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ORIGINAL ARTICLE

Stem cell transplantation for the Wiskott–Aldrich syndrome: a single-center experience confirms efficacy of matched unrelated donor transplantation

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The treatment of Wiskott–Aldrich syndrome (WAS), a once uniformly fatal disorder, has evolved considerably as the use of hematopoietic stem cell transplant has become more widespread. For the majority of patients who lack an human leukocyte antigen-identical sibling, closely matched unrelated donor bone marrow transplant (MUD BMT) at an early age is an excellent option that nevertheless is not uniformly chosen. We retrospectively analyzed our experience with transplantation in 23 patients with WAS from 1990 to 2005 at the University of Brescia, Italy, of whom 16 received MUD BMT. Myeloablative chemotherapy was well tolerated with median neutrophil engraftment at day 18, and no cases of grade III or IV graft-vs-host disease. Overall survival was very good with 78.2% (18/23) of the whole cohort and 81.2% (13/16) of MUD BMT recipients surviving. Among 18 survivors, full donor engraftment was detected in 12 patients, and stable mixed chimerism in all blood lineages in four patients. Deaths were limited to patients who had received mismatched related BMT or who had severe clinical symptomatology at the time of transplantation, further emphasizing the safety and efficacy of MUD BMT when performed early in the clinical course of WAS.

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Introduction

Hematopoietic stem cell transplantation (HSCT) has become the mainstay of treatment for the Wiskott–Aldrich syndrome (WAS). In the past, transplantation was performed only using related identical donors (RID) with mismatched related donors (MMRD) and matched unrelated donors (MUD) reserved for those patients with severe disease, whereas recently the use of MUD bone marrow transplant (BMT) and umbilical cord blood transplant has become increasingly common.[1–7] Despite published experience demonstrating excellent outcome of MUD HSCT in young patients,[8] as of a few years ago, the recommendation to pursue unrelated donor transplantation in patients with WAS was still far from uniform and less likely in centers with less transplantation experience, based on an international survey.[9]

We present here retrospective results of 23 WAS patients undergoing HSCT at a single institution over the last 15 years. Excellent survival was demonstrated in patients receiving MUD BMT. Furthermore, a detailed analysis of hematopoietic and immune reconstitution, lymphoid function and lineage-specific chimerism has confirmed the efficacy of the procedure.

Methods

Patients

From 26 June to 26 October 2005, 23 patients with WAS underwent HSCT in the Department of Pediatrics of the University of Brescia, Italy. Patient characteristics are summarized in Table 1. A previously described clinical scoring system was used to indicate the degree of symptomatology.[10] Initially, transplantation was reserved for those patients who had high clinical scores owing to the presence of repeated infection or autoimmunity; as data regarding the safety of alternate donor transplant for WAS accumulated, patients whose outcome was predicted to be poor based on the lack of normal WASP protein were transplanted regardless of clinical score (Table 1). Owing to the very poor outcome of MMRD transplant at this center, in agreement with reports from the literature, after 1996 patients without a genotypically identical donor, underwent closely MUD transplant. The clinical protocol of HSCT for WAS was approved by the local Ethical Committee of the Spedali Civili, Brescia. Informed consent was obtained from all children's parents.

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Table 1 Molecular and clinical features of 23 WAS patients treated with stem cell transplantation

Patient	*Age at diagnosis (months)*	*Mutation type*	*Gene defect*	*Effect*	*WASP protein expression*	*Score*	*Autoimmunity*	*Splenectomy*	*Age at BMT (months)*
1[a]	8	Splice-site	IVS10-2a>t	Exon skipping→fs	Reduced	2	No	No	10
2[a]	14	Complex	1115-1119insC; 1111-1182del	fs stop aa 494; in frame del 24 aa	ND	2–3	No	No	34
3[a]	17	Splice-site	IVS10-3delC	Exon skipping →fs	ND	2	No	No	20
4[b]	19	Nonsense	907delC	fs stop aa 291	Absent	2	No	No	30
5[c]	2	Nonsense	665C>T	R211X	ND	3	No	No	6
6[a]	9	Deletion	116delC	fs stop aa 44	Absent	5	AIHA	Yes	22
7[c]	9	Deletion	1028delG	fs stop aa 444	Reduced	3	No	Yes	31
8[c]	5	Splice-site	IVS8+1g>a	Exon skipping→fs	Absent	2–3	No	Yes	16
9[a]	5	Nonsense	155C>T	R41X	Absent	5	Vasculitis, colitis	Yes	50
10[c]	8	Insertion	1486-1487insTC	fs lack of stop codon	ND	5	AIHA	Yes	19
11[c]	5	Insertion	364insC	fs stop aa 121	Absent	4	No	Yes	41
12[c]	7	Nonsense	C995T	R321X	Absent	5	AIHA	Yes	47
13[c]	1	Splice-site	IVS7+1g>t	Exon skipping→fs	Absent	5	Colitis	No	22
14[c]	21	Deletion	1192delT	fs stop aa 445	Absent	5	AIHA	No	31
15[c]	9	Deletion	644delA	fs stop aa 261	Absent	5	AIHA, vasculitis	No	16
16[c]	15	Deletion	483-484delAG	fs stop aa 168	Absent	5	AIHA, vasculitis	No	68
17[c]	15	Missense	348T>C	L105P	Reduced	2–3	No	No	40
18	5	Missense	435C>T	A134V	Absent	5	AIHA	No	12
19	4	Deletion	271delC	fs stop aa 206	Reduced	5	Vasculitis	No	8
20	34	Deletion	755-757delC	fs stop aa 261	Absent	5	Vasculitis, arthritis	No	41
21	20	Deletion	1542-1545delGAGT	fs lack of stop codon	Absent	2	No	No	22
22	9	Deletion	1100-1103delC	fs stop aa 445	Absent	2	No	No	16
23	10	Splice-site	IVS4-2a>g	Exon skipping→fs	Very reduced	2	No	No	15

