Int J Hematol (2013) 98:206–213
DOI 10.1007/s12185-013-1399-4

ORIGINAL ARTICLE

Unrelated allogeneic bone marrow-derived mesenchymal stem cells for steroid-refractory acute graft-versus-host disease: a phase I/II study

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Received: 27 May 2013 / Revised: 5 July 2013 / Accepted: 9 July 2013 / Published online: 17 July 2013

Abstract We conducted a multicenter phase I/II study using mesenchymal stem cells (MSCs) manufactured from the bone marrow of healthy unrelated volunteers to treat steroid-refractory acute graft-versus-host disease (aGVHD). Fourteen patients with hematological malignancies who suffered from grade II (9 patients) or III aGVHD (5) were treated. Affected organs were gut (10 patients), skin (9 patients), and liver (3 patients). Seven patients had two involved organs. The median age was 52. No other second-line agents were given. MSCs were given at a dose of 2×10^6 cells/kg for each infusion twice a week for 4 weeks. If needed, patients were continuously given MSCs weekly for an additional 4 weeks. By week 4, 13 of 14 patients (92.9 %) had responded to MSC therapy with a complete response (CR; $n = 8$) or partial response (PR; $n = 5$). At 24 weeks, 11 patients (10 with CR and 1 with PR) were alive. At 96 weeks, 8 patients were alive in CR. A total of 6 patients died, attributable to the following: underlying disease relapse (2 patients), breast cancer relapse (1), veno-occlusive disease (1), ischemic cholangiopathy (1), and

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pneumonia (1). No clear adverse effects associated with MSC infusion were observed. Third party-derived bone marrow MSCs may be safe and effective for patients with steroid-refractory aGVHD.

Keywords Mesenchymal stem cells · GVHD · Steroid

Introduction

Allogeneic hematopoietic stem cell transplantation (AlloHSCT) is a curative therapy for hematological malignancies and hemopoietic stem cell disorders. Acute graft-versus-host disease (aGVHD), the most important complication associated with AlloHSCT, develops in a significant number of patients who receive AlloHSCT despite GVHD prophylaxis [1]. Levine et al. [2] showed using Cox regression analysis that GVHD grade had a significant impact on non-relapse mortality and overall survival (OS) in a phase II GVHD treatment trial. The relative risk of non-relapse mortality was 1.72 for patients with grade III–IV GVHD, compared to patients with grade 0–II GVHD. Significant factors on OS were aGVHD grade (0–II versus III–IV), donor type (related versus unrelated), and stem cell source (peripheral blood versus bone marrow versus cord blood).

In general, a steroid is first given to patients with aGVHD; however, about half of the patients do not respond to the therapy [3]. Unfortunately, second-line agents have not clearly shown effectiveness against steroid-refractory aGVHD, because they act as a non-specific immunosuppressant and reduce host immunity, leading frequently to infections caused by bacteria, fungi and viruses [4, 5]. Indeed, most patients with steroid-refractory aGVHD die of aGVHD itself, organ damage, and infections even if such second-line therapy is conducted. Recently, the American Society of Blood and Marrow Transplantation evaluated 29 studies in which agents were administered as secondary therapy in aGVHD [6]. Evaluated agents included mycophenolate mofetil, daclizumab, alemtuzumab, infliximab, etanercept, horse antithymocyte globulin, and so on, but excluded mesenchymal stem cells (MSCs). Importantly, the evaluation of complete response (CR) rates, overall response (OR) rates, and 6-month survival estimates did not support the choice of any specific agents for second-line therapy in aGVHD. Therefore, a new agent for steroid-refractory aGVHD is desirable.

MSCs have unique characteristics: specific immunosuppressive properties, no immunogenicity on its own, supportive activity for hemopoiesis, and differentiation abilities into fat cells, chondrocytes, and osteoblasts. Since the first dramatic report by LeBlanc et al., there have been several reports on the effectiveness of MSCs against steroid-refractory aGVHD [7–20]. However, there are several problems when evaluating MSCs against steroid-refractory aGVHD in these studies: before MSC administration, patients have already received one or more immunosuppressants other than for GVHD prophylaxis to steroid-refractory aGVHD and the follow-up time of the patients who received MSCs was relatively short. The source of MSCs used for steroid-refractory aGVHD in these studies was heterogeneous: HLA-identical siblings, HLA-haploidentical related donors, and HLA-mismatched unrelated (third-party) donors. The production of MSCs in different institutes leads to concern about purity and cell function. We report a phase I/II trial on steroid-refractory aGVHD using third party-derived bone marrow MSCs. Before MSC administration, patients only received steroids for aGVHD as a first-line therapy.

