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Outcomes of Hematopoietic Cell Transplantation in Adult Patients with Acquired Aplastic Anemia Using Intermediate-Dose Alemtuzumab-Based Conditioning

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A B S T R A C T

Graft-versus-host disease (GVHD) has no therapeutic benefit after hematopoietic cell transplantation (HCT) for patients with acquired aplastic anemia (AA), and its prevention is highly desirable. We designed a conditioning regimen using an intermediate dose of alemtuzumab (50 to 60 mg) and describe our institutional experience of 41 patients who underwent HCT for AA. The median age at HCT was 37 years (range, 17 to 59). The conditioning regimen was high-dose cyclophosphamide (n = 9) or fludarabine based (n = 32). Additional GVHD prophylaxis was with cyclosporine. With a median follow-up of 3.6 years, overall survival at 3 years was 85%. Survival in patients <40 years and ≥40 years was 96% and 67%, respectively ($P = .04$). Graft failure occurred in 4 (10%) patients; 2 primary and 2 secondary. The cumulative incidences of acute (grades 1 to 2) and chronic GVHD were 27% and 15%, respectively. No patients developed grade 3 to 4 acute GVHD or severe chronic GVHD. The following viral complications were frequent: cytomegalovirus reactivation (79%), herpes simplex (18%), varicella zoster (25%), and BK virus hemorrhagic cystitis (8%). The majority of patients had no significant long-term health issues. This intermediate-dose alemtuzumab-based conditioning regimen results in excellent survival with a favorable impact on GVHD and long-term health outcomes, but close monitoring for viral complications is important.

INTRODUCTION

The goals of hematopoietic cell transplant (HCT) in acquired aplastic anemia (AA) are sustained hematopoietic recovery, minimal regimen-related toxicities, and minimal graft-versus-host disease (GVHD). The most commonly used conditioning regimen in AA is cyclophosphamide and antithymocyte globulin (ATG) with cyclosporine and methotrexate as GVHD prophylaxis [1]. In addition, total body irradiation is often used for unrelated donor transplantations for patients with AA [2-4]. With these strategies, 30% to 40% of patients with AA develop acute and chronic GVHD, resulting in morbidity, mortality, and poor quality of life [1,3,5-7]. Unlike its effect in hematologic malignancies, GVHD has no therapeutic benefit in patients with AA, and prevention of GVHD is one of the most desirable goals of HCT in AA.

Previous studies reported a favorable effect on acute and chronic GVHD in HCT for AA using in vivo anti-CD52 monoclonal antibodies [8,9]. The main concerns with the use of anti-CD52 antibodies include the risk of graft failure and the increased risk of infectious complications [10-12]. In addition to using different dosing schedules, previous studies used a higher dose of anti-CD52 antibodies (75 to 100 mg) [8,9,13]. There is a suggestion that a lower dose of alemtuzumab may result in a lower risk of infectious complications and faster immune reconstitution [14].

To optimize the use of alemtuzumab in HCT for AA at Princess Margaret Cancer Centre, we designed a conditioning regimen with an intermediate dose of alemtuzumab (50 to 60 mg). We previously published preliminary outcomes of 17 patients treated with alemtuzumab-based GVHD prophylaxis [13,15]. This study reports on outcomes of an additional 24 patients, with updated follow-up on previously reported patients.

METHODS

Patients

A total of 41 consecutive patients who received HCT for AA between January 2005 and August 2013 at the Princess Margaret Cancer Center, Toronto, Canada were included in this study. Data were obtained from a blood and marrow transplantation database, and additional information

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was collected from computerized records and patient charts. The University Health Network-Research Ethics Board and Cancer Registry Data Access Committee approved this study.

Eligibility for Transplantation

Patients with severe AA (SAA) <40 years, and having a matched sibling donor (MSD) were offered upfront transplantation. The upper age limit for upfront therapy using MSD transplantation for patients with SAA was increased to 60 years in 2010. Patients with nonsevere AA (NSAA) were offered transplantation after failure of immunosuppressive therapy (IST) using a related or unrelated donor. Patients who only had a mismatched related donor (up to 1 antigen mismatch) or matched unrelated donor were eligible for transplantation if they were refractory to or had relapsed after ATG-containing IST.

Graft Source

Although the preferred source of stem cells was bone marrow (BM), peripheral blood grafts were accepted, based on the donor's choice. Thirty-four (83%) patients received BM grafts and 7 (17%) received peripheral blood grafts.

