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ORIGINAL ARTICLE

Long-term safety of umbilical cord mesenchymal stem cells transplantation for systemic lupus erythematosus: a 6-year follow-up study

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Abstract The aim of this study is to assess the long-term safety of allogeneic umbilical cord mesenchymal stem cells (UC MSCs) transplantation for patients with refractory systemic lupus erythematosus (SLE). Nine SLE patients, who were refractory to steroid and immunosuppressive drugs treatment and underwent MSCs transplantation in 2009, were enrolled. One million allogeneic UC MSCs per kilogram of body weight were infused intravenously at days 0 and 7. The possible adverse events, including immediately after MSCs infusions, as well as the long-term safety profiles were observed. Blood and urine routine test, liver function, electrocardiogram, chest radiography and serum levels of tumor markers, including alpha fetal protein (AFP), cancer embryo antigen (CEA), carbohydrate antigen 155 (CA155) and CA199, were assayed before and 1, 2, 4 and 6 years after MSCs transplantation. All the patients completed two times of MSCs infusions. One patient had mild dizzy and warm sensation 5 min after MSCs infusion, and the symptoms disappeared quickly. No other adverse event, including fluster, headache, nausea or vomit, was observed. There was no change in peripheral white blood cell count, red blood cell count and platelet number in these patients after followed up for 6 years. Liver functional analysis showed that serum alanine aminotransferase, glutamic-oxalacetic transaminase, total bilirubin and direct bilirubin remained in normal range after MSCs infusions. No newly onset abnormality was detected on electrocardiogram and chest radiography. Moreover, we found no rise of serum tumor markers, including AFP, CEA, CA125 and CA199, before and 6 years after MSCs infusions. Our long-term observational study demonstrated a good safety profile of allogeneic UC MSCs in SLE patients.

Keywords Mesenchymal stem cells · Umbilical cord · Systemic lupus erythematosus · Safety

Introduction

Mesenchymal stem/stromal cells (MSCs) are fibroblast-like multipotent cells that can be derived from a variety of adult and fetal tissues (e.g., bone marrow, fat, cord blood, umbilical cord, fetal lung, placenta, amniotic fluid, synovium and dental pulp). Currently, there are over 300 clinical trials evaluating MSCs therapeutic utility in a variety of diseases including osteoarthritis, wound healing, degenerative disk disease and autoimmune disorders [1, 2]. Systemic lupus erythematosus (SLE) is a remarkable and challenging disorder with diverse of clinical features and complex factors of causes (genetic, hormonal and environmental). In the past 10 years, we have observed the characteristics of MSCs from SLE patients and found some abnormalities of autologous bone marrow-derived MSCs [3]. And then we used allogeneic but not autologous MSCs transplantation (MSCT) for severe and drug-resistant lupus patients, which showed satisfactory clinical efficacy [4, 5].

Before the clinical application of MSCs, its safety profile should be strictly concerned. In animal model

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experiments, it was shown that 10 million UC MSCs per kilogram of body weight administered intravenously into cynomolgus monkeys did not affect the general health of the recipient [6]. Furthermore, a more dose of adipose tissue-derived MSCs (2.5×10^8 cells/kg body weight) infusion into SCID mice showed no adverse events [7], demonstrating a safety profile of allogeneic MSCs infusion. Our previous studies showed that both human bone marrow and umbilical cord-derived MSCs infused intravenously into MRL/lpr lupus mice were safe and no treatment-related death [8, 9]. However, the long-term safety profile of MSCs transplantation (MSCT) is unknown.

Allogeneic MSCT in lupus mice and humans could change the immune cell imbalance in vivo, including T helper 17 cells, regulatory T cells, plasma cells [10–12]. Recently, Liu et al. [13] showed that allogeneic MSCT could rescue autologous bone marrow MSCs function and ameliorate osteopenia in Fas-deficient-MRL/lpr mice, which could change the epigenetic cascade in vivo. So the long-term safety of MSCT in human must be emphasized and investigated.