Abbreviations: AIHA = autoimmune hemolytic anemia; BMT = bone marrow transplantation; del = deletion; fs = frameshift; ins = insertion; IVS = intervening sequence.
[a]Reported by Wengler *et al*.[29]
[b]Reported by Facchetti *et al*.[30]
[c]Reported by Jin *et al*.[12]

Donors

The majority of patients received bone marrow from RID ($n=4$), MMRD ($n=2$) or closely MUD ($n=16$). One patient[5] received RID cord blood. All bone marrow from RID and MUD before 2001 was infused unmanipulated. Since 2001, MUD patients with sufficient cell dose ($n=8$) also received additional $CD34^+$-selected bone marrow. Bone marrow from MMRD was T-cell depleted using Campath 1G (Wellcome Biotech, Beckenham, UK) *ex vivo* and with soybean lectin for patient 5 and patient 6, respectively.

Human leukocyte antigen (HLA) typing before 2001 was performed by serological methods for HLA-A and HLA-B, and by sequence-specific oligonucleotide probes for HLA-DRB1. After 2001, most patients had molecular typing performed for HLA-A, -B, -DRB1, -DQA1, -DQB1; HLA-C molecular typing was only available for one patient. Most MUD were fully matched by the typing available at the time of selection, although a few were mismatched at one or two class I antigens (Table 2).

Transplantation

The conditioning regimen used for the patients is shown in Table 2. All patients received myeloablative chemotherapy consisting of busulfan 16–20 mg/kg given orally for 4 days followed by cyclophosphamide 200 mg/kg total given intravenously for 4 days. In addition, of the 16 patients treated by MUD BMT, 13 also received anti-thymocyte globulin, whereas two patients received other drugs. MMRD patients (no. 5 and no. 6) also received thiotepa/Campath and thiotepa/ATG, respectively. Starting in 2001, busulfan levels were monitored and doses were adjusted by area-under-the-curve measurements.

For graft-vs-host disease (GVHD) prophylaxis, all patients received cyclosporine administered orally at 5 mg/kg/day divided into two doses starting day −1. Dosage was adjusted to blood levels. In addition to cyclosporine, RID patients no. 1–4 also received short-course methotrexate, according to the European protocols then in use. GVHD was graded according to Glucksberg criteria.[11]

All patients were transplanted in single rooms with laminar airflow. Intravenous broad-spectrum antibiotics were given prophylactically based on individual intestinal flora during the time of marrow aplasia. Intravenous immunoglobulins (IVIG) were infused weekly at 200 mg/kg/dose until discharge, then every 3 weeks at 400 mg/kg/dose. Intravenous acyclovir was given until immune reconstitution was complete. Patients were screened weekly for cytomegalovirus (CMV) reactivation by polymerase chain reaction (PCR) until T-cell reconstitution and were