Conditioning Regimen

Between 2005 and 2009, the institutional standard of care for AA patients <40 years with an MSD was conditioning with high-dose cyclophosphamide (50 mg/kg × 4 days from days −5 to −2) with alemtuzumab 50 mg (10 mg, 20 mg, 20 mg on days −8, −7, and −6 respectively) (n = 9, 23%), and those ≥40 years with an MSD or an MUD regardless of age received fludarabine (30 mg/m^2 × 4 days from days −5 to −2) with low-dose cyclophosphamide (10 mg/kg × 4 days from days −5 to −2) and alemtuzumab 60 mg (30 mg × 2 days on days −7 and −6) (Flu-Cy-Cam) (n = 28, 70%). In 2009, Flu-Cy-Cam became our institutional standard of care for all patients with AA receiving HCT (see Appendix in Supplementary Data for the complete protocol). Busulfan replaced low-dose cyclophosphamide in the Flu-Cy-Cam regimen in 3 patients included in this study. One of these patients had monosomy 7, with BM biopsy findings consistent with AA. The other 2 patients had SAA and symptomatic hemolytic paroxysmal nocturnal hemoglobinuria, and these 2 patients also received 200 cGy total body irradiation as a single fraction.

In the first 10 (25%) patients, alemtuzumab was used as an i.v. infusion; however, in 2006, we changed our institutional practice to subcutaneous (SC) alemtuzumab because of evolving data on its improved side effect profile [16–18] and, therefore, the subsequent 31 (75%) patients received SC alemtuzumab. Premedication for alemtuzumab (30 minutes to 1 hour before) included diphenhydramine 50 mg, acetaminophen 650 mg, and dexamethasone 20 mg orally.

GVHD Prophylaxis

Cyclosporin (CsA) was used for GVHD prophylaxis at a dose of 2.5 mg/kg/day i.v. every 12 hours, starting on day −1, titrated to therapeutic trough levels between 200 and 400 μg/L. When patients were able to tolerate oral medications, the i.v. dose was converted to the oral route. Slow CsA tapering was initiated at 6 months after HCT unless mixed chimerism or ongoing GVHD necessitated continuation of IST. Two patients received tacrolimus (titrated to therapeutic levels of 10 to 20 ug/L) instead of CsA because of a history of gum enlargement with CsA. One patient received mycophenolate mofetil instead of calcineurin inhibitors because of end-stage kidney disease at time of HCT [19].

Definitions

The burden of comorbidities was calculated using the HCT comorbidity index (HCT-CI) [20]. Performance status was assessed using the Karnofsky performance score.

The day of stem cell infusion was defined as day 0. Dates of *neutrophil* and *platelet recovery* were defined as the first of 3 consecutive days of an unsupported absolute neutrophil count ≥.5 × 10^9/L and unsupported platelet count ≥20 × 10^9/L, respectively. *Lymphocyte recovery* was defined as the first of 3 consecutive days of an absolute lymphocyte count of ≥.5 × 10^9/L. Patients were evaluable for engraftment if they survived >21 days after HCT. *Primary graft failure* was defined as the absence of absolute neutrophil count of ≥.5 × 10^9/L on 3 consecutive days after 6 weeks and *secondary graft failure* was defined as recovery followed by recurrent pancytopenia with a hypocellular BM in the absence of severe GVHD. *Time-to-RBC* and *platelet transfusion independence* were defined as the time from the date of HCT to the last date of transfusion for each.

Regimen-related toxicities were evaluated from the start of conditioning therapy until 6 weeks after HCT, defined according to the Bearman's criteria [21].

Patients who survived more than 21 and 100 days were evaluable for the occurrence of acute and chronic GVHD, respectively. Acute and chronic GVHD were graded according to modified Gluckberg's [22] and National Institutes of Health consensus criteria [23], respectively.

Bacterial and viral infections were based on culture and PCR, respectively. Invasive fungal infections were defined as per European Organization for Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) criteria [24].

