploHSCT where there is an intense T cell proliferation due to major HLA mismatch. Donor memory T cells are relatively protected from PTCy and provide donor immunity in addition to immune reconstitution post-transplant [13].

Reduced-intensity conditioning with fludarabine, reduced-dose busulfan, and TBI (200 cGy) is a low-toxicity regimen that induces sufficient immunosuppression to permit donor hematopoietic cells to consistently engraft despite the HLA-haplotype barrier. The feasibility of RIC in HaploHSCT in adult patients with hematological malignancies is well described [23–25]. The combination of fludarabine and busulfan with PTCy may potentiate the antineoplastic activity of the conditioning regimen which may lead to resulting in further disease reduction. Data in HaploHSCT using RIC and PBSC as the stem cell source is limited. Additionally, centers using PBSC in haploidentical transplants have shown rates of extensive chronic GVHD as high as 38% [16].

Table 4
Causes of Death

	n	%
TOTAL	19	38
Relapse	4	8
Graft failure	2	4
Infection	9	18
Acute GVHD	1	2
Multiorgan failure	1	2
Cardiac arrest	1	2
Respiratory failure	1	2

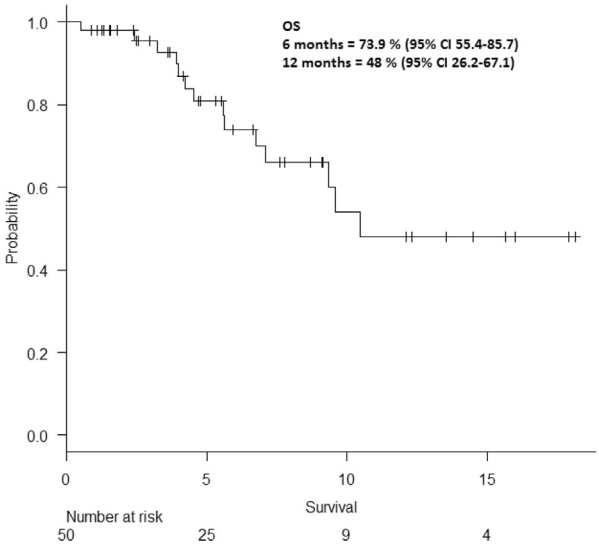

Figure 2. OS of 50 patients receiving haploidentical stem cell transplants for hematological malignancies. CI indicates confidence interval.

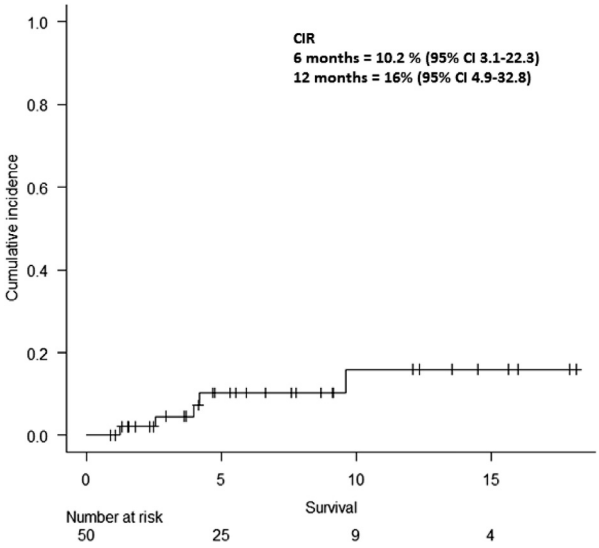

Figure 3. CIR.

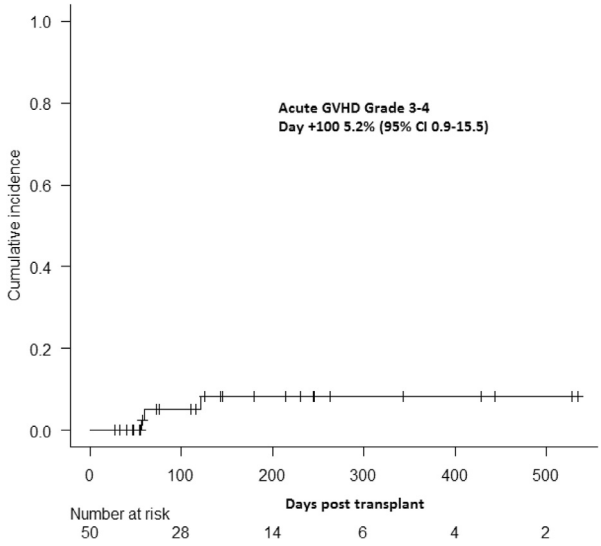

Figure 4. Cumulative incidence of acute GVHD grade III to IV.