Materials and methods

Patients' enrollment

To ensure that all the patients completed 6-year follow-up, nine SLE patients ranging from 20 to 46 years old were enrolled in this trial. They received allogeneic MSCs transplantation from January 2009 to September 2009. All enrolled patients met at least 4 of the 11 American College of Rheumatology criteria for SLE [14]. The eligibility criteria included treatment-refractory and active disease, as well as a Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) score of more than eight or at least one British Isles Lupus Assessment Group (BILAG) grade A or at least two BILAG grade B manifestations. Refractory treatment meant lack of response to treatment with monthly intravenous pulse cyclophosphamide (CYC, 500–750 mg/m^2) for $\geq$6 months or lack of response to treatment with oral mycophenolate mofetil (MMF, $\geq$1000 mg/day) or leflunomide (20 mg/day) for $\geq$3 months, or continued daily doses of $\geq$20 mg of prednisone or its equivalent [15, 16]. Patients were excluded from the study if they had the following conditions: (1) infection: including pneumonia, pulmonary tuberculosis, hepatitis, skin infection, central nervous system infection; (2) heart failure: New York Heart Association functional classification III or IV; (3) failure of one of the following vital organs: hepatic failure, renal failure, respiratory failure; (4) woman who was pregnant or lactating, and woman or man who intend to have a child in the following 6 months. Informed consent was obtained from each patient and donor.

Umbilical cord MSCs preparation and infusion

UC MSCs were prepared by the Stem Cell Center of Jiangsu Province, which is the National Stem Cell Institute in China and a member of the International Society for Cellular Therapy (ISCT). The Stem Cell Center was also certified by American Association of Blood Banks (AABB). The umbilical cords (UCs) were rinsed twice in phosphate-buffered saline (PBS) in penicillin and streptomycin, and the cord blood was removed during this process. The washed umbilical cords were cut into 1-mm^2 pieces and floated in low-glucose Dulbecco's modified Eagle's medium (DMEM) containing 10 % fetal bovine serum (FBS). The pieces of UCs were subsequently incubated at 37 °C in a humidified atmosphere consisting of 5 % CO_2. Nonadherent 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 about 10 days, the cells were trypsinized and passaged into a new flask for further expansion.

Cells from passage 2 were cryopreserved in dimethyl sulfoxide (DMSO, the ratio of DMSO to FBS was 1:9). When the patients were selected for MSCs transplantation in clinic, the cryopreserved MSCs were recovered by fast water bath and washed by PBS containing 10 % FBS for three times. Then the MSCs were cultured in DMEM containing 10 % FBS. Before clinical transplantation, the cells were trypsinized and cell viability was determined by trypan blue testing. The culture supernatant was analyzed for pathogenic microorganisms by direct cultivation analysis. Supernatant levels of alanine aminotransferase and endotoxins for each cell preparation were determined using an automatic biochemistry analyzer and by tachypleus amebocyte lysate analysis, respectively. In addition, supernatant virus indexes were determined by enzyme-linked immunosorbent assay. Cell surface labeling markers, including CD29, CD73, CD90, CD105, CD45, CD34, CD14, CD79 and human leukocyte antigen major histocompatibility complex class II molecule, DR haplotype (HLA-DR), were studied by flow cytometric analysis (FCM). We used good manufacturing practice conditions and clinical-grade reagents to prepare the cells, and the protocol was conducted in compliance with good clinical practice (GCP) standards. One million cells per kilogram of body weight were administered by intravenous infusion on days 0 and 7. The protocol was approved by the Ethics Committee at the Drum Tower Hospital of Nanjing University Medical School and registered in *ClinicalTrial.gov* (identifier: NCT01741857).

Clinical safety evaluations

All the patients were followed up after MSCT. Their clinical status and psychological questionnaire data were collected by two members of staff in our department. The possible adverse events, including immediately after MSCs infusion, and the long-term safety profiles were observed. All the patients were allowed to report any discomfort at any time to the doctors after MSCT. Blood routine test, liver function, electrocardiogram, chest radiography and serum levels of tumor markers, including alpha fetal protein (AFP), cancer embryo antigen (CEA), carbohydrate antigen 155 (CA155) and CA199, were determined before and 1, 2, 4 and 6 years after MSCT.

