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Marrow grafts from HLA-identical siblings for severe aplastic anemia: does limiting the number of transplanted marrow cells reduce the risk of chronic GVHD?

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Abstract

Twenty-one patients with severe aplastic anemia underwent marrow transplantation from HLA-identical siblings following a standard conditioning regimen with cyclophosphamide (50 mg/kg/day × 4 days) and horse antithymocyte globulin (30 mg/kg/day × 3 days). Post-grafting immunosuppression consisted of a short course of methotrexate combined with cyclosporine. The transplant protocol tested the hypothesis that the incidence of chronic graft-versus-disease (GVHD) could be reduced by limiting the marrow grafts to $\leq 2.5 \times 10^8$ nucleated marrow cells/kg. None of the patients rejected the graft, all had sustained engraftment and all are surviving a median of 4 (range 1–8) years after transplantation. Chronic GVHD developed in 16% of patients given $\leq 2.5 \times 10^8$ nucleated marrow cells/kg. Post-grafting immunosuppression has been discontinued in 20 of the 21 patients. In conclusion, limiting the number of transplanted marrow cells may have resulted in minimal improvement in the incidence and severity of chronic GVHD.

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Keywords

aplastic anemia; HLA-identical marrow grafts; conditioning regimen; chronic graft-versus-host disease

Introduction

Preclinical canine studies in the 1970s[1,2] led to the clinical introduction of the alternating cyclophosphamide (CY)/anti-thymocyte globulin (ATG) regimen to condition aplastic anemia patients for marrow transplantation from HLA-identical siblings. The regimen was developed to overcome the problem of transfusion-induced sensitization to non-HLA antigens and thereby reduce the high risk of graft rejection observed in early clinical trials.[3-6] It was first used to condition aplastic anemia patients for second marrow grafts following rejection of their first grafts.[7,8] Successful outcomes with second transplants encouraged using CY/ATG as conditioning regimen for first transplants.[9]

In 2005 we reported 88% survival among 81 aplastic anemia patients given HLA-matched related marrow grafts following CY/ATG with a median follow-up of 9.2 years.[10] Graft rejection was the exception. Acute graft-versus-host disease (GVHD), mostly grade 2, was seen at a rate of 24% using post-grafting immunosuppression with methotrexate (MTX) and cyclosporine (CSP).[11,12] The cumulative incidence of chronic GVHD was 26%. Closer analysis of the results showed a significant association between the dose of transplanted marrow cells and the risk of developing chronic GVHD. Specifically, the hazard ratio for developing chronic GVHD was 3.8 when 2.4–3.3 × 10^8 cells/kg were infused compared with ≤2.3 × 10^8 cells/kg; a further increase in the hazard ratio to 7.7 occurred with marrow doses of ≥3.4 × 10^8 cells/kg. The current prospective study tested whether targeting the marrow graft to ≤2.5 × 10^8 nucleated cells/kg reduced the risk of chronic GVHD. We also updated the survival of the previously reported patients with a median follow-up of 19 years.[10]

Materials and Methods

Twenty-one patients with severe aplastic anemia were treated with marrow grafts from HLA-identical sibling donors at Fred Hutchinson Cancer Research Center (FHCRC), Medical College of Wisconsin or Primary Children's Hospital of Utah between August 2006 and February 2015. The criteria for severe aplastic anemia were defined previously.[13] Definition includes marrow cellularity < 25 % with at least two of the following: 1) Absolute neutrophil count < 0.5×10^9/L; 2) platelet counts < 20×10^9/L; 3) absolute reticulocyte < 40×10^9/L. The research protocol and consent forms were approved by the Institutional Review Boards of the three centers. The trial was registered with Clinical trials.org. Patient and donor characteristics are listed in Table 1. The median age of patients was 15 (range 3–52) years. Patients received standard conditioning with CY at 50 mg/kg/day intravenously (IV) for 4 successive days (days −5, −4, −3, and −2). Horse ATG (ATGAM) was administered at a dose of 30 mg/kg recipient body weight IV 10 hours after each of the first 3 doses of CY (days −4, −3, −2). All patients received methylprednisolone, 1 mg/kg IV, before each dose of ATG. Donor bone marrow was infused IV 36 hours after the last dose of CY. The graft volume was adjusted so that no more than 2.5×10^8 nucleated marrow cells/kg