The combination of ATG and low dose PTCy was found to increase regulatory T cell reconstitution in an animal model leading to lower rates of acute GVHD in a murine experiment [26]. Our own experience with this combination in MUD transplants led to its use in the haploHCT setting [5]. We had also encountered a high incidence of severe acute and chronic GVHD with RIC for MSD and MUD transplants [27]. This led to a concerted effort to design a conditioning regimen that would reduce both acute and chronic GVHD.

RIC with low-dose busulfan and fludarabine and ATG + cyclosporine–based GVHD prophylaxis showed outcomes comparable with MUD with lower rates of cGVHD [18]. The inclusion of ATG in conditioning regimens results in faster achievement of donor chimerism, especially when using alternative donors [17,18]. However, the use of ATG is also associated with delayed immune reconstitution and increased infective complications, particularly viral reactivations with CMV or EBV [19–21].

Due to the higher degree of immunosuppression, haploHSCT recipients have an increased risk of infectious complications, particularly viral infections including CMV reactivation. High incidence of CMV reactivation was noted in our study; however CMV-related mortality was very low. T cell–replete haploHSCT recipients have a lower incidence of infections than T cell–depleted haploHSCT patients do because of improved immune reconstitution; the incidence of CMV reactivation has been reported to be 60% [12]. The best approach to prevent CMV reactivation in the setting of haploHSCT is not well established. The use of novel agents such as letermovir as prophylaxis may be potentially useful avenues of research [28].

EBV-related PTLD after allogeneic HSCT is associated with mortality rates as high as 50% to 90% [29,30]. Based on European data, the incidence of PTLD after allogeneic HSCT was 3.2%, varying from 1.2% in MUDs to 2.8% in mismatched related donors (including haploidentical and mismatched related donors). In the context of ATG-based conditioning, the incidence of PTLD has been reported to be 9.7% [31]. The risk of developing EBV-PTLD is predominantly related to the degree of T cell depletion or impairment, as EBV-infected B cells are held in check by cytotoxic T cell lymphocytes. This loss of this mechanism leads to uncontrolled B cell proliferation and the development of PTLD [32]. Although the incidence of EBV reactivation and PTLD in our patients was high, all patients responded well to rituximab monotherapy and no PTLD-related deaths occurred. The observed high rate of EBV reactivation and PTLD may be attributed to T cell impairment between donor and recipient secondary to HLA mismatch, the use of dual T cell depletion with PTCy and ATG, and the high rate of CMV reactivation. Patients were monitored closely with weekly PCR-based testing for EBV. This may have led to a higher rate of detection of EBV reactivation. EBV seromismatch has been described as a risk factor for PTLD development, and the selection of an EBV-matched donor may affect rates of reactivation in a manner similar to CMV [31]. There are no clear guidelines for the monitoring and preemptive treatment of EBV reactivation or PTLD in haploHSCT.

Our study's major limitation is the short duration of follow-up. The focus of this report is to demonstrate the applicability of this novel conditioning regimen in a broad selection of patients. While a longer follow-up would certainly be more informative for outcomes such as chronic GVHD and longer-term complications, the lower incidence of acute GVHD grade

II to IV was encouraging. The incidence of NRM was of considerable concern and has been attributed to infective complications, particularly after viral infections. We have implemented a reduction in the ATG dose in an attempt to mitigate the infection risk. Finally, the proportion of older (35% over 65 years of age) patients may explain lower tolerance to infectious and other post-transplant complications. Future modifications to this protocol may also include more aggressive antiviral prophylaxis and therapy.

To summarize, our experience indicates that T cell–replete haploHSCT after RIC with low-dose busulfan, fludarabine, TBI (200 cGy) combined with ATG, PTCy, and cyclosporine is a feasible and effective transplant regimen. This approach produced consistent donor cell engraftment with low rates of acute GVHD; however, this was tempered by high rates of viral reactivation and consequently, NRM. HaploHSCT may be used when a suitable HLA-matched donor is not available or when allogeneic HSCT is needed urgently. Prospective studies are needed to compare the outcomes of different conditioning regimens in haploHSCT and the outcome of allogeneic HSCT using alternative graft sources.

