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ORIGINAL ARTICLE

Cotransplantation of haploidentical hematopoietic and umbilical cord mesenchymal stem cells with a myeloablative regimen for refractory/relapsed hematologic malignancy

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Abstract Human leukocyte antigen haploidentical hematopoietic stem-cell transplantation (haplo-HSCT) is associated with an increased risk of graft failure and severe graft-versus-host disease (GVHD). Mesenchymal stromal cells (MSCs) have been shown to support in vivo normal hematopoiesis and to display potent immunesuppressive effects. We cotransplanted the culture-expanded third-party donor-derived umbilical cord MSCs (UC-MSCs) in 50 people with refractory/relapsed hematologic malignancy undergoing haplo-HSCT with myeloablative conditioning. We observed that all patients given MSCs showed sustained hematopoietic engraftment without any adverse UC-MSC infusion-related reaction. The median times to neutrophil $>0.50\times10^9$/L and platelet $>20\times10^9$/L engraftment were 12.0 and 15.0 days, respectively. We did not observe an increase in severe acute GVHD (aGVHD) and extensive chronic GVHD (cGVHD), too. Grade II–IV aGVHD was observed in 12 of 50 (24.0 %) patients. cGVHD was observed in 17 of 45 (37.7 %) patients and was extensive in 3 patients. Additionally, only five patients (10.0 %) experienced relapse at a median time to progression of 192 days. The probability that patients would attain progression-free survival at 2 years was 66.0 %. The results indicate that this new strategy is effective in improving donor engraftment and reducing severe GVHD, which will provide a feasible option for the therapy of high-risk hematologic malignancy.

Keywords Haploidentical transplantation · Myeloablative · Mesenchymal stem cells · Umbilical cord · Graft-versus-host disease

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is an effective therapeutic modality for a variety of malignant and nonmalignant disorders. However, fewer than 30 % of patients who require allogeneic HSCT have a human leukocyte antigen (HLA)-compatible sibling. In China, searching for HLA-matched donors is usually unsuccessful because no siblings are available for almost all young people. Fortunately, HLA haplo-identical hematopoietic stem cell transplantation (haplo-HSCT) provides a cure for patients with refractory/relapsed hematologic malignancy who are suitable for HSCT but lack an HLA-matched donor. To date, the results of haplo-HSCT have been disappointing and remain complicated by treatment-related mortality because of infection, bleeding, regimen-related toxicity, engraftment failure, and acute and chronic graft-versus-host disease (GVHD) [1–3]. Recently, several approaches, such as T cell or CD3/CD19 cell depletion, the use of a "megadose" of stem cells, and nonmyeloablative (NMA)-conditioning regimens, have been employed and have shown a significant decrease in GVHD [2, 3]. However, for patients with refractory/relapsed

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malignant disorders, NMA conditioning regimens may increase the incidence of tumor relapse and graft rejection and decrease the success rate of allogeneic haplo-HSCT.

Recently, some experimental and clinical data demonstrated that mesenchymal stem cells (MSCs) can support hematopoiesis, enhance the engraftment of HSCs, and reduce the incidence of GVHD following HSCT [4, 5]. MSCs are a heterogeneous subset of stromal stem cells that can self-renew and are multipotent. Furthermore, these cells express low levels of class I, but not class II, histocompatibility antigens and are not immunogenic in in vitro assays or preclinical models. MSCs have also been shown to suppress primary and ongoing mixed lymphocyte reactions [6, 7]. MSCs can be isolated from many adult tissues, including bone marrow (BM), periosteum, adipose tissue, fetal liver, cord blood, and umbilical cord (UC) tissues [8–10]. Currently, BM represents the major source of MSCs for cell therapy. However, the aspiration of BM involves invasive procedures, and the frequency and differentiation potential of BM-MSCs decreases significantly with age [11]. Recent studies have shown that a large number of MSCs can be easily isolated from the UC and collected using an accessible procedure [12]. Our recent data demonstrated that the haplo-HSCT combined with third-party donor-derived UC-MSCs for nine patients with refractory severe aplastic was not only safe and feasible but also effective for the improvement of donor engraftment and the lessening of severe GVHD (unpublished data). However, it remains unclear whether cotransplantation of UC-MSCs in haplo-HSCT recipients with refractory/relapsed hematologic malignancy can improve engraftment and reduce severe GVHD.

