Mesenchymal Stem Cells: The First Approved Stem Cell Drug in Japan

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Acute graft-versus-host disease (aGVHD) is one of the most frequent complications after allogeneic hematopoietic stem cell transplantation (HSCT). First-line therapy for aGVHD usually consists of corticosteroids, but almost half of patients fail to respond, and more than 60% eventually die. Although several treatment options have been developed as second-line therapy, currently, no established strategy for steroid-refractory (SR) aGVHD is available. Mesenchymal stem cells (MSCs) have unique immunoregulatory properties that make them attractive for use in salvage therapy for SR-aGVHD. Following the striking clinical course reported by Le Blanc's group, many clinical trials have shown favorable results of MSC infusions for SR-aGVHD. In Japan, two clinical trials were performed. Following the favorable results of clinical studies, MSCs have become the first approved stem cell drug for SR-aGVHD. This therapy is called TEMCELL. In this review, we briefly summarize the current status and problems with MSCs in Japan. Now that this cell drug is available to all eligible patients, a post-marketing study is needed to answer crucial clinical questions. (Journal of Hematopoietic Cell Transplantation 6(3): 125-132, 2017.)

Introduction

Various advances in the care of patients undergoing HSCT have improved the outcomes following transplantation.¹ Nevertheless, aGVHD remains the most frequent complication after allogeneic HSCT and is often fatal. First-line therapy for aGVHD usually consists of corticosteroids, such as 2 mg/kg/day methylprednisolone for 7–14 days, but 40–50% of patients fail to respond.² The prognosis of steroid-refractory aGVHD (SR-aGVHD) is quite dismal, with mortality of over 60%. Thus, effective second-line treatment for SR-aGVHD has been long awaited. Although promising results for several treatment options including anti-thymocyte globulin (ATG),³ mycophenolate mofetil (MMF),⁴ methotrexate (MTX),⁵ and tumor necrosis factor α antagonist⁶ were reported, no consensus has been established regarding the selection of salvage therapy for SR-aGVHD.

Mesenchymal stem cells (MSCs) have unique immunoregulatory properties that make them an attractive option for salvage therapy. Following the promising results of phase I /

II⁷ and phase II / III⁸ studies, MSCs have become the first approved stem cell drug for SR-aGVHD in Japan. An excellent, comprehensive review paper was published describing the sequence of events in achieving insurance approval of MSCs for SR-aGVHD treatment in Japan.⁹ In addition, a sophisticated review paper was published previously regarding the clinical application of MSCs.¹⁰ In this article, we briefly summarize the updated evidence and status of MSCs for SR-aGVHD.

Characteristics of MSCs

The definition of MSCs has three minimum requirements.^{11,12} 1) MSCs are spindle-shaped adherent cells, 2) MSCs are positive for the surface markers CD105, CD73, and CD90 and negative for the leukocyte markers CD34, CD45, CD11b, CD19, and HLA-DR, and 3) MSCs have tri-lineage multipotency. They can differentiate into osteocytes, adipocytes, and chondrocytes. Due to this property, MSCs are expected to be applicable in regenerative medicine.¹²

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MSCs have important characteristics that make them suitable for aGVHD treatment.¹³ First, they have low immunogenicity, because their expression levels of HLA class I and II are low, and they lack expression of co-stimulatory molecules.^{14,15} Results of in vitro studies revealed that MSCs suppress allo-reactive T cells, allowing therapeutic use of MSCs from a third-party donor. Second, they have immunomodulatory effects. In pro-inflammatory conditions, they induce M2 macrophages to reduce inflammation.¹³ They promote regulatory T cells and suppress helper T cells including the Th1, Th2, and Th17 lineages. Third, through the same mechanism as leukocytes, MSCs can migrate into inflammatory sites, and thus, they do not induce systemic immunosuppression.¹⁶ Another advantage of administration of MSCs early after HSCT is their ability to produce interleukin-6, interleukin-8, and granulocyte-macrophage colony-stimulating factor, which may recruit and activate neutrophils to enhance the antimicrobial effect and promote the local protective immune system.^{14,15} These properties of MSCs are advantageous for aGVHD treatment.