Materials and methods

Patients

During the period from January 2009 and November 2010, 14 patients were enrolled in a phase I/II trial using third party-derived bone marrow MSCs for steroid-refractory aGVHD across major transplant centers in Japan. This trial, sponsored by JCR Pharmaceuticals Co., Ltd (Ashiya, Japan) and designated JR-031-201, was approved by the ethics committee in each participating facility. Informed consent was obtained from all the patients.

The eligibility requirements included patients with steroid-refractory grade II to IV aGVHD and age over 6 months. Steroid-refractory aGVHD was defined as progression of aGVHD for 3 days with standard-dose steroid administration or no change in aGVHD for 5 days with the therapy. The standard steroid dose (prednisolone or methylprednisolone) was 1–2 mg/kg. Exclusion criteria were as follows: chemorefractory disease, severe infection, positive results of viral infections including human immunodeficiency virus, human T-lymphotropic virus type I, hepatitis B virus, and hepatitis C virus, severe organ damage including heart, lung, kidney, and liver except liver GVHD, uncontrolled hypertension, oxygen saturation at a steady state less than 94 %, and new immunosuppressive agents added other than steroids for aGVHD. In cases where attending physicians did not predict early relapse after AlloHSCT, no remission in acute leukemia, myelodysplastic syndrome, or hematological malignancies was included. All patients received prophylaxis against GVHD with a calcineurin inhibitor (tacrolimus or cyclosporine) alone or a combination of a calcineurin inhibitor and methotrexate or mycophenolate mofetil. The source of hemopoietic stem cell transplants was bone marrow, peripheral blood stem cells, or cord blood. Conditioning

was either myeloablative conditioning such as total body irradiation-based and intravenous busulfan-based regimens or non-myeloablative conditioning such as fludarabine-based regimens. aGVHD was defined according to the 1994 Consensus Conference on Acute GVHD Grading [21].

MSCs

MSCs were manufactured by JCR based on a license from Osiris Therapeutics Inc (Columbia, Merryland, USA) and named JR-031. JR-031 is almost the same as Prochymal produced by Osiris [12, 17]. Briefly, an aliquot of bone marrow obtained from healthy volunteers was cultured in a medium supplemented with 10 % fetal bovine serum from New Zealand (Life Technologies, New York, USA). The fetal bovine serum products were free of bacteria, viruses, mycoplasma, and endotoxins in the checking tests. The products met standards for Code of Federal Regulations 9CFR113.53 and the United States Department of Agriculture. Adherent cells were expanded by culture and used as MSCs. Before freezing, cells were examined in the terms of MSC characteristics [22]. Isolated cells showed positivity for CD73, CD90, CD105, and CD166 and negativity for CD34, CD45, and HLA-DR. The cells inhibited the mixed-lymphocyte reaction and differentiated to fat cells, chondrocytes, and osteoblasts. The cells had the ability to produce prostaglandin E2. Multicolor-fluorescence in situ hybridization showed that the cells had no chromosomal abnormalities. No infectious agents such as bacteria, mycoplasma, or viruses were detected in the supernatants of the cells or the cells themselves. No endotoxin was detected in the supernatant.

Treatment schedule and evaluation

Initially, patients received a dose of 2×10^6 MSCs/kg twice a week for 4 weeks. The first infusion of MSCs was given within 48 h of the diagnosis of steroid-refractory aGVHD. The interval between each MSC infusion was 3 or 4 days. The volume of one bag of JR-031 was 15 ml containing 100×10^6 MSC, 1.5 g DMSO, 750 mg of human albumin, and other electroride elements. A solution of 25 ml saline was added to thawed MSCs and they were infused at a speed of around 4 ml/min. Before infusion, either 100–200 mg of hydrocortisone or 5–10 mg of chlorpheniramine or both were given to prevent an infusion reaction. During the total course of MSC infusions, no increase in the dose of immunosuppressants given for GVHD prevention was allowed. As for the steroid dose for GVHD treatment, no increase of more than the initial dose of steroid was allowed. Steroid dose reduction including the start of tapering timing and the reduction dose was left to the physicians who took care of the patient.