Supportive Care

All patients received antibacterial, antiviral, and antifungal prophylaxis. Antibacterial prophylaxis with ciprofloxacin 500 mg twice daily orally was given until neutrophil engraftment. Antipneumocystis prophylaxis was initiated at the time of hospital discharge and consisted of oral cotrimoxazole 3 times per week orally or inhaled pentamidine 300 mg monthly (if allergic or intolerant to cotrimoxazole) and continued to 1 year after HCT or longer, if the patient was still on IST. Anti–herpes simplex virus (HSV) prophylaxis with acyclovir 400 mg once daily orally was given for 3 months. All patients received antifungal prophylaxis with itraconazole suspension (200 mg orally twice daily for 3 months) until January 2009, after which there was a change in our institutional practice to i.v. micafungin during the hospital stay and a switch on discharge to oral posaconazole suspension 200 mg 3 times a day for 3 months. Routine surveillance of cytomegalovirus (CMV) viremia was performed on a weekly basis for the first 100 days and subsequently on clinic visits up to 1 year or according to clinical need. This was done using pp65 antigenemia until 2011 and, thereafter, this was performed using PCR. Ganciclovir was used as preemptive treatment in patients testing positive for CMV viremia. Pulmonary function tests (PFTs) were routinely performed at baseline, 4 and 12 months after HCT, and annually thereafter. Hormone profiles including sex hormones, prolactin, and thyroid function tests were also done annually.

Chimerism and Disease Monitoring

Chimerism studies were performed on whole blood by short tandem repeat (STR)-PCR analysis. Testing was carried out at 30, 60, and 120 days after HCT routinely, and according to clinical need thereafter. Donor cells ≥90% were considered as predominantly donor chimerism (PDC) and <90% as mixed chimerism (MC). *Declining donor chimerism* was defined as a ≥10% decrease in donor chimerism on 2 consecutive measurements. In cases of MC, an increase in the intensity of IST was attempted.

Statistical Methods

Categorical variables were summarized with counts and percentages and continuous variables with medians and ranges or standard deviations, as appropriate. Time-to-reach hematologic endpoints were calculated using primary engraftment failure and death as competing endpoints. The incidence of infection was calculated using the cumulative incidence method with death and second HCT as a competing end point. The probability of overall survival (OS) was calculated with the Kaplan-Meier method. Univariate analysis was performed on OS using binary clinical factors with Kaplan-Meier curves and log-rank tests. Because of the small number of events, a multivariate analysis was not performed. All analysis was performed using R Version 3.0.1 (R Development Core Team).

RESULTS

Baseline patient-, disease-, and transplantation-related characteristics are summarized in Table 1. The median age at HCT was 37 years (range, 17 to 59). The majority of patients had SAA: 12 (29%) very severe AA, 19 (46%) SAA, and 10 (24%) NSAA. Patients with NSAA had failed IST and were transfusion dependent. Donors were HLA MSD in 29 (70%) and MUD in 12 (30%). Median follow-up of survivors was 3.6 years (range, .13 to 7.9).

Side Effects of i.v. and SC Alemtuzumab

Side effects of i.v. alemtuzumab (n = 10) were infusion-related rigors/chills (n = 6, 60%), fever (n = 4, 40%), generalized skin rash (n = 3, 30%), chest tightness (n = 1, 10%), and hypotension (n = 1, 10%). With SC alemtuzumab (n = 31), side effects were self-limiting erythema at injection site (n = 26, 84%), generalized skin rash (n = 3, 10%), and fever (n = 2, 6%). None of the patients discontinued alemtuzumab because of side effects.

Regimen-related Toxicities

The majority of patients tolerated the conditioning well. None of the patients had grade 3 toxicities. Bearman grade 1

Table 1
Patient, Disease, and Transplantation Characteristics

Characteristic	Value
No. of patients	41
Age at HCT, median (range), yr	37 (17-59)
<40	25 (61)
≥40	16 (39)
Gender	
Female	23 (56)
Male	18 (44)
Disease etiology	
Idiopathic	30 (73)
Chemical/drug exposure	4 (10)
Hepatitis	4 (10)
Pregnancy	3 (7)
Disease severity	
Very severe AA	12 (29)
SAA	19 (46)
NSAA	10 (24)
Paroxysmal nocturnal hematuria clone	
Negative	25 (61)
Positive	16 (39)
Cytogenetics	
Normal	26 (63)
Failed/unavailable	14 (34)
Monosomy 7	1 (2)
Prior IST	
None	20 (49)
1 course	11 (27)
≥2 courses	10 (24)
HCT-CI score	
0/1	33 (80)
2/3	8 (20)
Performance score at HCT	
≥80%	39 (95)
<80%	2 (5)
Time to HCT	
≥6 mo	26 (63)
<6 mo	15 (37)
Donor type	
MSD 6/6	29 (71)
MUD	12 (29)
10/10	11 (28)
9/10 (antigen DQ mismatch)	1 (2)
Female donor to male recipient	8 (20)
Graft source	
BM	34 (83)
PB	7 (17)
ABO mismatch	
Major	9
Minor	10
None	22
Recipient/donor CMV status	
Negative/negative	16 (39)
All others	25 (61)
Conditioning regimen	
CY(4)	9 (22)
FLU(30) + CY(40)	29 (71)
FLU(4) + BU(2)	1 (2)
FLU(4) + BU(2) + TBI 200	2 (4)
GVHD prophylaxis	
Alemtuzumab + cyclosporine	37 (90)
Alemtuzumab + tacrolimus	3 (7)
Alemtuzumab + mycophenolate	1 (2)
Alemtuzumab dose	
60 mg	35 (85)
50 mg	6 (15)
Alemtuzumab route of administration	
Subcutaneous	31 (76)
Intravenous	10 (24)