Disease activity assessments

The disease activity index was also assessed after MSCT. Complete clinical response was defined as SLEDAI score <3 and steroid requirement ≤ 10 mg/day of prednisone or its equivalent, combined with BILAG D scores or better in all organs but not hematological system. Complete remission for hematological system was defined as white blood cell count $>4000/\mu l$, hemoglobin >11 g/dl, platelet count $>100{,}000/\mu l$ and steroid maintenance ≤ 10 mg/day of prednisone or its equivalent. Partial clinical response was defined as achieving BILAG C scores or better and maintaining this response without a new BILAG A or B score. Disease relapse was defined as an increase in SLEDAI score ≥ 3 from the previous visit or experience 1 new domain with a BILAG A score or 2 new domains with a BILAG B score after a previous response [15, 16]. Lupus serologic changes, systemic evaluations such as renal functional indexes and hematological involvements were measured at each visit time. Transplantation-related mortality included all deaths associated with MSCs transplantation, except those related to recurrence of underlying disease.

Statistical analysis

Data were analyzed as of last data collection in September 2015. Patients were censored at the time of death or last follow-up. We used Fisher's exact test to compare distribution of categorical variables. Pairwise comparisons of pre- and post-MSCT variables were analyzed by paired *t* test analysis using statistical software (SPSS 13.0). All *P* values were two-sided, and $P < 0.05$ was considered statistically significant.

Results

Patients' baseline characteristics

All patients received two times of MSCs infusion with one-week interval. Patients' baseline characteristics are shown in Table 1. Before MSCs transplantation, no steroid or antihistamine drugs were used.

UC MSCs characteristics and quality control

All the infused UC MSCs were derived from passage 2 to passage 5, with rigorous purification and quality control. Cell viability of purified MSCs was >92 %, and each preparation was negative for pathogenic microorganisms, including aerobic and anaerobic bacterium, and negative for HBsAg, HBcAb, HCVAb, HIVAb-I and HIVAb-II, CMV-IgM and syphilis-Ab. FCM showed that CD29, CD73, CD90 and CD105 expressions were >95 %, in parallel with CD45, CD34, CD14, CD79 and HLA-DR expression <2 %. In addition, levels of alanine

Table 1 Clinical manifestation for each patient at baseline

Patients	Sex	Age	Disease duration, months	Baseline SLEDAI	Cell number (million)	Baseline BILAG	Clinical manifestations
01	F	46	40	17	51	12	LN, A, C, V, H, ANA+, anti-dsDNA+
02	F	37	41	12	50	12	A, LN, V, ANA+, anti-dsDNA+, H
03	F	21	50	11	62	9	V, LN, C, anti-SM+
04	F	28	98	9	52	9	V, A, alopecia, LN, C, ANA+, anti-dsDNA+
05	F	26	120	12	50	8	V, A, LN, ANA+, anti-dsDNA+
06	F	23	15	14	51	19	V, A, F, LN, P, ANA+, anti-dsDNA+
07	F	20	62	12	57	18	A, F, LN, C, P, ANA+
08	F	43	26	34	63	20	C, V, LN, A, seizures, ANA+
09	F	36	97	10	54	26	C, V, A, LN, P, ANA+

A arthralgia, *F* febrile, *H* hypocomplementemia, *LN* lupus nephritis, *V* vasculitis, *P* polyserositis, *C* cytopenia, *ANA* antinuclear antibody, *anti-dsDNA* anti-double-strand DNA antibody

aminotransferase and endotoxin in supernatant of each cell preparation were strictly controlled within 40 IU/L and 5EU, respectively. The capacity of MSCs that differentiate along adipogenic and osteogenic lineages was also assayed. MSCs were administered intravenously within 10 min each transplantation.

Immediate events associated with MSCs infusion

One patient felt mild dizzy and warm sensation 5 min after first MSCT, no headache, fever or other discomfort appeared. She was announced to have a rest, and the symptoms were spontaneously disappeared 10 min later. No discomfort appeared at the second infusion. No pruritus, headache, nausea, vomit, rash or chest tightness and pain occurred immediately and 48 h after MSCs infusions for all patients.