ACKNOWLEDGMENTS

Financial disclosure: The authors have nothing to disclose.

Conflict of interest statement: There are no conflicts of interest to report.

Authorship statement: A.L. and Q.S. collected and analyzed the data and contributed equally to the writing of this manuscript. W.L., S.T., J.L., F.M., R.K., D.K., H.M., and A.V. provided valuable input into the study design, analysis, and interpretation and reviewed the manuscript.

SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at doi:10.1016/j.bbmt.2018.07.008.

REFERENCES

1 Copelan EA. Hematopoietic stem-cell transplantation. *N Engl J Med*. 2006;354(17):1813–1826.
2 Gragert L, Eapen M, Williams E, et al. HLA match likelihoods for hematopoietic stem-cell grafts in the U.S. registry. *N Engl J Med*. 2014;371(4):339–348.
3 Besse K, Maiers M, Confer D, Albrecht M. On modeling human leukocyte antigen-identical sibling match probability for allogeneic hematopoietic cell transplantation: estimating the need for an unrelated donor source. *Biol Blood Marrow Transplant*. 2016;22(3):410–417.
4 Bolanos-Meade J, Fuchs EJ, Luznik L, et al. HLA-haploidentical bone marrow transplantation with posttransplant cyclophosphamide expands the donor pool for patients with sickle cell disease. *Blood*. 2012;120(22):4285–4291.
5 Deotare U, Atenafu EG, Loach D, et al. Reduction of severe acute graft-versus-host disease using a combination of pre transplant anti-thymocyte globulin and post-transplant cyclophosphamide in matched unrelated donor transplantation. *Bone Marrow Transplant*. 2018;53(3):361–365.
6 Kanda Y. Investigation of the freely available easy-to-use software 'EZR' for medical statistics. *Bone Marrow Transplant*. 2012;48:452.
7 Przepiorka D, Weisdorf D, Martin P, et al. consensus conference on acute GVHD grading. *Bone Marrow Transplant*. 1994;15(6):825–828. 1995.
8 Pavletic SZ, Martin P, Lee SJ, et al. Measuring therapeutic response in chronic graft-versus-host disease: National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: IV. Response Criteria Working Group report. *Biol Blood Marrow Transplant*. 2006;12(3):252–266.
9 Kernan NA, Collins NH, Juliano L, Cartagena T, Dupont B, O'Reilly RJ. Clonable T lymphocytes in T cell-depleted bone marrow transplants correlate with development of graft-v-host disease. *Blood*. 1986;68(3):770–773.
10 Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. *Biol Blood Marrow Transplant*. 2008;14(6):641–650.
11 Luznik L, Jalla S, Engstrom LW, Iannone R, Fuchs EJ. Durable engraftment of major histocompatibility complex-incompatible cells after nonmyeloablative conditioning with fludarabine, low-dose total body irradiation, and posttransplantation cyclophosphamide. *Blood*. 2001;98(12):3456–3464.
12 Ciurea SO, Mulanovich V, Saliba RM, et al. Improved early outcomes using a T cell replete graft compared with T cell depleted haploidentical hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2012;18(12):1835–1844.
13 Al-Homsi AS, Roy TS, Cole K, Feng Y, Duffner U. Post-transplant high-dose cyclophosphamide for the prevention of graft-versus-host disease. *Biol Blood Marrow Transplant*. 2015;21(4):604–611.
14 Bradstock KF, Bilmon I, Kwan J, et al. Single-agent high-dose cyclophosphamide for graft-versus-host disease prophylaxis in human leukocyte antigen–matched reduced-intensity peripheral blood stem cell transplantation results in an unacceptably high rate of severe acute graft-versus-host disease. *Biol Blood Marrow Transplant*. 2015;21(5):941–944.
15 Brunstein CG, Fuchs EJ, Carter SL, et al. Alternative donor transplantation after reduced intensity conditioning: results of parallel phase 2 trials using partially HLA-mismatched related bone marrow or unrelated double umbilical cord blood grafts. *Blood*. 2011;118(2):282–288.