Response to aGHVD was evaluated for each involved organ. CR was defined as the complete resolution of aGVHD; partial response (PR), as a decrease in organ stages of aGVHD; no response (NR), as no change in aGVHD; progression (PG), as progressive worsening of aGVHD; mixed response (MR), as a mixture of a decrease and increase in organ stages of aGVHD. Patients were dropped out of this JR-031-201 trial if there was PG after the infusion of 3 doses of MSCs or NR after the infusion of 5 doses of MSCs. After completing MSC infusion for 4 weeks, i.e., 8 doses of MSCs, the response was evaluated. When patients showed PR or MR, 2×10^6 MSCs/kg were further given weekly for 4 weeks. The response of MSC therapy to aGVHD was evaluated as follows: CR or PR by the end of 4, 12, and 24 weeks from the first MSC infusion, as wells as continuous CR for more than 28 days. Other evaluable factors associated with MSC therapy were survival, disease relapse, infection, chronic GVHD, and so on.

Monitoring of adverse effects

To monitor adverse effects associated with MSC therapy, laboratory studies, electrocardiogram (ECG), chest X-ray, and computed tomography (CT) of the chest and abdomen were done according to the schedule; ECG was conducted before the first MSC infusion, at 4, 12, and 24 weeks (the cessation of the study) from the first MSC infusion. Chest X-ray was performed before the first MSC infusion, at 4 and 24 weeks. CT was conducted before the first MSC infusion and at 24 weeks. Vital signs, including percutaneous oxygen saturation concentration, were measured before and after each MSC infusion.

Long-term follow-up

After completing JR-031-201, a long-term follow-up study, JR-031-202, was conducted. The observation period was from the week following the end of JR-031-201, i.e., 25 weeks after the first MSC infusion, to 96 weeks (2 years). Informed consent was obtained from each patient. Evaluated valuables included adverse effects associated with MSC therapy such as the status of aGVHD, development of chronic GVHD (cGVHD), disease relapse, ectopic tissue formation, and so on.

Statistical analysis

Survival was described as time from the first MSC infusion and calculated by the Kaplan–Meier method.

Results

Table 1 shows the characteristics of the patients who received MSCs. The median age was 52 years (range 4–62 years). Thirteen patients were adults, while only one was a child. All patients had hematological malignancies as follows: acute myeloid leukemia, 4 patients; acute lymphoblastic leukemia, 3; myelodysplastic syndrome, 3; chronic lymphocytic leukemia, 1; follicular lymphoma, 1; multiple myeloma, 1; and juvenile myelomonocytic leukemia, 1. Of these, 2 patients (no. 4 and 10) had MLL-related leukemia due to chemotherapy for breast cancer. Breast cancer in both patients was in complete remission before AlloHSCT. No patients with refractory disease to chemotherapy were included. Most patients received a transplant from HLA-mismatched unrelated donors after myeloablative or non-myeloablative conditioning. The source of hematopoietic stem cells for transplantation was bone marrow (9 patients), peripheral blood stem cells (1), and cord blood (4). HLA disparity was shown in the eight pairs. All except 3 patients received a combination of a calcineurin inhibitor and methotrexate as GVHD prophylaxis. All patients were first given either prednisolone or methylprednisolone to treat acute GVHD.