Delayed adverse events during follow-up

One patient had lupus nephritis for 16 years. She stopped all the medicine by herself and underwent disease flare with high levels proteinuria, hypoproteinemia, facial erythema, arthralgia and finger vasculitis. She received steroids (18 months) as well as immunosuppressive drugs treatment (CYC 0.6 g/2 weeks, for 9 months and then MMF 1.5 g/day, for 9 months), but had no clinical response. Umbilical cord MSCs were infused, and no discomfort occurred. Proteinuria slightly decreased and serum albumin increased. Six months later, she underwent pneumonia with fever, cough and expectoration, also had disease relapse with anasarca, proteinuria and cytopenia. She suffered from severe pneumonia and did not respond to antibiotics treatments. Ultimately, she died of respiratory failure 192 days after MSCs infusion.

There were 14 adverse events recorded in the remaining 8 recipients during 6-year visit, including 10 times upper respiratory infection, 2 times urinary tract infection and 2 times skin infection. Two patients had moderate herpesvirus infection 291 and 135 days after MSCs treatment, respectively. They were both treated by antiviral drugs. All adverse events resolved during follow-up. No headache or pain of neck, shoulder, muscular and chest was found. No patient experienced serious complications.

Routine test of blood and urine

Blood and urine routine tests were performed before and 1, 4 and 6 years after MSCs transplantation. We found there was no significant change in white blood cell count [before $(3.67 \pm 0.61) \times 10^9$/L, 1 year $(3.84 \pm 0.70) \times 10^9$/L, 4 years $(3.86 \pm 0.40) \times 10^9$/L, 6 years $(4.13 \pm 0.49) \times 10^9$/L, Fig. 1a], red blood cell count [before $(4.81 \pm 2.17) \times 10^{12}$/L, 1 year $(5.03 \pm 2.17) \times 10^{12}$/L, 4 years $(5.43 \pm 1.78) \times 10^{12}$/L, 6 years $(5.55 \pm 1.70) \times 10^{12}$/L, Fig. 1b] and platelet count [before $(164 \pm 53) \times 10^9$/L, 1 year $(175 \pm 75) \times 10^9$/L, 4 years $(221 \pm 69) \times 10^9$/L, 6 years $(186 \pm 56) \times 10^9$/L, Fig. 1c] before and after MSCT. Urine regular test showed all the patients with negative urine glucose, urobilinogen, urine bilirubin, urine acetone bodies and uric acid salt before and during all follow-up periods.