Clinical trials using MSCs as salvage treatment for SR-aGVHD

Following the results from basic research, MSCs have been applied in the clinical setting. In 2004, Le Blanc et al. published the first case report of a 9-year-old boy with grade IV aGVHD, which resolved dramatically after the infusion of MSCs from bone marrow (BM-MSCs).¹⁷ Following this promising result, they conducted a phase II study.¹⁸ Fifty-five patients were enrolled, 90% of whom had grade III-IV aGVHD, and the overall response (OR) rate and complete response (CR) rate were 71% and 55%, respectively. Overall survival (OS) at 2 years was better (CR vs. less than CR: 52% vs. 13%, $P=0.018$), and transplant-related mortality was lower (CR vs. less than CR: 37% vs. 72%, $P=0.002$). After this landmark report, many research groups conducted clinical trials evaluating the effect of MSCs for aGVHD.

However, controversial results were reported. Using Prochymal, which consists of BM-MSCs from a third-party donor,¹⁹⁻²² Osiris Therapeutics Inc. (Columbia, MD, USA) conducted a randomized double-blind study for treatment of SR-aGVHD (ClinicalTrials.gov NCT00366145), in which 76 transplantation centers in the US, Canada, Europe, and Australia participated. The primary endpoint was induction of a CR with a duration greater than or equal to 28 days. Although

Prochymal significantly improved the response of liver (76% vs. 47%, $P<0.05$) and gut GVHD (82% vs. 68%, $P<0.05$), the response of skin was not significantly different between Prochymal and placebo groups (78% vs. 77%, $P=0.9$) and thus, they could not demonstrate a superiority of MSCs over placebo in terms of the primary endpoint.²³

Clinical trials conducted in Japan

In Japan, using an aliquot of BM from healthy volunteers, JR-031 was developed by JCR based on a license from Osiris. The manufacturing process of JR-031 is basically the same as Prochymal. Using JR-031, a phase I / II study was conducted in Japan.⁷ Fourteen patients with grade II - III SR-GVHD were enrolled. Of the 14 patients enrolled, 12 donors were unrelated, and eight patients received grafts from an HLA-mismatched donor. GVHD prophylaxis regimens basically consisted of a calcineurin inhibitor and a short course of MTX. Nine had grade II aGVHD, five had grade III aGVHD, and none had grade IV aGVHD. As initial therapy, patients received 2.0×10^6 MSCs/kg twice a week for 4 weeks. The same dose of MSCs was administered weekly for 4 weeks as additional infusions (up to 12 infusions per patient). The unique parameters compared to other clinical trials were 1) no other second-line treatment was permitted, and 2) MSCs were given within 48 h of diagnosis of SR-aGVHD. Patients were observed for 24 weeks, and additional survival follow-up was done at 24 months. MSCs were first infused at a median of 47 days after HSCT. More than 90% of patients achieved a partial response or CR. The cumulative proportion achieving a CR by 24 weeks was also 92.9%. The estimated time to reach 50% CR was 3 weeks (**Figure 1A**). All but one patient maintained the CR for more than 8 weeks, suggesting that these responses were highly durable. Regarding adverse events, no infusion reaction in the early phase or ectopic tissue formation in the late phase was observed. Thus, no MSCs therapy-associated events occurred. OS at 2 years after transplant was 57.1% (**Figure 1B**). No significant difference in OS was reported between patients with grade II and grade III aGVHD. The results of this study indicate that administration of third party-derived BM-MSCs was safe and effective. However, the efficacy of this therapy for grade III-IV could not be fully assessed.

