Stem Cell Reports
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A Long-Term Follow-Up Study of Allogeneic Mesenchymal Stem/Stromal Cell Transplantation in Patients with Drug-Resistant Systemic Lupus Erythematosus

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https://doi.org/10.1016/j.stemcr.2018.01.029

SUMMARY

Allogeneic mesenchymal stem/stromal cells (MSCs) have been widely studied as an alternative cell source for regenerative medicine. Here, we report a long-term follow-up study of allogeneic bone marrow and/or umbilical cord MSC transplantation (MSCT) in severe and drug-refractory systemic lupus erythematosus (SLE) patients. Eighty-one patients were enrolled, and the 5-year overall survival rate was 84% (68/81) after MSCT. At 5-year follow-up, 27% of patients (22/81) were in complete clinical remission and another 7% (6/81) were in partial clinical remission, with a 5-year disease remission rate of 34% (28/81). In total, 37 patients had achieved clinical remission and then 9 patients subsequently relapsed, with 5-year overall rate of relapse of 24% (9/37). SLE Disease Activity Index scores, serum albumin, complement C3, peripheral white blood cell, and platelet numbers, as well as proteinuria levels, continued to improve during the follow-up. Our results demonstrated that allogeneic MSCT is safe and resulted in long-term clinical remission in SLE patients.

INTRODUCTION

Systemic lupus erythematosus (SLE) is a remarkable and challenging disorder that affects multiple organs including, but not limited to, the kidneys, CNS, lungs, and cellular blood components (Lisnevskaia et al., 2014). The general treatment approach for SLE includes corticosteroids and non-steroidal anti-inflammatory drugs, in combination with immunosuppressive drugs such as cyclophosphamide (CYC), mycophenolate mofetil (MMF), azathioprine (AZA), and leflunomide (LEF) (Murphy et al., 2013). Intravenous immunoglobulin (Ig) is also used in rare cases. However, over one-third of patients will relapse or flare or are resistant to these conventional therapies. In recent years, targeted biological therapies aimed at B cell activity and B cell-activating factors were developed for the treatment of refractory lupus patients. Short-term clinical studies showed encouraging results, but the long-term efficacy still warrants further investigations (Relle et al., 2015).

In this context, the use of either allogeneic or, more frequently, autologous hematopoietic stem cell transplantation (HSCT) represented the first application of cell-based therapies for treatment of refractory SLE patients. Several phase I/II studies and EBMT data registry analysis showed that autologous HSCT is a feasible treatment option leading to significant clinical responses, albeit limited to severely affected lupus patients and performed in expert centers according to good clinical practice guidelines after careful patient selection (Traynor et al., 2002). Nonetheless, the high rates of treatment-related mortality and relapse after HSCT have limited its clinical application (Wang and Sun, 2015). Another type of stem cells, mesenchymal stem/stromal cells (MSCs), primarily derived from adult bone marrow (BM), adipose tissue, or umbilical cord (UC), have shown promising potential for the treatment of human immune-mediated diseases. MSCs were first used clinically to treat acute graft-versus-host disease following allogeneic HSCT (Le Blanc et al., 2008). In the past 10 years, MSC transplantation (MSCT) was tried in refractory Sjögren's syndrome, systemic sclerosis, and dermatomyositis/polymyositis, with satisfactory clinical safety (Akiyama et al., 2012; Wang et al., 2011; Xu et al., 2012).

Beginning in 2007, we used allogeneic MSCs to treat severe and treatment-refractory lupus patients with our early reports showing that both allogeneic BM- and UC-MSCT induced disease remission and facilitated organ reparation (Liang et al., 2010; Sun et al., 2010). Later a multicenter and longer follow-up (12–48 months) analysis of all SLE patients treated in four centers in China with the same protocol confirmed the safety and efficacy of MSC cell-based therapy for lupus patients (Wang et al., 2013, 2014). In this study, we analyzed the outcome of 81 SLE patients treated in a single center after 5 years of follow-up.

Table 1. Patient Demographics and Pre-transplantation History

Variable	
Age (years)	32.7 (12–62)
Disease duration (months)	76.4 (6–264)
Sex (female/male)	76/5
Race (Asian/other)	81/0
Medication history (n)	
Corticosteroids	81
Cyclophosphamide	59
Mycophenolate mofetil	25
Leflunomide	17
Tripterygium	9
Azathioprine	7
Thalidomide	4
Vincristine	4
Cyclosporin A	4
Intravenous immunoglobulin	3
Methotrexate	2
Hemofiltration	2

RESULTS

Pre-MSCT Patient Demographics and Disease Manifestations

Eighty-one SLE patients were enrolled, and the clinical data collected and analyzed. All patients completed 5 years of follow-up except those that succumbed prior to the 5-year visit. The longest follow-up time was 8 years and the mean was 6.0 years (range, 5–8 years). Table 1 lists patient demographics and medication history. Detailed clinical information for each patient is shown in Table S1.