Table 2 Transplant characteristics and outcome

Patient number	*Donor*	*HLA matching*	*Conditioning regimen*	*Cell source*	*Additional CD34*	*Days to ANC >500/μl*	*aGVHD*	*Infections*	*Other*	*Outcome*	*Time post-transplant*
1	RID	6/6	Bu/Cy	BM	–	14	I, skin			A&W	5 years
2	RID	6/6	Bu/Cy	BM	–	31				A&W	11 years
3	RID	6/6	Bu/Cy	BM	–	16	I, skin			A&W, sensorineural deafness	9 years
4	RID	6/6	Bu/Cy	BM	–	23				A&W	8 years
5	RID	6/6	Bu/Cy	Cord	–	41	I, skin, liver			A&W	7 years
6	MMRD	Haplo	Bu/Cy, thio, MabCampath	TCD BM	–	—			Cerebral hemorrhage	Deceased	
7	MMRD	Haplo	Bu/Cy, ATG	TCD BM	–	11		EBV-PTLD		Deceased	
8	MUD	8/8	Bu/Cy	BM	–	54			TTP	A&W	9 years
9	MUD	7/8, A	Bu/Cy, thio	BM	–	14	II, skin, GI	OPSI		Deceased	
10	MUD	6/6	Bu/Cy, thio ATG	BM	–	16				A&W	8 years
11	MUD		Bu/Cy, ATG	BM	–	16		EBV-PTLD (R)	AIHA	A&W, hypothyroidism on replacement	6 years
12	MUD	6/6	Bu/Cy, ATG	BM	–	26	I, skin, liver			A&W, 2–3 infections per year, low plts	5 years
13	MUD	7/8[a], A	Bu/Cy, ATG	BM	–	40	I, skin, liver	PJP		Deceased	
14	MUD	8/8[a]	Bu/Cy, ATG	BM	–	20		CMV		A&W	4 years
15	MUD	8/8[a]	Bu/Cy, ATG	BM	+	19				A&W, low plts	4 years
16	MUD	8/8[a]	Bu/Cy, ATG	BM	+	18		CMV, EBV-PTLD (R)		Deceased	
17	MUD	8/8[a]	Bu/Cy, ATG	BM	+	14	I, skin			A&W	3 years
18	MUD	9/10[a], A	Bu/Cy, ATG	BM	+	18			AIHA, autoimmune hypothyroidism	A&W	29 months
19	MUD	10/10[a]	Bu/Cy, thio, MabCampath	BM	+	12	I, skin			A&W	24 months
20	MUD	8/10[a], A, C	Bu/Cy, ATG	BM	+	25		EBV (R)	TTP	A&W	21 months
21	MUD	10/10[a]	Bu/Cy, ATG	BM	+	17		CMV, EBV-PTLD (R)		A&W	16 months
22	MUD	10/10[a]	Bu/Cy, ATG	BM	+	18		EBV (R)		A&W	14 months
23	MUD	10/10[b]	Bu/Cy, ATG	BM	–	25		EBV (R)		A&W	6 months

Abbreviations: aGVHD = acute graft-vs-host disease; AIHA = autoimmune hemolytic anemia; ATG = anti-thymocyte globulin; A&W = alive and well; BM = bone marrow; Bu = busulfan; CMV = cytomegalovirus; Cy = cyclophosphamide; EBV-PTLD = Epstein–Barr virus post-transplant lymphoproliferative disease; haplo = haploidentical; MMRD = mismatched related donor; MUD = matched unrelated donor; OPSI = overwhelming post-splenectomy infection; PJP = *Pneumocystis jiroveci* pneumonia; R = rituximab; RID = related identical donor; TCD = T-cell depleted; thio = thiotepa; TTP = thrombotic thrombocytopenic purpura; A = HLA-A; C = HLA-C.

[a]Molecular typing performed for HLA-A, -B, -DRB1, -DQ, serologic typing for HLA-C.

[b]Molecular typing performed for HLA-A, -B, -C, -DRB1, -DQ.

treated with foscarnet, ganciclovir or cidofovir at the time of reactivation. Epstein–Barr viral (EBV) viral load by PCR testing of the blood was also performed weekly until T-cell reconstitution. Starting in 1999, patients exhibiting EBV positivity by PCR were treated pre-emptively with rituximab (Mabthera, Roche, Monza, Italy).

Mutational analysis and WASP protein assessment
Mutation analysis at the *WASP* locus was performed as described.[12] A number of these patients, their mutations and WASP levels have been previously reported as indicated in Table 1. To assess the relative contributions of WASP-expressing and non-expressing cells in different hematopoietic lineages, multicolor flow cytometry including detection of intracellular WASP was performed on 100 μl of peripheral blood of healthy control subjects and WAS patients. Peripheral blood leukocytes were initially stained for 10′ at room temperature (RT) with a mixture of anti-human CD14-FITC, CD19-PE and CD3-PerCP monoclonal antibodies (mAbs) or the appropriate isotype controls (all from Becton Dickinson, Mountain View, CA, USA). Blood samples were washed once with 3 ml of a phosphate-buffered solution (PBS) solution containing 5% of fetal bovine serum, before being analyzed for WASP expression using the Fix & Perm cell permeabilization kit (Caltag Laboratories, Burlingame, CA, USA) according to the manufacturer's protocol.

Analysis of WASP expression was carried out by incubating cell samples with purified mouse IgG_{2a} anti-human WASP mAb (Becton Dickinson) for 20′ at RT. After two washes with the PBS + fetal calf serum solution, samples were stained with a biotinylated goat anti-mouse IgG_{2a} secondary Ab (SouthernBiotech, Birmingham, AL, USA) for 15′ at RT. After having been washed twice, samples were finally incubated with conjugated streptavidin–APC (PharMingen, San Diego, CA, USA) for 15′ at RT and collected within an hour using a FACSCalibur (Becton Dickinson).