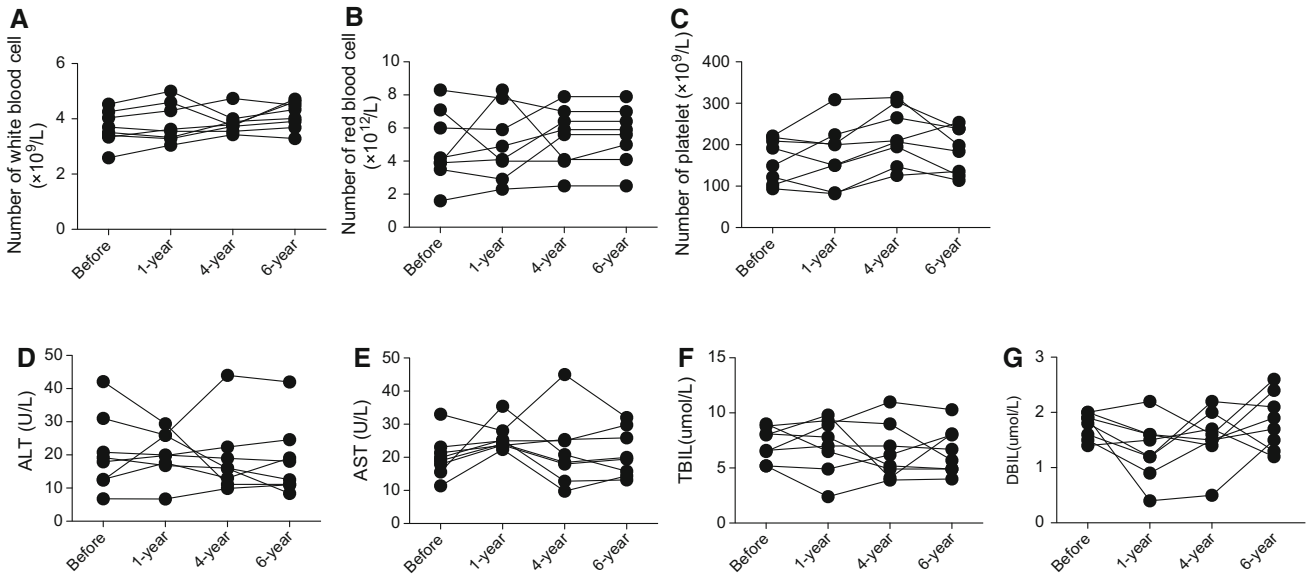

Fig. 1 Assessments of changes in peripheral white blood cell (**a**), red blood cell (**b**), platelet (**c**), alanine aminotransferase (*ALT*, **d**), glutamic-oxalacetic transaminase (*AST*, **e**), total bilirubin (*TBil*, **f**) and direct bilirubin (*DBil*, **g**) *before* and *after* umbilical cord mesenchymal stem cells (MSCs) transplantation

No patient had abnormal liver function before MSCT, and no newly onset liver injury was found after 6-year follow-ups. Liver functional tests showed that there was no change in serum alanine aminotransferase (before 20.35 ± 11.34 U/L, 1 year 20.28 ± 7.09 U/L, 4 years 18.91 ± 10.93 U/L, 6 years 18.35 ± 10.95 U/L, Fig. 1d), glutamic-oxalacetic transaminase (before 20.16 ± 6.31 U/L, 1 year 25.94 ± 4.17 U/L, 4 years 21.91 ± 10.77 U/L, 6 years 21.33 ± 7.13 U/L, Fig. 1e), total bilirubin (before 7.18 ± 1.52 μmol/L, 1 year 7.08 ± 2.48 μmol/L, 4 years 6.41 ± 2.49 μmol/L, 6 years 6.58 ± 2.11 μmol/L, Fig. 1f) and direct bilirubin (before 1.76 ± 0.23 μmol/L, 1 year 1.33 ± 0.54 μmol/L, 4 years 1.55 ± 0.50 μmol/L, 6 years 1.84 ± 0.51 μmol/L, Fig. 1g) before and after MSCT.

Chest radiography and electrocardiogram test

Before MSCT, chest radiography showed increased lung texture in both sides for six patients, and two other patients showed medium pericardial effusion. At 1-year follow-up, four patients showed increased lung texture, one patient showed old pathological changes in both sides, and no patient showed pericardial effusion. At 4- and 6-year follow-up, one and two patients showed increased lung texture, respectively. Electrocardiogram test showed ST-T changes for four patients before MSCT. At 1-year follow-up, two patients showed ST-T changes. At 4- and 6-year follow-up, one and two patients showed ST-T changes, respectively, also with one patient showing nodal tachycardia at 6-year visit. UCG demonstrated that two patients had pericardial effusion at baseline and disappeared at 1-, 4- and 6-year visits. No newly onset pericardial effusion, valvular regurgitation or inadequacy, or pulmonary hypertension was found.

Serum tumor-related markers test

In addition to the liver, renal, lung and heart functional test, we also detected serum tumor-related markers for our patients. Our results showed that serum alpha fetal protein (AFP, before 4.03 ± 3.22 ng/ml, 1 year 3.95 ± 2.44 ng/ml, 4 years 3.08 ± 2.10 ng/ml, 6 years 3.32 ± 2.18 ng/ml), cancer embryo antigen (CEA, before 1.14 ± 0.81 ng/ml, 1 year 1.14 ± 0.73 ng/ml, 4 years 1.16 ± 0.79 ng/ml, 6 years 0.93 ± 0.69 ng/ml), carbohydrate antigen 125 (CA125, before 14.48 ± 6.81 IU/ml, 1 year 17.84 ± 6.20 IU/ml, 4 years 14.66 ± 5.71 IU/ml, 6 years 15.56 ± 4.81 IU/ml) and CA199 (before 6.66 ± 4.56 U/ml, 1 year 6.75 ± 4.74 U/ml, 4 years 6.63 ± 3.93 U/ml, 6 years 5.30 ± 3.15 U/ml) had no change before and 6 years after allogeneic UC MSCs infusions (Fig. 2a–d).

Patients' clinical response to MSCT

Two patients showed complete clinical response after MSCT and another five patients showed partial clinical response, in which one patient relapsed. The remaining two patients showed no response to MSCT, in which one patient had renal dialysis 3 times a week 3 years after MSCs transplantation (Table 2). Because of the small subset of patients in each group, we did not analyze the relation between safety index and clinical response.