Table 2 shows the aGVHD severity and organ involvement before the first MSC infusion and response to aGVHD. The grade of aGVHD was grade II (9 patients) and III (5 patients). Grade IV aGVHD was not enrolled. The most affected organs were the skin (9 patients) and gut (10 patients). Seven patients had two involved organs. MSCs were first infused on the median 47 days after AlloHSCT. The median number of MSC infusions was eight. By 4 weeks after the first MSC infusion, 8 and 5 patients had achieved CR and PR, respectively. The OR rate was 92.9 % (13 of 14 patients). By 24 weeks, 4 of 5 patients with PR achieved CR. At 96 weeks, 8 patients were alive and in CR. As shown in Fig. 1, the estimated time to reach 50 % CR after the first MSC infusion was 3 weeks (MSC infusion six times). There was no difference in the time to reach CR between grade II and grade III aGVHD (data not shown). Relapse of aGVHD after MSC therapy occurred in one patient (no. 12). At 78 days after the first MSC infusion, he was admitted again because of bloody diarrhea. Endoscopic biopsy showed aGVHD in the cecum and colon. The patient was put on parenteral hyperalimentation with tacrolimus administration. His aGVHD gradually disappeared.

By the end of the follow-up (2 years), 6 patients had died (Table 2). Five of the 6 patients (no. 2, 3, 4, 9, and 10) died due to factors not directly related to aGVHD as follows: no. 9 patient, veno-occlusive disease on day 25; no. 7, hepatic failure on day 36; no. 2, pneumonia on day 82; no. 3 and 10, disease relapse on days 191 and 696, respectively; and no. 4, metastatic breast cancer on day

Table 1 Patient characteristics

Case no.	Age	Sex	Disease	HSCT				GVHD prophylaxis	First line therapy for acute GVHD
				Source	Donor	HLA disparity	Conditioning		
1	56	F	MDS/RCMD	BM	Unrelated	7/8	Myeloab	CyA + sMTX	mPSL
2	59	F	AML/2nd CR	BM	Unrelated	8/8	Myeloab	CyA	mPSL
3	44	M	AML/1st CR	BM	Unrelated	7/8	Myeloab	FK506	mPSL
4	36	F	ALL/2nd Rel	BM	Unrelated	6/8	Myeloab	FK506	mPSL
5	57	F	FL	PB	Sibling	8/8	Myeloab	CyA + sMTX	PSL
6	42	F	ALL/1st CR	BM	Unrelated	8/8	Myeloabl	FK506 + sMTX	PSL
7	29	M	MDS/RAEB	CB	Unrelated	4/8	Myeloab	FK506 + sMTX	mPSL
8	62	F	CLL/PR	CB	Unrelated	5/8	Non-myeloab	FK506 + sMTX	mPSL
9	55	M	ALL/1st CR	BM	Unrelated	8/8	Myeloab	CyA + sMTX	mPSL
10	49	F	AML/1st CR	BM	Unrelated	6/8	Myeloab	FK506 + sMTX	mPSL
11	4	M	JMML/1st CP	CB	Unrelated	5/6	Myeloab	CyA + sMTX	PSL
12	61	M	AML-MRC[a]	CB	Unrelated	4/6	Myeloab	FK506 + MMF	PSL
13	35	F	MM/1st CR	BM	Unrelated	8/8	Non-myeloab	FK506 + sMTX	PSL
14	61	F	MDS/RAEB	BM	Sibling	6/6	Non-myeloab	CyA + sMTX	PSL

HSCT hemopoietic stem cell transplantation, *GVHD* graft-versus-host disease, *F* female, *M* male, *MDS* myelodysplastic syndrome, *RCMD* refractory cytopenia with multilineage dysplasia, *AML* acute myeloid leukemia, *AML-MRC* acute myeloid leukemia with myelodysplasia-related changes, *ALL* acute lymphoblastic leukemia, *FL* follicular lymphoma, *RAEB* refractory anemia with excess of blasts, *CLL* chronic lymphocytic leukemia, *MM* multiple myeloma, *CR* complete remission, *PR* partial remission, *Rel* relapse, *BM* bone marrow, *PB* peripheral blood stem cell, *CB* cord blood, *Myeloab* myeloablative, *Non-myeloab* non-myeloablative, *CyA* cyclosporine, *FK506* tacrolimus, *sMTX* short-term methotrexate, *PSL* prednisolone, *mPSL* methylprednisolone