Data analysis was performed using Cell Quest software (Becton Dickinson). Specifically, lymphocyte and monocyte analysis gates were set on forward versus side-scatter, followed by the isolation of T- and B-lymphocyte and monocyte populations according to the positive expression of, respectively, CD3, CD19 and CD14.

Immune reconstitution
Lymphocyte subsets (CD3, CD4, CD8, CD19, CD16/56, CD45RA, CD45R0), immunoglobulins (IgG, IgM, IgA and IgE) were measured at 1, 3, 6, 9 and 12 months post-transplant, then yearly. Those patients independent of intravenous immunoglobulin who were at least 12 months post-transplant were vaccinated and antibodies against tetanus and hepatitis B antigen were measured. T-cell receptor (TCR) diversity analysis and T-cell receptor excision circle (TREC) quantitation were performed as described.[13]

Molecular analysis of chimerism
Chimerism was assessed on peripheral blood mononuclear cells, positively selected $CD3^+$ T lymphocytes and $CD19^+$ B lymphocytes, as well as on granulocytes, by microsatellite analysis using probes for DQ alpha, D1S80 and ApoB, as previously described.[14]

Results

Engraftment and hematopoietic reconstitution
All 22 patients who survived to day +30 experienced myeloid engraftment, defined as the first day that the absolute neutrophil count (ANC) was greater than 500/μl for three consecutive days (median 18 days, range 12–54 days, see Table 2). Patient 13 had only achieved an ANC of 300–400/μl as of day +33; at this time he received a boost of frozen bone marrow, then engrafted by day +40.

Platelet reconstitution was assessed in 18 patients surviving past 100 days. Most (16/18) discontinued platelet transfusions by day +30, with a median time to discontinuation of 18 days (7–483 days). The majority of survivors (16/18) have subsequently attained platelet counts above 150 000/μl. Patient no. 8 has mild thrombocytopenia with platelet count 98 000–104 000/μl at 6–9 years post-transplant. Patient no. 15 has more severe thrombocytopenia, with platelet counts 19 000–50 000/μl at 2–4 years post-transplant. The amount of WASP protein expressed in platelets in these two patients (50 and 0%, respectively) correlates with the degree of thrombocytopenia. Finally, patient no. 12, who had achieved a normal platelet count before HSCT after undergoing splenectomy, has maintained a normal platelet count post-HSCT in spite of autologous myeloid reconstitution and lack of WASP protein expression in platelets.

Comparison of mean platelet volume before transplant and at last assessment in 11 patients showed increases in all patients (mean 4.9 fl pre-transplant, 7.9 fl post-transplant, $P<0.0001$, Figure 1).

Chimerism and immune reconstitution
Detailed chimerism analysis of myeloid and lymphoid cells was performed in all 18 patients who are currently alive after transplant, and expression of WASP protein expression in different blood lineages was assessed in 16 of these

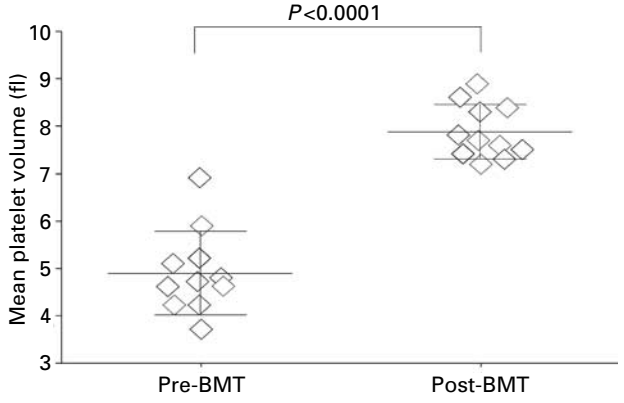

Figure 1 Normalization of platelet volume after HSCT for WAS. Mean platelet volume before (left panel) and after (right panel) HSCT in 11 patients with WAS.

patients (Table 3). All five patients who received RID transplants, attained full donor chimerism. Among 13 recipients of MUD BMT, seven had full donor chimerism in all blood lineages at the time of last evaluation. Of the remaining six patients, four (patients no. 8, 17, 18, and 22) showed stable mixed chimerism in all blood lineages, with a predominance of donor-derived cells. Two patients (no. 12 and 15) had split chimerism, with autologous reconstitution in the myeloid compartment.