Allogeneic MSCT

Of the 81 patients, 22 received allogeneic BM-MSCs. Four patients relapsed and were therefore treated a second time with UC-MSCs. Two other patients received two additional doses of UC-MSCT and one patient received three additional doses of UC-MSCT. Among the 59 patients first treated with UC-MSCs, 7 received a second UC-MSCT, 1 received two additional doses of UC-MSCT, and 1 received three additional doses of UC-MSCT. Thirty-nine patients were administered CYC (10 mg/kg/day) intravenously on days −4, −3, and −2; the other 42 patients did not receive CYC.

Overall Survival

Thirteen patients died in the first 5 years and another two patients died at 6 and 7 years, respectively, so the 5-year survival rate was 84.0% (68/81, Figure 1A). Eight patients died in the first year after MSCT. One patient died of pulmonary embolism in the second year and two patients died of disease relapse and end-stage renal disease (ESRD) in the third year. Four years after MSCT, one patient suffered bladder cancer and died. Another three patients died of disease relapse and progression, infection, and cryptococcal meningitis 5, 6, and 7 years after MSCT, respectively. The information for patient baseline characteristics, MSC origin, and reasons for death are shown in Table 2.

Disease Remission and Relapse

At 5-year follow-up, 22 patients (22/81, 27%) were in complete clinical remission (Figure 1B). Another 6 patients (6/81, 7%) were in partial clinical remission, and the overall complete and partial clinical remission at 5 years was 34%. In total, 37 patients had achieved clinical remission at the 5-year visits and thereafter 9 patients relapsed, with a 5-year overall rate of relapse of 24% (9/37). By Cox regression analysis, we found that disease relapse was not correlated with patient age ($p = 0.275$), disease duration ($p = 0.594$), MSCs source ($p = 0.747$), CYC pre-treatment ($p = 0.782$), baseline SLE Disease Activity Index (SLEDAI) score ($p = 0.057$), or proteinuria levels ($p = 0.104$).

Disease Activity and Serological Changes

SLEDAI scores significantly decreased and remained significantly lower ($p < 0.05$) 5 years after MSCT (Figure 2A). Serum albumin levels increased after MSCT, with statistical significance at 1-, 2-, 3-, 4-, and 5-year follow-up compared with baseline levels (all $p < 0.05$, Figure 2B). Serum complement 3 significantly increased (all $p < 0.05$, Figure 2C), while serum complement 4 slightly increased after MSCT, but with no statistical difference compared with baseline levels (all $p > 0.05$, Figure 2D).

Renal Functional Analysis

Sixty-six patients had increased proteinuria at baseline (>0.5 g/24 hr) and 8 patients died within the first year, and data for the remaining 58 patients were analyzed. After MSCT, 24-hr proteinuria significantly decreased at 1-, 2-, 3-, 4-, and 5-year follow-up (all $p < 0.05$, Figure 3A). Thirty-five, 33, and 30 patients had abnormal serum urea nitrogen, creatinine, and uric acid at baseline, respectively. However, these indices showed no obvious changes after MSCT (Figures 3B–3D). Seven patients who had normal renal function at baseline then suffered renal dysfunction 2–6 years after MSCT, and one patient recovered, one died of cryptococcal meningitis, one became dialysis dependent, and the other four patients still depended on

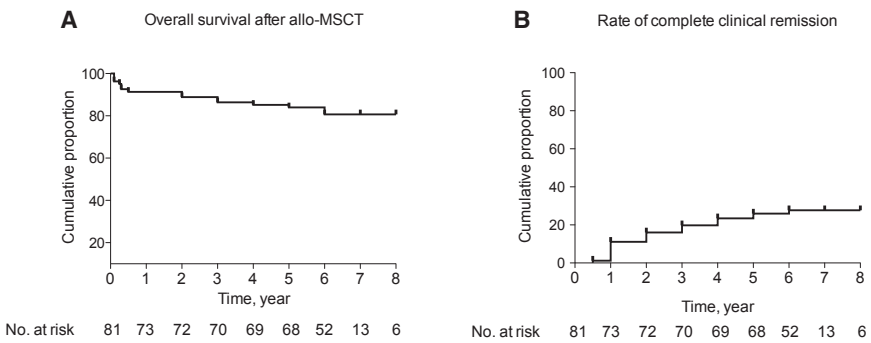

Figure 1. Patient Survival and Clinical Remission after Mesenchymal Stem/Stromal Cell Transplantation