PB indicates peripheral blood; CY, cyclophosphamide; FLU, fludarabine; BU, busulfan; TBI, total body irradiation.
Data presented are n (%), unless otherwise indicated.

toxicities were seen in 23 patients (56%), and 12 (29%) had a grade 2 toxicity. The most common regimen-related toxicity was mucositis (21 [51%] grade 1 and 9 [21%] grade 2). Two patients had grade 2 renal toxicity that resolved to baseline levels, 1 patient had grade 2 hepatotoxicity that resolved, 1 patient had hepatic veno-occlusive disease that responded to supportive care, and 1 patient had central line–related subclavian vein thrombosis.

Hematopoietic Recovery

The median times to neutrophil and platelet recovery were 17 days (range, 11 to 33) and 12 days (range, 2 to 33) respectively. The median times to RBC and platelet transfusion independence in survivors were 32.5 days (range, 0 to 1360) and 11 days (range, 0 to 373), respectively. The upper limit of time to RBC transfusion independence was due to 4 patients who required prolonged RBC transfusion support, 1 due to ABO mismatch, 1 developed an acquired factor VIII inhibitor requiring intensive care unit admission, 1 had chronic gastrointestinal bleeding due to gastric antral vascular ectasia, and 1 developed warm autoimmune hemolytic anemia in the absence of any GVHD signs or symptoms. Three of these patients required RBC transfusion support beyond 1 year.

Graft Failure

Graft failure occurred in 4 (10%) patients: 2 primary and 2 secondary. The 2 patients who had primary graft failure received second HCTs from a second donor; 1 died of HCT-related complications and the other is a long-term survivor. The 2 patients who had secondary graft failure (day 64 and day 85) received supportive care; 1 died of an intracranial hemorrhage and the other died of sepsis. All patients who had graft failure had failed IST before HCT.

Chimerism Data

Thirty-five (85%) patients had serial chimerism data available, 4 had a single result, and 2 patients died before routine testing. At 120 days after HCT, the median chimerism level was 96% (range, 62% to 100%).

At last follow-up of the 35 patients with serial results, 21 (60%) had attained and sustained stable PDC. Seven (21%) had stable MC with no intervention. One (3%) patient initially attained PDC but developed secondary graft failure and died.

GVHD

In 40 evaluable patients (1 patient died at day 3), acute GVHD was observed in 11 (28%) patients, all grade I and II. Of 33 evaluable patients, only 6 (15%) developed chronic GVHD; 5 had mild, 1 had moderate, and none had severe chronic GVHD. The cumulative incidences of acute and chronic GVHD were 27% (95% confidence interval [CI], 13% to 41%) and 15% (95% CI, 4% to 27%), respectively (Figure 1A,B). GVHD did not contribute to any of the deaths. Only 2 of 34 (6%) survivors were on corticosteroid treatment for GVHD treatment beyond 1 year. No patients had lung GVHD.

Infections

Culture-positive bacterial infection within the first 100 days after HCT occurred in 21 (51%) patients and sepsis was the primary cause of death in 2 (5%) patients. The most commonly cultured organism was coagulase-negative staphylococcus in 9 of the 21 (43%) patients. Bacterial infection beyond day 100 to 1 year occurred in 18 (50%) of 36 evaluable patients. The most commonly cultured organisms were pseudomonas and coagulase-negative staphylococcus; each cultured in 5 (28%) of the 18 patients.