Lymphoid reconstitution and function

The restoration of T- and B-cell numbers was examined by serial measurement of lymphoid subsets. Total $CD3^+$ cell counts rose to over 1000/μl on average at 3 months post-

Table 3 Chimerism and post-transplant WASP protein analysis

Patient number	*Donor*	*WASP protein expression (%) after HSCT*			*Time at last follow-up (years)*
		T cells	*B cells*	*Monocytes*	
1	RID	ND[a]	ND[a]	ND[a]	5
2	RID	ND[a]	ND[a]	ND[a]	11
3	RID	100	100	100	9
4	RID	100	100	100	8
5	RID	100	100	100	6
8	MUD	100	60	50	9
10	MUD	100	100	100	8
11	MUD	100	100	100	7
12	MUD	43	6	0	6
14	MUD	100	100	100	4
15	MUD	40	15	0	4
17	MUD	90	90	80	4
18	MUD	94	91	58	3
19	MUD	100	100	100	3
20	MUD	100	100	100	2
21	MUD	100	100	100	1.3
22	MUD	66	87	62	1.1
23	MUD	100	100	100	0.6

[a]Full chimerism was detected in peripheral blood mononuclear cells and granulocytes by molecular analysis.

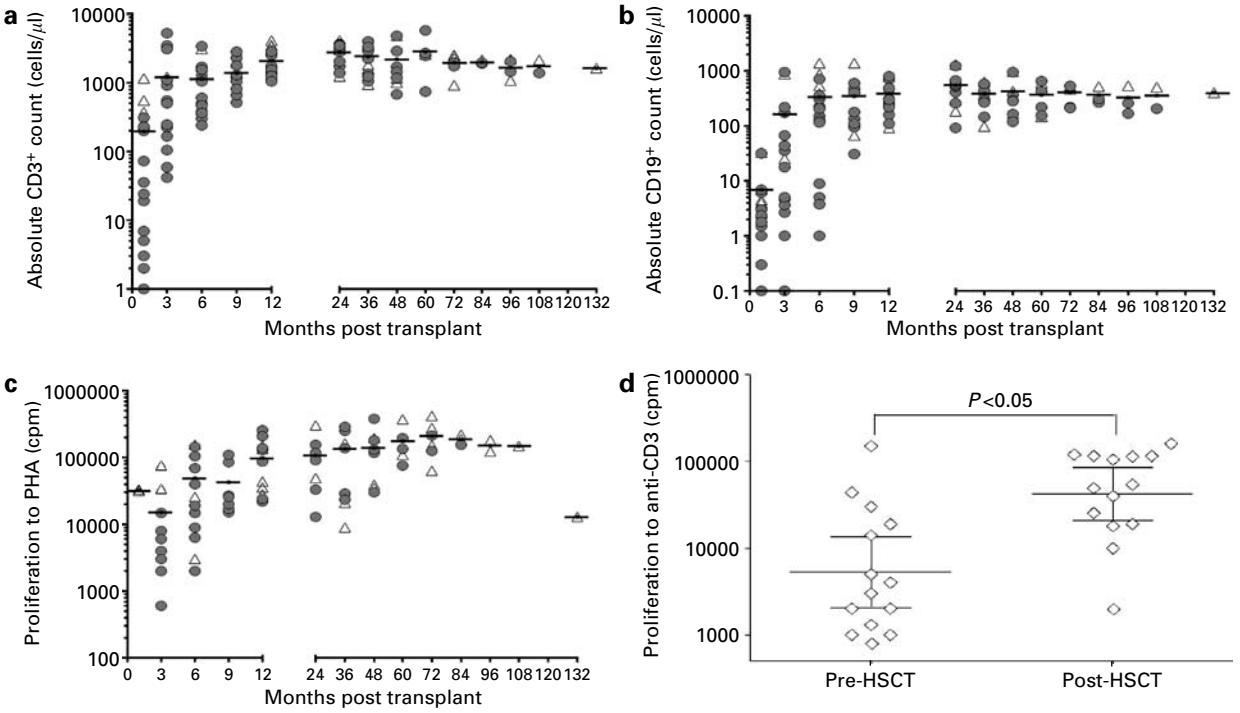

Figure 2 Immune reconstitution following HSCT for WAS. Reconstitution of circulating T lymphocytes (**a**), B lymphocytes (**b**), *in vitro* proliferative response to phytohemagglutinin (PHA) (**c**), and of *in vitro* proliferative response to anti-CD3 (**d**) is shown. Empty triangles and filled circles identify patients who have been transplanted with a matched related donor, and an MUD, respectively. In panels **a**–**c**, at each time point, horizontal bars represent geometric mean value $\pm$ standard deviation. c.p.m.: counts per minute.

transplant (Figure 2). The response to PHA normalized by 12 months on average, whereas the response to CD3 took longer, up to 5 years. The 18 surviving patients achieved $CD8^+$ cell counts greater than 200/μl by 1–3 months post-transplant, and $CD4^+$ cell counts greater than 1000/μl by 12 months post-transplant (Figure 3).