Probability of overall survival (A) and complete clinical remission (B) in lupus patients undergoing mesenchymal stem/stromal cell transplantation (MSCT), by Kaplan-Meier survival analysis.

drug therapy. Seven patients with lupus nephritis needed dialysis after MSCT, one of whom died of ESRD and pulmonary infection (Table 2). The other six patients also receive dialysis three times a week.

Hematological Follow-Up

Twenty-four, 41, and 26 patients had autoimmune leukocytopenia, anemia, and thrombocytopenia at baseline, respectively. The peripheral white blood cell count significantly increased at 1-, 2-, 3-, 4-, and 5-year follow-up (all $p < 0.05$, Figure 4A). Levels of hemoglobin increased, with statistical significance at the 2-year visit ($p < 0.05$, Figure 4B). Moreover, the peripheral blood platelet number increased after MSCT, with statistical significance at 2-, 3-, and 5-year follow-up ($p < 0.05$, Figure 4C).

Other Organ Function Changes

One lupus patient showed secondary Sjögren's syndrome and interstitial lung disease, and her interstitial pneumonia significantly improved after UC-MSCT. Four patients with diffuse alveolar hemorrhage improved as previously reported (Shi et al., 2012), and remained stable for more than 5 years of follow-up. Four other patients had no progression of interstitial lung disease after MSCT. One patient suffered drug-induced Stevens-Johnson syndrome before transplantation, and her skin ulcers gradually healed after UC-MSCT (Li et al., 2013). Seizures in three patients and headache in one patient did not recur following MSCT. Three other patients experienced seizures 3, 3, and 5 years after MSCT, respectively, whereby two resulted from active lupus and the other from cryptococcal meningitis. After MSCT, aminotransferase levels remained normal in three patients with autoimmune hepatitis, including no recurrence of upper gastrointestinal hemorrhage in one patient who had liver cirrhosis and had experienced two episodes of severe hemorrhage 1 month before MSCT. One patient suffered new-onset liver cirrhosis 3 years after MSCT, and she is now waiting for liver transplantation. All patients who had abnormal liver function were excluded hepatitis B or C virus infections before and after MSCT. The condition of one patient with severe protein-losing enteropathy markedly improved after one UC-MSCT and her serum albumin and body weight quickly increased. Eight patients had femoral head necrosis at baseline, two of whom were successfully given hip replacements; two other patients died and four had no signs of pain. One patient had a normal delivery 3 years after MSCT, and her daughter is now 3 years old with no abnormality.

Adverse Events

Two patients experienced diarrhea 3 and 4 months after MSCT, respectively. Three additional patients had moderate herpesvirus infection at 1 month, 6 months, and 59 months after MSCT, respectively. One patient experienced agranulocytosis and oral fungal infection 7 days after CYC pre-treatment, and her condition improved after conventional therapy. Tuberculosis infection in the lung was found in two patients 9 and 96 months after MSCT, respectively, and both patients were treated with anti-tuberculosis drugs. Two patients developed *Klebsiella pneumoniae* pneumonia and were treated with antibiotics. One patient developed cryptococcal meningitis and eventually died. One patient had an L3 vertebral fracture in an accident 2 months after her third MSCT. One patient suffered myocardial infarction at 38 months after MSCT and was treated by percutaneous coronary intervention. This patient also suffered chronic Guillain-Barré syndrome 66 months after MSCT and was treated with high doses of steroid and intravenous Ig. Eventually he developed diabetes and was treated with insulin. All adverse events are shown in Table S2.