[a] No chemotherapy before HSCT

Table 2 GVHD and outcome

Case	GVHD				1st MSC infusion	No. of MSC infusions	Response		Survival/ death	Cause of death (days)
	Grade	Skin	Liver	Gut	Days after HSCT		By 4 weeks	By 24 weeks	At 96 weeks	
1	II	3	0	0	38	8	PR	PR	Alive in CR	N/A
2	II	1	0	1	50	10	PR	CR	Dead	Pneumonia (82)
3	II	3	0	0	43	12	PR	CR	Dead	Relapse (696)
4	III	1	0	2	51	12	CR	CR	Dead	Breast cancer (244)
5	II	3	0	1	46	8	CR	CR	Alive in CR	N/A
6	II	3	0	0	48	8	CR	CR	Alive in CR	N/A
7	III	0	1	2	33	5	PG	–	Dead	IC (36)
8	III	3	0	3	57	12	PR	CR	Alive in CR	N/A
9	II	1	0	1	38	3	CR	CR	Dead	VOD (25)
10	II	0	0	1	45	8	CR	CR	Dead	Relapse (191)
11	III	0	2	4	78	12	PR	CR	Alive in CR	N/A
12	II	0	1	0	52	8	CR	CR	Alive in CR	N/A
13	III	3	0	4	44	8	CR	CR	Alive in CR	N/A
14	II	0	0	1	108	7	CR	CR	Alive in CR	N/A

GVHD graft-versus-host disease, *MSC* mesenchymal stem cell, *HSCT* hemopoietic stem cell transplantation, *CR* complete response, *PR* partial response, *PG* progression, *VOD* veno-occlusive disease, *IC* ischemic cholangiopathy, *N/A* not applicable