To assess the production of naïve T cells, absolute numbers of naïve $CD4^+$ T cells ($CD4^+$ $CD45RA^+$), memory $CD4^+$ T cells ($CD4^+$ $CD45R0^+$), TCR repertoire and TREC were enumerated. The number of memory $CD4^+$ T cells increased rapidly to over 200/μl at 3 months, peaked to around 400 cells/μl at 12 months after transplant, and then remained stable for up to 11 years post-transplant (Figure 3). In contrast, the number of naïve $CD4^+$ T cells rose more slowly, peaked at 2 years and appeared to decrease over time (Figure 3). Thus, at early post-transplant the ratio of naïve to memory phenotype cells was below 1, rising to 2.6 at 2 years. Importantly, at the time of last evaluation, the TREC levels of all surviving patients, who are beyond 1 year post-transplant, were within the normal range for our institution, and the T-cell repertoire was polyclonal in all patients but one (Table 4).

B-cell numbers represented by absolute numbers of $CD19^+$ cells were restored to greater than 100/μl by 3 months post-transplant (Figure 2). All 15 survivors who are more than 24 months post transplant are independent of immunoglobulin infusions, including three patients (no. 11, 16 and 20) who received rituximab for treatment of EBV reactivation, and the two patients (no. 12 and 15) who have a predominance of autologous B cells.

Outcome

As shown in Table 2, all patients treated by RID HSCT are cured with no WAS-related symptoms at the time of last follow-up. Of 13 survivors of MUD transplants, 10 (76.9%) have excellent clinical outcome with no signs attributable to WAS, including three with previous autoimmune disease (no. 10, 19, 20). In contrast, both patients treated by MMRD BMT died early after transplant.

Transplantation was tolerated extremely well in the majority of patients. There was little to no immediate regimen-related toxicity in this series. No patient developed veno-occlusive disease or bacterial infection during the pancytopenic period. Although eight of 23 patients developed acute GVHD, all were grade I or II and resolved with corticosteroid treatment; there were no cases of acute GVHD of grade III or above. Of the 19 patients who survived past day 100, none developed chronic GVHD with a follow-up of 9–133 months. No deaths were attributable to GVHD.

In contrast to the very low risk of serious GVHD, the incidence of infection, particularly viral reactivation of DNA viruses, was moderate, and in fact represented the major cause of death in our series (Table 2). Out of 18 patients treated by MUD or MMRD BMT, eight had reactivation of viral infections, owing to EBV in seven patients and to CMV in three patients (two patients had concurrent EBV and CMV reactivation). In three cases (patients no. 7, 11 and 16), EBV reactivation was associated with post-transplant lymphoproliferative disease (PTLD). Patient no. 7, who received an MMRD BMT and