16 Solomon SR, Sizemore CA, Sanacore M, et al. Haploidentical transplantation using T cell replete peripheral blood stem cells and myeloablative conditioning in patients with high-risk hematologic malignancies who lack conventional donors is well tolerated and produces excellent relapse-free survival: results of a prospective phase II trial. *Biol Blood Marrow Transplant*. 2012;18(12):1859–1866.
17 Shin DY, Lee JH, Park S, et al. Role of thymoglobulin in matched sibling allogeneic hematopoietic stem cell transplantation with busulfan and fludarabine conditioning in myeloid malignancies. *Bone Marrow Transplant* 2018;53:207-212;.
18 Lee KH, Lee JH, Lee JH, et al. Reduced-intensity conditioning with busulfan, fludarabine, and antithymocyte globulin for hematopoietic cell transplantation from unrelated or haploidentical family donors in patients with acute myeloid leukemia in remission. *Biol Blood Marrow Transplant*. 2017;23(9):1555–1566.
19 Kroger N, Solano C, Wolschke C, et al. Antilymphocyte globulin for prevention of chronic graft-versus-host disease. *N Engl J Med*. 2016;374(1):43–53.
20 Kalra A, Williamson T, Daly A, et al. Impact of donor and recipient cytomegalovirus serostatus on outcomes of antithymocyte globulin-conditioned hematopoietic cell transplantation. *Biol Blood Marrow Transplant*. 2016;22(9):1654–1663.
21 Chang YJ, Wang Y, Mo XD, et al. Optimal dose of rabbit thymoglobulin in conditioning regimens for unmanipulated, haploidentical, hematopoietic stem cell transplantation: long-term outcomes of a prospective randomized trial. *Cancer*. 2017;123(15):2881–2892.
22 Xu LP, Zhang CL, Mo XD, et al. Epstein-Barr virus-related post-transplantation lymphoproliferative disorder after unmanipulated human leukocyte antigen haploidentical hematopoietic stem cell transplantation: incidence, risk factors, treatment, and clinical outcomes. *Biol Blood Marrow Transplant*. 2015;21(12):2185–2191.
23 Ciurea SO, Zhang MJ, Bacigalupo AA, et al. Haploidentical transplant with posttransplant cyclophosphamide vs matched unrelated donor transplant for acute myeloid leukemia. *Blood*. 2015;126(8):1033–1040.
24 Ciurea SO, Shah MV, Saliba RM, et al. Haploidentical transplantation for older patients with acute myeloid leukemia and myelodysplastic syndrome. *Biol Blood Marrow Transplant*. 2018;24:1232–1236.
25 Kanate AS, Mussetti A, Kharfan-Dabaja MA, et al. Reduced-intensity transplantation for lymphomas using haploidentical related donors vs HLA-matched unrelated donors. *Blood*. 2016;127(7):938–947.
26 Wang Y, Chang YJ, Chen L, et al. Low-dose post-transplant cyclophosphamide can mitigate GVHD and enhance the G-CSF/ATG induced GVHD protective activity and improve haploidentical transplant outcomes. *OncoImmunology*. 2017;6(11) e1356152.
27 Sibai H, Falcone U, Deotare U. Myeloablative versus reduced-intensity conditioning in patients with myeloid malignancies: a propensity score-matched analysis. *Biol Blood Marrow Transplant*. 2016;22(12):2270–2275.
28 Marty FM, Ljungman P, Chemaly RF, et al. Letermovir prophylaxis for cytomegalovirus in hematopoietic-cell transplantation. *N Engl J Med*. 2017;377(25):2433–2444.
29 Reddy N, Rezvani K, Barrett AJ, Savani BN. Strategies to prevent EBV reactivation and posttransplant lymphoproliferative disorders (PTLD) after allogeneic stem cell transplantation in high-risk patients. *Biol Blood Marrow Transplant*. 2011;17(5):591–597.
30 Styczynski J, Gil L, Tridello G, et al. Response to rituximab-based therapy and risk factor analysis in Epstein Barr virus-related lymphoproliferative disorder after hematopoietic stem cell transplant in children and adults: a study from the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation. *Clin Infect Dis*. 2013;57(6):794–802.
31 Kalra A, Roessner C, Jupp J, et al. Risk factors for post-transplant lymphoproliferative disorder after thymoglobulin-conditioned hematopoietic cell transplantation. *Clin Transplant*. 2018;32(1):e13150.
32 Dierickx D, Habermann TM. Post-transplantation lymphoproliferative disorders in adults. *N Engl J Med*. 2018;378(6):549–562.