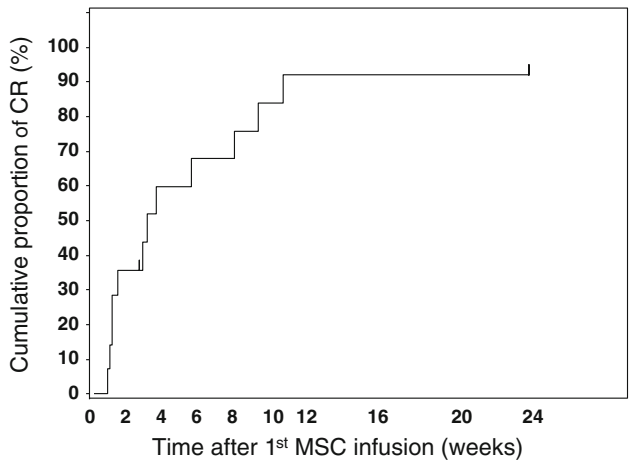

Fig. 1 Time to achieve complete response

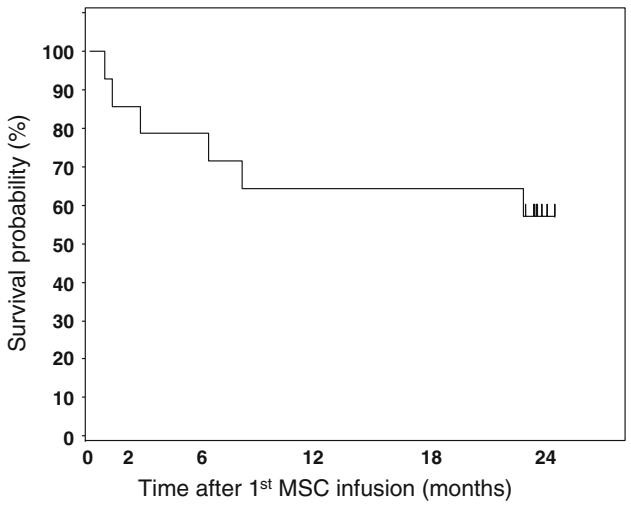

Fig. 2 Overall survival

244. The no. 2 patient showed pancytopenia caused by ganciclovir treatment for CMV antigenemia and died of pneumonia with massive pleural effusion. Autopsy findings showed pulmonary aspergillosis. Two patients with acute myeloid leukemia (no. 3 and 10) relapsed; the former received a second bone marrow transplant from another donor, but he died of sepsis. The latter died of septic shock after chemotherapy for disease relapse. Patient no. 4 maintained CR after MSC therapy, but a scheduled CT scan incidentally showed multiple low-density areas in both liver lobes on day 145. A liver biopsy demonstrated adenocarcinoma, leading to the suspicion of liver metastasis of breast cancer. Bone scintigraphy showed multiple isotope uptake regions in vertebrae and pelvic bone. The patient was diagnosed with recurrent breast cancer in the liver and bone and died of breast cancer. Patient no. 7 was evaluated as having PG of aGVHD. Serum liver enzyme levels and bilirubin values progressively worsened, leading to death. Necropsy of the liver showed ischemic cholangiopathy characterized by massive hepatocyte necrosis, marked congestion in the bile ducts, hyaline degeneration in the arterioles, disappearance of endothelial cells in the arterioles, and slight infiltration of lymphocytes. OS is shown in Fig. 2. There was no

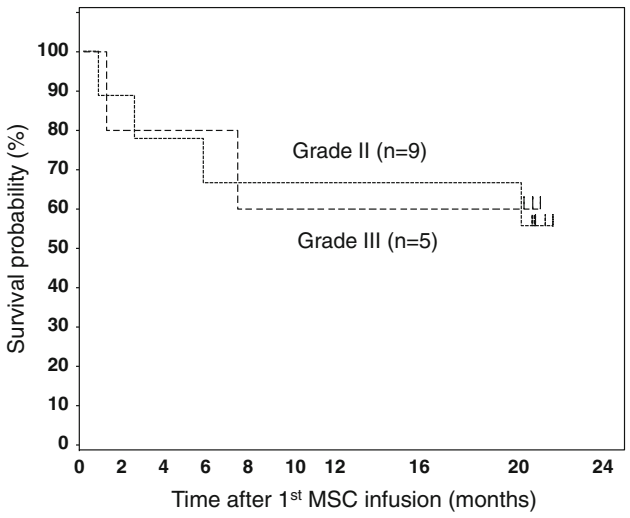

Fig. 3 Overall survival in the category of grade II and III acute GVHD. There was no difference between the two groups

difference in survival between groups of patients with grade II and grade III aGVHD (Fig. 3).

Adverse effects associated with MSC therapy were monitored. No infusion toxicity such as fever or decrease in oxygen saturation was observed. In the JCR-031-201 study, 27 events of infection episodes in 13 patients were collected as follows: bacteremia (3 events, 3 patients), pneumonia (4, 3), herpes zoster (3, 2), oral candidiasis (2, 2), infectious enterocolitis (2, 2), CMV antigenemia (2, 2), sepsis (1, 1), CMV colitis (1, 1), hemorrhagic cystitis (1, 1), and others (7, 7). In the JCR-031-202 study, 28 events in 9 patients were collected as follows: pneumonia (3 events, 3 patients), septic shock (3, 2), sinusitis (2, 2), upper respiratory tract infection (2, 2), oral herpes (4, 2), herpes zoster (1, 1), varicella (1, 1), infectious enterocolitis (1, 1), CMV antigenemia (1, 1), and others (8, 8). Ectopic tissue formation was not detected by scheduled CT scans. cGVHD developed in 7 patients; 4 patients (no. 8 at 24 weeks, 11 at 24 weeks, 12 at 48 weeks, and 14 at 36 weeks) with a limited form, and 3 patients (no. 1 at 24 weeks, 3 at 24 weeks, and 6 at 24 weeks) with an extensive form, of cGVHD.

Discussion

Reports of bone marrow MSCs used for steroid-refractory aGVHD are divided into two approaches; one approach used MSCs produced in the institution where patients were scheduled to receive the cells, while the another used MSCs manufactured in a company. In the former, the largest study was a phase II study conducted by the European Group for Blood and Bone Marrow [11]. Fifty-five patients with a median age of 22 years received MSCs for steroid-refractory aGVHD. Most patients had grade III or IV aGVHD. MSC donors were either HLA-identical, haploidentical, or HLA-mismatched unrelated donors. The median time from aGVHD onset to the first MSC infusion was 25 days. Of note, 33 patients had already received second-line therapy for aGVHD before MSC administration. Most patients received MSCs at a median dose of 1.4×10^6 MSCs/kg once or twice. The overall response rate was 71 % (CR, 30; PR, 9 patients). Twenty-four of these responders received MSCs from third-party donors. The overall estimated 2-year survival in this trial was 35 % and was significantly better in complete responders (53 %) versus non-complete responders (16 %). There was a better trend for 2-year estimated survival in the pediatric population compared to adults. No severe–adverse effects associated with MSC infusions were reported. Except for the report from the European Group for Blood and Bone Marrow, other reports were small-sized clinical studies including a phase I or a phase I/II study to treat steroid-refractory aGVHD with MSCs [7–10, 13–16, 18–20]. Importantly, in all of these studies, any second- or third-line immunosuppressive agent in combination with MSCs was allowed. Therefore, it is difficult to exactly evaluate the effects of MSCs on steroid-refractory aGVHD.