Herein, we report the results of our clinical trial of haplo-HSCT with myeloablative (MA) conditioning that was designed primarily to examine the safety and feasibility of the cotransplantation of culture-expanded third-party donor-derived UC-MSCs and donor HSCs in hematologic malignancy patients. Our secondary goal was to investigate the rates and kinetics of hematopoietic engraftment and the incidence and severity of GVHD.

Patients and methods

Patients and study design

Fifty patients with hematologic malignancy aged 9–58 (median 26 years), including 24 males and 26 females, who needed transplantation but lacked an HLA-identical sibling donor were enrolled in this study in our center from January 2007 to June 2012. The diagnoses were based on the French–American–British criteria and were confirmed by immunophenotype and cytogenetic analyses. Twenty-three patients had acute myelogenous leukemia (AML), 17 had acute lymphoid leukemia (ALL), 7 had non-Hodgkin lymphoma (NHL), and the other 3 patients had chronic myelogenous leukemia (CML) in blast crisis. Of the 50 patient/donor pairs, 19 (38.0 %) were mismatched in 3 HLA loci, 17 (34.0 %) were mismatched in 2 HLA loci, and 14 (28.0 %) were mismatched in 1 HLA locus [13, 14]. Detailed information about the patients is listed in Tables 1 and 2. The clinical protocol and consent forms were approved by the institutional review board for human investigation. Patients or their legal guardians provided written informed consent for their inclusion in the study. Patients were required to have responsive or nonprogressive disease and adequate visceral organ function, including a left ventricular ejection fraction of at least 50 %, forced expiratory volume in 1 s and diffusion of carbon monoxide >50 % of predicted, serum direct bilirubin <2.0 mg/dL, and an actual or calculated creatinine clearance >60 mL/min. Patients were excluded for major central nervous system dysfunction or active infection.

UC-MSCs preparation

UC-MSCs were purchased from the National Engineering Research Center of Cell Products, State Key Laboratory of Experimental Hematology. The immunophenotype of UC-MSCs included positivity for CD13, CD29, CD90, CD44, CD105 (SH2), CD106, CD73 (SH3), CD166, and HLA-ABC but negativity for CD14, CD34, CD38, CD45, CD31, and HLA-DR. CD106 and HLA-ABC were expressed at significantly lower levels than the other markers [12].

High-dose chemotherapy

The conditioning regimen was based on our previous protocol for HLA-identical sibling HSCT. Conditioning procedures included one of two regimens: (1) regimen 1, for patients with AML or CML in BC, 35 mg m^{-2} day^{-1} fludarabine (Flu) (days−10 to −8), 2 g m^{-2} day^{-1} cytarabine (Ara-C) (days−10 to −8), 0.8 mg/kg/6 h busulfan (Bu) (days−7 to −5), 300 mg-m^{-2} day^{-1} teniposide (VM-26) (day−4), 1.8 g m^{-2} day^{-1} cyclophosphamide (Cy) (days−3, −2), and 5 mg kg^{-1} day^{-1} rabbit anti-human T-lymphocyte immunoglobulin (ATG) (days−4 to −1); or (2) regimen 2, for patients with ALL or NHL, total body irradiation (TBI) with 1.8 Gy m^{-2} day^{-1} (days−9 to −7), 35 mg m^{-2} day^{-1} Flu (days −7 to −5), 2 g m^{-2}-day^{-1} Ara-C (days−7 to −5), 300 mg m^{-2} day^{-1} VM-26 (day−4), 1.8 g m^{-2} day^{-1} Cy (days−3, −2), and 5 mg kg^{-1} day^{-1} ATG (days−4 to −1) [15–17].

Allogeneic HSC infusion

At the start of the study, BM and peripheral blood stem cells (PBSCs) (the percentages of BM and PBSCs were 50 %, respectively) were both elected for HSCT as the source of cellular rescue. Donor allogeneic HSCs were obtained using