were infused. A median of 2 (range 1.1–3.5) × 10^8 nucleated cells/kg (corrected for white blood cell counts) was administered. In patients 3, 4 and 21, protocol violations occurred and 3.2, 3.5 and 3.1 × 10^8 marrow cells/kg were given, respectively. The median count of transplanted CD34+ cells was 4.4 (range 1.6–9.1) × 10^6/kg. The median counts of CD14+, CD3+, CD4+, and CD8+ cells (available in 16 patients) were 0.6 (range 0.2–1.3), 3.4 (range 1.4–6.6), 1.8 (range 0.7–3.7) and 1.5 (range 0.6–2.7) × 10^7/kg, respectively. Data on naïve T-cells were available in only 4 patients. Naïve CD4+ T-cells ranged from 0.1–0.3 × 10^7/kg and naïve CD8+ T-cells from 0.1–0.8 × 10^7/kg. Patients received MTX, 15 mg/m^2 on day 1 and 10 mg/m^2/day on days 3, 6 and 11, and CSP beginning one day before transplant at 1.5 mg/kg IV every 12 hours until complete resolution of gastrointestinal toxicity, when it was given at 6.25 mg/kg orally every 12 hours until day 50 and then tapered until day 180. Before the taper, CSP blood levels were targeted to a range of 200–400 mcg/L per standard practice.

Patients received red blood cell and platelet transfusions and antibacterial, antifungal and Pneumocystis Jiroveci prophylaxis per institutional standard practice guidelines (SPG). They received acyclovir for 1 year as herpes simplex virus (HSV) and varicella zoster virus (VZV) prophylaxis. Cytomegalovirus (CMV) surveillance with PCR for CMV DNA was performed weekly and preemptive therapy with ganciclovir or foscarnet was started per SPG. Grading of acute GVHD was performed using established criteria,[14] and the National Institute of Health criteria were used for assessing chronic GVHD.[15] The median follow-up after transplantation was 4 (range 1–8) years.

The 81 previously published patients had a median age of 25 (range 2–63) years, had genotypically or phenotypically HLA-matched related donors and received the same conditioning and postgrafting immunosuppressive regimens as the patients reported here.[10] Donor marrow cells contained a median of 2.8 (range 0.9–10.8) × 10^8 nucleated cells/kg. The patients were transplanted between July 1988 and June 2004 and their median follow-up was 19 (range 11–26) years.

Overall survival (Figure 1B) was estimated by the Kaplan-Meier method. Prevalence of chronic GHVD (Figure 1A) was estimated by methods previously described.[16]

Results

Engraftment

All 21 patients had prompt and sustained engraftment with median times of neutrophil recovery to >500/μL of 26 (range 21–36) days and of platelet recovery to >20,000 μL of 19 (range 10-35) days. None of the patients experienced graft rejection. Chimerism studies at 1 year after HCT using microsatellite markers showed peripheral blood CD3-positive and CD33-positive cells and nucleated marrow cells to be a median of 83%, 94% and 100% donor, respectively. At that time, peripheral blood recovery was complete.

Acute GVHD

The probability of developing acute GVHD was 47%, which was grade 2 in 38%, grade 3 in 9% and grade 4 in 0% of patients. The median day of onset of acute GVHD was 28 (range

22–73) days. All patients achieved complete resolution of acute GVHD in response to first line therapy with prednisone given at 1 or 2 mg/kg/day which was complemented by beclomethasone and budesonide in 6 patients who had gastrointestinal GVHD.

Chronic GVHD

Five patients developed chronic GVHD resulting in a cumulative incidence of 24%. If the 3 patients who received $>3 \times 10^8$ marrow cells/kg were removed from the analysis, the cumulative incidence of chronic GVHD was 16%. The median day of onset of chronic GVHD was 170 (range 83–213) days after transplantation. The median duration of immunosuppressive therapy for chronic GVHD was 17 (range 14–69) months. In all patients but one, immunosuppression has been discontinued as shown in the prevalence curves in Figure 1A.[16] One patient with well-controlled chronic GVHD of skin and joints has remained on immunosuppression with MTX, 15 mg once weekly and is undergoing a prednisone taper. Chronic GVHD of the lung was not seen in any of the patients. Acknowledging the limitations of small numbers of patients, no correlations could be identified between numbers of transplanted donor CD34+, CD14+, CD3+, CD4+, or CD8+ cells and risk of chronic GVHD.