MSCs manufactured by Osiris, Prochymal, were given to steroid-refractory GVHD patients. Kebriaei et al. compared a dose of 2×10^8 Prochymal cells/kg with 8×10^8 Prochymal cells/kg in combination with steroids to treat patients with de novo aGVHD. Thirty-one patients were evaluated: there was no difference between the two groups in terms of safety and efficacy [12]. Prasad et al. [17] showed the efficacy of Prochymal for pediatric patients with severe refractory aGVHD. Most patients received Prochymal at a dose of 2×10^8 cells/kg. Following positive results in these two studies, Osiris conducted a phase III trial investigating Prochymal for steroid-refractory aGVHD across transplant centers in the United States, Canada, and Australia [23]. This was a double-blind placebo-controlled study. Patients were randomized at a 2:1 ratio for either Prochymal or the placebo. The dose of Prochymal was 2×10^8 cells/kg. Of note, most patients had already received a second-line therapy before MSC therapy. This trial enrolled 260 patients. The primary endpoint was durable CR for 28 days. The preliminary analysis did not show a statistical difference between Prochymal and the placebo for the primary endpoint (Prochymal 35 % versus placebo 30 %). However, subpopulation analysis showed that Prochymal significantly improved the response in liver aGVHD (76 versus 47 %) and gastrointestinal aGVHD (82 versus 68 %). Infection rates were not different between the two groups. Rates of severe–adverse effects associated with MSC administration were not different in the two arms. Now, Prochymal is approved for use

in pediatric steroid-refractory aGVHD in Canada and New Zealand as a cell-based medicine [24].

We confirmed that third party-derived bone marrow MSCs are safe and effective for patients with steroid-resistant aGVHD. In our study, only MSCs were given to patients with steroid-refractory GVHD as soon as possible after the diagnosis of steroid-refractory GVHD. Since the cell dose of infused MSCs was constant and the number of MSC infusions was strictly scheduled, our results are reliable to estimate the effects of MSCs on steroid-resistant aGVHD. A high CR rate and good OS were obtained. Of note, gut aGVHD comprised 71 % (10 of 14 patients) of our patients and all the patients except one showed CR. These results are consistent with others, i.e., MSCs have a favorable clinical effect on gut aGVHD [7, 12, 23]. Our trials did not include patients with grade IV aGVHD. Therefore, the good results in our studies may have been overestimated. As in other reports, no apparent adverse effects associated with MSC therapy were observed in short-term and long-term observations.

The presence of fetal bovine serum is necessary for standard conditions for MSC expansion [25]. However, it is better not to use animal products to avoid unknown infections and other complications. von Bonin et al. and Lucchini et al. showed the usefulness of platelet-lysate-expanded bone marrow MSCs for steroid-refractory aGVHD [13, 15]. Alternatively, MSC donor serum can be used for MSC expansion. Arima et al. and Pérez-Simon et al. successfully treated steroid-refractory aGVHD with bone marrow MSCs expanded in a medium supplemented with autologous serum [16, 18]. It is not known which MSC culture is the best in terms of the safety and growth of MSCs. Ideally, a serum-free culture of MSCs should be introduced in a clinical setting [26].

After the completion of the JCR-031-201 and JCR-031-202 trials, we started a phase III trial using JR-031 focusing on steroid-refractory grade III or IV aGVHD. In the near future, the results of this study will be published.

Conflict of interest K. Muroi and K. Ozawa received payment for consultancy from JCR Pharmaceuticals Co., Ltd. Other authors declare no conflicts of interest.

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