Maintenance Therapy after MSCT

At the time of MSCT most patients were on prednisone at a low dose, and after MSCT the doses of prednisone as well as immunosuppressive drugs were tapered. During the last follow-up, 60 out of 66 patients had tapered their steroids to 2.5–10 mg per day, two patients were on higher doses of prednisone compared with baseline, and four patients were on the same dose as pre-transplantation. Fifteen

Table 2. Demographics and Reasons for Deaths

No.	Name	Age (years)	MSC Origin	Disease Duration (months)	Time to Death Post MSCT (months)	Cause of Death
1	HXX	55	UC	6	6	pulmonary bacterial infection
2	FXY	46	UC, UC	251	3	right heart failure
3	GHY	36	UC	97	6	pulmonary bacterial infection
4	JZP	35	UC	25	8	pulmonary hypertension, heart failure
5	DYF	53	UC	144	11	pulmonary infection, ESRD
6	ZYH	21	UC	39	0.2	acute heart failure
7	ZZY	62	UC	12	2	intracranial hemorrhage
8	LQX	45	UC	6	1	pulmonary infection, heart failure
9	CL	36	BM, UC	39	17	pulmonary embolism
10	ZXJ	27	UC	48	34	CNSL, ESRD
11	GGY	43	BM	7	31	ESRD
12	ZHX	43	UC	26	40	bladder cancer
13	LX	29	BM	40	52	ESRD, pulmonary infection
14	YLJ	39	BM, UC	60	65	cryptococcal meningitis
15	YYF	44	BM, UC	36	83	ESRD

BM, bone marrow; CNSL, CNS lupus; ESRD, end-stage renal disease; UC, umbilical cord; MSCs, mesenchymal stem/stromal cells.

patients discontinued immunosuppressive drugs, of whom four had complete disease remission. Four patients had lupus nephritis remission although with peripheral blood platelet count fluctuations (60–90 × 10^9/L). Another seven patients were dialysis dependent and discontinued immunosuppressants. For 51 patients who had immunosuppressive drugs at the last visit, 15 patients had CYC of 0.4–0.6 g per 1–4 months, 12 patients had MMF for maintenance therapy at doses of 0.5–1.5 g per day, 8 patients had LEF at a dose of 10–20 mg per day, 3 patients had AZA of 50–100 mg per day, 2 patients had cyclosporin A at 100 mg per day, 1 patient had tacrolimus at 2 mg per day, 8 patients had CYC combined with LEF, 1 patient had CYC combined with MMF, and 1 patient had LEF combined with methotrexate for maintenance therapy (Table S3).

DISCUSSION

Previously we reported phase I and phase II single-arm and multicenter short-term follow-up studies of MSCT in treating refractory severe lupus patients. The 1-year complete clinical remission was 32.5% by MSCT, and the overall clinical remission was 60%. Previous studies also showed good survival rates of 92.5%–94%. However, as not all of the patients completed 4 years' follow-up evaluation in our previous study (Liang et al., 2010; Sun et al., 2010; Wang et al., 2013, 2014), we report herein the longer-term follow-up of safety and clinically observed results in SLE patients.

Sixty-eight out of 81 patients in the present study all completed at least 5 years of follow-up with longest being 8 years. The 5-year survival rate was 84.0%, similar to that in the study of autologous HSCT (Burt et al., 2006). Recently a retrospective survey reviewed the efficacy and safety of autologous HSCT in 28 severe SLE patients refractory to all previous therapy from eight centers reported in the EBMT registry between 2001 and 2008. Although the 5-year overall survival was 81% ± 8% (Alchi et al., 2013), similar to our results, the follow-up time was also shorter (1–110 months, mean 38 months) than our study. For HSCT, the biggest challenge is the high rate of disease relapse as well as transplant-related mortality (TRM). Jayne et al. (2004) reported that although 66% of patients achieved clinical remission by 6 months, 32% subsequently relapsed, and TRM was 12% at 1 year. The EBMT data showed that the relapse incidence was 56% ± 11% and non-relapse mortality 15% ± 7% (Alchi et al., 2013). In the present long-term follow-up study of 81 highly refractory SLE patients treated by MSCT, there was no TRM. The 5-year rate of complete remission (27%) appeared