Table 1 Demographic and transplant characteristics of patients and donors

	Sex/Age	Diagnosis & Stage of disease	Donor/Age	HLA mismatch	Regemin	MNC 10^8/kg	CD34+ 10^6/kg
1	M/18	CML-BC	Mother/42	A B	R1	7.48	3.50
2	F/20	ALL- RF*	Uncle/36	A B	R2	8.80	9.62
3	F/26	ALL-RL2	Sister/30	A B	R2	7.95	14.42
4	M/28	AML-RF RL*	Brother/25	A B	R1	7.42	4.02
5	F/14	ALL-RL2	Father/38	A B DR	R2	9.27	4.20
6	M/16	ALL-RL2*	Father/40	A B DR	R2	6.22	5.69
7	M/21	ALL-RF	Brother/23	A B DR	R2	7.46	3.64
8	M/29	AML-RF	Sister/31	A	R1	6.02	2.56
9	M/16	NHL-RL	Mother/37	A B DR	R2	7.10	9.80
10	M/10	ALL- RF*	Mother/37	A B DR	R2	8.94	2.32
11	F/19	NHL-RF	Brother/25	A B	R2	6.88	7.41
12	F/25	ALL-RF	Brother/22	A B	R2	9.74	2.34
13	M/25	CML-BC	Mother/53	A B DR	R1	8.15	1.92
14	F/38	AML-RL	Brother/40	A B DR	R1	6.36	2.98
15	M/45	ALL-RL	Sister/44	B DR	R2	6.90	2.44
16	M/18	NHL-RL2#	Father/44	B DR	R2	6.06	3.40
17	F/19	AML-RF	Mother/45	A B DR	R1	7.40	2.72
18	M/19	ALL-RF	Mother/43	A B DR	R2	6.96	4.13
19	M/34	NHL-RL2#	Brothet/36	A B DR	R2	7.60	4.35
20	M/18	ALL-RL2	Father/43	A B DR	R2	6.48	3.14
21	F/53	AML-RL3*	Daughter/29	A B DR	R1	6.95	2.29
22	F/46	NHL-RL2#	Sister/49	A	R2	8.66	2.42
23	M/23	CML-BC	Father/46	A B DR	R1	6.80	2.76
24	F/40	NHL-RF	Sister/49	B	R2	7.86	2.15
25	M/25	AML-RF RL *	Father/51	B DR	R2	9.05	2.56
26	F/28	ALL-RF *	Brother/40	A	R2	8.70	3.04
27	F/45	AML-RL2	Brother/42	DR	R1	8.12	3.46
28	M/27	NHL-RF RL	Brother/25	B DR	R2	7.10	3.18
29	F/39	AML-RL	Sister/30	A DR	R1	7.64	3.40
30	F/48	AML-RF+	Sister/33	A	R1	9.60	6.38
31	M/33	AML-RF+	Sister/45	B DR	R1	8.63	4.68
32	F/29	AML-RL	Mother/53	A B DR	R1	6.78	3.28
33	F/53	AML-RL3+	Son/29	A B DR	R1	6.90	2.40
34	M/18	AML-RF+	Brother/32	A B DR	R1	7.82	2.26
35	M/58	AML-RF*	Brother/42	B	R1	5.95	2.30
36	F/23	ALL- RF	Mother/47	B DR	R2	8.29	3.42
37	F/29	AML-RF*	Sister/36	B DR	R1	8.53	6.48
38	F/16	ALL- RF	Brother/12	A DR	R2	6.66	7.52
39	M/9	ALL-RL2*	Father/35	B DR	R2	6.45	9.35
40	F/50	AML-RF+	Son/24	A B	R1	7.99	2.03
41	F/37	AML-RF+	Son/12	B	R1	7.20	9.64
42	M/36	AML-RL*#	Brother/39	DR	R1	9.21	4.94
43	M/13	AML-RF*	Mother/37	B	R1	9.05	4.51
44	M/20	ALL- RF	Mother/45	A B DR	R2	8.62	13.09
45	F/54	AML-RF*	Brother/51	B	R1	6.53	2.04
46	M/16	AML-RF	Brother/25	DR	R1	7.10	5.27
47	F/46	ALL- RF*	Son/22	A B DR	R2	7.60	2.21
48	F/26	AML-RF+	Brother/22	A B DR	R1	7.98	3.99
49	F/14	ALL- RF	Sister/21	DR	R2	7.80	2.16

Table 1 (continued)

	Sex/Age	Diagnosis & Stage of disease	Donor/Age	HLA mismatch	Regemin	MNC 10^8/kg	CD34+ 10^6/kg
50	F/42	AML-RF+	Sister/46	B	R1	7.70	2.69

MNC mononuclear cells, *F* female, *M* male, *AML* acute myelogenous leukemia, *ALL* acute lymphoblastic leukemia, *RL* relapsed leukemia *RL 2, 3* second or third relapse, *RF* refractory leukemia, *CML-BC* chronic myelogenous leukemia in blast crisis, *HLA* human leukocyte antigen *CMV* cytomegalovirus