Discussion

Over the past decade, accumulating evidence has shown that MSCs have the potential to exert protective and reparative effects in a variety of disease settings. Clinical trials are being increasingly established to investigate the therapeutic potential of these cells. Allogeneic MSCs have already been applied in clinical treatment for acute graft-versus-host disease (GvHD) following allogeneic hematopoietic stem cell transplantation (HSCT) [17, 18], ischemic cardiomyopathy [19, 20], and autoimmune diseases like systemic sclerosis [21], inflammatory bowel disease [22, 23], dermatomyositis/polymyositis [24], rheumatoid arthritis [25], Sjogren's syndrome [26] and type 1 or type 2 diabetes mellitus [27, 28]. The clinical efficacy varied among different studies; however, several safety concerns remain to be addressed.

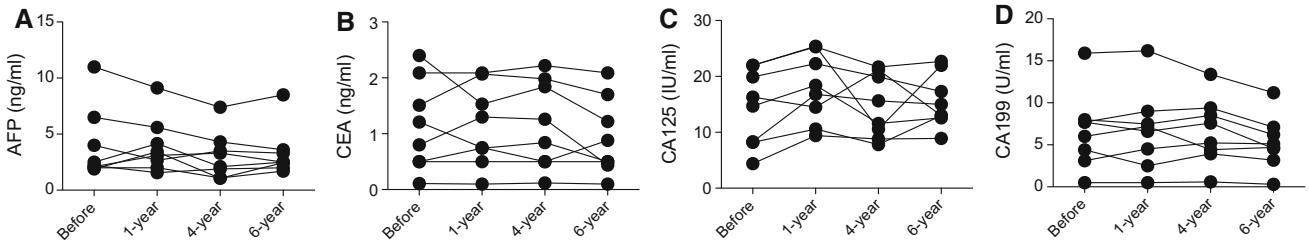

Fig. 2 Assessments of changes in serum alpha fetal protein (*AFP*, **a**), cancer embryo antigen (*CEA*, **b**), carbohydrate antigen 125 (*CA125*, **c**) and CA199 (**d**) *before* and *after* umbilical cord mesenchymal stem cells (MSCs) transplantation

Table 2 Treatments and patients' response before and after MSCT

Patients	Response to MSCT	SLEDAI at last visit	BILAG at last visit	Treatment at time of MSCT	Treatment at last visit
01	PR	4	7	Pred 20 mg/day, CYC 0.8 g/mo, LEF20 mg/day	Pred 5 mg/day, CYC 0.6 g/3mo, LEF 10 mg/day
02	PR	6	6	Pred 15 mg/day, CYC 0.8 g/mo, MMF 1.5 g/day	Pred 5 mg/day, CYC 0.6 g/mo
03	PR	4	6	Pred 20 mg/day, MMF 2.0 g/day	Pred 7.5 mg/day, MMF 1.0 g/day
04	CR	2	1	Pred 12.5 mg/day, MMF 1.5 g/day	Pred 5 mg/day, MMF 1.0 g/day
05	PR → R	8	12	Pred 20 mg/day, CYC 0.8 m/mo	Pred 5 mg/day, LEF 20 mg/day
06	NR	4	12	Pred 20 mg/day, CYC 0.8 g/mo	Pred 5 mg/day, renal dialysis
07	CR	2	1	Pred 20 mg/day, CYC0.8 g/mo, LEF 20 mg/day	Pred 10 mg/day, CYC0.6 g/mo
08	PR	NA	NA	Pred 15 mg/day, CYC1.2 g/mo, MMF 1.5 g/day	NA
09	NR	7	18	Pred 20 mg/day, CYC0.8 g/mo	Pred 15 mg/day, MMF 1.5 g/day

CR complete clinical response, *CYC* cyclophosphamide, *MMF* mycophenolate mofetil, *PR* partial clinical response, *Pred* prednisone, *NR* no clinical response, *NA* not available, *R* relapse