A phase II / III study was conducted of patients with grade III and IV aGVHD.⁸ The treatment protocol was basically the same as described above, and the observation time was pro-

Figure 1. Results of a phase I/II study using TEMCELL in Japan. (A) Cumulative proportion of CR. (B) OS in all patients. Reproduced from Ref. 7.

Figure 2. Results of a phase II/III study using TEMCELL in Japan. (A) Cumulative proportion of CR. (B) OS in all patients. (C) OS in patients who achieved OR (dotted line) was significantly better than that of patients who did not achieve OR (dashed line, $P < 0.0001$). Reproduced from Ref. 8.

longed to 52 weeks. A total of 25 patients were enrolled, including 3 patients with grade IV aGVHD. The median time from HSCT to the first MSCs infusion was 45 days. The primary endpoint of this study, which was durable CR over 28 days, was reached in 48% of patients. Six patients maintained a CR with MSCs alone, whereas 11 out of 25 patients received additional immunosuppressive agents including ATG, MMF, MTX, etc. The OR by 4 weeks was 60%, and the CR rate increased from 4 weeks to 24 weeks. The estimated time to reach 50% CR was 6 weeks (Figure 2A). At 52 weeks, 12 patients (48%) were alive with a CR (Figure 2B). The survival of patients showing an OR was significantly better than that of patients without an OR (Figure 2C). The steroid dose was reduced by two-thirds at 4 weeks and by half at 8 weeks.

Many prospective studies using MSCs for SR-aGVHD have been conducted (Table 1).^{7,8,18,20-22,24-34} As shown, the patients who were enrolled and the defined endpoints varied.

The heterogeneity of the treatment response criteria blurs the conclusion about the effectiveness of MSCs for aGVHD.³⁵ In a recently published meta-analysis evaluating 336 patients in 13 studies, CR and OR for SR-aGVHD at 6 months were 28% and 72%, respectively.³⁶ With survival analysis, 63% of patients were alive at 6 months. These results also indicate the efficacy of MSCs therapy for SR-aGVHD. Further prospective studies are warranted to truly evaluate the details of the efficacy of this novel cellular therapy. In 30–40% of patients who eventually achieved a CR, maximum responses were observed 4–8 weeks after MSCs administration.

The first approved cell drug, TEMCELL

Following these favorable clinical results, JR-031, named TEMCELL[®] HS, was approved by the Japanese government as one of the first Cellular and Tissue-based Products in September 2015 and was listed in the national health insurance