#With previous autologous peripheral blood stem cell transplantation

+Secondary acute leukemia with myelodysplastic syndrome

*White blood cell count >100×10^9 /L at diagnosis

either a standard BM harvesting technique or a PBSC apheresis procedure with standard pheresis equipment (CS-3000 plus, Baxter, USA) after the subcutaneous administration of recombinant human granulocyte-colony stimulating factor (Kirin Brewery, Tokyo, Japan) 5 μg kg^{-1} day^{-1} for 5 days. For BM harvest and PBSC collection, a minimum of 6×10^8 mononuclear cells and a minimum of 2×10^6 CD34 cells were required, respectively. PBSCs were cryopreserved using a controlled-rate liquid nitrogen freezer. BM harvest cells were usually not cryopreserved. BM stem cells were infused intravenously through a central venous catheter on day 0 and thawed PBSCs were infused on day 1.

UC-MSC infusion

The planned UC-MSC dosing scheme was 5.0×10^5/kg in all patients. Before a planned UC-MSC infusion, the cells were shipped in liquid nitrogen containers to our transplantation center. Cryopreserved UC-MSC units were thawed at the bedside in a 37 °C sterile water bath and infused intravenously over 30 min as described previously [18]. BM infusions were performed 4 h after the completion of UC-MSC infusion [18]. Vital and clinical signs and symptoms were monitored at the time of infusion and every 15 min thereafter for 3 h, followed by every 2 h for 6 h and every 8 h for 3 days.

Table 2 Patient demographic summary

Characteristic	Data
Age (y)	
Median	26
Range	9-58
Sex	
Male	24 (48.0 %)
Female	26 (52.0 %)
Patient/Donor paris	
3 HLA loci	19 (38.0 %)
2 HLA loci	17 (34.0 %)
1 HLA loci	14 (28.0 %)
ABO pairs	
Pairs	19 (38.0 %)
Un-pairs	31 (62.0 %)
CMV-positive donor	10 (20.0 %)
CMV-positive patient	18 (36.0 %)

Prophylaxis and treatment of GVHD

GVHD prophylaxis consisted of intravenous cyclosporine 3 mg-kg^{-1} day^{-1} in divided doses beginning the day before transplantation (day−3) and was continued thereafter. The oral mycophenolate mofetil (MMF) dose was 20 mg kg^{-1} day^{-1} from day−1 and was tapered off after 28 days if no acute GVHD (aGVHD) was observed. Rabbit ATG (Fresenius, Germany) was administered intravenously at a dose of 5 mg kg^{-1} day^{-1} only on days−4 to −1 before HSCT, and anti-CD25 antibody (Basiliximab, Novartis Pharma Stein AG, Switzerland) was administered intravenously at a dose of 0.5 mg kg^{-1} day^{-1} only on day 4 after HSCT. Patients were advanced to oral cyclosporine as tolerated. In the absence of GVHD, the oral cyclosporine dose was reduced weekly by approximately 5 % beginning on or near day 100, and therapy was usually discontinued by 6 months after transplantation. Acute and chronic GVHD were treated according to institutional practices.

Engraftment, toxicity grading, GVHD grading, and tumor assessment

All calculations were assessed from the day of HSC and UC-MSC infusions (designated as day 0). Neutrophil engraftment was defined as the first of three consecutive days of neutrophil counts >0.50×10^9/L, and platelet engraftment was defined as the first of seven consecutive days in which the platelet count exceeded 20×10^9/L without transfusion support. Toxic effects were graded using the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE version 4.0). Acute GVHD, which was defined as GVHD onset within 100 days of transplantation, was graded 0–IV (grades III to IV were considered moderate to severe) using a modification of the Glucksberg criteria [19]. Patients who survived more than 21 days after transplantation with evidence of engraftment were