Infections

Peripheral blood CMV reactivation, as assessed by PCR was observed in 9 patients (43%), at a median of 38 (range 20–66) days after transplantation. This percentage is consistent with findings in the general transplant population at the Hutchinson Center. One patient did not require therapy. In eight patients reactivation resolved after treatment with ganciclovir or foscarnet. Three patients developed bacteremia with Gram positive cocci on days +10, +48, +88, respectively, which resolved with vancomycin. Human herpes virus 6 reactivation occurred in two patients on days 14 and 42 after transplantation, respectively, and resolved with ganciclovir. Three years after transplantation one patient developed transient neutropenia presumed to be due to a parvovirus B19 infection of the marrow that responded promptly to granulocyte-colony stimulating factor and IV immunoglobulin treatment. These data are summarized in Table 2.

Survival

At a median follow up of 4 (range 1–8) years all 21 patients are alive (Figure 1B). The median time to discontinuation of post-grafting immunosuppression in all but one patient was 7 (range 5–69) months. No patient developed secondary cancer. No growth impairment was observed in children. One patient developed clinical hypothyroidism consistent with Hashimoto's thyroiditis 11 months after transplantation, with evidence of low free T4, elevated serum thyroid-stimulating hormone and high titers of anti-thyroid peroxidase and thyroglobulin antibodies. He was started on chronic replacement therapy with levothyroxine.

Figure 1B also shows an updated survival curve of the previously described 81 aplastic anemia patients.[10] With a median follow up of 19 (range 11–26) years, 81% of the patients are alive.

Discussion

Conditioning with CY/ATG and postgrafting immunosuppression with a short course of MTX and an extended course of CSP have been widely accepted for HLA-matched related marrow transplantation in patients severe with aplastic anemia.[9,17-23] With this transplant approach and the increasing use of leukocyte-poor and in vitro irradiated blood product transfusions before transplantation,[24,25] graft rejection has become infrequent. Acute GVHD has generally been only moderately severe. However, chronic GVHD, while seen only in a minority of patients, continues to require extended immunosuppressive therapy and has been associated with morbidity and even mortality.

The current, small, exploratory study hypothesized that limiting the marrow grafts from HLA-identical siblings to $\leq 2.5 \times 10^8$ nucleated cells/kg recipient body weight might reduce the risk of chronic GVHD. Unfortunately, protocol violations occurred and 3 patients received $>3.0 \times 10^8$ cells/kg. While graft rejection was not seen among the 21 patients reported here, and acute GVHD was limited to grades 2 and 3 which promptly responded to first-line therapy with prednisone, chronic GVHD occurred at a cumulative incidence of 24%, not different from our previous described 26 % incidence. When removing the 3 patients receiving $>3 \times 10^8$ marrow cells/kg from the analysis, the rate was 16%. Thus, limiting the number of transplanted marrow cells was safe but did not result in complete avoidance of chronic GVHD. Nevertheless, all but one of the current patients have responded to immunosuppressive therapy, which has been discontinued after a median of 19 months. All 21 patients survive with normal hematopoiesis between 1 and 8 (median 4) years, a follow-up period which is long enough for most chronic GVHD to have developed.

Restricting the number of marrow cells also restricted the graft content of T-cells. These included naïve T-cells which can cause chronic GVHD in response to minor tissue antigen disparities in the host.[26,27] The median numbers of CD3+, CD4+ and CD8+ T-cells in current grafts were 3.4, 1.9, and 1.5×10^7/kg, respectively. We did not find unusually high numbers of either of the three cell subsets in grafts of patients who developed chronic GVHD or unusually low numbers in grafts of patients who did not develop chronic GVHD. This lack of correlation with outcomes could be due to at least two unknowns. First, we lacked information on the content of naïve T-cells in the grafts except for a handful of patients, too few to be informative. Second, we did not know the degree of in vivo depletion of naïve donor T-cells imparted by residual ATG from the conditioning regimen. These unknowns remain to be addressed in future studies.