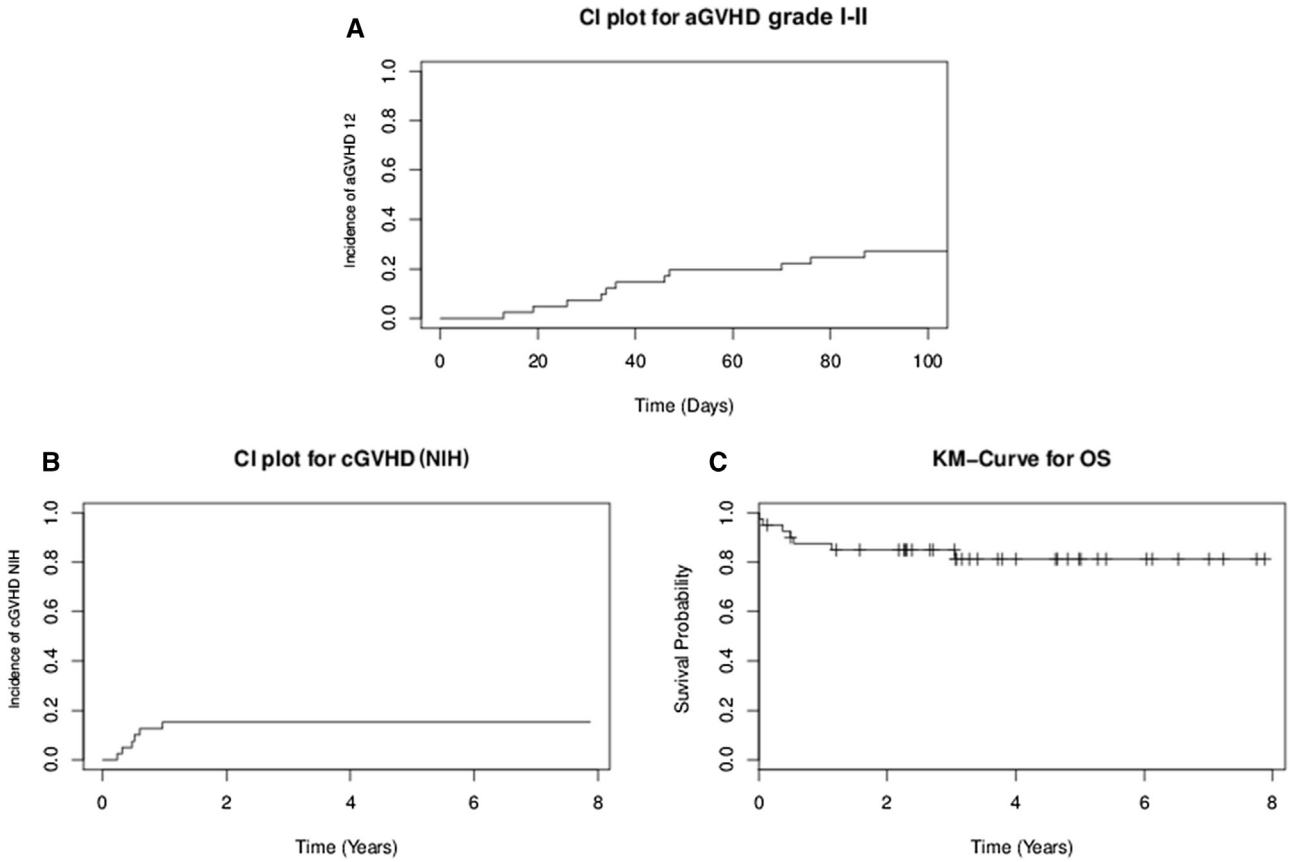

Figure 1. Transplantation outcomes in aplastic anemia HCT using alemtuzumab-based conditioning. (A) Shows cumulative incidence of acute GVHD (all grades I to II). (B) Shows cumulative incidence of chronic GVHD (all mild to moderate). (C) Shows 3-year overall survival Kaplan-Meier plot.

CMV reactivation occurred in 19 (76%) of the 25 at-risk patients. The median time to reactivation was 37 days (range, 21 to 129). Seven (28%) of these patients had a single reactivation, whereas the remaining 12 (72%) had recurrent reactivations and none developed CMV disease. HSV reactivation occurred in 7 (18%) of 38 evaluable patients. All of these patients responded to acyclovir. Varicella zoster virus reactivation occurred in 10 (26%) of 38 evaluable patients, and 2 patients developed postherpetic neuralgia. BK hemorrhagic cystitis occurred in 3 (8%) patients and resolved in all patients. The cumulative incidences of viral infections are presented in Figure 2A. None of the patients developed probable or proven invasive fungal infection.