Table 1. Prospective trials of BM-MSCs for aGVHD

Year	Author	n	Grade (n)	Dose (range) [$\times 10^6$ cells/kg]	Days from aGVHD diagnosis to 1st MSC infusion	Response	Overall survival	Adult or pediatric
2008	Le Blanc ¹⁸	55	II: 5, III: 25, IV: 25	1.4 (0.4–9.0)	27	CR 55%, OR 71%	N. A. [CR 52% vs. PR/NR 16% at 2 years]	Adult (25 pediatric)
2009	Kebriaeri ^{20*}	31	II: 21, III: 7, IV: 3	2.0 or 8.0	N. A.	CR 77%, OR 93%	71% at day 90 [CR 88% vs. PR/NR 14%]	Adult
2009	von Bonin ²⁴	13	III: 2, IV: 9	0.9 (0.6–1.1)	16	CR 15%, OR 54%	31% after median f/u of 8 months	Adult
2011	Prasad ^{22*}	12	III: 5, IV: 7	2.0 or 8.0	46	CR 58%, OR 75%	40% at 2 years [CR 68% vs. PR/NR 0%]	Pediatric
2011	Pérez-Simon ²⁵	10	III: 5, IV: 5	2.0 (1.0–2.0)	26	CR 10%, OR 70%	20% at 11 months	Adult
2012	Herrmann ²⁶	12	II: 1, III: 7, IV: 4	2.0 (1.7–2.3)	N. A.	CR 58%, OR 92%	55% at 30 months	Adult
2013	Muroi ^{7*}	14	II: 9, III: 5	2.0	2 [‡]	CR 50%, OR 93%	57.1% at 96 weeks	Adult (1 pediatric)
2013	Resnick ²⁷	50	II–III: 8, IV: 42	1.1 (0.3–2.3)	27	CR 34%, OR 66%	56.3% at 3.6 years [DFS]	Adult (25 pediatric)
2013	Ball ²⁸	37	III–IV: 37	2.0 (1.0–3.0)	13	CR 65%, OR 86%	37% at 2.9 years [CR 65% vs. PR/NR 0%]	Pediatric
2014	Introna ²⁹	31	II: 11, III: 17, IV: 3	1.5 (0.8–3.1)	24	CR 29%, OR 74%	50.0% at 1 year, 38.6% at 2 years	Pediatric
2014	Kurtzberg ^{21*}	75	B: 9, C: 21, D: 45 [†]	2.0	30	CR N. A., OR 61%	57.3% at 100 days [OR 76.1% vs. NR 27.6%]	Pediatric
2014	Sanchez-Guijo ³⁰	24	II: 7, III: 15, IV: 3	1.1 (0.7–1.3)	N. A.	CR 46%, OR 71%	N. A. [CR 72.7% vs. PR/NR 21.4% at 1 year]	Adult
2015	Zhao ³¹	28	II: 4, III: 8, IV: 16	1.0	17	CR 61%, OR 75%	N. A. [MSC 46.4% vs. Ctrl 26.3% at 3 years]	Adult
2016	Muroi ^{8*}	25	III: 22, IV: 3	2.0	2 [‡]	CR 24%, OR 60%	48% at 52 weeks	Adult
2016	von Dalowski ³²	58	III: 9, IV: 49	1.0	12	CR 9%, OR 47%	19% at 1 year	Adult
2016	Erbey ³³	33	III: 26 IV: 28	1.2 (0.5–2.8)	18	CR 55%, OR 76%	N. A. [CR 63.8% vs. PR/NR 29.4% at 1 year]	Pediatric

Abbreviations: BM, bone marrow; CR, complete response; OR, overall response; PR, partial response; NR, no response; aGVHD, acute graft-versus-host-disease; MSC, mesenchymal stem cell; N. A., not available. *Studies using Prochymal or the same protocol for preparing MSCs. †Interval between diagnosis of steroid-refractory aGVHD and MSC infusion. ‡aGVHD grades using the Center for International Blood and Marrow Transplant Registry grading scheme.³⁴

reimbursement in November 2015. The price of TEMCELL was set at ¥868,680 per bag of 7.2×10^7 cells in 10.8 ml (**Figure 3A**). Two bags are required for the average adult with a body weight over 36 kg. The bags must be transferred in ultra-

cold conditions (-150°C) using a specialty drug distribution unit (**Figure 3B**). One treatment course of TEMCELL in an adult patient will cost ¥13,898,880 (approximately US\$130,000) or up to ¥20,848,320 (approximately

Figure 3. Pictures of TEMCELL (A) and the specialty drug distribution unit (B)

Table 2. Potential concerns with MSC therapy in comparison to other treatments

	MSCs	rATG	MMF
Mechanism	Inhibition of Th lineage Promotion of Tregs and M2 macrophages (By direct cell contact)	Depletion of T, B, and NK cells	Inhibition of DNA and RNA synthesis in Th lineage cells
Immunosuppression	Local Mild (Anti-microbial?)	Systemic Severe	Systemic Moderate
Adverse effect	Ectopic tissue formation? Transformation? Pneumonia (pulmonary infection)?	Viral infection Allergic reaction Cytopenia	Diarrhea Cytopenia
Cost (BW 50 kg)	Extremely high ¥868,680/bag 2 bags/day, twice a week × 4 ¥13,898,880	Moderate to High ¥40,545/25-mg vial 1–5 mg/kg ¥81,090–¥405,540	Moderate ¥293.3/250-mg tab 20 mg/kg twice daily ¥70,392/month
Other considerations	Various sources (Umbilical cord, adipose tissue, and PB)	Prolonged immunosuppression	Oral medicine only in Japan Variation in blood concentration