A review of the literature does not allow definitive assessment of the incidence rates of chronic GVHD and, importantly of the impact on patient survival since the lower end of patient follow-up in most reports was often very brief (Table 3). A CIBMTR study from 2007 reported chronic GVHD rates of 21% and 32% and survivals of 74% and 80% among patients conditioned with either Cy alone or Cy/ATG, respectively; however, the shortest follow-up was only 3 months.[17] Of interest, this multicenter, prospective trial did not show a reduction in the chronic GVHD incidence when ATG was added to Cy in the conditioning regimen. A GITMO study in younger patients (median age 19 years) reported chronic GVHD incidences of 44% and 30% and survivals of 78% and 90%, respectively; the shortest

follow-up was 7 months.[18] A CIBMTR study from 2011 described chronic GVHD ranging from 10% to 43% (the latter after PBSC grafts) and survivals ranging from 72% to 80% (the latter in young patients); the shortest follow-up was 2.4 months.[20] Atta et al. described chronic GVHD incidences of 34% and 0%, respectively, comparing horse ATG to rabbit ATG in the conditioning regimen; the shortest follow-up was 8 days.[21] Of note, even though no chronic GVHD was seen with rabbit ATG, survival among patients given horse ATG was comparable, 65% versus 63%, respectively. Moreover, more than half of the patients were still at risk for developing chronic GVHD. An EBMT study from 2012 saw 11% chronic GVHD among 1,163 patients given marrow grafts, who were followed for a median of 2.1 years (range not given); survival was 90% for pediatric patients and 74% for adult patients.[28] Kim et al. reported a chronic GVHD incidence of 17% and survival of 78%; the shortest follow-up was a little more than 1 month.[22] We reported a chronic GVHD incidence of 11% and survival of 100% among pediatric patients who had a median follow-up of 6.1 (range 0.3–21.5) years.[23] Marsh et al. used fludarabine/CY/alemtuzumab conditioning in 50 patients with HLA-matched related or unrelated grafts, and reported an astonishingly low rate of chronic GVHD of 4% and a survival of 88%; the shortest follow-up was 2.5 months.[29] Survival was 88 %; however, as in other reported studies half of the reported patients were still at risk of developing chronic GVHD.

Taken together, chronic GVHD has remained a problem among aplastic anemia patients treated with HLA-matched related marrow grafts with incidence rates ranging from 0% to 44%. Historically, chronic GVHD has been associated with a case mortality rate of approximately 25 %.[30] Quite likely the case mortality rate has declined in recent years owing to improved diagnosis, more effective treatment and better supportive care for patients with chronic GVHD. It is therefore surprising to see that the very low chronic GVHD rates reported, for example, by the 2011 CIBMTR study[20] or by Atta et al. in 2012 did not result in significant improvements in survival. The 24 % rate of chronic GVHD in current patients (16 % among patients receiving the targeted marrow cell dose) is well within the range reported in the literature. Yet, all patients in the current, small prospective study are surviving and immunosuppressive drugs have been discontinued in all but one patient. Limiting the dose of transplanted marrow to $\leq 2.5 \times 10^8$ cells/kg has been safe. However, whether the nearly uniform response of chronic GVHD to therapy and the uniform survival can be attributed to targeting the marrow cell dose to a narrow range remains conjecture.