Post-transplantation Lymphoproliferative Disorder

Three patients developed an Epstein-Barr virus (EBV)–related lymphoproliferative disorder (PTLD) at days 72, 139, and 167 (Table 2). Two responded to rituximab-based treatment and 1 died because of progressive PTLD, despite therapy. All of these patients had failed IST before HCT; 2 failed 1 course of IST and the patient who died failed 2 courses of ATG-based IST. One patient, who had a second transplantation for primary graft failure, developed PTLD after the second HCT. Details of these 4 cases of PTLD are presented in Table 2 and Figure 2B.

Survival

With a median follow-up of 3.6 years, OS at 3 years was 85% (Figure 1C). At time of last follow-up, a total of 7 patients died. Of these, 5 (71%) had an MSD and 2 (29%) had an MUD. One of these patients received HCT as upfront treatment and died 3 years after HCT of an unknown cause after loss to follow-up. The remaining 6 patients had all failed ATG-based IST before HCT (1 failed 2 courses and 5 failed 1 course). The individual causes of death were secondary graft failure in 2 patients (days 137 and 199), sepsis in 2 (days 3 and 199), and PTLD in 1 (day 180). One patient died of complications of a second HCT for primary graft failure (day 414 after the first HCT).

In univariable analysis (including age, lines of pre-HCT IST, HCT-CI score, Karnofsky performance score, donor, graft source, CMV risk, and time to transplantation), age <40 years was the only factor associated with improved OS ($P = .04$) (Table 3). Nevertheless, 11 of the 16 (69%) patients over 40 years are alive with an overall probability of survival at 1 year of 67% (95% CI, 47% to 95%).

Long-term Health Outcomes

We evaluated the long-term health outcomes of all the survivors, which are summarized in Table 4. Relevant details are below.

Lung function

Of the evaluable 33 patients, 27 had evaluable PFTs at 1 year. Of these, only 1 patient had an asymptomatic drop in forced expiratory volume in 1 second $>20\%$, which returned to baseline at the second annual assessment. At last follow-up, 27 patients had serial PFTs available for review: all had forced expiratory volume in 1 second values similar to baseline (Table 4).

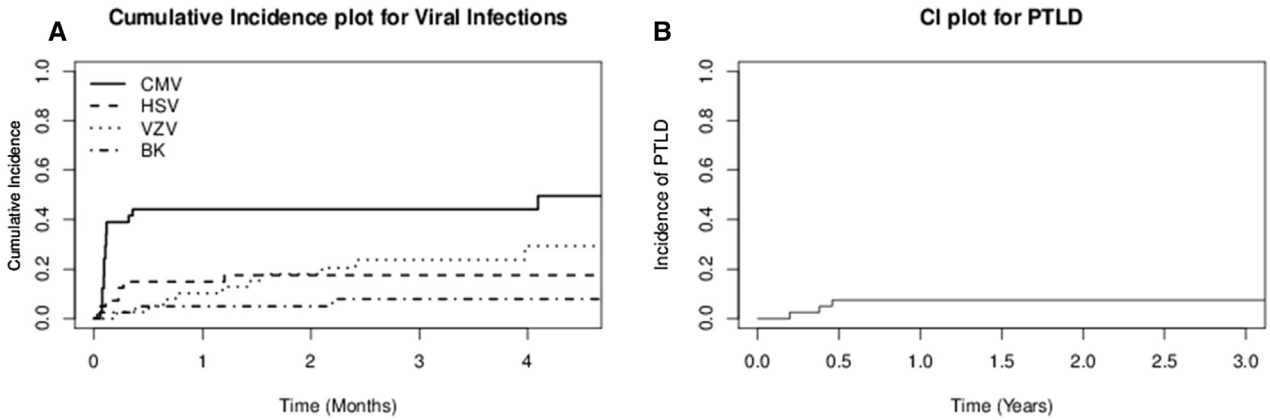

Figure 2. Viral complications and PTLD in aplastic anemia HCT using alemtuzumab-based conditioning. (A) Shows cumulative incidence of viral infections. (B) Shows cumulative incidence of PTLD.

Endocrine disturbances and autoimmune disorders

At last follow-up, 7 (21%) of the 33 survivors of the first HCT were requiring thyroxine replacement therapy. One patient had a major ABO mismatched donor and was not requiring transfusion but developed warm autoimmune hemolytic anemia at day 404 after HCT and became transfusion dependent, despite multiple lines of treatment. One patient developed Guillain-Barre syndrome at day 272 after HCT and made a full recovery after treatment with intravenous immunoglobulins. One patient developed an acquired factor VIII inhibitor at day 405 after HCT. One patient developed Graves hyperthyroidism 3.9 years after HCT that was thought to be transplant-mediated, as the donor had a history of this.