Table 3 The outcome of transplantations in 50 patients

Case	ANC>0.5×10^9/L (D)	PLT>20×10^9/L (D)	aGVHD grade	cGVHD	Relaspse (M)	Outcome (M)
1	15	17	No	Ext[c,d]	–	Dead 9, GVHD
2	11	12	I[a]	No	6	Dead 9, Relaspse
3	9	12	No	No	–	Alive 14
4	12	14	No	Lim[a]	–	Alive 8
5	10	11	III[b,c]	No	–	Dead 2, GVHD
6	12	16	No	No	2	Dead 5, Relaspse
7	16	18	No	No	–	Alive 58
8	15	19	No	No	–	Alive 57
9	16	16	No	No	–	Dead 9, infection
10	20	28	II[c]	No	–	Alive 53
11	14	14	No	Ext[a,d]	–	Dead 7, GVHD
12	14	14	No	No	–	Alive 6
13	11	14	III[b]	No	–	Alive 5
14	13	16	I[a]	Lim[c]	–	Alive 49
15	11	16	I[a]	Lim[a]	–	Alive 48
16	12	16	II[a,c]	Lim[c]	–	Dead 3, infection
17	16	26	IV[a,c]	No	–	Dead 3, GVHD
18	12	12	No	No	–	Alive 4
19	11	13	II[a,c]	No	–	Dead 3, infection
20	14	14	IV[b,c]	No	–	Dead 1.5, GVHD
21	15	18	II[a]	Lim[c]	–	Alive 42
22	12	15	I[a]	No	–	Alive 39
23	14	16	No	Lim[a]	–	Alive 42
24	15	17	No	No	3	Dead 5, Relaspse
25	13	16	No	Lim[c]	–	Alive 9
26	11	15	I[a]	Lim[c]	–	Alive 13
27	14	14	I[a]	Lim[a]	11	Dead 27, Relaspse
28	11	13	No	No	–	Alive 28
29	12	14	No	Lim[a]	–	Alive 36
30	11	13	No	No	6	Alive 35
31	12	14	No	Lim[c]	–	Alive 33
32	13	15	No	No	–	Dead 2, infection
33	14	18	IV[a,c]	No	–	Dead 2, GVHD
34	9	11	I[c]	No	–	Alive 32
35	10	11	No	No	–	Alive 28
36	10	14	IV[b,c]	No	–	Dead 2, GVHD
37	12	15	No	Ext[a,e]	–	Alive 21
38	9	11	II[b]	No	–	Alive 15
39	12	12	III[b]	Lim[c]	–	Dead 13, infection
40	16	21	No	No	–	Alive 20
41	15	19	No	No	–	Alive 21
42	9	11	No	No	–	Alive 15
43	20	24	No	Lim[a]	–	Alive 18
44	9	11	I[a]	No	–	Dead 4, infection
45	13	18	No	No	–	Alive 4
46	11	15	I[a]	Lim[a]	—	Alive 13
47	13	16	No	No	–	Alive 20
48	12	14	No	No	–	Alive 22

Table 3 (continued)

Case	ANC>0.5×10^9/L (D)	PLT>20×10^9/L (D)	aGVHD grade	cGVHD	Relaspse (M)	Outcome (M)
49	9	10	No	No	–	Alive 29
50	14	17	No	No	–	Alive 8

ANC absolute neutrophil count, *PLT* platelets, *aGVHD* acute graft-versus-host disease, *cGVHD* chronic graft-versus-host disease, *Ext* extensive, *Lim* limited, *D* day, *M* months

[a] Organ was affected by GVHD: skin

[b] Organ was affected by GVHD: intestinal

[c] Organ was affected by GVHD: liver

[d] Organ was affected by GVHD: lung

[e] Organ was affected by GVHD: eye

considered at risk for aGVHD. Chronic GVHD (cGVHD) was graded as limited or extensive according to previously published criteria in patients who survived more than 90 days with evidence of engraftment [20]. Patients underwent restaging with external imaging techniques and diagnostic marrow examinations as appropriate for each malignancy every 3 months after HSC infusion. Studies were not performed specifically to detect ectopic bone and cartilage formation. Relapse was defined as the recurrence of disease. Death in the absence of persistent relapse was categorized as nonrelapse mortality.

Supportive care

All patients had multilumen indwelling central venous catheters and were cared for in single rooms. Antibiotics were administered empirically for fever and neutropenia according to institutional guidelines, and all patients were supported with irradiated blood components in an attempt to maintain the hematocrit >25 % and the platelet count >10×10^9/L or to treat bleeding complications due to thrombocytopenia. Recombinant hematopoietic growth factors (Kirin Brewery, Tokyo, Japan) were administered routinely. Supportive care was given according to individual institutional practices and included cytomegalovirus infection prophylaxis with ganciclovir [21], *Pneumocystis carinii* pneumonia prophylaxis with trimethoprim-sulfamethoxazole, or inhaled pentamidine (in sulfa-allergic patients) at the start of the transplantation conditioning regimen. These treatments were withheld until the neutrophil count exceeded 0.50×10^9/L and were resumed until immunosuppressive therapy was discontinued. Antifungal agent prophylaxis included fluconazole, itraconazole, or liposomal amphotericin.

End points

Primary and secondary outcomes included the following factors: (1) determination of the short- and long-term safety of UC-MSC infusions, (2) donor-derived engraftment, (3) disease-/progression-free survival (i.e., survival without recurrent malignancy after transplantation), and (4) GVHD.