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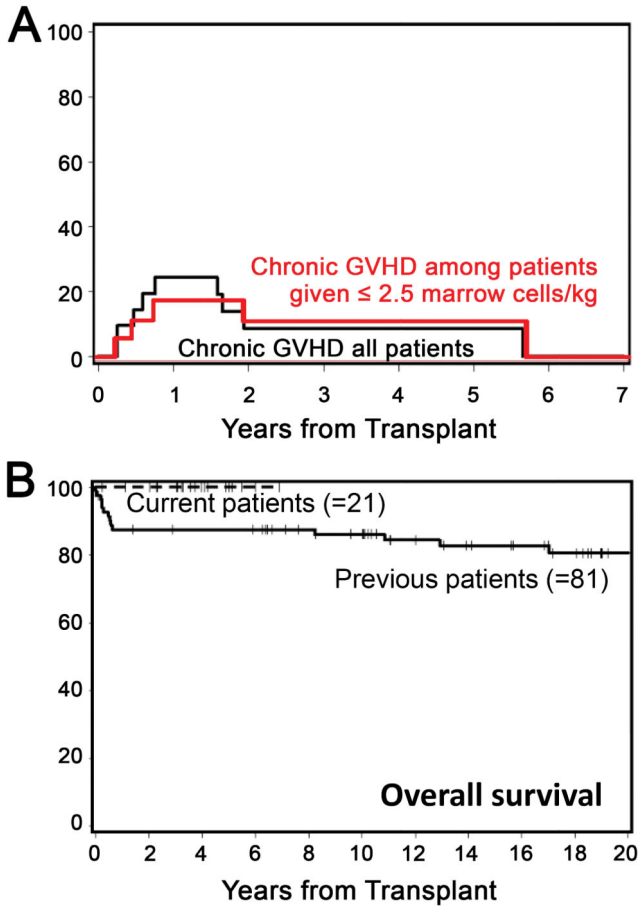

Fig 1.

(**a**) Cumulative incidence of chronic GvHD. (**b**) Probability of survival.

Table 1

Patient characteristics

Number of patients	21
Median age at transplantation, years (range)	15 (3–52)
Median time from diagnosis to transplantation, months	2 (1–9)
Sex (M/F) , *n*	16/5
Donor-recipient sex mismatch, *n*	8 (38%)
AB0 mismatch, *n*	
minor	3 (14%)
major	2 (9.5%)
bidirectional	1 (5.5%)
none	15 (71%)
CMV status, n	
R+/D+	7 (33.5%)
R+/D−	4 (19%)
R−/D+	3 (14%)
R−/D−	7 (33.5%)
Prior immunosuppressive treatment, *n*	
yes	1 (5%)
no	20 (95%)
Number of patients who received transfusions before transplantation, *n*	
platelets	21 (100%)
red blood cells	21 (100%)
Median (range) number of:	
nucleated cells in marrow graft $\times 10^8$/kg	2.0 (1.1–3.5)
CD34+ cells $\times$ 10^6/kg	4.4 (1.6–9.1)
CD3+ cells $\times$ 10^7/kg	3.4 (1.5–6.7)

CD4+ cells × 10^7/kg	1.8 (0.7–3.7)
CD8+ cells × 10^7/kg	1.5 (0.6–2.7)
CD14+ cells × 10^7/kg	0.6 (0.2–1.3)
Engraftment, median day (range)	
neutrophils >500/μL	26 (21–36)
platelets >20.000/μL	19 (10–35)

Abbreviations used: AB0: blood group antigens, CMV: cytomegalovirus, D: donor; F: female, M: male, R: recipient.