Performance Status at Last Follow-up and Return to Work

Of the 34 survivors, only 2 patients had Karnofsky performance scores <80%. One patient had severe autoimmune hemolytic anemia and required a splenectomy and the other developed smoldering myeloma 2 years and 9 months after HSCT, had cryptogenic fulminant hepatic failure, and received a cadaveric liver transplant at 3 years and 10 months after HCT.

Fertility

Seventeen women were ages 16 to 45 years of age at time of HCT; 2 of them died and 1 was lost to follow-up. Of the remaining 14 women, 13 (93%) had return of their regular menses after HCT. Six women conceived at 8, 11, 21, 22, 34, and 89 months after HCT. Three women delivered healthy babies, 1 is yet to deliver, and 2 had medical terminations. Thirteen of these women were more than 1 year after HCT and at last follow-up; 12 (92%) had normal estradiol levels.

Secondary Malignancy

Only 1 long-term survivor developed a secondary malignancy: smoldering myeloma. This patient had failed 2 rounds of ATG-based IST before HCT.

DISCUSSION

We report the findings of a simplified conditioning regimen for patients with AA. Compared with previously published studies [1,8,9,13,15,25-28], we show that an intermediate dose of alemtuzumab (50 to 60 mg) in combination with high-dose cyclophosphamide or fludarabine and low-dose cyclophosphamide is effective in reducing the risk of significant acute and chronic GVHD, and the impact on survival has been favorable (3-year OS of 85% in adult patients). The rate of graft rejection is comparable to existing studies [8,9,13,15,26-28]. As this study only involved adult patients, the median age of 37 years is higher than previous published reports [1,3-6,8,9].

In this study cohort, we also attempted to further explore the detailed clinical course of patients receiving alemtuzumab-based conditioning to identify tolerability in older patients, infection risks, long-term health, and quality-of-life outcomes. Patients older than 40 years old composed 39% of this study cohort, with a 1-year OS probability of 67%. Although this appears inferior to survival of patients younger than 40, it appears comparable or better than previously reported studies [1,25]. The HCT-CI was previously identified as a predictor of outcome in the study by Marsh et al. [13], but we were not able to confirm that the HCT-CI score or performance status were predictive of outcome in this study, likely because of the small number of patients.

There is a concern that the use of alemtuzumab is associated with an increased risk of viral infection compared with ATG [29,30]. Previous studies using alemtuzumab-based

Table 2
Post-Transplantation Lymphoproliferative Disease

Patient No.	No. of lines of IST before HCT	Time to HCT, mo	Donor	Time from HCT to PTLD, d	Treatment of PTLD	Outcome
1	2	191	MUD	167	1 x R-CHOP	Died
2	2	11	MUD	72	1 x R-CHOP then 4 x rituximab	Remission
3	1	8	MSD	139	4 x rituximab	Remission
4	1	8	MUD	307*	4 x rituximab	Remission

R-CHOP indicates rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone.
* PTLD occurred on day 307 after HCT; however, this patient had a second HCT on day 85 because of primary graft failure.

Table 3
Univariable Analysis for OS

Variable	n (%)	*P* Value	3-Year Survival Probability (95% CI)
Age		.041	
≤40	25 (61)		96 (89-100)
>40	16 (39)		67 (47-95)
Lines of pre-HCT IST		.084	
0	20 (49)		95 (86-100)
1	11 (27)		61 (38-100)
≥2	10 (24)		89 (71-100)
HCT-CI		.66	
0-1	33 (80)		84 (72-98)
2-3	8 (20)		88 (67-100)
Performance score		.10	
≥80%	39 (95)		87 (77-98)
<80%	2 (5)		50 (13-100)
Donor		1	
Matched sibling donor	29 (71)		86 (74-100)
Matched unrelated donor	12 (29)		82 (63-100)
Graft source		.29	
BM	34 (83)		88 (78-100)
PB	7 (17)		67 (38-100)
CMV donor/recipient		.44	
-/-	25 (61)		84 (70-100)
All others	16 (39)		88 (73-100)
Time to transplantation		.54	
≥6 mo	26 (63)		80 (65-97)
<6 mo	15 (37)		93 (82-100)

conditioning in AA only reported variable incidences of CMV, EBV, and adenovirus. In this study, we tried to study the infectious complications in detail and show that viral complications are common: CMV reactivation (79%), HSV (18%), varicella zoster virus (25%), and BK virus hemorrhagic cystitis (8%). It is noteworthy that although CMV reactivation is common, none of the patients developed CMV disease with screening and preemptive treatment strategies. Several novel effective CMV preventive strategies are on the horizon, and hopefully integration of these strategies in alemtuzumab-based conditioning may help in decreasing the risk of CMV reactivation [31].