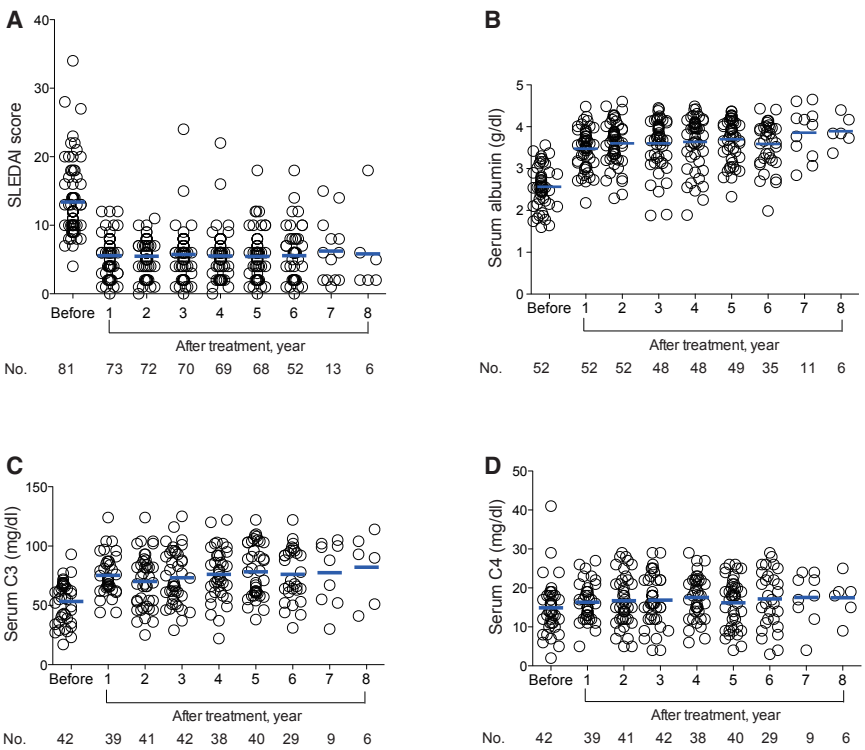

Figure 2. Disease Activity and Serologic Changes Before and After Mesenchymal Stem/Stromal Cell Transplantation

SLE Disease Activity Index score significantly decreased (A), and serum albumin (B) complement 3 (C3; C), and serum complement 4 (C4; D) increased, but with no significant difference compared with pre-MSCT levels.

higher than reported with the use of HSCT (21%). Twenty-four percent of MSCT patients relapsed after 5 years of follow-up, a rate of relapse also lower than that of HSCT.

In the United States experience, 7 infection events occurred in 50 patients during HSC mobilization, 20 infection events during HSCT hospitalization, and another 9 infection events after HSCT (Burt et al., 2006). In the European experience, 22 infection events occurred in 53 patients (Jayne et al., 2004). However, in the present long-term follow-up report of MSCT, 11 infection events occurred in 81 patients. Just a small proportion of patients was given CYC for pre-treatment. For the safety of the patients and also because most of the patients were unresponsive to CYC before MSCT, we chose a much lower dose of CYC compared with the HSCT protocol. The lower rate of infection may due to the taper of low-dose CYC before MSCT, or because only a small number of patients received low-dose CYC as pre-treatment while the others did not, so they were less prone to be infected compared with HSCT.

Another potential concern is tumor formation. A meta-analysis published by the Canadian Critical Care Trials Group showed no association between autologous or allogeneic MSC administration and tumor formation in the 36 studies reviewed (Lalu et al., 2012). In a normal environment, no new-onset tumor formation occurred after MSCT (Wang et al., 2012). Our long-term follow-up study showed that one patient suffered bladder cancer 4 years after MSCT, and she eventually died of cancer. It is reported that long-term therapy with immunosuppressive drugs, such as CYC, is correlated with tumor formation, especially bladder cancer (Baydar et al., 2009), and this patient had a total dose of 22.4 g of CYC before MSCT. Because of the lack of a control group, it is uncertain whether the occurrence of bladder cancer is related to MSCT or immunosuppressive therapy.

The present study has some intrinsic limitations. We still lack a control group of patients with conventional therapies but not combined with MSCT. Therefore, the current data only provide evidence that MSCT could induce disease remission on the basis of other drugs taken by patients enrolled in this study. On the other hand, however, all the enrolled patients suffered severe and/or conventional drug-resistant disease at baseline, and at that time if the patients were assigned to the control group, the disease would have further progressed. In the coming phase II–III and controlled study, we will recruit new-onset and active lupus patients and further confirm whether the addition of MSCT is prior to conventional therapies only. Second, 39 patients had CYC pre-treatment before MSCT, and it is true that pre-treatment by CYC may interfere with clinical results. However, our previous animal studies had demonstrated that the addition of CYC before MSCT did not enhance clinical efficacy in MRL/lpr lupus mice (Zhou et al., 2008). Furthermore, in a previous single-center analysis, we had shown that there was no difference in efficacy between SLE patients with and without CYC treatment