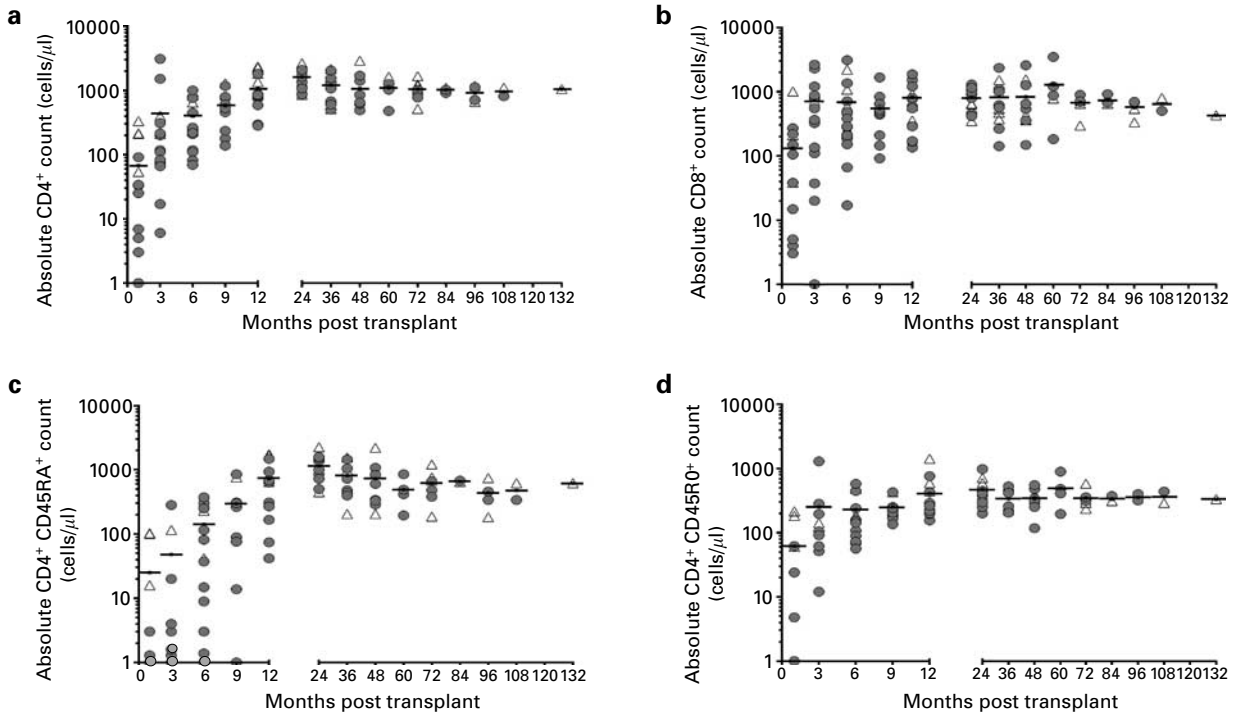

Figure 3 Reconstitution of naïve and memory T cells after HSCT for WAS. Reconstitution of circulating $CD4^+$ T lymphocytes (**a**), $CD8^+$ T lymphocytes (**b**), naïve $CD4^+$ T cells (**c**), and memory $CD4^+$ T cells (**d**) following HSCT for WAS. Empty triangles and filled circles identify patients who have been transplanted with a matched related donor, and a MUD, respectively. At each time point, horizontal bars represent the mean value.

Table 4 T cell chimerism, reconstitution and repertoire

Patient	*T-cell chimerism*	*$CD4^+$ $CD45RA^+$ (cells/μl)*	*$CD4^+$ $CD45R0^+$ (cells/μl)*	*RA/R0 ratio*	*TRECs ($\times 10^{-6}$ lymphocytes)*	*Clonality*
1	D	ND	ND	ND	ND	ND
2	D	624	336	1.86	56 829	P[a]
3	D	620	288	2.15	49 632	P[a]
4	D	183	359	0.51	28 586	P[a]
5	D	904	358	2.52	163 994	P
8	D	342	438	0.78	45 814	P
10	D	1361	1181	1.15	77 030	O
11	D	384	358	1.07	48 978	P
12	M	196	196	1.00	59 897	P
14	D	294	301	0.98	183 581	P[a]
15	M	320	257	1.24	114 562	P[a]
17	90% D	401	204	1.97	58 766	P
18	94% D	1458	416	3.50	440 654	P[a]
19	D	996	298	3.34	140 353	P
20	D	1581	413	3.83	42 993	P[a]
21	D	671	266	2.52	52 153	P[a]
22	M	770	286	2.69	155 598	P

Abbreviations: D = donor; M = mixed; ND = not done; O = oligoclonal; P = polyclonal; TRECs = T-cell receptor excision circles.
[a]Some clonal expansion.

developed EBV-PTLD before the availability of rituximab, died on day +86. Patient no. 16, who developed EBV-PTLD after MUD BMT, died of multiple organ failure and cerebral hemorrhage in spite of rituximab. The remaining five patients with reactivation of EBV infection after transplant cleared their infection with rituximab; in four of these, rituximab was used as pre-emptive treatment, in the absence of clinical symptoms. Patient no. 13, who developed *Pneumocystis jiroveci* pneumonia, died early after transplant (day +33). Patient no. 9, who had been splenectomized 4 years before MUD BMT, attained complete donor engraftment, but died 25 months after transplant of fulminant meningococcal sepsis despite oral antibiotic prophylaxis.

Overall survival with a follow-up of 3 months to 132 months was 78.2% (18/23) in the whole cohort. All recipients of matched sibling bone marrow (5/5) and 81.2% of unrelated donor recipients (13/16) are surviving. Neither patient receiving mismatched related transplant survived. Hemorrhage and infection were the predominant causes of death (Table 5). Notably, no transplant-related deaths beyond day 100 have occurred.

Table 5 Causes of death in WAS patients undergoing BMT

Patient	*Donor*	*Cause of death*	*Time post-BMT*
6	MMRD	Cerebral hemorrhage	20 days
7	MMRD	EBV-PTLD	86 days
9	MUD	Meningococcemia	2 years
13	MUD	PJP	65 days
16	MUD	Cerebral hemorrhage, EBV-PTLD, MOF	74 days

Abbreviations: EBV-PTLD = Epstein–Barr virus-related post-transplant lymphoproliferative disease; MMRD = mismatched related donor; MOF = multiple organ failure; MUD = matched unrelated donor; PJP = *Pneumocystis jiroveci* pneumonia.