In the present study, one patient underwent mild dizzy and hot 5 min after the first MSCs infusion, and no discomfort appeared after the second infusion. This may be assessed as immediate infusion adverse event and not related to MSCs infusion. Other studies also reported transient fever [29], skin rash, headache, nausea and vomiting [30] for intravenous MSCs infusions. MSCs isolation, expansion, harvesting and cryopreservation should be performed under strict good manufacturing practice (GMP) conditions. In the different laboratories, final cell products have to undergo control quality tests before release, including viability, sterility, endotoxin content, mycoplasma contamination, FCM analysis, and tests to ensure genetic stability. Tarte et al. [31] showed the occurrence of aneuploidy in MSCs cultivation in vitro, but it was not related to culture process and had no transformation feature. In addition, clinical studies should be performed under ethically approved protocols. Serious adverse events (SAEs) and suspected unexpected serious adverse reactions (SUSARs) should be carefully observed and recorded [32].

The long-term adverse events reported in our study included one death and 14 infection events. However, it is clearly that the patient died of uncontrolled disease activity and organ failure. Lupus patients often took steroids as well as immunosuppressive drugs and were more likely to suffer infection, including upper respiratory infection, urinary tract infection and skin infection. In randomized clinical study of MSCs treating for multiple systematic atrophy, there was no significant difference for the infection events between treatment and control groups [30]. So the infection adverse events were not considered related to MSCs transplantations. Recently, Yang et al. [33] showed in their study that MSCs could defend against gammaherpesvirus infection via the cGAS-STING pathway. In pulmonary infection caused by Pseudomonas aeruginosa, adipose tissue-derived MSCs attenuate infection via inhibiting overproduction of prostaglandin E2 [34]. Moreover, human bone marrow MSCs are resistant to HBV infection during differentiation into hepatocytes in vivo and in vitro [35], so there is no evidence to support infection increase related to MSCs infusion.

The more concerning safety of allogeneic MSCT is tumor formation. Human bone marrow-derived MSCs increased the growth of ERAα-positive breast cancer cell lines in an in vitro three-dimensional tumor environment, but have had no effect on an ERAα-negative cell line [36]. Another study has shown that both human fetal MSCs and human adipose-derived MSCs transplanted subcutaneously into BALB/c-nu/nu mice alone or together with tumor cell lines F6 or SW480 (ratio 1:1 or 1:10), favored the growth of these tumor cell lines [37]. On the other hand, some antitumorigenic effects of MSCs were reported. Fetal skin or adipose tissue-derived MSCs inhibited the growth of human liver cancer cell lines, breast cancer (MCF-7), and primary leukemia cells by reducing their proliferation, colony formation and oncogene expression [38]. A meta-analysis published by Canadian Critical Care Trials Group showed no association between autologous or allogeneic MSCs administration and tumor formation in the 36 studies reviewed [39]. In a tumor microenvironment, MSCs were believed to play both, a pro-tumorigenic role and an anti-tumorigenic role. However, this is dependent on a host of factors like types of MSCs, its source, type of cancer cell line under investigation, in vivo or in vitro conditions, factors secreted by MSCs and interactions between MSCs, host's immune cells and cancer cells [40]. In a normal environment, no newly onset tumor formation occurred after MSCs infusions. On the other hand, our lupus patients were all refractory cases. Before MSCT, they all had long

time and high dose of different immunosuppressive drugs like CYC, MMF. These drugs, especially CYC, are tightly associated with tumor formation in patients [41]. So we need controlled and long-term follow-up study to further examine the influence of MSCT on tumor formation in patients. In the present study, we observed no tumor formation event after 6-year follow-up by two times of UC MSCs transplantations and no change in serum tumor biomarkers before and after transplantation, which partly illustrate the safety of clinical application of UC MSCs.

There are also some limitations of this study. Firstly, the number of this study is very small, and we need a much larger scale of study involving more patients to assess the safety profile of allogeneic MSCT. Secondly, the safety indexes that we used are somewhat limited, and we should apply the system organ class (SOC) for adverse event according to the Common Terminology Criteria [42].

In conclusion, here we demonstrate the long-term safety profile of UC MSCs infusion for drug-resistant SLE patients. Our data confirm that UC MSCs treatment is safe and no tumor-related adverse events related to the therapy in 6-year observation. A large number of patients treated with allogeneic MSCT and longer follow-up time need to be investigated before widespread clinical application of MSCT in SLE therapy.

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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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