Table 4
Long-Term Health Outcomes after Transplantation

Outcomes in Long-Term Survivors	n = 34
cGVHD requiring steroid therapy beyond 1 year after HCT (evaluable = 6)	2 (33)
>20% FEV1 drop from baseline at time of last follow up (evaluable = 27)	0
Autoimmune disorders	
Gillian-Barre syndrome at day 272 after HCT	1 (3)
Warm autoimmune hemolytic anemia at day 404 after HCT	1 (3)
Acquired factor 8 deficiency at day 405 after HCT	1 (3)
Graves hyperthyroidism at 3.9 years after HCT	1 (3)
Performance score at time of last follow-up (evaluable = 34)	
≥80%	32 (94)
<80%	2 (6)
Employment	
Return to full-time work/education (evaluable = 28)	25 (90)
Unable to return to work (evaluable = 28)	2 (10)
Continued retirement (evaluable = 7)	7 (100)
Female fertility in survivors ages 16-45 years (evaluable = 14)	
Return of menses	13 (93)
Normal estradiol levels at last follow-up	12 (86)
Conceived	6 (43)
Delivered a healthy child	3
Yet to deliver	1
Terminated pregnancy	2
Secondary malignancy (evaluable = 34)	
Shouldering multiple myeloma	1 (3)

Data presented are n (%), unless otherwise indicated.
cGVHD indicates chronic graft-versus-host disease; FEV1, forced expiratory volume in 1 second.

Three patients developed EBV-related PTLD and another developed this after a second HCT. These patients had also failed immunosuppression therapy before HCT. In a retrospective analysis of patients with AA who received HCT, the strongest predictive factor for the development of PTLD was failure to respond to repeated courses of ATG-based IST, which suggests the need for monitoring of EBV-PCR levels in patients who have failed IST before transplantation [32].

Long-term health outcomes using this conditioning regimen appear to be excellent. The majority of patients had pulmonary function that returned to baseline, regained their performance status, and were able to return to work if previously working. In females, fertility appears to be preserved, based on the women who were able to conceive. Preservation of fertility is an important aspect of AA transplantation, as a large proportion of patients diagnosed with AA are within their reproductive years. In addition to regimen-related damage to gonads, risk of infertility is higher in patients who get GVHD. It is plausible in our study that prevention of significant GVHD may have contributed to preserved fertility.

We observed 4 patients who developed autoimmune disorders, which, to our knowledge, has not been widely reported. Autoimmune disease after transplantation is becoming increasingly recognized but data are still only based on single cases and single-center, retrospective studies [33]. It is thought that failure of central tolerance mechanisms during de novo lymphopoiesis after conditioning-induced lymphopenia leads to the generation of new autoreactive T and B cells and, if uncontrolled, to the development of autoimmune disease [34]. In the setting of allogeneic HCT, autoimmune cytopenias are the most common manifestations of autoimmune disease, but there is no evidence linking T cell depletion as a risk factor [33]. As implicated in 1 of the cases included in this study (Graves disease), transfer of autoimmune diseases from the donor to the recipient has also been reported [33].

There is a debate whether there should be an upper age limit for offering an MSD as upfront therapy for SAA. In the beginning, our center adopted the British Guidelines, and upfront transplantation were offered to patients under the age 40 years with an MSD [35]. Encouraged by the ability to perform transplantation with minimal risk of significant GVHD, we extended the upper age limit of transplantation to 60 years as upfront therapy for those who have an MSD. Use of SC alemtuzumab has also further simplified the protocol. We demonstrated in this study that this protocol can be used for related or unrelated donors, regardless of age.

In conclusion, intermediate-dose SC alemtuzumab–based conditioning in AA is a simplified approach for conditioning for AA that resulted in excellent survival and has a favorable impact on GVHD. The majority of survivors had good long-term health outcomes, were able to return to work, and female fertility appears to be preserved. Nevertheless, close monitoring for viral complications, such as CMV reactivation and EBV, particularly in patients who fail IST before HCT, is very important.

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.bbmt.2014.06.033.

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