Abbreviations: MSC, mesenchymal stem cell; rATG, recombinant anti-thymocyte globulin; MMF, mycophenolate mofetil; Th, helper T cell; Tregs, regulatory T cells; NK, natural killer cell; BW, body weight; aGVHD, acute graft-versus-host-disease; PB, peripheral blood.

US\$200,000). The product was launched on February 24, 2016 with temporary usage restriction in several institutions that participated in the clinical trials. For post-marketing drug surveillance, all patients who received TEMCELL have been registered to allow analysis of their outcomes for evaluation of the efficacy and adverse events.

Issues of concern

Although MSC therapy is a promising treatment, several concerns have been identified (Table 2). First are the safety issues associated with the stem cell properties of MSCs. Use of these cells involves the possibility of ectopic tissue formation or malignant transformation following many passages, which was suggested by in vivo and in vitro pre-clinical stud-

ies using murine MSCs.³⁷ However, this susceptibility to malignant transformation of human MSCs has not been directly confirmed.¹³ Furthermore, recent studies indicated that allogeneic donor MSCs may not completely be immune-privileged and infused MSCs may eventually be rejected by the recipient.³⁸ Past histological studies of specimens from autopsies and biopsies of patients with SR-aGVHD who received MSCs revealed no evidence confirming these safety concerns,³⁹ although further long-term observation may be required. Second, the cost of this treatment is surprising at over ¥800,000 per bag, requiring ¥14,000,000 per entire course. Third, due to their immunosuppressive effect, MSCs may increase the risk of opportunistic infection in highly immune-deficient recipients.⁴⁰ The prospective registration of all patients will help address these issues.

Compared with other agents available in Japan, MSCs treatment carries distinct properties. Currently, MMF^{4,41-44} and ATG^{3,45-47} are the two most frequently used second-line treatments for SR-aGVHD.⁴⁸ In Japan, rabbit ATG was approved in 2008 for SR-aGVHD. However, its long-term efficacy is yet to be confirmed. In terms of the mechanism, MSCs migrate into locally inflamed sites and inhibit helper T cells or promote regulatory T cells, mainly via direct cell contact. Thus, adverse events derived from severe immunosuppressive effects may be reduced compared to other agents. The cost for MSCs is substantial (**Table 2**), and criteria to select patients who will benefit from this treatment will be crucial. Another consideration is various sources of MSCs for clinical use. Although most MSCs used for aGVHD treatment were derived from BM in previous reports, other tissues including adipose tissues,⁴⁹ umbilical cord blood (CB),^{50,51} and placenta^{52,53} are potential sources of MSCs and have different properties compared to BM-derived MSCs. Fang et al. reported promising results of a clinical study using adipose tissue-derived MSCs in which 5 out of 6 patients with grade III-IV SR-aGVHD achieved a CR.⁴⁹ Nagamura et al. have revealed that Wharton's Jelly-derived MSCs do not express HLA-DR, even in the presence of the pro-inflammatory cytokine, interferon-gamma.^{50,51} A phase I study using CB-derived MSCs for SR-aGVHD is currently under preparation in Japan. These differences in MSC sources may affect the efficacy and should be evaluated in the near future.

Conclusion

MSCs have unique characteristics that are advantageous for aGVHD treatment. Results of clinical studies in Japan were favorable enough to achieve insurance approval. However, no data are available that compare the use of MSCs with other options. Thus, now that this treatment is publicly available, registration of all cases for post-marketing study is likely to produce pivotal data.

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Conflict of interest disclosure

The authors have no conflicts of interest to report.

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