Statistical analyses

Statistical analysis was performed with SPSS 13.0 statistical software, version 18.0. Estimates of disease-/progression-free and overall survival were obtained using an analysis of life-table methods according to the Kaplan–Meier method.

Results

Engraftment and chimerisms

In Table 1, the median hematopoietic cell doses infused were 7.60×10^8 mononuclear cells (range, 5.95–9.74×10^8/kg) and 3.40×10^6 $CD34^+$ cells (range, 1.92–14.42×10^6/kg) for transplants. Data for all patients who survived at least 30 days after transplantation and were evaluable for engraftment are summarized in Table 3. Overall, the median time to achieve neutrophil engraftment (absolute neutrophil count, ≥0.50×10^9/L) was 12.0 days (range, 9.0–20.0 days). Overall, the median time to achieve platelet engraftment ≥20×10^9/L was 15.0 days (range, 10.0–28.0 days). Delayed platelet recovery (>30 days) was not observed in any of the 50 patients. All 50 patients achieved full donor chimerism.

Graft-versus-host disease

Table 3 indicates the incidence and severity of GVHD. Overall, 21 of 50 (42.0 %) patients experienced aGVHD between 15 and 83 days after transplantation, including 9 (18.0 %) with grade I, 5(10.0 %) with grade II, 3 (6.0 %) with grade III, and 4 (8.0 %) IV aGVHD. The other 29 patients had no aGVHD. Seventeen of 45(37.7 %) evaluable patients who survived at least 90 days after transplantation

experienced cGVHD, and which was limited in 14(31.1 %) patients and extensive in 3 (6.6 %) patients.

Relapse

Five of the 50 (10.0 %) patients experienced tumor relapse or progression at a median of 192 days (range, 65–385 days; Table 3). Two of these patients achieved a second complete remission after the withdrawal of immunotherapeutic agents: one of these patients survived, and the other patient later died of severe pulmonary fungal infection. The remaining one patient received regular chemotherapy with no responsiveness.

Survival

Upon follow-up for 1–58 months, 33 of the 50 (66.0 %) patients were alive well. As shown in Fig. 1, the probabilities of 1- and 2-year disease-/progression-free survival were 77.8 and 66.0 % in the 50 patients, respectively. Seventeen patients died, six patients died as a result of infection, seven patients died as a result of GVHD, and four patients died as a result of relapse. The probability of 2 year LFS in AML and CML patients was 75.4 %, which was significantly higher than that in the ALL and NHL patients (47.3 %, P=0.024).

Clinical safety outcomes

As anticipated for the HSC transplant recipients that were given MA therapy, all 50 patients experienced at least one adverse event during the study period, and 39 of 50 (78.0 %) patients developed at least one grade IV adverse event (Table 4). Only nine patients (18.0 %) demonstrated adverse events that were considered treatment related (Table 5). However, no patients experienced infusional toxicity during the UC-MSC infusion.

Discussion

Some study reported that MSCs were infused into patients 1 or 24 h after BM or 4 h before BM infusion [18, 22]. In this study, there was no immediate or delay toxicity related to IV UC-MSCs. None of the 50 patients experienced allergic reactions or respiratory symptoms. Furthermore, in comparison with other reports [18, 22] in which large amounts (over 1×10^6/kg) of MSCs were usually infused intravenously, we administered 5×10^5 cells/kg of UC-MSCs to all of the patients in this study, and the clinical outcome was better, with milder GVHD and stable engraftment. Our results indicate that this dose of UC-MSCs retained an effective role.

Haplo-HSCT is still largely disappointing mainly because of high mortality from T cell-mediated alloreactions in lethal GVHD even T cells depletion or "megadose" of stem cells