Discussion

Since the first report of the cure of WAS by BMT,[1] the definition of suitable patients and donors for this curative procedure has evolved considerably. This single institution experience of 23 patients undergoing transplantation from RID, MMRD and MUD parallels the trend in donor selection reported in the literature. In addition, evidence showing tight correlation between the absence of WASP protein and poor outcome[12] has driven us to transplant patients with a suitable RID or MUD as soon as the diagnosis and absence of WASP protein have been confirmed; thus, clinical severity is no longer the single factor considered in the decision process at our institution. With this approach, MUD BMT has excellent outcome, with 81.2% of patients treated at our center by MUD BMT surviving.

Previous to identification of a genotype–phenotype correlation in WAS, patients without a suitable RID were treated by HSCT only when their clinical manifestations were sufficiently severe to warrant transplantation. However, this may affect the outcome of the procedure. Notably the three patients treated by MUD BMT who died post-transplant (patients no. 9, 13 and 16) all had previous autoimmune disease, and were steroid-dependent. All 12 patients with clinical scores of 4 or less, who have received RID or MUD transplantation, are surviving with no clinical manifestations of WAS. The strong genotype–phenotype correlation in WAS, the severe clinical course seen in patients who completely lack WASP protein expression and the excellent outcome of patients transplanted early can now be used to guide decisions regarding the timing of transplantation.[12]

Resistance to the use of MUD for the treatment of WAS, despite clear evidence from a large series that RID and MUD transplants for WAS leads to equally good outcome,[8] may be based on earlier reports of extended life expectancy following splenectomy.[15] Our detailed analysis of 16 MUD transplants revealed that MUD BMT was tolerated, in general, extremely well, with little acute

GVHD, none of high severity. In this small series, no patient developed chronic GVHD. Indeed, a regimen of cyclosporine (without methotrexate) was sufficient to prevent GVHD, allowing for prompt engraftment. Lymphoid reconstitution was similar to that reported in the literature[16,17] and all vaccinated patients have evidence of helper-dependent specific antibody production.

During the last years, the infusion of positively selected bone marrow $CD34^+$ cells in addition to unmanipulated marrow has been consistently used at our center for infants with severe primary immunodeficiency who have a MUD. This strategy allows to increase the stem cell dose without increasing the amount of $CD3^+$ T cells infused with the graft; this may facilitate engraftment without increasing the risk of GVHD.

Infections represent a major risk factor for the outcome of HSCT in WAS. Patients with WAS are highly susceptible to EBV-PTLD, particularly if MMRD are used.[3,6] Our series was similar in that seven patients had evidence of EBV reactivation after transplant. This was associated with EBV-PTLD in three cases, two of which died of this complication. In five cases (four of which were asymptomatic), EBV reactivation responded promptly to the treatment with rituximab. Because most patients were treated pre-emptively, whether the high incidence of EBV PCR positivity in MUD patients predicts a high incidence of EBV-PTLD also in this setting, is impossible to ascertain from this study; larger prospective trials would be required to answer this question. On the other hand, that the majority of viral infections occurred in patients receiving transplants from MUD may be due to ascertainment bias, because patients treated by MUD-HSCT were routinely screened for CMV and EBV by PCR.

Mixed and split chimerism have been reported previously in WAS patients undergoing BMT;[3,18] however, little information are available on the long-term stability of the chimerism and its correlation with WASP protein expression in different lineages. We found that mixed chimerism or recipient reconstitution was more apt to develop in the myeloid lineage or B lineage than in the T-cell lineage (Table 3), and that myeloid autologous reconstitution correlated, not surprisingly, with poor platelet reconstitution. In the two patients who lost donor chimerism, myeloid conversion was detected first, followed by loss of donor platelets reconstitution and of donor B-cell chimerism.

The relative resistance of the T-cell compartment to autologous conversion leads us to speculate that within this compartment expression of normal WAS protein confers a selective advantage in the competitive setting, as also suggested by experience with spontaneous revertants.[19] Conversely, the relative sensitivity of the myeloid/platelet compartment to autologous reconstitution, may imply that nonmyeloablative approaches are unlikely to consistently cure WAS patients of thrombocytopenia. This may also impact on future therapeutic strategies for WAS such as gene therapy. A number of investigators have pursued gene replacement as an alternative strategy for WAS using mouse models[20–24] or by transducing human T cells.[25–27] However, given that the animal model fails to fully recapitulate the platelet defects seen in humans,[28] the direct relevance of these approaches to correction of human WAS is not yet clear. Likewise, the role of WASP, if any, in human stem cell survival and competition for niche remains uncertain.

Our institutional experience has been largely successful, with a majority of patients receiving MUD BMT. The improvement in clinical symptomatology, particularly for those patients with clinical scores of 4 or less, was consistently excellent. That all deaths occurred in patients with clinical score of 5 further underscores our recommendation that WAS patients with absence of WASP protein expression owing to severe *WASP* gene mutations should be transplanted expeditiously with an RID or closely matched MUD if available. Overall, this experience confirms that significant progress has been achieved in stem cell transplantation for WAS, even with use of alternative donors.

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