Table 2

Transplantation outcomes

Patient number (Age, years)	Number of nucleated marrow cells × 10^8/kg	CD34 × 10^6/kg	CD3 × 10^7/kg	Acute GVHD day / grade	Chronic GVHD day of onset	Discontinuation of Immunosuppression (months)	Outcome/years after transplantation	CMV reactivation day /therapy	Bacterial or other viral infections/ day/therapy
1 (15)	1.4	3.0	2.0	+56/2	+89	69	Alive /8	-	-
2 (6)	1.7	7.00	6.7	+29/2	-	6	Alive /6	-	HHV6/+42/Ganciclovir
3 (7)	3.2	6.4	6.0	+28/2	+91	20	Alive /8	+24/Foscarnet	Strepto. Viridans/+88/Vancomycin
4 (8)	3.5	6.2	4.3	+24/2	-	7	Alive /4	-	HVV6/+14/Ganciclovir
5 (12)	2.5	3.1	3.4	-	-	6	Alive /4	+ 18/Foscarnet	-
6 (11)	2.5	8.5	3.2	+40/3	-	10	Alive /3	-	Staph coag. Neg./+48/Vancomycin
7 (16)	2.5	6.3	3.1	-	-	6	Alive /6	-	-
8 (12)	1.9	2.8	3.6	+28/2	-	14	Alive /7	+ 10/Ganciclovir	Parvovirus /2 years/IVIG
9 (50)	1.9	5.2	2.1	+24/3	-	7	Alive /6	+50/Ganciclovir	-
10 (17)	2.5	4.4		+29/2	-	8	Alive /3	+20/Foscarnet	Staph coag.Neg./+10/Vancomycin
11 (3)	2.5	9.1	5.2	-	-	6	Alive /4	-	-
12 (16)	1.1	2.9	1.5	+72/2	-	12	Alive /4	-	-
13 (12)	1.6	2.6	4.4	-	-	5	Alive /4	+42/spontaneous resolution	-
14 (52)	2.1	2.6	2.9	+22/2	+ 175	24	Alive /3	+54/Ganciclovir	-
15 (35)	1.4	3.7		-	+ 170	ongoing	Alive /3	+66/Ganciclovir	-
16 (42)	2.5	5.5	3.3	-	-	8	Alive /2	-	-
17 (23)	2.5	4.9	3.4	-	-	6	Alive /1	-	-
18 (6)	1.6	4.6	4.6	-	-	7	Alive /1	-	-
19 (9)	1.4	3.6		-	-	7	Alive /5	-	-
20 (16)	1.6	3.5		-	-	6	Alive /3	+38/Ganciclovir	-
21 (26)	3.1	1.6		-	+213	13	Alive /8	-	-

Abbreviations used: CMV: cytomegalovirus, F: female, GVHD: graft-versus-host disease, HHV6: human herpes virus 6, IVIG: intravenous immunoglobulin M: male.

Table 3

Selected reports of hematopoietic cell transplantation from HLA-identical siblings for severe aplastic anemia

Transplant Team	Number of Patients	Median Patient Age (Range) Years	Conditioning Regimen	Graft Source Median (Range) # of cells/kg×10^8	GVHD Prevention	% Chronic GVHD	% Survival	Range (Median) Follow-up, Years
CIBMTR (Champlin et al., 2007)[17]	130	24 (1–51)	CY (n=60) CY/ATG (n=70)	Marrow 2.4 (0.01–5.0) 2.7 (0.01–7.0)	MTX/CSP	21 32	74 80	0.4–10.2 (6.3)
GITMO (Locjatelli et al., 2000)[18]	71	19 (4^46)	CY	Marrow 3.7 (1.1–10.4) 4.1 (1.4–12.5)	CSP vs. MTX/CSP	44 30	78 94	0.6–7.8 (4.0)
CIBMTR (Chu et al., 2011)[20]	547 78	18 25	CY/ATG + other	Marrow Blood	MTX/CSP + other	16 10	80 72	0.2–10.4 (5.2) 0.2–10.2 (3.7)
Atta et al., 2012[21]	20 20	1 (4–48)	CY/horse ATG CY/rabbit ATG	Marrow 3.4 (1.16–5.4) 2.49 (1.01–5.15)	MTX/CSP	34 0	65 63	0.02–14 (4.5) 0.03–4.8 (0.7)
EBMTR (Bacigalupo et al., 2012)[28]	1163 723	18 (1–68) 24 (1–69)	CY/ATG+ other	Marrow Blood	MTX/CSP + other	11 22	≤20 years 90 >20 years 74 ≤20 years 76 >20 years 64	median 2.0
Kim et al., 2012[22]	50	35 (15–60)	CY/ATG	Marrow	MTX/CSP	17	78	0.1–7.1 (1.5)
Burroughs et al., 2012[23]	31	12.8 (1.8–19)	CY/ATG	Marrow 2.8 (0.7–6.2)	MTX/CSP	10	100	0.3–21.5 (6.1)
Marsh et al., 2011[29]	21	35 (8–62)	FLU/CY/alemtuzumab	Marrow	CSP	4	88	0.2–9.8 (1.5)

Abbreviations used: ATG: anti-thymocyte globulin, CSP: cyclosporine; CY: cyclophosphamide, GVHD: graft-versus-host disease, MTX: methotrexate