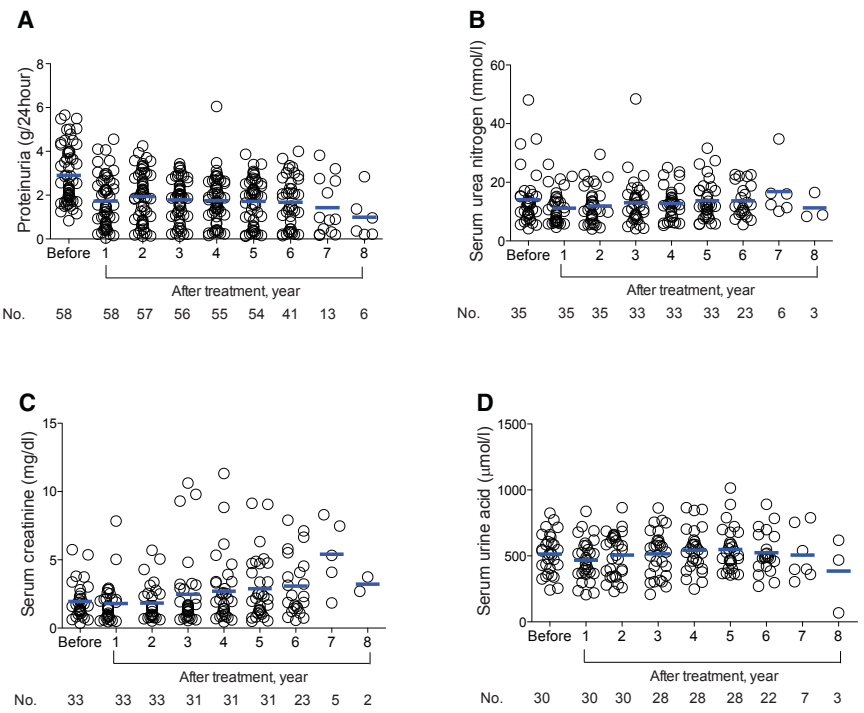

Figure 3. Changes of Proteinuria and Renal Function Before and After Mesenchymal Stem/Stromal Cell Transplantation

Twenty-four hour proteinuria significantly decreased at 1, 2, 3, 4, 5, 6, 7, and 8 years' follow-up (A). Serum urea nitrogen (B), creatinine (C), and urine acid (D) showed no significant change before versus after MSCT.

before MSCT (Wang et al., 2013). So whether the low dose of CYC pre-treatment indeed influences the effect by MSCT is not clear. Third, among all the 66 patients in the last follow-up, 51 were on low-dose immunosuppressive drugs combined with glucocorticoid. It is possible that maintenance therapy may affect MSCT efficacy. However, the doses of steroid as well as immunosuppressive drugs were tapered after MSCT, so the clinical efficacy may likely not be due to concomitant drug but to MSCT. Some cases in previously published studies were included in the present study; however, the present study is more focused on the long-term follow-up results, notably the survival rate and disease remission rate at the 5-year time point. The specific inclusion criterion here is that all patients completed at least 5 years of follow-up and all the results were not published previously. Two types of MSCs were used and the clinical data were analyzed together. The inclusion criteria for both BM- and UC-MSCT are the same. For the setup of this clinical trial, we first used BM-MSCs for patient treatment, but finally found that many patients did not have appropriate BM donors. We then tried to use UC-MSCs based on the beneficial effect in lupus mouse models by using the same patient inclusion criteria. We consulted an earlier publication and analyzed the results of two different MSCTs; however, we did not find any difference in ameliorating disease activity between BM- and UC-MSCT (Wang et al., 2013). Therefore, in the present long-term follow-up analysis, we did not analyze these separately.

In conclusion, this long-term follow-up study provides evidence that allogeneic MSCT had at least comparable if not better clinical efficacy than HSCT, but with fewer adverse events and significantly lower cost in treating drug-refractory active SLE patients.

EXPERIMENTAL PROCEDURES

Patient Enrollment

This was a pre-designed open-label phase II clinical trial to observe the long-term safety, as well as efficacy, of BM- or UC-MSCs in treating conventional treatment-refractory SLE patients. From March 2007 through October 2010, 81 SLE patients refractory to conventional therapies were enrolled in an MSCT trial at The Drum Tower Hospital (Nanjing, China) after signing informed consent. The study was approved by the Ethics Committee at the Drum Tower Hospital of Nanjing University Medical School and registered in ClinicalTrials.gov (identifiers: NCT00698191 and NCT01741857). All enrolled patients met at least 4 of the 11 American College of Rheumatology criteria for SLE, with an SLEDAI score of more than or equal to 8 or with at least one British Isles Lupus Assessment Group (BILAG) grade A or at least two BILAG grade B manifestations. All the patients were unresponsive to previous treatment with one or more successive or simultaneous conventional immunosuppressive drugs (CYC 500–750 mg/m^2/month, mycophenolate mofetil $\geq$1,000 mg/day, leflunomide 20 mg/day, azathioprine 100 mg/day, alone or in combination for more than 6 months) or had continuing requirement for a daily dose of $\geq$20 mg of prednisone or its equivalent. Patients were excluded from the study if they had the following