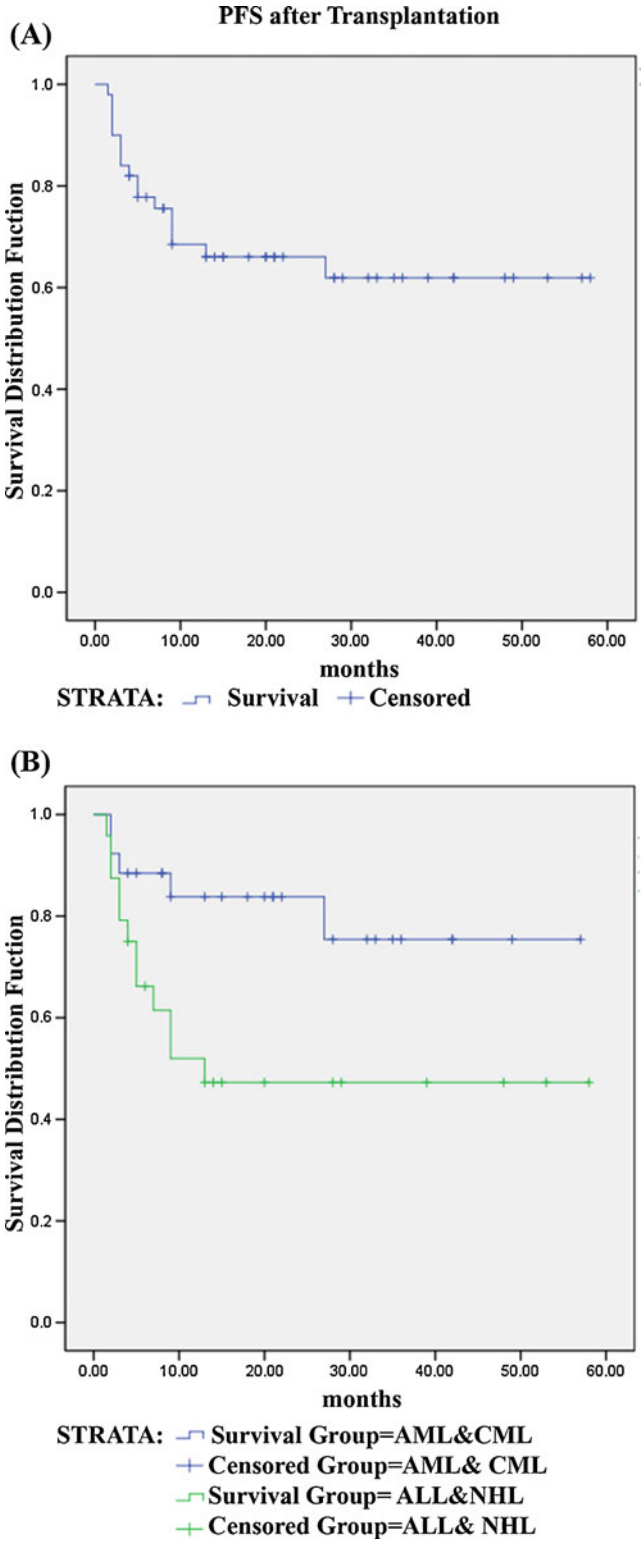

Fig. 1 Disease-/progression-free survival (*PFS*) after transplantation. **a** The probability of 2-year PFS for all patients was 66.0 %. **b** The probability of 2-year PFS in AML and CML patients was 75.4 %, which was significantly higher than that in ALL and NHL patients (47.3 %, P=0.024)

Table 4 Summary of adverse and severe adverse events for all patients with ma therapy

Variable	Total (N=50)
≥1 adverse event	50
≥1 severe adverse event	39
Fatal severe adverse event	11
Highest NCI grade or severity	
Grade 1	4
Grade 2	3
Grade 3	4
Grade 4	39
Relationship[a]	
Not related	41
Related	9

At each level of summation (except for the row for ≥1 adverse event), the patients who reported more than 1 event were counted only once. Data are the number of patients. NCI indicates National Cancer Institute

[a] Not related indicates an unrelated or unlikely relationship; related indicates a possible, probable, or definite relationship