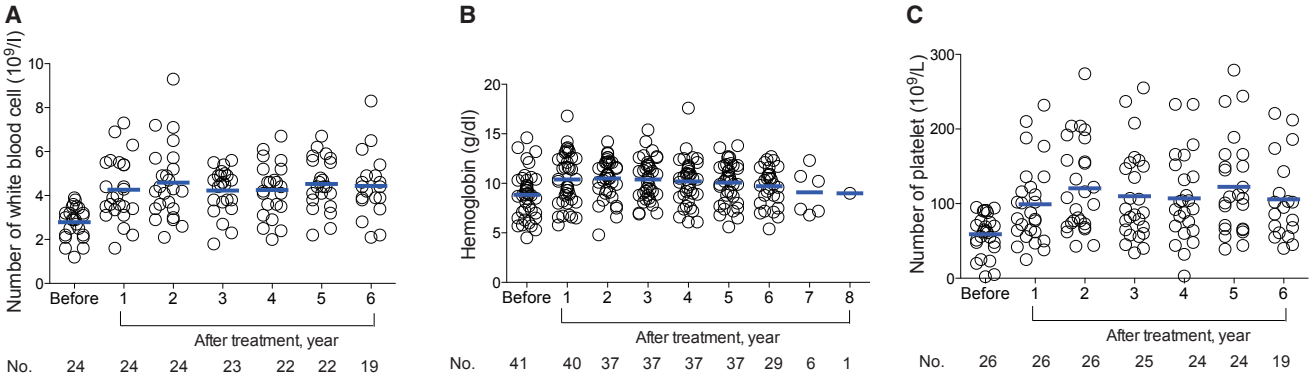

Figure 4. Changes of Hematologic Indices Before and After Mesenchymal Stem/Stromal Cell Transplantation
Peripheral white blood cell count significantly increased at 1, 2, 3, 4, 5, and 6 years' follow-up (A). Levels of hemoglobin increased, with statistical significance at 2 years (B). Peripheral blood platelet number was significantly elevated at 2, 3, and 5 years' follow-up (C).

conditions: (1) contraindication to MSCs at the time of screening such as infection, including pneumonia (bacterial, virus, or fungal), pulmonary tuberculosis, hepatitis B and C, skin infection, CNS infection; (2) severe organ dysfunction such as heart failure New York Heart Association functional classification III or IV, hepatic failure, renal failure, or respiratory failure; and (3) woman who were pregnant or lactating, or a woman or man who intended to initiate a pregnancy in the following 6 months. Both BM- and UC-MSCT patients had to meet the same inclusion and exclusion criteria.

MSC Preparation

BM-MSCs were isolated from bone marrow aspirates obtained from healthy donors with informed consent (22 donors: 9 female and 13 male, average age 30.7 ± 9.1 years; range 17–43 years). The donors were usually the patient's relatives. Each recipient had one BM-MSC donor. BM mononuclear cells were separated and then cultured with low-glucose DMEM (DMEM-LG) containing 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin in a humidified incubator at 37°C under 5% CO_2. Non-adherent cells were removed when the medium was exchanged on the third day. When the primary MSCs had expanded to 80% confluence, they were harvested and expanded to reach the treatment dose based on the body weight of the recipient.

UC-MSCs were prepared by the Stem Cell Center of Jiangsu Province (Beike Bio-Technology). UC-MSCs for each recipient were from one donor, but if the patient relapsed and received another UC-MSCT, the cells came from a different donor. The cords were rinsed in PBS with added penicillin-streptomycin, the cord blood being removed during this process. The washed cords were cut into 1-mm^2-sized pieces and floated in DMEM-LG containing 10% FBS. The pieces of cord were subsequently incubated at 37°C in humid air with 5% CO_2. Non-adherent cells were removed by washing. The medium was replaced every 3 days after the initial plating. When well-developed colonies of fibroblast-like cells appeared after 10 days, the cultures were trypsinized and passaged into a new flask for further expansion.

Cells of passages 2–5 were used for clinical treatment. Before infusion, cell viability was determined by trypan blue testing. The culture supernatant was analyzed for pathogenic microorganisms. Supernatant levels of alanine aminotransferase, endotoxins, and virus indices were determined. Cell surface labeling markers, including CD29 (catalog no. [Cat#] 11-0291-82), CD73 (Cat#11-0739-42), CD90 (Cat#11-0909-42), CD105 (Cat#17-1051-82), CD45 (Cat#12-9459-42), CD34 (Cat#12-0349-42), CD14 (Cat#12-0149-42), CD79 (Cat#12-0792-42), HLA-DR (Cat#17-9956-42), and their negative controls IgG2ak (Cat#17-4732-81) and IgG1k (Cat#12-0792-42), were studied by flow-cytometric analysis. We used good manufacturing practice conditions and clinical grade reagents to prepare the cells, and the protocol was conducted in compliance with GCP standards.

Criteria for release of MSCs for clinical use included spindle-shaped morphology, absence of visible clumps, and absence of cell supernatant contamination by pathogens as well as by virus for hepatitis B surface antigen, hepatitis B core antibody, hepatitis C virus antibody, HIV antibodies I and II, cytomegalovirus IgM, and syphilis antibody, and cell viability greater than 92%. Immunophenotype analysis indicated that the cultured MSCs had positive expressions of CD73, CD105, CD90, and CD29 (>90%) and negative expressions of CD45, CD34, CD14, CD79, and HLA-DR (<2%).

MSC Transplantation

Before MSCT, patients were administered CYC (10 mg/kg per day) intravenously on days −4, −3, and −2. If the patient had severe baseline disease conditions such as low serum albumin (<2.5 g/dL) or high serum creatinine (>3.4 mg/dL), or severe leukopenia (white blood cell count <2,000/μL), CYC was not used. All patients underwent MSCT and 1 million cells per kilogram of body weight were administered by intravenous infusion, without adding steroid or other immunosuppressive drugs. Multiple infusions of MSCs were permitted if disease relapsed after the previous infusion, employing the same dose (but not necessarily the same source) of cells for each infusion. When the patient received a repeated MSCT, no CYC for pre-treatment was given. After MSCT, patients returned for scheduled follow-up at 1, 3, 6, and 12 months and then yearly thereafter. Medical history, physical examination, serologic testing, and necessary imaging studies were

performed during follow-up visits for evaluation of SLEDAI scores. The investigators assessed and recorded adverse events and their severity throughout the study.

Outcome Characteristics

The primary endpoint was 5-year overall survival. Secondary endpoints included complete and partial clinical remission, and disease relapse at 5-year follow-up. The definitions of complete clinical remission, partial remission, and disease relapse are given in Supplemental Experimental Procedures and Jayne et al. (2004).

Statistical Analysis

Rates of overall survival, complete clinical remission, and disease relapse at different visit times were analyzed by a Kaplan-Meier survival curve. Pairwise comparisons of pre- and post-MSCT variables were analyzed by paired t test analysis using statistical software (SPSS 13.0; IBM, Armonk, NY, USA). To find the possible factors that affect disease relapse, we used the univariate Cox proportional hazards model. Statistical significance was set at $p < 0.05$ and was adjusted by the Bonferroni method to allow for multiple comparisons.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and three tables and can be found with this article online at https://doi.org/10.1016/j.stemcr.2018.01.029.

AUTHOR CONTRIBUTIONS

L.S. and S.S. had full access to the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. G.G., D.F., S.S., and L.S. designed the study; D.W., J.L., H.Z., H.W., B.H., X.F., and L.S. acquired patients' clinical data; D.W., H.Z., J.L., and L.S. analyzed and interpreted the data; D.W. and H.Z. performed the statistical analysis; D.W., S.S., G.G., D.F., and L.S. wrote the manuscript.

ACKNOWLEDGMENTS

The study was supported by the Major International (Regional) Joint Research Project (81720108020), the National Natural Science Foundation of China (91442119 and 81401347), the Jiangsu Province Major Research and Development Program (BE2015602), Jiangsu Province 333 Talent grant (BRA2016001), The National Key Research and Development Program of China (2017YFC0909003), the Jiangsu Province Six Talent Project (WSN-156), and the Nanjing Outstanding Youth Foundation (JQX17004).

Received: June 17, 2017
Revised: January 24, 2018
Accepted: January 24, 2018
Published: February 22, 2018

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