are used [2, 23]. In our center, the results of haplo-HSCT were very disappointed from January 2006 to December 2007, too. Fifteen patients received haplo-HSCT with the same GVHD prophylaxis except UC-MSCs. The incidences for grades II to IV and III/IV aGVHD was up to 66.7 and 40 %, respectively. At the same time, extensive cGVHD occurred in 4 (36.3 %) of 11 evaluable patients who survived at least 90 days after transplantation. The most disappointed results were that only seven (46.7 %) patients were alive in the following 24 months. In this present trial, all of the 50 patients received haplo-HSCT with the GVHD prophylaxis and only 14.0 % of them developed grade III-IV aGVHD, although the overall rate of aGVHD was 42.0 %, which was better than our previous results. Additionally, cGVHD occurred in 17 patients (limited, n=14; extensive, n=3). Many studies have shown that the incidences for grades II–IV and III/IV aGVHD range from 44 to 64 % and from 16 to 26 %, respectively. Extensive cGVHD rates range from 30 to 46 % [16, 24, 25]. Acute and chronic GVHD rates identified in the trial fall within these ranges and would be a little better than the abovementioned. The results indicate that the new GVHD prophylaxis with ATG, CD25, CSA, MMF, and UC-MSCs was effective for the lessening of lethal aGVHD and extensive cGVHD. Some studies have been well investigated that ATG may remain in vivo for a much longer time, which is beneficial for in vivo T cell depletion and consequently for prevention of aGVHD, whereas anti-CD25 antibody has similar capacity in treating and preventing GVHD [26]. UC-MSCs do not express the immune costimulatory molecules or HLA-DR and express low levels of HLA-ABC [12] and which possess inhibitory effects on a variety of immune cells (T cells, B cells, NK cells, and DC) in terms of their proliferation, differentiation, maturation, and secretion [27]. Le Blanc reported that refractory GVHD was significantly alleviated in a patient who received third-party donor-derived MSCs. Le Blanc et al. [5, 28] also demonstrated that the infusion of MSCs was an effective therapy for patients with steroid-resistant GVHD in a phase II clinical trial. Similar results were also observed in our recent studies of nine patients with severe aplastic anemia of haplo-HSCT, which showed that the cotransplantation of HSCs with third-party donor-derived UC-MSCs had much sustained donor engraftment and milder GVHD (unpublished data). All of these results suggest that the infusion of third-party donor-derived UC-MSCs may play a considerable role in the lessening of severe GVHD. In addition, seven patients died as a result of gut GVHD in the study. Therefore, we should use UC-MSC not only for prevention of GVHD but also for therapy of GVHD in the short future. A randomized double-blind clinical trial evaluating the use of third-party donor-derived UC-MSC infusion for the prevention and lessening or treatment of GVHD will be required to establish the efficacy of this treatment regimen.

High-dose immunosuppressive agents are usually employed to overcome the HLA barrier. However, the benefit of a decrease in the incidence of GVHD is often accompanied by the cost of higher rates of graft rejection, leukemia relapse, and

Table 5 The list of adverse events for all patients

Adverse event	Grade 1	Grade 2	Grade 3	Grade 4	Relationship	
					R	NR
Oral ulcer	4		1	6	1	10
Diarrhea			1	10	3	8
Gastrointestinal hemorrhage		1		4	1	4
Hemorrhagic cystitis		1		6	1	6
Interstitial pneumonia			1	0	1	0
Liver dysfunction			1	10	2	9
Kidney dysfunction		1		3	0	4
Total (N=50)	4	3	4	39	9	41

R related, *NR* not related

infection, particularly for patients with refractory/relapsed hematologic malignancy [2, 3, 29]. In this study, all patients achieved sustained full donor engraftment and prompt hematopoietic recovery. The median times to a neutrophil recovery of 0.50×10^9/L and a platelet recovery 20×10^9/L for all patients were 12.0 days (range, 9.0–20.0 days) and 15 days (range, 10–28 days), respectively. Some studies have suggested that MSCs produce cytokines that support hematopoiesis, which could potentially enhance marrow recovery [12, 30, 31]. The results of this study suggest that the MA conditioning regimen was sufficient to achieve sustained donor engraftment in which the third-party donor-derived UC-MSCs may have played an important role.

In vitro, MSCs suppress T cell proliferation both by cell/cell interactions and via soluble factors [32–34]. Conversely, suppressed T cell function has the potential to abrogate graft-versus-leukemia activity in the allograft setting. Several groups have demonstrated that BM-MSCs facilitate tumor growth in in vitro and preclinical models [35]. In this study, we did not observe an increase in the number of patients who developed tumor progression. Fortunately, all 50 patients had a low relapse rate (10.0 %) and a high 2-year survival (66.0 %), among whom 23 AML and 3 CML patients had a higher 2-year survival rate (75.4 %) than that in the 17 ALL and 7 NHL patients (47.3 %), despite all of them belonged to refractory/relapsed hematologic malignancy. At the same time, interesting observation is that none of the patients who relapsed developed extensive cGVHD. This result was more favorable than those of other studies that used haploidentical transplantation by MA or NMA conditioning [36, 37].

In conclusion, our data demonstrate that the haplo-HSCT combined with third-party donor-derived UC-MSCs for patients with refractory/relapsed hematologic malignancy was not only safe and feasible but also effective for the improvement of donor engraftment and the lessening of severe GVHD. Therefore, this treatment regimen may provide a feasible option for the treatment of high-risk hematologic malignancy.

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